Growth and survival of the abalone Haliotis asinina fed seaweed Gracilariopsis heteroclada, formulated diet, and a combination of both

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Abstract

This paper compares the effect of feeding the abalone *Haliotis asinina* with seaweed (SW), formulated (FD), and mixed (SW + FD) diets. The feeding experiment, in six replicates, was conducted in a tank facility and lasted for ten months. At the end of the experiment, abalone fed mixed diets had significantly the highest mean shell length and body weight (45.90 ± 0.19 mm, 22.82 ± 0.31 g), followed by those fed seaweeds (43.47 ± 0.17 mm, 18.25 ± 0.26 g), and lastly, by those fed formulated diet (41.78 ± 0.18 mm, 15.76 ± 0.22 g). Mean growth rates were significantly higher when abalone were fed with mixed diets at 1.48 ± 0.03 mm and 1.69 ± 0.04 g mo^{-1}, respectively, than those fed seaweeds (1.26 ± 0.03 mm, 1.27 ± 0.06 g mo^{-1}) or formulated diet (1.06 ± 0.09 mm, 1.00 ± 0.11 g mo^{-1}). Survival was consistent one month from the start of culture until harvest—highest in abalone fed mixed diets (78.81 ± 2.48%), followed by those fed seaweeds (70.12 ± 4.07%), and lastly formulated diet (64.53 ± 4.61%). Moreover, feed conversion ratios (FCR) were significantly lower in abalone fed mixed diets (15.48 ± 0.69) and formulated diet (18.07 ± 3.50) compared with those fed seaweeds (41.31 ± 2.36). Pearson correlation analysis did not show any correlation between growth rates and the environmental variables tested, except for survival in all treatment diets, which showed significantly moderate positive correlations with temperature. Based on the results of this experiment, giving abalone a mix of formulated and natural diets is the best feeding regime. The importance of a balanced and more nutritional diet on the well-being of the abalone was observed in this study.

1. Introduction

The donkey's ear abalone, *Haliotis asinina*, is one of the most commercially-important gastropods in the Philippines. However, overharvesting and poor management of stocks, and the loss of habitat due to anthropogenic pressures have led to the dwindling of resources, which may not only be observed in the Philippines (Prieto-Carolino et al. 2018) but also worldwide (Cook 2019; FAO 2021). With a very promising potential for large-scale commercial production, the culture of this species should be encouraged to minimize the decline of natural stocks, which remain the major source of abalone fished for consumption and trade. However, the culture of *H. asinina* has been dependent on the red alga, *Gracilariopsis heteroclada*, considered the best-suited diet for abalone farming in the Philippines (Capinpin and Corre 1996). Dependence on *G. heteroclada* as the sole feed for the abalone is not sustainable since it is also being harvested or farmed for human consumption and agar production (Santander-Avanceña et al. 2015; Endoma et al. 2019). Despite the availability of technology to produce abalone in captivity, this dependence on natural food as *H. asinina* feed hindered the acceptance of culture in the aquaculture industry resulting in sporadic small-scale cultures in the country. For the country to go into large, commercial-scale culture, it has to start using formulated diets (Buss et al. 2015) that are more nutrition-balanced, economical, and convenient to use (Kirkendale et al. 2010; Choi et al. 2018).

For abalone culture to become sustainable, it is important to understand the feeding preferences of the species, before an acceptable diet or feeding regime can be developed and recommended to the industry. It is on this premise that the Aquaculture Department of the Southeast Asian Fisheries Development

Center (SEAFDEC/AQD) in Tigbauan, Iloilo, Philippines, has been testing different diets for *H. asinina* from formulated (Bautista-Teruel and Millamena 1999; Bautista-Teruel et al. 2003; Bautista-Teruel et al. 2011; Bautista-Teruel et al. 2016; Lebata-Ramos et al. 2021) to natural (Lebata-Ramos and Solis 2021) or both (Capinpin and Corre 1996; Bautista-Teruel et al. 2001) for more than two decades. Despite all these efforts, however, natural food remains the preferred diet because formulated diets are more expensive, less stable in the water, and are not readily eaten by the abalone. Considering the nature of the abalone, known to forage by actively grazing on attached benthic algae (Shepherd 1973), Lebata-Ramos et al. (2021) developed thin flaked diets, found to be more acceptable and palatable to the abalone compared with the usual noodle type formulated diet used for decades. Aside from being more palatable to the abalone, when the unrefined formula of the abalone grow-out culture feed of Bautista-Teruel et al. (2016) is used, this flaked diet is 80% cheaper, hence more cost-efficient (Lebata-Ramos et al. 2021).

Earlier, Lebata-Ramos et al. (2021) compared the growth and survival of *H. asinina* when fed noodle and flaked diets using the refined and unrefined formulations of Bautista-Teruel et al. (2016) in a three-month (nursery phase) feeding trial. This study compared in a ten-month (grow-out phase) feeding trial the use of a single diet of seaweeds (SW) or formulated diet (FD) and mixed seaweeds and formulated diet (SW + FD). The pre-existing diet formulation for abalone grow-out culture (Bautista-Teruel et al. 2016) prepared in the form of flakes (Lebata-Ramos et al. 2021) was used as the formulated diet and *G. heteroclada* as the seaweed diet. To check on what has been done on the use of mixed feeding regimes for the abalone, Google Scholar was searched using the keywords "*Haliotis asinina*" and "mixed diet" (Google 2022). This resulted in only 59 articles, mostly on the use of mixed species of algae and/or seaweeds as feed for *H. asinina* (Tahil and Juinio-Menez 1999; Angell et al. 2012; Capinpin et al. 2015) or on other species of *Haliotis* (Qi et al. 2010; Robertson-Andersson et al. 2011; Viera et al. 2011). None has been reported on the use of formulated flaked diet and seaweeds in a mixed feeding regime for *H. asinina* in grow-out culture. This study aimed to determine which feeding regime is the best for abalone in grow-out culture—seaweeds only, formulated diet only, or a mix of seaweeds and formulated diet.

2. Materials And Methods

2.1. Experimental Animals

Abalone *H. asinina* juveniles were obtained from the Abalone Hatchery of SEAFDEC/AQD and transferred to the tank facility where they were acclimated and the feeding experiment conducted. They were stocked in perforated PVC tube containers (1 m in length, 0.2 m dia) at the recommended stocking density of 140 ind tube^{-1} (Lebata-Ramos 2018). The tubes were then suspended inside concrete tanks provided with sand-filtered, flow-through seawater and aeration. Abalone were fed with alternate rations of seaweed *G. heteroclada* and formulated flaked diet daily at 1600 h, one month prior to the start of the experiment.

