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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

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An experiment was conducted to determine the effect of using only a single species diet, *Hydropuntia edulis* (= *Gracilaria edulis*) and a diet of mixed algae (*H. edulis, Eucheuma arnoldii*, and *Halymenia durvillaei*), which are all locally abundant in Tondol, Anda, Pangasinan on the growth of abalone *Haliotis asinina*. Small abalone (40.38±0.31 mm shell length and 14.69±0.39 g body weight for Trial 1 and 37.66±0.33 mm SL and 13.37±0.35 g BW for Trial 2) found in the area were collected by fishers and stocked in cages (50 cm X 50 cm X 20 cm). Two trials with two treatments were conducted with five replicates per treatment. Stocking density was 15 abalone per cage. Results of Trial 1 showed that those fed with a single species diet grew from 40.30±0.45 mm to 44.67±0.38 mm in shell length and 14.71±0.57 g to19.73±0.44 g in weight whereas those fed with mixed algal diet grew from 40.45±0.47 mm to 46.74±0.79 mm in length and 14.67±0.61 g to 23.20±1.08g in total weight after 135 d in culture. There was a significant difference in length and weight after 135 d using the t-test (P<0.05). Results for trial 2 were similar in that a significant difference was observed between the two treatments after 120 d using the t-test (P<0.05). In trial 2, abalone fed with a single-species diet grew from 37.48±0.56 mm to 43.72±0.35 mm in shell length and from 13.47±0.66 g to 19.00±0.59 g in weight whereas those fed with mixed-algae grew from 37.84±0.40 mm to 44.85±0.20 mm in shell length and from 13.27±0.32 g to 20.73±0.36 g in weight. In both cases, mixed-species of red algae produced better growth in shell length and weight. This may be because abalone in the wild rarely feed on single species and they frequently encounter a variety of drift algae. It may also be due to the fact that nutrients lacking in single-species diets may be provided and/or fortified using a variety of seaweeds, which are locally abundant. Survival rate was 100% in all cages.

Key Words: Abalone, Cage culture, *Eucheuma arnoldii*, Growth, *Haliotis asinina*, *Hydropuntia edulis, Halymenia durvillaei*,

INTRODUCTION

The abalone (*Haliotis asinina* Linné) fishery of Anda, Pangasinan, northern Philippines is artisanal or small-scale, typified by fishers gleaning or free-diving on shallow rocky areas. Due to unregulated harvesting, local abalone populations easily became overfished (Capinpin 2013). The high demand for the species led to a "boom-and-bust" fishery in the 1970s to the 1980s, according to earlier interviews among fishers in Carot, Anda, Pangasinan (Capinpin 2013).Their life history makes them especially vulnerable to overfishing, which poses a great challenge to fishery management (Capinpin 2012). In areas where abalone are found very few and widely apart, chances of successful fertilization becomes

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limited. Capinpin (2013) surveyed the abalone fishery and introduced the culture of abalone in sea cages in Carot, Anda, Pangasinan as a resource conservation strategy, and recommended to replicate the mariculture of abalone in cages in other areas to routinely create dense breeding populations which can help in enhancing the existing breeders and the periodic release of abalone in sanctuaries.

Meanwhile, the Philippine National Aquasilviculture Project (PNAP) was a government program through the Bureau of Fisheries and Aquatic Resources (BFAR) and the Commission on Higher Education (CHED) and was implemented in partnership with State Universities and Colleges (SUCs). One of its program components was the establishment of community-based multi-species hatchery per SUC which would serve as "lying-in" center for gravid high-value species to allow them to spawn and repopulate the surrounding area. For its part, the Pangasinan State University – Binmaley Campus research team focused on setting up community-based hatcheries for blue crab in Sual and abalone in Anda, Pangasinan.

