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MULTI-TROPHIC AQUACULTURE OF THE GREEN ABALONE *HALIOTIS FULGENS* AND THE WARTY SEA CUCUMBER *APOSTICHOPUS PARVIMENSIS* ENHANCES PRODUCTION

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ABSTRACT Around the world, abalone species are a highly valuable marine resource. In recent years, abalone fisheries in the Northeast Pacific have suffered massive mortalities because of environmental factors. Aquaculture has been proposed as a solution to stop the collapse of this multimillion-dollar resource. Moreover, it has been shown widely that coculturing two or more species can increase the productivity of the resources cultured. The aim of this study was to test whether the growth rates of the green abalone *Haliotis fulgens* would increase when cocultured with the warty sea cucumber *Apostichopus parvimensis*. Growth rates of juvenile abalone increased by 29% in the presence of medium densities of *A. parvimensis*; however, coculture settings are not common in the Northeast Pacific. To our knowledge, this is the first coculture reported of these two species. Thus, these findings provide relevant information to promote innovative strategies for sustainable production of food.

KEY WORDS: *Haliotis fulgens*, *Apostichopus parvimensis*, aquaculture, juvenile abalone, sea cucumber, coculture, growth rates, Baja California

INTRODUCTION

Abalone species are considered a delicacy and one of the most valuable seafood worldwide. In recent years, its global production has drastically switched from fishing to farming (FAO 2017). At present, 95% of the abalone consumed globally comes from aquaculture and its production has increased 500% in the last 10 y, from 24,400 t in 2006 to 129,000 t in 2015 (Gordon & Cook 2013, FAO 2017). This growth in abalone production has been driven largely by Asian countries, such as China and South Korea, with only 3% by the rest of the word (Cook 2016).

In Mexico, about 380 t of abalone are fished annually from deteriorated natural populations (Guzmán del Proo & Monte Luna 2017), whereas aquaculture production fluctuates around 60 t per annum (Sosa-Villalobos et al. 2016). Hence, there is a significant potential to invest in further developing the production capacity of abalone through innovative aquaculture strategies which could also bolster populations in the context of overfishing and climate change.

One way to enhance the growth of abalone in an aquaculture setting is through coculturing with detritivore organisms. For example, implementing a coculture of sea cucumbers is relatively easy; there is no need to drastically modify the culturing process or infrastructure. Moreover, cocultures with sea cucumbers can improve the water quality of tanks because they ingest organic matter (Kang et al. 2003, Qi et al. 2013, Yuan et al. 2015), which can reduce operational costs.

This type of aquaculture could be an alternative to fishermen who live off declining populations of abalone. This study tests the success of a coculture in the fishing cooperative Buzos y Pescadores de la Baja California at Isla Natividad, in western Mexico. The abalone fishery at Isla Natividad was profitable until 2006. After two mass mortalities and a hypoxia event recorded in 2009, the abalone fishery was closed (Micheli et al. 2012). After the mass mortalities, the cooperative considered diversifying their efforts by applying polyculture techniques involving abalone and sea cucumbers, in the hope to enhance productivity. The cooperative has 20 y of experience in cultivating abalone for repopulation efforts. Therefore, increasing abalone growth rates, resulting in a decreased harvest time, would be beneficial to the fishermen to optimize their productivity. To our knowledge, no one has tried to perform coculture experiments in this area.

The aim of this study was to explore if the coculture of green abalone and warty sea cucumber could enhance production: specifically, to determine whether (1) coculture would increase juvenile abalone growth rates and (2) what the optimal ratio of warty sea cucumber to abalone is for enhancing the productivity.

