Queen Conch Aquaculture: Hatchery and Nursery Phases

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Source: Journal of Shellfish Research, 39(3) : 731-810

Published By: National Shellfisheries Association

URL: https://doi.org/10.2983/035.039.0319

Journal of Shellfish Research, Vol. 39, No. 3, 731–810, 2020.
DOI: 10.2983/035.39.0319

QUEEN CONCH AQUACULTURE:

HATCHERY AND NURSERY PHASES

User Manual

First Edition

Megan Davis and Victoria Cassar

Florida Atlantic University
Harbor Branch Oceanographic Institute
Queen Conch Lab

Conservación ConCiencia

HARBOR BRANCH
FLORIDA ATLANTIC UNIVERSITY

NOAA FISHERIES

THE NAGUABO FISHING ASSOCIATION

Preface

The 'Queen Conch Aquaculture: Hatchery and Nursery Phases User Manual' was designed and written by Florida Atlantic University Harbor Branch Oceanographic Institute Research Professor, Megan Davis* and Science Communicator and Designer, Victoria Cassar.

This Manual is a deliverable of the *Saltonstall-Kennedy NOAA Fisheries grant (NA19NMF4270029)* 'Development of a Fishers Operated Pilot-Scale Queen Conch (*Strombus gigas*) Hatchery and Nursery Facility for Sustainable Seafood Supply and Restoration of Wild Populations in Puerto Rico', Megan Davis, PI (FAU Harbor Branch), Raimundo Espinoza, Co-PI (Conservación ConCiencia) and Carlos Velazquez, Collaborating Organization (Naguabo Fishing Association).

The manual includes the science and art of growing queen conch (*S. gigas*) that Davis has developed over her 40-year career designing, implementing and operating experimental size aquaculture facilities, as well as production-scale facilities, in Florida and throughout the Caribbean. In addition, Robinson Bazurto provided advice on Chapter 4: Growing Microalgae.

This edition is written for the Puerto Rican fishers of the Naguabo Fishing Association who are learning to operate the Naguabo Queen Conch Hatchery and Nursery; however, the majority of the information presented in this manual can be applied to other queen conch hatchery and nursery projects to produce conch for sustainable seafood, conservation and restoration. This manual is also available in Spanish.

The 'Queen Conch Aquaculture: Hatchery and Nursery Phases User Manual' was sponsored by *Florida Atlantic University Harbor Branch Oceanographic Institute, Saltonstall-Kennedy NOAA Fisheries award NA19NMF14270029, and the National Shellfisheries Association.*

Cover illustration by NOAA Fisheries FishWatch.

Citation: Davis, M. and V. Cassar. 2020. Queen Conch Aquaculture: Hatchery and Nursery Phases User Manual. Journal of Shellfish Research, Vol. 39, No. 3, 731–810.

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I. Introduction: The Queen Conch

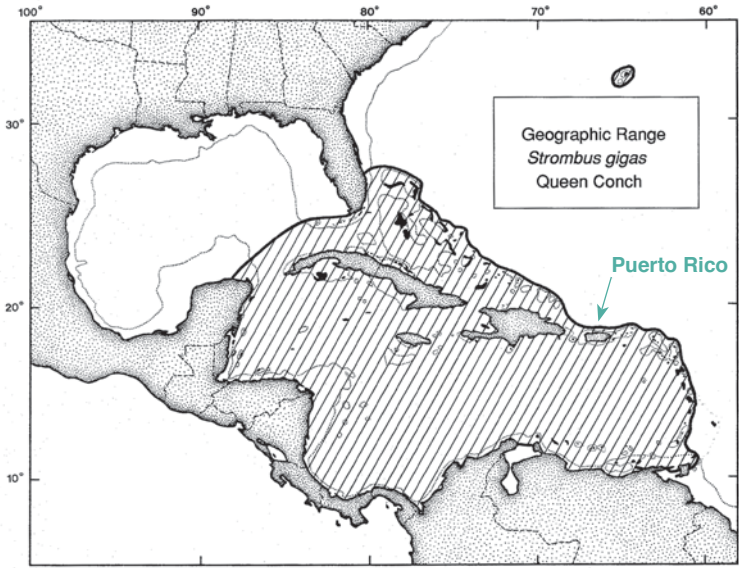


Illustration by Bonnie Bower-Dennis

The queen conch, *Strombus gigas*, is deeply rooted in the way of life in the Caribbean region. It is one of the most commercially important fisheries and many island communities depend on it for sustenance and their livelihoods. Intensive fishing and habitat degradation, however, have caused conch populations to significantly dwindle.

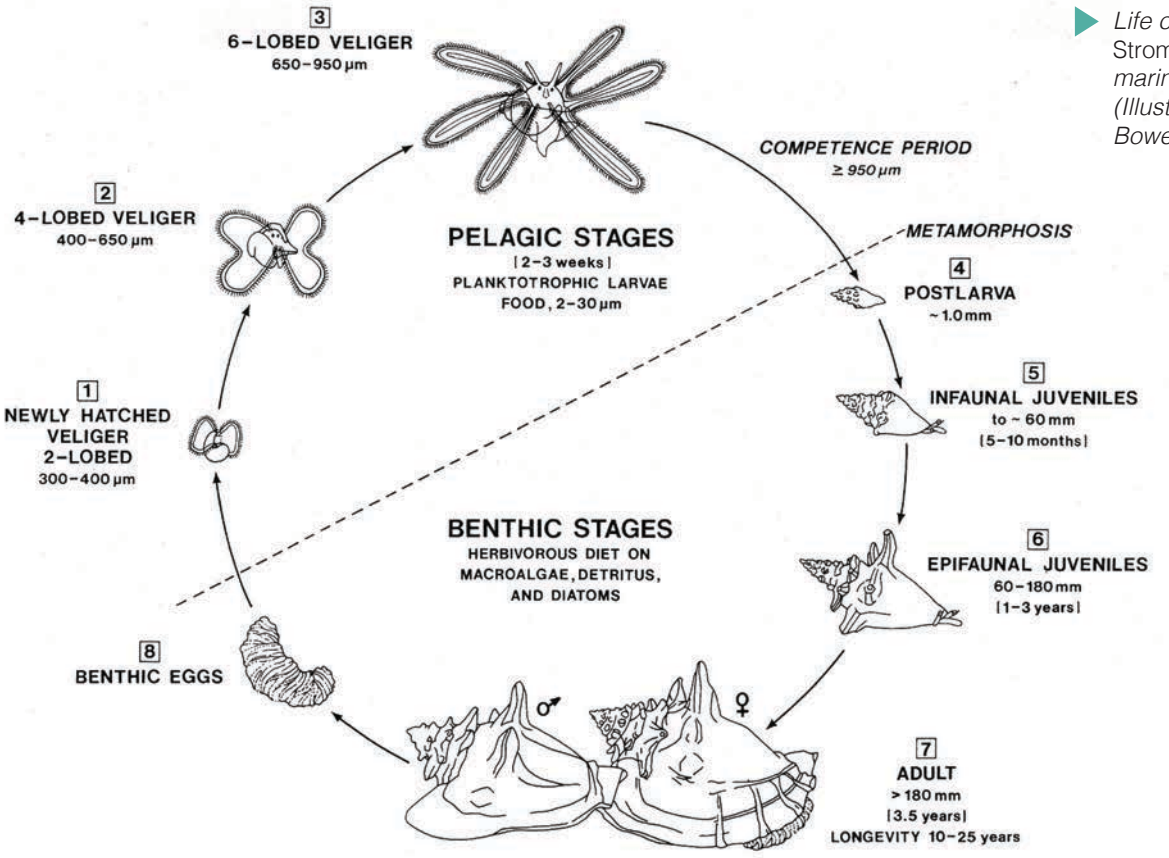
The Queen Conch Resources Fishery Management Plan established a program to help rebuild conch populations in the U.S. Caribbean. For Puerto Rico this includes a minimum harvest size of 9 inches (22.9 cm) in shell length or 3/8 inches (9.5 mm) in lip thickness.

In Puerto Rico, daily bag limits are 150 conch per licensed commercial fisher or 300 per vessel and the closed season is during the peak reproductive months (August 1 to October 31) in jurisdictional waters (0 - 9 nm). Conch harvest has been prohibited in the U.S. Exclusive Economic Zone (EEZ) off of Puerto Rico since 1997. The Puerto Rico Department of Natural and Environmental Resources (DNER) manages the state conch fishery and the Caribbean Fisheries Management Council manages the federal conch fishery. In Puerto Rico the conch, locally known as 'carrucho', is one of the main fisheries species with most of the conch consumed locally with little export. The annual landings are 300,000 to 350,000 pounds (DNER 2016 - 2017) and fishers receive $8 to $12 per pound.

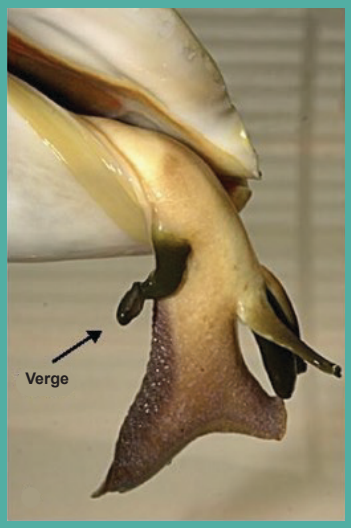
In addition to their socio-economic importance, queen conch play a crucial ecological role. Most people associate a beautiful pink shell or a culinary dish to the name queen conch, but rarely do people know about the animal that lives inside. Queen conch are a marine gastropod, or snail, and are herbivorous. Using their proboscis, or snout, they graze upon microscopic algae and other epiphytes that grow on seagrass blades and the sandy bottom. Queen conch adults aggregate in 'herds' in seagrass meadows and sand flats to reproduce during the summer months. They lay egg masses which hatch into veligers that drift in the ocean currents for three to four weeks and then settle as benthic snails in the seagrass. It takes approximately four years for the queen conch to become mature with a well-developed flared lip.

Image by Shane Gross

LIFE CYCLE



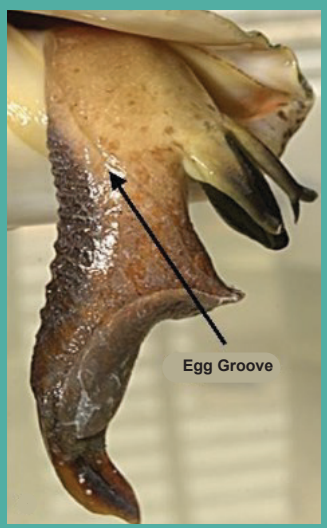
Life cycle of Strombus gigas, *a marine* ***gastropod***. *(Illustration by Bonnie Bower-Dennis)*



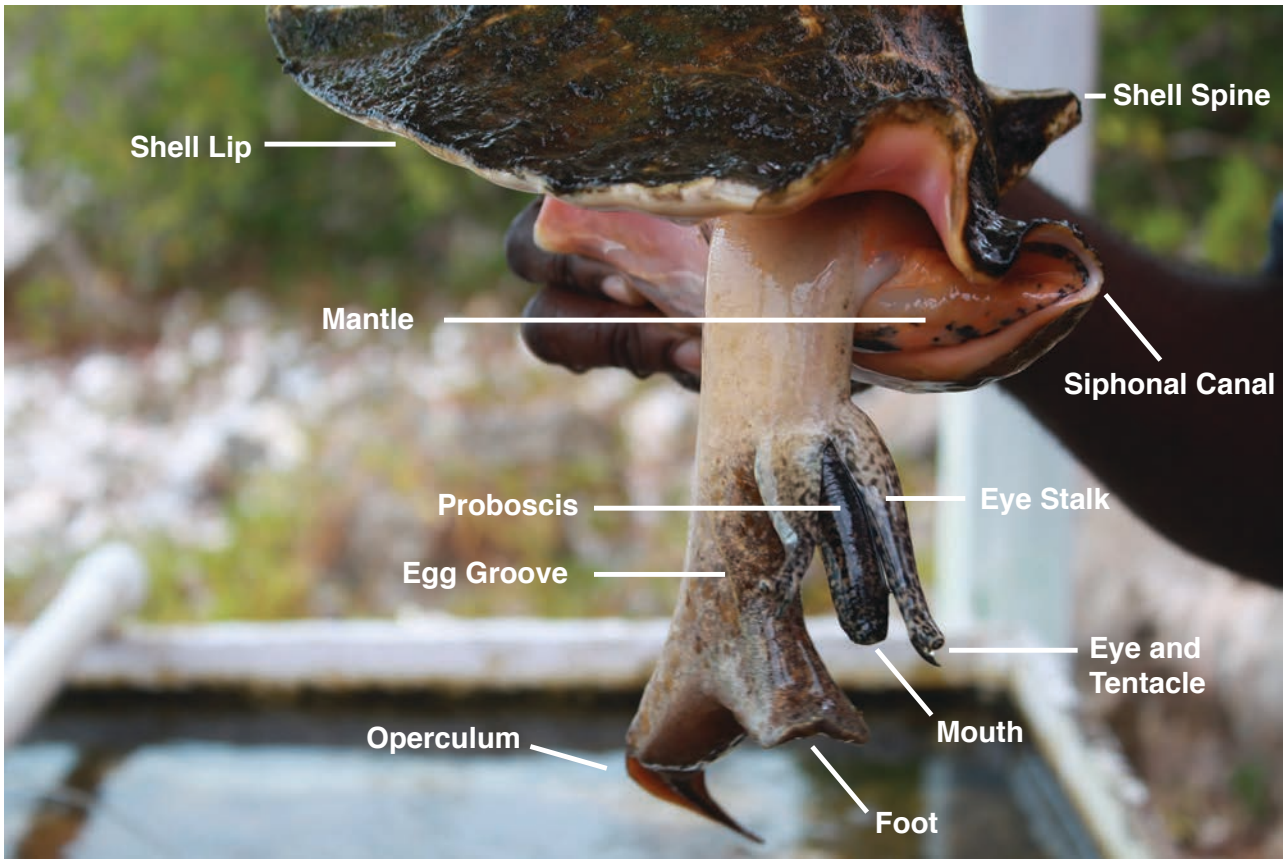
Male reproductive organ.

(Image by Michiel van Nierop)

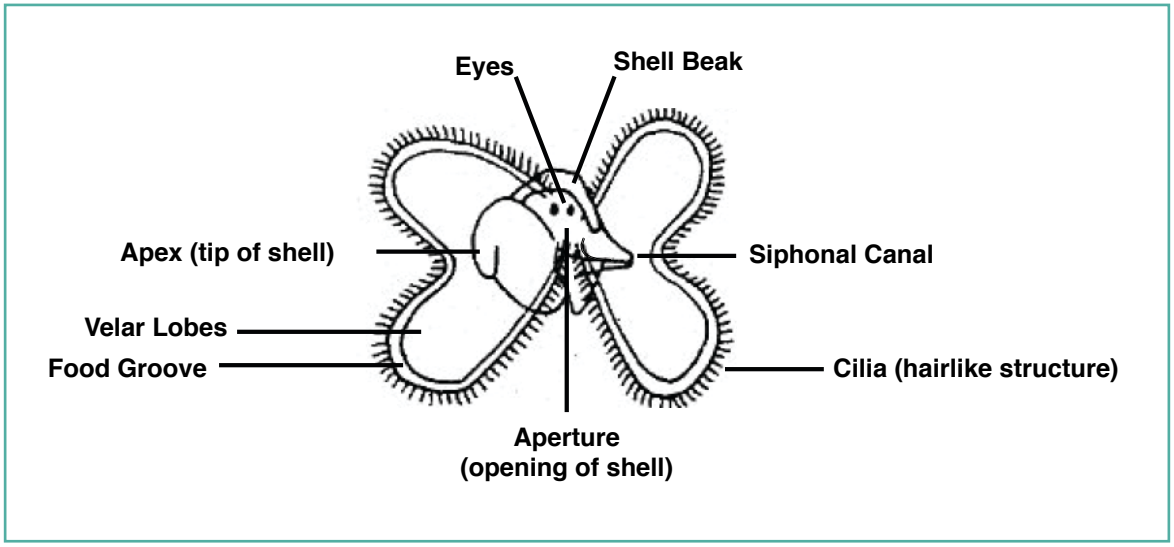
Male and female copulating. Males are always positioned behind.



Female reproductive organ.

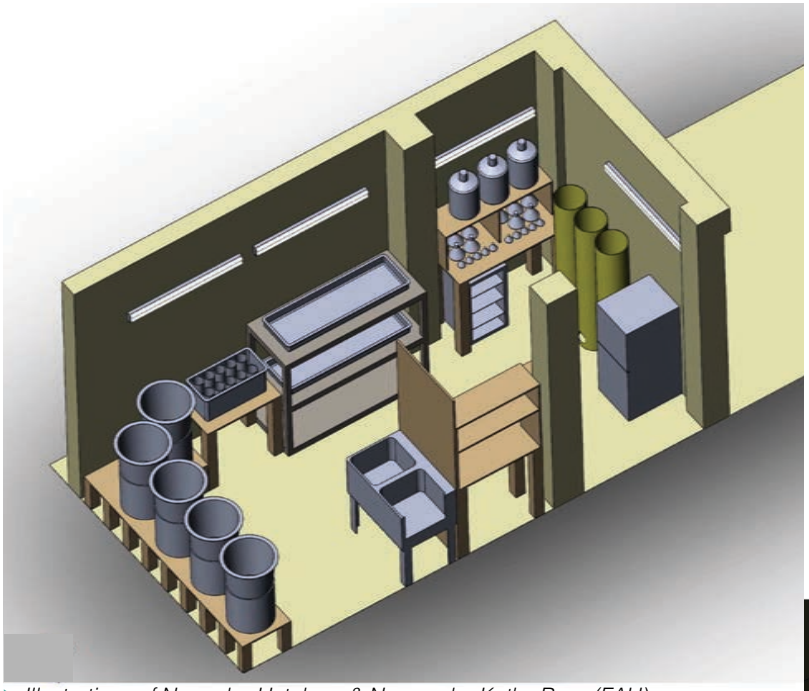


Anatomy of an adult female queen conch. Male is similar except has verge (see previous page). (Image from Caicos Conch Farm)



*Anatomy of a 4-day-old queen conch **larva** (veliger).*

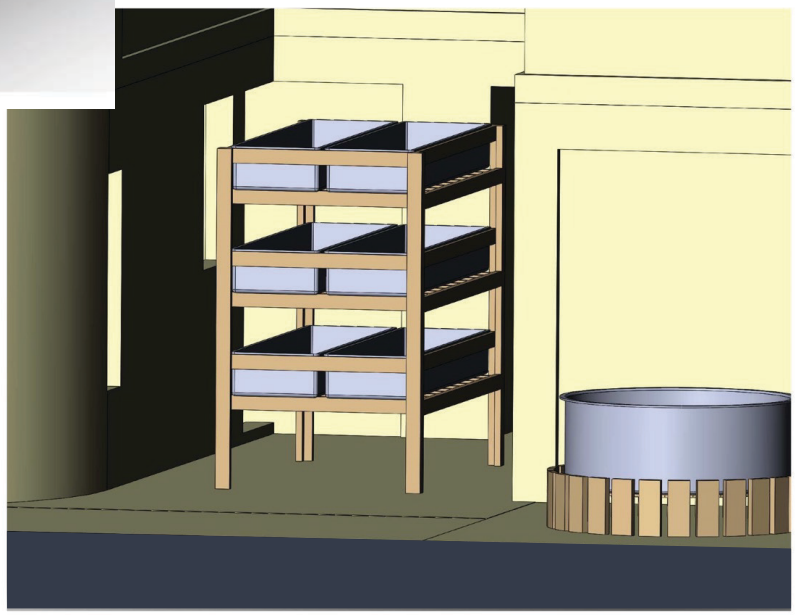
II. Overview: Aquaculture Facilities



Illustrations of Naguabo Hatchery & Nursery by Kathy Russ (FAU)

Hatchery:

The hatchery is where conch eggs hatch and are ***cultured*** (farmed) from egg to larvae, to metamorphosis. It also includes the microalgae area (food for conch).



Nursery:

The nursery is where the small juvenile conch are grown until they are large enough to be released for ***restocking*** wild populations, or for growout of sustainable seafood.

Note: All new fiberglass and plastic tanks and PVC piping should be filled with seawater or fresh water for a minimum of 24 hours before using. This leaching process removes any manufacturing residues. Toxic items which should not be used in the systems are: brass, copper, zinc, lead, and some cleaning agents. Insecticides (mosquito spray) and sunscreen should not be used on hands and arms when working in the facilities.

NAGUABO HATCHERY AND NURSERY

The Naguabo Fishing Association building provides space for the fisher-operated Queen Conch Hatchery and Nursery.

This facility is also a showcase for the wider community to learn about queen conch biology, fisheries, aquaculture, and restoration.

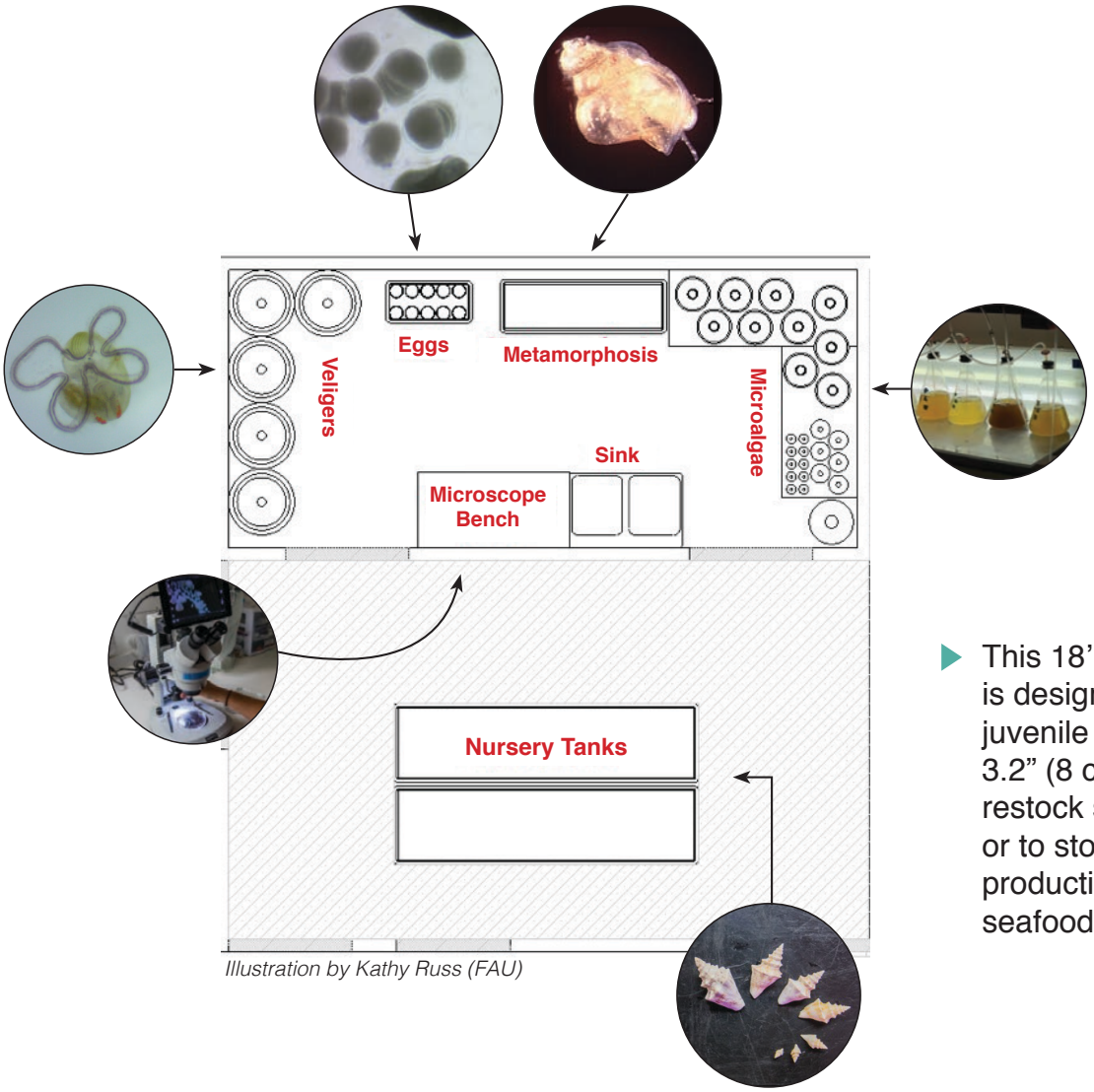
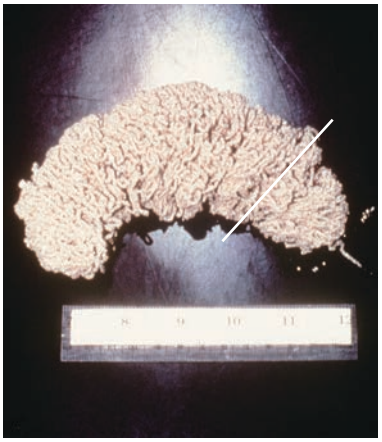


Illustration by Kathy Russ (FAU)

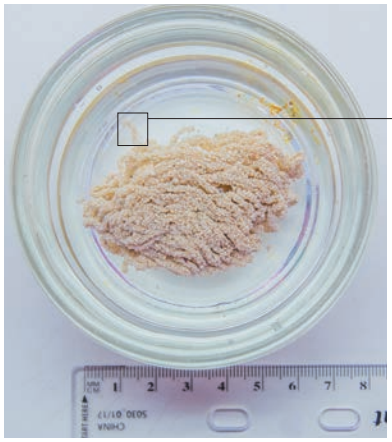
This 18' x 18' (324 ft^2) facility is designed to grow 2,000 juvenile conch per year to 3.2" (8 cm) in shell length to restock seagrass meadows or to stock coastal pens for production of sustainable seafood.

