Fecundity and condition factor of abalone *Haliotis asinina* broodstock conditioned in banana leaf and "buho" slat substrates

Argie L. De Guzman and Lota A. Creencia*
College of Fisheries and Aquatic Sciences
Western Philippines University
*Corresponding author: lotacreencia@gmail.com
https://doi.org/10.69721/TPS.J.2014.6.1.01

ABSTRACT

This study was conducted to document the spawning behaviour and determine the fecundity and condition factor of the female abalone *Haliotis asinina* broodstock conditioned using two indigenous substrates, the banana (*Musa* spp.) leaf and "buho" (*Schizostachyum lumampao*) slat, for three months at Western Philippines University-Binduyan Marine Research Station (WPU-BMRS). Those abalone conditioned with no substrate served as control. Forty-five female broodstocks with 5-7cm shell length were used in the study. The spawning episode of the three treatments did not correlate with the lunar cycle. They spawned either later or earlier than the full moon and new moon. Spawning happened 3-5 nights in a row with an interval of 8-10 days. There was no significant difference ($P>0.05$) among the three treatments on the number of individuals that spawned within the four spawning episodes. Abalone conditioned in banana leaf substrate had an average fecundity of 259,353.21±39,307.63 eggs. However, there was no significant difference among treatments ($P>0.05$). The initial and final condition factors of each treatment were significantly different ($P<0.01$) but there were no significant differences on the condition factor among treatments. The indigenous substrates, banana leaf and buho slat are potential alternative substrates for broodstock conditioning. However, spawning performance and fecundity of abalone broodstock were not influenced by the substrates. Similar long-term studies are recommended.

Keywords: abalone, broodstock, condition factor, fecundity, *Haliotis asinina*, substrates

INTRODUCTION

Throughout many parts of Asia, abalones are highly prized seafood (Butterworth 2010). They have been collected more than 7,000 years ago along the Pacific coast for food and for the manufacture of shell implements and mother-of-pearl decorations (Olin 1994). Among the five species of tropical abalone in Philippine marine waters, *Haliotis asinina* is the biggest which is being cultured in the country (SEAFDEC 2000, 2008).

The abalone *H. asinina* achieves sexual maturity within 6-8 months of culture at shell size range of 35-40mm (SEAFDEC 2000). The smallest individual with a mature gonad from the wild is about 40mm shell length while hatchery reared abalone is about 35mm (Capinpin et al. 1998). Tank-reared broodstock can spawn year-round (SEAFDEC 2000). There is no

need for induction for *H. asinina* broodstock for they are able to spawn in the hatchery. Moreover, gonadal maturity and readiness to spawn can be assessed visually. Mature abalone which are ready to release gametes are generally creeping near the water surface. They are active but relaxed and their feet felt soft and flabby (Setyono 2006).

Various studies have used artificial substrate in rearing *H. asinina* such as plastic cages and polyvinyl chloride (PVC) pipes, both offshore and in indoor tanks (Capinpin et al. 1999, Setyono 2007, Minh et al. 2010). There are also unpublished studies that used indigenous materials as culture substrate such as bamboo (Villapa 2009), wood (Valdestamon 2009) and coconut stalk (Lota 2009). However, indigenous substrates have not been tried as conditioning substrate for abalone broodstock. In this study, two indigenous materials, banana leaf and "buho" slat were used to determine the spawning pattern, fecundity and condition factor of abalone broodstock.

MATERIALS AND METHODS

Experimental Area and Duration of Study

This study was conducted at the Western Philippines University – Binduyan Marine Research Station (WPU–BMRS) in Bgy. Binduyan, Puerto Princesa City from July to September 2012. The site is 79km away from the city proper of Puerto Princesa. Nine tank compartments were used in this study. Each compartment was placed with one conditioning basket (Figure 1). The abalone broodstocks were conditioned at 25-29^{o}C temperature and 30-37ppt salinity in wooden tanks. Four spawning episodes occured during the study period.

Figure 1. Photograph of the wooden tank compartments (left) and conditioning basket with *Gracilaria* sp. as abalone food (right).

