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RESEARCH

Coculture of abalone (*Haliotis midae*) and sea cucumber (*Neostichopus grammatus*) to reduce tank cleaning frequency in abalone farming

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Abstract
Abalone farming produces nutrient-rich sludge, and the frequent cleaning and removal of sludge from abalone tanks is labour and capital-intensive. This study aimed to assess the effect of culture methods and tank cleaning frequency on abalone growth, water quality, and sludge characteristics. The study was conducted for 16 weeks. Four treatments were used, namely, abalone cocultured with sea cucumber cleaned once (AS1) and twice weekly (AS2); abalone monoculture cleaned once (A1) and cleaned twice weekly (A2). Abalone (initial avg weight = 40.9 g) were maintained under commercial stocking conditions in flow through systems at a salinity of 35 ppt and fed a commercial formulated diet (Abfeed™) and fresh seaweed (*Ulva lacinulata* and *Gracillaria gracilis*). The stocking densities were 200 abalone m^{-2} and 50 sea cucumbers m^{-2}. The sea cucumbers (initial average weight = 12.5 g) fed on the abalone faeces and leftover feed, which settled at the abalone tank's bottom (sludge). There was a significant interaction between the type of culture and the frequency of cleaning on abalone weight ($F_{(1,476)} = 12.41$, $p < 0.001$). Abalone in the A2 treatment group showed higher growth ($p = 0.006$) than those of the A1 treatment group, while abalone in AS1 had significantly higher growth ($p < 0.001$) than those in the AS2 treatment group. Also, abalone in AS1 showed higher growth ($p = 0.026$) than abalone in the A1 treatment. However, abalone in AS1 had growth similar ($p = 0.53$) to those in A2. The survival rate of the sea cucumbers was high, however, the sea cucumbers experienced a reduction in weight regardless of the treatment. This study showed that growth optimisation in abalone farming can be achieved by cleaning tanks twice instead of once weekly. However, when abalone are cocultured with sea cucumber, tanks need only be cleaned once a week without compromising abalone growth or water quality. The coculture of abalone and sea cucumber results in an additional aquacultured product, a significant reduction in labour and other associated costs of cleaning, without an additional cost of production.

Keywords Carbon · Growth · Abalone · Nitrogen · Sea cucumber · Sludge · Water quality

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Introduction

In aquaculture, wastes rich in nutrients such as nitrogen, carbon, phosphorous, and sulphur are derived from uneaten food, faeces, and other excreta of animals (Dauda et al. 2019). Solid wastes are sometimes referred to as sediment or sludge and settle at the bottom of tanks, ponds, or the benthos in open systems. The accumulation of sludge is often detrimental to the culture system and the animals therein. For example, excess sludge can reduce water quality by stimulating the proliferation of pathogenic bacteria (Rajkowski 2009). Infectious diseases are frequently caused by sludge build-up in the culture unit (Chen et al. 1997; Hossain et al. 2016; Khan 2018; Jasmin et al. 2020). In open systems, sludge impacts the receiving environment negatively and has led to criticism concerning aquaculture sustainability, including that of economically important abalone culture (Piedrahita 2003; Cao et al. 2007; Bao et al. 2019; Ahmad et al. 2022).

Abalone is one of the most prized seafood delicacies in the world and is highly sought after, commanding a high monetary value (Cook 2016; Suleria et al. 2017). The depletion of abalone in the wild due to over-harvesting has led to abalone farming becoming established in many countries to supplement wild catches while contributing to food security (Hobday et al. 2000; Dichmont et al. 2000; Shepherd et al. 2001). South Africa is the third-largest producer of farmed abalone worldwide (HKTDC Research 2017). *Haliotis midae* is one of the abalone species endemic to South Africa and the only farmed species (DAFF 2018).

Farmed abalone are intensively fed with pellets and algae (Troell et al. 2009; Naidoo et al. 2006; Kirkendale et al. 2010). Abalone produce large amounts of feed waste (especially when fed on pellets) due to the animal's mode of feeding, scraping food particles before sending them to the mouth. In the process, food is broken into pieces (Mai et al. 1995). Waste food and faeces settle to form a layer of sludge at the base of abalone tanks. After some time, the sludge decomposes, affecting the tank's water quality, which is detrimental to abalone growth and health. This may result in stress, disease, and even death (Akinwole et al. 2016; Jasmin et al. 2020). Hence, cleaning abalone holding tanks frequently to remove sludge is a necessity. Traditional cleaning practices used in abalone farming include removing animals from the tank, draining rearing water, scrubbing the tank wall with a sponge and brush, and flushing out settled sludge with clean seawater. Traditional cleaning of abalone tanks is cumbersome, can lead to stress or cause physical harm to animals in culture, and a high percentage of production cost is spent on payment of labour for washing and tank cleaning (Lee et al. 2021). Traditional tank cleaning is especially laborious in the Eastern Cape of South Africa, located along the upper limit of the distribution of abalone, where water temperatures are usually high (average 21 °C) (Britz et al. 1997). These warmer temperatures lead to rapid bacterial proliferation in the sludge, resulting in tanks needing to be cleaned more frequently than in farms where the water is cooler.

Any innovation that reduces cleaning rates in abalone farming while maintaining animal growth and welfare without otherwise adding to the cost of production would be economically beneficial. Integrated multi-trophic aquaculture has been proposed as a possible ecological solution to reprocess and reduce nutrient release from aquaculture (Chopin et al. 2012; Zamora et al. 2018; Chary et al. 2020)."Integrated multi-trophic aquaculture (IMTA) is the farming, in proximity, of aquaculture species from different trophic levels, and with complementary ecosystem functions, in a way that allows one species' uneaten feed and wastes, nutrients, and by-products to be recaptured and converted into fertilizer, feed, and energy for the other crops, and to take advantage of synergistic

interactions between species" (Chopin et al. 2012). The benefits of IMTA are increased efficiencies in terms of diversification, leading to increased profitability. A typical proposed potential IMTA candidate is the deposit-feeding sea cucumber. Sea cucumbers are valued food in Asia, commanding high prices (Prescott et al. 2017; Purcell et al. 2018). They are generally detritivores and are able to feed on aquaculture waste (Slater et al. 2009; Zamora and Jeffs 2012; Onomu et al. 2023). Sea cucumbers have been used as a coculture candidate in conjunction with abalone, shrimp, oysters, fish, scallops, mussels, and sea urchins (Kang et al. 2003; Slater and Carton 2007; Ren et al. 2012; Qi et al. 2013; Yokoyama 2015; Zhou et al. 2017; Neofitou et al. 2019; Grosso et al. 2021). Sea cucumbers possess bioremediation ability and can reduce waste's total organic matter, nitrogen, and carbon content via their feeding activity, thereby improving water quality (McTavish et al. 2012; Watanabe et al. 2012; Neofitou et al. 2019; Onomu et al. 2023). The cocultureof abalone and sea cucumber has reportedly led to improved growth in abalone (Kang et al. 2003; Bauer et al. 2019).

One factor that must be considered in IMTA is the compatibility of the species to be cocultured in order to prevent stress (Chopin et al. 2012). Stress or stress response refers to the reaction of an animal to the stressor. Stressors of aquatic animals include high stocking density, fluctuations in temperature, salinity, pH, and oxygen; inadequate food supply; and exposure to toxins (Cheng et al. 2004a, b; Lee et al. 2019). In vitro tests used as an indicator of stress or disease in cultured invertebrates, such as sea cucumber and abalone, include the haemocyte count, phenoloxidase measurement, superoxide production, phagocytosis assay, protein, glucose, and glycogen (Hooper et al. 2007).

