Parhyale hawaiensis culture

Equipment

1. **Crushed coral**: For Parhyale, the best size is small pieces roughly 5-10 mm each. The finer grind, which looks like coarse sand, is too fine since it creates unnecessary difficulty in collecting animals from the tanks. We use Tropic Marin brand. The coral does not need to be sterilised when it comes new out of the package, but if I reuse it to form a new culture with different animals from the previous culture, in between the tap water and the salt water washes (see "Setting up"), I sometimes pour boiling water over it to kill off any previous life forms, that I may not want in my new tank. The coral provides a substrate for Parhyale to climb and hide, and also act as a niche for microorganismal life which helps keep culture metabolism stable and the tank clean.
2. **Tupperware**: Glass fish tanks, terraria, or just large plastic tubs with lids can be used. Parhyale will live in anything. Glass is not porous and therefore easier to clean, but heavier and more of a pain to move around, also risk of cutting yourself on sharp edges. Plastic containers are absolutely fine, as long as they have *never* been washed with soap, bleach, or detergents. When cleaning the Tupperware between cultures, *only* use hot water to scrub them out, and take care that the sponge or rag you use is kept exclusively for that purpose, and has *never* had soap or bleach on it.
3. **Air Pumps**: these do not have to be heavy duty as the volume of water in any given culture will probably not exceed 10 or 20 L. The small ones commonly found in pet or aquarium supply stores are fine. Check these frequently to make sure they are running smoothly. *Aeration is perhaps the only critical factor for Parhyale culture – if a culture goes for 24 hours without aeration, all animals will almost surely die.*
4. **Rubber tubing for air pumps**: flexible latex tubing with an inner diameter of approximately 5 mm can be found at pet or aquarium supply stores. The most important thing about the tubing is that it fits snugly around the air supply hole of the pump. Those parts of the tubing in direct, constant contact with the salt water will eventually get hard, brittle, and may discolour. When they get too hard to remove easily from the air pump, simply cut off the hardened part. Check the tubing every now and then for holes, tears or other leaks, since this will prevent air from reaching the culture. *Aeration is perhaps the only critical factor for Parhyale culture – if a culture goes for 24 hours without aeration, all animals will almost surely die.*
5. **Air stones**: these are optional. It is not necessary to use them, but they do have the advantage of weighing down the tubing so that the bubbles are released into the water. If you don't have any, you will have to fix the open end of the tubing so that it does not float up out of the water while it is bubbling. You can do this by using small suction cups with hooks for the tubing attached to them, which can be stuck on to the inside of the Tupperware beneath the water level. You can buy these at pet and aquarium supply stores.
6. **Artificial Sea Water**: I use Tropic Marin sea salt, available from aquarium supply stores. Keep the container well closed since it is quite hygroscopic. Dissolve in deionised water at a concentration of about 33g/L. Use a hydrometer to check the salinity of the water – it should be between 1.023 and

1.026 (32-34 ppt). Always check the salinity before changing the water of an existing culture. It is a good idea to also check the salinity of the water in the culture you are changing. The salinity tends to rise as water evaporates with time, so if you always add clean water at exactly the same salinity as the culture, after a few months you will have an extremely high salinity culture. Although Parhyale are tolerant of a wide range of salinities, in the lab culture, do not subject them to radical (over 0.003) changes in salinity, and keep them in the 1.023 to 1.026 range. If the salinity has risen a lot since the last time you changes the water, bring it back down by lowering the salinity of the fresh water you are adding. This is important because salinity changes can affect the morphology of the embryos during dissection, and you don't want to have a critical batch of embryos compromised because they were born into a higher salinity than the standard fixatives we use, which are isotonic with salinity of approximately1.024 approx.

7. **Heating Filaments**: if you have a temperature controlled room to store the cultures in, then these are not necessary. If you want to keep your tanks at room temperature and do not live at the equator, then you may want to use a heating filament to keep the temperature relatively steady. Parhyale will tolerate a temperature range of at least 18 to 29 ºC, but for embryological studies, is important to keep the culture at a constant 18 or 25 ºC for constancy of developmental timing.
8. **Phosphate absorber bags**: these look like large tea bags filled with gravel, available from aquarium supply stores, and help keep dense (>500/L) animals cultures cleaner for longer, as they absorb some of the waste products. In sparser populations that have frequent water changes (at least every 3-4 weeks), they are not necessary but cannot do any harm.

Setting up

1. Wash crushed coral thoroughly through a few changes of tap water, then again through at least three changes of artificial sea water (ASW). This washes any extra dirt and dust off of the stones.
2. Put the coral in the Tupperware you plan to use. You should have enough coral to form a layer about 1cm thick on the bottom.
3. Add ASW to cover the coral to a depth of at least 3 cm. More water is not strictly necessary, but will not hurt the culture.
4. Add Parhyale. As few or as many (up to about 750 animals/L) animals can be added as you like, but keep in mind that very dense cultures will require more care as they will foul the water faster.