Experimental Design

This study used indigenous materials as test substrates, the banana (*Musa* spp.) leaf and "buho" (*S. lumampao*) slats. Those with no substrates served as control. Each treatment had three replicates. The treatments and replicates were distributed using Completely Randomized Design (CRD). Test substrates were placed inside the conditioning baskets.

Test Abalone Management

The female abalone broodstocks were procured from Bgy. Tagburos, Puerto Princesa City. They were kept in a fiberglass tank filled with sea water and provided with aeration while in transit from Tagburos to Binduyan. At BMRS, the abalone broodstock were conditioned in concrete tank for two weeks before the experiment. Conditioning gave the abalone time to acclimate to a contained environment and to prepare its body for spawning. Forty-five individuals were used in this study with shell length ranging from 5-7cm. Each replicate had five individuals of female abalone broodstock. Each female abalone broodstock was tagged (e.g. 1A1, 1B1, 1C1…) using a dymo plastic tag attached with epoxy on abalone shell to monitor each individual's performance.

Tank and Substrate Preparation and Management

Nine wooden tank compartments (97cm × 80cm × 60cm) were used for all replicates. Each compartment was cleaned before filling it up with sea water. Nine conditioning baskets (60cm diameter × 55cm high) were used. They were made of cut PVC pipes as frame and plastic (Amazon®) net as enclosure which were tied with polyamide nylon.

Slats of "buho" were tied with polyamide nylon while banana leaves were sewed with monofilament nylon "kuralon". Fabricated substrates were placed in tanks and soaked in sea water for 10-15 days to condition the indigenous materials. Each substrate had a dimension of 40cm × 25cm. Five pieces were stacked together to form a holder which was placed inside the conditioning basket. Change of water was done every other day in the holding tanks.

Broodstock Management

Female abalone broodstocks were placed in wooden compartments after they had been conditioned in concrete tank. Before stocking, the shell length (mm) was measured using Vernier caliper and the total weight (g) by digital weighing scale. The broodstocks were fed *ad libitum* with seaweeds (*Gracilaria* spp.). Monitoring was done every morning and afternoon for water quality parameters such as temperature (thermometer), salinity

(refractometer) and checking of aeration. Siphoning of dirt was done every morning for the maintenance of good water quality. At least 60% of the water was replaced every other day.

Broodstock Selection and Spawning

Materials such as glass and plastic containers, beakers, plastic cups, pipettes, stirring rods, portable aerators and spatula were prepared before the time of spawning. Spawning was checked and monitored from 10:00pm to 3:00am. The abalone was separated when it was creeping toward the water surface and ready for spawning (Setyono 2006). Its gonad development was assessed before placing it in the glass container for spawning observation (Table 1). Upon inspection, the abalone with gonad stages 2 -3 was selected to be placed in the glass container.

Table 1. Stage of gonadal development of *H. asinina* (after Singhagraiwan and Doi 1992).

Stages	Criteria used for observation by the naked eye
0	No gonad development
1	Pre-matured gonad covering a little portion of hepatopancreas
2	Partially matured gonad covering about 25% of hepatopancreas
3	Fully matured gonad covering about 50% of hepatopancreas

The glass container that contained 5L of seawater had an aeration from a portable aerator. One glass container was used for each abalone. The individual female abalone that crept towards the top of the container and about to spawn was monitored closely to avoid spilling of eggs outside the container. Sometimes, the glass container was covered with basin to capture spawned eggs. After spawning was completed, each broodstock was lifted out of the container and put back in the conditioning basket. The water inside the spawning container was stirred gently to distribute the eggs well in the water column. A 250ml beaker or plastic cup was used to scoop egg samples, from which 1ml aliquot sample was extracted using a glass pipette. The aliquot was placed in the Sedgewick chamber and viewed under the microscope with high power objective (HPO) (40x) magnification.

The eggs present were counted and sampling was repeated for five times. Fecundity (F) was computed using the formula:

$$F = \frac{\text{total number of eggs counted}}{\text{volume of sample}} \times \text{total volume of container}$$

Spawning Performance

Each spawner was checked if it had fully spawned or partially spawned every spawning night. All female abalones with stage 3 gonads were spawners while some of those with stage 2 gonads were partial spawners. Handling from experimental basket to glass container did not interrupt the spawning. The abalones that spawned and did not spawn were recorded as well as their fecundity.