2.2. Experimental Diets

Formulations for unrefined diets developed earlier for abalone grow-out culture by Bautista-Teruel et al. (2016) and used in this experiment are shown in Table 1. This formulated diet satisfied the optimum protein and lipid requirements of the juvenile *H. asinina* (Bautista-Teruel and Millamena 1999; Bautista-Teruel et al. 2011; Bautista-Teruel et al. 2016). Following the prescribed standards in FDS (1994), the SEAFDEC/AQD Feed Mill Plant prepared the irregularly-shaped flaked diet, ranging 2–3 cm^2 and 310–315 µm thick (Lebata-Ramos et al. 2021) using the flaking machine. Diets were placed in sealed containers and kept inside the Feed Mill cold room, where feed ingredients and other diets are being stored. On the other hand, *G. heteroclada* was obtained from the regular seaweeds supplier of the Abalone Hatchery and kept live in concrete tanks provided with sand-filtered, flow-through seawater and aeration. Samples of both flaked diets and seaweeds were submitted to the Laboratory Facilities for Advanced Aquaculture Technology of SEAFDEC/AQD for proximate composition analysis and analyzed following the Official Methods of Analysis of AOAC International (Latimer 2016). Table 2 shows the results of the proximate composition analysis.

Table 1
Composition (dry weight basis) of formulated flaked diet, lifted from Bautista-Teruel et al. (2016).

Ingredients	**Volume (g/kg feed)**
Fish meal	150
Defatted soybean meal	200
Acetes	120
Wheat flour	200
Seaweed	210
Common ingredients*	120

*Vitamin mix (20), mineral mix (10), di-calcium phosphate (9.95), vitamin C (50), BHT (0.05), soybean oil (15), and fish oil (15). Vitamin and mineral mixes, commercial brand: retinol (1.2 MIU/kg), cholecalciferol (0.2 MIU/kg), α-tocopherol (0.02 MIU/kg), thiamin (8 g/kg), riboflavin (8 g/kg), pyridoxine (5 g/kg), cobalamin (0.002 g/kg), niacin (40 g/kg), calcium pantothenate (20 g/kg), biotin (0.04 g/kg), folic acid (1.8 g/kg), iron (40 g/kg), manganese (10 g/kg), zinc (40 g/kg), copper (4 g/kg), iodine (1.8 g/kg), cobalt (0.02 g/kg), selenium (0.2 g/kg).

Table 2
Proximate composition (%) of formulated flaked diet and seaweed *Gracilariopsis heteroclada* used as feeds in the experiment.

Proximate composition (%)	Feed	
	Formulated flaked diet	Gracilariopsis heteroclada
Crude protein	34.36	10.89
Crude fat	2.57	0.16
Crude fiber	3.31	4.56
NFE*	35.04	47.43
Crude ash	24.39	36.97
Estimated energy (kcal/kg)**	3007. 30	2347.22
*Nitrogen-free extract, calculated by difference		
**Based on 9 kcal/g fat, 4 kcal/g protein and carbohydrate as typical physiological fuel values (Brett and Groves 1979).		

2.3. Feeding Experiment

Acclimated abalone were all measured [shell length (SL): mean = 31.05 ± 0.08, range = 26.20–36.90 mm; body weight, (BW): mean = 5.85 ± 0.05, range = 2.80-10.65] and randomly stocked in 18 perforated PVC tube culture containers at 140 ind $tube^{-1}$. Six tubes, two from each treatment (SW = seaweeds only; FD = formulated diet only; SW + FD = mixed diets), were randomly distributed in each of the three concrete tanks provided with sand-filtered, flow-through seawater and aeration. Temperature ranged 26.00–33.00°C (means ± S.E. = 29.76 ± 0.09°C), salinity 29.00–34.00 ppt (means ± S.E. = 31.93 ± 0.06 ppt), and dissolved oxygen 3.60–6.60 mg L^{-1} (means ± S.E. = 5.26 ± 0.02 mg L^{-1}) (Fig. 1). Abalone were fed daily at 1600 h with the designated diets: *ad libitum* of the seaweed *G. heteroclada*, at 3% body weight (dry weight) of the formulated diet, and alternately every other day of seaweed and formulated diet for the SW, FD, and SW + FD treatments, respectively. Mean initial shell lengths and body weights of abalone for each treatment diet are shown in Table 3. Monitoring of growth parameters and survival was done every 30 days. All abalone were gently removed from each tube using a thin plastic spatula, counted, and 30 individuals were randomly picked and measured for shell length to the nearest tenth of a millimeter (mm SL) using a caliper and for body weight to the nearest tenth of a gram (g BW) using the Ohaus CS200 digital balance. After ten months, on 9 March 2022, the experiment was terminated. All remaining abalone were counted to determine final survival, and each was measured for SL and BW.

Table 3
Means ± S.E. of shell length (mm) and body weight (g) of abalone *Haliotis asinina* during the start (initial) and termination of the experiment (final); means ± S.E. of growth rates in terms of length and weight; means ± S.E. of final survival (%); and means ± S.E. of feed conversation ratios (FCR) from each treatment diet (SW = seaweed *Gracilariopsis heteroclada*; FD = formulated diet; SW + FD = mixed seaweed and formulated diet). Means with different superscripts are significantly different.

Treatments	**Shell length (mm)**			**Body weight (g)**			**Survival (%)**	**Feed Conversion Ratio (FCR)**
	Initial	**Final**	**Growth rate (mm mo^{-1})**	**Initial**	**Final**	**Growth rate (g mo^{-1})**		
Seaweed (SW)	30.95 ± 0.13	43.47 ± 0.17[b]	1.26 ± 0.03[b]	5.76 ± 0.09	18.25 ± 0.26[b]	1.27 ± 0.06[b]	70.12 ± 4.07[ab]	41.31 ± 2.36[a]
Formulated diet (FD)	31.28 ± 0.13	41.78 ± 0.18[c]	1.06 ± 0.09[b]	5.96 ± 0.08	15.76 ± 0.22[c]	1.00 ± 0.11[b]	64.53 ± 4.61[b]	18.07 ± 3.50[b]
SW + FD	30.92 ± 0.14	45.90 ± 0.19[a]	1.48 ± 0.03[a]	5.83 ± 0.09	22.82 ± 0.31[a]	1.69 ± 0.04[a]	78.81 ± 2.48[a]	15.48 ± 0.69[b]

2.4. Statistical analysis

Statistical analysis and graphs were done using the Minitab 17.0 software package (Minitab, State College, Pennsylvania, U.S.A., http://www.minitab.com). Growth parameters and FCR from different treatment diets (SW = seaweeds only; FD = formulated diet only; SW + FD = mixed diets) were tested for normality using the Anderson-Darling Test and compared using the one-way analysis of variance (ANOVA). Tukey's Test was used to compare the differences between diets where growth parameters and FCR significantly differed at $p < 0.05$ statistical significance. Survival data were arcsine-transformed before proceeding with the same analyses used for the growth parameters. Monthly growth rates and survival were correlated with mean monthly readings of temperature, salinity, and D.O. using Pearson correlation at $p < 0.05$ statistical significance.