Neostichopus grammatus is a species of sea cucumber endemic to South Africa and has been identified as a potential aquaculture species (Onomu et al. 2023). *Neostichopus grammatus* is not exploited in South Africa, i.e., it is not eaten, fished, or farmed. It is distributed from Cape Agulhas in Cape Town (34 "50'S/20"00'E) to Cape Vidal in Kwazulu Natal (28.3726°S, 32.4142°E) (Thandar 1987). The only study available on the culture of *N. grammatus* is that by Onomu et al. (2023) which reported the waste (faeces and leftover food) of abalone as an acceptable food to *N. grammatus*. The study also showed that *N. grammatus* can reduce the nitrogen, carbon and total organic matter content of waste via their feeding action. Thus *N. grammatus* was proposed as a potential IMTA candidate with abalone.

This study aims to assess the feasibility of the coculture of abalone (*H. midae*) and sea cucumber (*N. grammatus*) and apply principles of waste utilisation and integration in the same production system to address specific challenges encountered in abalone farming. The effect of tank cleaning frequency and method of culture (coculture/ monoculture system) on growth, water quality, sludge characteristics, and health are assessed.

Materials and Methods

Collection of experimental animals

Sea cucumbers (*N. grammatus*) were collected by Scuba divers west of Nahoon Reef (-32.98715°S, 27.96607°E), East London, Eastern Cape, South Africa. They were placed in 20 L buckets containing seawater and then transported to the study site (Wild Coast Abalone Farm) approximately two hours from the collection site. Hatchery-produced abalone*, H. midae*, were donated by Wild Coast Abalone Farm, Haga Haga, Eastern Cape.

Experimental design

The experiment ran for 16 weeks, with the experimental design consisting of four treatments with four tank replicates each. The treatments were: -

- Abalone cocultured with sea cucumber with tanks cleaned once weekly (AS1);
- Abalone cocultured with sea cucumber with tanks cleaned twice weekly (AS2);
- Abalone monoculture with tanks cleaned once weekly (A1); and
- Abalone monoculture with tanks cleaned twice weekly (A2).

Sixteen tanks were used for the study, which was constructed from Intermediate Bulk Container (IBC) referred to as "flow-bins"; the top of the flow-bin was cut off, and the remaining "tank" plumbed. The dimensions of the flow-bin tanks were 1 m x1m × 0.69 m (L x B x H). The tank had a water volume of 0.65 m^3 and an inflow rate of two litres of seawater per minute, resulting in 0.31% of the total tank's volume being replaced per minute; the total tank volume was exchanged every 5 h. This exchange rate per basket of abalone was the same as that used in commercial abalone farms. Fourteen months old abalone was stocked in oyster mesh production baskets (L = 900 mm; W = 480 mm; H = 500 mm) according to standard abalone farm procedure (Fig. 1). Each tank contained an oyster mesh basket containing ca. 200 abalone with an average shell length of 59 mm and an average weight of 40.9 g or approx. 9.5 kg of abalone per basket resulting in a stocking density of 200 abalone m^{-2} and a biomass of 9.5 kg m^{-2} (abalone commercial stocking density). Each basket contained a rack that served as a substrate for the attachment of abalone.

A total of four hundred sea cucumbers with an average weight of approximately 12.5 g were stocked at the bottom of the abalone tanks at a density of 50 sea cucumbers (average weight = 628.59 ± 1.95 g) per tank. This resulted in a stocking density of 50 seacucumbers m^{-2} and a sea cucumber biomass of 628.59 ± 1.95 g m^{-2}. The sea cucumber density was selected based on the feeding rate of the sea cucumber and the estimated waste and faeces produced by abalone as determined by Onomu et al. (2023). The sea cucumbers were provided with a dome-shaped PVC pipe in the tank base as a shelter.

Feeding

Abalone were fed as per standard farm procedure, which included being fed with a commercial abalone pellet- Abfeed™ 32S (32% protein; pellet size -10 mm x 10 mm × 1.2 mm; sinking feed), and fresh farm-produced seaweeds *Ulva lacinulata,* and *Gracilaria gracilis*. The animals were fed pellets daily (0.36% of body weight; feeding rate prescribed by the feed manufacturer) and seaweed (0.29% of body weight) thrice a week per standard farm procedure; this was adjusted as abalone increased in size. Of the three times feeding of seaweed, *Ulva lacinulata* was fed twice and *Gracilaria gracilis* once weekly. The amount of food given to the abalone daily was measured and used to calculate the average daily food provided, and if there was leftover food in the tanks, that particular tank was not fed. The sea cucumbers were not fed during the experimental period but had access to faeces and pellets that fell through the abalone basket onto the bottom of the tanks.

Fig. 1 Image of experimental tanks used in the experiment

Cleaning and maintenance of tanks

Tanks were cleaned once or twice weekly, depending on the treatment. The cleaning routine involved moving the abalone baskets out of tanks and spraying with water from a hosepipe to unclog the mesh holes of debris, moving the washed basket into a recently filled tank with clean seawater, draining the original tank to about 20% to pick and transfer sea cucumbers underneath the tank into the new, cleaned tanks. Empty tanks were cleaned by scrubbing with a sponge and a brush, while baskets were sprayed only once weekly, irrespective of the cleaning frequency treatment. For tanks receiving twice-weekly cleaning, the second cleaning did not involve spraying the abalone basket but rather simply moving the basket into a washed tank containing clean seawater. In this manner, abalone and sea cucumbers from each treatment moved tanks in synchronization with each cleaning.

Survival

Survival of both abalone and sea cucumbers was monitored during the experiment. Survival was calculated as

$$\text{Survival rate} = \frac{\text{number of animals at the end of the experiment}}{\text{number of animals at the beginning of the experiment}} \times 100$$

Weight measurement

Wet weight (g) and shell length (mm) of 30 abalone (a representative number) were measured at the beginning of the study and every four weeks during the study using a weigh balance (Mettler PE 3600) and Caliper (Tork Craft). The sea cucumbers were starved for 48 h before the commencement of the study to evacuate guts content, according to Zamora and Jeffs (2012). Thereafter, the sea cucumbers were weighed. The sea cucumbers were placed on a sponge for a few seconds to expel excess coelomic fluid before being weighed. Growth was calculated as the mean tank change in weight.

Abalone feed conversion ratio (FCR)

The abalone baskets were monitored daily and fed only when no food was left in the basket (depicting that all feed was consumed as the pellet had high stability in water). The dry weight equivalent of the feed given to each (pellet and seaweed) was recorded and used for the FCR calculation. The FCR of abalone was estimated as

$$\text{FCR} = \frac{\text{Amount of food provided (dry weight)}}{\text{Weight gain}}$$

Amount of waste/ sludge generated

During each cleaning session (ca. every week and every three to four days—for once and twice cleaning, respectively), a mesh bag (180 μm) was tied to the outlet of the tanks. The faeces of abalone, either processed or unprocessed by the sea cucumber, and food that fell through the mesh of the abalone baskets were trapped by the mesh bag. The waste (faecal waste) was stored in a -20 °C deep freeze until it was ready to be processed for drying. The waste was dried using an oven to a constant weight at 70 °C, and the dry weight was measured using a weighing balance. Waste samples were not rinsed with fresh water to remove salt as some waste may be lost in the rinsing process, making the estimation of waste produced inaccurate. The salt in the waste was thought to be very minimal to influence the estimation accuracy of waste produced. To estimate the amount of waste generated per day, the dry weight of the sludge in each tank was divided by the cleaning session of the tank. That is, the dry weight of sludge derived from tanks cleaned once weekly was divided by seven, and the dry weight of those cleaned twice weekly were accumulated and divided by seven.

Proportion of feed turned waste per day (PFW)

The PFW was estimated as

$$\mathrm{PFW} = \frac{\text{average sludge per day}}{\text{average feed provided}} \times 100$$

Water quality

Water temperature was measured hourly with a hobo temperature logger UA-001–64 (Onset, USA). Dissolved oxygen was measured twice weekly with an OxyGuard Handy Polaris probe (OxyGuard International, Denmark); ammonia, nitrite, nitrate, and phosphorus were measured using a Palintest photometer 7100 (United Kingdom) every two weeks before routine tank cleaning.