The spawning performance (SP) was computed following the formula:

$$SP = \frac{\text{number of individuals that spawned}}{\text{total number of individuals}} \times 100$$

Condition Factor

The condition factor of each individual was computed by using the formula:

Condition Factor or *K* = $(W/L^{2.99}) \times 5575$ (see Dlaza 2006).

where: W – body weight (g), L – shell length (mm)

Data Analyses

The data were encoded in the Microsoft Excel program and analysed using ANOVA or T-test and descriptive statistics. The computer program included a sort routine which automatically generated the stand tables and corresponding charts which were presented in means ± standard error.

RESULTS AND DISCUSSION

Spawning Performance

Spawning performance during each spawning episode refer to the number of individuals that spawned per treatment. All female abalones that spawned during the duration of the study have contributed to the overall spawning performance. Within four spawning episodes, the abalone conditioned in no substrate had 93.3% (14 of 15) spawning performance, in banana leaf substrates was 86.6 % (13 of 15) and in buho slat substrates was 73.3 % (11 of 15) (Table 2).

Table 2. Spawning performance of abalone in three treatments (T) during the first, second, third, fourth episodes and the overall spawning data.

T	First spawning		Second spawning		Third spawning		Fourth spawning		Overall	
	No.	%	No.	%	No.	%	No.	%	No.	%
1 (BLS)	7	46.7	9	60.0	10	66.7	9	60.0	13	86.6
2 (BS)	8	53.3	11	73.3	10	66.7	4	26.7	11	73.3
3 (NS)	12	80.0	11	73.3	12	80.0	3	20.0	14	93.3

Within the four spawning episodes, abalone conditioned in buho substrates and no substrate showed high performance on the 1st to the 3rd spawning episodes but low on the 4th spawning. On the other hand, abalone conditioned in banana leaf substrates showed a maintained performance within the four spawning episodes. There was no significant difference (P>0.05) among the three treatments on the number of individuals that spawned during the four spawning episodes. The first and second spawning episodes happened within 1-3 nights after the new and full moon, respectively. The third spawning episode was within the lunar cycle but the 4th episode was earlier than the lunar cycle compared to the three spawning episodes (Figure 2).

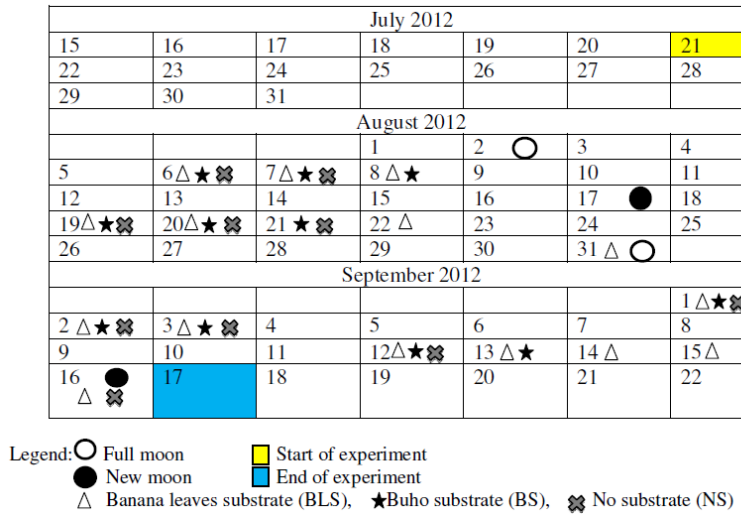

Figure 2. Spawning calendar of abalone broodstock during the four spawning episodes.

The spawning performance showed that abalone conditioned in each treatment did not always follow the lunar cycle, hence not predictable. However, Capinpin & Hosoya (1995) observed that spawning coincided with new and full moon for recently captured *H. asinina* held in tanks. This lunar

periodicity lasted for two months after which spawning continued every two weeks but no longer coinciding with the lunar cycle. In other words, spawning cycle is entrained with the new and full moon periods. In another study, the time interval between successive spawning of hatchery-reared abalone provided with optimal rearing conditions and adequate algal food was 13-15 days (Capinpin et al. 1998). Abalone spawn at night during 11:00 PM to 3:00 AM. Males usually spawn first then followed by the females. According to Capinpin (pers comm.), abalone kept too long before being used in the experiment may lose spawning synchronicity with the lunar cycle as cues and being associated with new and full moon can no longer be experienced by the abalone.