MATERIAL AND METHODS

Study Site

The study was conducted at the Buzos y Pescadores aquaculture facility at Isla Natividad situated off the Pacific Coast of Baja California, Mexico (Fig. 1). The green abalone *Haliotis fulgens* used in the experiment were 6-mo-old, aquaculture-raised individuals with an average shell length (SL) of 10.66 ± 1.69 mm. The warty sea cucumber *Apostichopus parvimensis* were collected at La Guanera (27° 53.110′ N, 115° 10.243′ W; Fig. 1), from a depth of 12 m on April 23, 2015. The collected sea

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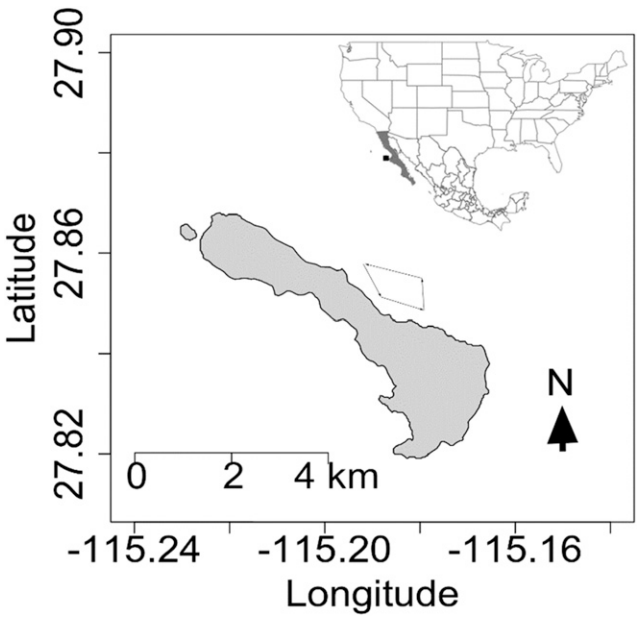

Figure 1. Isla Natividad, Baja California Sur, Mexico. The marked area, La Guanera, is where the sea cucumbers and algae were collected.

cucumbers were placed inside individual plastic bags underwater. Once at the surface, the sea cucumbers were placed in coolers and transferred to the laboratory within one hour, taking utmost care to avoid evisceration. Once in the laboratory, the sea cucumbers were weighed and placed in the experimental tanks.

From April 22 to July 19, 2015, the experiment was conducted in three tanks of 1,200 L (2.5 m long, 1.1 m wide, and 0.6 m deep), with an open system and continuous seawater flow of 4.35 ± 1.59 L min^{-1}. Each tank had three baskets, measuring 90 cm long, 40 cm wide, and 45 cm high (Fig. 2). Each basket had 30 corrugated plastic plates where the juvenile abalone were adhered. The sea cucumbers were placed in the bottom of the tanks, outside of the baskets, and were unable to enter the baskets.

Experimental Design

To test the effect of sea cucumber presence on abalone growth rates, different sea cucumber densities were used (medium density, high density, and a control with no sea cucumbers). In the medium sea cucumber density tank (MD), seven sea cucumbers were placed ($1{,}812 \pm 34.11$ g; 210.69 g m^{-2}), resulting in a weight ratio of ~2:1 abalone to sea cucumbers, after the recommended density by Battaglene et al. (1999) for sea cucumber monoculture. In the high sea cucumber density tank (HD), 14 sea cucumbers were placed ($3{,}360 \pm 21.22$ g; 390.69 g m^{-2}), resulting in a ratio of ~1:1 abalone to sea cucumber. The control tank did not have any sea cucumbers.

Each one of the experimental tanks housed 4,500 juvenile abalone (SL = 10.66 ± 1.69 mm), equivalent to ~3,600 g in total biomass of abalone. To know whether potential heterogeneity within each tank influenced abalone growth, the organisms were evenly distributed in the three baskets within each tank during the experiment. Differences in abalone shell length among baskets within tanks were not detected, except in the second month, between two baskets in the control tank (Bonferroni *post hoc* test, t2, basket 2 versus 3, $P = 0.040933$). Thus, further statistical comparisons between treatments were carried out pooling all organisms per tank.