1. Egg Mass Collection and Incubation

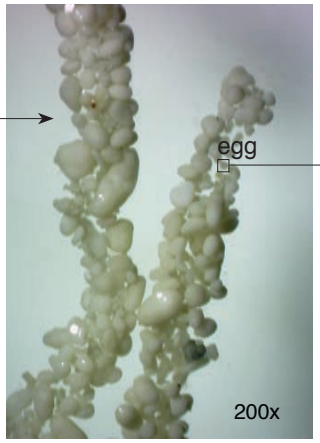
*The first step to culturing conch in a hatchery is to collect sections of **egg masses** from the wild. A full egg mass has approximately 500,000 eggs - much more than what is needed in the hatchery. This is why only a 1/4 or less of each egg mass is collected. An egg mass is one long, coiled strand.*



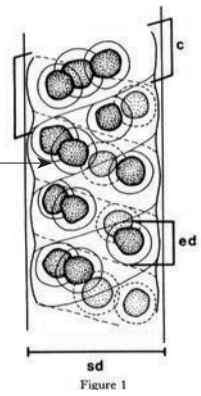
A whole egg mass marked to show 1/4 of the mass. (Image by LeRoy Creswell)



A 1/4 of an egg mass covered in sand. (Image by H. Forrest Thomas)



Close up of egg mass strand covered in sand. Eggs visible inside of the strand.



c: coil of the strand
ed: egg capsule diameter
sd: strand diameter
(*Illustration by Bonnie Bower-Dennis*)

FIELD KIT

To collect egg mass sections, it is necessary to bring a field kit on the boat containing the following:

- 5-gallon bucket with lid
- Snorkeling gear
- Data sheet with clipboard
- Pencil with eraser, and sharpener
- Thermometer
- Refractometer (to measure salinity)
- Resealable bags (like Ziploc) numbered 1 to 6 (quart size; freezable)

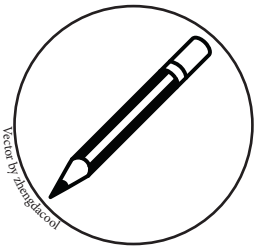
Note: A permit is required to collect egg masses from the wild.

STEP 1:
During the breeding season (April - October), look for egg masses underneath the lip of the female. Newly laid egg masses are hardier for transport because the embryos are not as developed compared to ones found on the sand without a female. A female typically takes 24 to 36 hours to lay an egg mass.

Image by H. Forrest Thomas

STEP 2:
Once located, gently break off 1/4 or less of the egg mass from the middle of the mass or close to the foot of the female. Place it in a resealable bag with seawater and seal. Make sure there is only one egg mass section per bag.

STEP 3:
Back on the boat, carefully place the resealable bag with the egg mass into the 5-gallon bucket, which should be 3/4 filled with seawater, and close the lid. Keep the bucket out of the sun and gently refill every two hours to maintain a stable temperature in the bucket.



Vector by zhengdacool

STEP 4:
Fill out the **Egg Mass Collection Data Sheet** after each egg mass section is collected. Repeat the process until the desired number of egg mass sections is obtained. Generally, only six egg masses are needed per month for a hatchery of this size. This provides options for selecting the best egg masses to hatch, as well as for ***genetic diversity***.

Name: Fisher 1

Date: 06.18.2020

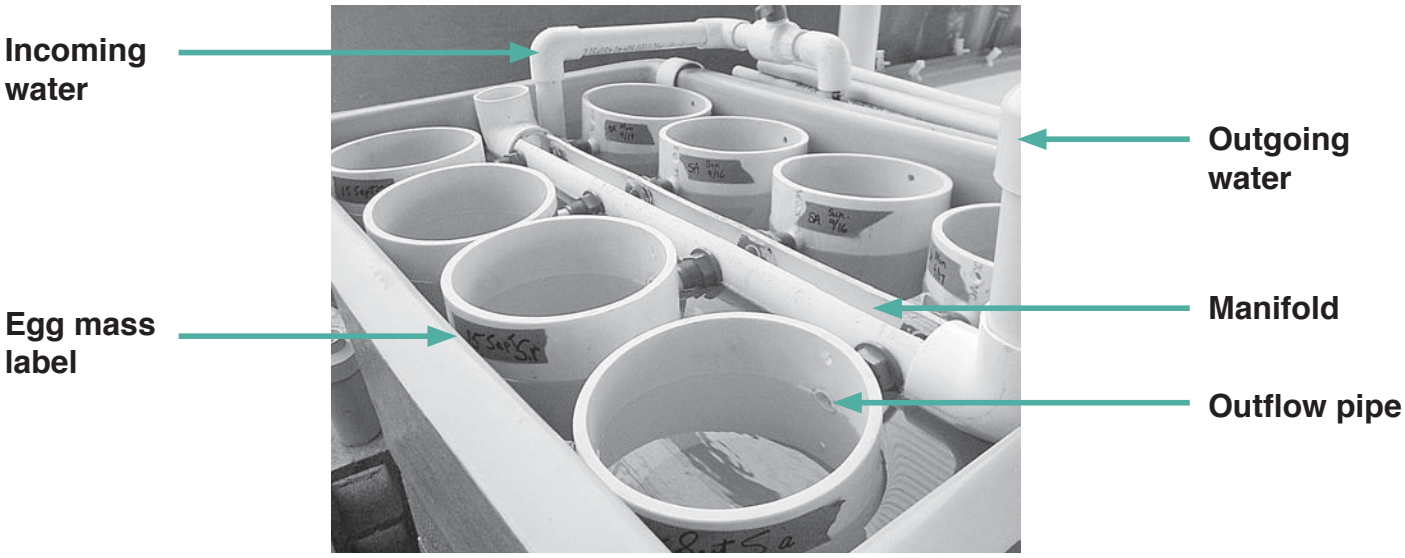
Weather: Sunny, 0 - 1ft waves

Resealable Bag #	Location	Time Collected	Under Female	Without Female	Temp (°F)	Salinity	Approx Depth (ft)
1	*Naguabo*	*12:30 pm*	✓		*80*	*36*	*15*

Egg Mass Collection Data Sheet

INCUBATION SYSTEM

*Now that the egg mass sections have been transported to the hatchery, they must be transferred to the **incubation** tank, which holds up to eight incubation cylinders. Egg masses will stay in this system until the day the embryos are ready to hatch. Temperature plays an important role in the development of the embryos. The temperature range to incubate the egg masses is 26 - 30 °C, with 28 °C being ideal. At this temperature the eggs will hatch in three to four days after collection.*



An incubation tank with eight incubation cylinders.

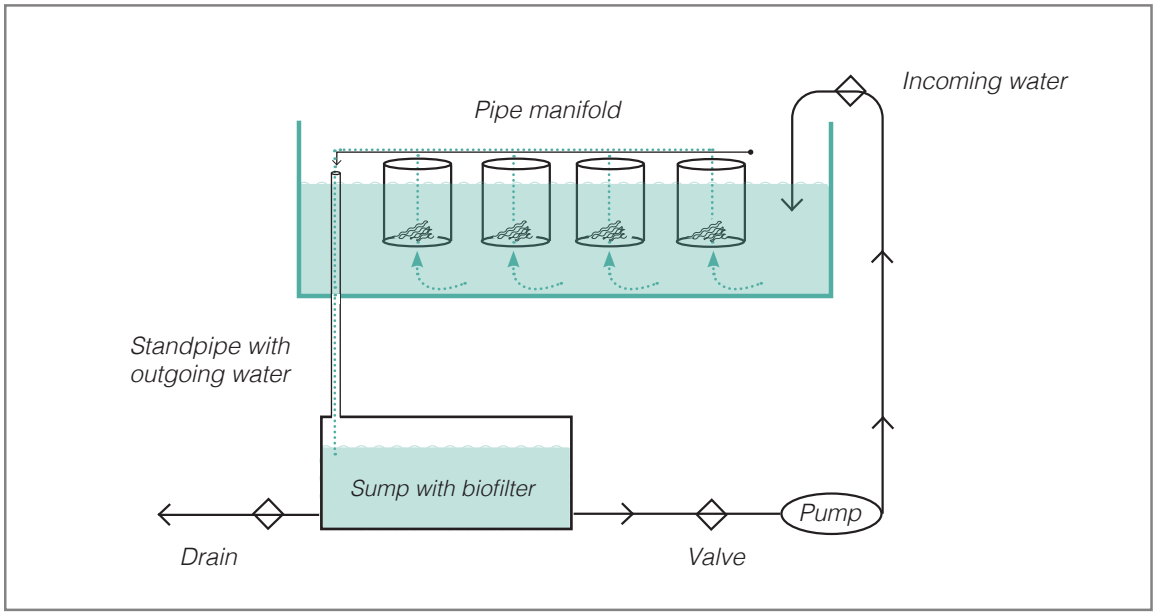


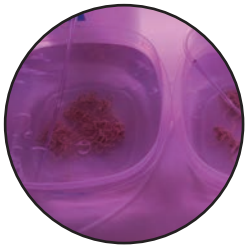
*Illustration of an **upwelling system**. The bottom of each cylinder has a screen mesh (60 - 70 µm) which allows water to upwell through it and drain into a pipe manifold. This provides even water flow over the developing egg mass strands. The incubation tank is on a **recirculating system**, meaning the water is being used over and over again.*

INCUBATION OF EGG MASSES

STEP 1:
As soon as the egg mass sections are in the hatchery, it is important to know the development stage of the ***embryos*** to determine when they will hatch. Gently detach a small piece of strand (1 - 2 in; 2 - 3 cm) from the middle of the egg mass section. Place it on a depression slide or a small Petri dish keeping the strand immersed in seawater. Using a dissecting microscope, and referring to the egg development section (next page) record the observations on the **Egg Development Data Sheet.**

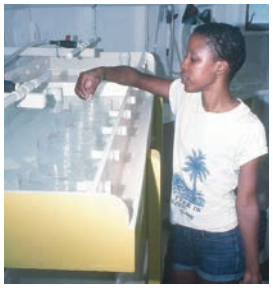
STEP 2:
Fill up the incubation recirculating system (tank and ***sump***) with ***filtered*** and ***UV-sterilized*** seawater and place the appropriate number of incubation cylinders into the tank. For example, six egg masses will need six incubation cylinders.

STEP 3:
Check the water temperature in the egg mass collection bucket and the temperature of the incubation recirculating system. If there is more than a 2 °C difference, place the egg masses (still in their resealable bags with seawater from the field) into a bowl with water from the incubation tank until the temperatures equalize. This will ***acclimate*** the eggs to their new environment.



STEP 4:
Fill one or two shallow containers with seawater. Place an egg mass inside each container and gently massage it using fingers to loosen the strand. Repeat this step with every egg mass and change the seawater in the container between each one. Loosening strands removes debris and will ensure good water circulation through the egg mass while in the incubation cylinder.

STEP 5:
Using hands, gently place each loosened egg mass section into its own cylinder in the incubation tank. Make sure to label each cylinder according to the number on the resealable bag and the date. For example, if an egg mass section was collected on 06.18.2020 and put into the resealable bag number 1, the label would read: 1 - 06.18.2020.



Egg mass strands are gently massaged in shallow bowls and then transferred to incubation cylinders.

Egg mass sections in upwelling incubation cylinders.

STEP 6:
Each morning, collect a small piece of the egg mass strand from each incubation cylinder. Using a dissecting microscope and referring to the images below, record the Stage of the embryos on the **Egg Development Data Sheet:**

Egg Mass #: 1

Date of Collection: 06.18.2020

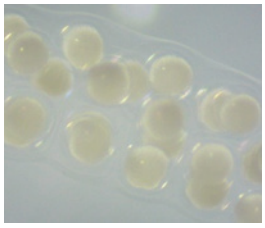
Collection Site: Naguabo

Today's Date	* Days in Hatchery	Stage	Temperature in tank (°C)	Observations	Initials
06.20.20	2	3	28	Shell and lobes are visible	MD

Note: Day 0 is the day of collection.

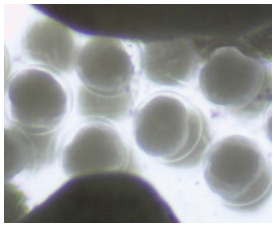
Egg Development Data Sheet

EGG DEVELOPMENT



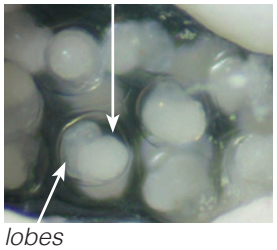
Stage **1**

The eggs look round and smooth.



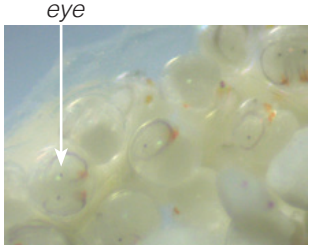
Stage **2**

The eggs look round and bumpy.



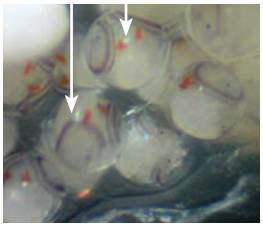
Stage **3**

The shell and lobes of the embryo become visible.



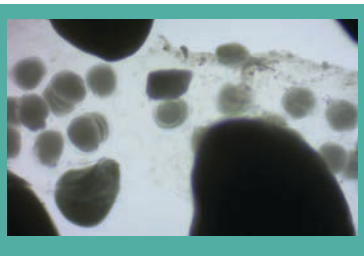
Stage **4**

The black eyes and orange foot become visible.The edge of the lobes darken. Embryos begin to slowly rotate.

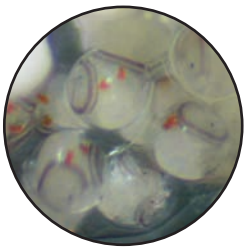


Stage **5**

Day of hatch. The edge of the lobes become darker. The rotation of the embryos is obvious.



Note: If a strand contains empty egg capsules like this one, keep an eye on it to ensure that the embryos develop correctly.



STEP 7:
Once the eggs are ready to hatch, the embryos inside of the egg capsule will be in Stage 5 (see previous page) and steadily rotate.

9:00 PM

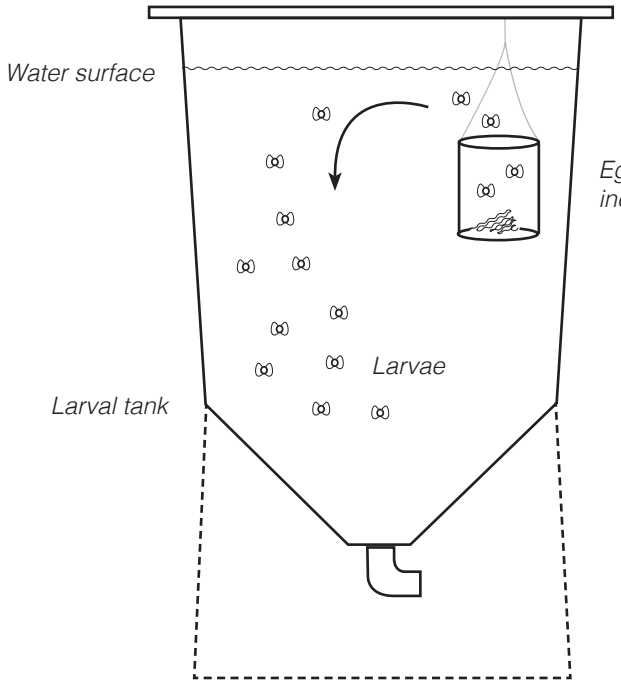
STEP 8:
Based on many years of observation, the embryos usually hatch in the evening at approximately 9:00 PM. About 90 - 100% will hatch the first night, but some egg masses require two nights to hatch completely.

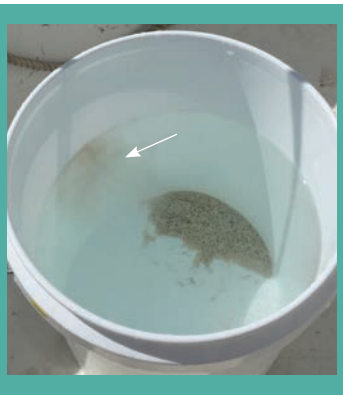
PREPARING FOR HATCH

In the afternoon, place the egg mass with Stage 5 eggs inside a clean incubation cylinder and hang it inside the larval tank about four to six inches below the water surface.

As the veligers hatch in the evening they swim out of the container into the larval tank.

The next morning, gently remove the incubation cylinder and residual egg mass from the larval tank to avoid the egg mass debris from falling into the larval tank.





If there are extra egg masses, they can be released into the ocean.
Place the egg mass with Stage 5 eggs inside a clean bucket to hatch. The follwing morning there will be veligers swimming at the surface and the egg mass will look like a pile of sand on the bottom. Gently pour the veligers from the bucket into the ocean.

If there are more veligers than needed in the larval tanks, they can also be released during water changes.

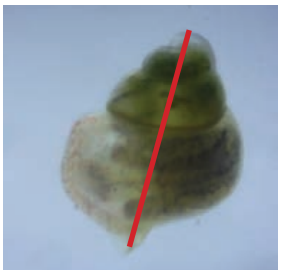
2. Larval Rearing

*Once the eggs have hatched, there will be thousands of larvae, also known as **veligers**, swimming in the larval tanks. Larval rearing is the most intensive part of the queen conch culturing process.*

*This chapter contains how to successfully **rear** larvae using specific techniques, and is also where the art of rearing queen conch will come into play. By paying close attention to the animals, an innate understanding about their needs will occur over time.*

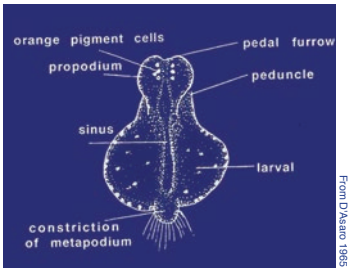
CHAPTER OVERVIEW

Optimizing veliger density in tanks



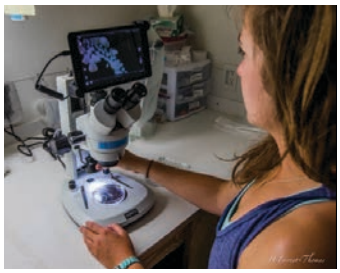
Measuring shell length

Detailed larval development



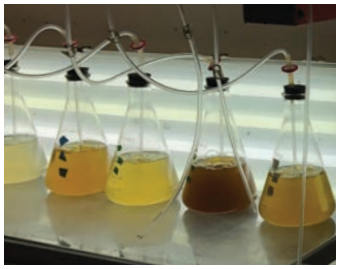
From D'Asaro 1965

Larval foot development



Microscope observations

Water changes

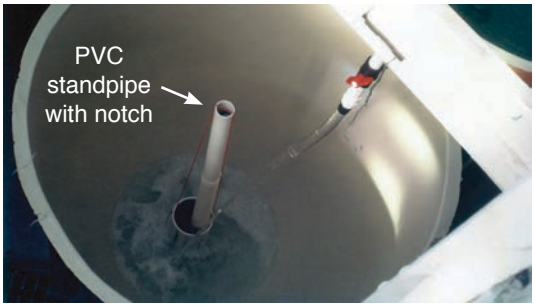


Feeding veligers

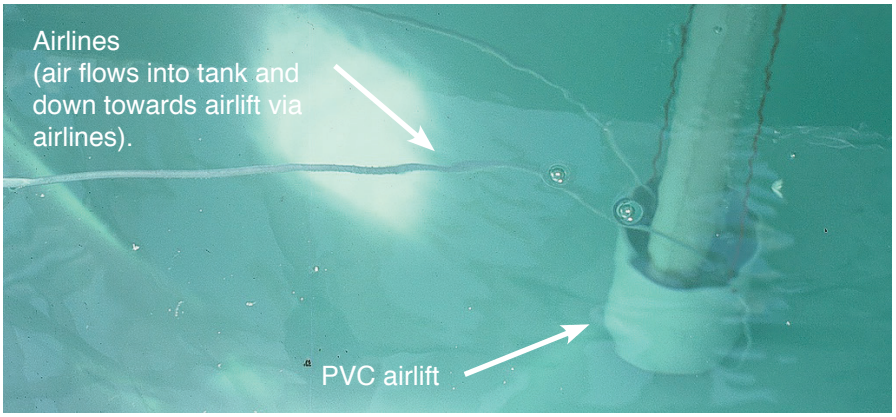
LARVAL TANK SYSTEM

Water in the larval tanks is considered static, meaning the tank is filled once with filtered, UV-sterilized seawater, and does not leave the tank until a manual water change is done (p. 27). Optimal culturing temperature inside a larval tank is 28 °C (82 °F) and a salinity of 36. Veligers, however, can be grown at temperatures 24 - 32 °C (75 - 90 °F) and salinities 26 - 40.

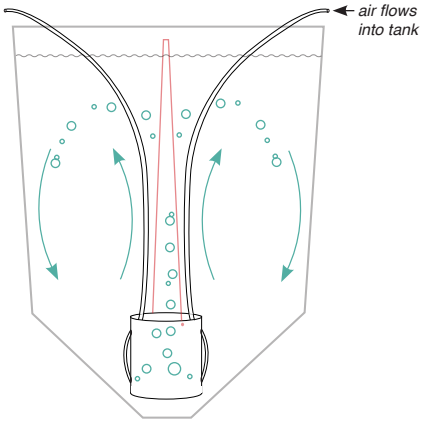
- Larval tanks have a conical bottom with a one-inch diameter drain at the lowest point. The conical bottom should be a gentle slope angled at 30 to 45 degrees. If the slope is too steep the conch larvae will continually touch the sides causing them to retract their lobes or damage their shells. Larval tanks come in many sizes, but they operate more or less the same way. Here a 1,000-L tank is set up for a water change.



- Each tank is set up with a one- to two-inch diameter PVC standpipe that stands two to three inches above the water level. The standpipe, prevents veligers from getting trapped in the drain hole.The top of the standpipe should be even with the tank rim, or slightly below it, so that a lid can be placed on top. The lid prevents bugs and dust from falling into the tank.



- To keep the veligers in suspension, a PVC airlift (6” dia x 6” high; 15 cm x 15 cm) with two airlines (no airstones) attached to either side is used. The airlift hangs on a thin polypropylene line from a notch in the top of the standpipe and hovers right above the bottom of the tank (0.5 cm).



- *Illustration of airlift and airlines. Air flows in a circular motion inside of the tank. This keeps the veligers from sinking to the bottom.*

DENSITY IN THE LARVAL TANKS

Each larval tank is 68 liters or 18 gallons. It is very important that the conch larvae are stocked initially at a relatively low density (~ 200 / L; 760 / gal) equaling approximately 13,600 veligers per larval tank. Throughout the larval cycle, the density in the tanks is reduced as the veligers get older and larger. The ending density should be 10 - 20 veligers per liter meaning 680 - 1,360 veligers per larval tank.

STEP 1:
Dip the density wand (20 ml) vertically into the larval tank and seal the top of the wand with thumb. Take the wand out of the tank and place it over a 105-μm sieve. Remove thumb to release the sample. Repeat five times. If a minimum of five veligers has not been collected, continue this process. Remember to record the number of times this step has been repeated to have an accurate density count. This process is done for each tank using one individual sieve per tank and cleaning the wand in between tanks to avoid cross-contamination.

STEP 2:
Place each sieve into its own shallow sieve container with seawater. Count the veligers again and make sure there are at least five veligers per sieve.

For example, if there were five samples taken with the 20-ml density wand, the total sample size would be 100 ml. If there were 15 veligers counted in the 100-ml sample, there would be 150 veligers per liter (100 ml x 10 = 1,000 ml or 1 L).

15 (amount of veligers per 100 ml) x 10 = 150 (amount of veligers per L)

→ *Since the initial stocking density should be approximately 200 larvae per liter, the density in this tank is the correct density according to the Larval Density Table.*

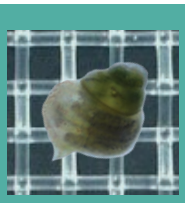
STEP 3:
Every water change day, check the *Larval Density Table* (next page) to determine what the larval density should be according to the age and shell length of the veliger. If necessary, during the water change, adjust the density by either reducing it, or combining low density tanks together.

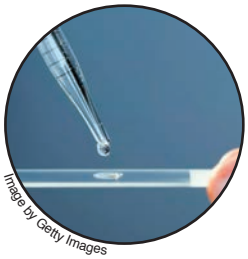
LARVAL DENSITY TABLE

This table is based on a 21-day larval cycle, but veligers can grow faster or slower depending on the environment (ex: feeding, density, temperature). Monitoring shell length and development will assist in determining the correct sieve mesh size to use for a water change. Growth rate is usually 35 - 50 µm per day. This table assumes a 40 µm average daily growth rate or 80 µm for a two day period.

Age	Shell Length (µm)	Density (Larvae / L)	Sieve Mesh Size (µm)
2	350	100 - 200	105
4	430	60 - 80	150
6	510	50 - 60	
8	590	40 - 50	200
10	670	30 - 40	
12	750	30 - 40	250
14	830	20 - 30	
16	910	20 - 30	
18	990	10 - 20	300
20	1070	10 - 20	
21	1150 - 1200	Metamorphosis	

Note how small the sieve mesh size is compared to the veliger shell. Sieve mesh size takes into consideration both shell length and shell width to ensure that the veliger will not go through the mesh no matter its orientation. If a mesh size is too big, veligers can get caught in the screen openings, which may damage their shell beak or apex.