The mariculture of abalone in Tondol, Anda, Pangasinan was explored to address both resource conservation and the provision of additional livelihood to fishers. As a resource enhancement activity, mariculture guarantees that the cultured organisms with economic value are allowed to grow to sexual maturity before they are harvested. Since mariculture makes possible the aggregation of individuals, the probability that fertilization would take place is increased. Hence, the Donkey's ear abalone is a good candidate to achieve the objectives of the PNAP project as (1) *H. asinina* attains sexual maturity at 35 mm shell length and spawns every 15 days coinciding with the lunar cycle (Capinpin & Hosoya 1995; Capinpin et al. 1998; Counihan et al. 2001) and (2) abalone is a high-value commodity and its culture can help augment the income of the coastal communities (Capinpin 2013). In this project, two trials were conducted using small abalone (40.38±0.31 mm shell length and 14.69±0.39 g total weight for Trial 1 and 37.66±0.33 mm SL and 13.37±0.35 g TW for Trial 2)fed with locally abundant seaweeds for 120-135 days. Growth and survival was evaluated using two feeding regimes – using a single-species diet (*Hydropuntia edulis* only) and a mixed diet (*H. edulis, Eucheuma arnoldii*, and *Halymenia durvillaei*).

MATERIALS AND METHODS

Study Site

The study was conducted in Barangay Tondol in Anda, Pangasinan in northern Philippines. *Haliotis asinina* and all the red seaweeds used in the study were found naturally in the study site.

Experimental Animals

Small abalone (40.38±0.31 mm shell length and 14.69±0.39 g body weight for Trial 1 and 37.66±0.33 mm SL and 13.37±0.35 g BW for Trial 2)found in the area were collected by fishers and stocked in polyvinyl chloride (PVC) cages covered with netting material. It was very difficult to collect large numbers of abalone smaller than this size due to their cryptic behavior and coloration. Moreover, at this size, some of the collected abalone are already sexually mature. *Haliotis asinina* attains sexual maturity at about 35 mm shell length (Capinpin et al. 1998). A total of 300 small abalone were used in two feeding trials.

Feeding Experiment

Twenty cages measuring 0.5 m x 0.5 m X 0.2 m similar to that used by Capinpin et al. (1999) were used in the feeding trials. Two pieces of halved PVC pipes were placed inside each cage as shelters. An experiment was conducted to determine the effect of growing abalone using only a single species diet, *Hydropuntia edulis* (= *Gracilaria edulis* (S.G. Gmelin) P.C. Silva) versus a mixed red algal diet (*H. edulis, Eucheuma arnoldii* Weber-van Bosse, and *Halymenia durvillaei* Bory de Saint-Vincent).The two feeding ration were given *ad libitum* in approximately equal amounts and were replaced weekly. The mixed diets were composed of equal parts of the three red algae. Cage culture protocols and feeding rates are available from Capinpin et al. (1999).

Five replicate cages were made per treatment and stocking density in each cage was 15 individuals. Diet treatments were randomly allocated to each cage and the cages were suspended in hanging long lines using a completely randomized design. The net cages were suspended about 1.5 m below the water surface. Abalone mariculture was conducted for 135 and 120 d for trials 1 and 2, respectively. Trial 1 commenced on 10 January 2013 whereas trial 2 began on 23 February 2013.

Abalone length and weight were measured every 15 d using a Vernier caliper and a kitchen weighing balance. Mean shell lengths were taken from all the stocked individuals to at least one decimal whereas mean weights were determined by taking the total biomass in each cage divided by the total number of animals. The abalone were blotted dry prior to weighing to decrease the variability in weights.

Daily growth rates in terms of weight (DG_W) and shell length (DG_{SL}) were calculated based on formulas used by Capinpin et al. (1999):

$$DG_W\ (mg.d^{-1}) = 1000G_W/n$$

$$DG_{SL}\ (\mu m.d^{-1}) = 1000G_{SL}/n$$

where G_W is increase in weight (g); G_{SL} is increase in shell length (mm); and *n* is days of rearing.

Data Analysis
Data were analyzed using independent t-test (SPSS version 15.0), provided that parametric assumptions were satisfied. Significance was set at 95% level (=0.05).

