CULTURE OF SEA CUCUMBERS IN KOREA:

A guide to Korean methods and the local sea cucumber in the Northeast U.S.

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In an effort to develop suitable culture techniques for sea cucumber (*Cucumaria frondosa*) in the Northeast, this guide reviews the current knowledge of *C. frondosa* biology and reports on techniques for the hatchery culture of the Japanese sea cucumber *Apostichopus japonicus* learned during a research exchange between the United States (NOAA Sea Grant) and South Korea (National Fisheries Research and Development Institute). The final portion of the guide discusses the potential adoption of the culture techniques for *A. japonicus* for use with *C. frondosa*.

Supported by Sea Grant and NOAA-MOF Joint Project Agreement on Integrated Multi-Trophic Aquaculture, through the Joint Coordination Panel for Aquaculture Cooperation for US-Korea.

Pietrak, M., J.K. Kim, S. Redmond, Y.D. Kim, C. Yarish, and I. Bricknell. 2014. Culture of Sea Cucumbers in Korea: A guide to Korean methods and the local sea cucumber in the Northeast U.S. Orono, ME: Maine Sea Grant College Program. seagrant.umaine.edu/extension/korea-aquaculture







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Introduction

Sea cucumbers are a valuable fishery around the globe. The United Nations Food and Agriculture Organization (FAO) estimated the global production from both aquaculture and capture fisheries to be 153,183 metric tonnes in 2011 (FAO 2013a). Of that production, roughly 85% is the Japanese sea cucumber *Apostichopus japonicus* (1). Sea cucumbers are developed into a variety of products including dried muscle, fermented guts, and dried gonads (FAO 2013b). Wild stocks have been heavily fished in many areas around the world. As a result of overfishing, sea cucumber culture techniques have been developed for variety of species. Hatchery production has centered in the Pacific Rim and is used both as a source of juveniles for various culture activities and for stock enhancement and restoration efforts. Currently hatchery production exists for approximately a dozen different tropical and temperate species (Purcell et al. 2012, Table 1).

Integrated Multi-trophic Aquaculture (IMTA) efforts in northeastern North America have focused on various combinations of fish, shellfish, and algae. Sea cucumbers have been proposed as one potential species that could be added as a consumer of benthic deposits. Within the Gulf of Maine, the predominate sea cucumber species, *Cucumaria frondosa* (2), has been fished commercially in Maine since 1990. Landings peaked in 2004 with a harvest of just over 10 million pounds (ME DMR 2013). The value of the fishery in Maine fluctuated between \$66,000 and \$562,000 from 1994 until the peak in 2004. The price

per pound has more than tripled since the peak harvest in 2004. The economic value for the Maine fishery is based on the boat price paid to fishermen, and does not represent the significant increase in value that occurs with processing into various final consumer products.

Sea cucumbers from the Maine fishery traditionally have gone to the Asian food market, however the potential for various nutraceuticals may represent a higher value use. For example, trials are underway to examine the potential for Frondoside A, a compound extracted from *C. frondosa*, to treat cancer (Attoub et al. 2013). At least one company in the region is processing sea cucumbers for the extraction of various nutraceuticals.

A recent review of the potential for culture of *C. frondosa* in the Northeast (Nelson et al. 2012a) highlighted advantages and challenges for commercial culture of this species, in particular under IMTA. Strengths include an existing market for the product with good potential for increased nutraceutical use, a well-understood reproductive biology (especially for populations in the St. Laurence River area), and evidence that *C. frondosa* would be a suitable species for integration in IMTA farms as a benthic filter feeder. Culture of this species faces several challenges. These include a relatively low market price, even when the fishery was at its peak, due to the thinner muscle wall, a long grow-out period, and a lack of published techniques for culturing this species.

