UQBR Aquaria SOP 4 – Production and Maintenance of Zebrafish Diets

REQUIREMENT:

1. To ensure that the requirements and regulations as set out by the following are met as far as practicable:
    - AEU UQ
    - The Code
    - OGTR
    - Department of Agriculture and Fisheries (DAF)
    - QLD Workplace Health and Safety, and
    - UQ OH&S

2. To standardise practice for all UQBR staff and researchers within UQBR facilities.

3. Annual review is required to maintain best practice and usability of this SOP.

RESPONSIBILITY:

It is the responsibility of the individual performing animal handling procedures and techniques to ensure they have been assessed as competent.

Please Note:
This UQ Biological Resources (UQBR) SOP expands upon UQ Animal Ethics Unit SOPs. This document outlines the procedures followed by UQBR and should not be referenced in Animal Ethics Applications.

No changes or deviations from this SOP are to occur unless the Director of UQBR gives prior authorisation.

NB: The use of (*) indicates this statement is dependent on the facility procedures
NB: The use of (**) indicates this statement is dependent on AEC Approvals

OBJECTIVE:
Ensuring that Zebrafish are raised on the appropriate diet for their current stage of ontogeny, and fed an appropriate quantity of food, will ensure that they grow at the best rates possible and become active breeders at the earliest age possible

I. EQUIPMENT

Equipment Items

- Scales.
- Mixing Bucket.
- 1L Mating Tank.
- Food storage buckets.
- Falcon Tubes.
- Scissors.
- 5L plastic Beaker.
- Food clickers + tools.

Consumables

- NRD 3/5 (Commercially available dry marine diet)
- O.range Wean-S (Commercially available dry marine diet)
- O.range Start (Commercially available dry marine diet)
- Organic Spirulina powder
- Rotifers (Type-L marine (*Brachionus plicatilus*))
- Rotigrow Plus

Rotifer Equipment

- 25L Culture tub with tap and lid.
    - Lids should have clean filter pads attached to the bottom so that are suspended in the middle of the tub.
- Aerator and hosing.
- Marine Salt.
- Live, Type-L (*Brachionus plicatilus*) marine Rotifers for initial culture stock.
- Rotigrow Plus for maintaining cultures.
- RO water supply.
- Sieve 60µm and 120µm.
- 2 x 5L beakers/tubs.
- Virkon 1% solution. (10g virkon powder per liter to make 1% solution)

II. PREPARATION

III. PROCEDURE

Rotifer Background.

Type-L marine rotifers (*Brachionus plicatilis*) are small invertebrates capable of internally capsulizing algal cells in a process called bio-encapsulation. Their small size and ability to transfer a chosen nutrient profile, make them ideal for usage in larval polycultures to sustain larvae through the difficult first-feeding period. All of the rotifers cultured for usage in the UQBR Zebrafish aquatics facility are currently being produced on site to ensure fresh unlimited supply.

Figure 1- Rotifer Draining

RotiGrow Plus.

RotiGrow Plus OmegaBlend is a blend of 5 microalgae designed to produce a highly productive and very clean rotifer culture – while dramatically boosting Omega Fatty Acids and providing a diversity of other microalga nutrients.

Figure 2- Prepared mating tank for straining of adult and baby rotifers.

- **Procedure – Maintenance of Rotifers**

Note - Rotifers are best maintained with limited disturbance, this means limited harvesting and limited environmental manipulation.

Weekdays Rotifer Maintenance (Monday to Thursday)

Morning routing (AM):

1. Rotifer harvest, you need to drain 5L from each tub (refer to Figure 1)
    a. Open the tap, running the solution through a 120 µm sieve into a 5L plastic beaker.
    b. Carefully remove the sieve and pour contents into a second sieve inside a mating tank. (Refer to figure 2 and 3).
    c. Rinse out the remaining contents of the solution at the bottom of the first sieve by flushing with **3ppt** water into the mating tank.
    d. Top up with 3ppt to the ridge marks (400mls) this will dilute the concentration.

Figure 3- Straining adult rotifers into a prepared tank

2. Add 2mls of Algae (water will turn a bright green)
    a. Label these as ***Adults*** and set this tank aside in the main holding room for use in **polycultures.**

3. Pour remaining liquid from the beaker into the second 5L plastic beaker (See Appendix Photo 4)
    a. Setup the 60 µm sieve in one of the 5L plastic beakers.
    b. Pour remaining liquid.
    c. Rinse contents in the sieve with 3ppt water into mating tank.
    d. Pour remaining water down the drain.
    e. Complete for all tubs.