To quantify the mean change in shell size as an indicator of growth, monthly photographs of 450 randomly picked

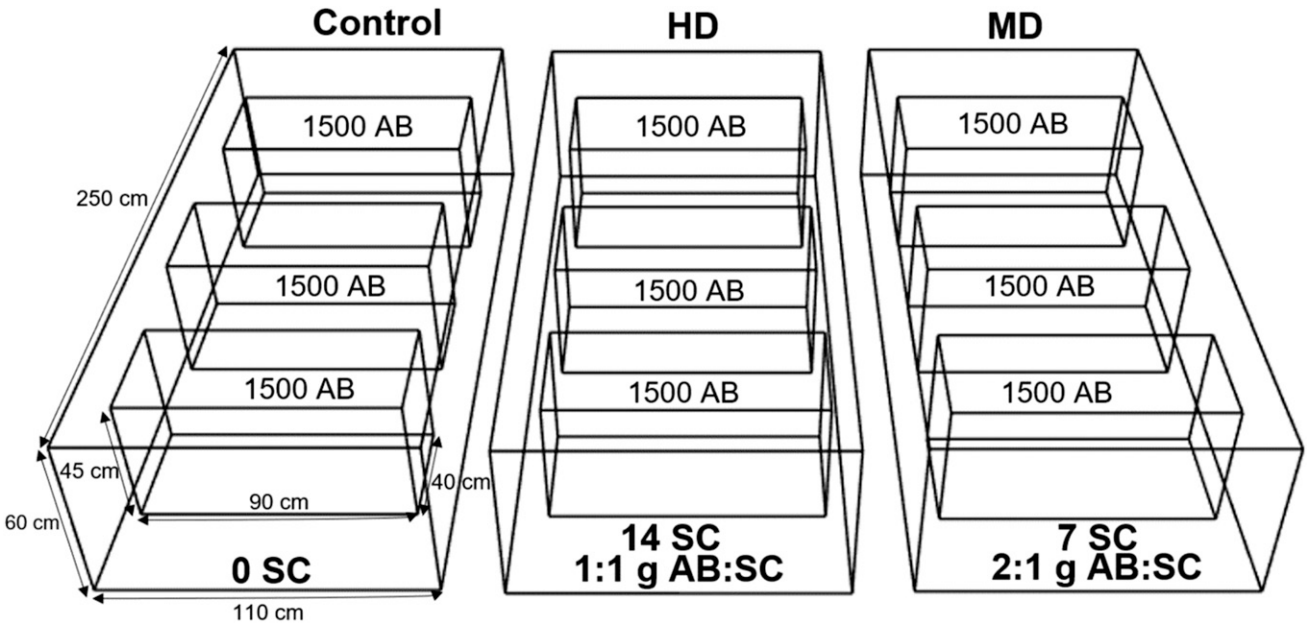

Figure 2. Experimental design for the coculture abalone (AB) and sea cucumber (SC). HD = high sea cucumber density treatment, 14 cucumbers, 390.69 g m^{-2}; MD = medium sea cucumber density treatment, seven cucumbers, 210.69 g m^{-2}; control (without sea cucumber). Each tank had 4,500 juvenile green abalone. The area on which the sea cucumbers could feed was 8.6 m^2, which includes the sides and bottom of the tanks and the exterior of the baskets. Model made with https://www.vectary.com.

abalone were taken per tank placed in a plasticized grid of 15 × 10 cm. The photographs were taken with a Fujifilm JV100 camera mounted on a tripod and postprocessed using ImageJ software (https://imagej.nih.gov/ij/). To minimize the lens distortion, horizontal and vertical calibrations were performed with a 1-cm grid next to the abalone. Once the scale was defined in ImageJ, abalone shell length was then estimated using the ruler tool.

To test for differences in the growth of *Haliotis fulgens*, a factorial ANOVA was used, with growth (mm) as a dependent variable and time and tanks as independent categorical variables, followed by a Tukey HSD test ($\alpha = 0.05$). A Kolmogorov–Smirnov ($P < 0.05$) normality test and a Cochran variance heterogeneity test were applied *a priori*; as the latter was rejected, the experiment continued as suggested by Lindman (1974). The total growth of abalone (final length – initial length) was also calculated.

