Density effects on growth and survival rates of Donkey's Ear Abalone, *Haliotis asinina* (Mollusca, Gastropoda: Haliotidae) grown in submerged PVC pipes in Carot, Anda, Pangasinan

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ABSTRACT

This study was conducted to compare growth and survival rates of abalone (*Haliotis asinina*) at three stocking densities in PVC pipes on a seagrass bed in Carot, Anda, Pangasinan. PVC pipes cut into 0.75-m lengths were used to culture abalone fed with local seaweed *Hydropuntia edulis* for 120 days. Three densities were tested – 20, 40, and 80 abalone per pipe, corresponding to 53, 107, and 213 abalone/m^2, respectively. Abalone juveniles grew from 2.04 ± 0.38 cm to 3.93 ± 0.08 cm in length and from 2.02 ± 1.10 g to 13.63 ± 1.13 g in weight after 120 days in Treatment 1, the lowest density. One-way ANOVA showed no significant differences in growth and survival rates up to 90 days, but density-dependent effects on the growth and survival rates were observed on Day 105 onwards. Treatment 3 showed lowest survivorship at Day 120 (49.17 ± 13.13%), which was significantly different from Treatment 1 (95.00 ± 5.00%; p=0.019). The slow growth and low survival in Treatment 3 during the latter stages of culture were probably due to large amounts of seaweeds added weekly which hindered free flow of water. PVC pipes for rearing abalone have great potential but proper density should be followed (50-100/m^2) with thinning of stocks as they grow larger.

Keywords: Haliotis asinina; PVC pipes; seagrass bed, growth, survival rates

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INTRODUCTION

The Donkey's ear abalone, *Haliotis asinina*, is the largest tropical abalone species in the Indo-Pacific region and has the most potential for culture because of its larger size and body weight compared to other tropical species. It is also the fastest growing among the tropical abalone species (Capinpin et al. 1999; McNamara and Johnson 1995).

Different cage designs were evaluated for the culture of abalone in the nursery phase (11-15 mm size) and grow-out phase (25-30 mm) in hanging long lines or suspended from a floating raft (Encena et al. 2013). Lebata-Ramos et al. (2021) compared the growth and survival of *H. asinina* reared in different culture containers such as cages, trays, recycled oil containers, and tubes for nursery and grow-out culture on a reef flat in Agho Island, Concepcion, Iloilo. They concluded that the use of tubes or pipes which are stable, durable, and reusable in growing abalone on reef flats is the cheapest and the most convenient option for commercial production since cultured animals were readily accessible during low tide. In Anda, Pangasinan, the culture of abalone in hanging net cages was done in Carot, Anda and in Tondol, Anda, Pangasinan with promising results (Capinpin 2013; Capinpin et al. 2015). We hypothesized if the culture of abalone in submerged PVC pipes in the subtidal seagrass beds would yield comparable results in terms of growth and survival rates with those cultured earlier in hanging net cages in the same locality.

This study was conducted to compare growth and survival rates of abalone (*Haliotis asinina*) cultured at three stocking densities in submerged PVC pipes on a subtidal seagrass bed in Carot, Anda, Pangasinan.

MATERIALS AND METHODS

Study Area

The grow-out area (16°20'52.8" N, 119°59'38.9" E) was accessible to the cooperators and visible from their residences, allowing them to guard the site against poaching. The site is also a natural seagrass area (Figure 1). Barangay Carot shares a common border with Barangays Cabungan and Dolaoan.

