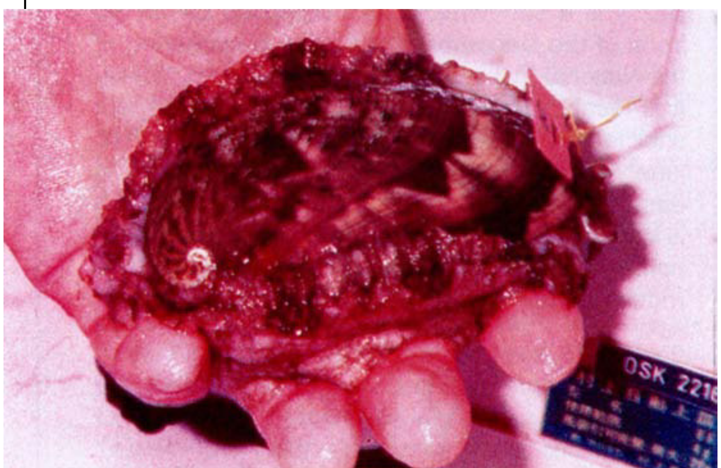
Abalone R&D at AQD

By **M Castaños**



Haliotis asinina

"A major factor limiting the expansion of abalone cultivation in temperate countries is the ready and economic availability of suitable algal rations," explains Emmanuel 'Manny' Capinpin, a researcher spearheading the abalone culture effort at AQD. Abalone feed on seaweed.

"There is also the environmental considerations in harvesting large quantities of macroalgae," Manny says. "In the Philippines, however, cultured seaweed is available and may be used to feed abalone. *Gracilariopsis heteroclada* has a high protein content, promotes fast growth, and can maintain growth of *Haliotis asinina* over extended periods. Moreover, this alga is abundant, farmed in drainage canals and brackishwater ponds, and available year-round."

"In other countries, better growth of abalone was observed when using mixed algal diets but in the Philippines, using a single-species algal diet is sufficient," Manny further explains. He emphasized that suitable diet is important to the success of abalone culture. Abalone grow slowly, about 2-3 cm per year. Growth is also very heterogenous (abalone seldom grow at the same rate). It all depends on the kind of food abalone eat.

"For now, farms must rely on cultured seaweeds," he says, "and there are already a number of studies done on the culture of *G. heteroclada."* But he hopes this dependence won't last long. Researchers around the world are trying to develop cost-effective artificial diets (see related article on page 24).

In addition to food quality, it is also important for farmers to use the right culture system design. Grow-out culture starts with 1.5 cm abalone. Culture systems vary, from intertidal ponds (see page 15, this issue) to land-based tanks and hanging net cages and barrels. "Farmers must be aware that an ideal culture system is one that promotes an even distribution of abalone, ready access to feed, minimum contact among abalone and feed with fecal wastes, good waterflowand exchange," Manny elaborates. "And minimum human intervention, too."

AQD is working on the abalone species with the highest aquaculture potential in the Philippines. *Haliotis asinina* was chosen because of its large size and body weight. So far, AQD researchers were able to spawn the donkey's ear abalone artificially, and raised the spats in the hatchery. Cage culture trials are underway. An artificial diet study has also been initiated by Myrna Teruel and Oseni Millamena. Researchers will also study the efficient mass culture of diatoms for larval feed in addition to settlement and survival patterns of abalone larvae.

AQD research results on abalone are presented below:

REPRODUCTIVE BIOLOGY

AQD researchers have studied reproductive biology (gonad histology, gonad indices, sex ratio, spawning period and fecundity, time interval between successive spawnings and minimum size at sexual maturity).