Maintenance

1. **Feeding**: Parhyale are greedy, and will eat anything except their own waste, including each other.
    a. **Fish Flake Mix**: We feed them on a mixture of fish flakes (in my hands, tropical or freshwater fish flakes both work well), freeze-dried Tubifex, and wheat germ pellets (for fish ponds). Grind a mixture of these components to a coarse powder in a mortal and pestle kept exclusively for this purpose. *Only* wash the mortar and pestle with hot water, *never* with bleach, soap or detergents. I keep the ground food in a small tin at room temperature, and sprinkle in a generous pinch per culture container. You can also just sprinkle fish flakes over the water, crushing them slightly with your fingers as you do so. To maintain the culture at a constant level, feed once or twice a week. To amplify the culture, feed every one or two days, but monitor carefully to make sure you are not overfeeding. If you see many food particles floating around, or the water is brown, do not feed, but wait until the water has cleared up again. If you are feeding hatchlings to raise to adulthood, be *very, very* spare with the feeding, as it is extremely easy to overfeed and kill them. Grind the fish flakes very finely for the babies, and monitor the water quality daily.
    b. **Carrots**: these are another option for feeding. Cut the carrots into chunks and just drop a chunk or two into each culture box. Replenish when finished and remove if rotting and slimy.
    c. **Rich Mix**: Bill Browne uses the following formula. Mix the ingredients together into a slurry and store at 4ºC. Keep the powdered stocks at -20ºC and the liquid stocks at 4C.
        - 150ml Zoe Marine vitamins (Kent) (approx. volume)
        - 3 PostIt scoops larval diet (Ziegler 240-450um)
        - 3 PostIt scoops beta-meal (MTI)
        - 3 PostIt scoops plantkton (Artificial Plankton, OSI)
        - 5-6 PostIt scoops spirulina (any powdered form)
        - 10-20ml Super Selco (INVE Aquaculture)
    d. **Extavour Lab Rich Mix**: we have modified Bill's formula slightly as follows. Mix the ingredients together into a slurry and store at 4ºC. Keep the powdered stocks at -20ºC and the liquid stocks at 4C.
        - 150ml Azoo marine vitamins
        - 25g Ziegler 250um larval diet
        - 25g HUFA protein
        - 50g spirulina
        - 20ml Super Selco
        - 100ml ASW
2. **Water changing**: The frequency of water changing depends on the density and feeding frequency of the culture, and on whether you want to amplify or simply maintain the culture. A culture with about 250 animals/L, fed once a week, can go without water changes for as long as six weeks, if you want to maintain the animals levels constant. If you want to amplify the culture and/or collect couples frequently, change the water weekly. To change the water, stop the bubbler and stir the culture around vigorously with your hand or a

clean stick/glass pipette, so that the waste or brought up from the bottom and suspended in the water. Pour half to two thirds of the water off into a bucket. Pass the old water through a sieve with pores small enough to trap hatchlings, but large enough to allow most waste particles to pass through – a pore size of approximately 300 microns is probably ok. Wash the animals trapped in the sieve in this way, back into the culture with clean water. Top up the tank to its former water level with clean water. If you need to feed and change water on the same day, first change the water, then feed them.

3. **Aeration**: Aeration is perhaps the only critical factor for Parhyale culture – *if a culture goes for as little as 24 hours without aeration, all animals will almost surely die*. For this reason, it is a good idea to check the cultures every day, even if you don't have to feed them, to make sure that the bubblers are still going.
4. **Temperature**: Standard cultures are kept at 25ºC. Embryogenesis takes about 10 days at 25ºC, and 14-16 days at 18ºC. Parhyale will tolerate a temperature range of at least 18 to 29 ºC, but for embryological studies, is important to keep the culture at a constant 18 or 25 ºC for constancy of developmental timing.

Troubleshooting

The water is cloudy
The culture has been overfed, is too dense, or has died. If the animals are still moving around and you see food particles in the water, overfeeding was likely the problem. Death should be obvious. If the animals are all dead, you will have to abandon the culture. Pour off and discard all of the water, and wash the coral through several changes of tap water to remove the carcasses. Then you can sterilise the coral with boiling water and use it to start a new culture (see "Setting up"). If there are still animals alive, change the water, but instead of changing only part of it as you would normally, change as much of it as you can. If the culture seems too dense (a random scoop through the culture with a 50 ml falcon tube comes up with more than 20 animals), thin it out by splitting it into subcultures. If you prefer to keep it at high density, then you will have to add a phospho-absorber bag, and change the water more frequently.

The water is not cloudy but the animals are dead
Check the bubbler – it probably stopped and the animals suffocated due to lack of aeration. Proceed as for "cloudy water" problem by abandoning the culture.

There are lots of animals but not many mating couples
The water is probably clean enough to sustain normal development, but not clean enough for them to feel comfortable mating. Increase the frequency of your water changes and they should start to mate again.

I am changing the water often to amplify the culture, but the culture density seems to stay the same
You may be throwing out the babies when you change the water. Check some of the water after you have passed it through the sieve to see if babies are falling through and being discarded. Change to a finer sieve if necessary.

There is a lot of waste in the culture
Keep in mind that Parhyale eat a lot and produce a lot of waste. If you feel the amount of waste in the culture is excessive, make sure to stir the water thoroughly before pouring it off, when you change the water, so that the waste is brought up from the bottom of the culture and resuspended.

I frequently see the animals eating each other
Feed them more often.

Schedule

Amplification:

- Change water once a week
- Feed every two days

Maintenance:

- Change water once a month
- Feed once or twice a week

References

Browne, W., Price, A., Gerberding, M., Patel, N. (2005) Genesis 42: 124-149

Extavour, C. (2005) Developmental Biology 277(2): 387-402

Gerberding, M., Browne, B., & Patel, N. (2002) Development 129(24): 5789-801