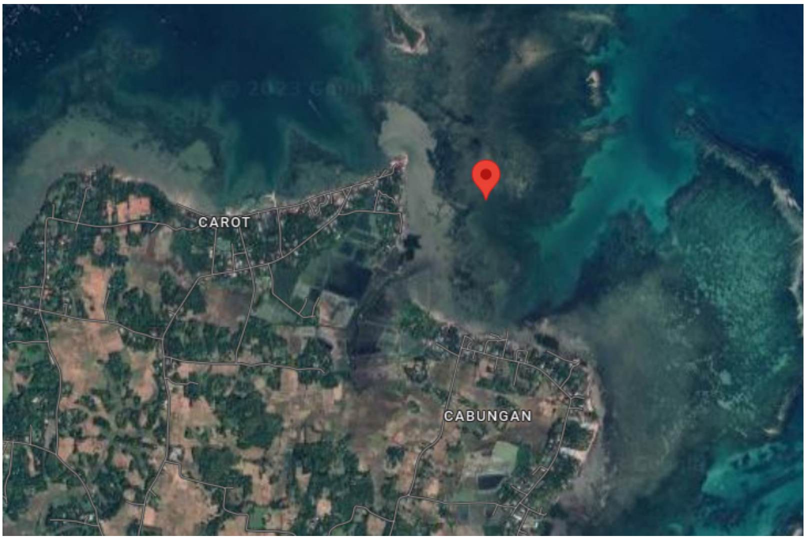

Figure 1. Location of the study site in Carot, Anda, Pangasinan.

Experimental Animals

The abalone juveniles used in this study was produced at the Bureau of Fisheries and Aquatic Resources - Regional Mariculture Technology Demonstration Center in Lucap, Alaminos, Pangasinan. A total of 320 hatchery-produced abalone juveniles were used in the study. Twenty five percent of juvenile abalone were initially sampled and measured 2.04 ± 0.38 cm in shell length and 2.02 ± 1.10 g in total weight. They were transported to the culture site in Carot, Anda, Pangasinan in oxygenated plastic bags within 1 hour of sampling and temperature-acclimated prior to distribution to their respective treatment containers.

Experimental Units

Black-colored PVC pipes with a 16-cm diameter and cut into 0.75-m lengths were used to culture abalone, which were fed with a local seaweed *Hydropuntia edulis* (S.G. Gmelin) Gurgel & Fredericq for 120 days. The pipes had 5-mm holes drilled at 5-cm intervals for water exchange. The inside surface area of the pipes was 0.375 m^2 and the ends were covered with nets to facilitate water flow in and out of the pipes. The pipes were secured using bamboo pegs and nylon ropes at depth of about 2.13-2.44 m in a subtidal seagrass bed (Figure 2).

Experimental Design

Three stocking densities were used as the experimental treatments: 20 abalone (Treatment 1), 40 abalone (Treatment 2), and 80 abalone (Treatment 3) per PVC pipe, which corresponds to 53, 107, and 213 abalone/m^2, respectively. A prior report of Capinpin et al. (1999) revealed density-dependent effects on growth rates with different stocking densities, 43, 88, 130, and 175 abalone/m^2, during grow-out of 3.5 cm abalone in cages. Hence, we tested 50, 100, and 200 abalone/m^2, which corresponds to 20, 40, and 80 abalone per pipe, or 53, 107, and 213 abalone/m^2 based on inside surface area of the pipes. Further, the present study was a straight run culture encompassing nursery phase (2 cm) to grow-out (>3 cm).

Three replicates were made for each experimental treatment. Hence, a total of 9 PVC pipes were used. The pipes were placed at the sea bottom amongst the seagrass bed in Carot, Anda, Pangasinan and laid out using a completely randomized design (Figure 2).

Figure 2. The PVC pipes placed at the bottom amongst the seagrass beds at 2.13-2.44 m deep.

Feeding Experiment

The grow-out trial was conducted from February 1 to June 2, 2023, for a total of 120 days. The experimental animals were fed *ad libitum* with a predetermined amount of red macroalga *Hydropuntia edulis* (S.G. Gmelin) Gurgel & Fredericq for 120 days. Each PVC pipe was checked weekly to replenish consumed feeds and to clean them from fouling organisms. The estimation of weekly feed was based on the feeding rates of abalone suggested in an earlier study (Capinpin et al. 1999).