On the other hand, spawning of *H. asinina* in Heron Reef, Australia did not correlate precisely with the lunar cycle but much relate to the tidal cycle (Counihan et al. 2001). Abalone in this study shows an asynchrounous spawning pattern which is similar to the report of Capinpin et al. (1998). There are instances that an individual may spawn partially during the spawning episode but does not spawn its eggs in one release. In Heron Reef, Australia, *H. asinina* are considered as extremely synchronous spawners (Counihan et al. 2001).

In this study, spawning episode happened within 3 - 5 nights. This was longer than those in Heron Reef, Australia with spawning episode of 1 to 3 nights (Counihan et al. 2001). The spawning interval between two successful spawning episodes varied from 8 - 10 nights. This was however, shorter than in Heron Reef, Australia which was 12 to 15 days (Counihan et al. 2001) and hatchery-reared abalone which was 13 to 15 days (Capinpin et al. 1998). As observed during the study, abalones conditioned in banana leaf substrates have longer and earlier spawning episodes. The other treatments (buho & no substrate) have shorter spawning night and sometimes behind scheduled date (Figure 2). During spawning events, the abalone would creep near the water surface and move its muscular foot above the water level (Setyono 2006). On spawning nights at 10pm – 3am, the abalones were actively crawling and creeping up and down the water column. Some would even creep and show a cue of spawning even they did not possess mature gonad.

Fecundity

Abalone on banana leaf substrates had high average fecundity during the first spawning episode with 417,457.14±110,01554 eggs. Those in no substrate had high average fecundity of 664,666.7±197,333.3 eggs on the fourth spawning episode. Those in buho substrates had generally low average fecundity and the lowest at 74,000±28,011.9 eggs during the fourth spawning episode (Figure 3). However, there was no significant difference ($P > 0.05$) among the three treatments within the four spawning episodes.

Most abalone from different treatments spawned during the four spawning episodes (Figure 3). There were a few that did not spawn throughout the four spawning episodes. Individual abalone spawned differently during each spawning episode. Those that spawned partially had either decreased or increased its fecundity in the succeeding spawning event. Similarly, Counihan et al. (2001) reported that each individual abalone spawned within one or two nights, or not at all, and 78.1% spawned on at least one night during the spawning event.

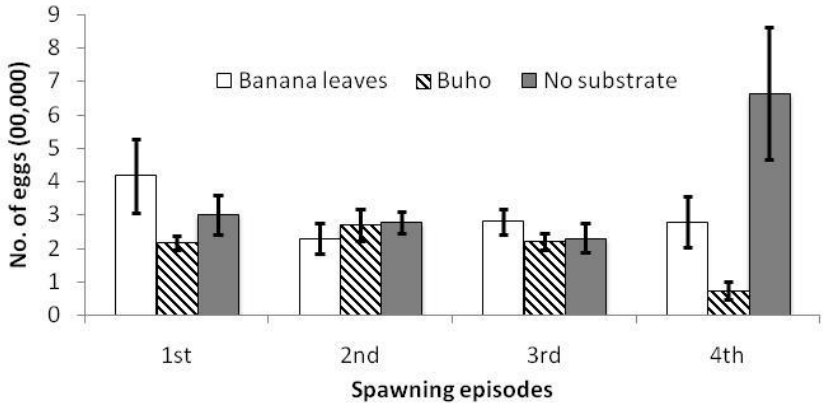

Figure 3. Average (±SE) fecundity of abalone broodstock conditioned in three treatments during the four spawning episodes.

Abalone conditioned in banana leaf substrates had an average fecundity of 259,353.21±39,307.63 eggs during the four spawning events. Those in no substrate had 237,265.5±32,161.21 eggs and those in buho substrates had 214,830.303±28,603.29 eggs (Figure 4). However, fecundities were not significantly different ($P>0.05$) among the three treatments.

