Slime Lines

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Seaweeds: Feeding the Laboratory Model

The successful laboratory culture of many marine invertebrates depends upon consistently providing the appropriate nutritional source for the various stages of the organism's life cycle. For *Aplysia californica*, seaweeds play two essential roles: 1) the induction of metamorphosis at the end of the planktonic stage and 2) post metamorphic nutrition for juveniles and adults.

The first description of a metamorphic inducing substrate for *Aplysia californica* was *Laurencia pacifica*, by Kriegstein, Castellucci and Kandel in the early 70's. The prevailing concepts suggested that the metamorphic inducing relationship between a marine invertebrate and algal species was quite unique and singular. Consequently, it became essential to the successful development of the *Aplysia* program that *Laurencia pacifica*, a seasonal cold-water rhodophyte found on the west coast of the United States, be brought into continuous year- round culture. Numerous attempts to isolate a laboratory strain resulted in minimal quantities of algal production and repeated subculturing failed to produce sustainable cultures of any significance. *Laurencia's* cold water prerequisite, fastidious culture requirements and seasonal growth patterns limited its potential for a continuous culture environment.

By 1979, the successful intensification of the larval phases led to the production of large numbers of metamorphically competent larvae. The increasing number of competent animals further emphasized the need for a readily available metamorphic substrate and made possible the evaluation of alternative algae as well as extracts. While in Woods Hole, MA., trials with five northeast coast rhodophytes demonstrated that the transformation from a planktotrophic larval stage to a benthic macroalgae grazer could not only be accomplished with *Laurencia* but with all five of the seaweeds. Unlike the seasonal limitations imposed by dependency on *Laurencia*, at least one of these five east coast algal species was available throughout the year. In addition, several species readily developed sustainable non-reproductive cultures providing 30-50g/week of actively growing substrate. The most prolific and consistent for metamorphosis and growth, *Agardhiella subulata*, strain A1, triggered metamorphosis of *A. californica* at >90% and grew in continuous culture without replacement for more than eight years.

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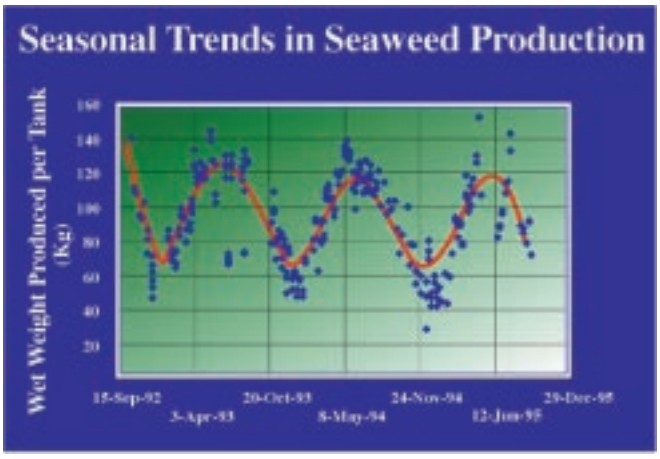

Three years of data (above) clearly demonstrate the seasonal trends in seaweed production.

WHO WE ARE!

Pamela Konoval, the laboratory's most recent arrival, was born and raised in Mansfield, OH. Pam joined the *Aplysia* laboratory a year and a half ago after four years at Bowling Green University where she received a B.S. in Biology with an emphasis was on marine biology. Recently married, Pam's husband, George, works as a dive supervisor for the NOAA Facility across the street.

After the animals metamorphose, Pam oversees the growout process which includes the daily care and feeding of the animals in the main wetlab (below). In addition, she conducts the monthly bacterial swabs and weekly water quality monitoring process required by the NIH Grant. Somehow she still finds time to pack and ship 10,000 sea hares a year.

If you have some questions about maintaining or feeding your animals, give the lab a call and ask for Pam.

What Do You Think Of Our Slime?