Figure 4- Straining of remaining water (left) before rinsing the strainer into the prepared tank (Right)

4. Add 1.5mls of Algae (water will turn a bright green)
    a. Label these as ***Babies*** and set this tank aside in the main holding room for use of **feeding fry/juveniles.**

5. Remove the lid from each tub and rinse with R.O water the attached filter pad (flock trap) to remove the built-up detritus and mulm.

6. Top up the 4 rotifer tubs with 5L of **15ppt** water, replacing what we have harvested.

7. Feed the tubs with Rotigrow plus algae, 12.5ml.

<u>Afternoon routine (PM</u>**<u>)</u>**

1. Feed 25ml of Rotigrow Plus to each tub.

<u>Weekly Rotifer Maintenance (Friday).</u>

Every Friday one tub receives a full clean. As the setup currently has 4 tubs in rotation, each tub therefore gets a full clean once every 4 weeks. Each tub is labeled with the date it was last cleaned.

1. For all 4 tubs follow rotifer maintenance Monday – Thursday. Follow steps 1-5 with all tubs and follow steps 6-7 with the 3 "youngest tubs."

2. For the oldest tub. You will repeat steps 1 and 3 until tub is empty (Refer to Figure 5). Note: This harvest process **must** be done with 15ppts (instead of the usual 3ppt) in order to minimize shock to the rotifers that will be used to seed the cleaned tub. Instead of only doing a single pass through each sieve you will do 2. **Do not** discard the old water as we need to keep 15L of the old water to re-seed the culture.
    a. Keep aside the *Adult*s labeled ***Tub # and date*** in a separate tub from the ones harvested for daily maintenance.

3. Once drained, disconnect the tub from the aerator:
    a. To disconnect, simply unplug the hosing at the small black regulator tap and turn off the air supply.

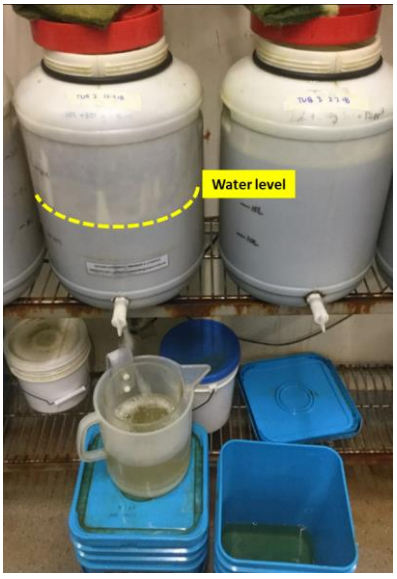

Figure 5- Complete draining of rotifer tub

4. Begin rinsing the tub out in a sink with RO water.

5. Using RO water and old pre filter pad from the tub to remove all detritus and mulm from the sides and bottom of the tub.

6. Using RO water spray the air diffuser (black cross piece that weights down airline) to remove any build up within the tubes.

7. Fill the tub with RO and virkon to make a 1-2% virkon solution. Leave for at least *10* minutes.

8. Rinse out the culture tub with RO water to completely remove any residue of Virkon. Make sure to run RO through the tap.
9. Return tub to the culture rack and reattach the air hose, to keep the aerator at the bottom of the tub insert on of the ends into the tap.

10. To re-seed the tub:
    a. Add 15L of the old water.
    b. Top up with 5L of fresh 15ppt. (15g per 1L of RO)

11. Pour the contents of the mating tank marked ***Adults (Tub #)*** back into the culture tub.

12. Return air to the tank, ensuring to set the regulator to a moderate bubbling.

13. Add in 12 ml of algae.

14. Replace pre-filter pad:
    a. Undo the zip tie/screw and discard the old pad and
    b. Replace with new pre-filter.

15. On Sunday, add 5L of fresh 15ppt to top up to 25L.

    Note: Do not harvest from this tub until Monday.

Weekend Maintenance

1. Harvest rotifers from 3 x “old culture tubs” as per Mon-Thursday.
2. Do not discard the harvest water, instead use this to top-up the tubs that have been harvested.
3. Clean the flock trap with R.O water.
4. Feed 25ml to all rotifer culture tub.
5. For the tub cleaned on Friday; top-up with 5L of fresh 15ppt on **Sunday.**

Procedure - Setup New Rotifer Culture

1. Decontaminate empty tub containing rotifers,
    a. Place Virkon powder within the tub and fill with RO water to create a 1-2% virkon solution.

2. Rinse tub thoroughly with RO water before use as any chemical residue will kill the rotifers.

3. Reseed the cleaned tub with old culture.
    a. At least 50% of the total volume needs to be “old water” to start the new culture.
    b. And add 50% of fresh 15ppt marine salt solution to the culture.

    NOTE: Rotifers seem to prefer slight ammonia levels and do poorly in completely clean water. To avoid any major colony crashes, never do a complete water change on a tub – always re-use at least 50% of the ‘old’ water in the newly cleaned tub.