Sludge assays

Three and six day old sludges collected from the base of abalone tanks at cleaning days (three replicates per treatment from separate tanks) were analysed for total organic matter, carbon, nitrogen, phosphorous, and sulphur content at weeks one, eight, and sixteen.

Total organic matter (TOM)

TOM was analysed using the combustion method (Byers et al. 1978). The samples were oven-dried at 60 °C for 48 h and weighed. Afterward, they were combusted in a furnace at 500 °C for 6 h. The samples were weighed, and TOM calculated as

$$\mathrm{TOM} = \frac{\text{Initial dry weight of sample} - \text{final weight of sample after calcination}}{\text{Initial dry weight of sample}} \times 100$$

Carbon and nitrogen

Carbon and nitrogen were determined by the Dumas combustion method using a C: N:S analyser (Carlo Erba Instruments NA Model 1500, USA). This was done by placing one mg of the sample in the tin container. The tin container was ignited at a high temperature of 1020 °C in oxygen, with the sample temperature rising to over 1500 °C on a second ignition of the tin container. An automatic analyzer equipped with a thermo-conductivity detector and a gas chromatographic separation column was used.

Phosphorous and sulphur

To analyse for phosphorus and sulphur, sludge samples were digested with nitric acid and perchloric acid using the open vessel wet digestion method, according to Zasoski and Burau (1977).

Health Indices

Collection of haemolymph and coelomic fluid

Abalone haemolymph (0.5 mL), was collected from the pedal sinus of abalone using 2 mL syringes and 26 G×1/2″ needles, according to Macey and Coyne (2005). The haemolymph was placed into a microcentrifuge tube and placed on ice. The sea cucumbers were left on a sponge for a few seconds to expel sea water accumulated in the body, after which the sea cucumber was opened longitudinally and about 1 mL of coelomic fluid was collected according to the procedure of Zhang et al. (2021) and Luparello et al. (2019). The coelomic fluid was collected at the beginning of the study (control), and at the end of the study. The hemolymph and coelomic fluid samples were stored at -80 °C until required; and were used for glucose and protein analysis in abalone and sea cucumber, respectively. Coelomocytes of the sea cucumber coelomic fluid were separated by centrifuging at 3000×*g* for 10 min at 4 °C. 400 µL coelomocytes were resuspended in 100 µL cold 0.85% saline and then sonicated at 22 kHz for 25 s, then centrifugation at 4000 g for 10 min, according to Chen et al. (2018). The resultant supernatant was used to determine superoxide dismutase.

Glucose

Abalone haemolymph was deproteinized by mixing 100ul of haemolymph with 100ul of 5% Trichloro acetic acid (TCA), which was left to react for 30 min. The mixture was then centrifuged at 13,000×*g*. The supernatant was decanted for glucose analysis using the glucose oxidase procedure (GAGO-20, Sigma -Aldrich assay kit, USA). D-Glucose (Sigma-Aldrich, USA) was used as the standard.

Superoxide dismutase (SOD)

SOD was determined using a commercially available assay kit (Sigma-Aldrich: 19,160). The microplate was incubated at 37 °C for 20 min, and the colour was measured at 450 nm using a microplate reader (BioTek: SYNERGY Mx). One unit of SOD activity was defined as the amount of enzyme required for inhibiting superoxide-induced oxidation by 50%.

$$\text{SOD inhibition rate } (\%) = \frac{\left[\left(A_{\text{Blank1}} - A_{\text{Blank 3}}\right) - \left(A_{\text{Sample}} - A_{\text{Blank 2}}\right)\right]}{\left(A_{\text{Blank 1}} - A_{\text{Blank 3}}\right)} \times 100\%$$

$$\text{SOD activity (U/mL)} = \text{SOD inhibition rate } (\%) \div 50\% \times (240\mu\text{L} \div 12\mu\text{L}) \times \text{dilution factor}$$

Protein

Protein was determined according to Bradford's method (Bradford 1976), with bovine serum albumin (Sigma-Aldrich: A7906) as the protein standard. A 5 µl volume of each sample and 250 µl of Bradford's reagent (Sigma-Aldrich: B6916) were added to a 96-well flat bottom plate (in triplicate). After incubating for 20 min, absorbance was measured at 595 nm using a microplate reader (BioTek: SYNERGY Mx).

Analysis of data

Sigma Plot Version 11 statistical software was used for all data analysis. At the commencement of the experiment, a one-way analysis of variance (ANOVA) was used to compare the mean tank weight of abalone to ensure that animal sizes and weights were statistically similar across the various treatments. T-test was used to compare the mean weight of sea cucumbers in co-culture. All data were tested for homogeneity (Levene's test) and normality of variances (Shapiro–Wilk's test). A two-factorial ANOVA was used to analyse the effect of both factors (culture method and tank cleaning frequency) on abalone weight, water quality parameters, sludge characteristics, and health parameters. Where a significant result was found ($p < 0.05$), Tukey's post hoc test was used for pairwise comparison.

Results

Survival

The survival rate of abalone in both monoculture and co-culture cleaned either once or twice was high over the duration of the study and showed no significant difference. The survival rate of abalone was $100 \pm 0.00\%$, $95.5 \pm 3.37\%$; $98.5 \pm 0.58\%$ and $99.5 \pm 0.5\%$ (mean $\pm$ SD) for AS1; AS2; A1, and A2, respectively. The survival rate of sea cucumbers was 92% for AS1 and AS2.

Water quality parameters

The culture method and tank cleaning frequency had no significant effect on the ammonia, dissolved oxygen, and pH levels. There were, however, differences in the levels of phosphorus, nitrate, and nitrite (Table 1). AS1 treatment had a significantly higher phosphorous level in water than AS2, A1 and A2. The frequency of cleaning influenced the nitrate level in the water such that tanks cleaned once (AS1 and A1) had a higher nitrate level compared to those cleaned twice (A2 and AS2). However, tanks cleaned twice (AS2 and A2) had a higher nitrite level in water than tanks cleaned once (AS1 and A1) (Table 1).

Abalone weight

When considering the target variable abalone weight, the two-way ANOVA revealed that the interaction term between the two factors, culture method and cleaning frequency, was significant ($F_{(1,476)} = 12.41$, $p < 0.001$); i.e. differences among treatments cannot be attributed to only one or the other main factor. The frequency of cleaning alone had no effect on abalone weight ($F = 0.136$; $p = 0.71$); however, the culture method significantly influenced abalone weight ($F = 6.93$; $p = 0.009$). The results of Tukey's post hoc test show that abalone in the AS1 and A2 treatment groups had significantly higher weight ($p < 0.001$; $p = 0.006$ respectively) than abalone in the A1 treatment group (Fig. 2). Similarly, abalone in the AS1 treatment group had significantly higher weight ($p = 0.026$) than those in the AS2 treatment group. However, the weight of abalone in AS2 did not differ significantly ($p = 0.53$) from that of abalone in A2 treatment groups (Fig. 2).