1) Having problems with our animals? Let us know, we're committed to providing the highest quality animals to meet your needs.

2) Have any special needs? Aged animals, special diets, or large numbers of cohorts for longitudinal studies? We can help.

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Metamorphosing large numbers of larvae led to substantial numbers of early juveniles initially feeding on laboratory cultured seaweed which resulted in a sizable demand for macroalgae to feed the later juvenile phases. As early as 1986, weekly consumption requirements reached 100-200 lbs of field collected *Gracilaria tikvahiae*. Field collection from Cape Cod bays readily supported the need during the spring, summer and early fall. Winter months, however, left some of the embayments ice covered and inaccessible. Even more important, animal growth was dramatically reduced when fed algae collected during these months. Attempts to cultivate large quantities of macroalgae at Woods Hole using techniques developed by Dr. John Ryther in Florida, proved unreliable at best in the temperate climate. Winter light levels and a water temperature of > 10°C brought algal growth to a standstill.

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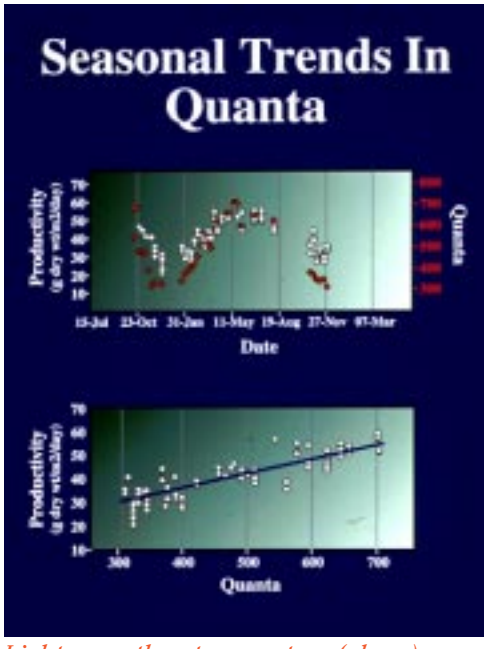
Limited field collection and small batch cultures of selected strains had demonstrated its feasibility. However, the model was growing in worldwide acceptance and by 1987 required 350-400 lbs/week of seaweed to support the demand.

Intensive seaweed culture was the only option with the potential to support the food and metamorphic requirements year round. Serendipitously, the facility was moved to its present location in Miami, Florida in the fall of 1989. Light and temperature no longer limited outdoor cultures and large scale food production became a reality. Numerous isolates of *Gracilaria*, *Agardhiella* and a local species of *Laurencia* were all evaluated for growth and sustainability of vegetative strain in large scale culture (reproductive strains will produce gametes and in some cases the plants are lost). A South Florida vegetative strain of *Gracilaria tikvahiae*, (Strain SB), supported metamorphosis and growth as well as year round biomass production. Trials during the past six year period have resulted in a maximum production of 1000 lbs /week in May/June and 400lbs/week during February. Yearly production has reached 12 tons and naturally more than needed for the sea hare culture. Notably all production came from an original inoculum of less than 10 pounds.

Successful intensive algal culture provides a means of manipulating the plant by varying the culture media. By reducing available nitrogenous sources, the seaweed will utilize interstitial protein, most noticeably its phycoerythrin. If maintained in a low nitrogen media for 2 weeks the plant will eventually become pale yellow to straw colored (stripped algae) and lose approximately 50 % of the available protein. Similarly, exposing the plants to elevated levels of amino acid or labeled compounds may enhance the corresponding levels in the animals. Manipulating the plant and animals may have some important and useful applications in your laboratory. If you would like some assistance with a trial project give us a call.



Light more than temperature (above) proved to be the major contributing factor for seaweed production.