STEP 4:
On the morning prior to the water change, use the eye dropper to collect the veligers from the sieve used from the density determination process (p. 14) and place them onto a depression slide with a drop of seawater. Using the dissecting microscope and referring to pages 17 - 19, carefully observe and measure the larvae. Record observations on the **Larval Rearing Data Sheet**:

Egg Mass # / Larval Batch #: 1 - 06.18.2020

Date	Age	Temp (°C)	Water Change	Starting Tank #	Ending Tank #	Larval Density (per L)	# of Lobes	Shell Length (µm)	Food in Gut (P, D, G)	Food Fed (cells/ml)
06/24/20	2	28	Yes	1	2	150	beg.4	350	G	5,000

Larval Rearing Data Sheet

SUPPLIES:

- Eye dropper
- Depression slides
- Dissecting microscope
- Ocular micrometer
- Wash bottle
- Sieve
- Sieve container
- Calculator
- Data sheet and clipboard
- Pencil, eraser, and sharpener

MICROSCOPE OBSERVATIONS

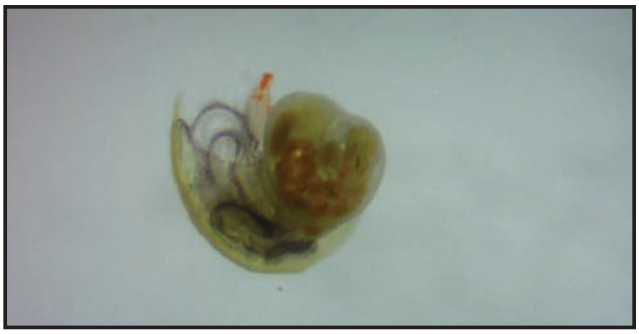
There are three columns in the **Larval Rearing Data Sheet** *that require using a* ***dissecting microscope,*** *also known as a stereo microscope. The 'number of lobes' and 'food in gut' can be observed after the veligers have been placed on a depression slide. The 'shell length' is determined with the use of a small ruler built into one of the microscope eyepieces called an ocular micrometer. Here is information to help fill out these three sections.*

# of Lobes	Shell Length *(µm)*	Food in Gut *(P, D, G)*
beg.4	*350*	*G*

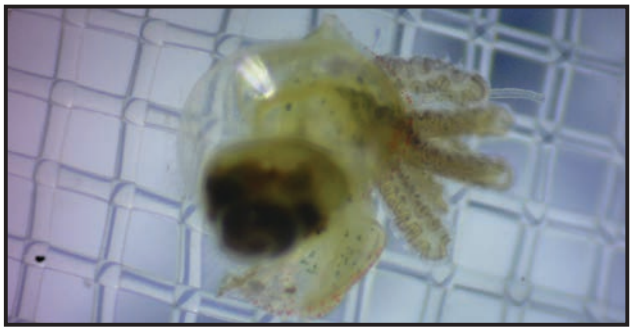
While looking through the microscope, consider the following:

- Are the veligers coming out of their shells?
- Are they alive?
- Are their lobes moving?
- Are the cilia on the lobes moving?
- Are their lobes extended and rounded?
- How many lobes do they have?
- Do their guts look pale, dark, or golden?

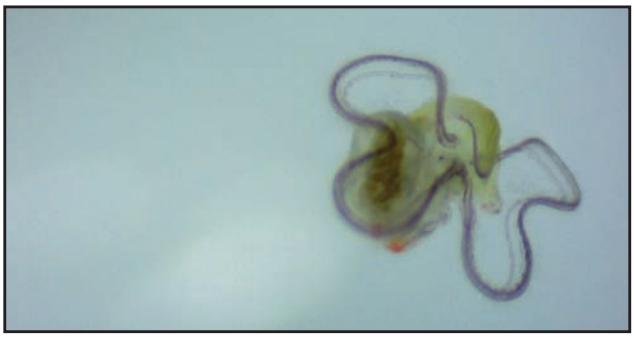
SIGNS OF HEALTHY & UNHEALTHY LARVAE



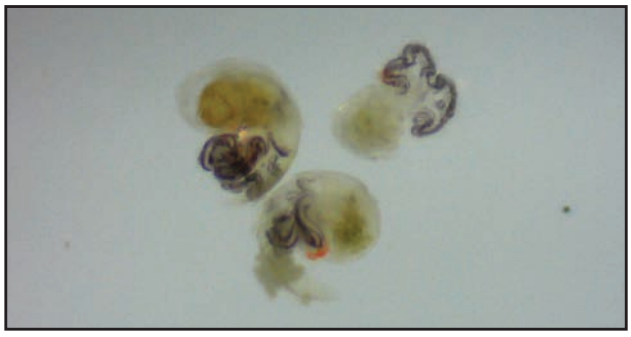
▶ *Golden gut typical for younger veligers (healthy).*



▶ *Dark gut typical for older veligers (healthy).*



▶ *Round, fullly extended lobes (healthy).*

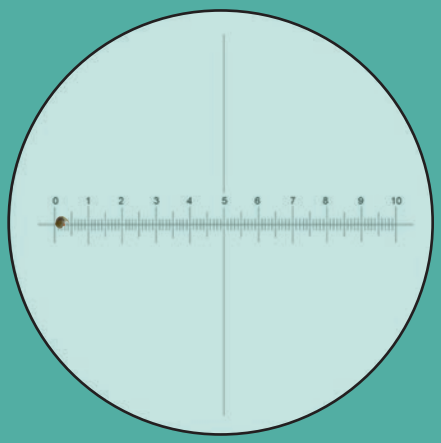


▶ *Stunted lobes and pale guts (unhealthy).*

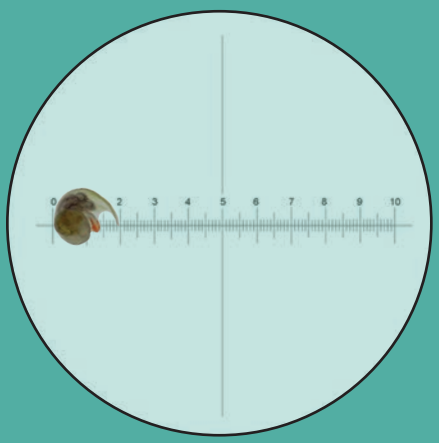
MEASURING SHELLS WITH MICROMETER

*The next step is to determine the shell length of five individuals. Conch veligers, however, are tiny and even at the end of their larval cycle they are only about the size of a pinhead (1,000 µm = 1 mm). It would be impossible to measure them with a regular ruler, this is why an **ocular micrometer** is used. The micrometer disk fits into the eyepiece of the dissecting microscope for use with the dominant eye.*

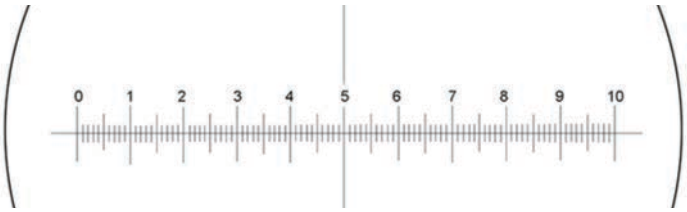
▸ *Refer to p. 68 for detailed microscope instructions.*



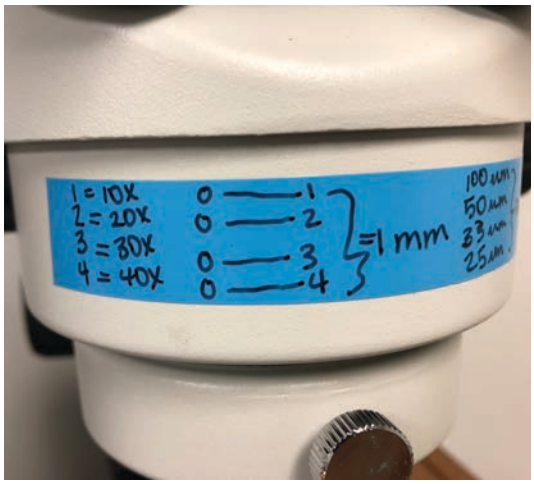
10X - A micrometer is positioned over a veliger. Despite being 10 times bigger than what is visible with the naked eye, it is still pretty small. 40X is a preferred magnification.



40X - The micrometer is in position to measure the shell length of the veliger. Note how the apex and siphonal canal (which is behind the beak) are aligned with the units.



▸ *There are 10 units in this micrometer and each unit has 10 subunits.*



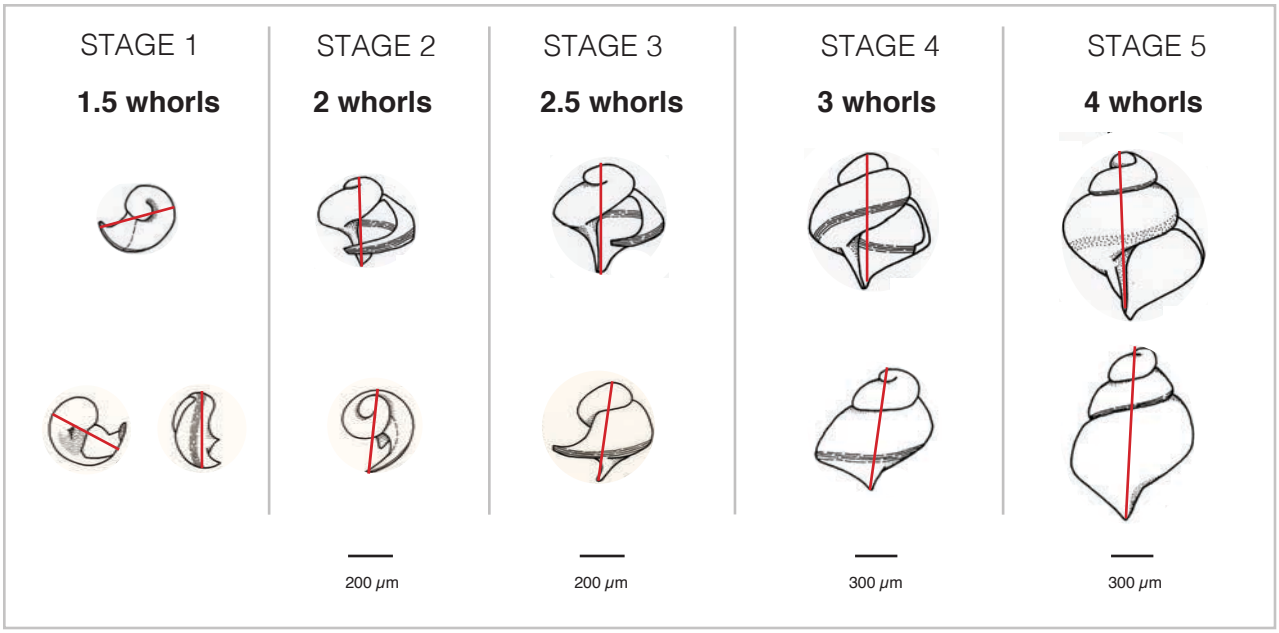
Magnification	Number of units per mm	Microns per subunit
10X	1 = 1mm	100
20X	2 = 1mm	50
30X	3 = 1mm	33
40X	4 = 1mm	25

▸ Since the eyepieces are 10X magnification, and the objectives on the microscope are 1X through 4X, the total magnifications when looking through the microscope are 10X through 40X.

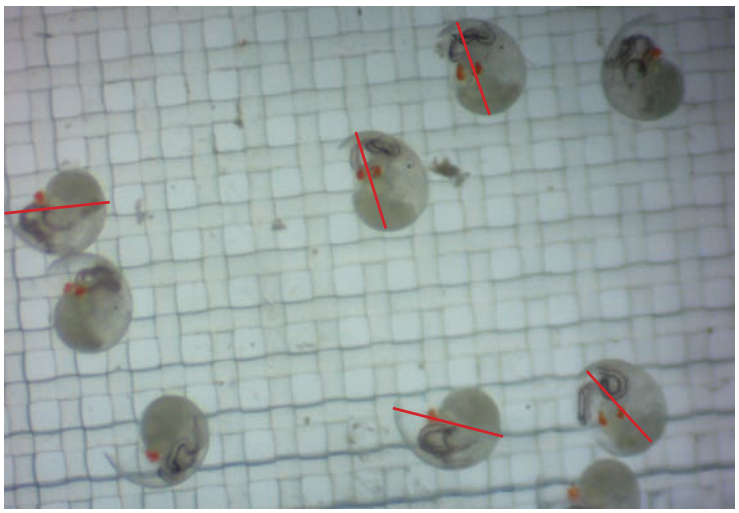
MEASURING SHELLS WITH MICROMETER

Although the veligers do not look like adult queen conch yet, they do in fact have a tiny shell, the same one they will keep their entire lives. When looking at an adult conch, the very tip of their ***apex*** *is the initial larval shell from which it grows more and more* ***whorls****. As the veligers develop over the course of their larval cycle their shells will grow from 1.5 whorls (Stage 1) to 4 whorls (Stage 5).*

The illustrations below show how to measure shell length, from apex to siphonal canal. Stage 1 and Stage 2 veligers sometimes need to be measured from their apex to their beak due to their shape and the way they lay on the slide. This will still give an accurate measurement.



Illustrations by Bonnie Bower-Dennis in Davis et al. 1993 (modified)

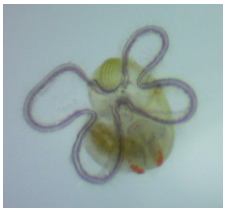


To measure the shell of veligers, pipette a few individuals using the eye dropper. This commotion causes their lobes to retract into their shell, making the veligers easier to measure. One by one, rotate the eyepiece with the micrometer to position it to measure the shell length. Repeat on five veligers. In this image you can see some veligers are marked with a red line as an example of how to measure newly-hatched veligers.

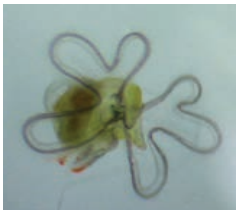
*Now that shell development has been described, this next section will discuss the **morphology** of veligers from the moment they hatch (Stage 1) to when they are ready to metamorphose (Stage 5). Conch larvae are considered **zooplankton**, microscopic marine animals that live in the water column. To accomodate this environment and lifestyle they have **lobes** which allow them to swim, feed, and breathe until they settle and become **benthic** snails.*

VELIGER DEVELOPMENT

STAGE 1:
2 lobes

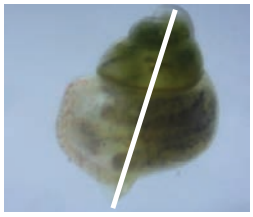


STAGE 2:
4 lobes



STAGE 3:
6 short lobes

STAGE 4:
6 elongated lobes



STAGE 5:
Shell length is 1.0 - 1.2 mm

SHELL DEVELOPMENT

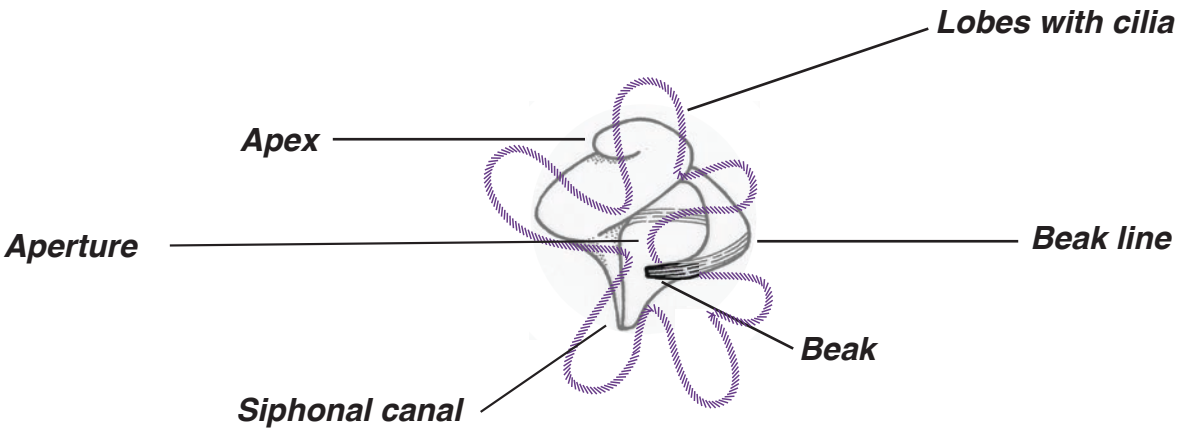
1.5 whorls

2 whorls

2.5 whorls

3 whorls

4 whorls



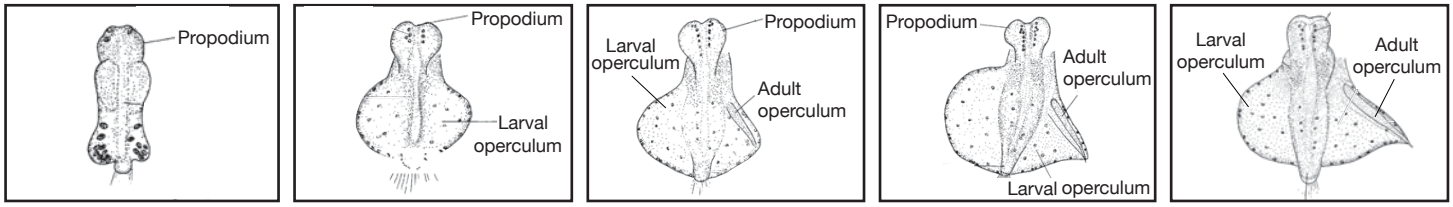
LARVAL FOOT DEVELOPMENT

*The veliger foot has two parts: the **propodium** (front of foot) and the **metapodium** (back of foot), which has an **operculum** (larval, and later on adult clawlike structure). The foot can be used as an indicator organ for determinig the stage of larval development and has many functions during the larval cycle such as:*

1. Removal of rejected food from the mouth
2. Balancing organ for swimming
3. Mucus secreting cells (triggered when stressed)
4. Protection with operculum
5. Locomotion organ (during swim-crawl)
6. Excretory organ

FOOT DEVELOPMENT

Illustrations from D'Asaro 1965



A B C D E

A: **DAY 1 (STAGE 1)**
Orange pigments on the propodium.

B: **DAY 4 (STAGE 2)**
Orange pigments multiply, propodium and the larval operculum widens.

C: **DAY 6 - 10 (STAGE 3)**
Adult operculum can be seen within the borders of the larval operculum.

D: **DAY 12 - 16 (STAGE 4)**
Adult operculum is now very visible and is beginning to extend over the larval operculum forming a claw. Some of the orange pigment cells on the larval operculum change to dark green.

E: **DAY 18 - 21 (STAGE 5)**
When lobes are retracted, the foot is very active. The claw continues to extend past the larval operculum. Operculum is used to orient the position of the shell when the conch is in the swim-crawl phase. The majority of orange pigment cells change to dark green on the larval operculum and the propodium.

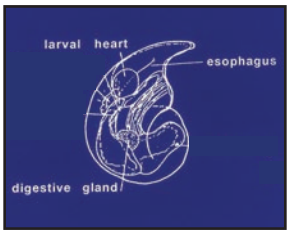
POST-METAMORPHOSIS:
The adult operculum takes over from the larval operculum. Now that the lobes have wrinkled and disappeared, there is total use of the foot and adult operculum.

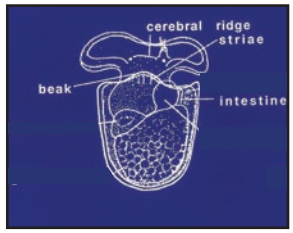
LARVAL DEVELOPMENT: DETAILED

STAGE 1
DAY 1

Newly-hatched veligers have a distinct, elongated ***beak***, two velar lobes, and a light-colored gut. Although they hatch with a ***yolk reserve*** they are ***planktotrophic*** and will need to feed on phytoplankton. The black eye spots and orange pigments on the foot are visible. Shell length is 300 - 350 µm and the shell has 1.5 whorls.

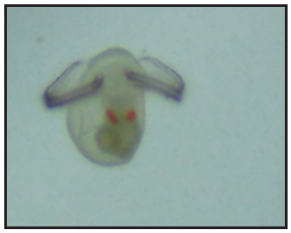
Profile view





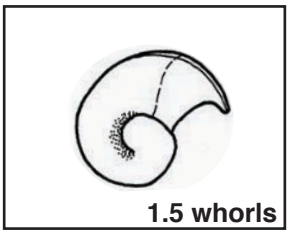
Side view

Profile view



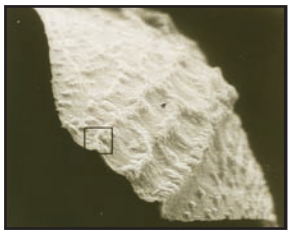
Side view: foot

Profile view



Frontal view: eyes

Close-up of beak: SEM

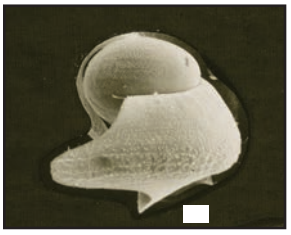


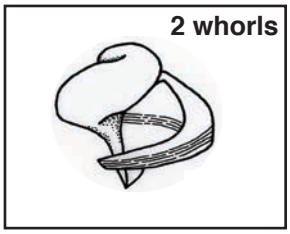
Close-up of shell: SEM

STAGE 2
DAY 4

The shell has an elongated beak that projects over the ***aperture***. The velar lobes have indented to form 4 lobes. ***Siphonal canal*** is visible. The foot has expanded and the orange pigments multiply near the bottom of the foot. Phytoplankton makes the veliger digestive glands look golden. Shell length is 430 - 470 µm and the shell has 2 whorls.

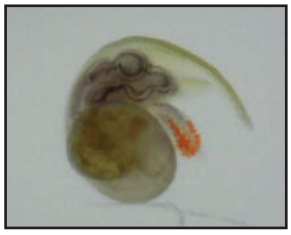
Elongated beak: SEM

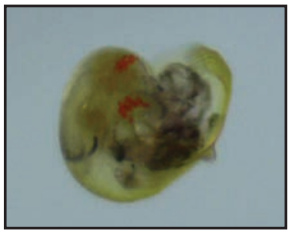




Aperture and beak line

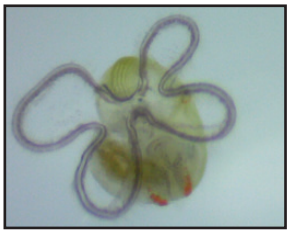
Aperture down: orange foot visble

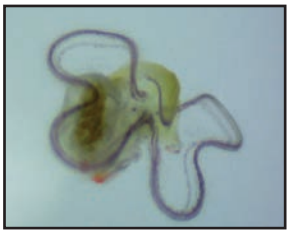




Aperture up: lobes retracted

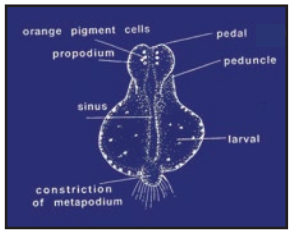
Four short lobes





Elongated beak, eye spots visible

Close-up: foot



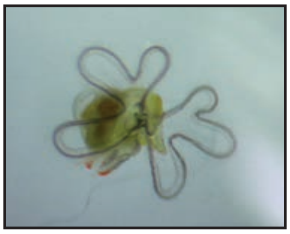
*Close-up: **cilia** on lobe and food groove*

P. 22-24: Blue illustrations by D'Asaro 1965
White illustrations by Bonnie Bower-Dennis

STAGE 3
DAY 6 - 10

Cleavage of the third pair of lobes completed by day ten. The digestive area has expanded and is golden brown with phytoplankton. The shell continues to have an elongated beak. The shell length is 510 - 670 µm and the shell has 2.5 whorls.

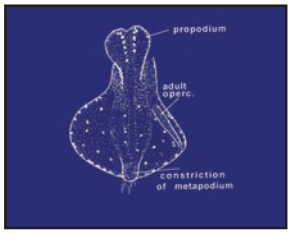
Frontal view: 6 lobes

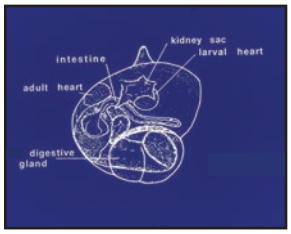


Frontal view: beak

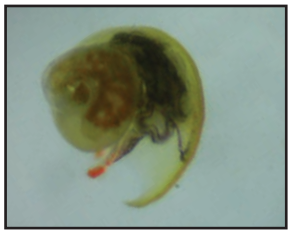
Frontal view: foot

Foot: close up

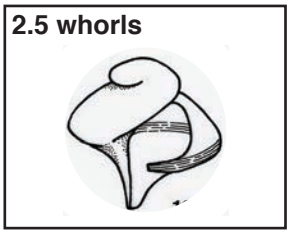




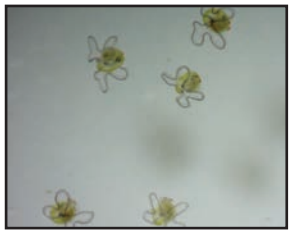
Apex in foreground



Apex view: golden digestive gland



Aperture view: beak



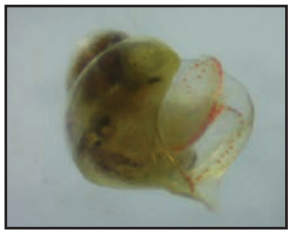
Swimming veligers

STAGE 4
DAY 12 - 16

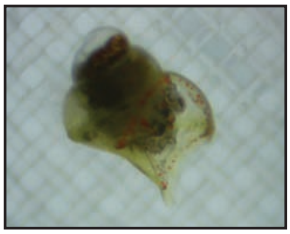
The 6 lobes have elongated and the shell beak has mostly receded. The digestive area is dark brown. The foot has greatly expanded and the adult operculum claw is visible. Some of the orange foot pigments have turned to dark geen spots on the metapodium. Some veligers exhibit swim-crawl behavior. The shell length is 750 - 910 µm and the shell has 3 whorls.