RESULTS AND DISCUSSION

Trial 1
Results of Trial 1 showed that those fed with a single species diet grew from 40.30±0.45 mm to 44.67±0.38 mm in shell length and 14.71±0.57 g to19.73±0.44 g in weight whereas those fed with mixed red algal diet grew from 40.45±0.47 mm to 46.74±0.79 mm in length and 14.67±0.61 g to 23.20±1.08g in total weight after 135 d in culture. In terms of shell length, a significant difference was observed between treatments after 105 d which persisted until the end of the trial at 135 d (Figure 1). On the other hand, in terms of weight, a significant difference was observed between treatments at day 135, the end of the feeding experiment (Figure 2). Tables 1 and 2 show significant differences in length and weight after 135 d using the t-test ($P<0.05$).

Trial 2
Results of trial 2 showed that those fed with single-species diet grew in length from 37.48±0.56 mm to 43.72±0.35 mm whereas those fed with a mixed diet grew from 37.84±0.40 mm to 44.85±0.20 mm (Figure 3). In terms of total weight, abalone fed the single-species diet grew from 13.47±0.66 g to 19.00±0.59 g whereas those fed with a mixed-algal species grew from 13.27±0.32 g to 20.73±0.36 g after 120 days (Figure 4). Significant differences in shell length for both treatments were observed beginning on day 105 up to day 120 ($P<0.05$) (Table 3) whereas significant differences in weights for both treatments was observed only at day 120 ($P<0.05$) (Table 4).

In both trials, temperature ranged from 26-32°C and salinity from 32-34 ppt throughout the experimental period.

Growth Rates
Table 5 shows the faster growth rates of abalone fed the mixed-algal species as compared to those fed with a single-species diet in this experiment for both trials 1 and 2.

The faster growth rates of abalone on mixed diets of fresh seaweeds were also observed by other authors (Naidoo et al. 2006; Stuart & Brown 1994; Yu et al. 2014). Day & Fleming (1992) and Stuart & Brown (1994) suggested that a variety of algae is better able to meet the preferences and nutritional requirements of cultured abalone over extended periods. Other authors also observed higher growth rates of abalone fed artificial diets supplemented with other algae than artificial diets alone (Dang et al. 2011) or faster growth rates on seaweeds supplemented with diatoms (Simental-Trinidad et al. 2004).Abalone in the wild rarely feed on single species and they frequently

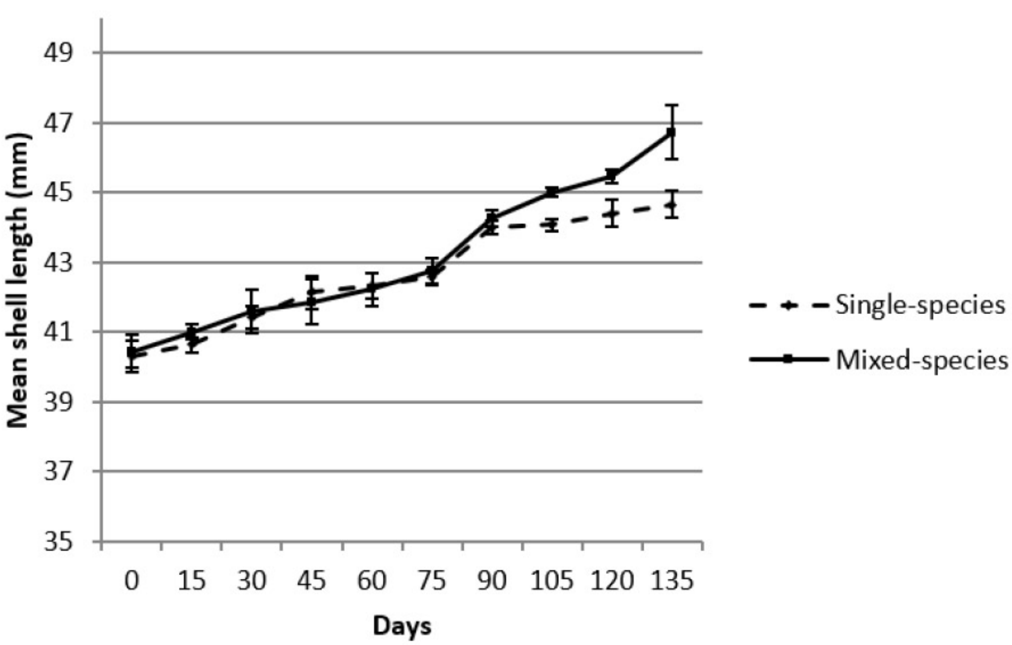

Figure 1. Mean shell length (mm) of abalone (*Haliotis asinina*) fed with single-species and mixed-species red algae in sea cages for 135 days in Tondol, Anda, Pangasinan (Trial 1).