Monitoring of growth parameters in terms of shell length, weight, and survival was conducted every 15 days, wherein 25% of the total stocks in each pipe were sampled to avoid stress. The samples consisted of mixed sizes randomly collected by hand. The shell length of each abalone was measured using an electronic caliper and the total weight using a digital balance. Survival was determined by deducting the number of collected empty shells from the total number of stocked abalone per pipe. Final survival was determined at Day 120 upon termination of the experiment by counting the number of live abalone harvested from each pipe.

Temperature and salinity were measured during sampling using a laboratory mercurial thermometer and a refractometer, respectively.

Growth Evaluation

Daily growth rates in terms of weight (DG_W) and shell length (DG_{SL}) were also calculated as follows (Capinpin 2013):

$$DG_W \text{ (g/day)} = G_W/n$$

$$DG_{SL} \text{ (mm/day)} = G_{SL}/n$$

where G_W is increase in weight (g) calculated as final weight minus initial weight, G_{SL} is increase in shell length (mm) calculated as final length minus initial length, and n is days of culture.

Statistical Analysis

Data on growth and survival were tested for homoscedasticity to satisfy parametric assumptions using Levene's test and analyzed using one-way analysis of variance using Statistica Version 8.0 (Statsoft, Inc. 2007). Tukey's was used as post-hoc test to compare differences between treatment means which significantly differ at $p<0.05$ level.

RESULTS AND DISCUSSION

Growth in Terms of Shell Length and Total Weight

Figures 3 and 4 show the growth curve of *H. asinina* cultured in PVC pipes and placed along the seagrass bed. Growth in terms of shell length and total weight was not significantly different in the different treatments up to Day 90 (p>0.05).

On Day 105, there was still no significant differences in shell lengths among the different treatments, however, in terms of body weight, one-way ANOVA showed significant differences among the different treatments (p<0.05). Treatment 3 (highest stocking density at 8.68 ± 0.87 g) was significantly different from Treatment 1 (lowest stocking density, 12.7 ± 0.80 g) (F=8.40, p=0.018, df=8).

On Day 120, there were observed significant differences in shell lengths and weights among the different treatments (p<0.05). In terms of shell length, Treatment 3 (highest density, 34.92 ± 0.66 mm) was significantly different from Treatment 1 (lowest density, 39.29 ± 0.78 mm) (F=10.30, p=0.011, df=8). The same trend was observed in terms of total weight, with abalone from Treatment 1 (13.63 ± 1.13 g) becoming significantly heavier than those in Treatment 3 (9.02 ± 0.56 g) after 120 days (F=9.69, p=0.013, df=8).

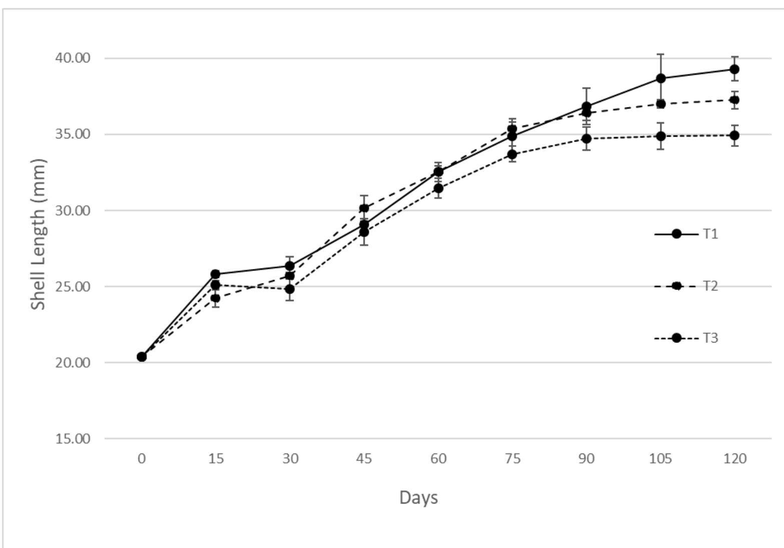

Figure 3. Growth curve of *H. asinina* cultured in PVC pipes in terms of shell length for 120 days. Error bars represent standard error of the means.