Aperture view: SEM

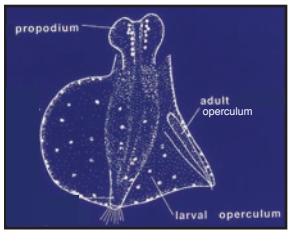
Operculum acts as door to protect veliger

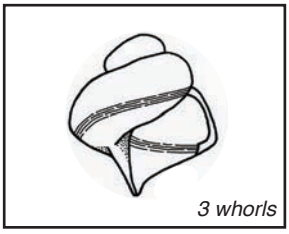


Brown digestive gland

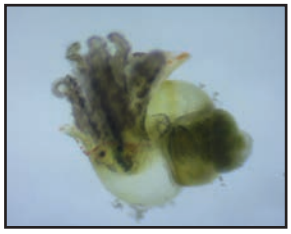


Foot: close up

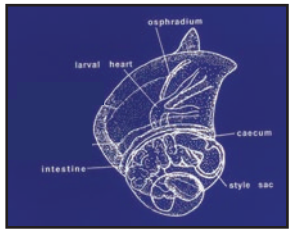




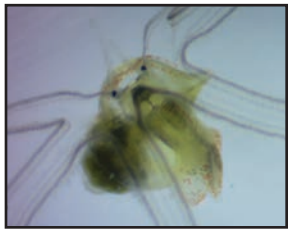
Receding beak



Aperture up: lobes retracted



Digestive gland in apex

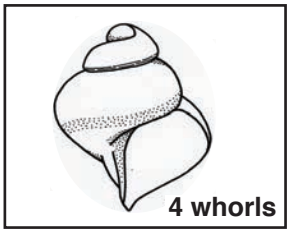


Eyes, foot, and lobes visible

STAGE 5
DAY 18 - 21

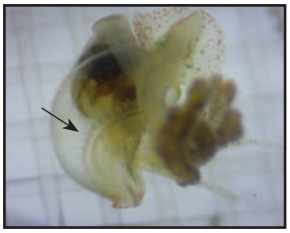
The eyes of competent veligers are at the base of their tentacles. Pigments on the foot have changed from orange to dark green. ***Ctenidium*** (gill) and ***osphradium*** are visible. ***Buccal mass*** is developing. The digestive area is dark brown to green. The larval shell has no beak, and has reached terminal larval shell length of 1,000 - 1,200 µm. The shell has 4 whorls. Many veligers exhibit swim-crawl behavior.

Aperture view

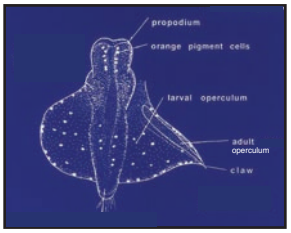


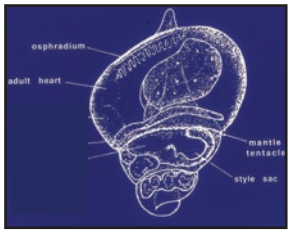
6 elongated lobes

Gill near the siphonal canal

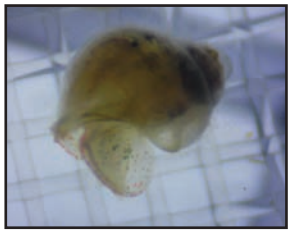


Foot: close up

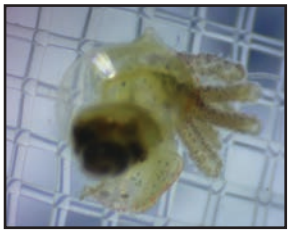




Gills and adult heart are visible



Dark green spots on the operculum



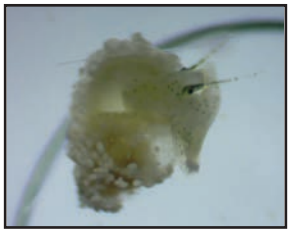
Swim-crawl position

Competent veliger

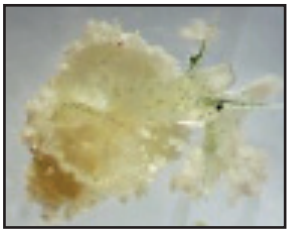
METAMO-RPHOSIS
DAY 21

The metamorphosed conch has lost its velar lobes and crawls with its foot. The eyes have partially migrated up the ***tentacles*** and the ***proboscis*** is used for grazing. The shell goes from smooth whorls to ridged whorls. The shell length two days after ***settlement*** is 1,300 - 1,600 µm.

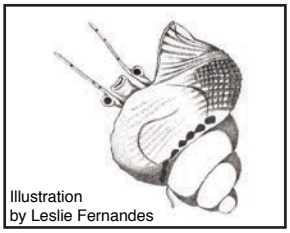
Newly-metamorphosed



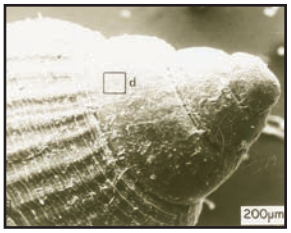
Proboscis protrudes



Smooth & ridged whorls



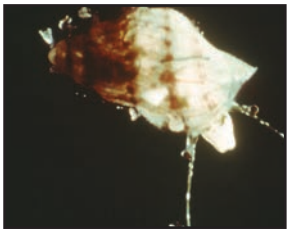
Whorl textures: SEM



Body and foot have dark green pigments

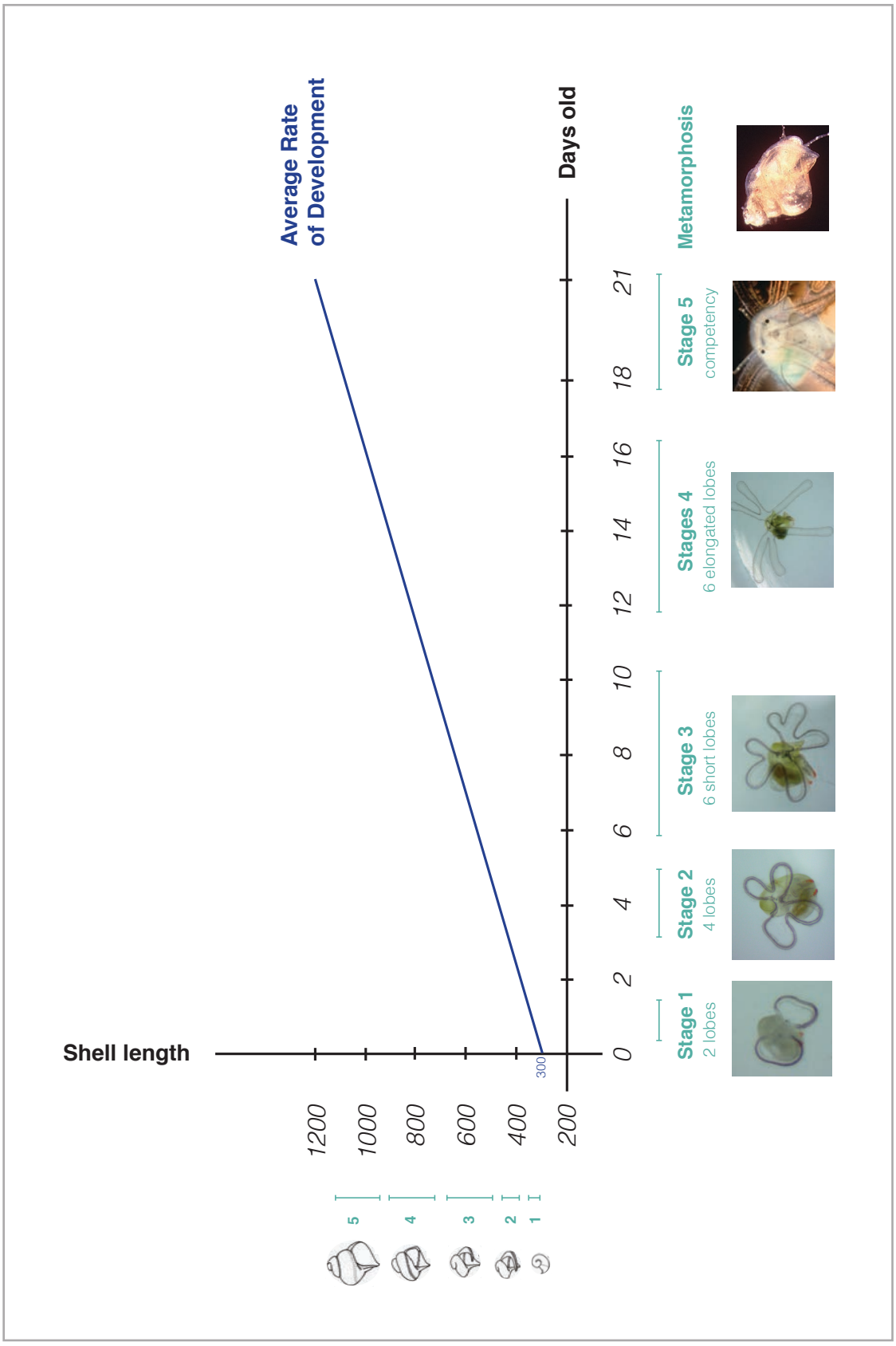
Siphonal canal lengthens
(Image by LeRoy Creswell)

Debris sticks to shell



1 to 2 weeks after metamorphosis
(Image by LeRoy Creswell)

LARVAL DEVELOPMENT SUMMARY



▲ *This figure is based on development during a typical 21-day larval cycle from hatch to metamorphosis. The larval cycle length varies depending on conditions such as temperature, density of the culture, and feeding quantity.*

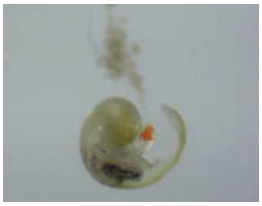
Improper handling: It is extremely important to handle veligers gently during water changes and when the larvae are in the tanks. If the water change is done too fast the veligers may land roughly on the sieve, which can cause breakage of the shells. Veligers will then need to spend time repairing their shells rather than growing their shells larger. If the veligers are kept on the water change sieve too long, the aeration in the tank is too fast, and/or the veligers are overfed this will cause stress and they will secrete mucus chains.

Inadequate food supply: Veligers will begin to feed on microalgae the next morning after hatching (day 1), however, veligers will not fully begin to utilize this food source until day three or four. By day four the embryonic food in the yolk cells in the stomach has decreased completely and by day ten the albumen cells have decreased completely. Sufficient microalgae early in development will make the veligers more robust for the later stages. Therefore, it is recommended that veligers are fed starting on day one.

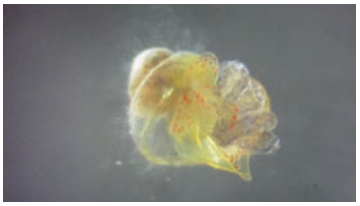
Correct amount of food supply: It is important to follow the *Microalgae Daily Feeding Table* (p. 31) to determine how much microalgae to feed the veligers each day. Feeding amounts are also determined based on veliger density, development stage, and how much food they have in their digestive gland. Too much food will cause mucus chains, which can cause clumping of the veligers.

Contamination: Bacteria and protozoans (ciliates) will proliferate under unhealthy conditions, which can be caused by not removing the hatched egg mass soon enough or introducing a contaminated egg mass to a tank, too much food or poor-quality microalgae, and not removing debris or dead veligers during water changes. Additionally, beginning with the swim-crawl stage, the veligers will tend to spend time at or near the bottom of the tank. It is important to keep the bottom clean or these late stage veligers will come in contact with metabolic waste. Keep the aeration higher to keep the late stage veligers in suspension.

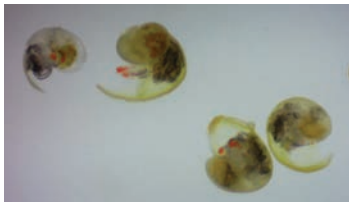
Disease vs. toxicity: Disease is caused by an organism, typically a bacterial infection such as *Vibrio*. The larvae will grow normally but show a high mortality usually between day seven and ten. Bacteria can be introduced from the microalgae, aeration system, or from the seawater. Toxicity usually occurs from the system such as new tanks and piping. It could also come from the air or water. The larvae do not grow and mortality is high, usually starting on day four. Both of these situations can cause 100% mortality also known as a crash of the larval batch.



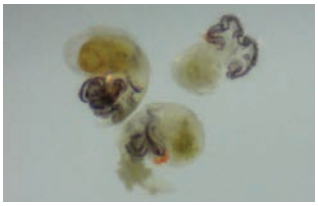
Mucus chain is a sign of stress from handling or overfeeding. It is not necessarily fatal but should be monitored.



An overgrowth of 'fuzz' can occur on the outside of the larval shell. The source is typically from the seawater or microalgae. Some growth is acceptable, but these sources of potential contamination should be checked.



These 4-day-old veligers are healthy except for the one on the far left. It is smaller, has less food in its gut and is considered a runt. Most runts will get culled out of the culture during water changes when the sieve size mesh is increased.



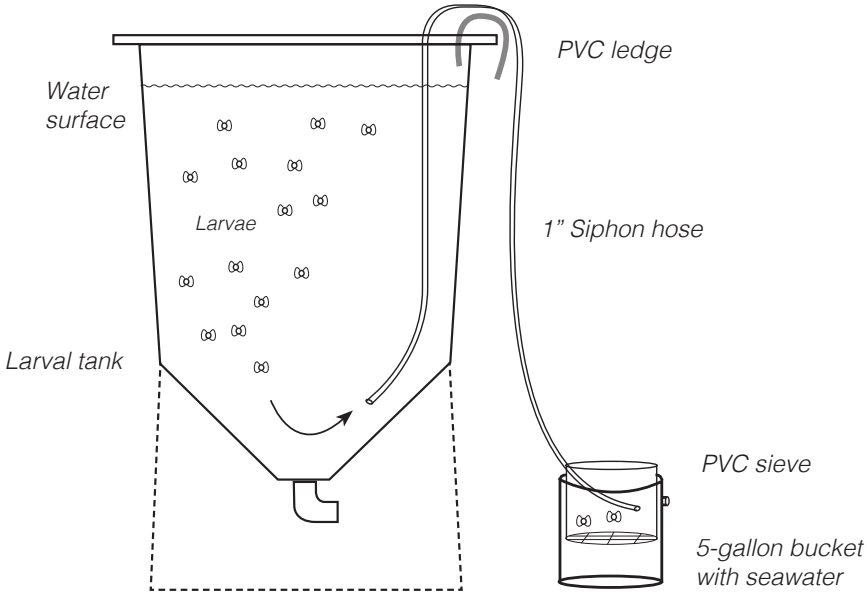
These 4-day-old veligers did not develop well. Lobes are shrivelled and stunted and their guts are pale.These veligers will not survive.

The water in the larval tank should be changed every other day, starting on day 2, to allow the tank surfaces to be cleaned. This removes bacteria films and prevents harmful bacteria and protozoans to proliferate in the tanks. During a water change the veligers are siphoned from the tank into a sieve (10" dia x 12" h) with the appropriate mesh size (p. 15), to cull out the stunted and dead veligers.

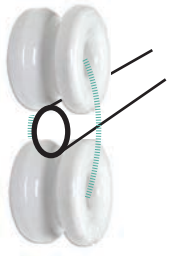
Water changes also allow larval density to be adjusted. For example, if the density is double the recommended amount, the water in the tank should be siphoned only half-way to collect the veligers needed. The other half should be transferred into another tank with lower density or be released into the wild.

Only start a water change once the **Larval Rearing Data Sheet** (p. 16) *is filled out because the status of the larval culture must first be determined such as larval density and microscope observations of the vital signs of the veligers.*

Larval tank set up for water change.



The tank walls of each larval tank are cleaned with a mop.



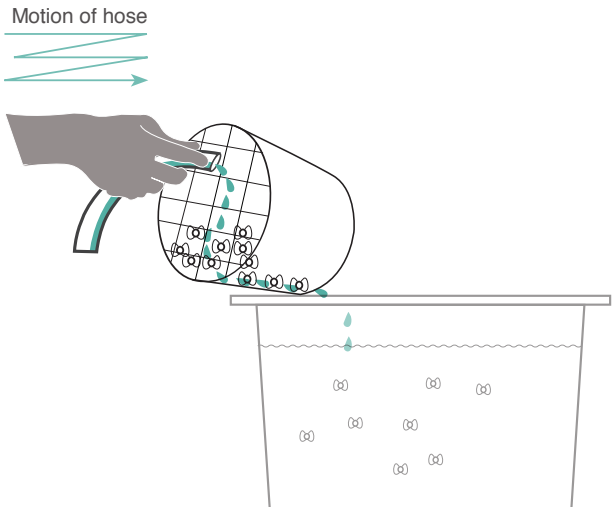
There are typically two ceramic weights zip-tied together and put over the end of the siphon hose that is placed inside of the tank. The weights keep the ***siphon hose*** *from moving around and prevent it from floating up.*

Note: All water change gear (buckets, siphons, sieves, tanks, and airlifts) should be cleaned with a mild muriatic acid solution (10 ml muriatic acid / 1 L freshwater) and then rinsed with freshwater, after each water change.

STEP 1:
Conch larvae cannot be dry-sieved. Therefore, the appropriate mesh-sized sieve is placed into a 5-gallon bucket that is filled with seawater prior to starting the water change. Make sure that the end of the siphon hose is secured under the sieve handle and start the siphon. The dead and slow-growing veligers will pass through the screen, while healthy, fast-growing larvae are retained. The siphon method is less stressful to the veligers compared to draining them from the bottom of the tank. Often the bottom of the tank has some dead larvae and it is best not to siphon the tank completely.

Every two to three days depending on shell length, gradually transition to a sieve with a larger mesh size. Starting with the 105-µm mesh and ending with the 300-µm mesh. See *Larval Density Table* on page 15. *Note that some of the mesh screens of the sieves are so small the grid pattern can barely be seen.*

STEP 2:
Using the PVC handle, gently remove the sieve with a slight rocking motion from the 5-gallon bucket. Carry the sieve to the tank where veligers are going to be transferred to. Lean the sieve on the tank edge and tilt the opening down towards the water. Using very low water pressure, hose the mesh working from the top to the bottom. This will create a stream to guide the veligers down and out of the sieve as shown in the illustration.

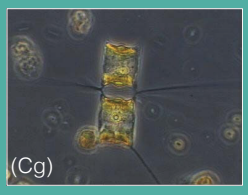


STEP 3:
Once the veligers are transferred to the new tank, observe them as they come back into suspension. Check that the air is gentle but high enough to keep the veligers in circulation with their lobes extended. Sometimes it will be necessary to turn the air up a little bit until they have their lobes extended, and then the air is turned back down. Once the larvae are fully dispersed, they are fed microalgae and the tank is covered with a lid.

FEEDING VELIGERS

Veligers hatch with yolk reserves, and will begin feeding on single-celled microalgae six to eight hours after hatching. Therefore, to increase larval survival and vigor, the veligers should be fed the morning after they hatch. From then on, feed veligers daily and it is recommended to feed them shortly after water changes as this stimulates them to swim and be active feeders. See Chapter 4: Growing Microalgae for more details.

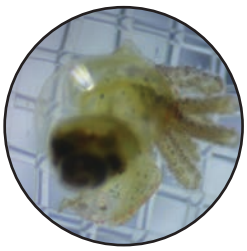

(Iso)


(Cg)

The two preferred species of microalgae (phytoplankton) that are cultured for feeding veligers are:

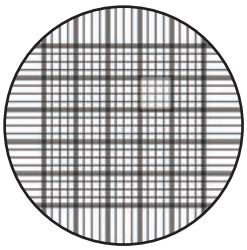
1. Caicos *Isochrysis* or Tahitian *Isochrysis galbana* (Iso), a flagellate alga that is fed every day of the larval cycle.

2. *Chaetoceros gracilis* (Cg), a diatom that is fed in the later larval stages, typically starting on day 16, when the veligers are about 900 µm.

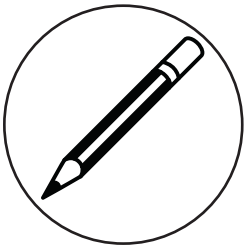


STEP 1:
Prior to feeding the veligers, it is necessary to take into consideration age, stage of development, gut color, amount of feed, and water change dates. Consider the following:

- Are they developing according to the Stages?
- Are they looking healthy?
- Are their digestive glands full of the golden to dark brown microalgae?
- Do they have fecal mucus chains as a sign they were overfed the day before?
- Was the water changed the day before?



STEP 2:
Every day, cell counts (p. 54) are done using a ***hemocytometer***, for the microalgae cultures that will be fed to the larvae. These counts along with the observations from Step 1 are used to determine how much microalgae is needed to feed the veligers in each larval tank.

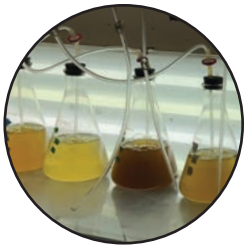


STEP 3:
Once the cell count is done, refer to the *Microalgae Daily Feeding Table* (next page) to fill out the **Microalgae Feeding Data Sheet:**

Egg Mass # / Larval Batch #: 1-06.18.2020

Date	Larval tank #	Larval age *(day)*	Algae type *(Iso or Cg)* and culture #	(A) Size of larval tank *(ml)*	(B) Algae cells needed per ml of tank water	(C) Algae cell count *(cells/ml)*	(D) Total algae cells needed for each larval tank (A x B)	(E) # of mls to feed larval tank (D/C)
06.23.20	*2*	*1*	*Iso 6.19-A*	*68,000* 68×10^3	*5,000* 5×10^3	*6,000,000* 6×10^6	*340,000,000* 340×10^6	*56.7* *57 ml*
07.13.20	*2*	*21*	*Iso 7.09-A*	*68,000* 68×10^3	*10,000* 10×10^3	*6,000,000* 6×10^6	*680,000,000* 680×10^6	*113* *113 ml*
07.13.20	*2*	*21*	*Cg 7.09-A*	*68,000* 68×10^3	*5,000* 5×10^3	*4,000,000* 4×10^6	*340,000,000* 340×10^6	*85* *85 ml*

Microalgae Feeding Data Sheet



STEP 4:
Veligers are fed after a water change and tanks that are not scheduled for a water change also receive microalgae to feed the veligers at this time.

STEP 5:
Pour the correct amount of microalgal culture (ex: 57 ml) from the flask that was used for the cell count (ex: Iso 6.19-A) into a graduated beaker. Slowly pour the beaker contents into the appropriate larval tank in a circular motion around the standpipe. The rising air bubbles from the airlift will help disperse the microalgal culture throughout the tank. Feed larvae only once per day. Repeat the feeding process every day until the veligers become competent for metamorphosis.

MICROALGAE DAILY FEEDING TABLE

This feeding table is used to determine how much microalgae to feed veligers in each 68-L larval tank.

Age of Veliger	Cell count Iso in larval tank (cells/ml)	Cell count Cg in larval tank (cells/ml)	Total Iso cells fed daily per tank	Total Cg cells fed daily per tank
Day 1	2,500		170,000,000	
Day 2 *(water change)*	5,000		340,000,000	
Day 3	2,500		170,000,000	
Day 4 *(water change)*	6,000		408,000,000	
Day 5	3,000		204,000,000	
Day 6 *(water change)*	6,000		408,000,000	
Day 7	3,000		204,000,000	
Day 8 *(water change)*	7,000		476,000,000	
Day 9	3,500		238,000,000	
Day 10 *(water change)*	7,000		476,000,000	
Day 11	3,500		238,000,000	
Day 12 *(water change)*	8,000		544,000,000	
Day 13	4,000		272,000,000	
Day 14 *(water change)*	8,000		544,000,000	
Day 15	4,000		272,000,000	
Day 16 *(water change)*	9,000	3,000	612,000,000	204,000,000
Day 17	4,500		306,000,000	
Day 18 *(water change)*	9,000	3,000	612,000,000	204,000,000
Day 19	4,500		306,000,000	
Day 20 *(water change)*	10,000	5,000	680,000,000	340,000,000
Day 21	5,000		340,000,000	

3. Metamorphosis

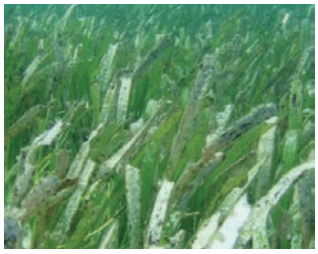
*Around day 18 - 21, veligers should start showing signs of **competency**, but will not go through metamorphosis without a natural **cue** - such as the presence of their food. In the wild, this cue are the epiphytes comprised of benthic diatoms that cover seagrass blades, macroalgae, and sand. **Epiphytes** trigger veligers to settle into seagrass beds, their juvenile habitat.*

*When veligers are metamorphically **competent**, they have a 'swim-crawl' behavior. They still have their lobes and can continue to swim or drift but they can also use their foot to test the substrate to see if it is the right place to settle.*

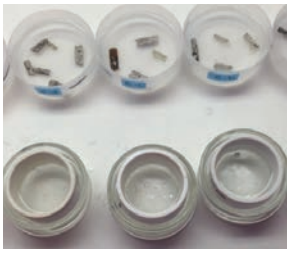
This chapter will demonstrate how to guide veligers through this key transformation.

FROM VELIGER TO SNAIL:

Competent veligers wait for a cue to trigger settlement.



Natural cues found in seagrass and sandy substrate habitat are used to trigger metamorphosis.



A subset of larvae are presented this cue in the hatchery setting.

The swimming larvae metamorphose into bottom-dwelling snails. *(Image by LeRoy Creswell)*

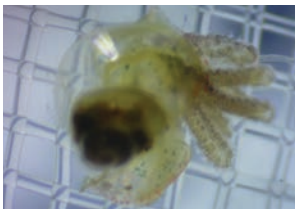
MORPHOLOGICAL AND BEHAVIORAL CHANGES

As the conch go through metamorphosis, many changes occur in their method of movement, feeding, and respiration. During these changes the veligers use a lot of energy, therefore, it is important to make sure that they are well fed and cared for prior to metamorphosis. For instance, a few days prior to competency, veligers are fed the lipid-rich ***diatom*** Chaetoceros gracilis *to give them extra energy. Here are the key changes the conch go through as they transition from larvae to benthic snails.*

	Larvae	Benthic Snails
Changes in Movement	Swim with lobes. (Velar lobes shorten, wrinkle, and are eventually absorbed or discarded during metamorphosis.)	Crawl with foot.
Changes in Feeding	Collection of microalgae cells (phytoplankton) with lobes.	Development of buccal mass to form snout, also known as proboscis, used for grazing epiphytes.
Changes in Respiration	Exchange of oxygen with lobes. Larval heart and adult heart both beat.	Gill, also known as ctendium, is fully functional. Larval heart ceases to function and adult heart beats strongly.
Other Changes in Morphology	Eyes are at the base of tentacles. Foot has a larval operculum and an adult claw operculum. The foot and the body have orange and green pigment spots.	Eyes have migrated partially up the tentacles. Adult operculum becomes a harder and more prominent claw over the foot region. All pigment spots on the foot and body have turned dark green / black.
Changes in the Shell	Shell has uniform color and minimal debris.	Spots occur on outside of shell. Shell becomes sticky and accumulates debris, algae filaments, and diatoms.