Table 1 Mean phosphorus, nitrate, nitrite, ammonia, and dissolved oxygen content of the rearing water of the various coculture [sea cucumber (*Neostichopus grammatus*) and abalone (*Haliotis midae*)] and monoculture (abalone only) systems over the duration of the study

	Treatments				Within culture method		Within frequency of cleaning		Interactions	
	AS1	AS2	A1	A2	F	*p*	F	*p*	F	*P*
Phosphorus (mg/L)	0.219 ± 0.029^{a}	0.120 ± 0.020^{b}	0.142 ± 0.017^{b}	0.100 ± 0.014^{b}	5.157	0.026	10.744	0.002	1.772	0.187
Nitrate (mg/L)	1.112 ± 0.078^{a}	0.827 ± 0.096^{b}	1.145 ± 0.088^{a}	0.823 ± 0.084^{b}	0.026	0.871	12.091	< 0.001	0.046	0.830
Nitrite (mg/L)	0.017 ± 0.002^{a}	0.022 ± 0.001^{b}	0.015 ± 0.002^{a}	0.022 ± 0.002^{b}	0.115	0.735	11.625	0.001	0.0231	0.880
Ammonia (mg/L)	0.203 ± 0.054	0.130 ± 0.019	0.128 ± 0.037	0.121 ± 0.020	1.372	0.245	1.279	0.261	0.888	0.349
Dissolved oxygen (mg/L)	8.216 ± 0.081	8.167 ± 0.122	12.01 ± 3.817	8.156 ± 0.102	0.981	0.325	1.043	0.310	0.992	0.323
pH	8.365 ± 0.115	8.336 ± 0.124	8.388 ± 0.116	8.307 ± 0.118	0.001	0.980	0.216	0.643	0.049	0.826

Where AS1 = Abalone + sea cucumber + once weekly tank cleaning; AS2 = Abalone + sea cucumber + twice weekly tank cleaning; A1 = Abalone monoculture + once weekly tank cleaning and A2 = Abalone monoculture + twice weekly tank cleaning). Data are presented as mean ± SE ($n = 21$). Different superscript letters in each row indicate a significant difference between treatments (Tukey, $p < 0.05$).

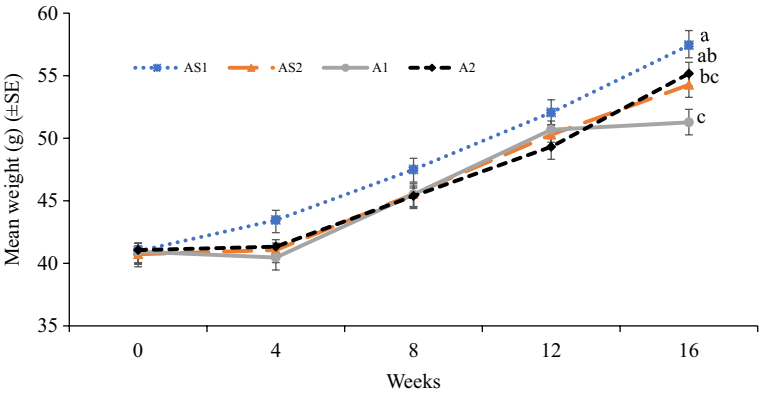

Fig. 2 Mean weight (g) of abalone in coculture and monoculture systems over 16 weeks of study. Where AS1 = abalone + sea cucumber + once weekly tank cleaning; AS2 = abalone + sea cucumber + twice weekly tank cleaning; A1 = abalone monoculture + once weekly tank cleaning, and A2 = abalone monoculture + twice weekly tank cleaning. Different letters above lines are significant (Tukey test, $p < 0.05$). Data are presented as mean ± SE (n = 30)

Abalone shell length

The interaction term between the two factors, culture method and cleaning frequency on abalone shell length, was significant ($F_{(1,476)} = 11.12$; $p < 0.001$), meaning that differences among treatments cannot be attributed to only one or the main factor. The frequency of cleaning did not affect the shell length of the abalone ($p = 0.43$); however, the culture method significantly affected the shell length of the abalone ($p = 0.004$). The shell length of abalone in the AS1, AS2, and A2 treatment groups was similar to one another ($p > 0.05$) and significantly higher ($p < 0.05$) than that of A1 (Fig. 3).

Sea cucumber growth

The weight of sea cucumbers in co-culture cleaned either once or twice was not significantly different at the end of the study ($t = 0.242$; $p = 0.81$). The sea cucumbers in both treatments showed a decrease in weight over the study period (Fig. 4).

Feed conversion rate (FCR) and proportion of feed turned waste per day (PFW)

The FCR, average daily food provided, average waste produced per day, and PFW of abalone in the various treatment were similar and were not influenced by the culture method or the tank cleaning frequency (Table 2). However, the interaction between the culture method and the frequency of tank cleaning was significant for PFW.

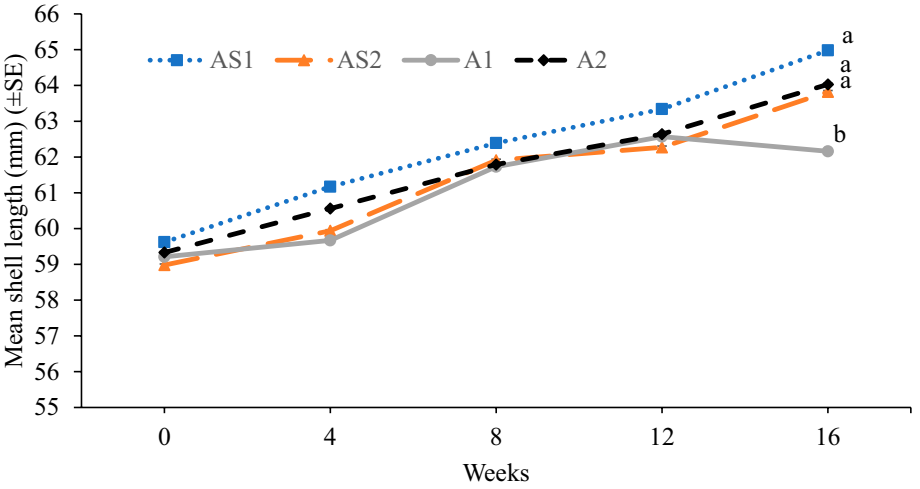

Fig. 3 Abalone's mean shell length (mm) in coculture and monoculture systems over 16 weeks of study. Where AS1 = abalone + sea cucumber + once weekly tank cleaning; AS2 = abalone + sea cucumber + twice weekly tank cleaning; A1 = abalone monoculture + once weekly tank cleaning, and A2 = abalone monoculture + twice weekly tank cleaning. Different letters above lines are significant (Tukey , $p < 0.05$). Data are presented as mean ± SE ($n = 30$)

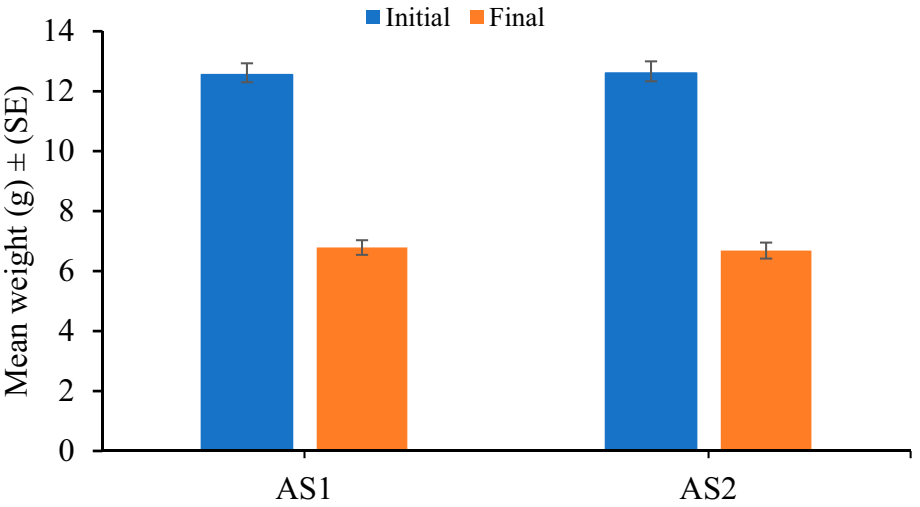

Fig.4 Initial and final mean weight (g) of the sea cucumber *Neostichopus grammatus* in coculture with abalone (*Haliotis midae*) over 16 weeks. AS1 = abalone + sea cucumber + once weekly tank cleaning, and AS2 = abalone + sea cucumber + twice weekly tank cleaning. Data are presented as mean ± SE ($n = 50$)

