Planarian Care – Tal Shomrat, 2011

General:

The planarians are being held mainly in1.8 Liter, plastic containers (manufactured by Rubbermaid, *Durable*)

The containers are kept **closed with lids** in order to reduce evaporation. I found this **very important** since evaporation will cause increase in the salinity which could kill the worms.

All containers are labeled and in general are divided to 2 **groups I &II**.

The worms are kept in 2 incubators with different conditions. Some are kept in **18°C** (the same temperature as the room) with cycle of weak neon light (9:00 am – 6:00 pm) and dark (6:00 pm – 9:00 am). The other are kept in **10°C** / 24hr Dark.

In contrast to the worms kept in 18°C, The worms that are being kept at 10°C, are less active (short exploratory phase during training, 30-60 minutes instead of ~3hr) and less likely to "drop" their tails. They will grow faster or save their body size, for few weeks, in case they won't be fed.

* When changing water **it's important** to take care that the water temp' of the fresh water will be equal to the old water in the containers. A way to do it is to take out the containers and place them outside in room temperature for a while or place container with fresh water inside the incubator.

Feeding:

Food

Liver: the main type of food we are using. We feed the worms with processed Organic Beef liver kept frozen in -80°C. The worms are readily fed on it and will grow fast. "If you want to fatten your worms up in a hurry, by all means feed them fresh liver …The difficulty with using liver or some other bloody meat is that the juice rapidly contaminates the water in which the planarians are kept. Thus, is you feed the animals liver, you must remove the residue and change the water within two or three hours after the meat is put in" (McConnell's Manual.1965,p.18).

Egg-Yolk: “The planarians readily ate the egg yolk and turned a beautiful yellow color after each meal. Most of the animals grew smaller and smaller rather than larger and larger, just as Hyman said they would. A few of the planarians (the ones that probably couldn't read) did seem to grow and thrive on a diet of egg yolk” (McConnell’s Manual.1965,p.18). I fed a group of dorotocephala with organic egg yolk and as it seems to me they do get smaller although readily ate the egg yolk and become yellowish.

Artimia (fresh-hatched brine) & Daphnia: Probably the most healthy and natural type of food for planarians. I found that they are readily fed on them and catch them by their slime. This kind of food is less polluting the water and can be left inside the planarians’ continuer for long time (even overnight and even longer). The Artimia are kept in **high salinity**, therefore be sure to rinse them with Rodi-water or Poland-spring before feeding the planarian. The Artimia will survive for at least 30 minutes in the fresh spring water (I’ve seen them survivor for even overnight, in spring water).

Arctic Copepods powder: from http://www.brineshrimpdirect.com/Arctic-Copepods-c195.html The worms didn’t find interest in this died food.

“**Planarians can be starved for months before die… A large, healthy planarian can live for six or more months without any food whatsoever”** (McConnell’s Manual.1965,p.18).

Feeding Procedure:

1. Place one small petri dish with organic beef liver (stored in the -80°C freezer) at room temperature till it defreezes (½-1hr).
2. Dispense small pieces of liver by 1-2 ml syringe with 18G needle. Make sure it sink and not float on the water surface.

- After most of the worms finish eating (it takes them 2-3 hours), **but before** their water get dirty, **BE SURE** to exchange their water. If you forget, the water will be contaminated and the worms will die.

--After feeding, their body color will change relative to the food. Liver will give them pinkish color and hardboiled egg yolk will make them yellowish. The change in body color could serve as good indication that the worm ate.

* write down on the calendar (on the wall) the date and the group of worms that have been fed.

Water Exchange:

1. Remove the livers by using plastic pipettes. Always use **new pipette** for each containers.
2. Push the floating worms down to the bottom of the tray by pipetting and wait for the worms to settle down. Pour out the water *carefully* into the chemical-free sink or bucket. If some worms get floating, pick them up (return into the trays later) or push them down by pipetting.
3. Pour small amount of fresh **species specific** water into the tray. Wash the bottom of the tray by pipetting. If some particles of livers are remaining, remove as completely as you can. And then pour water out carefully.
4. Drain the old water into the waste plastic jar which when full should drain into one of the lab sinks. Take care of cleaning the sink from liver waste.
5. By pipetting shift and concentrate worms in one corner/side of the box.
6. using unbleached paper towel (the brown towels), wipe down the bottom/sides of the container.
7. Refill with fresh water.

* Make sure **NOT** to wash away any worms. Use transfer pipette to keep them in the box.

In case that worm washed to the sewage bucket pull it out into a petri dish by pipette and rinse it **3 times** with fresh water before put it back to the worms containers. This procedure is done in order to avoid transference of contamination to the worms' containers.

8. Always left an area (one corner/side) with slime, never sweep it all. The slime contain protected factors that important for the worms health.

9. **2 days after the feeding day,** Change and clean all the containers (also the ones that haven't been fed). In that cleaning I change (usually, but if not sure it's better to clean) just the

water without cleaning the slime, depend on the thickness of the slime and the water condition in term of smell and clearance.

The Species we have and their specific requirements

Dugesia ***japonica*** (the main species I am using)

Advantage: easy to breed and maintain in the lab.

Possess outstanding regenerative capabilities

Very active and tolerant training procedures.

Disadvantage: strong tendency for spontaneous fission

In order to suppress spontaneous fission when kept the worm close to room temperature they need to be kept in continuous darkness and be fed at least once a week.