The highest fecundity of abalone conditioned in banana leaf substrate with 2,431,000 eggs was obtained by an individual with a shell length of 73mm. Generally, abalone with shell length of 57-73mm had high fecundity (Figure 5).

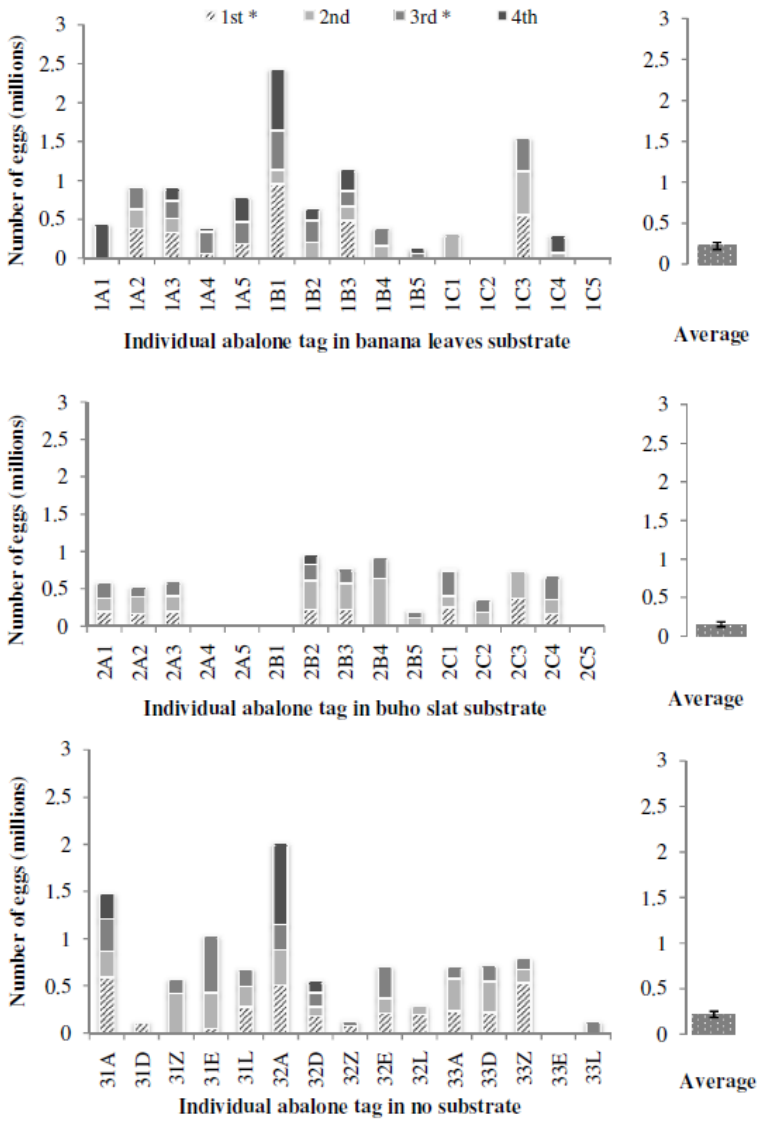

Figure 4. Average fecundity of individual abalone in three treatments during four spawning episodes.

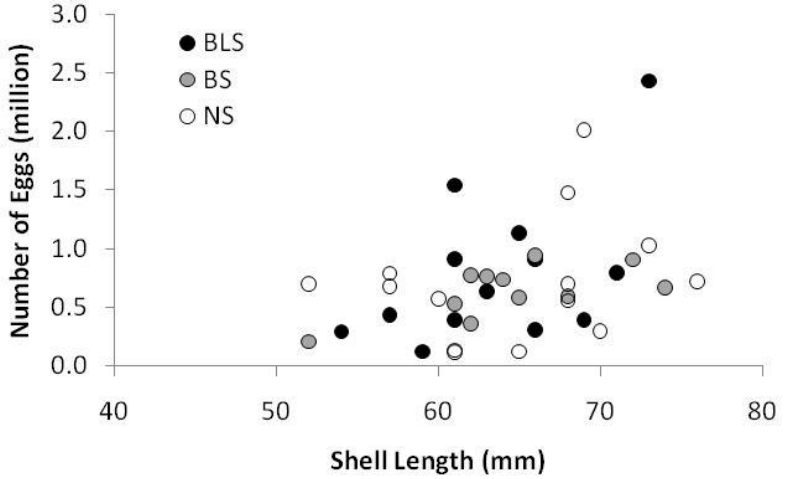

Figure 5. Distribution of abalone fecundity in relation to shell length during the four spawning episodes.