Apostichopus japonicus



Michael Pietrak

Cucumaria frondosa



Hamel & Mercier (SEVE)

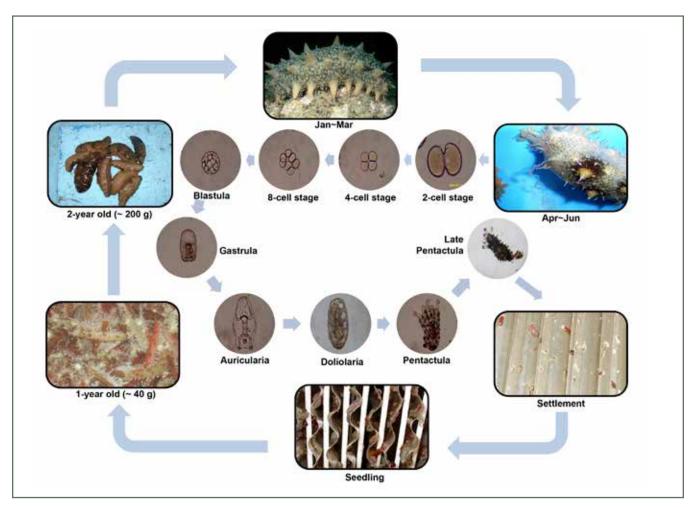
Table 1: Global hatchery production of sea cucumber juveniles. Modified from Table 1 in Purcell et al. 2012.

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Solomon IslandsH. scabran/aExperimental1996-2000USA (Alaska)P. californicusn/aExperimental2010-ongoing	Philippines (Iloilo)	H. scabra	11,000	Experimental	2010-ongoing
USA (Alaska) P. californicus n/a Experimental 2010-ongoing	Saudia Arabia	H. scabra	n/a	Sea ranching	n/a
	Solomon Islands	H. scabra	n/a	Experimental	1996-2000
Vietnam H. scabra 200,000+ Pond farming 2001-ongoing	USA (Alaska)	P. californicus	n/a	Experimental	2010-ongoing
			200,000+		

LIFE HISTORY AND BIOLOGY OF CUCUMARIA FRONDOSA

Mature adult sea cucumbers "broadcast spawn" their gametes. Fertilized embryos develop into pentacula, the five-tentacled larval stage in the life history of echinoderms. After a period of time in the plankton, the larvae settle to the sea floor and metamorphose into juveniles. *C. frondosa* average 20 cm in length, although they are capable of growing up to about 50 cm, and live in temperatures between -1.8 °C and 8 °C. Levin and Gudimova (2000) provide a good description of *C. frondosa*'s morphology.

Adult animals reach sexual maturity in about five years and around 80-100 mm in length. Adult animals display sexual dimorphism and can be easily distinguished when the feeding tentacles are extended. Females display a gonopore approximately 4 mm in diameter and a papilla approximately 7 mm in length with a single genital pore. Males possess a larger gonopore approximately 8-10 mm in diameter surrounded by between five and 22 papillae (Hamel and Mecier 1996).



Life history of sea cucumbers and milestones for their culture in Korea. Figure from NFRDI.

Spawning typically occurs in the St. Lawrence around mid-June (Hamel and Mercier 1996), while populations near Mount Dessert Island in Maine spawned between the end of March and the end of May (Ross 2011). Day length is likely an important cue to start the process of producing gametes (Hamel and Mercier 1996, 1999), however cues in the mucus appear to be more important in synchronizing the spawning of animals in a given locality (Hamel and Mercier 1999). Following fertilization, the embryos develop into pentacula larvae. According to Hamel and Mercier (1996) this took nine days at 12 °C and 26 psu (practical salinity units; Table 2). Ross (2011) does not report embryo development timing beyond the 64-cell stage, which could take up to 48 hours. Ross did report that in one sampling season "elongated embryos" were found in the early plankton tows, and that lab observations showed that this stage was reached after four to seven days. Larvae prefer to settle on rock or gravel substrate after approximately 48 days after fertilization (Hamel and Mercier 1996).

Few have looked at growth rates, but Hamel and Mercier (1996) reported that the mean growth rate in the wild was 2 mm a month, while the maximal growth rate during the spring was 5-6 mm per month. Studies have also shown that the rate an individual *C. frondosa* inserts its tentacle into its mouth is related to the feeding rate of the animal (Holtz and MacDonald 2009). It has also been shown that *C. frondosa* are capable of feeding on high organic content materials, such as feces and fish feeds, from IMTA salmon farms (Nelson et al. 2012a). The absorption rate increased with increasing organic content, suggesting that *C. frondosa* might be cultured on salmon-mussel-algae IMTA farms.