Intensive seaweed production, the final component limiting the large scale year-round production of Aplysia has consistently supported *Aplysia* culture for the past 7 years. The successful culture of *Gracilaria* brought to fruition the early vision of Kandel, Castelluci, and Krigestein of making large numbers of laboratory reared *Aplysia* at any stage continuously available for neurobiological and behavioral research.

Seaweed Care and Feeding:

Note the difference between the two portions of algae from the same strain, shown above. The pale-colored clump on the left has been raised in a low nutirient environment, while the clump on the right has been maintained under ideal growing conditions.

Seaweed cultivation requires a continuous source of good quality sea water which can prove to be a serious limitation to many Aplysia facilities. Fortunately, laboratory cultured seaweed is available throughout the year from the NIH-University of Miami Aplysia Resource Facility. The algae can be shipped via Federal Express along with the animals. To preserve algae quality in your laboratory, transfer the algae immediately upon arrival to 5-10 gallon aquarium with freshly filtered seawater, if available, or newly mixed sea salts if you are located inland. In either case, the aquarium should be aerated and maintained at room temperature (20-24°C) and the salinity adjusted to 30-36 ppt. Ambient light conditions are normally sufficient for holding the seaweed for one week. Due to possible transfer of diseases, avoid storing food and animals in the same tank. If the plant tips turn pink or green the algae is decomposing and should be discarded. Plant and water should also be replaced weekly to avoid using old material and possibly contaminated water. Depending on the animals and the experimental protocol, animals can be fed an amount readily consumed in 5-10 minutes every other day. Limiting feeding will keep your animals healthy, active and maintain water quality as well as minimizing the possibility of bacterial problems.

If you are planning on holding the animals for a long period, bear in mind that they were raised on this algae and changing their diet may adversely affect them. If you would like more details on seaweed culture, feeding or handling, I can be reached at tcapo@rsmas.miami.edu

Research Focus:
The Trying Teens

An important research focus of many Aplysia scientists is development. Certain aspects of behavioral development can be used as a straightforward and noninvasive production tool. On our Production line here at the Resource, we use Aplysia behavioral development as an indicator of maturation state for each successive animal cohort as it moves down the line. Checking the on-time appearance of certain behaviors ensures that developmental times are stable from batch to batch. For example, at 30-35 days after hatching, when they become competent to enter the metamorphic phase, larvae will change their adversive behavior toward the algal species onto which they will settle and then metamorphose. We also use behaviors such as righting, siphon and parapodial withdrawl as behavioral indicators of animal health at all stages.

One behavior we watch for very carefully on our line is the appearance of mating behavior. Copulation means the animal has entered the reproductive phase that soon leads to senescence. Thus once mating behavior is observed in a cohort in the facility we know that cohort has ended its useful life on the line.

One of our Resource investigators, electrophysiologist Lynne Fieber (Research Assistant Professor), has begun studying a neurodevelopmental period related to mating. Lynne is studying the development of the capability to release egg laying hormone. Sexual behavior and egg laying functionally define sexual maturation in Aplysia. Yet Aplysia are considered adults much earlier in development when the reproductive system is newly formed, at stage 13, about 120 days after hatching. An important developmental feature of this stage

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is the appearance of the bag cell clusters on the abdominal ganglion. Early stage 13 animals are 4-6 months away from their first copulatory behavior, yet these bag cells already contain egg laying hormone, and the cellular machinery necessary to release it. Although early stage 13 Aplysia are equipped to reproduce, they clearly do not. Lynne is searching for features of the bag cells which inhibit the release of egg laying hormone in *Aplysia* that are not reproductively active.

Lynne hopes that her results will be of use on the production line. Understanding aspects of the hormonal control of reproductive development may allow us to define maturation stage on a finer level. Currently, no developmental stage separates the 20 g early stage 13 animals from 200 g animals which are 4-6 months older and beginning to engage in reproductive behavior. Lynne is hopeful that an electrophysiological measurement relevant to hormone release may occur during this long interval. If so, we would have an additional developmental point to mark the passage from nonreproductive to reproductive adults.

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