"Abalone may spawn throughout the year," Manny and his colleagues report. They collected their aba-

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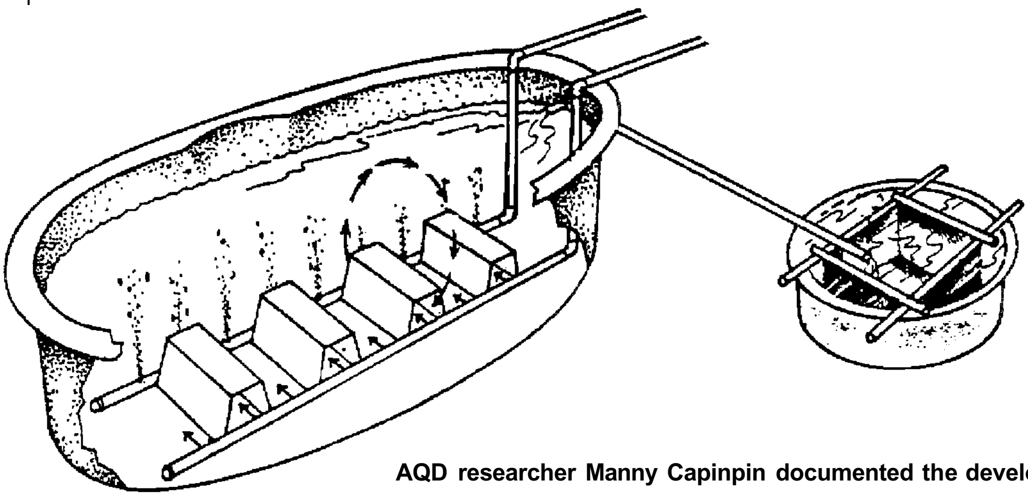
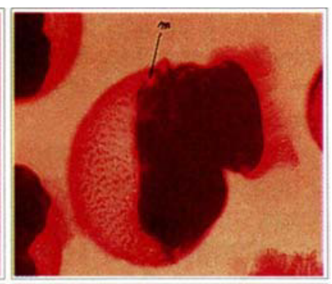


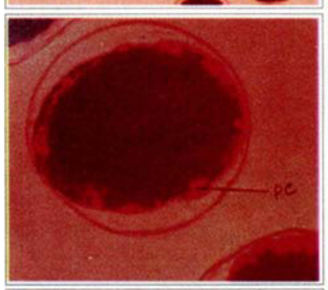
This is the 1 -ton oval fiberglass broodstock conditioning tank at AQD. This set-up is similar to the set-up at the Oyster Research Institute in Japan. It has artificial shelters, well-circulated water, and a device for harvesting newly hatched larvae.

AQD researcher Manny Capinpin documented the development of *Haliotis asinina* larvae

fertilized egg 180 µm

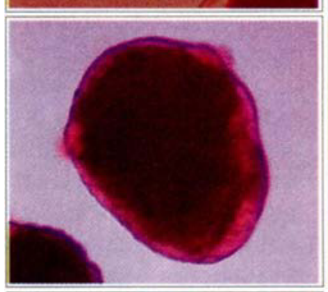


veliger larva before torsion of foot mass (m, mantle)

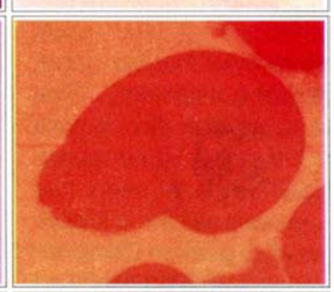


trocophore before hatch-out

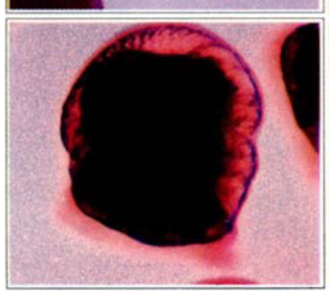
veliger larva ready for settlement



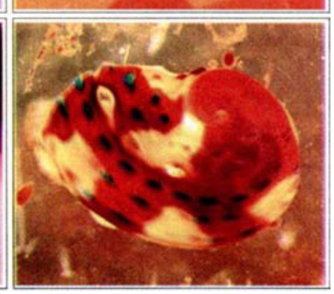
trocophore after hatch-out



creeping larva



trocophore showing larval shell secretion



juvenile with first respiratory pore

lone samples from shallow coral reefs in Panagatan Cays off Antique in west central Philippines.