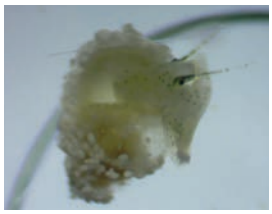
TRANSFORMATIONS

LARVAE: Six elongated lobes.



SWIM-CRAWL: Lobes and foot are visible.

SWIM-CRAWL: Gill (see arrow) is visible.



BENTHIC SNAIL: Lobes absorbed, snout and foot are visible.

PREPARING THE METAMORPHOSIS CUE

Seaweed extract of the red ***macroalga*** Laurencia poitei *or a small dose of hydrogen peroxide are both reliable cues that trigger metamorphosis in the hatchery setting. These cues should induce approximately 75% of the veligers. The success of metamorphosis for a batch of larvae is dependent on the uniformity of the veligers in the larval culture. Therefore, it is important to cull the slow growers during water changes. Also, testing a small group of veligers before inducing the whole tank of larvae will ensure that the culture is ready for metamorphosis.*

Laurencia poitei is a red bushy macroalga (seaweed) with branches that have knobby tips. The *Laurencia* extract is made by hand. Start with collecting older thick red-brown stalks of the macroalga from shallow, sandy, seagrass flats into mesh bags such as dive bags. The younger stalks, yellow-orange in color, are slightly toxic to the veligers and will cause a low percentage of veligers to metamorphose.

In the hatchery, the macroalgal fronds are gently rinsed with seawater and sorted to remove coral pieces, sponges, excess sand, and other algal species.The silt or epiphytes on the macroalgal branches are part of the cue for metamorphosis, therefore, do not clean them off too much.

In an industrial blender, a ratio of 2 g of *Laurencia* to 1 ml of seawater is blended for approximately two minutes. The solution is frozen for a minimum of two days to lyse**,** or burst**,** the cells and release the molecular algal-associated cue.

The frozen solution is thawed overnight, filtered through a 200-µm polyester screen, and the resulting extract is refrozen. This filtering is typically done by squeezing the thawed blended solution by hand with the screen mesh. The extract is collected in 10 small 500-ml containers for ease of use later.

5 kg (2.25 lbs) of collected macroalgae produces 8 L (2.1 gal) of blended slurry, which yields approximately 3.75 L (1 gal) of extract.

To determine the potency of the extract, a test set is done:

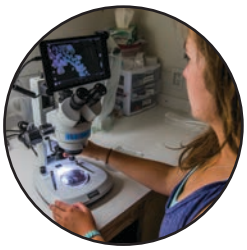
Before any new batch of *Laurencia* extract is used on the large-scale, the dosage is determined by placing 25 metamorphically competent veligers in three different extract concentrations: 7, 10, 15 ml of *Laurencia* extract / L of seawater for four hours.

This ***test set*** can be done in small 50- or 100-ml polypropylene tri-beakers. After removing the conch from the extract, percent metamorphosis is determined using a dissecting microscope. A minimum of 60% metamorphosis is considered effective to select a given dosage of *Laurencia* extract for use on the large-scale. The test set only needs to be done once for each new batch of extract.

A more cost-effective cue is to expose the competent veligers to a mild solution of 3% pharmaceutical grade hydrogen peroxide (0.06 ml of H_2O_2 / 1 L of seawater) for four hours. Although this is an effective cue, there can be a margin of error when making up the solution since it is used in such a low concentration. If this cue is selected, a test set of veligers should be conducted with the hydrogen peroxide cue prior to the entire culture being induced.

PREPARING FOR METAMORPHOSIS

Veligers need to go from a swimming stage to a benthic stage and this is a big transition for them. If the veligers are not developed enough, or too developed, they will not be able to complete metamorphosis and will die. There is only about a five day window when they can be successfully induced, typically beginning around day 21.



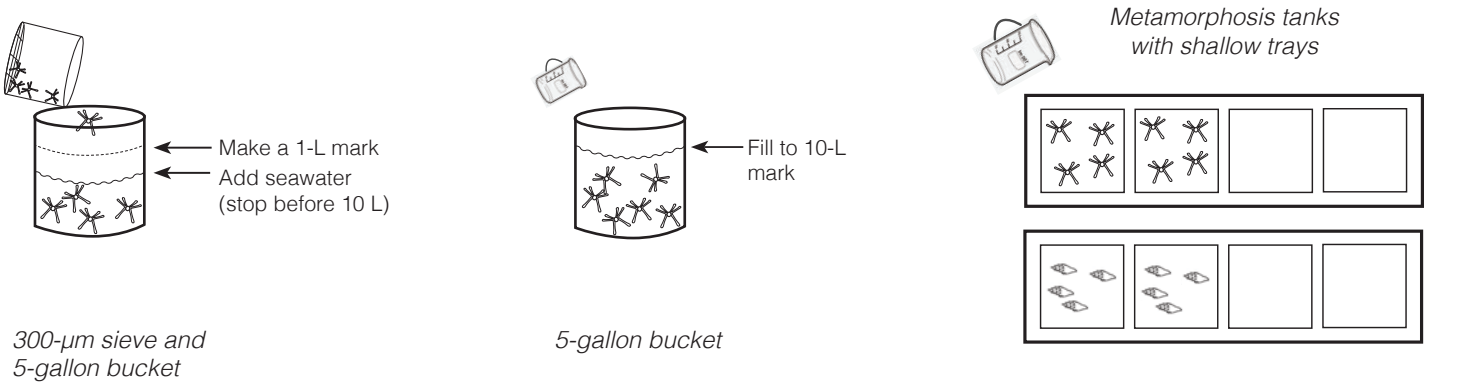
STEP 1:
Starting at Stage 4, use the microscope to see if the majority of larvae have signs of competency for metamorphosis. If they are not ready, check again each day until day 21 or older. If the veligers are ready (Stage 5), remove the *Laurencia* extract from the freezer if this is the cue being used to induce metamorphosis. Otherwise make sure that hydrogen peroxide has been purchased from the pharmacy.

STEP 2:
When larvae seem competent, test set a sample of veligers to be absolutely sure they are ready. Skim the surface of the larval tank with a small sieve until 10 - 20 veligers are collected. Expose them to hydrogen peroxide or the *Laurencia* extract at the preferred dosage for four hours. If 60 - 75% of the larvae go through metamorphosis, prepare the entire larval tank to be induced the next day. If a low percentage of veligers metamorphose, try a test set again the following day and possibly the day after.

STEP 3:
Prepare the metamorphosis tanks (38 L each) by filling them with 28 L of filtered, UV-sterilized seawater and the correct dosage of *Laurencia* extract or hydrogen peroxide. Mix the solution well with hands, and then place four shallow screen trays (250 µm) each with an area of 12" x 16" (0.12 m^2 or 1.3 ft^2) into the tank. The tanks are now ready to receive competent veligers.

STEP 4:
Metamorphically competent veligers are siphoned from the larval rearing tank (see p. 27) and concentrated into 10 L of aerated seawater in a 20-L (5-gal) bucket. The new density is estimated with the mean of three 50-ml subsamples. Gently mix the veligers in the bucket with a small round flat plastic or fiberglass disk, then use a beaker to collect subsamples to determine veliger density (see p. 14).

Transferring correct amount of veligers to metamorphosis trays:



a) *Gently hose the veligers from the 300-µm sieve into the 5-gallon bucket.*

b) *Calculate the new concentration of veligers by taking 3 beaker subsamples.*

c) *Transfer veligers from the 5-gallon bucket to the metamorphosis trays.*

Assuming that there are 10 veligers / L in the larval tanks, there would be 680 veligers per larval tank since the larval tanks are 68 L.

a) If 680 veligers (v) in one larval tank are ready for metamorphosis, and if those 680 are concentrated in a bucket filled with 10 liters of seawater, then the density would be 68 veligers per liter:
680 v / 10 L = 68 v / L

b) Considering that in real life the density in this larval tank will not be exactly 10 v/L, it will be necessary to take three 50-ml subsamples using a 50-ml tri-beaker from the bucket. These samples will provide a more accurate density count of the veligers.

Subsample 1: five veligers are counted in the beaker
Subsample 2: three veligers are counted in the beaker
Subsample 3: four veligers are counted in the beaker

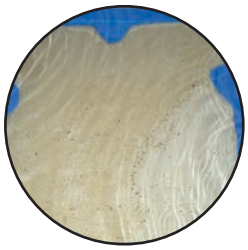
Thus, on average, there are four veligers per 50 ml (0.05 L): **4 v / 0.05 L = 80 v / L = 800 v / 10 L**
Therefore, the larval tank was holding 800 veligers, and so is the bucket with the concentrated veligers.

c) If each metamorphosis tray can hold 420 veligers (p. 37) and there are 800 veligers total, then half of the bucket contents should be put into one tray and the other half in another:
800 v / 2 trays = 400 v and 10 L in the bucket / 2 = 5 L of the bucket per tray

Thus, only two trays out of the four will be used for inducing metamorphosis for this one larval tank. If there is another larval tank with veligers ready for metamorphosis, the same procedure can be done and the veligers can be added to the remaining two trays.

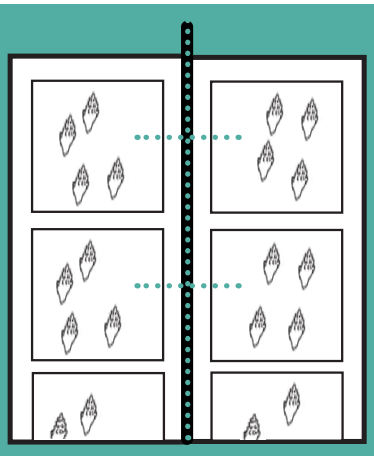
STEP 1:
The veligers are distributed using a 500-ml or 1-L graduated beaker with a handle into the pre-determined number of trays in the metamorphosis tank. The competent larvae are stocked at 3,500 / m^2 (325 / ft^2), which is equivalent to approximately 420 veligers per tray or 1,680 per tank. It is recommended to keep the veligers in subdued light to mimic the lower light that would be found when they metamorphose in the seagrass beds. Veligers typically stop swimming 10 - 30 minutes after they are exposed to the metamorphic cue.

STEP 2:
After four to five hours, water flow is slowly introduced in the shallow tank trays to eliminate the cue from the system. Approximately 60 - 75% of the veligers will have metamorphosed. Additional veligers may metamorphose within the next 24 hours, but it is not guaranteed. Veligers cannot be re-induced that is why it is so important to carefully time the induction of the veligers for metamorphosis.



STEP 3:
The newly-metamorphosed conch are fed after the cue has been diluted. The dilution takes approximately 30 minutes. They are benthic grazers now and, therefore, unable to feed on planktonic algae. At this point in their life cycle, the conch must be fed a ***flocculated*** algae (p. 58) which they can graze on using their proboscis. In this image, a row of newly-metamorphosed conch can be seen grazing their way through the flocculated algae (dark beige) from the lower right hand corner towards the center of the tray.

Image by Michiel van Nierop



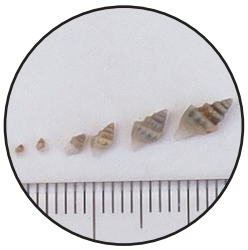
Examples of shallow water metamorphosis tanks and mesh screen bottom trays on a recirculating system. A small stream of water enters the trays at an angle to create water motion in the downwelling tray. The desired culture temperature is 28 – 30 °C (82 – 86 °F).

CARING FOR METAMORPHOSED CONCH

Following metamorphosis, the conch will look like tiny snails under the microscope and will grow rapidly over the next three to four weeks. The conch are maintained on screen trays in the metamorphosis tanks until they reach an average size of 3 - 4 mm in shell length. Typically, 50% of the conch survive from the competent veliger stage to the post-metamorphosed stage (4 mm). To ensure good survival, growth, and development it is important to care for the conch daily. This includes observing and feeding the conch, and cleaning their environment which will all be recorded on the **Metamorphosed Conch Observation Data Sheet.**

Date	Days after meta.	Weekly size of conch *(mm)*	Spray trays	Food remaining *(%)*	Food fed per tray *(ml)*	General observations	Temperature *(°C)*
7/15/20	1	1.2	No	10	44	Conch spread out and active	28
8/11/20	28	3.5	Yes	0	74	Feed more	27

Metamorphosed Conch Observation Data Sheet



STEP 1

Every day, observe the conch in the metamorphosis trays and pay attention to their behavior and consider the following: are they spread out, clumped in one area, or along the walls of the tray? Are they active or lethargic? Do they need more or less feed?

Each week, 10 conch per tray are measured using a dissecting microscope with an ocular micrometer. When the conch are small, an eye dropper pipette is used, and as they get larger a plastic pipette with the end cut off can be used.

STEP 2

Visually check the conch density in the metamorphosis trays to ensure optimal density for efficient feeding and maintenance. If the density of conch in a tray is low, gently hose the conch off and combine them into other trays. Some trays may have a low density due to poor metamorphosis induction, water quality, amount of feed, and/or contamination.

STEP 3

Prior to feeding, use a seawater spray wand to gently remove any uneaten food and feces, and redistribute the conch on the tray. Every other day the metamorphosis tanks will need to be partially drained to remove any uneaten food and feces that might have accumulated underneath the trays. The tanks should then be refilled with filtered, UV-sterilized seawater and then the conch are ready to be fed.

FEEDING GUIDE FOR METAMORPHOSED CONCH

Each day the conch are fed flocculated *Chaetoceros*. Prior to feeding there are several observations that need to be taken into consideration: shell length, conch density, mortality, growth rate per day, and amount of left-over feed. Too much feed in the tanks can cause bacteria to flourish, not enough feed and the conch growth can be stunted. One sign that there is not enough feed, is observing the conch crawling up the sides of the trays in search of more food. The table below is a guide for feeding 1.0 mm to 4.0 mm conch. With proper feeding, they will grow approximately 0.18 - 0.22 mm per day.

Shell length (mm)	**Estimated* total cells per conch per day**	**Total number of conch per tray**	**Estimated* total number of cells per tray per day**	***Amount of flocculated alga needed for each tray**
1.0 - 1.2	6×10^7	420	2.5×10^{10}	44 ml
1.2 - 2.0	8×10^7	250	2.0×10^{10}	35 ml
2 - 3	12×10^7	250	3.0×10^{10}	53 ml
3 - 4	20×10^7	210	4.2×10^{10}	74 ml

** Amount of flocculated algal cells needed for each tray assuming an initial cell count of 6×10^6 cells per ml prior to flocculation and flocculation concentrate fills a 1-L container.*

Use the **Metamorphosed Conch Feeding Data Shee**t to keep track of the volume of flocculated algal cells needed to feed to the metamorphosed conch on a daily basis.

Date	Days after meta.	Number of cells/conch/ day	Cells/ml *(before flocculation)*	Cells/ml *(after flocculation)*	Number of conch	Total cells needed	Amount of feed per tray *(ml)*
7/15/20	1	6×10^7	6×10^6	57×10^{10}	420	2.5×10^{10}	44
8/11/20	28	20×10^7	6×10^6	57×10^{10}	210	4.2×10^{10}	74

Metamorphosed Conch Feeding Data Sheet

FEEDING NEWLY-METAMORPHOSED CONCH (0 - 2 weeks old)

Newly-metamorphosed conch are fed 6 x 10^7 cells/conch/day of flocculated Chaetoceros *and the feeding amount will increase over time. The concentration and volume of the flocculated microalgae to feed the conch needs to be determined from the cell count of the* Chaetoceros *culture prior to flocculation. Here is an example of the calculations used to determine how much to feed the newly-metamorphosed conch:*

Assuming the *Chaetoceros* cell count is: 6 x 10^6 cells/ml
Amount of conch per tray: 420
Conch feed needs: 6 x 10^7 cells/conch/day

= (420 conch per tray) x (6 x10^7 cells per conch per day)
= 2,520 x 10^7 cells
= 2.5 x 10^{10} cells

This is the total number of cells that are fed to the 420 conch in one tray for one day.

Knowing that a 95-L suntube of microalga before flocculation has a cell count of 6 x 10^6 cells/ml:

= (6 x 10^6 cells/ml) x (95L x 1,000 ml/L)
= (6 x 10^6 cells/ml) x (9.5 x 10^4 ml)
= 57 x 10^{10} cells

This is the amount of cells in a 95-L suntube. All of these cells have been concentrated through the flocculation process and now fit into a 1-L container.

How many ml of flocculated microalga from the 1-L container need to be fed to the conch in each metamorphosis tray?

57 x 10^{10} cells / 1,000 ml (the 1-L container volume)
= 0.057 x 10^{10} cell/ml

= (2.5 x 10^{10} cells needed to be fed to the tray of conch) /
(0.057 x 10^{10} cells/ml of the flocculated cell count in the 1-L container)
= 44 ml of flocculated microalga

Thus, 44 ml of the 1 L flocculated microalga must be used to feed the conch in one metamorphosis tray.

44 ml x 8 trays
= 352 ml total to feed

Therefore, 352 ml of the 1 L flocculated microalga is needed to feed the conch in all eight metamorphosis trays, if both tanks are full of conch.

FEEDING POST-METAMORPHOSED CONCH (2 - 4 weeks old)

Two to four weeks after metamorphosis, feed amount increases up to 20 x 10^7 cells/conch/day. Here is an example of the calculations used to determine how much to feed the post-metamorphosed conch that are 3 - 4 weeks old after metamorphosis.

Assuming the cell count is: 6 x 10^6 cells/ml
Conch per tray (assuming a 50% survival rate: 420 x 0.50 survival) = 210 conch per tray
Conch feed needs: 20 x 10^7 cells/day

= (210 conch per tray) x (20 x 10^7 cells per conch per day)
= 4,200 x 10^7 cells
= 4.2 x 10^{10} cells

This is the total number of cells needed to feed the 210 conch in one tray for one day.

Knowing that a 95-L suntube of microalga before flocculation has a cell count of 6 x 10^6 cells/ml:

= (6 x 10^6 cells/ml) x (95 L x 1,000 ml/L)
= (6 x 10^6 cells/ml) x (9.5 x 10^4 ml)
= 57 x 10^{10} cells

This is the amount of cells in a 95-L suntube. All of these cells have been concentrated through the flocculation process and now fit into a 1-L container.

What is the new cell count (cells/ml) in the concentrated 1-L (1,000 ml) container?

57 x 10^{10} cells / 1,000 ml (the 1-L container volume)
= 0.057 x 10^{10} cell/ml

= 4.2 x 10^{10} cells needed to be fed to the tray of conch / 0.057 x 10^{10} cells/ml of the flocculated cell count in the 1-L container
= 74 ml of flocculated microalga

Thus, 74 ml of the 1 L flocculated microalga must be used to feed the conch in one metamorphosis tray.

74 ml x 8 trays
= 592 ml total to feed

Therefore, 592 ml of the 1 L flocculated microalga will be needed to feed the conch in all eight metamorphosis trays, if both tanks are full of conch.

4. Growing Microalgae

*As mentioned in Chapter 2: Larval Rearing, conch veligers are considered zooplankton and they feed on **phytoplankton** - also known as microalgae - during their entire larval cycle.*

Phytoplankton are microscopic algae that live in marine environments. These organisms cannot be seen with the naked eye, but they are the foundation of the ocean food web and produce an estimated 80% of the world's oxygen.

Isochrysis galbana and *Chaetoceros gracilis* are two of the many species of phytoplankton, and are the preferred food source for conch.

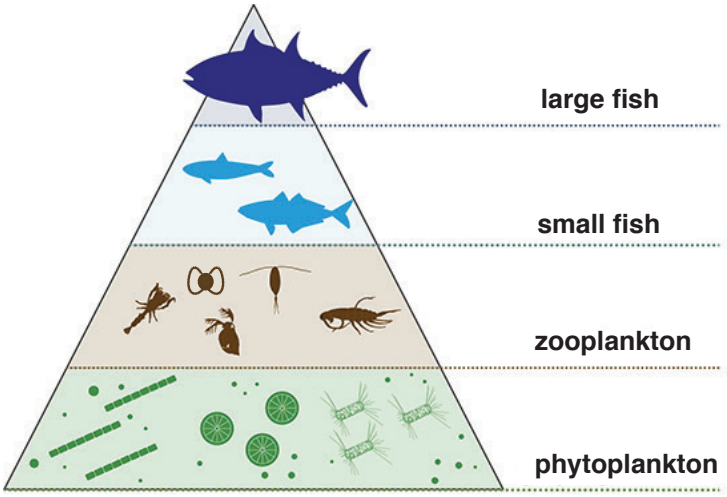


Illustration adapted from JAMSTEC 2016

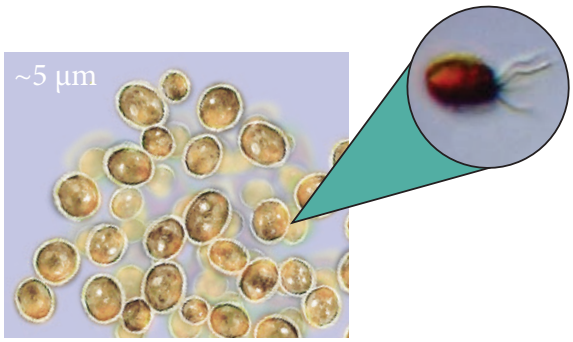


Image by Sustainable Aquatic; Close-up: Wikipedia Commons

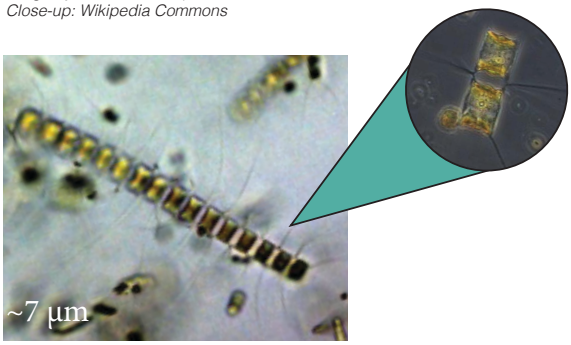


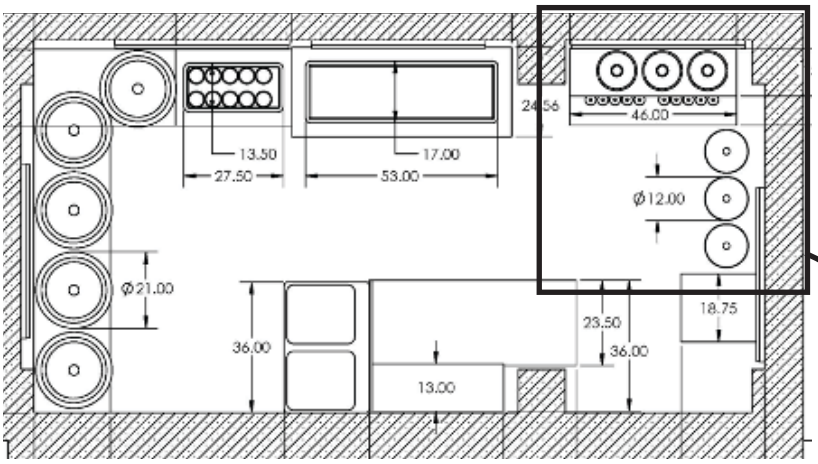
Image by Vistey; Close-up: Science of Earth

Isochrysis galbana is a golden brown flagellate, meaning it has 2 tails (flagella), and moves in a spiral motion. It is fed to veligers exclusively up to day 16, and on day 18, *Chaetoceros gracilis* is added to the diet.

Chaetoceros gracilis is a diatom meaning it contains ***silica*** in its cell walls. In calm conditions they form chains, but in aerated systems in hatcheries, they are usually seen individually or in pairs.

UNDERSTANDING THE LAYOUT AND VESSELS

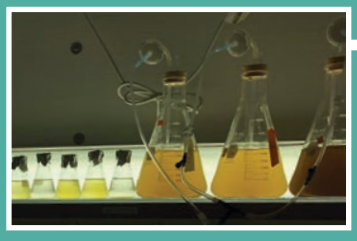
To have a steady supply of food for the conch veligers, a section of the hatchery is designated for growing microalgae. There are four types of vessels in the microalgae room: test tubes, flasks, carboys, and suntubes (from smallest to largest). Each play an important role in the ***inoculation*** *sequence known as 'scaling up'.*



Blueprint by Kathy Russ (FAU)

Blueprint of a small conch hatchery including a microalgae room.