Sea cucumbers were wet-weighed every two weeks, using an Accu-Weight electronic scale model DSY-1100 to the nearest 2 g, removing excess water with a sponge before weighing them (Zhou et al. 2006, Shannon & Mustafa 2015). The sea cucumbers were handled carefully to avoid stress. To test for differences in the growth of *Apostichopus parvimensis* in the two treatments (high density versus medium density), a factorial ANOVA was used, with weight (g) as the dependent variable and treatment and time as independent categorical variables, followed by Tukey's test for significant pairwise differences ($\alpha = 0.05$). The data were evaluated *a priori* for normality with a Shapiro–Wilk's test ($P < 0.05$) and for homogeneity of variances with a Cochran test. Survivorship of sea cucumbers was also calculated. The data were analyzed using STATISTICA 8 (StatSoft Inc., Tulsa, OK).

Finally, daily increment and specific growth rates (SGR) were estimated for each species according to Hopkins (1992).

Husbandry of Abalone and Sea Cucumbers

The abalone were fed *ad libitum* every 48 h with the giant kelp *Macrocystis pyrifera* and the palm kelp *Ecklonia arborea* collected from the La Guanera sample site, and *Navicula incerta* grown in the laboratory. Food was added into the baskets and no food was provided outside of the baskets. Although sea cucumbers were not able to access the interior of the baskets, they could pass their oral tentacles through the holes, so the baskets were considered as the feeding surface. The total area on which sea cucumbers could feed was 8.6 m^2. The tanks were cleaned every week.

Physicochemical Parameters

Water temperature was recorded every two hours for the duration of the experiment with a HOBO U22 Water Temp Pro v2 sensor with an accuracy of ±0.2°C. Dissolved oxygen (DO) was measured three times a day (at 07:00, 12:00, and 17:00 h) with a YSI-550A sensor calibrated at a salinity of 33.4 ppm, to the nearest 0.3 mg/L. The pH was registered at the same time as DO with a Thermo Scientific sensor model Orion Star A121 with a 3-point calibration (4.01, 7.00, and 10.01) to the nearest 0.01 unit. Finally, the flow was examined manually at the beginning of the experiment with a 2,000-mL test tube with an accuracy of ± 20 mL and set equal in the three treatments. To test for differences among the tanks, a one-way ANOVA was performed. There were no statistically significant differences in the physicochemical parameters examined among the three tanks (°C, $F_{30,\ 2108.1} = 0.8456$, $P = 0.1625$; pH, $F_{10,\ 720} = 0.92143$, $P = 0.51261$; DO, $F_{10,\ 720} = 1.3907$, $P = 0.17989$).

RESULTS

Growth of the Abalone Haliotis fulgens

At the end of the experiment, the abalone in the medium-density (MD) sea cucumber treatment were 18% larger (total growth of 8.23 ± 2 mm SEM in shell length) than the abalone from the high-density (HD) sea cucumber treatment (total growth of 6.73 ± 1.9 mm SE) and 29% larger than the control group (total growth of 5.85 ± 2.17 mm SE). The abalone in the HD tank were 13% larger than those in the control group (Table 1) (factorial ANOVA, $F_{11,\ 5388} = 826.139$, $P = 0.00001$; tanks, $F_{2,\ 5388} = 79.728$, $P = 0.00001$; time, $F_{3,\ 5388} = 2{,}923.1$, $P = 0.00001$; tanks × time, $F_{6,\ 5388} = 26.448$, $P = 0.00001$; Fig. 3). Tukey's test indicates that during the first two months, the abalone in the MD and control tanks were significantly larger than the abalone in the HD tank, whereas in the third month, the abalone in the MD tank were significantly larger than the abalone in the HD and control tanks ($P < 0.05$). The mean daily increase in shell length of abalone was 99 ± 7 μm, 76 ± 5 μm, and 67 ± 8 μm in the MD, HD, and control tanks, respectively (Fig. 4, Table 1). The specific growth rates were 0.78% ± 0.11%, 0.61% ± 0.04%, and 0.47% ± 0.05% for the abalone from the MD, HD, and control tanks, respectively (Table 1).