Monthly growth rates, both for length (GR_L) and weight (GR_W), were obtained using the following equations:

$$GR_L = \frac{\left(L_f - L_i\right)}{\left(T_f - T_i\right)} \times 30$$

1

$$GR_W = \frac{\left(W_f - W_i\right)}{\left(T_f - T_i\right)} \times 30$$

2

where, GR = growth rate

L = length (mm)

W = weight (g)

T = sampling date

f = present sampling

i = preceding sampling

30 = constant, days in a month

Survival (%) and feed conversion ratio (FCR) were obtained using equations 3 and 4, respectively:

$$Survival(\%) = \frac{N_f}{N_i} \times 100$$

3

where, N_f = number of abalone in each container during monthly samplings and harvest

N_i = number of abalone initially stocked per container (N = 140)

$$FCR = \frac{F_t}{W_t}$$

4

where, F_t = total feed given per container (g)

W_t = total weight gained per container, $W_f - W_i$

W_f = total final weight of abalone per container (g)

W_i = total initial weight of abalone per container (g)

3. Results

Significant differences in mean shell length and body weight of abalone between treatment diets were first observed on the second and the first month of culture (ANOVA: $p < 0.001$, $F = 31.25$, $df = 2$; $p < 0.013$, $F = 4.34$, $df = 2$), respectively. Abalone fed with mixed diets were consistently the largest (ANOVA: $p < 0.001$, $F = 130.31$, $df = 2$) and the heaviest (ANOVA: $p < 0.001$, $F = 173.39$, $df = 2$) one month from the start of culture until harvest. From the first to the third month of culture, abalone fed formulated diet were the second largest and heaviest to those fed mixed diets, but they were outgrown on the fourth month by those fed seaweeds, and this trend remained until harvest on the tenth month (Fig. 2).

Mean growth rates of abalone, both in terms of length and weight, were significantly highest when fed with mixed diets at 1.48 ± 0.03 mm mo^{-1} and 1.69 ± 0.04 g mo^{-1}, respectively, while these did not significantly differ between those fed seaweeds and formulated diet (ANOVA: $p = 0.001$, $F = 13.08$, $df = 2$; $p < 0.001$, $F = 21.95$, $df = 2$; Table 3). The highest growth rates, both for length (3.04–5.12 mm mo^{-1}) and weight (2.90–5.41 g mo^{-1}), were observed on the fourth month, which marked the start of a significantly distinct trend in growth, with those abalone fed mixed diets exhibiting the highest shell length and body weight.

Survival was consistently highest in abalone fed mixed diets, followed by those fed seaweeds, and lastly by formulated diet, one month from the start of culture until harvest. However, significant differences in survival were only observed in the last four months of culture (Fig. 3). During harvest, abalone fed mixed diets had significantly higher survival (78.81 ± 2.48%) than those fed formulated diet (64.53 ± 4.61%), but both did not significantly differ from those fed seaweeds only (70.12 ± 4.07%) (ANOVA: $p = 0.045$, $F = 3.85$, $df = 2$; Table 3).

Feed conversion ratios (FCR) were significantly lower in abalone fed mixed diets (range: SW = 9.97–13.09, FD = 3.46–4.48; SW + FD mean = 15.48 ± 0.69) and formulated diet (range: 9.24–31.45; mean = 18.07 ± 3.50) compared with those fed seaweeds (range: 32.49–48.88; mean = 41.31 ± 2.36) (ANOVA: $p < 0.001$, $F = 38.23$, $df = 2$; Table 3).

Moreover, abalone fed formulated diet developed a distinct turquoise shell color, while those fed seaweeds and mixed diets retained their natural color. The striations on the foot muscle of the abalone fed with seaweeds and mixed diets were brownish, grayish-green on those fed formulated diet (Fig. 4).

Pearson correlation analysis did not show any correlation between growth rates and the environmental variables tested. However, survival of abalone fed seaweed, formulated, and mixed diets showed a significantly moderate positive correlation with temperature (Pearson correlation: $p < 0.05$, $r = 0.70$; $p < 0.05$, $r = 0.62$; $p < 0.05$, $r = 0.68$, respectively).

4. Discussion

In this ten-month grow-out culture trial, a mixed diet proved to be better than a single diet, whether using natural or formulated diets. Abalone were significantly larger and heavier when fed mixed diets, as observed from the early months of culture until harvest. This effect, when feeding abalone with mixed diets, had already been reported in other species of *Haliotis*. *Haliotis fulgens*, grown under commercial culture conditions for 329 days, had the highest growth rate in terms of length and weight when fed with mixed diets, then with seaweeds, and lastly, formulated diet (Durazo-Beltrán et al. 2003). In a study by Naidoo et al. (2006), *Haliotis midae* fed with mixed diets also showed the best growth. In a feeding experiment comparing the performance of two natural diets versus a formulated feed on weaning red abalone *Haliotis rufescens*, the highest growth was obtained with *Porphyra columbina* (3.3 mm $month^{-1}$), followed by the mixed diet of *P. columbina* and formulated diet, then *Macrocystis pyrifera*, and lastly, the formulated diet (Hernández et al. 2009). Consistent with the result of the present study, the findings of these different feeding experiments on different species of *Haliotis* seemed to promote the use of mixed diets but not the sole use of formulated diet. According to Mercer et al. (1993), generally, high balanced levels of protein (> 15%), lipid (3–5%), and carbohydrate (20–30%) are essential for the optimal growth performance of abalone. Feeding abalone with seaweeds or formulated diet only may not be enough to meet their nutritional requirements for optimal growth, but a mix of these diets could, hence better growth may be attained using both diets. And even with the use of all-natural diets, mixing them improved the growth rate of *H. asinina* (Capinpin et al. 2015), *Haliotis diversicolor* (Wang et al. 2010), and *H. midae* (Robertson-Andersson et al. 2011) compared with feeding them monospecific diet.

Survival was consistently highest one month from the start of culture until harvest in abalone fed mixed diet, followed by those fed seaweeds, and lastly, by formulated diet. These results concur with the findings of Durazo-Beltrán et al. (2003), where *H. fulgens* showed a significantly highest survival of 53.7% after 329 days of culture when fed mixed seaweeds and formulated diets. However, contradictory to the result of the present study, they observed higher survival in abalone fed with formulated diet than those fed seaweeds. In a study on *H. rufescens* that also lasted for ten months, survival was significantly lowest in abalone fed artificial feed using a feeder (94.2%), almost similar in other treatment diets; highest in those fed with mixed *M. pyrifera* and artificial feed (99.1%) and mixed *Gracilaria chilensis* and artificial feed (98.8%) (Venegas et al. 2016). In another feeding trial for *H. rufescens* that lasted for 162 days, survival was lowest in those fed formulated diet only (78.3%), followed by those fed macroalgae (86.6%), and highest in a combination of these two (90%) (Kemp et al. 2015). The effect of mixed diets on better survival observed in *H. asinina* in the present study corroborates the results of other studies on other species of *Haliotis*.