<u>However, Still</u> worms which are being kept in 10°C even if fed once every 2 weeks are fission less.

- Use **'Poland Spring' Natural SPRING WATER' (**pH 6-7), not distilled water or tap water.

Dugesia ***dorotocephala***

Advantage: Quite active (But less than *D.jap*) and the main model animal at the 60th

Disadvantage:

- Not so easy to maintain in the lab and not very tolerant training procedures (whith negative reinforcement) compare to *D.jap*.
- I found them very sticky and thus very hard to handle with a pipette or even with a paintbrush as suggested by McConnell. Frequently the worms stick to the inner side of the pipette and it's very hard to get them out, without disrupting the ATA chambers' meniscus, which could affect the Tracking quality.

*** Cannibalize** very readily; should be sorted to different sizes or fed frequently (once every 1-2 weeks).

The water I use is spring water with 0.6gr Alkaline Buffer /Liter.

http://www.seachem.com/Products/product_pages/AlkalineBuffer.html

***Water acidity should be pH 8-9**

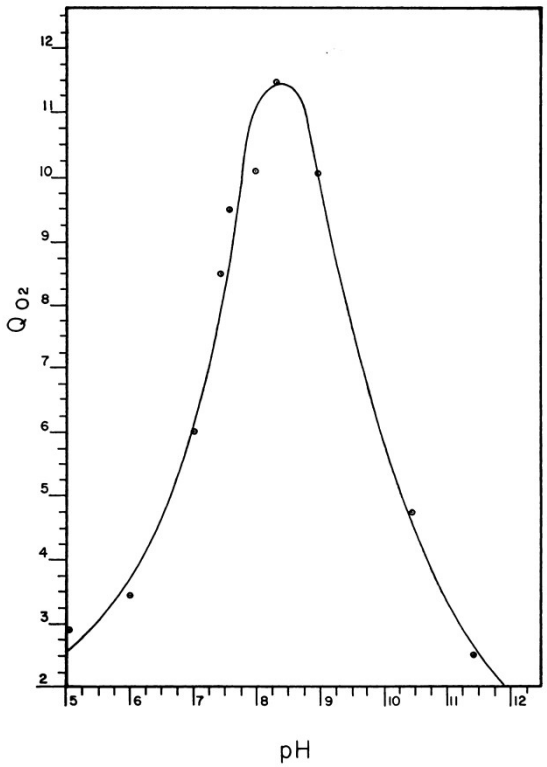

FIGURE 1. The effect of pH on succinoxidase activity in whole body homogenates of *Dugesia dorotocephala*.

in terms of optimum conditions for enzyme activity would be expected at the cellular level. It is possible, too, that the ability of planarians to convert their external medium to a constant pH may be correlated with the optimum activity of succinoxidase in these animals.

SUCCINOXIDASE ACTIVITY IN HOMOGENATES OF DUGESIA DOROTOCEPHALA. *Biol Bull* **127:** 317-323. (October 1964)

WILLIAM L. MENGEBIER and MARIE M. JENKINS

1. The presence of succinoxidase activity in homogenates of *Dugesia dorotocephala* was investigated, utilizing the Warburg technique.
2. Succinoxidase activity was found to be greaterin fed animals than in those starved for 48 hours.
3. Enzyme activity was found to be proportional to enzyme concentration; the activity of the enzyme was inhibited by the addition of KCN and urethane.
4. Optimum enzyme activity occurred at a pHof 8.3, a value considerably higher than the figure quoted forvertebrate tissue.

*For D. *dorotocephala,* I found it necessary (in order to keep them healthy and to prevent twisted tails) to exchange water more often (every second to third day). However, the slime should be cleaned just once a week (3/4 area).

planarians supplier: I ordered dorotocephala from **Carolina** (http://www.carolina.com/product/dugesia+dorotocephala%2C+living.do?keyword=dorotocephala&sortby=bestMatches) and from **Nasco** (http://www.enasco.com/product/LM00031M)the

worms from Carolina were more healthy than the worms from Nasco. The worms from both venders came with a very dark (black) color but after several days in the lab they became more brownish.

This phenomenon have been identified by other lab too:

"The dorsal surface is brown to almost black (animals kept in laboratory culture become lighter) "

Roman Kenk, The fresh-water triclads of Michigan,Ann Arbor,University of Michigan Press, Jan,1944. (**This paper is the best guide I've found for the taxonomy of the American planarians**).

Twisted-tail: Disease noticed in D.dorotocephala after reaching to a large size (>1.5cm when rest). The planarians develop a twisted-tail that seems to be dragged more than really controlled and moved by and with the worm's body. After a while the worm will dropped the tail which won't survive and regenerates.

In addition after chopping the worm none of the fragments except the Head survive. However chopped fragments from large worm (>1cm) even from healthy one with normal tail <u>usually</u> won't regenerate except of the head (personal experience).

*Schmidtea **mediterranea***

Use **specific** water made by Wendy/ Junji, can be found in their worms' room (worms 1) in plastic 15Liter carboys. **Shake well** the carboy before use in order to mix the added salts.

Advantage: easy to breed and maintain in the lab.

Have a small and stable diploid genome, which makes them the most suitable species for genetic approaches.

Disadvantage: less tolerant for training.

Not so active compare to the other two species.