Table 2 Food provided, waste production, proportion of food turned waste per day (corresponding to food available to sea cucumbers) and feed conversion ratio per basket of abalone (*Haliotis midae*) during the study (9.5 kg of abalone)

	Treatments				Within culture method		Within frequency of cleaning		Interactions	
	AS1	AS2	A1	A2	F	*p*	F	*p*	F	*p*
Average food provided (g/d)	55.139 ± 1.097	53.415 ± 1.176	55.549 ± 0.744	52.864 ± 1.155	0.004	0.948	0.207	0.657	4.344	0.059
Average sludge produced (g/d)	7.117 ± 0.331	7.689 ± 0.362	6.775 ± 0.167	7.270 ± 0.040	2.141	0.169	4.212	0.063	0.021	0.88
Proportion of food turned waste (%/d)	12.900 ± 0.499	14.429 ± 0.818	12.211 ± 0.429	13.774 ± 0.334	1.488	0.246	0.001	0.976	7.87	0.016
Feed conversion ratio	1.594 ± 0.026	1.438 ± 0.043	1.869 ± 0.260	1.491 ± 0.062	1.455	0.251	0.665	0.431	3.844	0.074

Where AS1 = Abalone + sea cucumber + once weekly tank cleaning; AS2 = Abalone + sea cucumber + twice weekly tank cleaning; A1 = Abalone monoculture + once weekly tank cleaning and A2 = Abalone monoculture + twice weekly tank cleaning

Sludge assays

Total organic matter (TOM)

At the end of the first week of the study, the culture method significantly influenced the sediment mean TOM concentration ($F = 8.806$; $p = 0.018$). However, there was no significant effect of cleaning frequency ($F = 2.489$; $p = 0.153$) on TOM, nor was there any interaction ($F = 0.813$; $p = 0.394$) between the two factors for TOM. However, at week 8, the culture method ($F = 4.272$; $p = 0.073$) and frequency of cleaning ($F = 0.004$; $p = 0.953$) had no significant effect on the mean TOM content of the sediment. Neither was a significant interaction term observed between factors ($F = 0.913$; $p = 0.367$). At week 16, the culture method had no significant effect ($F = 0.586$; $p = 0.466$) on the TOM of the sediment; however, the cleaning frequency significantly influenced the sediment's mean TOM. Tanks cleaned once had a higher sludge TOM content than those cleaned twice ($F = 5.523$; $p = 0.047$). The interaction term between the two factors' culture method and cleaning frequency on the sediments' TOM was not significant ($F_{(1,8)} = 0.057$; $p = 0.817$) (Fig. 5).

Carbon

The carbon content of the sediments was similar ($p > 0.05$) among treatments in the first week of the study. Neither the culture method nor the frequency of tank cleaning used in the study showed a significant effect on the carbon content of the sediment in the first week (frequency

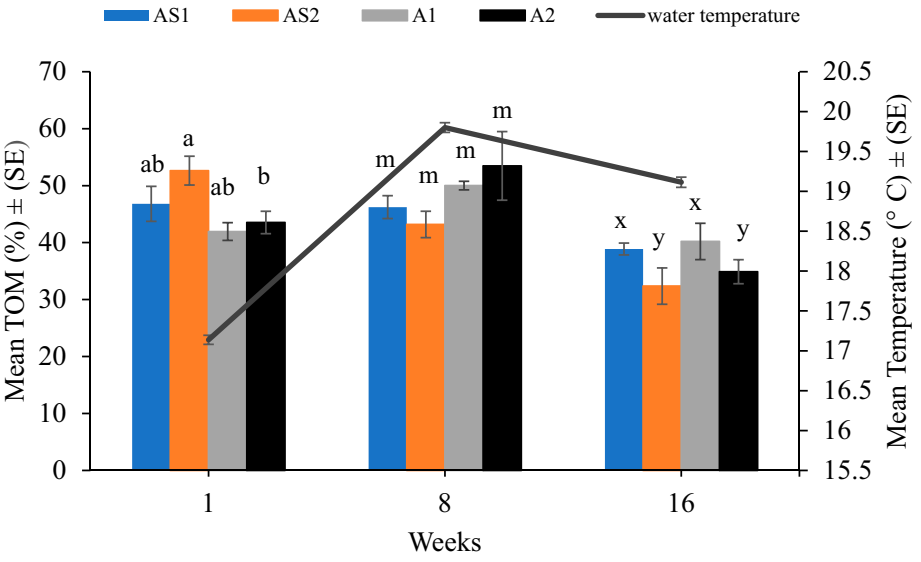

Fig. 5 Mean TOM (%) of sludge from the coculture or monoculture systems over 16 weeks of study. Where AS1 = Abalone + sea cucumber + once weekly tank cleaning; AS2 = Abalone + sea cucumber + twice weekly tank cleaning; A1 = Abalone monoculture + once weekly tank cleaning and A2 = Abalone only + twice weekly tank cleaning). Data are presented as mean ± SE (n = 3). Different letters above each bar indicate a significant difference between treatments ($p < 0.05$)

of cleaning - F = 0.502; $p = 0.499$; culture method - F = 2.553; $p = 0.149$). The interaction term between the two factors, culture method and tank cleaning frequency on the carbon content, was insignificant ($F_{(1,8)} = 0.855$; $p = 0.382$). Similarly, at week eight, the sediment carbon content showed no difference ($p > 0.05$). The culture method and the frequency of cleaning had no significant effect on the carbon content of the sediment at week eight (culture method - F = 0.159; $p = 0.701$; frequency of cleaning - F = 1.051; $p = 0.336$). Likewise, the interaction term between the two factors was insignificant ($F_{(1,8)} = 0.0501$; $p = 0.828$).

However, at week 16, the frequency of cleaning had a significant effect on the sediment carbon content ($F_{(1,8)} = 14.845$; $p = 0.005$) but not the culture method ($F_{(1,8)} = 0.013$; $p = 0.912$). Tukey's post hoc test showed that tanks cleaned once had lower carbon content compared to those cleaned twice (Fig. 6).

Nitrogen

At the end of the first week of the study, the nitrogen content of sediment was not significantly affected by either the culture method (F = 0.404; $p = 0.543$) or the tank cleaning frequency (F = 1.850; $p = 0.211$). The interaction term between the two factors culture method and cleaning frequency was not significant ($F_{(1,8)} = 0.272$; $p = 0.616$). Similarly, at the eighth week of the study, the nitrogen content of sediment was not significantly affected by either the culture method (F = 0.970; $p = 0.354$) or the tank cleaning frequency (F = 1.964; $p = 0.199$). The interaction term between the factors was not significant ($F_{(1,8)} = 1.125$; $p = 0.320$). However, at week sixteen, the frequency of cleaning had a significant effect on the mean nitrogen content of the sediment but not the culture method (F = 0.082; $p = 0.782$). Tanks cleaned once had a lower sludge nitrogen content (F = 7.380; $p = 0.026$) than tanks cleaned twice at week 16. The interaction term between the factors, culture method and cleaning frequency on the nitrogen content of the sediment was not significant ($F_{(1,8)} = 0.283$; $p = 0.609$) (Fig. 7).