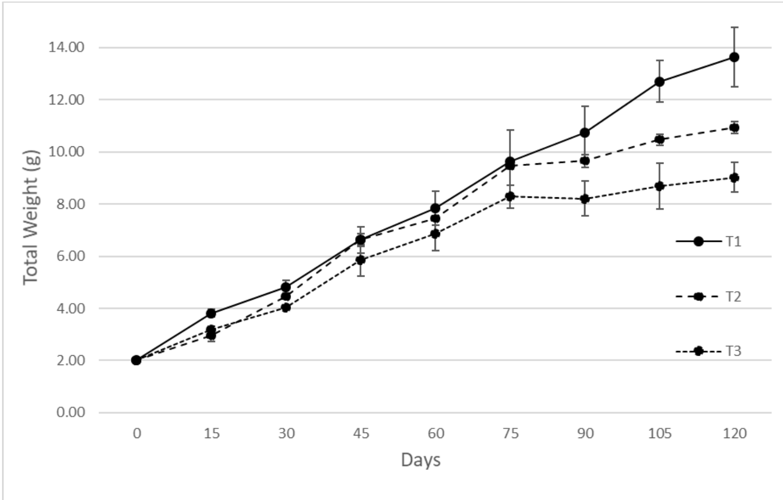

Figure 4. Growth curve of *H. asinina* cultured in PVC pipes in terms of total weight for 120 days. Error bars represent standard error of the means.

The computed growth rates in the present study were compared with results from other studies done either in the same culture area and using the same type of seaweed or same culture unit or type (Table 1).

Table 1. Growth rate of *H. asinina* cultured in PVC pipes as compared to similar studies.

Culture Unit	Culture Area	Seaweed	DG_{SL} (mm/d)	DG_{W} (g/d)	Reference
PVC pipes	Carot, Anda, Pangasinan (Seagrass bed)	*Hydropuntia edulis*	0.121-0.157	0.058-0.097	Present study
PVC cages	Carot, Anda, Pangasinan	*H. edulis*	0.076	0.09	Capinpin (2013)
PVC cages	Tondol, Anda, Pangasinan	*H. edulis*	0.032-0.052	0.037-0.046	Capinpin et al. (2015)
PVC cages	Guimaras, Iloilo	*Gracilariopsis heteroclada (=G. bailinae)*	0.124-0.167	0.087-0.140	Capinpin et al. (1999)
PVC pipes	Concepcion Iloilo (Reef flat)	*G. heteroclada*	0.217 (nursery phase) 0.121 (grow-out phase)	0.069 (nursery) 0.143 (grow-out)	Lebata-Ramos et al. (2021)

The resulting growth rates were faster in the present study (0.121-0.157 mm/day, computed range for T1-T3) as compared to an earlier study done in the same area (0.076 mm/day) using the same type of seaweed as feed (Capinpin 2013). This was due to the larger initial sizes of abalone used in the earlier study wherein they were already mature. Generally, smaller abalone grows at a faster rate than larger abalone. *H. asinina* matures at around 35 mm in shell length for both sexes (Capinpin et al. 1998). When *H. asinina* reaches this size, there is a reduction in growth rates of abalone due to the requirement for energy during gonad maturation. The slowing of growth rate following sexual maturity in abalone is well known and has been attributed to the channeling of energy into gonad development (Capinpin and Corre 1996, Mercer et al. 1993). The results of the present study also showed faster growth rates both in terms of shell length and weight to those cultured in a nearby culture site in Tondol, Anda, Pangasinan (Capinpin et al. 2015).

