

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 replenish 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)