Growth of the Sea Cucumber Apostichopus parvimensis

The total mean growth of sea cucumbers was negative, with values of –5.42 ± 14.15 g and –25.62 ± 13.55 g for the MD and HD tanks, respectively. However, wet-weight (g) growth rates during the first six weeks were positive, but after that, body weights decreased in both tanks for the remaining 6 wk (Fig. 5). During the positive growth period, sea cucumbers from the MD tank grew 60% more than sea cucumbers from the HD tank, with growths of 35.43 ± 15 g and 14.14 ± 6.06 g at the MD and HD tanks, respectively (factorial ANOVA, $F_{7,\ 76} = 2.556$, $P = 0.0202$; tank, $F_{1,\ 76} = 13.128$, $P = 0.0005$; time, $F_{3,\ 76} = 1.4802$, $P = 0.2266$; tank × time, $F_{3,\ 76} = 0.3789$, $P = 0.7685$; Fig. 5).

TABLE 1.

Total growth (mm), average of daily increase in shell length (DISL) (μm), and the average specific growth rate (SGR) (%) of the abalone in the three treatments during the 90 days of the experiment.

Treatment	Total growth (mm)	DISL (μm)	SGR (%)
MD	8.23 ± 2	99 ± 7	0.78 ± 0.11
HD	6.73 ± 1.9	76 ± 5	0.61 ± 0.04
Control	5.85 ± 2.17	66 ± 8	0.47 ± 0.05

MD, medium density of *Apostichopus parvimensis*; HD, high density of *A. parvimensis*; Control, without *A. parvimensis*.

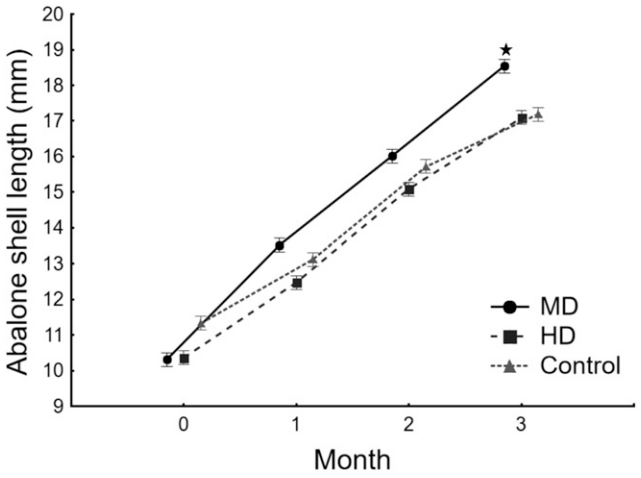

Figure 3. Growth of the green abalone *Haliotis fulgens* (mm) in the treatments during the three months of experimentation. MD = medium sea cucumber density (210.69 g m^{-2}), HD = high sea cucumber density (390.69 g m^{-2}), control (without sea cucumber). Circles represent medium density (MD) of sea cucumbers; squares represent high density (HD) of sea cucumbers; and triangles represent the control (without sea cucumbers). Vertical bars denote confidence intervals at 0.95.