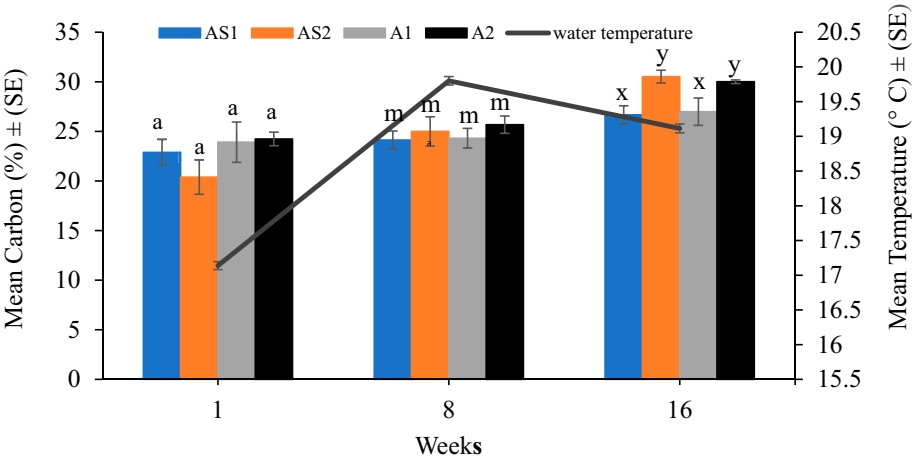

Fig. 6 Mean carbon (%) of sludge from the coculture or monoculture systems over 16 weeks of study. Where AS1 = Abalone + sea cucumber + once weekly tank cleaning; AS2 = Abalone + sea cucumber + twice weekly tank cleaning; A1 = Abalone monoculture + once weekly tank cleaning and A2 = Abalone monoculture + tanks cleaned twice weekly). Data are presented as mean ± SE ($n = 3$)

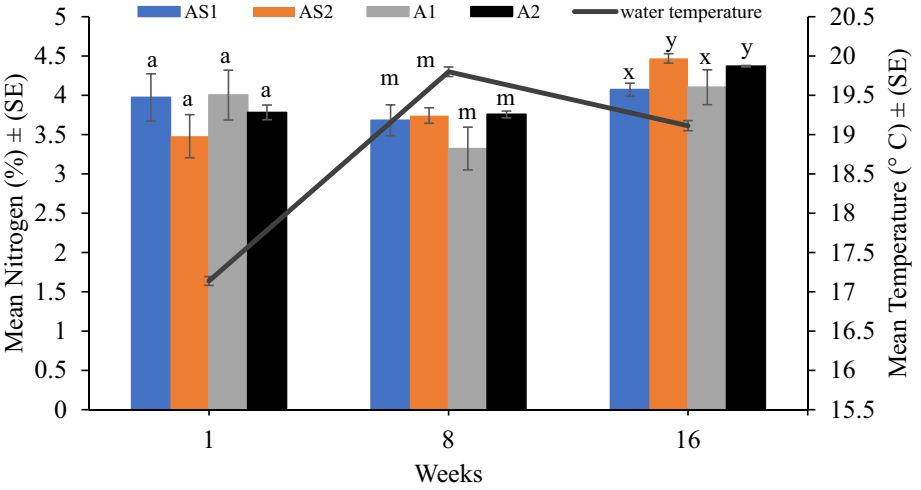

Fig.7 Mean nitrogen (%) of sludge from the coculture or monoculture systems over 16 weeks of study. Where AS1 = Abalone + sea cucumber + once weekly tank cleaning; AS2 = Abalone + sea cucumber + twice weekly tank cleaning; A1 = Abalone monoculture + once weekly tank cleaning and A2 = Abalone monoculture + twice weekly tank cleaning). Data are presented as mean ± SE ($n = 3$). Different letters above each bar indicate a significant difference between treatments (Tukey, $p < 0.05$)

Phosphorus

After the first week, the culture method (F = 0.826, $p = 0.390$) and the frequency of cleaning (F = 5.163; $p = 0.053$) had no significant effect on the mean phosphorus content of the sediment. The interaction term between the two factors was not significant ($F_{(1,8)} = 0.242$; $p = 0.636$). At week eight also, the culture method (F = 0.001; $p = 0.981$) and the frequency of cleaning (F = 0.001; $p = 0.981$) showed no significant effect on the phosphorus content of the sediment. The interaction effect between the two factors was not significant (F = 0.253; $p = 0.628$). Similarly, at week 16, the culture method (F = 0.834; $p = 0.388$) and the frequency of cleaning (F = 1.166; $p = 0.312$) had no effect on the mean phosphorus content of the sediment, and neither was the interaction term between the factors significant (F = 0.007; $p = 0.936$) (Fig. 8).

Sulphur

The culture method significantly affected (F = 5.384; $p = 0.049$) the mean concentration of sulphur in sediments in the first week of the study, unlike the frequency of cleaning, which was not significantly different (F = 1.253; $p = 0.296$). The mean sulphur content of sediment in co-culture was higher compared to those in the monoculture group, while no significant interaction was observed between the culture method and the tank cleaning frequency ($F_{(1,8)} = 0.913$; $p = 0.367$). However, at weeks 8 and 16, the culture method (week 8 – F = 1.217; $p = 0.302$; week 16 – F = 1.112; $p = 0.322$) and the frequency of cleaning (week 8 – F = 2.565; $p = 0.148$ and week 16 – F = 4.085; $p = 0.078$) had no significant effect on the mean sulphur content of the sediment. There were also no significant interactions between these factors at week eight ($F_{(1,8)} = 0.923$; $p = 0.365$) and week 16 ($F_{(1,8)} = 0.069$; $p = 0.799$) (Fig. 9).

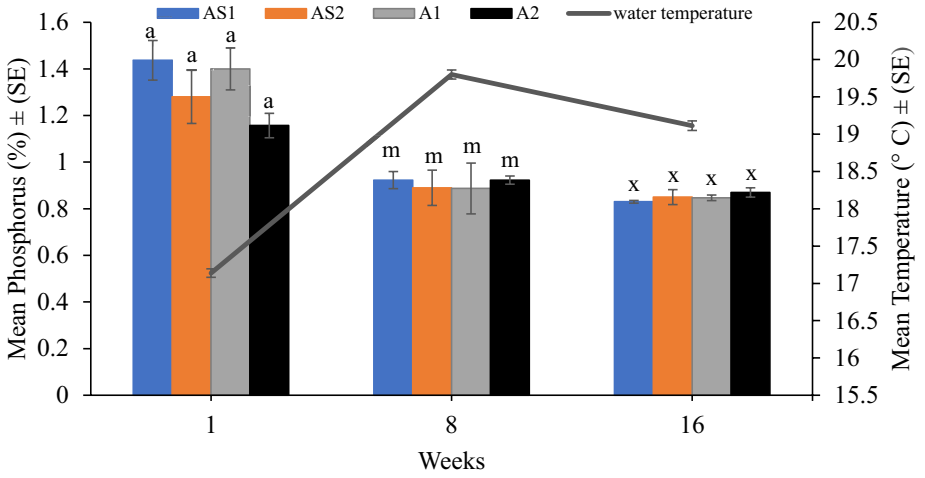

Fig. 8 Mean phosphorus (%) of sludge from the coculture or monoculture systems over 16 weeks of study. Where AS1 = Abalone + sea cucumber + once weekly tank cleaning; AS2 = Abalone + sea cucumber + twice weekly tank cleaning; A1 = Abalone monoculture + once weekly tank cleaning and A2 = Abalone monoculture + twice weekly tank cleaning). Data are presented as mean ± SE ($n = 3$)

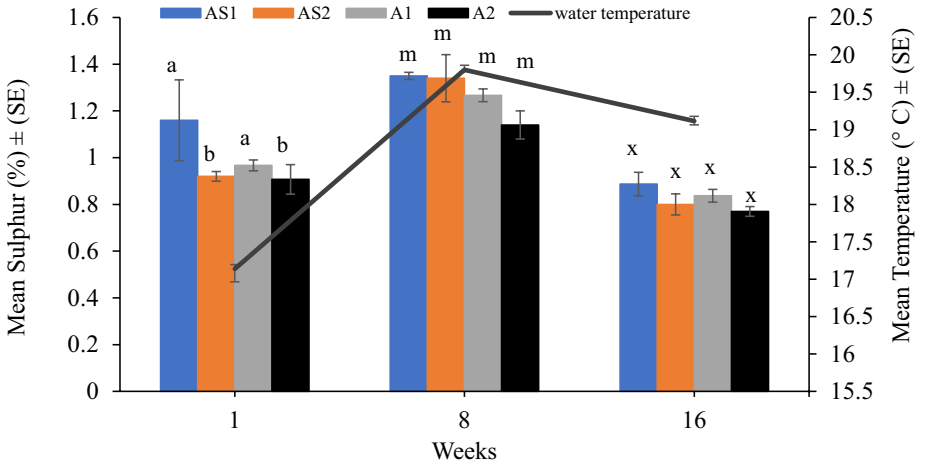

Fig. 9 Mean sulphur (%) of sludge from the integrated multi-trophic aquaculture or monoculture systems over 16 weeks of study. Where AS1 = Abalone + sea cucumber + once weekly tank cleaning; AS2 = Abalone + sea cucumber + twice weekly tank cleaning; A1 = Abalone monoculture + once weekly tank cleaning and A2 = Abalone monoculture + twice weekly tank cleaning). Data are presented as mean ± SE ($n = 3$). Different letters above each bar indicate a significant difference between treatments (Tukey, $p < 0.05$)

Health indices

Abalone

The culture method and the frequency of cleaning had no significant effect on the haemolymph glucose, superoxide dismutase, and total protein (Table 3).