Carboys: *20 L*



Flasks: *125 ml (left) and 1,000 ml (right)*

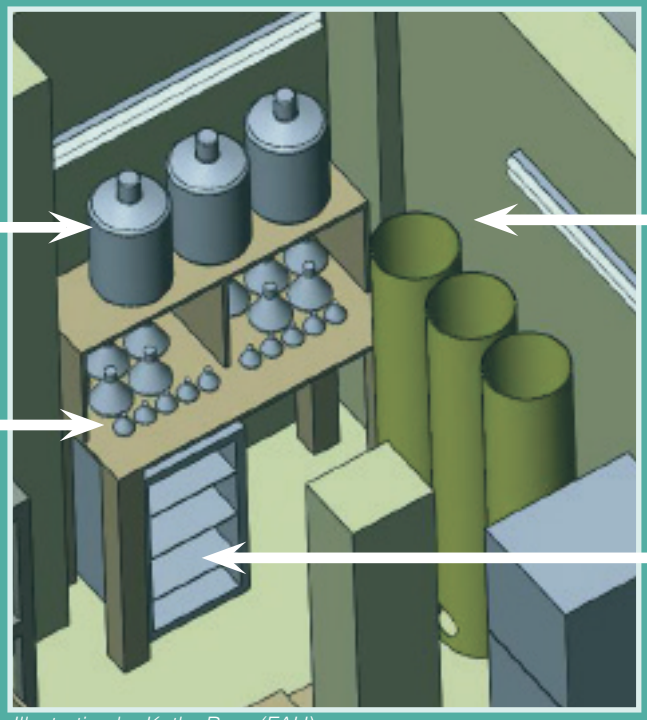


Illustration by Kathy Russ (FAU)

Image by Solar Components Corp

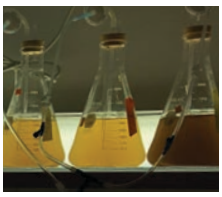
Suntubes: *95 L (left) and 250 L (right)*

Test tubes: *25 ml (inside incubator)*

Each ***agar plate*** contains the microalgal starter culture. See Appendix 2: Supply Sources.

Test tubes are kept in racks and covered with either screw-on caps or push-on caps. Each test tube is 25 ml and is made of glass (Pyrex or Kimex). Approximately 50 test tubes with caps are needed.

The small Erlenmeyer flasks are 125 ml each and are made of glass (Pyrex or Kimex). Each flask is covered with a 50-ml plastic (polypropylene) tri-beaker cap as show in this photo. Eighteen of these flasks with caps will be needed.



The large Erlenmeyer flasks are 1,000 ml each and are made of glass (Pyrex or Kimex). Each flask is covered with a 100-ml plastic (polypropylene) tri-beaker cap. It is not necessary to have aeration as shown in this photo. Eighteen of these flasks and caps will be needed.

The plastic carboys are 20 L each and three are needed. They will have a 250-ml plastic (polypropylene) tri-beaker cap customized to fit aeration tubing as shown in this photo. The flexible aeration tubing is connected on the inside of the carboy to a rigid plastic tube to distribute air throughout the container. See below for more detail.

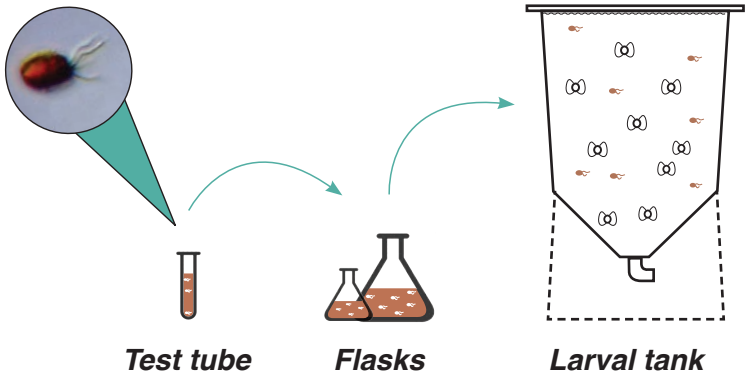
The fiberglass suntubes are 95 L each and three are needed. They have a conical bottom and a flat plastic lid customized with a hole for the aeration tubing. The flexible aeration tubing is run almost to the bottom of the suntube and a porcelain weight holds it in place.

The aeration used in carboys and suntubes allows densely packed cells to circulate constantly in the vessels. This ensures that cells are evenly exposed to light and, therefore, all have a chance to photosynthesize.The flexible tubing also has an in-line air filter to ensure that no contaminants enter the culture via air.

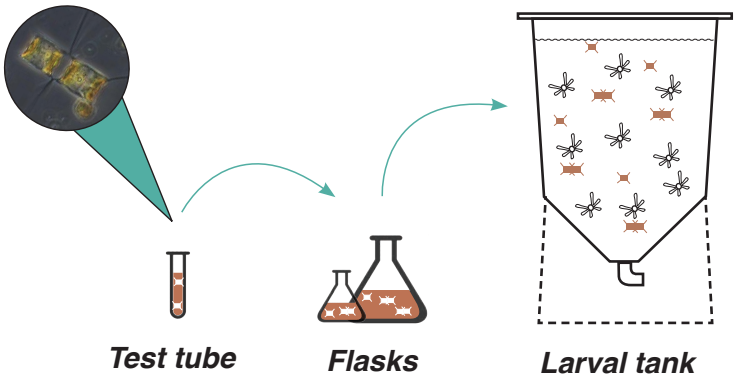
Photo left: aeration set-up for carboys.
Photo right: close-up of rigid tubing.
Photo bottom: air filters.

SCALING UP OVERVIEW

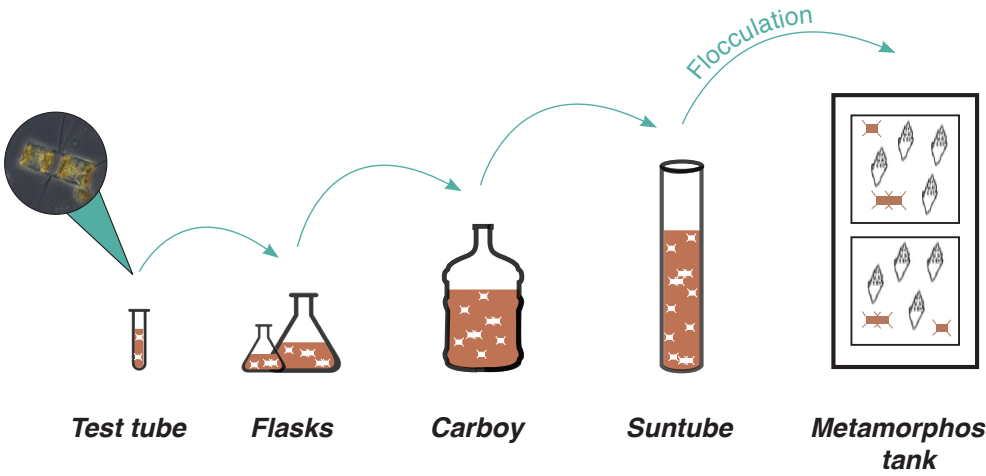
The process of 'scaling up', is more formally described as a progressive batch culture. It begins with single-cells which multiply exponentially into millions of identical cells as they are transfered to progressively larger vessels. This is how both Isochrysis *and* Chaetoceros *are grown. Each of these microalgal species are grown as* ***monocultures*** *meaning they are grown separately.*



- The *Isochrysis* monoculture is used to feed conch veligers throughout the entire larval cycle.



- The *Chaetoceros* monoculture is used to feed conch veligers from Stage 4 (usually around day 16) through Stage 5. *Chaetoceros* is also grown in a progressive batch culture sequence.

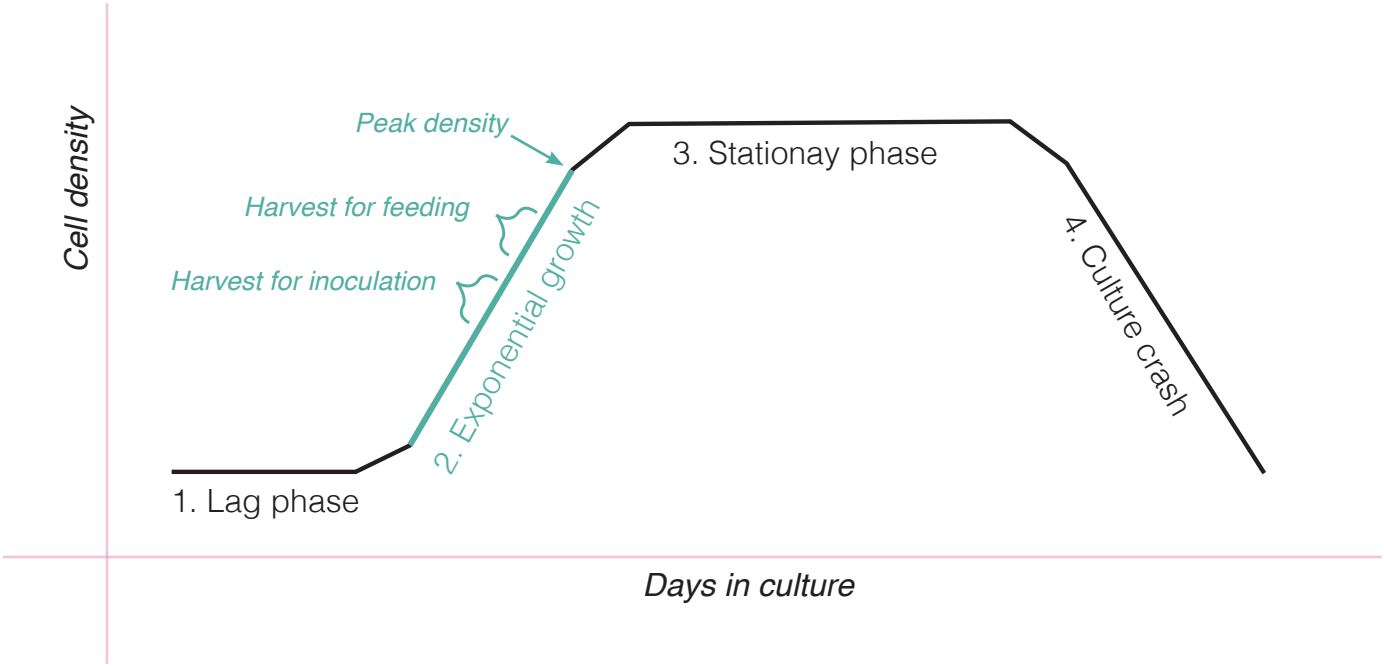


- To feed metamorphosed conch, repeat the same process with the *Chaetoceros* culture, and grow it even further up to the 95-L suntubes. Finally, flocculate the microalga (see p. 58).

EXPONENTIAL GROWTH CURVE

The microalgal monocultures follow the growth curve depicted below starting with the lag phase. If left alone, it will reach a stationary phase (determined by the size of the vessel and the amount of ***media*** *available), and will eventually crash.*

It is important to understand this growth curve because the optimal time to harvest the microalgae is during the exponential growth phase when it is at its highest nutritional value. Harvest microalgae to either feed veligers or as part of scaling up for the progressive batch culture.



1. Lag phase: Lasts a few hours and occurs right after inoculant is transfered to a new vessel.

2. Exponential growth phase: During the first 4 - 5 days, the culture should be experiencing rapid cell division as the cells grow logarithmically in the available media and vessel space. The culture must be harvested and transferred right before peak density.

3. Stationary phase: Following peak density, cell division begins to slow down and plateau because the media has been used up. The cells have low nutritional value in this phase.

4. Crash phase: As the cells are starved of nutrients, the cell density decreases rapidly. During this phase, recovery is near impossible. The culture turns pale brown and smells sour. If a crash occurs unexpectedly early on, it may be due to contamination, low pH, light levels, and/or low oxygen.

INOCULATION: DETAILED

*Microalga grown in each culture vessel serve as the **inoculant** for the next larger vessel, until the quantity of cells required for feeding is reached in the exponential growth phase.*

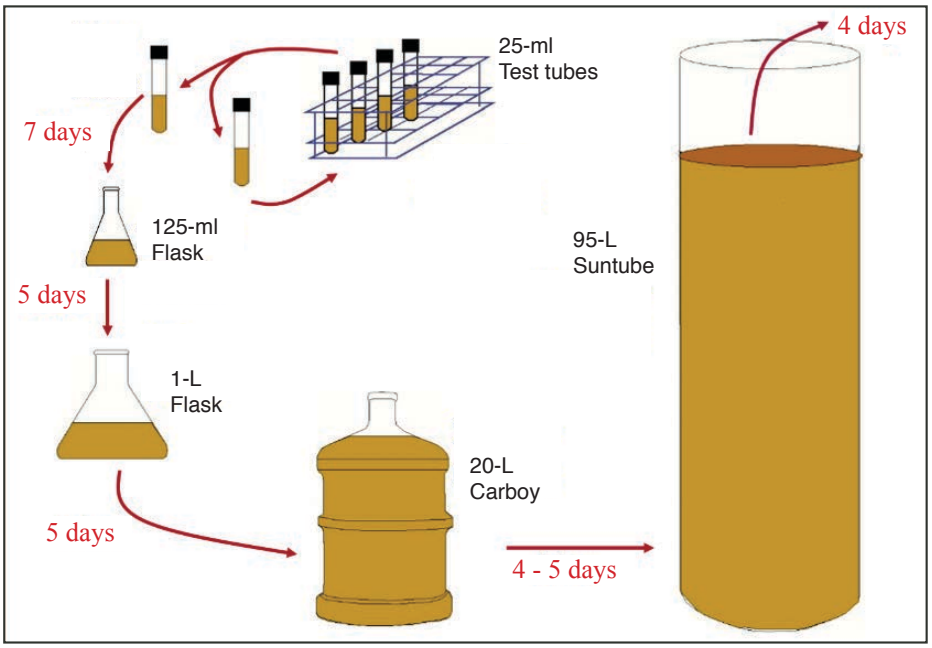


Illustration by Peter Van Wyk (modified)

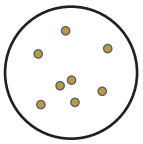
Day 0 is considered as the first day when the inoculant is added.

The general rule for scaling up is to use 10% inoculant for smaller vessels and 5 - 7% for larger vessels. This level of inoculation results in faster exponential growth and the cultures are less prone to contamination. Inoculation begins with a pure stock culture, which is used to make the backup stock and the working stock.

Vessel type	Vessel size	Media content + culture	Inoculant needed	Receiving vessel
Test tube P	25 ml	10 ml	1 ml	Test tube pure stock (P) Test tube working stock (W)
Test tube W	25 ml	20 ml	10 ml	125-ml Flask (90 ml of media)
Flask	125 ml	100 ml	100 ml	1,000-ml Flask (900 ml of media)
Flask	1,000 ml	1,000 ml	1,000 ml (1 L)	16-L Carboy (15 L of media)
Carboy	20 L	16 L	5 L	Suntube (85 L of media)
Suntube	95 L	90 L		Flocculate

PRODUCTION SCHEDULE

The microalgal production schedule is determined by knowing the maximum amount of microalgae needed to feed the veligers and metamorphosed conch. It is advised to culture extra in case of slow growth or crashes. Cultures should begin at least three weeks before bringing in the first egg masses, and are grown in a repetitive cycle throughout the hatchery phase.



Each pure monoculture arrives on agar plates and/or in test tubes, which need to be placed into the incubator. The next day, cells from one vessel (agar plate or test tube) must be transfered into two test tubes:
- One for a pure culture (serves as backup); inoculated once per week
- One for a working culture

During the highest feeding demand (day 21) inoculate one 25-ml test tube every other day and two 25-ml test tubes every other day. A total of 10 test tubes will be needed in culture since it takes seven days for the culture to reach peak density for inoculation to the 125-ml flask. This is done both for *Isochrysis* and *Chaetoceros*.

For *Isochrysis* during the highest feeding demand (day 21), inoculate one 125-ml flask every other day and two 125-ml flasks every other day with one test tube per flask. Up to seven flasks will be needed in culture since it takes five days to reach peak density for inoculation to the 1-L flask. For *Chaetoceros* during the highest feeding demand (day 21) inoculate one 125 ml per day. In total five flasks will be needed at once.

For *Isochrysis* during the highest feeding demand (day 21), inoculate one 1-L flask every other day and two 1-L flasks every other day with one 125-ml flask per 1-L flask. Up to seven flasks will be needed in culture since it takes five days to reach peak density for feeding veligers. For *Chaetoceros* during the highest feeding demand (day 21) inoculate one 1 L per day with one 125-ml flask. This means five flasks in culture will be needed at once. They will be used for feeding veligers and inoculating carboys.

Inoculate one carboy of *Chaetoceros* every four days with one 1-L flask per carboy. In total, three carboys will be needed to inoculate the suntubes.

For *Chaetoceros*, inoculate three suntubes every four days with one carboy. At peak density (4 - 5 days) the microalga will be flocculated to feed metamorphosed conch.

VESSEL CLEANLINESS AND STERILIZATION

When beginning the microalgal cultures and when transferring inoculant from vessel to vessel, it is important to always be thinking about cleanliness and ***sterilization****.*

Cleaning techniques for:

1. Glassware (test tubes, flasks, and pipettes):
Use a small test tube or flask brush to wash vessels and caps with liquid alconox, rinse with fresh water, and dip in a mild muriatic acid solution (10 ml acid / L of freshwater.) Rinse well with freshwater and let dry, ideally for a day. Before use, rinse with seawater. After inoculation, keep the used Pasteur pipettes in a mild muriatic acid solution, rinse with fresh water and let dry. *Note: For glassware, the muriatic acid solution can be kept and reused for one week.*

2. Plastic and Fiberglass (carboys and suntubes):
Pour about half a liter of mild muriatic acid solution (10 ml acid / L of freshwater) into carboys and swirl around. If necessary, use a carboy brush to clean out any remaining algae. Pour out, rinse well with fresh water, and let dry. The same can be done for suntubes using two liters of the mild acid solution and a mop to distribute the solution. Before use, rinse with seawater.

Sterilization techniques for:

1. Glassware (test tubes and flasks):
Using a 700-watt microwave with a turn table, microwave loosely capped test tubes and flasks with their contents of seawater and media for 8 - 10 minutes per 1 - 1.5 L of liquid. For example, when test tubes and small flasks are all microwaved together, this can add up to 1 L. Let sit for 24 hours prior to inoculating with microalga. Glass pipettes in reusable bags can be microwaved for 10 minutes.

2. Plastic and Fiberglass (carboys and suntubes):
Fill carboys and suntubes with seawater, chlorinate at 5 ppm (5 ml household chlorine per 1 L of seawater), cover and leave overnight. Dechlorinate with vitamin C (1 ml vitamin C solution / ml of chlorine used). It is advised to prepare vitamin C stock solution ahead of time: 165 grams of vitamin C per one liter of freshwater. After vitamin C is added, turn on aeration in carboys and/or suntubes. Wait 30 seconds. Using a pool chlorine test kit make sure all of the chlorine is gone.

To begin and/or continue a culture, vessels must be prepared to receive microalgal inoculant in the following order:

Test Tubes and Flasks (glassware):

1. Clean (acid solution)
2. Fill vessels with seawater and media
3. Sterilize (microwave)
4. Let sit for one day
5. Add microalgal inoculant

Carboys and Suntubes (plastic/fiberglass):

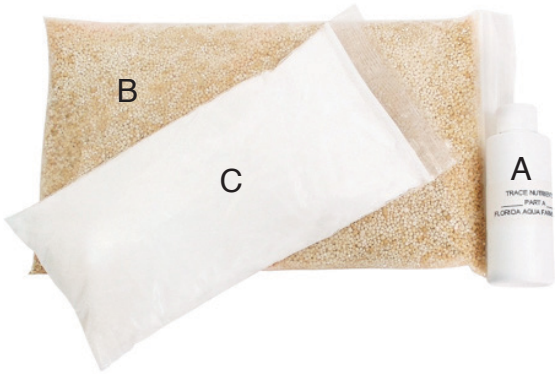
1. Clean (acid solution)
2. Fill with seawater
3. Chlorinate
4. Let sit overnight
4. Decholorinate with vitamin C
5. Add media
6. Add microalgal inoculant

PREPARING THE MEDIA

Microalgae will not grow on their own in the vessels. They need a growth media to encourage cells to multiply. The media functions as a fertilizer (nutrients) and is always introduced into the vessels before adding the microalgal inoculant. In nature, high concentrations of nutrients can cause algal blooms, which are not always desirable. In culture, these nutrients are in high concentrations to optimize microalgal cell growth. Here is an example of media that can be used:

Microalgae Grow Mass Packs Instruction Guide

(Part Nos. F2A6–F2A7)



The 'Microalgae Grow Mass Pack' is a modified Guillard's f/2 formulation for aquaculture application and comes from *Pentair Aquatic Eco-Systems.* It contains three parts:

Part A: trace metals and vitamins (copper, zinc, cobalt, magnesium, B_{12}).

Part B: macronutrients (nitrates and phosphates).

Part C: silicates for the diatom *Chaetoceros* to build their box-like cell walls.

Mixing Instructions

1. To obtain a balanced nutrient media, both PART A and PART B must be completely mixed together as one mixture.
2. Add PART B (primary nutrients) to 3 liters (101.5 fluid ounces) of sterile fresh water. Distilled and/or deionized water are preferred, but not necessary.
3. Mix for about five minutes until totally dissolved. Apply heat if necessary, but not more then 35–38ºC (95–117ºF) is usually needed.
4. Slowly add PART A (trace nutrients) and mix. Heat should not be necessary and should be avoided if possible.
5. If you wish to use metric measurements add about 600 mL more water to obtain 4 liters of nutrient solution. If you want to use fluid ounces, add about 13 fluid ounces of water to obtain one gallon of fertilizer.
6. Dosage level is about 1 mL per 2.5 l (2.65 l actual) of culture water, or about 1 fluid ounce per 21 gallons (6 tsp/21 gallons or 1/4 tsp/3.4 quarts). Note that this f/2 level will normally sustain a dense culture for 7–10 days. If you culture algae only 3 to 5 days, then you may want to try f/4, which is half the dose. Ideally, algae harvested for direct feeding should have nutrient levels as low as possible to reduce excessive nutrient loading in invertebrate cultures.

Instructions by Pentair Aquatic Eco-Systems.

Mixing Silicate (F2A7 Only)

7. SILICATE MUST BE MIXED AND STORED SEPARATELY. Add contents of PART C to 3 liters (101.5 fluid ounces) of sterile water and mix for about 5 minutes. Apply heat if needed, but not more than 38ºC (117ºF).
8. If you wish to use metric measurements, add about 850 mL more water to obtain 4 liters of silicate solution. Add about 21 fluid ounces of water to obtain one gallon.
9. Dosage level is about 1 mL per 2.5 l (2.65 actual) of culture water, or 1 fluid ounce per 21 gallons (6 tsp/21 gallons or 1/4 tsp/3.4 quarts) of culture water. Use only in diatom culture.

Storage

10. Nutrient solutions should be stored in clean dark glass or plastic containers in a cool, dark area. Precipitate will develop as the mixture ages. Shake well before adding to culture water.
11. To aid against media contamination and provide longer shelf life, adjust the pH to 3.0 using hydrochloric acid. Further sterilization is not normally needed. High heat sterilization is not recommended, but microwave sterilization can be used (7 min/liter). Vacuum filtration through a 0.45 micron cellulose nitrate membrane can also be used.

WARNING: STORE IN SAFE PLACE AND KEEP OUT OF REACH OF CHILDREN.

Temperature:
The hatchery as a whole should be kept at 27 °C (80 °F) and certain vessels such as test tubes and small flasks should be kept in the incubator at 24 - 25 °C (75 - 77 °F).

pH:
As microalgae grow, the pH of the cultures increase. It is best to start a culture with a pH of 7.9 - 8.0. When silicates are used to grow *Chaetoceros*, the pH typically goes up to 9.0. Muriatic acid drops should be added to bring the pH back down.

Alkalinity:
Alkalinity is the capacity of the water to resist changes in pH. It should be 200 - 250 ppm.

Salinity:
Full strength seawater from the ocean can be used (salinity 36), however, a slightly lower salinity (30 - 32) minimizes bacterial contamination from *Vibrio.*

Overall water quality:
The seawater used for microalgal cultures should be pre-filtered with mechanical filters (5 - 10 µm) and UV-sterilized to kill unwanted microalgae, bacteria, and other potential pathogens and contaminants.

Aeration:
Cultures that are in test tubes and flasks will not have a direct air source, therefore, they will need to be swirled once or twice a day. Carboys and suntubes have an airline, with no air stone, to circulate the microalgal culture. Place an air filter of 0.22 µm on each carboy and suntube culture to minimize bacteria entering the culture.

Light:
Microalgae should be grown with a diurnal cycle (12 hours light : 12 hours dark), however, 24 hours of light may be advantageous. Natural or artificial lighting can be used. LED lights work very well and can be placed on an automatic timer to come on in the morning around 8:00 AM and off at 8:00 PM.

Example of LED lights for growing microalgae.

Fluorescent lights can also be used to grow microalgae.

Seawater filter and UV-sterilization system.

SAFELY TRANSFERRING INOCULANTS

The biggest threat to microalgal cultures is contamination. This is why vessels are always cleaned, sterilized, and kept covered. Transferring inoculants is when the cultures are at greatest risk of contamination, as lids are temporarily removed. Transfers must be done with attention to detail.

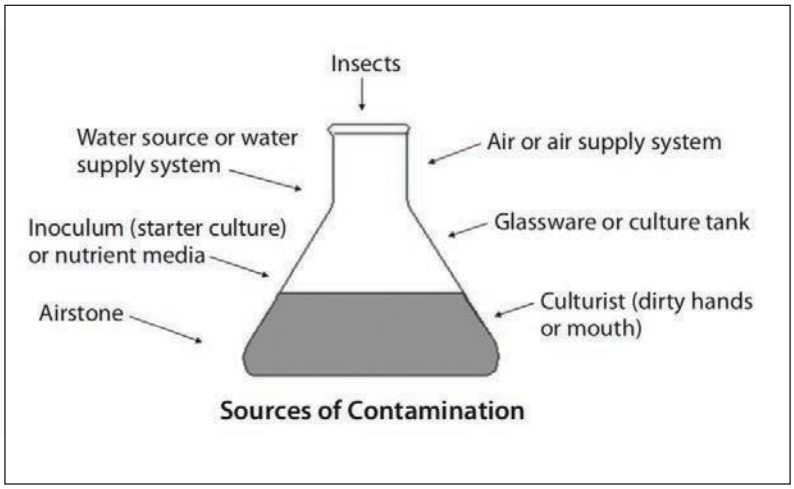


Illustration from LeRoy Creswell 2010

Another threat is improper growing conditions. Microalgae need certain amounts of light and air space for photosynthesis and respiration to take place. Vessels are never filled to their maximum volume to allow for this air exchange.