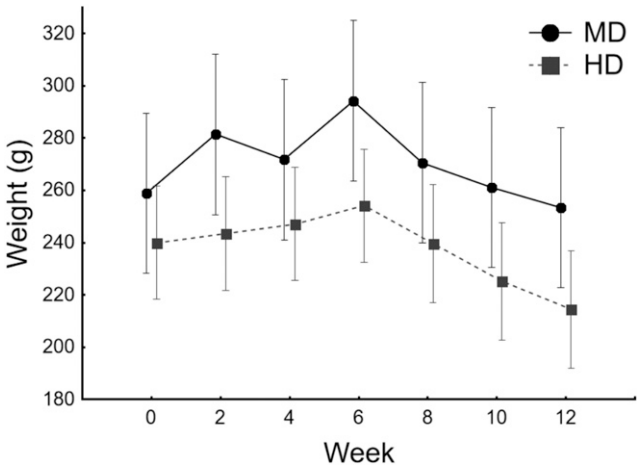

Figure 5. Average body weight values of *Apostichopus parvimensis* (g) in coculture with *Haliotis fulgens*, medium-density tank (MD; 210.69 g m^{-2}) and high-density tank (HD; 390.69 g m^{-2}). Circles represent medium density (MD) of sea cucumbers; squares represent high density (HD) of sea cucumbers. Vertical bars denote confidence intervals at 0.95.

During the rest of the time , sea cucumbers from the MD and HD tanks lost 40.86 ± 17.73 g and 39.76 ± 17.3 g, respectively (factorial ANOVA, $F_{5, 54} = 2.016$, $P = 0.09$; tank, $F_{1, 54} = 7.736$, $P = 0.007$; time, $F_{2, 54} = 0.928$, $P = 0.402$; tank*time, $F_{2, 54} = 0.034$, $P = 0.967$; Fig. 5). The mean daily increase in body weight was –0.06 ± 1.27 and –0.29 ± 0.67 g day^{-1} for the sea cucumbers from the MD and HD tanks, respectively (Table 2). The mean specific growth rates were –0.02% ± 0.46% day^{-1} and –0.13% ± 0. 28% day^{-1} for the sea cucumbers in the MD and HD tanks, respectively (Table 2). The survival of *A. parvimensis* was 100% and 92.86% in the MD and HD tanks, respectively (Table 2).

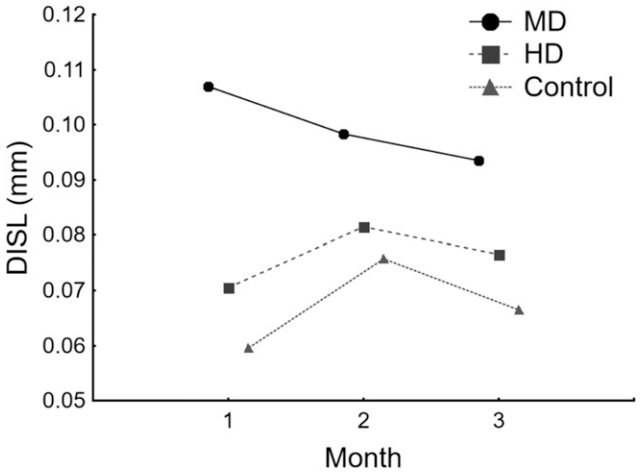

Figure 4. Average daily increase in shell length (DISL, mm) of the green abalone in the three treatments during the experiment. Circles represent medium density (MD) of sea cucumbers; squares represent high density (HD) of sea cucumbers; and triangles represent the control (without sea cucumbers).

DISCUSSION

Growth of the Abalone Haliotis fulgens

The juvenile green abalone *Haliotis fulgens* raised in coculture with the warty sea cucumber *Apostichopus parvimensis* grew more than those without sea cucumbers. On average, abalone had 29% larger shells when raised with a medium density of sea cucumbers than those raised without sea cucumbers. This result is similar to results from other studies in Asia of cocultures with different species of abalone and sea cucumbers (Table 3). For example, Kang et al. (2003) reported a 55% growth increase of the juvenile charm abalone *Haliotis discus hannai* when cocultured with the Japanese sea cucumber *Apostichopus japonicus*, compared with control tanks without the holothurian. Comparisons of growth rates between the present and previous studies should be interpreted with caution because different species were used in the experiments, there were a variety of weight measurements applied, different developmental stages were examined, and cultivation methods varied (Table 3).