Condition Factor

After four spawning episodes, the abalone in all three treatments increased in condition factor (K). Those in no substrates had increased in *K* from 1.17±0.02 to 1.31±0.04g mm^{-1}, in banana leaf substrates from 1.12±0.03 to 1.24±0.04g mm^{-1} and in buho substrates from 1.14±0.03 to 1.19±0.09g mm^{-1} (Table 3). Moreover, the condition factors of the three treatments between initial and final measurements were significantly different ($P<0.01$). On the 4th spawning episode, mortality of abalone was observed in buho substrate where a part of the flesh was accidentally cut during the removal of broodstock in one spawning event.

Table 3. Average shell length, total weight, condition factor and number of individual survived during the four spawning episodes of abalone conditioned in banana leaves substrate, buho slat substrate and no substrate.

Treatment	Initial			Final			No. survived
	Shell length (mm)	Total weight (g)	Condition factor (K) (g mm^{-1})	Shell length (mm)	Weight (g)	Condition factor(K) (g mm^{-1})	
BLS	64.27±1.44	52.43±4.03	1.12±0.03	67.00±1.40*	64.92±4.36*	1.24±0.04*	15
BS	63.80±1.58	51.51±3.32	1.14±0.03	67.07±1.54*	67.46±4.79*	1.19±0.09*	14
NS	64.53±1.70	55.45±3.91	1.17±0.02	65.27±1.47	63.11±3.52*	1.31±0.04*	15

*-values are significantly different ($P<0.05$) from initial sampling.

Majority of the abalone conditioned in three treatments had increased their *K* within the conditioning period (Figure 6). Only a few individuals have a decreased K after four spawning episodes. One factor that could have affected their *K* is the absence of mature gonads which contributed to the weight of the abalone. Mature gonads are sometimes more than 75% of the entire digestive gland which becomes bulky and can often be seen without lifting the animal's body (Setyono 2004b). This condition was not observed after the fourth spawning episode which indicates that the gonads are spent and need to re-mature for the next spawning episode.

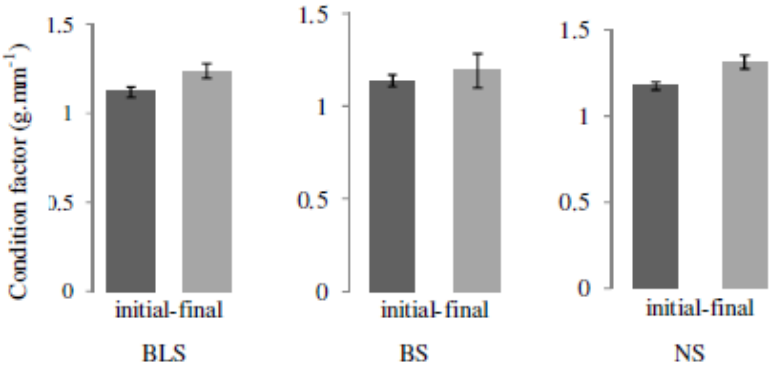

Figure 6. Mean (±SE) initial and final condition factor of broodstock abalone conditioned in banana leaves substrate (BLS), buho substrate (BS) and no substrtae (NS).

The increased *K* after four spawning episodes shows that conditioning is favorable to abalone broodstock. However, there was no significant difference (P>0.05) among the treatments on final *K*. This result is similar to the report of Setyono (2004a), where abalone conditioned within 14 weeks had a significantly different *K* between initial and final *K*. In addition, abalones conditioned in no substrate were observed to have grouped together in one part of the conditioning basket. Due to lack of substrate inside the baskets, the shell of other abalone served as their substrate for attachment. It is important to condition the abalone spawners to increase its condition factor.