Parallel to the result of the present study, previous studies have shown that abalone fed natural food or seaweeds had higher FCR than those fed formulated diet. In a 90-day feeding trial for *H. asinina*, those fed seaweeds had an FCR of 6.98, while only 1.5–2.3 for those fed formulated diets (Bautista-Teruel and Millamena 1999). In a ten-month feeding trial in tanks on *H. rufescens*, FCR was highest with the mixed natural food of *M. pyrifera* (16.0) and *G. chilensis* (12.7) diet, whereas lowest using artificial feed with (1.4) and without a feeder (1.6) (Venegas et al. 2016). Moreover, extended culture periods also increase FCR, especially when using natural food as feed. Capinpin et al. (1999), when using *Gracilariopsis* sp., reported an FCR of 12.58–15.54 in *H. asinina* cultured for 150 days, while 19.63–22.57 at 180 days. In a 90–180 day culture of the same species, using the same feed, FCR ranged 7.56–22.57, with higher FCR during longer culture duration (Encena et al. 2013). Surprisingly, however, in just 90 days of feeding *H. asinina* with seaweeds, Fermin and Buen (2000) obtained a very high FCR of 31–40. FCR could greatly vary, especially when natural food is used. However, the supplementation of natural foods can help improve the FCR. A 62-day feeding experiment on *H. asinina* revealed that *Gracilaria* sp. supplemented with probiotics (*Bacillus amyloliquefaciens* MA228, *Enterobacter ludwigii* MA208, and *Pediococcus acidilactici* MA160) resulted in significantly lower FCR compared with the unsupplemented seaweed feed (Amin et al. 2020). Abalone, as natural grazers, feed on a mixed species of algae and other plants growing in their natural environment. Given a monospecific diet, their feed intake may vary depending on the culture conditions or the sufficiency of nutrients in the diet needed to promote growth or support reproduction.

Abalone fed formulated diet developed a distinct turquoise shell color, while those fed seaweeds and mixed diets retained their natural color. The light blue-green shell color in *H. asinina* fed formulated diet had been reported earlier by Capinpin and Corre (1996) and Bautista-Teruel and Millamena (1999). Feeding abalone with formulated diet to change the shell color was eventually adopted as a tagging mechanism for sea ranching and stock enhancement (Gallardo et al. 2003; Okuzawa et al. 2008; Lebata-Ramos et al. 2013; Salayo et al. 2020). However, not all formulated diets will change the color of the abalone shell. In Lebata-Ramos et al. (2021), only the unrefined diet formulation of Bautista-Teruel et al. (2016) resulted in a turquoise abalone shell, while the refined formulation, which has *Spirulina* spp. and sodium alginate, did not cause any distinct changes on the natural shell color of the abalone. Aside from the presence of some feed ingredients, a certain amount or a daily ration of such may be required for the shell color to change. In the present experiment, using mixed diets of formulated feed and natural food, given at alternating rations every other day, maintained the natural shell color of *H. asinina*.

Temperature is among the most potent abiotic factors influencing marine mollusks (Kong et al. 2017). In the present study, survival of abalone, regardless of treatment diets, showed a significantly-moderate positive correlation with temperature. Although an increasing temperature can be detrimental to any organism, the water where *H. asinina* were cultured and showed a positive correlation with their survival had a narrow temperature range of 26.00–33.00°C. Even temperate species, like the *Haliotis diversicolor supertexta*, have the ability to tolerate temperatures as high as 36.8°C when maintained at 30°C or as low as 3.5°C when maintained at 20°C (Chen and Chen 1999). Hence, for the tropical species *H. asinina*, temperatures at low to mid 30s°C may be optimum for survival. Also, the lower temperatures during the culture period were recorded during days of heavy rains, which had caused the water to become silted and resulted in more abalone deaths.

5. Conclusion

Abalone fed mixed diets were significantly the largest and heaviest than those fed seaweeds, then formulated flaked diet. In terms of mean growth rates, both in length and weight, theirs were also significantly higher compared with those fed solely with either seaweeds or formulated diet; those of the latter two did not differ significantly. Aside from better growth, survival was also significantly highest in abalone fed mixed diets, while FCR was the lowest; significantly lower than those fed seaweeds, but did not significantly differ from those fed formulated diet only. The findings of this study confirm the importance of a balanced and more nutritional diet on the general well-being of the abalone.

While most commercially-important seafood species in the country are being produced in captivity in aquaculture facilities, despite the established hatchery technology for the abalone, they remain being sourced from the wild. Aquaculture may greatly help boost abalone production and minimize dependence on the dwindling natural stocks. Although *H. asinina* has a strong potential for large-scale culture, dependence on the seaweeds as feed has put it on the sideline of the aquaculture industry. These promising results on the use of mixed diets in the grow-out phase (this study) and solely of formulated diet in the nursery phase (Lebata-Ramos et al. 2021) may encourage stakeholders to venture into the large-scale commercial culture of abalone. Moreover, this new development will help minimize, if not completely eliminate, the dependence of the abalone industry on *G. heteroclada* as feed, which by itself is also a commercially important aquaculture species in the Philippines.

Declarations

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Availability of data and material - The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability - Not applicable

Authors' contributions - All authors contributed to the conception and design of the study. Material preparation was performed by Joseph B. Biñas and Ellen Flor D. Solis. Data collection and analysis were done by Ma. Junemie Hazel L. Lebata-Ramos and Ellen Flor D. Solis. The first draft of the manuscript was written by Ma. Junemie Hazel L. Lebata-Ramos and all authors have commented on the previous versions of the manuscript. All authors have read and approved the final manuscript.