Table 2: The time and size evolution of selected developmental stages of *Cucumaria frondosa* up to young sea cucumbers, under natural environmental conditions reproduced in the laboratory. Table modified from Hamel and Mercier 1996.

Stage of development	Time	Size (mm)
Fertilized oocytes	0 min	0.90±0.2
64-cell	24.0±2.0 h	1.40±0.5
Blastula	48.0±3.6 h	1.35±0.3
Hatching	62.0±8.0 h	1.30±0.4
Young gastrula	72.0±8.5 h	1.30±0.3
Late gastrula	4.3±0.5 d	1.40±0.5
Elongation	5.5±0.5 d	1.60±0.5
Vitellaria (uniformly ciliated	8.0±1.0 d	1.55±0.3
Young pentactula (5 tentacles)	11.0±1.5 d	1.30±0.3
Appearance of the anal pore	13.0±1.0 d	1.35±0.4
Late pentactula (with pair of ambulacral podia)	17.0±1.4 d	1.40±0.5
Settlement (cilia loss)	46.0±2.0 d	1.40±0.4
Young sea cucumber with 2 pairs of ambulacral podia	3.0±0.2 months	1.70±0.5
Young sea cucumber with 5 pairs of ambulacral podia	4.0±0.2 months	1.90±0.5

Note: The temperature recorded throughout the development varied from 0 $^{\circ}$ C to 13 $^{\circ}$ C as reported in Figure 1 of Hamel and Mercier 1996. A new stage was considered attained when 50-60% of the embryos reached it.

KOREAN HATCHERY CULTURE TECHNIQUES

Hatchery culture systems

Culture facilities

In March 2013, a NOAA Sea Grant-sponsored delegation from the United States visited three different sea cucumber hatcheries near Gangneung in Gangwon Province, South Korea.

One of the facilities was a private hatchery that used discharged cooling water from a nearby coal-fired power plant as a heat source. This water was mixed approximately 50-50 with local seawater and sand filtered prior to use in the facility.

The second hatchery visited was a provincial government hatchery that reared various species for stock enhancement. A main seawater line supplied storage tanks for the entire facility. Incoming water was filtered and heated as required in the individual species culture buildings. Neither the private nor the provincial facility sterilized incoming water.

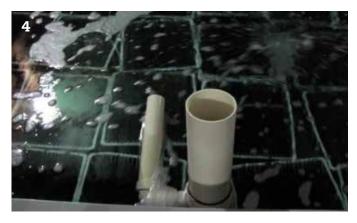
The final hatchery visited was an NFRDI research hatchery.

Culture tanks

All three hatcheries used similar rectangular culture tanks approximately five meters long and three meters wide, with a water depth of about one meter (3). A water inlet was located at the far side of the tanks; the bottom of each tank sloped slightly toward a drain at one end (nearest the walkway) to facilitate regular cleaning.

An internal standpipe of four-inch PVC acts as an emergency drain (4). The private hatchery attached a two- to four-inch "T" just below the water level in the tank with an unglued two-inch elbow that actually set the water level by having the pipe angled up, shown here. To partially drain the tank for the twice-daily water changes, the pipe is turned down, submerging it, draining the tank in a limited fashion.

A similar drain was installed between the tanks so that when moving the animals to the adjacent tank in order to clean the bottom, some of the already heated water could be saved. It is important to take the surface water, which is relatively free of sediments, and not the bottom water. At all locations the culture tanks were operated as static systems with partial water replacement twice a day, although the provincial hatchery had access to flowing seawater and recirculation systems.