Sea cucumber

The coelomic protein, glucose, and coelomocyte superoxide dismutase values were similar between sea cucumbers in co-culture tanks cleaned once or twice and those from the control treatment (initial sea cucumber coelomocyte before the start of the experiment) (Table 4).

Discussion

Overall effect of tank cleaning frequency in relation to water quality and sludge parameter

Water quality parameters such as nitrite, nitrate, dissolved oxygen, and phosphorus are of great importance in aquaculture as they affect the growth, health, welfare, and well-being of aquatic animals (Boyd 2017; Xia et al. 2017; Liu et al. 2019; Motta et al. 2020).

The frequency of tank cleaning affected the nitrate and nitrite content of the rearing water such that tanks cleaned once weekly had higher nitrate and lower nitrite content when compared to tanks cleaned twice weekly, irrespective of the culture methods. The phosphorus content of the rearing water was influenced by the culture method and the tank cleaning frequency. Co-culture tanks cleaned once had a higher phosphorus content than co-culture tanks cleaned twice, and monoculture tanks cleaned either once or twice. The frequency of cleaning follows a natural phenomenon with some reactions that may either reduce or increase the concentrations and activeness of some chemical elements.

The frequency of cleaning used in this study significantly impacted the sludge characteristics in week 16 and not week eight or week 1 in both sections. Sludge characteristics not affected by the frequency of cleaning in weeks 1 and 8 could be due to the low water temperature experienced in these weeks (17.13 ± 0.06 °C; mean ± SE). However, as the water temperature began to show an increase from the eighth week (19.8 ± 0.06 °C; mean ± SE) difference in the sludge characteristics was observed. This result is similar to Prema et al. (2020), who stated that water temperature influences the decomposition rate as decomposition is faster at high temperatures than at low temperatures.

The sludge characteristics affected by the tank cleaning frequency were carbon and nitrogen. The sludge of tanks cleaned once weekly had lower levels of carbon and nitrogen than those cleaned twice weekly, irrespective of the presence/absence of sea cucumbers in tanks. This result could be because tanks cleaned once underwent more decomposition levels than those cleaned twice, resulting in more microorganisms to remineralize nutrients (Moriarty 1997; Liang and Blaser 2011; Boyd 2017). For example, (Blagodatsky et al. 2010) stated that soil decomposition rate is influenced by the size of the soil organic matter, the biomass of microorganism present, and the microbial activities. Similarly, (Jasmin et al.

Table 3 Mean glucose, total protein of abalone (*Haliotis midae*) haemolymph and superoxide dismutase content of tissue within the various treatments. Where AS1 = Abalone + sea cucumber + once weekly tank cleaning; AS2 = Abalone + sea cucumber + twice weekly tank cleaning; A1 = Abalone monoculture + once weekly tank cleaning and A2 = Abalone monoculture + twice weekly tank cleaning). Data are presented as mean ± SE ($n = 8$)

	Treatments				Within culture Method		Within frequency of cleaning		Interactions	
Parameters	ASH1	ASH2	A1	A2	F	*p*	F	*p*	F	*p*
Glucose (mg/mL)	24.690 ± 5.10	28.478 ± 5.79	31.928 ± 13.98	32.934 ± 9.280	0.506	0.485	0.085	0.773	0.029	0.867
Total protein (mg/mL)	10.473 ± 1.402	15.27 ± 1.337	13.752 ± 1.280	14.303 ± 1.337	0.743	0.394	3.980	0.053	2.511	0.121
Superoxide dismutase (U/mL)	0.209 ± 0.007	0.200 ± 0.007	0.199 ± 0.011	0.224 ± 0.007	0.600	0.442	0.882	0.351	3.879	0.054

Table 4 Mean glucose, total protein, and superoxide dismutase of sea cucumber within the various treatments. Where AS1 = Abalone + sea cucumber + once weekly tank cleaning; AS2 = Abalone + sea cucumber + twice weekly tank cleaning; control (initial sea cucumber coelomocyte). Data are presented as mean ± SE ($n = 12$)

Parameters	ASH1	ASH2	CONTROL	ANOVA RESULT	
				F	*P*
Glucose (mg/mL)	8.501 ± 1.255	7.084 ± 1.397	12.556 ± 2.505	2.551	0.095
Total protein (mg/mL)	0.37 ± 0.053	0.401 ± 0.113	0.189 ± 0.040	2.506	0.098
Superoxide dismutase (U/mL)	0.183 ± 0.009	0.197 ± 0.026	0.198 ± 0.004	1.101	0.352

2020) also stated that microorganisms play a role in the decomposition and mineralization of sludge and uses nutrient available in the sludge as nutrients and energy for growth.

Abalone growth

In aquaculture, the cleanliness of the culture environment is known to affect water quality, affecting the animal's welfare and growth (Lee et al. 2021). In the present study, abalone in monoculture tanks cleaned twice (A2) exhibited higher growth than those cleaned once (A1), possibly due to the frequency of cleaning (twice cleaning). Tanks cleaned twice may have a cleaner environment, composed of few microorganisms and lower microbial activity compared to those cleaned once. This is evidenced by the significantly increased water nitrate and nitrite contents in monoculture tanks cleaned only once weekly in the current study. Conversely, abalone in the co-culture tank cleaned once (AS1) exhibited significantly higher growth than those in the monoculture cleaned once (A1). This result is similar to previous findings on abalone and sea cucumber coculture, which report that abalone in coculture exhibits higher growth than those in monoculture (Kang et al. 2003; Bauer et al. 2019). The phenomena behind the presence of sea cucumber in an abalone tank cleaned once, resulting in higher growth of the abalone, has been linked not only to the consumption of waste but also the consumption of protozoans, bacteria, benthic diatoms, and macroalgae by the sea cucumber (Michio et al. 2003; ChávEz et al. 2011; Gao et al. 2011; Yokoyama 2015).

Abalone in the co-culture tank cleaned once (AS1) had similar growth to abalone in the monoculture tank cleaned twice (A2). It could be that the feeding activities of the sea cucumber in the co-culture tank cleaned once acted as a substitute for the cleaning effect achieved by cleaning tanks twice. While one might expect significantly improved abalone growth in the co-culture tank cleaned twice weekly (AS2) compared to those cleaned once weekly (AS1) because cleaning tanks twice means a healthier tank environment coupled with the presence of sea cucumbers since sea cucumbers play a role in improving growth while consuming waste. The reverse was the case.