References

1. Amin M, Bolch CJS, Adams MB, Burke CM (2020) Growth enhancement of tropical abalone, *Haliotis asinina* L, through probiotic supplementation. Aquac Int 28:463–475. https://doi.org/10.1007/s10499-019-00473-4
2. Angell AR, Pirozzi I, de Nys R, Paul NA (2012) Feeding preferences and the nutritional value of tropical algae for the abalone *Haliotis asinina*. PLOS One 7:e38857. https://doi.org/10.1371/journal.pone.0038857
3. Bautista-Teruel MN, Fermin AC, Koshio SS (2003) Diet development and evaluation for juvenile abalone, *Haliotis asinina*: animal and plant protein sources. Aquaculture 219:645–653. https://doi.org/10.1016/S0044-8486(02)00410-6
4. Bautista-Teruel MN, Koshio SS, Ishikawa M (2011) Diet development and evaluation for juvenile abalone, *Haliotis asinina* Linne: Lipid and essential fatty acid levels. Aquaculture 312:172–179. https://doi.org/10.1016/j.aquaculture.2011.01.004
5. Bautista-Teruel MN, Maquirang JRH, dela Peña MR, Balinas VT (2016) Test of refined formulated feed for the grow-out culture of tropical abalone *Haliotis asinina* (Linnaeus 1758) in concrete land-based tanks. J Shellfish Res 35:633–639.
6. Bautista-Teruel MN, Millamena OM (1999) Diet development and evaluation for juvenile abalone, *Haliotis asinina*: protein/energy levels. Aquaculture 178:117–126. https://doi.org/10.1016/S0044-8486(99)00121-0
7. Bautista-Teruel MN, Millamena OM, Fermin AC (2001) Reproductive performance of hatchery-bred donkey's ear abalone, *Haliotis asinina*, Linne, fed natural and artificial diets. Aquac Res 32:249–254. https://doi.org/10.1046/j.1355-557x. 2001.00022.x
8. Brett JR, Groves TDD (1979) Physiological energetics. In: Hoar WS, Randall DJ, Brett JR (eds) Fish Physiology: Bioenergetics and Growth, vol 8. Academic Press, Inc., New York, pp 280–352.
9. Buss JJ, Jones DA, Lumsden A, Harris JO, Bansemer MS, Stone DAJ (2015) Restricting feed ration has more effect than diet type on the feeding behaviour of greenlip abalone *Haliotis laevigata*. Mar Freshw Behav Physiol 48:51–70. https://doi.org/ 10.1080/10236244.2014.990701
10. Capinpin Jr. EC, Corre KG (1996) Growth rate of the Philippine abalone, *Haliotis asinina* fed an artificial diet and macroalgae. Aquaculture 144:81–89. https://doi.org/ 10.1016/S0044-8486(96)01332-4
11. Capinpin Jr. EC, 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.

12. Capinpin Jr. EC, Toledo JD, Encena VC, Doi M (1999) Density dependent growth of the tropical abalone *Haliotis asinina* in cage culture. Aquaculture 171:227–235.
13. Chen J-C, Chen W-C (1999) Temperature tolerance of *Haliotis diversicolor supertexta* at different salinity and temperature levels. Comp Biochem Physiol Part A Mol Integr Physiol 124:73–80. https://doi.org/10.1016/S1095-6433(99)00092-6
14. Choi DG, Kim J, Yun A, Cho SH, Jeong HS, Lee KW, Kim HS, Kim PY, Ha MS (2018) Dietary substitution effect of fishmeal with tunic meal of sea squirt, *Halocynthia roretzi*, Drasche, on growth and soft body composition of juvenile abalone, *Haliotis discus*, Reeve 1846. J World Aquac Soc 49:1095–1104. https://doi.org/10.1111/ jwas.12537
15. Cook PA (2019) Worldwide abalone production statistics. J Shellfish Res 38:401–404.
16. Durazo-Beltrán E, Toro-Vázquez JF, Vásquez-Peláez C, Viana MT (2003) Effect of the seaweed *Macrocystis pyrifera* and a formulated diet on growth and fatty acid composition in the green abalone, *Haliotis fulgens*, under commercial culture conditions. Cienc Mar 29:645–654.
17. Encena II VC, de la Peña 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 32:21–27.
18. Endoma LF, Nacional LM, Luhan RJ (2019) Solar irradiation as an alternative bleaching process for agar extracted from *Gracilariopsis heteroclada* in Iloilo, Philippines. Bot Mar 62:605–613.
19. FAO (2021) Global Capture Production: Abalone. Food and Agriculture Organization of the United Nations, Rome. http://www.fao.org; accessed 5 May 2021
20. FDS (1994) Feed Development Section Manual: Feeds and feeding of milkfish, Nile tilapia, Asian sea bass, and tiger shrimp, vol 21. SEAFDEC Aquaculture Department, Tigbauan, Iloilo. SEAFDEC Aquaculture Extension Manual pp 1–97
21. Fermin AC, Buen SM (2000) Feeding, growth and survival of abalone (*Haliotis asinina* Linnaeus 1758) reared at different stocking densities in suspended mesh cages in flow-through tanks. Philipp Sci 37:31–41.
22. Gallardo WG, Bautista-Teruel MN, Fermin AC, Marte CL (2003) Shell marking by artificial feeding of the tropical abalone *Haliotis asinina* Linne juveniles for sea ranching and stock enhancement. Aquac Res 34:839–842.
23. Google (2022) Google Scholar. Google LLC, Mountain View, California. https://scholar. google.com.ph/scholar?hl = en&as_sdt = 0%2C5&as_vis = 1&q=%E2%80%9CHaliotis + asinina%E2%80%9D+%E2%80%9Cmixed + diet%E2%80%9D&btnG=; accessed 17 March 2022
24. Hernández J, Uriarte I, Viana MT, Westermeier R, Farías A (2009) Growth performance of weaning red abalone (*Haliotis rufescens*) fed with *Macrocystis pyrifera* plantlets and *Porphyra columbina* compared with a formulated diet. Aquac Res 40:1694–1702.
25. Kemp JOG, Britz PJ, Toledo Agüero PH (2015) The effect of macroalgal, formulated and combination diets on growth, survival and feed utilisation in the red abalone *Haliotis rufescens*. Aquaculture 448:306–314. https://doi.org/10.1016/j. aquaculture.2015.06.016