4. Feed start-up stock culture 0.5mls per liter of Rotigrow Plus in the morning and 1ml per liter in the afternoon until step 5 is complete.

5. Monitor the population dynamics within the tub.
    a. Let the culture to sit for 2-3 days without any water top ups, maintaining daily feeding routine.
    b. After 3 days, density should have doubled. Begin to increase the water volume by 50% (with fresh 15ppt) per day until you reach your target culture volume (25L).
    c. After siting at target volume for a day you can begin to harvest. (This generally takes about a week).

6. Now that the culture has been established, continue the “Daily rotifer maintenance” procedures as required for daily operational use. Alternatively, this culture can now be used to seed a second culture.

    Note:
    - As a visual guide, water removed from the tubs should be bright green after feeding. If the water has a green coloration the following morning, then the culture has been overfed (or there may be a problem with the culture). Alternatively, if the water is completely yellowed, then the culture was underfed. Ideally there should be some residual algae within the tubs the following morning to result in very pale green water.
    - The amount of food going into the tubs will need to increase as rotifer numbers increase until appropriate stocking densities are met.

Procedure - Making Polyculture

Larvae are ready to be setup in polycultures once they have inflated their swim bladders and are able to swim freely in the water column. (usually between 4 and 5 dpf)

To Setup:

1. Harvest rotifers.
2. Setup a 1.2 L Techniplast tank with 1 x 500 µm screen baffle.
3. Add 400 ml of 3ppt water.
4. Add 15 ml of the dense, harvested Adult rotifers.
    a. Ensure rotifers are swimming around in the tank before adding in the larvae.
    b. Acclimatize Rotifers in 3ppt water for 10-15 minutes before setting up the polyculture.
5. Transfer the label from the lid of the Petri dish onto the setup polyculture tank.
6. Carefully transfer the larvae from the Petri dish to the polyculture tank.
    a. If necessary, gently flush the petri dish with system water/ embryo media to ensure ALL larvae is transferred into the tank.(Do not use RO)
7. Place a lid firmly on the tank.
8. Place the tank on the allocated rack in the Seddon quarantine/nursery room.

Procedure – Maintenance of Polyculture (Refer to table 1)

1. Although the rotifers initially seeded into the tank will remain active and begin breeding within the polycultures, **5 ml of harvested rotifers** to the polyculture need to be added daily to keep up with the feeding demand of the fish.
2. Check rotifer density daily. If the number of live rotifers within the polyculture has dropped, 10mls of rotifers need to be added instead of the usual 5 mls.
3. Monitor water quality closely whilst tanks are in static polycultures and adjust water conditions (by adding fresh water) if/when necessary.
   Note: Larvae become susceptible to ammonia stress around 8-9 dpf, so be mindful of potential toxicity effects (increased larval mortality, sudden bloom & subsequent crash of rotifers)
4. After approximately 7 days on polyculture, fish should be developed enough to be moved onto the recirculating system- Drip flow.
   Note As a general rule, complete ‘Maintenance of Polyculture’ procedure should not extend past 1 week of static culture as this increases the risk of larval mortality due to poor water conditions

Procedure - **Making Dry Food**

- Small Fry – 1L tanks nursery/drip flow/Otto (Skuttle green Bottle) (See Appendix Photo 6)
  - 50:50
    - Spirulina
    - O.range start (100um)
- Juvenile – 3L Babies/Otto (Skuttle Red Bottle) (See Appendix Photo 7)
  - 50:50
    - NRD 2/4
    - O.Range Wean-S (200-400um)
- Sub Adult and Adult (See Appendix Photo 8)
  - 50:50
    - O.Range Wean-S (200-400um) (3kg Bag)
    - NRD 3/5 (3Kg bag)
  - Plus 50g/Kg Spirulina (i.e. 300g)

Figure 6- O.Range Start-S (left) and Spirulina (Right)

Figure 7- O.Range Wean-S (left) and already made Fry Diet (right)