A study by Lebata-Ramos et al. (2021), which used pipes set in intertidal flats, reported comparable results in terms of shell length at 0.121 mm/day during the grow-out phase (i.e., >3 cm), but described slower rates during the nursery phase (0.217 mm/d, juveniles <3 cm). In terms of body weight however, the results were similar or within the growth rates reported either during the nursery or the grow-out phase. Smaller abalone grows in shell length faster than in larger abalone during the nursery phase, but during grow-out phase the growth rate in terms of weight is faster in larger abalone than smaller ones. This means that in the nursery phase abalone spend their energy in growing their shells, while in the grow-out phase their energy is spent more on developing their somatic and reproductive tissues in preparation for reproduction (Lebata-Ramos et al. 2021). Slowing of growth upon reaching sexual maturity is well-known phenomena in fish and shellfish. It is for this reason that triploidization is being used to enhance the productivity and yield of several fish species because of channeling the energy required from gonadal development to somatic growth (Park et al. 2016).

Slowing of growth can also be caused by density-dependent effects, as shown by an earlier study using net cages (Capinpin et al. 1999), due to competition for space or food. High density in the cage reduces the mobility of abalone at the bottom of the stack, thus affecting their feeding rates.

Growth rates of *H. asinina* may greatly vary depending on the culture condition but with appropriate culture container, proper density, ample seaweeds, and ideal culture environment, enhanced growth and maximum survival are attainable. Lebata-Ramos et al. (2021) showed that pipes or tubes produce fast growth rates and high survival rates compared to other structures like trays, recycled containers, and cages during the nursery and grow-out culture of abalone in a reef flat in Concepcion, Iloilo.

Survival Rates

The computed survival rates are shown in Figure 5. No significant differences were observed in survival rates in the different treatments which remained high up to 90 days of culture (p>0.05). Beginning on Day 105 however, there was an observed drop in survival rates in Treatment 3, the highest stocking density. Highly significant difference was observed between Treatment 3 (52.92 ± 8.43%) and the two other treatments (95 ± 2.89 in T1 and 95.83 ± 0.83% in T2) (F=22.57, p=0.0016, df=8). However, no significant difference was observed between Treatments 1 and 2 (p>0.05).

On Day 120, there was also an observed decrease in survival rates in Treatment 2. Tukey's test revealed significant difference between Treatment 3 (49.17 ± 7.58) and Treatment 1 (95 ± 2.89%, p<0.05), but no significant difference between Treatments 1 (95 ± 2.89%) and 2 (78.33 ± 11.58, p>0.05) as well as Treatments 2 and 3 (p>0.05). Survival rates in those cultured at the lowest density remained high all throughout until Day 120 (95 ± 2.89%).

The slow growth and low survival in Treatment 3 during the later stages of culture were probably due to the large amounts of seaweeds added at weekly intervals which hindered free flow of water within the PVC pipes, based on calculations using the earlier feeding guide (Capinpin et al. 1999), wherein the amount of seaweeds may be thrice as much in the highest density. Water flow is a significant factor affecting growth and survival rates. In an experiment, Wassnig et al. (2010) showed that increased oxygen availability with increasing flow is thought to have enhanced abalone performance. In that experiment, they tested whether the negative impact of high stocking density in shallow raceway tanks could be ameliorated by optimizing water flow. Indeed, they were able to increase density and resulting biomass gain by increasing flow.

Water parameters such as temperature ranged from 27-32°C and salinity from 34-35 ppt throughout the experimental period.

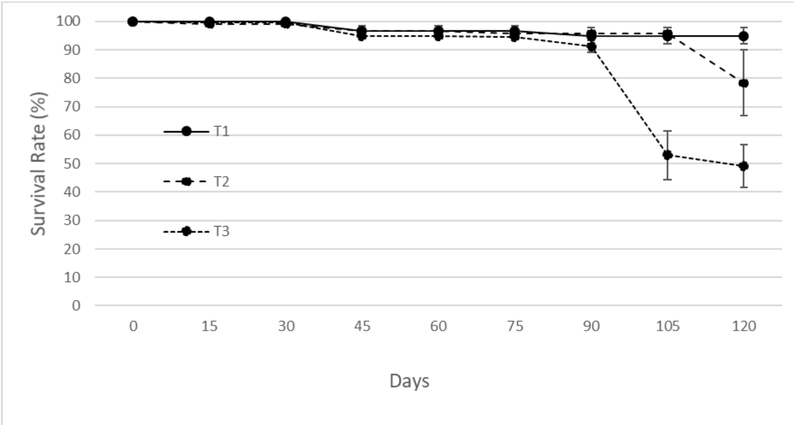

Figure 5. Survival rates of *H. asinina* cultured in PVC pipes for 120 days. Error bars represent standard error of the means.