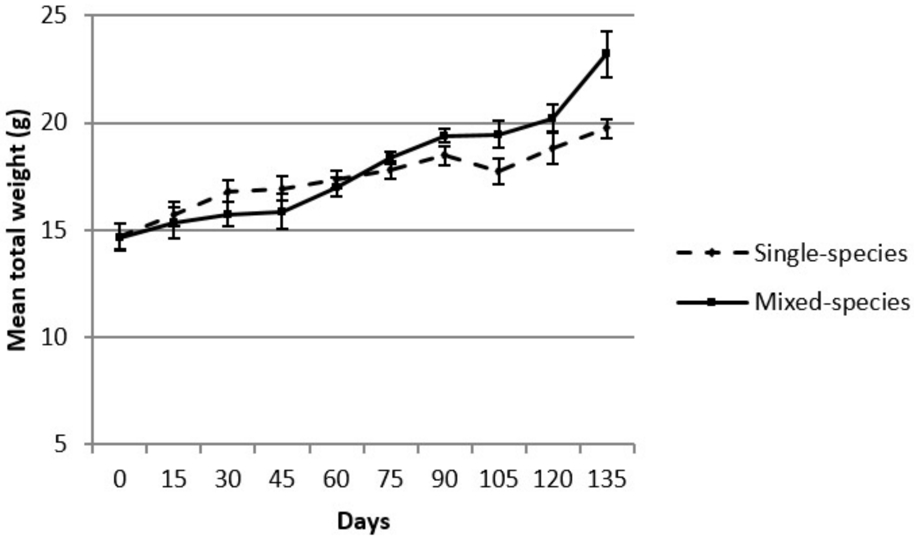

Figure 2. Mean total weight (g) of abalone (*Haliotis asinina*) fed with single-species and mixed-species red algae in sea cages for 135 days in Tondol, Anda, Pangasinan (Trial 1).

Table 1. Comparison of length t-test of abalone (*Haliotis asinina*) after 135 days in Trial 1 (5% level).

	N	Mean	SD	SEM	t	df	Sig (2-tailed)
Single-species diet	5	44.67	0.85	0.38	-2.354	8	0.046
Mixed-species diet	5	46.74	1.77	0.79	-2.354	5.752	

Table 2. Comparison of weight t-test of abalone (*Haliotis asinina*) after 135 days in Trial 1(5% level).

	N	Mean	SD	SEM	t	df	Sig (2-tailed)
Single-species diet	5	19.73	0.98	0.44	-2.974	8	0.018
Mixed-species diet	5	23.20	2.41	1.08	-2.974	5.301	