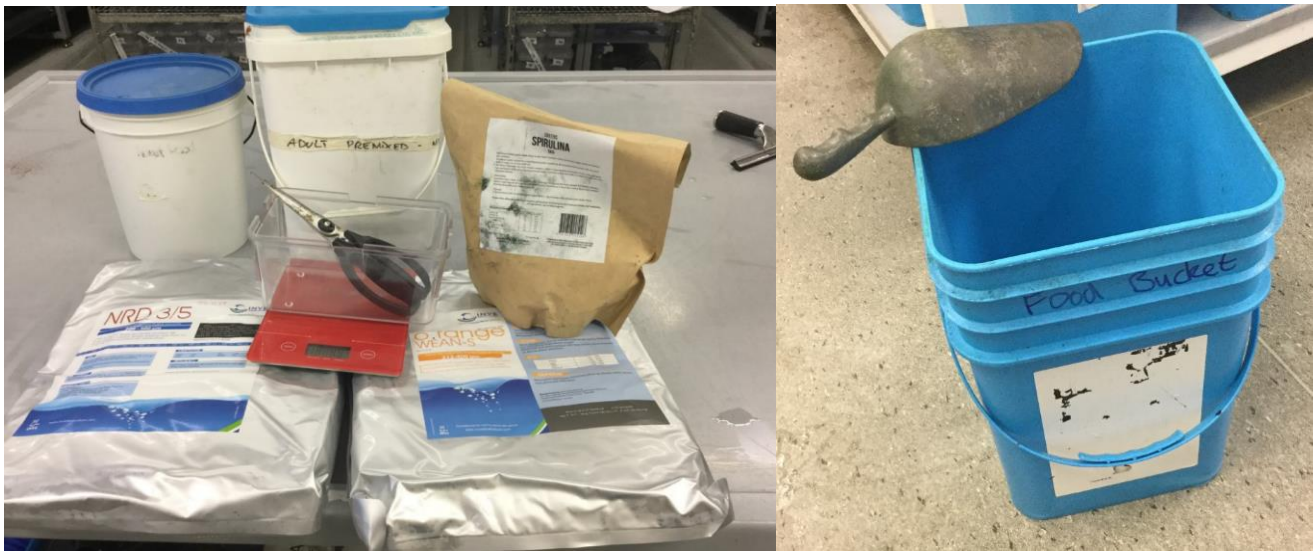

Figure 8- Equipment and food required to make adult diet

APPENIDX

Developmental Stage	Avg. body length	dpf	Diet	Delivery Method	Feeding Frequency	Feeding Times
Larvae	~3mm	~5-12	Larvae	Ad libitum	Grazing	Continual
Fry	~5mm	~12-19	Fry	Hand Fed	3x / day	8am, 12pm, 5pm
Small Juvenile	~10-15mm	~20-26	Juvenile	Hand Fed (T5 Bottle 4)	3x / day	8am, 12pm, 5pm
Large Juvenile	~10-20mm	~26-35	Adult + Juvenile	Hand Fed (Tritone)	3x / day	8am, 12pm, 5pm
Sub Adult	~15-25mm	~33-63	Adult	Hand Fed (Tritone)	3x / day	8am, 12pm, 5pm
Adult	25mm	63+	Adult	Hand Fed Tritone	1x / day 3x / day	12pm 8am, 12, 5pm

Table 1- Zebrafish Rearing Protocols

IV. CONSIDERATIONS

- Adverse events should be referred to UQBR SOP 22 UQBR Veterinary Care Protocol
- Zebrafish should be fed 5% body weight per day to ensure maintenance diet requirements are met
- Food clickers manually dispense dry food, the clicker to be used depends on each tank stocking density.
- Ensure Constant, clean air supply into rotifer tubs
- The use of inadequate air-stones may create fine air bubble that's will trap and kill rotifers (if this happens you will see "rafts/boats" of rotifer groups on the water surface). The stones also create a perfect environment for bad bacteria to colonisation.
- L-type Rotifers reach their optimal growth at 18-25°C.
- Ensure all dry food is mixed through thoroughly.

V. SAFETY

1. PPE use is essential when completing this task.
2. All accidents, injury or near misses are to be reported immediately to the Facility Manager and recorded on a UQ OHS Incident Report Form

VI. REFERENCES

1. Australian code for the care and use of animals for scientific purposes (8th Edition, NHMRC 2013): https://www.nhmrc.gov.au/guidelines/publications/ea28
2. Department of Agriculture and Fisheries (DAF): http://www.daf.qld.gov.au/
3. Guidelines to promote the wellbeing of animals used for scientific purposes (NHMRC, 2008): https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/ea18.pdf
4. OGTR PC2 work requirements and regulations: http://www.ogtr.gov.au
5. QLD WH&S Act 2011: https://www.worksafe.qld.gov.au/laws-and-compliance/workplace-health-and-safety-laws/laws-and-legislation/work-health-and-safety-act-2011
6. UQ Animal Ethics Unit SOPs: http://www.uq.edu.au/research/integrity-compliance/standard-operating-procedures-sops UQ OHS Unit: http://www.uq.edu.au/ohs/
7. UQ OHS Incident Report Form: http://www.uq.edu.au/ohs/index.html?page=141331
8. UQBR SOPs: V:UQBR/SOPs/Common/UQBR SOPs and http://biological-resources.uq.edu.au/secure/uqbr-sops