The use of tubes, which are stable, durable, and reusable in growing abalone on reef flats is the cheapest and the most convenient option for commercial production since cultured animals are readily accessible during low tide in a reef flat (Lebeta-Ramos et al. 2021) and subtidal seagrass beds. The use of submerged PVC pipes as rearing units for abalone has great potential but proper stocking density should be followed (50-100/m^2) during later part of the growing period (i.e., 3 cm shell length until 5 cm harvest size) with thinning of stocks as they grow larger.

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REFERENCES

Capinpin EC Jr. 2013. Status of abalone fishery and experiential mariculture as a resource conservation strategy in Carot, Anda, Pangasinan. Sci Diliman. 25(1):51-70.

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

Capinpin EC Jr, Encena VC II, Bayona NC. 1998. Studies on the reproductive biology of the Donkey's ear abalone *Haliotis asinina* Linné. Aquaculture. 166(1-2):141-150. https://doi.org/10.1016/S0044-8486(98)00275-0.

Capinpin EC Jr, Toledo JD, Encena VC II, Doi M. 1999. Density dependent growth of the tropical abalone *Haliotis asinina* in cage culture. Aquaculture. 171(3-4):227–235. https://doi.org/10.1016/S0044-8486(98)00490-6.

Capinpin EC Jr, Parreno SC, Abalos RS. 2015. Growth of abalone *Haliotis asinina* fed with *Hydropuntia edulis*, singly or in combination with other red algae in sea cages in Tondol, Anda, Pangasinan, Northern Philippines. Philipp J Sci. 144:43–49.

Encena VC II, de la Pena M, Balinas VT. 2013. Evaluation of density and cage design for the nursery and grow-out of the tropical abalone *Haliotis asinina* Linne 1758. J Shellfish Res. 32(1):21–27. https://doi.org/10.2983/035.032.0105.

Lebata-Ramos MJHL, Mediavilla, JP, Solis, EFD, Sibonga RC, Alicante FPL, Dionela CS. 2021. Nursery and grow-out culture of the abalone *Haliotis asinina* on a reef flat: A comparison of growth and survival using different culture containers. Aquaculture. 541:736786. https://doi.org/10.1016/j.aquaculture.2021.736786.

McNamara DC, Johnson CR. 1995. Growth of the ass's ear abalone (*Haliotis asinina* Linne) on Heron Reef, tropical eastern Australia. Mar Freshw Res. 46(3):571–574. https://doi.org/10.1071/MF9950571.

Mercer JP, Mai KS, Donlon J. 1993. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* Linnaeus and *Haliotis discus hannai* Ino: I. Effects of algal diets on growth and biochemical composition. Invert Reprod Dev. 23:75–88. https://doi.org/10.1080/07924259.1993.9672298.

Park IS, Gil HW, Lee TH, Nam YK, Kim DS. 2016. Comparative study of growth and gonad maturation in diploid and triploid marine Medaka, *Oryzias dancena*. Dev Reprod. 20(4):305-314. https://doi.org/10.12717/DR.2016.20.4.305.

StatSoft, Inc. 2007. Statistica (Data analysis software system), version 8.0. StatSoft, Inc.

Wassnig M, Roberts RD, Krsinich A, Day RW. 2010. Effects of water flow rate on growth rate, mortality and biomass return of abalone in slab tanks. Aquac Res. 41(6):839-846. https://doi.org/10.1111/j.1365-2109.2009.02361.x.

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