26. Kirkendale L, Robertson-Andersson DV, Winberg PC (2010) Review on the use and production of algae and manufactured diets as feed for sea-based abalone aquaculture in Victoria. University of Wollongong, Shoalhaven Marine & Freshwater Centre, Nowra, Victoria. pp 1–198
27. Kong N, Liu X, Li J, Mu W, Lian J, Xue Y, Li Q (2017) Effects of temperature and salinity on survival, growth and DNA methylation of juvenile Pacific abalone, *Haliotis discus hannai* Ino. Chin J Oceanol Limnol 35:1248–1258.
28. Latimer Jr. GW (ed) (2016) Official Methods of Analysis of AOAC International, 20th ed. AOAC International, Rockville, Maryland. p 3172
29. Lebata-Ramos MJH (2018) Grow-out culture of abalone in small islands and/or community. SEAFDEC Aquaculture Department, Tigbauan, Iloilo. pp 1–114
30. Lebata-Ramos MJH, Doyola-Solis EFC, Abrogueña JBR, Ogata H, Sumbing JG, Sibonga RC (2013) Evaluation of post-release behavior, recapture, and growth rates of hatchery-reared abalone *Haliotis asinina* released in Sagay Marine Reserve, Philippines. Rev Fish Sci 21:433–440.
31. Lebata-Ramos MJHL, Biñas JB, Solis EFD, Balinas VT (2021) Flakes versus noodles as feed for the abalone *Haliotis asinina*, does form matter? Aquac Nutr 27:1880–1887. https://doi.org/10.1111/anu.13325
32. Lebata-Ramos MJHL, Solis EFD (2021) Can *Ulva reticulata* replace *Gracilariopsis heteroclada* as natural food for the abalone *Haliotis asinina*? J Appl Phycol 33:1869–1872. https://doi.org/10.1007/s10811-021-02407-1
33. 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. Invertebr Reprod Dev 23:75–88. https://doi.org/10.1080/07924259.1993.9672298
34. Naidoo K, Maneveldt G, Ruck K, Bolton JJ (2006) A comparison of various seaweed-based diets and formulated feed on growth rate of abalone in a land-based aquaculture system. J Appl Phycol 18:437–443.
35. Okuzawa K, Maliao RJ, Quinitio ET, Buen-Ursua SMA, Lebata MJHL, Gallardo WG, Garcia LMB, Primavera JH (2008) Stock enhancement of threatened species in Southeast Asia. Rev Fish Sci 16:394–402.
36. Prieto-Carolino A, Pilapil-Anasco C, Mediodia HJP, Gelvezon RPL, Gabunada FM (2018) Prospects of community-based co-management of Philippine abalone fishery. Ocean Coastal Manage 163:185–191. https://doi.org/10.1016/j.ocecoaman.2018. 06.006
37. Qi Z, Liu H, Li B, Mao Y, Jiang Z, Zhang J, Fang J (2010) Suitability of two seaweeds, *Gracilaria lemaneiformis* and *Sargassum pallidum*, as feed for the abalone *Haliotis discus hannai* Ino. Aquaculture 300:189–193. https://doi.org/10.1016/j. aquaculture.2010.01.019
38. Robertson-Andersson DV, Maneveldt GW, Naidoo K (2011) Effects of wild and farm-grown macroalgae on the growth of juvenile South African abalone *Haliotis midae* Linnaeus. Afr J Aquat Sci 36:331–337. https://doi.org/10.2989/16085914. 2011.636910

39. Salayo ND, Azuma T, Castel RJG, Barrido RT, Tormon-West DHM, Shibuno T (2020) Stock enhancement of abalone, *Haliotis asinina*, in multi-use buffer zone of Sagay Marine Reserve in the Philippines. Aquaculture 523:735138. https://doi.org/10. 1016/j.aquaculture.2020.735138
40. Santander-Avanceña SS, Luhan MRJ, Felera-Panizales J (2015) Improved growth performance of *Gracilariopsis heteroclada* via short-term nitrogen enrichment. Bot Mar 58:457–463.
41. Shepherd SA (1973) Studies on Southern Australian abalone (Genus *Haliotis*). 1. Ecology of five sympatric species. Aust J Mar Fresh Res 24:217–257.
42. Tahil AS, Juinio-Menez MA (1999) Natural diet, feeding periodicity and functional response to food density of the abalone, *Haliotis asinina* L., (Gastropoda). Aquac Res 30:95–107. https://doi.org/10.1046/j.1365-2109.1999.00294.x
43. Venegas AG, Cárdenas TC, Aguilar RF, Bernal RS, Ferreira FH (2016) Effects of diet and feeding regime on growth and survival of the red abalone *Haliotis rufescens* in land-based tank cultures in southern Chile. J Shellfish Res 35:969–979.
44. Viera MP, de Vicose GC, Gómez-Pinchetti JL, Bilbao A, Fernandez-Palacios H, Izquierdo MS (2011) Comparative performances of juvenile abalone (*Haliotis tuberculata coccinea* Reeve) fed enriched vs non-enriched macroalgae: Effect on growth and body composition. Aquaculture 319:423–429. https://doi.org/10.1016/j. aquaculture.2011.07.024
45. Wang B, Weiwei Y, Xiangyuan W (2010) Effects of different diet treatments on the growth and survival of juvenile small abalone *Haliotis diversicolor*. J Xiamen Univ Nat Sci 49:286–292.