CONCLUSION AND RECOMMENDATIONS

In abalone hatchery, broodstock conditioning is important to increase its condition factor. Spawning performance and fecundity of abalone broodstock are not influenced by the substrates. Both banana leaves and

buho slat substrates are potential alternative substrates for the conditioning of abalone broodstock.

Similar studies should be done for a longer period to know the advantages and disadvantages of conditioning with and without substrates.

REFERENCES

Butterworth A. 2010. Integrated Multi-Trophic Aquaculture Systems Incorporating Abalone and Seaweeds. Nuffield Australia Project No. 0914: 21, 23.

Capinpin EC Jr and M Hosoya. 1995. Spawning and larval development of a tropical abalone, *Haliotis asinina* Linné. Philippine Journal of Science, 124: 215-232.

Capinpin Jr EC, Encena II VC and NC Bayona. 1998. Studies on the reproductive biology of the Donkey's ear abalone *Haliotis asinina* Linne. Aquaculture, 166: 141-150.

Capinpin EC Jr, Toledo JD, Encena II VC and M Doi. 1999. Density dependent growth of the topical abalone *Haliotis asinina* in cage culture. Aquaculture, 171: 227-235.

Counihan RT, Mcnamara DC, Souter DC, Jebreen EJ, Preston NP, Johnson CR and BM Degnan. 2001. Pattern, synchrony and predictability of spawning of the tropical abalone *Haliotis asinina* from Heron Reef, Australia. Marine Ecology Progress Series, 213: 193-202.

Dlaza TS. 2006. Growth of juvenile abalone under aquaculture conditions. MSc Graduate Thesis. Department of Biodiversity and Conservation Biology, University of the Western Cape, South Africa.

Lota BH. 2009. Growth and yield of abalone (*Haliotis asinina*) fed with seaweeds and seagrasses using coconut stalk as substrate in a floating cage. BS Fisheries, Western Philippines University-Puerto Princesa Campus, Palawan, Philippines.

Minh ND, Petpiroon S, Jarayabhand P, Meksumpun S and S Tunkijjanukij. 2010. Growth and survival of abalone, *Haliotis asinina* Linnaeus 1758, reared in suspended plastic cages. National Science, 44: 621-630.

Olin P. 1994. Abalone Culture In Hawaii. University of Hawaii, Honolulu, HI 96822, Fact sheet no. 3.

SEAFDEC/AQD. 2000. Abalone Seed Production and Culture. SEAFDEC – Aquaculture Department, Iloilo, Philippines.

SEAFDEC/AQD. 2008. Abalone culture. A sustainable, environment-friendly opportunity for coastal communities. SEAFDEC – Aquaculture Deparment, Iloilo, Philippines, no.20.

Setyono DED. 2004a. Broodstock conditioning of the tropical abalone ***(****Haliotis asinina)* in the laboratory. Factsheet 36: 1-13.

Setyono DED. 2004b. Abalone (*Haliotis asinina* L.): 2. Factors affect gonad maturation. Oseana, 29(4): 9-15.

Setyono DED. 2006. Induction spawning for the tropical abalone (*Haliotis asinina*) in the laboratory. Indonesian Aquaculture Journal, 1(1): 20-23.

Setyono DED. 2007. Stocking density for juvenile tropical abalone, *Haliotis asinina* reared in structures suspended offshore. Research Centre for Oceanography - Indonesian Institute of Sciences, 33: 213–226.

Singhagraiwan T and M Doi. 1992. Spawning pattern and fecundity of the donkey's ear abalone, *Haliotis.asinina* Linne, observed in captivity, Thailand Marine Fisheries Bulletin, 3: 61-69.

Valdestamon CA. 2009. Growth and yield of abalone (*Haliotis asinina*) fed with seaweeds and seagrasses using wood as substrate in a floating cage. BS Fisheries, Western Philippines University-Puerto Princesa Campus, Palawan, Philippines.

Villapa AS. 2009. Growth and yield of abalone (*Haliotis asinina*) fed with seaweeds and seagrasses using bamboo as substrate in a floating cage. BS Fisheries, Western Philippines University-Puerto Princesa Campus, Palawan, Philippines.