They calculated gonad bulk indices and made histological cross-sections. They found that gonads of both male and female abalone become ripe in January. Abalone spawn from February to May, become spent in June to July (specimens collected were at resting stage) but rematured after that.

The smallest individual from the wild with a mature gonad is about 40 mm shell length. Hatchery reared abalone reach maturity at about 35 mm.

The sex ratio of abalone Manny and his colleagues collected from Panagatan is approximately 1:1.

"Abalone is a partial spawner with an asynchronous spawning behavior," the researchers noted. Ripe females measuring 58-70 mm shell length release about 150,000 to 600,000 eggs per spawning.

Manny also compared the reproductive performance of wild-caught and hatchery-reared abalone broodstock. Multiple spawnings occur when both groups were induced to spawn by totally draining the tank and replacing water after 45 minutes of desiccation. But wild-caught female broodstock spawn more frequently and produce more eggs than hatchery-bred broodstock. Hatchery-reared abalone have short time intervals between successive spawnings of 13-15 days.

INDUCED SPAWNING

Manny and JICA Expert Masahiro 'Mike' Hosoya noted the spawning of the abalone in the laboratory. "The abalone spontaneously spawned several days before or during the new moon and full moon," they report. "Natural spawnings seem regular, at least every two weeks following a lunar cycle. *Haliotis asinina* is known to spawn year-round with a monthly peak in October." Their broodstock (10 males and 35 females) had shell lengths ranging from 54 to 108 mm and weights ranging from 35 to 187 grams.

As a general rule, males spawn slightly earlier than the females. Abalone spawn from about 10 PM to 3 AM.

The broodstock conditioning tank at AQD is a 1-ton oval fiberglass tank (see diagram previous page). "This set-up is similar to what the Oyster Research Institute in Japan is using," Mike says. "It has artificial shelters, well-circulated water, and a device for harvesting newly hatched larvae called trocophores." The adult abalone were fed *Gracilariopsis heteroclada* ad libitum. Spawning occurred at 28-30°C and 30-32 ppt. The researchers then tracked the developing larvae (see photos, previous page).

"Our success in spawning *H. asinina* in captivity supports the strong potential of donkey-ear abalone as an aquaculture species," Manny explains, "however, more studies should be focused on laboratory-controlled conditioning (maturation) and the factors that trigger spawning. Farmers in other countries have experienced considerable mortalities up to 90-95% in the first few weeks after settlement. We observed the same at AQD, and will focus future studies on environmental and nutritional requirements of newly-settled abalone larvae."

With JICA Expert Hosoya, Manny also tried desiccation, thermal shock, ultraviolet irradiation of the seawater, and hydrogen peroxide treatment to induce abalone to spawn. This was supposed to make it easier for abalone to spawn year-round in captivity.

"We were successful only in one occasion when milt from a single male induced three females to spawn," Manny says. "The release of gametes from an abalone can induce or trigger spawning of conspecifics. We were unsuccessful because we failed to recognize ripe specimens at that time. But we can easily recognize these now and place them in separate containers. There is no need to induce them because abalone spawn naturally."

A male and a female are usually placed in one tank. Sperm density is critical to successful fertilization. "High sperm density during fertilization causes lysis of the vitelline layer of the eggs which render the eggs unviable," Manny explains. "If these eggs do hatch, there is a high percentage of abnormal trocophores." But sometimes, the eggs are unfertilized because of very low sperm concentration or the delay of the male to spawn. This phenomenon of polyspermy in abalone is well-known.

The option is separating ripe male and female abalone, and fertilizing the eggs artificially. But further basic research is needed, particularly on the factors that control maturation and spawning.