For example, 25-ml test tubes only contain 20 ml of liquid (seawater with media and inoculant) allowing for 5 ml of air space.

Transferring inoculants between test tubes and flasks:

1. Wipe working surfaces and hands with 70% alcohol. Minimize any movement of air in the area where the transfers are taking place.

2. Place all of the flasks and test tubes necessary for transfers on the working surface. Make sure receiving vessels are labeled with a piece of tape (species name + date of inoculation).

3. a. When transferring from **test tube to test tube**, ignite a small alcohol burner. Hold the glass pipette in the dominant hand, and the two test tubes in the other hand. Remove caps with ring finger, pinky, and thumb, with dominant hand, and do not put down on work bench. Flame the tip of the pipette as well as the neck of the test tubes. Draw 1 ml of inoculant from the transfer test tube with the pipette, flame necks of the two test tubes again, and place the contents of the pipette into the receiving test tube. Flame the necks of the test tubes and put the caps back on. If additional receving test tubes will be inoculated with the transfer test tube, repeat the process.

   b. When transferring from **test tube to small flask** or **small flask to large flask**, ignite a small alcohol burner and hold one vessel in each hand. Remove cap from the transfer vessel (put down on the bench), and remove cap from the receiving vessel (keep in opposite hand). Flame the neck of each vessel by slowly rotating it into the flame. In one motion, pour the entire content of the smaller vessel into the receiving vessel. Flame its neck and close with cap.

3.a *Test tube 1 on the left has inoculum. Test tube 2 on the right is recipient.*

3.b *Test tube on the left has inoculum. Small flask on the right is recipient.*

3.b *Small flask on the right has inoculum. Large flask small on the left is recipient.*

4. Turn off the burner and transfer all new vessels to the incubator and shelves in the algae area.
5. All vessels that are empty and their caps should be properly cleaned (see p. 49).
6. Remove all materials from working area and wipe surfaces with 70% alcohol.

Transferring Inoculants to Carboys and Suntubes:

1. Always bring transfer vessels as close as possible to receiving vessels. A step stool might be needed when transferring flasks to carboys and carboys to suntubes.
2. When transferring from a 1-L flask to a carboy, remove flask cap, tilt the carboy cap back, and pour the entire flask inoculant into the carboy. This should all be done swiftly.
3. When transferring from a carboy to suntube, remove carboy cap, slide suntube lid slightly off to the side, pour 1/3 of the carboy inoculant into each suntube, and close suntube.

2 *Flask on the right has inoculum. Carboy on the left is recipient.*

3 *Carboy on the left has inoculum. Suntube on the right is recipient.*

All images on page by Robinson Bazurto

CELL COUNTS

Hemocytometers are typically used for counting human blood cells and are perfect for counting microalgal cells. It is important to count microalgal cells to ensure that the culture is healthy and to determine how much of the culture is needed to feed to veligers and metamorphosed conch.

A hemocytometer is a thick glass slide with a mirrored surface which has precisely etched grids defining a known volume (Fig. A). A special cover slip is placed on top of the mirrored surface and a drop of the algal sample is added (Fig. B). The sample is drawn under the coverslip by capillary action.

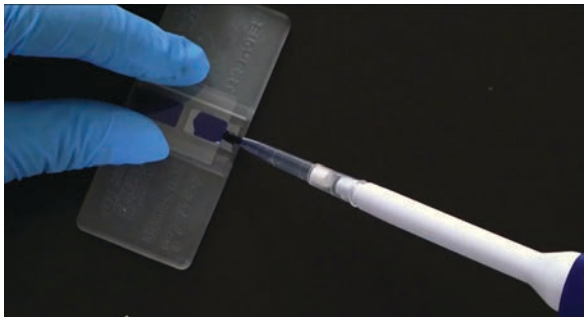
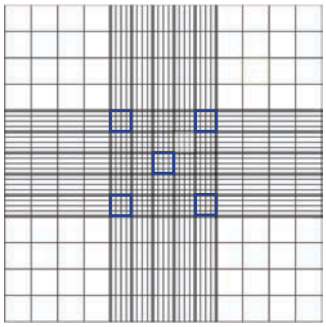


Fig. B - Example of capillary action. Blue liquid is absorbed under the coverslip. (Image by BioNetwork)

Materials Needed:

- Compound microscope (70X, 100X, 400X, 1000X)
- Hemocytometer with cover slip
- Pasteur pipettes
- Pipette bulb
- Small beaker (25 ml)
- Wipes to clean glassware
- Hand-counter
- Data sheet
- 70% alcohol



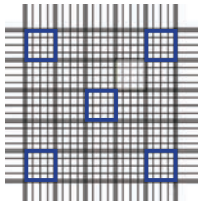
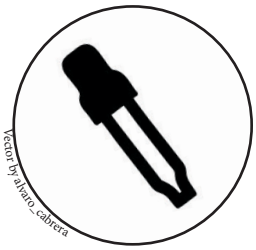


Fig. A - Full grid (left) Close-up of grid (right)

For high density cultures, use the central square. For lower density cultures use the outter squares.



Step 1:
Collect a culture sample approximately (2 ml) with a Pasteur pipette from the 1-L flask for feeding the veligers or from the suntube in preparation for flocculation.

Step 2:
Place the cover slip on the hemocytometer so that both central counting grids are covered, but the V shaped grooves are exposed for easy access.

Step 3:
With a Pasteur pipette give the culture sample a swirl and then place the tip of the pipette next to the V groove until capillary action is observed filling the chamber. If the chamber is under-filled or overfilled, the process will need to be started over, because these conditions will result in inaccurate cell counts.

Step 4:
Observe the monocultures using a compound microscope starting at 100X and moving up to 400X.

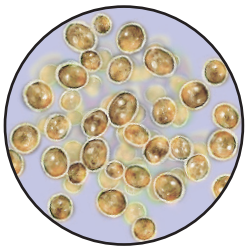
WHAT TO LOOK FOR:

Isochrysis galbana (Motile, 5 - 7 µm)

- The culture should look rich golden brown in color.
- About 70 - 80% of the algal cells should be moving in a helical (spiral) movement. If a large portion of the cells is not moving, take another sample. If nothing changes, there is an issue with the culture. Select another culture to observe for cell counts and feeding.
- There should be minimal clumping of the microalgal cells. If there is a lot of clumping there may be a bacterial contamination. Bacterial cells are very small so it is unlikely to see them. This is why it is important to note symptoms such as clumping.
- Protozoans, like ciliates, can be similar in size to algal cells, and seen in the culture. It is alright to have a small number, however, if there are a lot in the culture they may contaminate the larval tank and compete with the veligers for food. They dart and move differently than *Isochrysis*.

Chaetoceros gracilis (Non-motile, 8 - 10 µm)

- The culture should look rich golden brown in color.
- The cells should be dividing. This will look like two or more cells latched together.
- *Chaetoceros* does not move, so if a lot of movement is observed, then some other contaminants are present, such as ciliates.
- There should be minimal clumping in the culture. If there is a lot of clumping then there may be a bacterial contamination and it should not be fed to veligers. If bacteria contamination is found in the suntube, but the health of the cells look good overall, it should be fine to flocculate the culture and feed to the metamorphosed conch.



Step 5:
Prepare microalgal culture for counting cells. Since *Isochrysis* is ***motile***, the cells need to be stopped in order to count them. This is done by adding a very small amount of alcohol (< 1 ml) to the sample.

Step 6: Using the example on the following page as reference, determine the amount of cells in 1 ml of the microalgal culture (the size of a drop of water). Plug into the equation below:

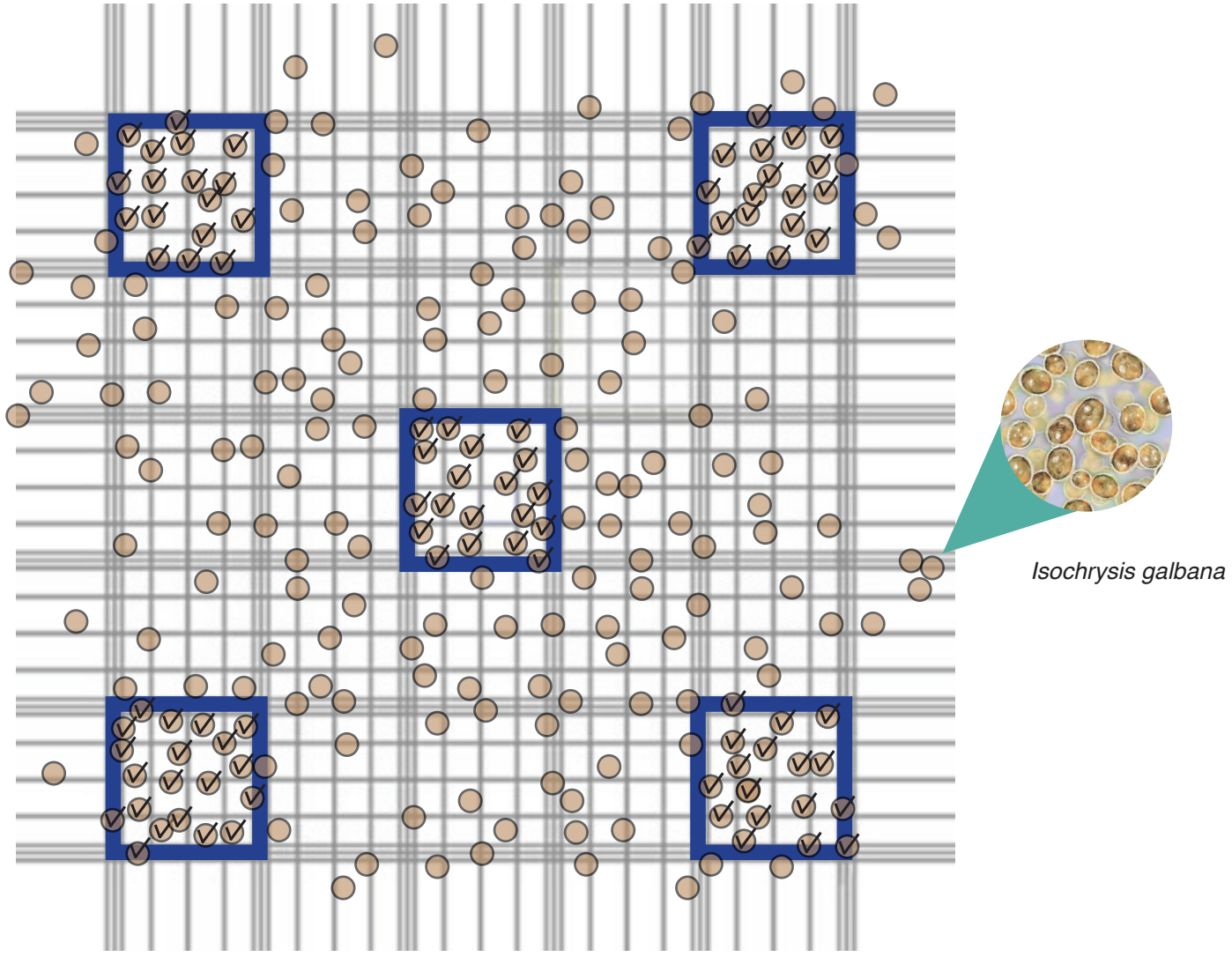
_____ (cells counted inside the 5 blue squares) x 5 = ___y___
___y___ x 10,000 = _____ cells/ml

In order to accurately count microalgal cells, focus on five specific squares marked in blue below.

In this example, all of the 'checked-off' cells that are within the blue border, including the cells on the border, are counted. If two thirds or more of the cell is outside of the border then it is not counted. Below, 90 cells were counted.

Multiply this result by five and then multiply by 10,000 to calculate how many cells are in 1 ml of the culture:
90 cells x 5 counts = 450 cells
450 x 10,000 = 4.50 x 10^6 cells/ml

There are 4,500,000 algal cells in 1 ml (approximately one drop) of this culture. Typical cell counts are 4.0 to 8.0 x 10^6 cells/ml for *Isochrysis,* and 3.0 to 6.0 x 10^6 cells/ml for *Chaetoceros.*



▶ *Close-up of the central square grid of a hemocytometer with* Isochrysis *cells counted.*

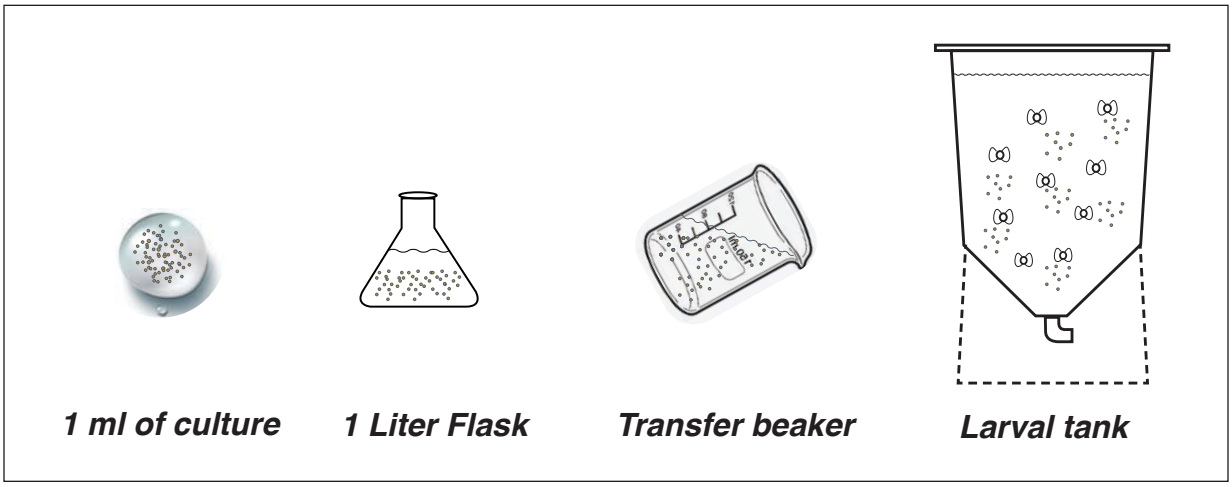
How many ml of microalgal culture are needed to feed a (68-L) tank full of veligers?
As the veligers grow larger they will require more food. The quantity of microalgal cells needed to feed veligers is therefore determined by the age of the veligers. Refer to the *Microalgae Daily Feeding Table* (p. 31) and the **Microalgae Feeding Data Sheet** below to find out how much to transfer to the larval tank.

Date	Larval tank #	Larval age *(day)*	Algae type *(Iso or Cg)* and culture #	(A) Size of larval tank *(ml)*	(B) Algae cells needed per ml of tank water	(C) Algae cell count *(cells/ml)*	(D) Total algae cells needed for each larval tank (A x B)	(E) # of mls to feed larval tank (D/C)
06.23.20	2	1	Iso 6.19-A	68,000 68×10^3	5,000 5×10^3	6,000,000 6×10^6	340,000,000 340×10^6	56.7 57 ml
07.13.20	2	21	Iso 7.09-A	68,000 68×10^3	10,000 10×10^3	6,000,000 6×10^6	680,000,000 680×10^6	113 113 ml
07.13.20	2	21	Cg 7.09-A	68,000 68×10^3	5,000 5×10^3	4,000,000 4×10^6	340,000,000 340×10^6	85 85 ml

Microalgae Feeding Data Sheet

Example of calculations:

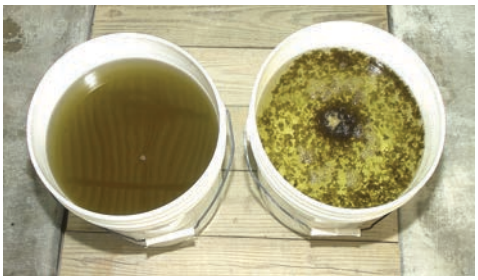
- The size of the laval tank is 68 L or 68,000 ml (A).
- According to (B) 5,000 algal cells are needed per ml of tank water on day 1 of the larval cycle.
- Using the hemocytometer it was determined that the *Isochrysis* cell count was 6 x 10^6 cells/ml (C)
- To determine how many cells are needed for each larval tank (D), multiply column (A) times (B).
- To determine the amount of microalgal culture to feed the larval tank (E), divide column (D) by (C).



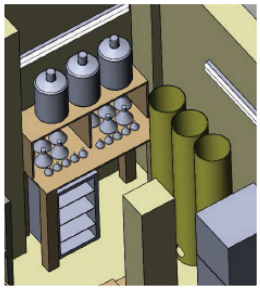
Sequence from cell counts to feeding veligers. (Vector by Graphics RF)

FLOCCULATION: FOOD FOR METAMORPHOSED CONCH

The conch are now benthic grazers, therefore, they are unable to feed on planktonic microalgae. A process known as flocculation is used to cause floating particles, such as the microalga Chaetoceros, *to clump together and settle out in a thick mass that the conch can graze upon with their proboscis.*



Microalga before (left) and after floccuation (right).

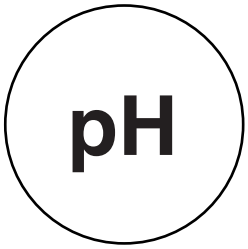


A 95-L suntube which is kept in the microalgae area.

Image by NOAA

The flocculation procedure begins with ***chitosan***, a macromolecule from the exoskeleton of crustaceans (see Appendix 2: Supply Sources). The positive charge of the chitosan bonds with the negatively charged diatoms such as *Chaetoceros*. The efficiency of the flocculation is related to pH, temperature, mixing technique, and the condition of the diatom cells

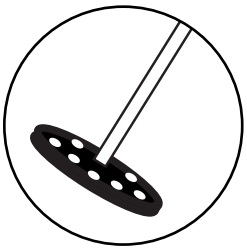
Step 1:
The *Chaetoceros* culture in the conical bottom suntubes should be ready in four to five days after innoculation with the microalga from the carboys (see p. 47). Once the culture is ready, it is important to do a cell count prior to flocculation.



Step 2:
Adjust the microalgal culture to a pH of 6.0 - 7.0 with the addition of muratic acid (*~ 0.1 - 1.2 ml of muratic acid / L of culture*). Mix the cells for two to three minutes.

Image by Tidal Vision

Step 3:
Prepare a stock solution of chitosan (*10 g chitosan to 1% acetic acid*) in advance. It can be stored for several weeks in the refrigerator. To make a stock solution do the following: If the acetic acid is 80% strength then add 12.5 ml of acetic acid to 987.5 ml of freshwater. That equals 1% acetic acid. Add 10 g of chitosan to the 1 L of the 1% acetic acid. Mix well.



Step 4:
Add the chitosan solution into the microalgal culture at a dose of 5 ml of chitosan solution per liter of microalga, which is equivalent to 475 ml in a 95-L conical bottom suntube. Use a pole with a disk at the end of it to mix everything together.

A research technician uses a pole with a disk to mix the chitosan solution into the microalgal culture.

Step 5:
Next sodium hydroxide (*5% or 5 g NaOH/100 ml freshwater*) is added to raise the pH to 8.5 - 9.0 (~ 1.0 - 1.5 ml/L of microalga). The microalgal cells are stirred again for several minutes until flocculation is observed. The flocculated cells settle at the conical bottom of the suntube and are separated from the 'clear' water on top (this can take 45 - 60 minutes). Open the valve slowly to collect the flocculated algae. In this case, 95 L will result in 1 L of flocculated algal cells (the ratio is typically 100:1). If necessary flocculated algal cells can be stored in the refrigerator for up to one week.

A research technician feeds flocculated microalgal cells to metamorphosed conch.

5. Juvenile Rearing

*Once the conch reach 3 - 4 mm in shell length, they are moved to the **nursery**. The tanks here are set up in a recirculating system, meaning the water is reused over and over again. The small conch will be grown on sand and will be **cryptic** during the first couple of months. Gradually, they will grow large enough to be handled with fingers. This is the longest phase for growing conch onshore and takes approximately one year.*

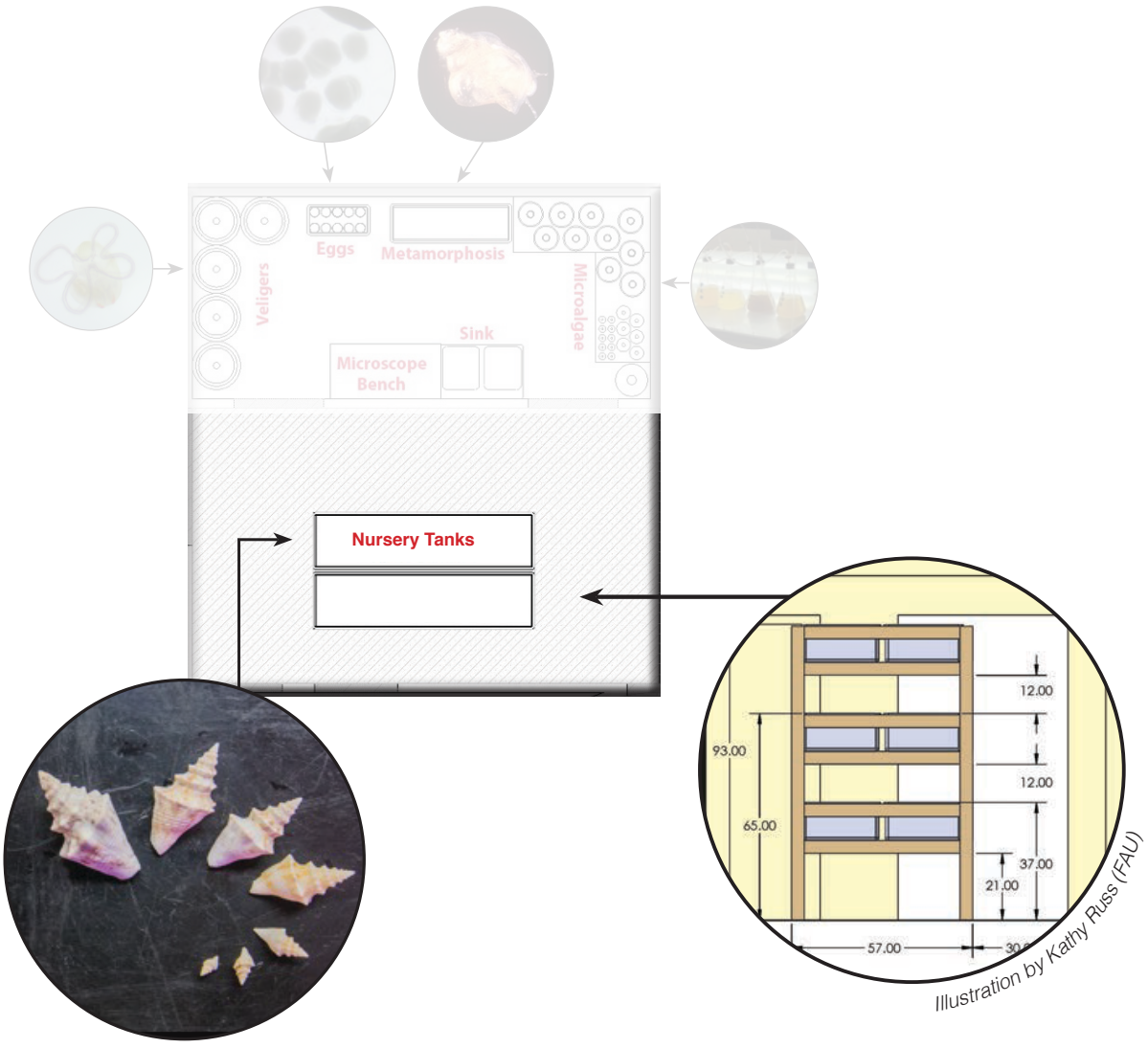
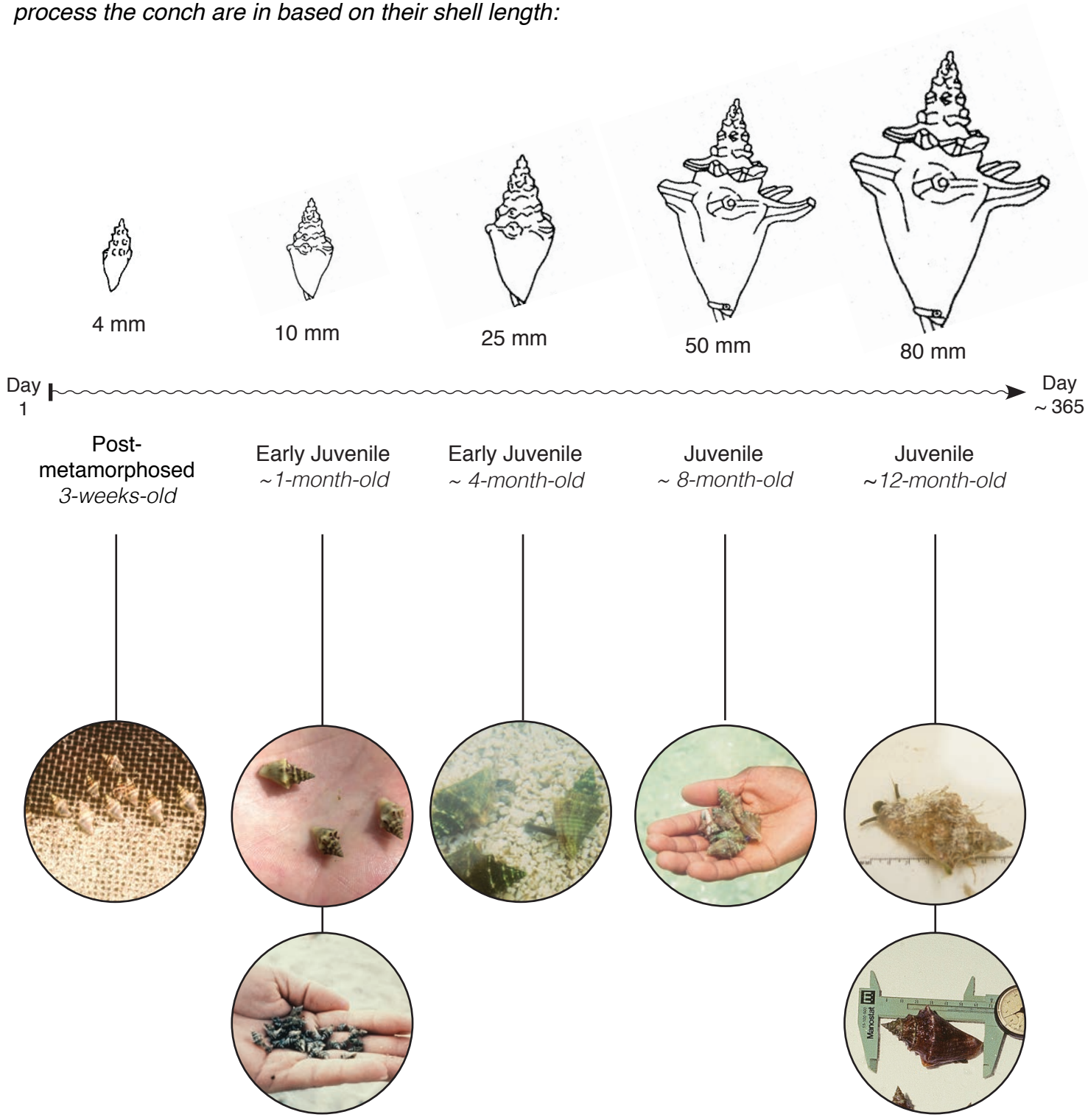