This may indicate that the positive impact of the sea cucumbers on abalone in the co-culture tank cleaned twice (AS2) was not evident. This could be as a result of the increased cleaning frequency. The increased cleaning frequency and handling could cause stress to abalone, which impacted the growth but was too little to be detected by the health indicators used in this study (Morash and Alter 2016). Results of the current

study revealed that an interaction exists between the culture methods and the frequency of tank cleaning applied. It can be argued that the frequency of tank cleaning affects the sea cucumber, which reduced feeding activity such that the bioremediation impact of the sea cucumber was not apparent. It could also be that frequent handling of abalone (twice instead of once) affected the growth performance of abalone in co-culture tanks cleaned twice (Morash and Alter 2016). This is corroborated by abalone growth in the co-culture tank cleaned twice (AS2) being similar to that of monoculture cleaned twice (A2). This raises questions such as:

1)What impact does twice cleaning have on sea cucumber in an abalone-sea cucumber co-culture system? And (2) what type of interaction/ reaction occurs in sea cucumber–abalone co-culture tanks cleaned twice weekly?

Sea cucumber growth

Several factors are known to affect the growth of cultured sea cucumbers, including spawning, stocking density, temperature, and the presence of sand substrate in tanks (An et al. 2007; Slater et al. 2009; Robinson et al. 2013; Bauer et al. 2019). Findings from the present study show that the sea cucumbers in co-culture tanks cleaned either once or twice lost weight in a way that did not significantly differ between treatments over the study period. The conditions in the tank, such as the tank bottom being bare, may have been unsuited to the sea cucumbers' longer-term growth and could be the reason for the negative growth experienced by the sea cucumbers.

Nevertheless, the spawning event observed at the beginning of the experiment might have contributed to the weight loss experienced. The experimental period coincides with the period of natural spawning events in this species (September to November), during which the gonads of the sea cucumbers are reabsorbed, leading to weight loss (Foster and Hodgson 1995). The species of sea cucumber used in this study are naturally small and had attained sexual maturity as (Branch et al. 2016) reported that *N. grammatus* found in the wild does not attain more than 15 cm in size. Indices such as ingestion, faecal production and assimilation rate of sea cucumbers measured by Onomu et al. (2023) could have been beneficial in monitoring the welfare of the sea cucumbers. However, the present study was done on a larger scale compared to those of Onomu et al. (2023), which made measuring the indices not feasible. Also, since the sea cucumbers fed only on the waste (uneaten food and faecal waste) produced from the abalone, it was impossible to measure the amount of food ingested by the sea cucumber. Nevertheless, the bottom of the tanks was monitored during each cleaning event for faeces of sea cucumber, which served as evidence of waste consumption.

Health Indices

The superoxide dismutase, protein, and glucose content of abalone hemolymph and sea cucumber coelomocytes in the treatment groups were similar across all treatments. These results, compared to previous studies, indicate that the animals were not stressed. The values of SOD for abalone hemolymph recorded in this study are similar to Lange et al. (2014) and Vosloo et al. (2013a), who reported a SOD range of 0.09 – 0.13 U/mL for *Haliotis laevigata* and 0.08 – 2.18% mg/ protein for *Haliotis midae*, respectively. The result was, however, dissimilar from (Nam et al. 2020), who reported a range of 30 – 35 U/mL for

the Pacific abalone. The results of the total protein of abalone recorded in this study were similar to that of Goosen et al. (2014), who reported a value of 8 – 10 mg/mL.

Glucose values of 23 – 25 mg/mL were recorded for *Haliotis diversicolor* (Cheng et al. 2004b) and 16- 20 mg/mL for *Haliotis iris* (Nollens et al. 2004). Similarly, Carefoot et al. (1993) showed 23- 25 mg/mL values for *Haliotis kamtschatkana*. However, values of 41.4 -99 mg/mL have been reported by (Vosloo et al. 2013a, b) for *H. midae*. The values of 24.69 -32.93 mg/mL recorded in this study are comparable and strongly indicate that the abalone in the current study were not stressed.

The glucose value of the sea cucumber coelomic fluid recorded in this study was similar to that of Chen et al. (2018), who recorded a glucose content of 9 mg/mL for *A. japonicus*. However, the SOD values recorded in this study were dissimilar to those reported by (Yan et al. 2014; Yu et al. 2020) (100 and 90 u/mL, respectively) for *A. japonicus*. Similarly, the values for the sea cucumber coelomic total protein reported in this study were lower than values obtained by (Hawa et al. 1999) (7.24 mg/mL, 2.05 mg/mL, and 1.80 mg/mL) for *Bohadschia mamorata, Stichopus variegatus* and *Stichopus badiontus*, respectively. The dis-similarities of the SOD and the total protein values compared to this study could be due to the different species of sea cucumber used. The values of the total protein and SOD recorded in this study show that the sea cucumber was not stressed, as the values were not different from those of the control recorded before the commencement of the experiment.

Indication of sea cucumber bioremediation

The culture methods (presence/ absence of sea cucumber) used in this study had no significant effect on the water quality parameters. This may be due to the high-water exchange rate (two litres per minute) used in this study. This exchange rate was used as it was the standard operating procedure of the farm where the study was carried out. This result is contrary to the findings of Kang et al. (2003), which stated that the co-culture of abalone (*Haliotis discus*) and sea cucumber (*Stichopus japonicus*) led to a reduction in inorganic nitrogen. However, it should be noted that Kang et al. (2003) used a static system which made it feasible to more clearly determine co-culture effect on water quality, unlike the present study with a high exchange rate.

The result shows that the culture methods (the presence/ absence of sea cucumber) had no significant effect on the sludge characteristics, regardless of string-like faeces observed in the tank as evidence that the sea cucumber utilized the sludge. The presence of sea cucumber showing little to no impact on the sludge could be due to the low water temperatures recorded in the study from week 1 to week 8. Similarly, sludge characteristics being unaffected by the presence of sea cucumber regardless of the increase in water temperature experienced after the eighth week of the study could be due to the increased abalone biomass, feeding rate and faecal production rate compared to the reduced sea cucumber biomass (half the initial weight), making the impact of the sea cucumber on the sludge unapparent. It could also be due to the short time frame the sea cucumber had (a maximum of 6 days) to feed on and impact the sediments before they were flushed out of the tank. This result is consistent with MacDonald et al. (2013), who reported similar carbon and nitrogen content of sediment impacted by *Holothuria forskali* and the control (without *H. forskali*) after five days of daily deposition of *Dicentrarchus labrax* waste. However, after an additional five days of non-deposition of *D. labrax* waste, the sediment impacted by *H. forskali* had a lower nitrogen content compared to the control (without *H. forskali*). In a

similar experiment by MacDonald et al. (2013), the sea cucumber *H. forskali* was reared for eight weeks and fed with *D. labrax* waste. By the end of the experiment, lower carbon was reported for the sediment impacted by the sea cucumber than the control without sea cucumber.

Conclusion

This study aimed to assess the feasibility of co-culturing abalone (*H. midae*) and sea cucumber (*Neostichopus grammatus*) and the impact of tank cleaning frequency on abalone growth, water quality and sludge characteristics,

The results of this study confirm that growth optimisation in abalone farming (monoculture) can be achieved by cleaning tanks twice instead of once weekly. However, when abalone are cocultured with sea cucumbers, tanks need only be cleaned once a week without compromising abalone growth.The co-culture of the sea cucumber and abalone species led to improved abalone growth at the detriment of sea cucumber growth. Should the aim of co-culture be to obtain maximum growth of both species, including bioremediation, then the coculture of both species in this study might not be regarded as feasible. However, should the aim be to use sea cucumbers to enhance abalone growth and reduce labour costs involved in abalone tank cleaning (irrespective of the sea cucumber's growth), this form of coculture may be considered feasible.

Contrary to expectations, the frequency of tank cleaning proved to be the most crucial factor influencing the water quality and sludge characteristics. These findings could be beneficial not just to the abalone industry but to the aquaculture industry, where tank cleaning is a necessity. It is recommended that future research use smaller sea cucumbers at a low density which are provided a substrate (sand) to optimize their living conditions when in co-culture with abalone.

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Data availability The data that support the findings of this study are available on request from the corresponding author, A.J. Onomu.

Declarations

Competing interests The authors declare no competing interests.

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