

## Wet Lab Protocol

This laboratory has a number of students/technician working in it. Since I do not always have time to teach each student these procedures, I rely on previous students to help pass on the methods. Hence, when I ask one student to show another student how to do something, I expect that student that is learning to learn the procedures as shown to him. I DO NOT WANT STUDENTS CHANGING PROCEDURES. Once you have been working with a procedure for a time and you have an idea on how to improve it then you are welcome to come talk to me. If you think there is an error in the methods (as sometimes occurs) you are welcome to talk with me.

Remember, each experiment is extremely costly to run and any small oversight in procedures has the potential to ruin good research. Additionally, a good experiment is based on the ability to anticipate problems and deal with them prior to them becoming a problem. Hence, I have established standard procedures for all work, including but not limited to the following:

### Water quality

#### Daily Measurements

Dissolved Oxygen: **DO is determined using a YSI meter which must be calibrated for oxygen and salinity prior to use.** If you are having problems with the meter, check the batteries and the membrane. DO should be  $> 90\%$  saturation. Adjust the air flow rates as needed.

Salinity: YSI Conductivity Meter

Temperature: Record Temperature from either the Salinity or DO meters. A HOBO data logger may also be used to record system temperature, however you still must record daily temperatures as a backup.

#### 2-3 times per week:

Total Ammonia-nitrogen (TAN) and Nitrite-nitrogen: See biochemical procedures. **Note:** when preparing blanks, be sure to use reagents

pH: pH meter must be calibrated before use. pH should be  $\geq 7.5$ . If the pH is low, you can bring it up by adding sodium bicarbonate, soda ash or baking soda. Remember, pH is also dependent on  $\text{CO}_2$  buildup so make sure there is adequate aeration if you have pH

problems.

### **Feeding**

In general, feeds are offered based on the biomass of the replicate (tank or pond) and corrected for mortality on a daily basis (using average weight). For the first feedings of an experiment, the overall average biomass may be used, **after the next weighing then the individual replicate biomass is used.** Most of the time feeds are fed on a dry weight basis (not as is or wet weight basis).

Fry or PL's continually to 4 times per day

Fish juveniles, 2x per day

Fish, ponds 1-2 times per day

Shrimp, 4 time per day

Shrimp, ponds 2 times per day

Daily rations should be weighed out precisely, however daily feedings can be separated by eye. Fish are generally fed  $\frac{1}{2}$  ration on the day that they are weighed. Make sure you are aware of how much feed is available. Making additional feed can be a 2-3 day process, and it can take much longer if it must be ordered and/or shipped.

### **Weighing**

Weighing of critters is generally done every 1-2 weeks. Weighing should be done in a cool environment. Animals should be weighed in the mornings when possible in order to avoid the hottest part of the day, and to allow for an evening feeding. Items needed in order to weigh animals should be prepared **BEFORE** the time you plan to weigh animals. Items generally used include: scale, buckets, nets (keep in mind that catfish spines get caught in mesh nets), data sheet.

**Shrimp** are weighed using a reverse weight determination as a towel dry weight. To do this you first put a dry paper towel in the bottom of the weighing container, you then place the shrimp (after most of the water is allowed to drain from them in the net) into the container. The container, towel and shrimp are tarred (balance zeroed). The shrimp are then transferred into a container with water and the towel and container placed on the scale. The negative weight is the shrimp biomass.

- If you do not need an accurate weight (eg intermediate weight) the shrimp can be de-watered (in net) then transferred into a tarred container and the weight determined.
- For PL's the shrimp are placed on a paper towel, then individually weighed on an analytical balance (n> 30).
- Note: air temperature is very important so do this at a cool time of the day.

**Fish** are weighed into a container filled partway with water that has been tarred. The fish are first de-watered (in a net or shrimp basket) then carefully added to the water without splashing any out. The weight is then read and recorded. Fish should be anesthetized with hypno @ 0.43 ppm prior to weighing in water. The type of net used to catch the fish should be appropriate for the size of fish as well as any scale and fin problems. For catfish metal or nylon nets should be used.

- Marine fish are dipped in fresh water (for approximately 30 seconds) during the weighing process. Marine fish should also be treated with chloroquine (Marex; Aquatronics, Oxnard, CA, USA) at 21.1 mg/L, as a preventative measure against *Amyloodinium ocellatum*, at least twice a month (generally a couple days before weighing).

### **System Maintenance**

Tanks: Siphoning should be performed routinely to remove particulate matter from tanks. Tanks should also be cleaned (scrubbed and siphoned) when animals are removed for weighing. Tanks should always remain securely covered in order to prevent animals from jumping out.

Water exchange: routine water exchange (10-15%) should coincide with siphoning. Remember, if the system is turned off the system may not have enough water to restart. It will take 30 minutes before water levels will stabilize so make sure you add water to the sump and watch the water level for at least 30 minutes after the system has been turned on.

Sand/Bead Filters: Filters should be back washed routinely (frequency dependent upon system loading).

Heaters: Heaters should be placed in systems BEFORE it gets cold. At best, rapid fluctuations in

temperature can cause animals to go off feed, and also can be fatal to animals intolerant of colder temperatures. Also, be sure submersible heaters are unplugged when removed for tank cleaning or when exchanging water.

### **System Volumes**

#### **North Auburn**

Square Blue Poly: 61 cm x 61 cm x 50 cm: approximate volume 190 L (depending on length of standpipe)

Round Blue Poly: 1 m<sup>3</sup>: approximate volume 1000 L

Aquaria: 60 cm x 30 cm x 25 cm: approximate volume 40 L (depending on length of standpipe)

Biofilters: 305cm x 62 cm x 50 cm: approximate volume 950 L

#### **Claude Petet**

Square Blue Poly: 61 cm x 61 cm x 50 cm: approximate volume 190 L (depending on length of standpipe)

Vats (wetlab) One vat system (i.e. Vats 1 & 2) = 15,000 L

A&B line tanks (Greenhouse 1) - 1 m<sup>3</sup>

C line (Greenhouse 1) - 1 m<sup>3</sup>

A,B,&C line Sumps - approximately 400 L

### **General Observations**

It is critical to be observant in the lab. Any problems with equipment or animals should be investigated and reported immediately. You should always check pumps, tanks, and other equipment daily for leaks and other problems. Also, routine observation of animals is important in order to catch signs of disease, infection, and aggression.