Illustration by Kathy Russ (FAU)

JUVENILE STAGES AND GROWTH RATE

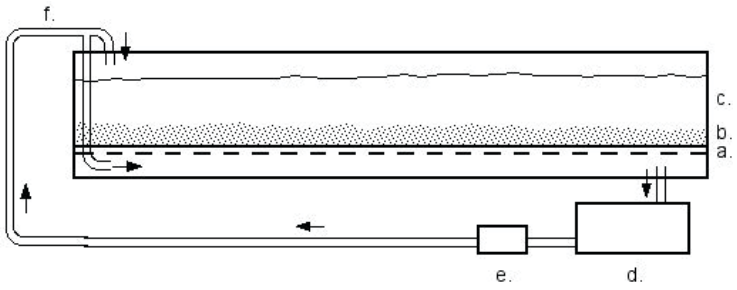
After metamorphosis, the conch will be 1.0 - 1.3 mm in shell length and look more like a tiny sea snail. The conch will grow at an ideal rate of 0.22 mm per day which means it will take about one year to reach 80 mm (3.2 in) in shell length (80 mm / 0.22 mm per day = 363 days). This is the size that can be used for restoration of seagrass beds or for growout in sea pens to produce sustainable seafood. Throughout the nursery phase, different terminology is used to describe which part of this year-long process the conch are in based on their shell length:



The post-metamorphosed conch (3 - 4 mm; 0.12 - 0.16 in) are placed into fiberglass tanks at 1,700 conch / m² (160 conch / ft²). The tanks are set up to have a thin layer of elevated coral aragonite sand ***substrate*** *on top of a window screen to allow for good water flow.*

- Three pairs of juvenile tanks stacked on top of each other on a shelving unit. This system is located outdoors next to the hatchery and is protected from the weather with a fiberglass roof. The roof has a transparent section that allows sunlight to enter to help promote natural diatom growth used to supplement the feed that the conch receive. Each tank is 1.5 m² (16 ft²; 96" L x 24" W x 12" D) and contains four screen trays filled with sand. *(Image by Raimundo Espinoza)*

- Each tank has four screen trays filled with sand for the early juveniles (4 - 40 mm). Eventually the juveniles will be moved into the tank directly with a sand bed on the bottom. The conch will stay in this system from when they are 40 mm to 80 mm in shell length.



- The seawater comes into each tray with a small hose (f) and circulates through the sand using a ***downwelling*** action (a, b, c). For the raised sand bed, the small hoses that went into each tray will now be used over the length of the tank. The seawater leaves the tank through an external standpipe and into a sump (d) that is filled with biofiltration media and then the water is pumped (e) back into the tanks. There will be three tanks on one recirculating system, therefore, there will be two recirculating systems in the nursery.

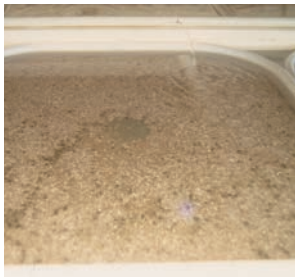
STOCKING DENSITY AND FEEDING TABLE

This table is based on the conch juveniles growing 0.22 mm per day with a survivorship of 75% from 4 mm to 80 mm shell length. Use this table as a reference when determining stocking density based on the size of the conch and daily feeding rate per conch.

Stocking Density *(per m^2)*	***Stocking for 1.5 m^2 tank*** *(stocking density x 1.5)*	***Shell Length***	***Amount of Feed*** *(per conch per day)*	***Duration kept at that density***
1,700	2,550	4 - 20 mm	10 mg	10 weeks
800	1,200	20 - 40 mm	120 mg	16 weeks
400	600	40 - 50 mm	500 mg	8 weeks
150	225	50 - 80 mm	1 - 2 grams	16 weeks

ADJUSTING STOCKING DENSITY

Step 1:
Fill the appropriate number of nursery tanks with filtered seawater about two weeks prior to stocking each tank to establish the biofilter and to allow some natural diatoms to establish on the sand substrate. The screen on the bottom of the trays can be window screen and the coarse sand should be 1 - 3 mm in diameter. It is best to have a thin layer of sand in each tray (0.5 cm).



Step 2:
According to the *Stocking Density and Feeding Table* on the previous page, each tank can hold 2,550 post-metamorphosed conch (4 mm). To stock the nursery trays, the conch from the metamorphosis tanks are gently hosed off the screens into a shallow container. It is assumed that 50% of the original 420 veligers that were metamorphosed and grown to 4 mm will survive. Therefore, three metamorphosis trays of conch can be combined and stocked into one sand filled tray. If all eight metamorphosis trays have 4 mm conch, there will be approximately 1,680 conch (210 conch per tray x 8 trays). These conch will fill three sand trays in the nursery system.

Step 3:
The nursery system will be filled with conch over time as more conch are raised in the hatchery. As the juvenile conch get larger the density in the trays will need to be reduced. The first reduction in density occurs when the conch are 20 mm in shell length. Their density is reduced to 800 conch per m^2 or 1,200 per tank or 300 per tray. To do this, take the nursery tray with the sand and conch and gently hose the contents into a sorting screen. The sand goes through the screen and the conch stay on the screen. Since the density is approximately half of what it was, transfer half the conch into one tray and the other half into a second tray. The sand that was sorted can be reused.

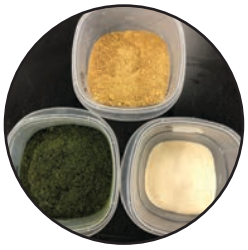
Step 4:
When the conch reach 40 mm in shell length the tanks are set up with a raised sand bed and no trays. A thin layer of sand (0.5 - 1 cm) is elevated from the bottom using a grate which is then covered with window screen. As the conch get larger, the density can be reduced by counting the conch by hand. Refer to the *Stocking Density and Feeding Table* on the previous page. Repeat this process until the conch are large enough for restoration purposes or for stocking sea pens for seafood production (80 mm).

PREPARING THE GEL DIET

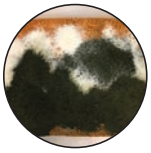
During the nursery phase, conch are fed daily with a gel-based diet. The gel diet can be prepared in bulk ahead of time and stored in the freezer for several months. Here are the ingredients and steps to prepare the gel diet.

Ingredients and Supplies:

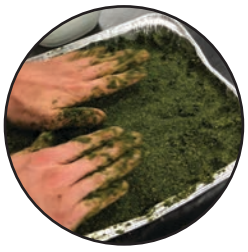
- Hot freshwater	**1500 ml - 1500 g**	(60%)
- Fish pellets (such as cat fish chow)	**600 g**	(24%)
- Gelatin (like what is used to make jello)	**100 g**	(4%)
- Dried *Ulva* seaweed (also known as sea lettuce)	**300 g**	(12%)
- Measuring cup		
- Kitchen scale		
- Aluminum reusable trays		
- Food processor		
- Spatula (or mix with hands)		



STEP 1:
Blend the appropriate amounts of fish pellets and dried *Ulva* (seaweed) separately using a food processor.



STEP 2 :
Mix all of the dry ingredients together: pellets, *Ulva*, and gelatin.



STEP 3:
Slowly add hot freshwater to the mixture while using a spatula or hands to stir the mixture. The hot water activates the gelatin and will turn the mixture into a thick paste. Quickly fill the aluminum trays with the paste before it sets. The paste should be pressed firmly into the trays using fingers and palms and should be approximately ½ to 1 inch thick. Refrigerate the mixture in the trays overnight.

STEP 4:
Cut the gel diet into small cubes that are approximately one inch by one inch and store in labelled quart-sized resealable bags. Label with the date the gel diet was made. The gel diet can stay in the refrigerator for one week and can be stored in the freezer for several months.

STEP 5:
Depending on the size of the conch, the sand trays are sprayed down every two to four weeks to remove accumulated waste products from the conch and uneaten food. During this process, the sand and the conch are left intact and the conch lie dormant. The cleaning is done by using a spray wand similar to the wand used for cleaning the trays with the metamorphosed conch. The water level in the tanks are lowered and the sand with the conch are sprayed down and this wastewater is removed instead of going back into the system.

STEP 6:
Once the amount of gel diet is determined (p. 63), use the kitchen scale to weigh out the appropriate amounts of feed for each tray or tank. Let the gel diet cubes defrost, and then crumble the cubes evenly throughout the trays or tank for the conch to graze on.The gel in the diet will help the feed to stay stable in the nursery tank for about 24 - 48 hours. After this time it will begin to mold. In order not to waste gel diet, it is best not to overfeed the conch.

A single nursery tank is 1.5 m^2 and the stocking density for 4 - 20 mm conch is 1,700 conch per m^2.

1,700 conch per m^2 x 1.5 m^2 = 2,550 conch per tank
2,550 conch per tank x 10 mg of feed per conch = 25,500 mg
25,500 mg / 1,000 mg per g = 25 g

Therefore, the daily feeding for a nursery tank containing 2,550 post-metamorphosed conch to early juvenile conch (4 - 20 mm) would be 25 grams (~ 3 - 4 cubes).

A single nursery tank is 1.5 m^2 and the stocking density for 80 mm conch is 150 conch per m^2.

150 conch per m^2 x 1.5 m^2 = 225 conch per tank
225 conch per tank x 2 g of feed per conch = 450 g

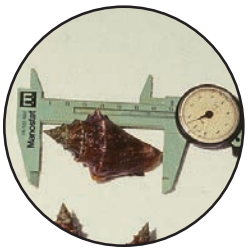
Therefore, the daily feeding for a nursery tank containing 225 juvenile conch (80 mm) would be 450 grams (~ 50 - 70 cubes).

The maximum amount of gel diet daily that would be needed to feed six nursery tanks stocked at full capacity with juvenile conch 80 mm in shell length would be:

= 450 g per day per tank x 6 tanks
= 2,700 g per day or 2.7 kg (6 lbs)

Therefore, the mixture on the previous page is approximately enough for one day of feeding all six tanks for this size and number of juvenile conch.

JUVENILE OBSERVATIONS



Shell length:

A subsample of five to ten conch should be measured once per week for each tray or tank. Once the conch reach a shell length of 10 mm, switch from using the dissecting microscope to using a set of calipers.

Healthy juvenile conch:

- The thin leading edge of the shell aperture of a healthy juvenile conch is usually covered with feed, sand, and feces.The healthy conch animal fills their shell and their ***mantle***, foot, and operculum are easily seen in the aperture.

- Small juvenile conch are most active at night. Typically, during the day conch lay dormant with their aperture towards the sand substrate, however, it is not unusual to see them feeding during the day time. They might tend to be partially buried during the daytime, which is normal behavior.

Unhealthy juvenile conch:

- An undernourished conch recedes back into the shell making it difficult to see the foot and operculum. When a conch is slow growing the shell becomes covered with a green or blue-green algae and the shell may get thick and eroded.

- Juvenile conch in poor health are often observed on the sand surface, positioned with their aperture upwards and their bodies partially extending out of the their shell. In this state, response to a stimulus is often sluggish. This behavior could be a result of stagnant water flow, low oxygen, high temperatures, overfeeding, or stress from handling. Remedies include flushing the tank with new seawater, feeding less, and/or adjusting water flow rate. The kicking behavior seen by conch is usually a sign of stress and has been observed in low-oxygen situations or possibly from bad food.

Date	Days in Nursery	Weekly size of conch *(mm)*	Tank sand sprayed	Food remaining *(%)*	Food fed per tray *(grams)*	General Observations	Temperature *(°C)*
8/11/20	1	4	No	10%	25	Conch spread out active	29
8/10/21	365	80	Yes	0%	450	Feed more	30

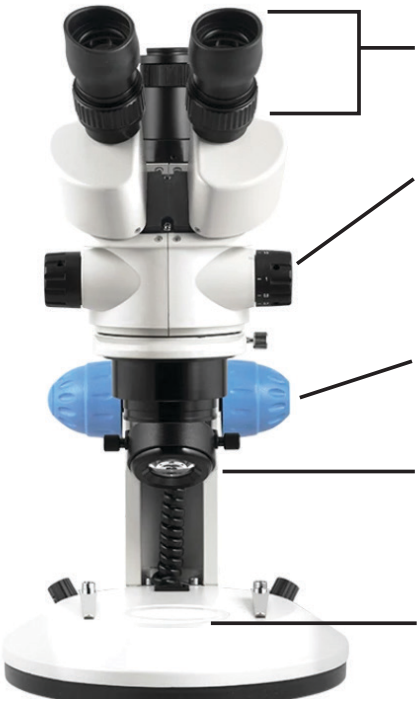
Juvenile Conch Observation Data Sheet

Appendix 1: Microscope Care & Maintenance

When using the microscope always:

- Pick it up with both hands. Never drag it.
- Wipe it down with a freshwater damp cloth and dry.
- Cover it when finished.

Eye piece Magnification	Zoom	Magnification
10X	0.7X	7X
10X	1.0X	10X
10X	2.0X	20X
10X	3.0X	30X
10X	4.0X	40X
10X	4.5X	45X



Micrometer disk fits into the eyepiece of dominant eye.

Eye piece automatically magnifies 10X

The black knob controls the **zoom magnifier** which goes from 0.7X to 4.5X. There are markings on the knob to show the magnification.

The blue knob moves the whole scope up and down to **focus**.

Top light

Bottom light

(*Image by LW Scientific*)

Appendix 2: Supply Sources

Category	Potential Vendors
Fiberglass Tanks	- Red Ewald LLC - Dolphin Fiberglass Products Inc. - Aquatic Equipment and Design - Pentair Aquatic Eco-Systems - Solar Components Corp.
Lab Supplies	- Fisher Scientific - Aquatic Equipment and Design - Pentair Aquatic Eco-Systems - Carolina Biological Supply - Florida Aqua Farms - U.S. Plastic
Filtration	- Pentair Aquatic Eco-Systems - Aquatic Equipment and Design
Shelving	Intermetro Industries Corp.
Microscopes	Nils's Microscopes
Microalgal Isolates (phytoplankton)	- AlgaGen LLC - NCMA Bigelow Laboratory - UTEX Culture Collection of Algae
Ulva Seaweed	Grown locally or FAU Harbor Branch
Fish Feed Pellets	Purina Mills, Zeigler Brothers, or Cargill
Mesh Screens	Miami Aquaculture Inc.
Chemicals	- Tidal Vision (Chitosan powder) - Fisher Scientific (Sodium hydroxide) - Sigma-Aldirch (Sodium hydroxide) - PC NetwoRx Inc. (Ascorbic acid) - Household chlorine and muratic acid - Pentair Aquatic Eco-Systems (Algae media) - Florida Aqua Farms (Algae media) - American Spice Company (Gelatin)

Appendix 3: Glossary and Measurements

The following glossary terms are in large part defined in the context of conch aquaculture.

A

Acclimation: The process in which an individual organism adjusts to a change in its environment (such as altitude, temperature, humidity, photoperiod, or pH), allowing it to maintain performance across a range of environmental conditions.
Agar plate: A Petri dish that contains agar, a jelly-like substance obtained from red algae, as a solid growth medium used to culture microorganisms such as microalgae.
Aperture: The main opening in gastropod shells, where the foot and head of the animal emerges for locomotion and feeding.
Apex: The pointed tip of the shell. It is the oldest part of the shell where the first whorl, or spiral, begins.

B

Beak: A projecting structure of the larval conch shell that wraps over the aperture and ends in a point.
Benthic: Anything associated with, or occurring on, the bottom of a body of water like an ocean, lake, and stream. The benthos are the bottom-dwelling plants and animals found on or in the sediments.
Buccal mass: Is the mouth part of molluscs that is first seen in the larvae once they are competent. It forms the proboscis, or snout, which is used for grazing.

C

Chitosan: Is made from treating the hard outter skeleton (exoskeleton) of shrimp and other crustaceans with an alkaline substance. It is used in pharmaceuticals and is used to flocculate microalgal cells for feeding conch juveniles in aquaculture.
Cilia: The hairlike structure along the edges of the lobes of veligers. Moves in a wavelike motion to propel veligers and are also used to capture food particles and oxygen exchange.
Competency / Competent: Larvae are considered competent when they are morphologically and physiologically ready to undergo metamorphosis.
Cryptic: Camouflage of an animal in their environment.
Ctenidium: A comblike structure, which is a respiratory organ (gill) in a mollusc. First seen in the larvae once they are competent. The gill will replace the cilia that aided the veliger with oxygen exchange.
Cue: A smell, chemical, temperature or other external factor that triggers a change such as metamorphosis.
Culture: The maintenance of plantlife or sealife in conditions suitable for growth. Aquaculture is the rearing of aquatic plants and animals specifically.

D

Diatom: Diatoms are a major group of phytoplankton found in the oceans, waterways and soils of the world. Their cell walls are made of silica, a glasslike material.
Dissecting microscope: This microscope is also known as a stereo microscope and is designed for low magnification observation of a sample.

Downwelling system: Is used in aquaculture tanks to move water from the surface downwards through a screen tray.

E

Egg mass: The female conch lays crescent-shaped egg masses in the sand. Each egg mass is comprised of a long sticky strand that is covered in sand and contains thousands of eggs.
Embryo: The early stage of development of a multicellular organism. It is the part of the life cycle that begins just after fertilization and continues through the formation of body structures, such as tissues and organs.
Epiphytes: Benthic diatoms and other organisms that grow on the surface of seagrass, macroalgae, sand, and rocks. The term is derived from Greek epi meaning 'upon', and phyton, meaning 'plant'.

F

Filter: A porous device that mechanically removes impurities or particles from liquid such as seawater.
Flocculation: The aggregation of cells, that were once in suspension, by the addition of an agent such as chitosan.

G

Gastropod: A mollusc of the class Gastropoda, such as a snail, slug, or whelk. Most gastropods have a single spiral shell into which the body can be withdrawn.
Genetic diversity: The total number of genetic characteristics in the genetic makeup of a species. It serves as a way for populations to adapt to changing environments.

H

Hemocytometer: A counting-chamber device originally designed for counting blood cells, which can be used to count microalgal cells.

I

Incubation: The phase of keeping eggs in favorable environmental conditions until hatch.
Inoculation: Is used for the continuous production of microalgae using the batch culture technique.
Inoculant: A small volume of a dense microalgal culture which is typically transfered into a larger vessel.

L

Larva(e): For marine animals, the larval stage starts after hatch and ends at metamorphosis. Larvae are usually planktonic and spend most of their time in the water column.
Lobes: Protrusions that are characteristic in queen conch larvae. They are used for locomotion, respiration, and feeding.

M

Macroalga(e): Unlike microalgae, macroalgae are visible without a microscope. They are also known as seaweed.
Mantle: A layer of tissue in molluscs which secretes the shell.
Media: Nutrient-rich substance used for cultivation of microorganisms.

Metapodium: The posterior portion of the foot of some molluscs.
Motile: Capable of motion.
Monoculture: The cultivation of a single crop such as a single plant or algal species.
Morphology: The study of the form and structure of organisms.

N
Nursery: A place where young plants and organisms, like juvenile conch, are grown to a certain size.

O
Ocular micrometer: A glass disk that fits in a microscope eyepiece that has a ruled scale. It is used to measure the size of magnified objects.
Oligotrophic: A term used to describe environments of water with relatively low nutrient levels.
Operculum: A structure resembling a lid or a small door that protect gastropods while inside of their shell.
Osphradium: An olfactory organ in certain molluscs, linked with the respiratory organ. The main function of this organ is thought to be used for testing incoming water for silt and possible food particles.

P
Phytoplankton: A group of free-floating microscopic algae that drift with the water currents. They form an important part of the ocean food web. Derived from the Greek phyton, meaning 'plant', and planktos, meaning 'wanderer' or 'drifter'.
Planktotrophic: Refers to the development of larvae that must feed on plankton in order to develop to metamorphosis.
Proboscis: An elongated sucking mouthpart that is typically tubular and flexible.
Propodium: The anterior portion of the foot of a mollusc.

R
Rear: To raise and care for animals in a particular manner or place until fully grown or until a certain development stage.
Recirculating system: A type of aquacuture system, which operates by filtering the water from the fish or conch tanks so that the water can be reused. This dramatically reduces the amount of water used and helps to control water quality.
Restocking: The raising of animals in aquaculture to release them into a river, lake, or ocean to supplement existing populations or to create a population where none exists.

S
Settlement: Refers to when some planktonic organisms, such as conch, find a place in the benthic zone to settle for metamorphosis.
Silica: An oxide of silicon with the chemical formula SiO_2, most commonly found in nature as quartz and in various living organisms such as diatoms, sea sponges, and hydroids. It is one of the components of glass.

Siphonal canal: A semi-tubular extension of the aperture of the shell.
Siphon hose: A device that involves the flow of liquids through tubes without a pump.
Sterilization: The process of making something free from bacteria. For example, microwaving glassware with media or using an alcohol burner for flaming vessels during microalgae transfers.
Substrate: The surface on which an organism lives.
Sump: A tank that is positioned below the culture tanks, which collects seawater in a recirculating system and returns the water back to the culture tanks with a pump.

T
Tentacles: A flexible, mobile, and elongated sensory organ present in many molluscs. They are receptive to touch, vision, and smell or taste of particular foods or threats. Examples of such tentacles are the eyestalks of various kinds of snails.
Test set: A group of smaller tests that are done to predict and achieve the desired outcome on a larger scale.

U
Upwelling system: When water rises up (upwells) to replace the water that was displaced at the surface. In aquaculture, the water in tanks can artificially create this upwelling motion.
UV-sterilized: A disinfection method that uses short wavelength ultraviolet light to kill unwanted microorganisms such as bacteria.

V
Veliger: The larval stage of certain molluscs that have ciliated lobes for swimming and feeding.

W
Whorls: A pattern of spirals, like that of a snail shell.

Y
Yolk reserve: Certain species of animals, usually those with short incubation periods, hatch with a yolk reserve. This gives them the energy they need for the first several hours of their lives until they are able to feed on their own.

Z
Zooplankton: Plankton consisting of small animals and the immature stages of larger animals. The word zooplankton is derived from the Greek zoon, meaning ‘animal’, and planktos, meaning ‘wanderer’ or ‘drifter’.

Volume:
1 gallon = 3.78 liters
1 liter = 1,000 milliliter

Linear measurements:
1 inch = 2.54 centimeter
1 meter = 3.3 feet
1 meter = 100 centimeters
1 centimeter = 10 millimeters
1 millimeter = 1,000 micrometers

Area:
1 square meter = 10.8 square feet

Temperature:
Celcius - Fahrenheit

to convert,
C x 1.8 + 32 = F
F - 32 x 0.556 = C

0^{o} C = 32^{0} F
28^{o} C = 82^{o} F (Ideal temperature for raising conch.)

Exponent examples:
1 x 10^{6} = 1,000,000
1 x 10^{4} = 10,000
1 x 10^{3} = 1,000

Abbreviations and Symbols:

Liter = L
Milliliter = ml
Micrometer (micron) = µm
Centimeter = cm
Millimeter = mm
Gallon = gal
Inches = in
Grams = g

Appendix 4: Production Schedule

Example of a production schedule for a small queen conch hatchery and nursery for each one-year growing season.

Life Cycle Phase	Total Number/ Season	Time	Size (SL)	Stocking Density	Survival	Number of Tanks	Size of Each Tank
Egg Mass	36	3 - 4 days until hatch		1/ container		1 incubator tank with 8 containers	60 L
Larvae	14,400	3 weeks	300 - 1,200 microns	Start with 100 - 200/L end with 10 - 20/L		5 conical tanks	68 L
Meta-morphosis	7,200	3 - 4 weeks	1.0 - 4.0 mm	Start with 3,500/m^2 end with 1,750/ m^2	50%	2 rectangular tanks with screen trays	0.5 m^2; 38 L
Juvenile	5,400	12 months	4 - 80 mm	Start with 1,700/m^2 end with 150/m^2 or less	75%	6 rectangular tanks with sand	1.5 m^2; 314 L

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User Manual

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Citation: Davis, M. and V. Cassar. 2020. Queen Conch Aquaculture: Hatchery and Nursery Phases User Manual. *Journal of Shellfish Research*, Vol. 39, No. 3, 731–810.

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