**Making Platymonas Media**

To make 1 L (I made 7.5 L in 3000 mL flasks):

> Dissolve 30g Instant Ocean (IO) in 1L dH2O

> Pasteurize by slowly heating to 73°C for 10-15 minutes on two consecutive days

> Add 20mL (one tube) of concentrated Alga-Gro (Carolina #153751) **per 1 L** (wait to add Alga-Gro until starting cultures)

* Do in volumes that make sense for culture sizes

* Can be pasteurized ahead of time - dispense and use as needed

**Making Artificial Sea Water**

To make 1 L:

> Dissolve 25g IO into 1L dH2O

Divide into 6 - 500 mL flasks/section

**Spring 2018 Needs (separate from shrimp culture):**

**For Platymonas Media (7.2 L total):**

- 1 pack of Alga-Gro concentrate (12 tubes)
- 216g Instant Ocean
- Water bath to go to 73°C (~8 on our water bath)

**For Artificial Sea Water (21 L total):**

- 525g IO

- 42 - 500 mL flasks (not really - don’t have to be used at the same time – make as labs need them, leave note letting instructors know to save leftovers for next lab)


**Culturing Platymonas**

From the 10ml stock tube of Platymonas obtained through Carolina (#152475) make a 1 L stock culture:

- Add the tube to 200 mL Platymonas media in a 500 mL flask w/ foam stopper
- Place under lights on a stir plate at low-med speed for 4-5 days at ~22°C until growing rapidly
- Add this culture to 800 mL Platymonas media in 1500 mL flask w/ foam stopper, return to lights and stir plate for 4-5 days at ~22°C

**This is the 1L stock culture**

**From 1 L stock culture make 7 - 1 L cultures (1/section)**:

- Add 140 mL stock Platymonas culture to 860 mL Platymonas media in 1500 mL flask
- Place under lights on a stir plate at low-med speed w/ foam stopper for 4-5 days at ~22°C until growing rapidly

**These are the cultures that will be given to labs, so plan the days they need to be started**

***Make a mini-culture as you go to use for shrimp feeding (100 ml/day), add on w/ IO each time some is used to replen for feeding the next day

**Spring 2018**

> The 200mL culture should be started on 3/13
> The 1 L stock culture should be started on 3/17 or 3/18 (see what 4 days of growth looks like)
> - Will be six days old on 3/24
> The first set of lab cultures for classes on M (3/26) and T (3/27) should be started on 3/22
> (three total) from the 1 L stock culture
> The second set of lab cultures for classes on W (3/28) and R (3/29) should be started on 3/24
> (four total) from the 1 L stock culture

**Spring 2018 Needs:**

- 7.2L pasteurized Platymonas media, divided (see Making Platymonas media)
- 500 mL flask
- 8 - 1500 mL flasks
- 9 stir bars, autoclaved in appropriate flasks
- 9 foam stoppers, autoclaved
- 8 stir plates
- 1 tube Platymonas culture (Carolina #152475)