Figures

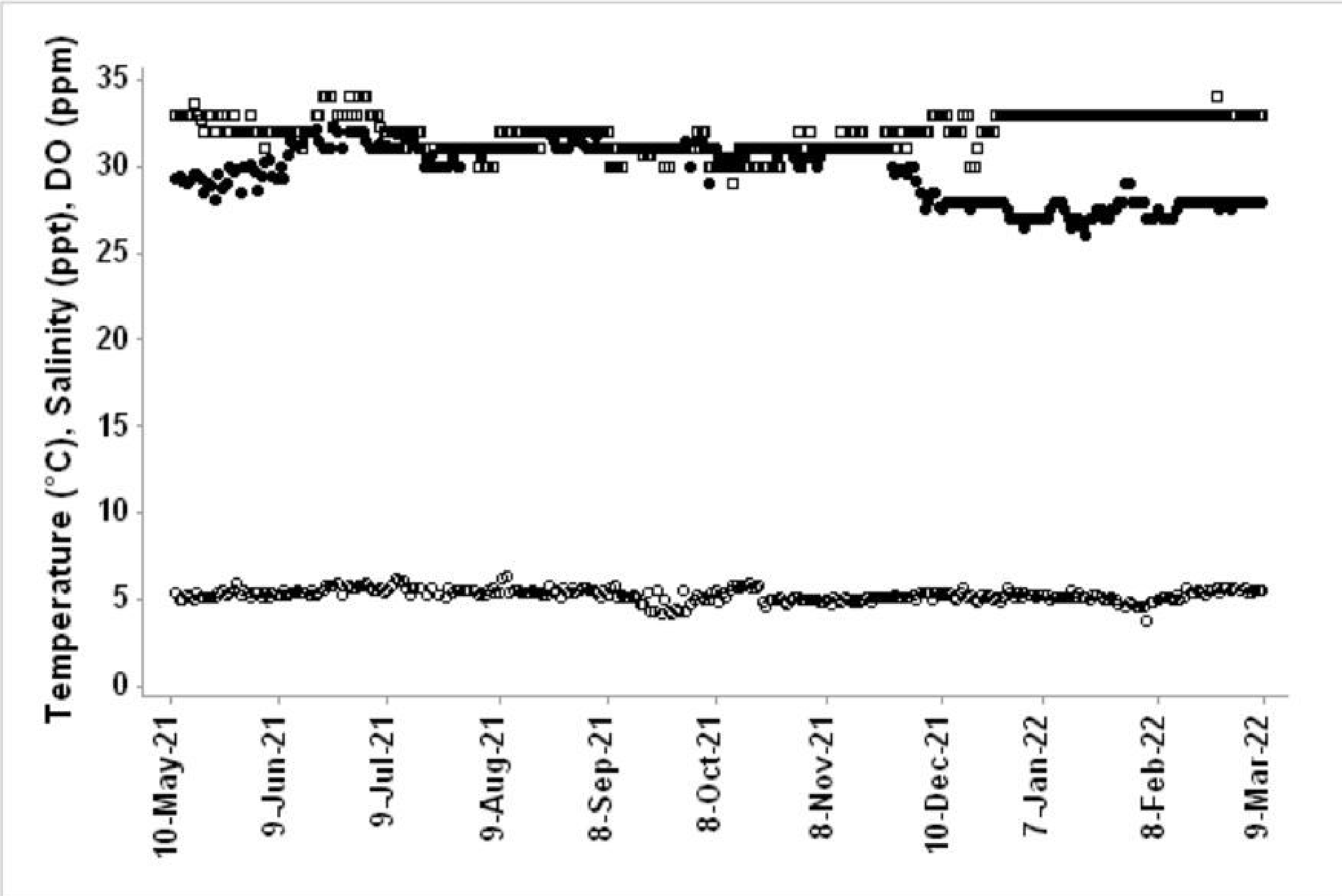

Figure 1

Daily temperature (°C, black-filled circles), salinity (ppt, white-filled squares), and dissolved oxygen (ppm, white-filled circles) taken during the duration of the abalone *Haliotis asinina* feeding experiment

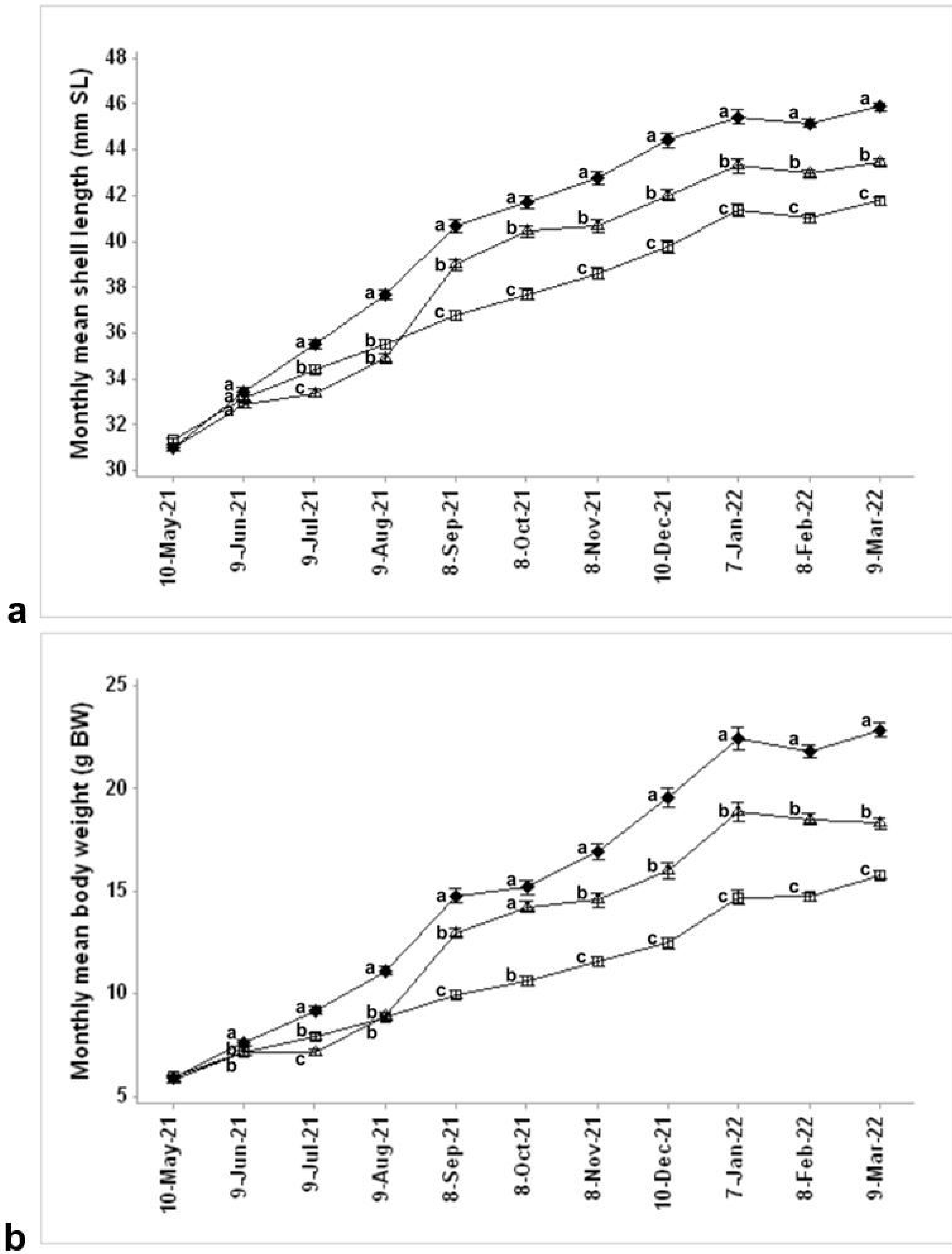

Figure 2

Monthly means±S.E. of (a) shell length (mm) and (b) body weight (g) of abalone *Haliotis asinina* fed seaweeds *Gracilariopsis heteroclada* (white-filled triangles), formulated flaked diet (white-filled squares), and a mix of the two diets (black-filled diamonds). Error bars represent 95% confidence interval from the mean. Means with different superscripts are significantly different