RAISING ABALONE IN THE HATCHERY

When Manny obtained 3-month old abalone juveniles, he collaborated with AQD researcher Kaylin Corre to determine the growth rate of donkey's ear abalone if fed an artificial diet (produced by a company, Nihon Nosan Kogyo, in Japan) and cultured red seaweeds *Gracilariopsis heteroclada* and *Kappaphycus alvarezii.*

The researchers do not worry about using *G. heteroclada* and *K. alvarezii* because AQD has already very good technologies for propagating them. Farmers even raise *heteroclada* in drainage canals.

The researchers initially fed diatoms (*Navicula* and *Nitzschia*) to the abalone, and then the red seaweed after the abalone reach 10 mm in shell length. They experimented on 180 juveniles having weights of 0.48 ± 0.01 g and shell lengths of 14.52 ± 0.12 mm. The artificial diet was given to abalone at 5% body weight which was later adjusted according to consumption. Seaweed were given *ad libitum.*

"In our experiment, we stocked 20 abalone juveniles in one plastic tray (about 5 x 20 x 24 cm). The tray is perforated and enclosed in a net," Manny elaborates. "Each tray has PVC pipes as shelters, and the trays were suspended in a 1 ton oval fiberglass tank. We cleaned the tank every 2 days." The researchers maintained water flow at 300-350 liters per hour, and gently aerated the tank. Temperature was about 28-31°C, salinity 28-32 ppt.

The experiment lasted for 4 months. "The abalone fed the artificial diet and *G. heteroclada* grew faster than the abalone fed *K. alvarezii,"* Manny reports. "This did not surprise us because *alvarezii* had the lowest protein and fat content (see table above)."

"Between *G. heteroclada* and the artificial diet, the seaweed win hands down," Manny says, consulting the table on growth rates shown below. "Although it might appear that abalone grow better with artificial diets in the first 90 days, *heteroclada* still give long-term growth gains."

"Growth (in terms of shell length) slowed down after 90 days probably because the abalone were maturing then," Manny explains. "Abalone might have channelled more of its energy towards reproduction. We found that all juveniles fed artificial diet and *heteroclada* were sexually mature at the end of the experiment, but not juveniles fed *alvarezii."*

There were also shell color differences in the abalone. Those fed artificial diet have light bluish green shells, those fed the red seaweed have brown shells. The artificial diet probably contained brown algae.

Survival of abalone fed the three diets range 98-100% after 4 months.

Although their experiment ended at 120 days, Manny and Kaylin continued to monitor the growth of abalone fed *heteroclada* up to 1 year. *H. asinina* fed solely *G. heteroclada* grew well, reaching a mean of 24 g and 46 mm during its first year of growth. This is considerably faster than the 43 mm reported for the same species in Thailand. "In other countries, there is no need to feed abalone with a variety of algae to meet the preferences and nutritional requirements of cultured abalone over extended periods," Manny explains. "*G. heteroclada* can promote good growth over extended periods."

Proximate composition of the abalone diets (in %)

	Artificial diet	Seaweed Gh	Seaweed Ka
Crude protein	32.40%	17.32	5.35
Crude fat	3.74	1.70	1.23
Crude fiber	5.04	4.79	4.38
Nitrogen-free extract	41.99	54.45	70.08
Ash	16.83	4.74	18.96
Moisture	7.50	3.54	9.10

Gh, *Gracilariopsis heteroclada;* Ka, *Kappaphycus alvarezii*

Average daily growth of the abalone *Haliotis asinina fed* three diets

Diet	Weight (mg per day)		Shell length (μm per day)	
	0-90 days	90-120 days	0-90 days	90-120 days
Artificial diet	73.8	66.0	191.8	81.7
G. heteroclada	67.1	112.7	192.9	133.3
K. alvarezii	9.2	9.3	59.4	52.7

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CAGE CULTURE TRIALS

Manny is testing the use of hanging net cages or barrels in the grow-out culture of donkey-ear abalone. He thinks this is the more viable alternative for tropical abalone considering the lengthy culture.