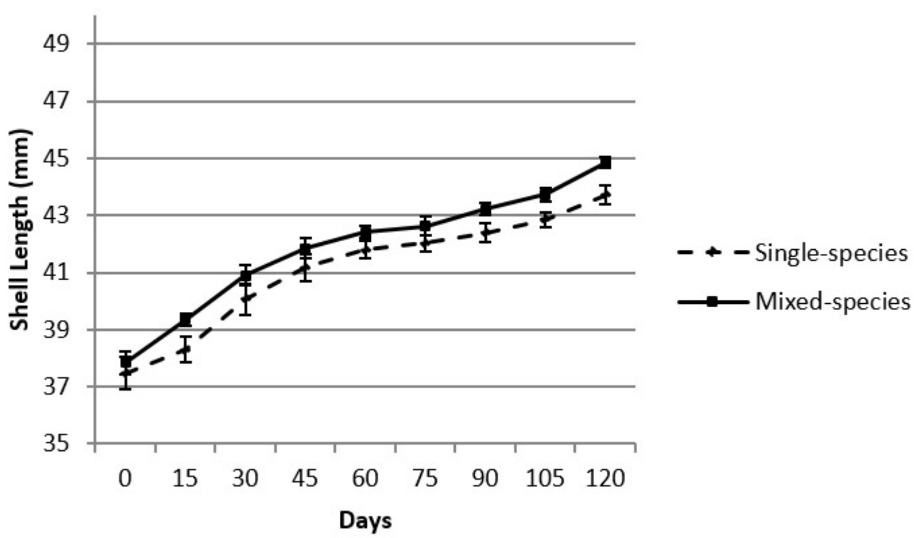

Figure 3. Mean shell length (mm) of abalone (*Haliotis asinina*) fed with single-species and mixed-species red algae in sea cages for 120 days in Tondol, Anda, Pangasinan (Trial 2).

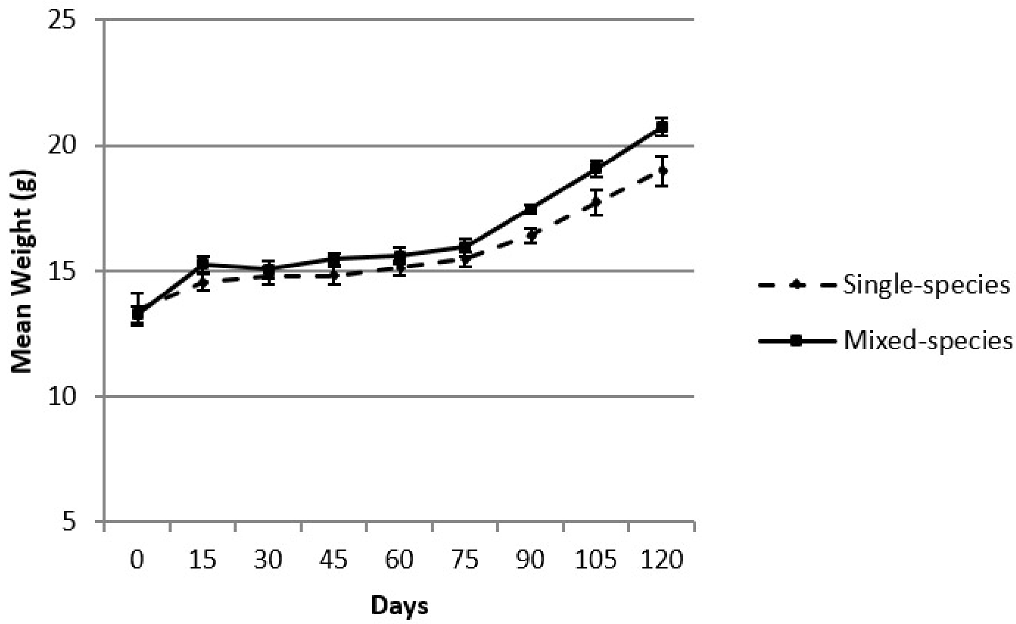

Figure 4. Mean total weight (g) of abalone (*Haliotis asinina*) fed with single-species and mixed-species red algae in sea cages for 120 days in Tondol, Anda, Pangasinan (Trial 2).

Table 3. Comparison of length t-test of abalone (*Haliotis asinina*) after 120 days in Trial 2 (5% level).

	N	Mean	SD	SEM	t	Df	Sig (2-tailed)
Single-species diet	5	43.72	0.78	0.35	-2.845	8	0.022
Mixed-species diet	5	44.85	0.44	0.20	-2.845	6.326	

Table 4. Comparison of weight t-test of abalone (*Haliotis asinina*) after 120 days in Trial 2 (5% level).

	N	**Mean**	**SD**	**SEM**	**t**	**df**	**Sig (2-tailed)**
Single-species diet	5	19.00	1.31	0.59	-2.520	8	0.036
Mixed-species diet	5	20.73	0.80	0.36	-2.520	6.597	

Table 5. Mean growth rates in length (DGSL) and weight (DGW) of abalone (*Haliotis asinina*) in Trials 1 and 2.