According to Gómez-Montes et al. (2003), an acceptable growth for *Haliotis fulgens* in aquaculture is 80 μm day^{-1}, and Turrubiates (1989) reported 96 μm day^{-1} in the wild at Tortugas Bay, approximately 7 km from Isla Natividad. In this study, the abalone in the MD tank presented an optimum growth with an

TABLE 2.

Total growth (g), average daily increase in body weight (DIBW) (g), and the average specific growth rate (SGR) (%) of *Apostichopus parvimensis* during the experiment.

Treatment	Total growth (g)	DIBW (g)	SGR (%)
MD	– 5.43 ± 14.15	–0.06 ± 1.27	–0.02 ± 0.46
HD	–25.62 ± 13.55	–0.29 ± 0.67	–0.13 ± 0.28

MD, medium sea cucumber density; HD, high sea cucumber density.

TABLE 3.

Summary of published studies examining the coculture of abalone and sea cucumbers.

AB + SC	Coordinates and study area	Culture type	System and time	Organism	Initial size	Final size	SGR (% day^{-1})	DI (μm d-^{1}) (g day^{-1})	Survival (%)
Kang et al. (2003)		Land based laboratory		*Haliotis discus hannai*	AB = 13.32 ± 2.40 mm	AB = 16.19 ± 0.43 mm	0.22	31 μm	90.0 ± 2.0
	Lat: 34.617176 Lon: 127.721695		Control AB			**AB** + SC = 19.30 ± 0.27 mm	0.41	66 μm	95.3 ± 1.2
			Tank AB + SC	*Apostichopus japonicus*	SC = 5.0 ± 1.2 g	**SC** + AB = 15.21 ± 1.66 g	1.23	1.13 g	100
	Dolsan, Jeollanam-do, South Korea	Static tanks	Tank AB + SC + S			**AB** + SC + S = 19.71 ± 0.38 mm	0.43	71 μm	96.0 ± 2.0
						SC + AB + S = 18.35 ± 0.63 g			100
			90 days				1.44	1.48 g	
Qi et al. (2013)		Mariculture	Cages suspended for a year		AB = 75.98 ± 2.22 mm	**AB**:SC=3:1 = 88.08 ± 5.10 mm; 103.00 ± 10.16 g	0.12 ± 0.008	33 μm	100
					62.43 ± 2.18 g	**AB**: SC = 6:1 = 88.22 ± 4.32 mm;	0.16 ± 0.009		
				H. discus hannai		101.26 ± 8.64 g	0.12 ± 0. 013	34 μm	100
	Lat: 37.01–37.09						0.16 ± 0.012		
	Lon: 122.24–122.35			*A. japonicus*	SC = 25.3 ± 2.68 g	**SC**: AB = 1:3 = 47.88 ± 6.22 g		0.61 g	100
							0.18 ± 0.009		
	Sanggou Bay, China					**SC**: AB = 1:6 = 48.07 ± 4.38 g	0.18 ± 0.013	0.62 g	100
Kim et al. (2014)					AB = 28.80 ± 1.53 mm 2.43 ± 0.45 g	AB (CP) = 34.66 ± 2.20 mm 4.74 ± 0.82 g	0.33 ± 0.19	27 μm	97.8
		Land based laboratory		*H. discus hannai*		AB (CT) = 35.69 ± 2.78 mm	0.37 ± 0.34	32 μm	98.9
	Lat: 34.626357 Lon: 127.715271		Cages with tubes (CT)			5.18 ± 1.09 g			
				A. japonicus	SC (CP) = 3.37 ± 1.31 g	SC (CP) = 7.50 ± 1.16 g	0.40 ± 0.15	0.19 g	93.0
	Gamak Bay, South Korea	36 t tanks with flow-through system	Cages with plates (CP)						
			7 mo		(CT) = 3.61 ± 1.33 g	SC (CT) = 1.40 ± 0.89 g	–0.47 ± 0.17	–0.11 g	52.0