Culture equipment

Very little equipment is required for spawning and rearing sea cucumbers in the hatchery. In addition to the culture tanks described above, it would be useful to have some smaller tanks for conditioning broodstock. The size and style of the tanks is not critical, but conditioning requires an ability to slowly raise the water temperature to 15 °C. Nets of the appropriate mesh size (200 μm for A. japonicus or 800 μm for C. frondosa) are required to transfer the fertilized eggs to the culture tanks described above, which are used for the entire culture cycle in the hatchery. Access to a dissecting microscope in order to view the development of

the larvae would be advantageous, however it is likely that an experienced operator will know the larval stage based on the degree-days since fertilization.

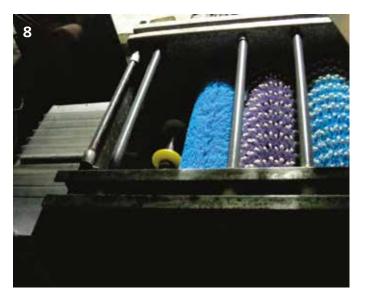
The most important pieces of equipment are the settling plates and the juvenile cucumber shelters (**5**). The shelters consist of an open plastic crate designed to hold file folders (**6**). Settling plates made from clear corrugated PVC sheets are cut to dimensions to fit inside the crate (**7**). In one of the hatcheries, the settling plates were treated with a vitamin mix prior to the first use, but this practice was not consistent at all of the hatcheries visited.







There is a need to clean the settling plates on a regular basis by hand washing/scraping or, with very dirty plates, an acid wash. One hatchery had a commercial plate washing machine that consisted of internal rollers that feed the individual panels and rotating brushes that scrub them clean (8).

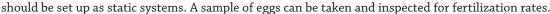


Korean sea cucumber culture process

Broodstock conditioning and spawning

Adult *A. japonicus* are collected from the wild approximately 60 days in advance of the spawning season and placed into conditioning tanks at ambient seawater temperatures ($\mathbf{9}$). The temperature is increased 1 °C per day until it reaches 15-18 °C. The animals are held at 15 °C for 800 degree days, at which point they should be ready to spawn. They can be fed a commercial diet during this time, which typically consists of powdered algae such as *Spirulina* spp. or *Dunaliela* spp. Feeding must be stopped the day before spawning to avoid contamination by sea cucumber feces.

Spawning is initiated through temperature shock: conditioned animals are moved from the holding tank directly into water that is 3-5 °C warmer. After release of both sperm and eggs, fertilized eggs can be removed with a net and placed into rearing tanks that have appropriately sized mesh covering the drains. The tanks





Larval culture

Fertilized eggs are collected from the spawning tanks and placed into the culture tanks at a density of 0.25 individuals mL⁻¹ (Kang et al. 2012). No specific density was provided, but the NFRDI culture tank shown here holds approximately one million larvae (**10**). Larvae are fed a diet of microalgae. For the first two days a 50-50 mix of *Isochrysis galban* and *Pavlova lutherii* is recommended. After day two they can be fed *Cheotoceros calcitrans*. Samples of larvae should be taken daily in order to monitor development. After approximately seven to eight days, larvae should be close to the pentacula stage and settlement. At this point shelters with clean settling plates can be placed in the larvae rearing tanks.

Upon settlement the new juveniles are transitioned to a feed mixture of mud or bentonite, yeast, and powdered algae (11).

Predation of larvae by zooplankton was reported to be a problem in at least one facility visited. This can likely be managed through good water filtration.





Juvenile culture

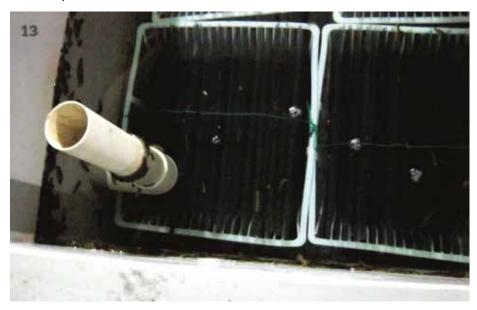
Juveniles are cultured on the settling plates for about a year until they are transferred to cage systems for grow-out at sea. Maintaining good husbandry practices in the hatchery is critical for preventing disease, which can be a significant issue. All of the systems visited maintained static water in the culture tanks, with twice-daily partial water changes at feeding times. In one facility, the water was changed before feeding in the morning and after feeding in the afternoon. The water temperature is maintained around 15 °C. Conservation of water heat was given as the reason for maintaining static systems. It is possible to use a flow-through system, as was observed at the NFRDI research lab, although animals were maintained under ambient water temperatures. It should be noted that the juvenile sea cucumbers are mobile and will move within and between the shelter and throughout the tank. Appropriate screening must be maintained on drains when conducting water changes or if using a flow-through system.