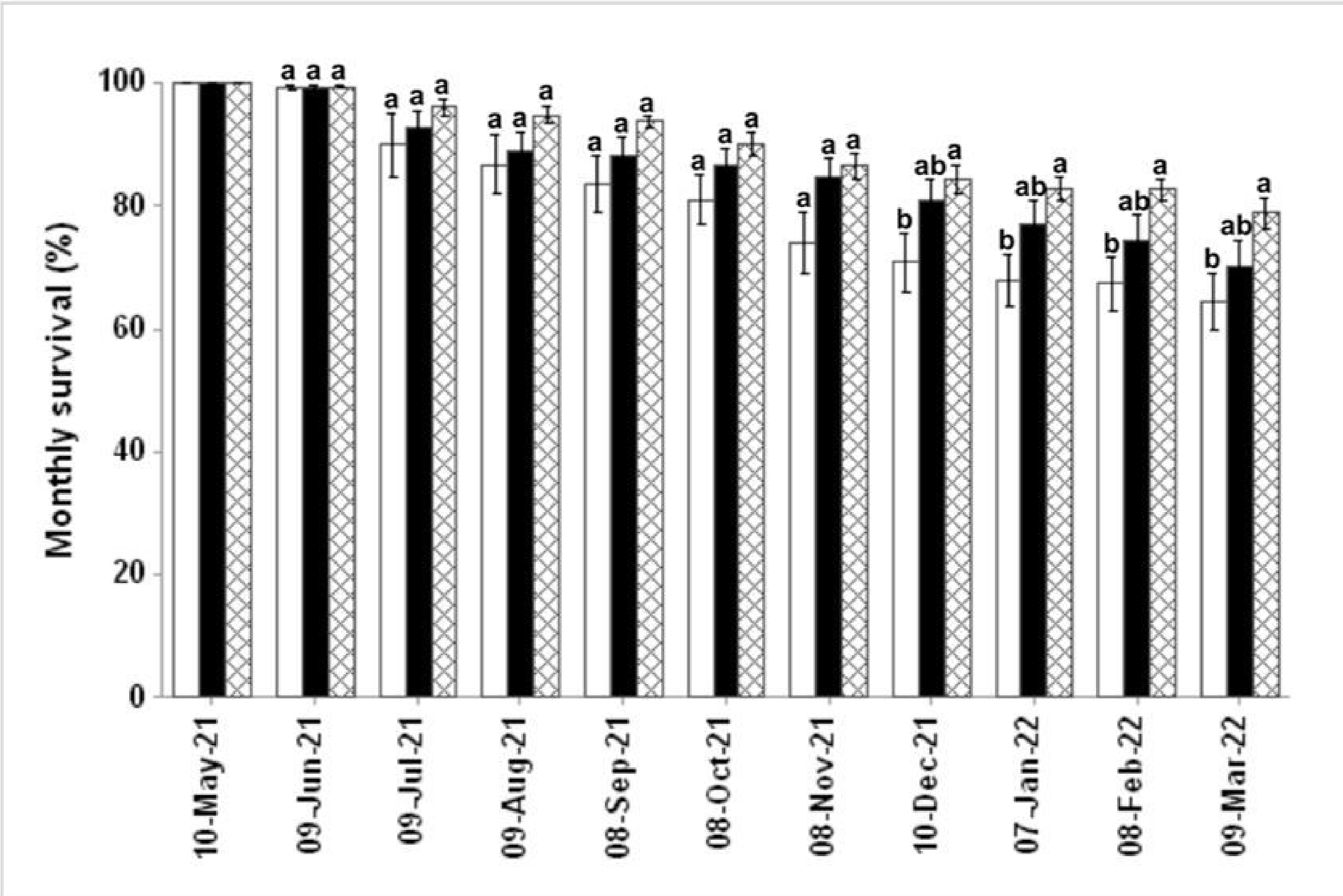

Figure 3

Monthly means±S.E. of survival (%) of abalone *Haliotis asinina* fed seaweeds *Gracilariopsis heteroclada* (black-filled bars), formulated flaked diet (white-filled bars), and a mix of the two diets (cross-hatched bars). Error bars represent 95% confidence interval from the mean. Means with different superscripts are significantly different

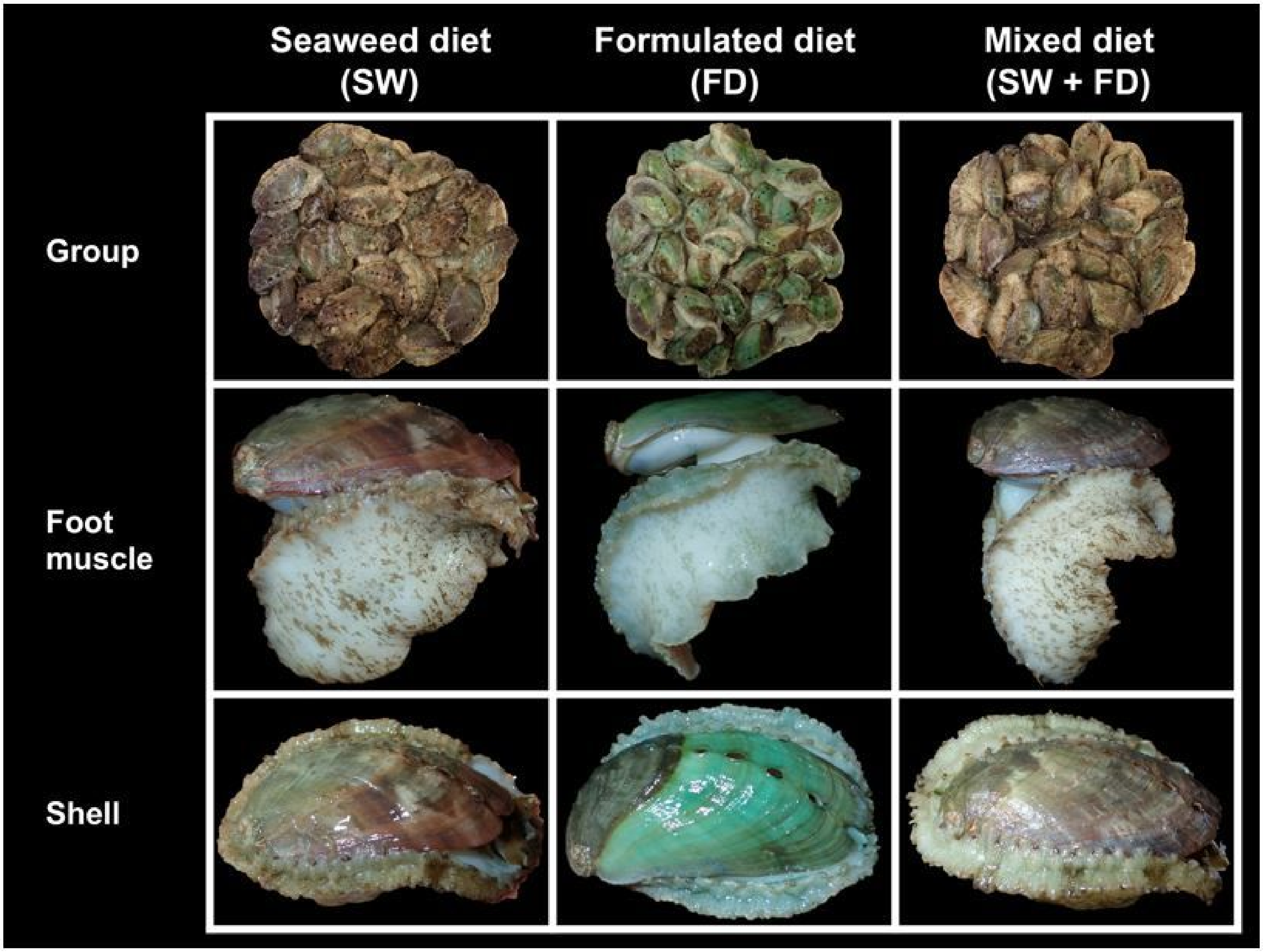

Figure 4

Differences in the shell and foot muscle color of abalone *Haliotis asinina* fed seaweeds *Gracilariopsis heteroclada* (SW, first column), formulated flaked diet (FD, second column), and a mix of the two diets (SW+FD, third column)