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TABLE 3.
continued

AB + SC	Coordinates and study area	Culture type	System and time	Organism	Initial size	Final size	SGR (% day^{-1})	DI (µm d-1) (g day^{-1})	Survival (%)
Present study					MD tank:	MD tank:			
					AB = 10.31 ± 1.56 mm	AB = 18.54 ± 2.36 mm	0.67	99 µm	–
					SC = 258.85 ± 34.11 g	SC = 253.43 ± 46.34 g	0.18	0.48 g	100
	Lat: 27.855658	Land-based laboratory	4,500 abalone per tanks	*Haliotis fulgens*	HD tank:	HD tank:			
	Lon: 115.171899				AB = 10.35 ± 1.57 mm	AB = 17.09 ± 2.3 mm	0.57	77 µm	–
		1,200 L tanks with flow-through system	MD tank: 2:1 g AB:SC	*Apostichopus parvimensis*	SC = 240 ± 21.22 g	SC = 214.38 ± 50.69 g	0.02	0.05 g	92.86
	Isla Natividad		HD tank: 1:1 g AB:SC		Control tank:	Control tank:			
	Baja California Sur, Mexico		Control tank: 0 sec 90 days		AB = 11.33 ± 1.73 mm	AB = 17.18 ± 2.51 mm	0.47	66 µm	

AB, abalone; SC, sea cucumber; SGR, specific growth rate; DI, daily increase; S, sediment. (Note: The values belong to the species (either AB or SC) highlighted in bold in the first two treatments).

average of 107 µm day^{-1} reached in the first month and an average value of 99 µm day^{-1} during the whole experiment. Thus, the results suggest the potential benefit of coculturing green abalone and warty sea cucumbers to enhance juvenile abalone growth rates.

More experiments are needed to understand the mechanisms and explain why juvenile abalone grew faster in the presence of sea cucumbers. A conjecture is that sea cucumbers improve water quality by ingesting not only the accumulated debris but also bacteria (Gao et al. 2011), protozoa (Yingst 1976), cyanobacteria (Chávez et al. 2011), macroalgal detritus (Michio et al. 2003), and benthic diatoms (Yokoyama 2013) from the bottom of the tanks and baskets. Further experiments could determine the optimum ratio of warty sea cucumber and abalone needed for coculture. For example, in this study, the lesser growth in the HD tank may be due to densities above the optimum carrying capacity of the system.

Growth of the Sea Cucumber Apostichopus parvimensis

Higher growth rates of *Apostichopus parvimensis* were recorded during the positive growth period in the MD tank than the HD tank. This result coincides with Battaglene et al. (1999) who found the growth of the tropical sea cucumber *Holothuria scabra* in monoculture decreased in densities higher than 225 g m^{-2} (MD 210.69 g m^{-2} and HD 390.60 g m^{-2}); however, the ideal density for sea cucumbers in any coculture will depend on the amount of waste produced by the other organisms. For example, MacDonald et al. (2013) reported that a density of 400 g m^{-2} of *Holothuria forskali* can process the solid waste (8.76 g m^{-2} day^{-1}) of the European seabass *Dicentrarchus labrax* in culture. Hence, sea cucumber density is food quantity dependent.

The negative growth rates recorded in sea cucumbers during the last 6 wk of the experiment were likely due to a spawning event in the tanks and then a reproductive rest phase, which in nature begins at the beginning of June. During spawning events, sea cucumbers reabsorb their gonads and stop feeding, losing body weight (Espinoza-Montes 2000).

CONCLUSIONS

Recent extreme weather events related to climate change have resulted in massive declines in fishing. This study proposes a simple and effective alternative to fishing using coculture of two important commercial species. The results on the coculture of the green abalone *Haliotis fulgens* with the warty sea cucumber *Apostichopus parvimensis* are promising, which could lead to potential profitable increases in abalone production when cultured in the presence of sea cucumbers.

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