Trial 1/Replicate	Treatment 1 (Single-Species)		Treatment 2 (Mixed Species)	
	DG_{SL} (µm.d^{-1})	DG_W (mg.d^{-1})	DG_{SL} (µm.d^{-1})	DG_W (mg.d^{-1})
1	29.93	32.15	37.56	53.33
2	24.22	19.70	32.74	41.93
3	30.52	38.07	56.74	76.52
4	42.00	51.85	28.89	42.96
5	35.04	44.44	77.04	101.26
Mean	**32.34±2.96**	**37.24±5.48**	**46.59±8.99**	**63.20±11.37**
Trial 2/Replicate				
1	50.67	38.92	66.92	69.42
2	42.67	50.00	53.08	50.00
3	59.17	55.58	59.25	61.08
4	41.75	33.33	51.00	69.42
5	65.67	52.83	61.83	61.08
Mean	**51.99±4.65**	**46.13±4.27**	**58.42±2.90**	**62.20±3.57**

encounter a variety of drift algae. It may also be due to the fact that nutrients lacking in single-species diets may be provided and/or fortified using a variety of seaweeds. Survival rate was 100% in all cages.

The growth rates observed in the present study were lower as compared to earlier studies on *H. asinina* using *Gracilariopsis bailinae* (=*G. heteroclada*) as feed (Capinpin & Corre 1996; Capinpin et al. 1999). The low growth rates in this study may be due to the lower protein content of *Hydropuntia edulis (=Gracilaria edulis*) as compared to *G. bailinae*. No biochemical composition was done in this study but Capinpin and Corre (1996) and Capinpin et al. (1999) reported higher crude protein content of >15% for *G. bailinae* as compared to <10% for *G. edulis* (Boobathy et al. 2010; Jayasankar et al. 2005; Krishnapriya et al. 2014). Furthermore, the seaweed (*H. edulis*) used in the present study were shorter and tougher than that of *G. bailinae*. It is recommended to have the seaweeds used in this study analyzed for biochemical composition.

Sexual maturity for *H. asinina* is reached at a size of 35 mm for both male and female (Capinpin et al. 1998). 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 & Corre 1996; Mercer et al. 1993; Shepherd & Hearn 1983).

The present study confirms that abalone fed solely *Hydropuntia edulis (= Gracilaria edulis)* and other *Gracilaria* spp. can support the continuous growth of *H. asinina* over the long term (Capinpin & Corre 1996; Capinpin et al. 1999). However, it was also shown in the present study that supplementation of *H. edulis* with other red algae can promote better growth of abalone.Hence, it is better to supplement *Gracilaria* spp.with other red algae for better results. The present findings could also serve as baseline growth rates in this region for future commercial and/or restocking activities.

As mentioned earlier, sexual maturity for this species is reached at a size of 35 mm for both male and female and can spawn every 13-15 days interval (Capinpin et al. 1998). Thus, the cultured stocks had the potential to contribute in repopulating the surrounding areas as the mariculture cages serve as reproductive reserves. During sampling, we observed some abalone with mature gonads and at some other times, we also observed spent gonads, indicating that spawning had taken place.

Encena et al. (2013) recommended the use of multitier trays in commercial grow-out of abalone, but for community-based projects, a simple net cage design such as the one used in the present study, is sufficient because it is low-cost and provides potentially good water flow and high surface-to-volume ratio (Capinpin et al. 1999). Capinpin (2013) earlier presented a simple cost and return analysis for a similar cage culture experiment in Carot, Anda, Pangasinan. A small income was attained for the three-month culture period, which can be increased when done on a larger scale and by improving survival rates and searching for new market opportunities.

By growing abalone in sea cages, one can increase the size and weight of the abalone and thus the selling price and profitability of the subsistence fishermen. In addition, by growing the abalone out to the recommended 5 cm shell length, the proposed legal size limit in Anda, Pangasinan, it allowsthe captive abalone to spawn prior to harvest and supports the repopulation of surrounding areas (Capinpin 2012; Capinpin 2013).

CONCLUSION

Sea cage culture of abalone is an excellent tool for the conservation of abalone fishery and can promote a sustainable subsistence livelihood. In the culture of *H. asinina* in net cages, the use of a basal diet of locally available *Gracilaria* spp., supplemented with other available algae is preferable because it produces better growth rates than single species diets.

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