He determined the effect of different stocking densities on the growth rate, feed conversion, and survival of the donkey-ear abalone. Although the details will be published in a scientific journal, Manny shared his results.

"We have conducted three trials. The first two trials used 15-20 mm juveniles, the third used 35-40 mm juveniles," Manny explains. "We know that the hatchery reared donkey-ear abalone reach maturity at about 35 mm, hence, we assume that 16-20 mm abalone have not reached maturity yet." These abalone will use the energy from its food intake to somatic (or muscle) growth and not for reproductive growth. Although Manny expects that mature abalone has a lower growth rate following sexual maturity, he wants to know this for sure.

"We would like to determine the growth characteristics of the two size groups and to find out the growth of abalone following sexual maturity," Manny says.

The net cages used in the experiment measure 40 x 40 x 20 cm and are made of PVC pipes fitted together and covered with nets. Two pieces of PVC gutter were placed inside as shelters. The cages were suspended about 1.5 m below the sea surface (see photos).

Preliminary results clearly showed that growth rate of abalone decreased as stocking density increased. "The results conform with many others concerning the inverse relationship between growth and stocking density in shellfish culture," Manny explains. "The high density in the cage makes it difficult for abalone at the bottom of the stack to move and reach the food. This restricts the feeding rates so that food availability becomes a limiting factor even though enough food was given. " The results of the study

Growth rate, feed conversion, and survival rate of two size groups of abalone cultured for 150 days in cages (mean of three replicates).

Size group	Stocking density (no./cage)	Growth rate		Feed conversion rate	Survival rate (%)
		Weight: mg per day	Length: μm per day		
15-20 mm	45	140	160	14	96
	90	113	149	14	95
	135	91	134	16	93
	180	87	124		92
35-40 mm	17	280	132	18	98
	35	225	117	17	98
	52	196	108	21	94
	70	168	89	21	94

AQD is conducting studies on the cage culture of the abalone Haliotis asinina *at its Igang Marine Substation in Guimaras Island. Another study on the use of barrels for grow-out culture is on-going. Results are promising.*

also confirm that feeding rates are higher at lower stocking densities.

The rapidly growing small juveniles measuring 16-20 mm have feeding rates of 35-40% body weight. Feeding rate decreased to about 5-10% with bigger abalone weighing more than 50 mm. Growth and survival were higher at lower stocking densities (table on page 22).

"The increased tendency of abalone to stack at higher densities due to lack of primary surface restricts movement of animals in search of food," Manny notes. "We can conclude that food limitation is one of the main factors affecting growth at higher densities."

Another factor may be the rate of water flow. The abalone were fed weekly in the study, and the cages stocked at higher densities naturally receive more loads of feeds which in turn restricts water movement.

"Water movement stimulates feeding behavior and therefore growth of abalone," Manny explains. "The design of the grow-out system should provide good water flow to stimulate abalone to feed at a higher rate. I believe that the net cage is a good design because water can flow from any direction and has a high surface-to-volume ratio."

Abalone grown in cages can reach marketable size of about 50 g body weight or 60 mm shell-length in one year. This is a shorter period compared to temperate countries where abalone grows at a rate of 2-3 cm per year and reaches market size in 3-5 years.

"This study shows the potential of abalone farming in the Philippines," Manny concludes.

SOME OF THE ABALONE RESEARCH STUDIES HAVE BEEN PUBLISHED

EC Capinpin Jr and KG Corre. 1996. *Growth rate of the Philippine abalone,* Haliotis asinina *fed an artificial diet and macroalgae.* **Aquaculture** 144: 81-89.

EC Capinpin and M Hosoya. 1995. *Spawning and larval development of a tropical abalone* Haliotis asinina *(Linne).* **The Philippine Journal of Science** 124 (3): 215-232.