To maintain good water quality it is important to clean accumulated feed and feces from the bottom of the tanks approximately every five days. This is accomplished by moving the shelters to an adjacent tank that is empty of animals (**12**).

Surface water can be moved over to reduce the energy needed to heat the water. The image here shows a drain in the wall between adjacent



tanks (13). Tilting the standpipe down allows clean, warm surface water from one tank to partially fill the adjacent tank where animals are moved while the old tank is cleaned. Mesh bags or screens should be placed on drains to capture loose animals in the tank being drained. Once drained, the tanks can be scrubbed clean and readied for the next transfer of animals. In addition to cleaning the tanks on a regular basis, the settling plates must also be changed out and cleaned about once every three months.



CULTURE POTENTIAL FOR CUCUMARIA FRONDOSA IN THE NORTHEAST

Cucumaria frondosa appears to be a potential candidate for culture in the northeastern United States. In particular, C. frondosa will likely make a good addition to IMTA sites being developed in the region, given its ability to feed on particulate organic wastes. Nelson et al. (2012b) reviewed the potential for culturing *C. frondosa* in the Northwest Atlantic and found it to be a potentially viable candidate species. Several major hurdles are immediately apparent to the culture of this organism. First, the current market for the wild caught *C. frondosa* does not appear to be valuable enough to make aquaculture economically viable. This situation is likely to change as higher-value uses of various extracts and nutraceuticals are further developed for both humans and animals. Second, a better understanding of some aspects of the basic biology is needed, in particular growth rates under various conditions and feeding levels. Finally, culture techniques will need to be adapted and optimized for this species.

A good starting point may be provided by the techniques observed in Korea, such as use of temperature shock to induce spawning. Nelson et al. (2012b) point out that hatchery production of juvenile sea cucumbers will likely be required as fishing for mature broodstock will compete with the wild fishery and juveniles are very difficult to find and collect in the wild. The long planktonic larval stage seen in *C. frondosa* compared to species raised in Korea may also pose additional problems. Hatchery producers will need to evaluate costs and feasibility of using the same tanks for juvenile culture and larval production. The long larval phase will also pose issues associated with water quality and treatment. Using static systems for the duration of the larval phase will likely not be possible, as a regular exchange of water will be needed to maintain water

quality. Prevention of larvae loss or damage will be an issue to contend with. Larval settlement onto the plate culture system used in Korea should be tried, however the spacing of the plates may need to be adjusted. The ability to grow sea cucumbers on a vertical surface will greatly increase space efficiency. As *C. frondosa* remains a planktonic feeder all of its life, their culture should not experience the transition to deposit feeders that was hinted at as a bottleneck in production in Korea, and might permit for the use of continuous flow culture tanks that will help to improve water quality and remove uneaten feed. If neutral to slightly negatively buoyant diets can be developed, flow through the tank should allow feed to be carried to all animals.

It will be necessary to develop grow-out techniques that can incorporate sea cucumbers onto IMTA sites. While the Korean design of a net pen cage that has a false bottom to separate the fish from the sea cucumbers might be feasible, other ideas should be considered. It may be more practical to culture sea cucumbers in submerged trays or cages similar to those used for shellfish culture. Most importantly, husbandry techniques will need to be developed that allow for the grow-out to occur in two and a half years or less. It would not be advisable to culture sea cucumbers for much longer than the grow-out period of the other organisms on the site, so that the entire site may be fallowed before starting the next crop of animals.

The development and optimization of culture techniques for *C. frondosa* is likely to take five to 10 years of research and grow-out experience. Because current medical trials may appear to be successful or other economic factors could change in a manner that will improve economics of sea cucumber culture, work on culture techniques should begin immediately.

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