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Feeding indicators and bioremediation ability of warty sea cucumber *Neostichopus grammatus* fed potential wastes from abalone *Haliotis midae* farming

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ABSTRACT: The reuse of aquaculture waste to produce valuable sea cucumber biomass promises to reduce environmental impacts of aquaculture and increase incomes. This study aimed to assess the suitability of potential waste from abalone farming as feed for the warty sea cucumber *Neostichopus grammatus* and infer bioremediation effects from sea cucumber feeding. Four diets: abalone waste, fermented algae (sea lettuce *Ulva lacinulata*) mixed with sand (U+S), abalone pelleted feed, and sand, were fed to *N. grammatus* under controlled conditions for 6 wk. Sea cucumbers fed sand exhibited a significantly higher ($p < 0.001$) ingestion rate (1.35 ± 0.04 g ind.$^{-1}$ d^{-1}) than other diets. Those fed U+S (1.03 ± 0.02 g ind.$^{-1}$ d^{-1}) had a significantly higher ingestion rate ($p < 0.001$) than those fed abalone waste and pellet. However, the ingestion rate was similar for pellets (0.67 ± 0.07 g ind.$^{-1}$ d^{-1}) and abalone waste (0.54 ± 0.02 g ind.$^{-1}$ d^{-1}) ($p = 0.138$). Ingestion and faecal production rates were inversely influenced by the diet's total organic matter content (TOM). The faecal TOM of sea cucumber fed abalone waste, pellet, and U+S decreased from the TOM of the diet by 37.53 ± 1.63 % ($p < 0.001$), 31.39 ± 0.53 % ($p < 0.001$), and 48.83 ± 9.81 % ($p = 0.03$), respectively. There was a decrease (37.74 ± 0.12 %, $p < 0.001$) in the carbon content of sea cucumber faeces fed pellet compared to the feed, while there was no difference in nitrogen ($p = 0.08$) content. The carbon content of sea cucumber faeces fed U+S was not significantly different ($p = 0.11$) from that of the feed. However, the nitrogen content decreased by 63.00 ± 8.27 % ($p = 0.03$). This study shows that wastes from abalone farming are acceptable food for *N. grammatus*, affirms the bioremediation potential of *N. grammatus* through integrated multitrophic aquaculture, and recommends its coculture trial with abalone.

KEY WORDS: Integrated multitrophic aquaculture · Ingestion rate · Total organic matter · Carbon · Nitrogen

1. INTRODUCTION

Fed aquaculture is generally characterised by the release of particulate organic matter (faeces, unconsumed feed) and inorganic nutrients that cause environmental deterioration (MacDonald et al. 2013). One method proposed to reduce its negative impact is integrated multitrophic aquaculture (IMTA) (Chopin et al. 2012). IMTA involves culturing species of different trophic levels in the same system such that the waste from one serves as food for the other (Chopin et al. 2012). This reduces the nutrient load discharged to the environment while maximising production output.

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Sea cucumbers are valued as food and traditional medicine in China and Southeast Asia (Cai et al. 2020). The demand for sea cucumbers is high, which supply cannot meet (Juinio-Meñez et al. 2017, González-Wangüemert et al. 2018, López-Rocha & Velázquez-Abunader 2019). Consequently, all possible sea cucumber sources are being scrutinised, including those of less commercial importance to supplement wild catches (Han et al. 2016). Sea cucumbers have been widely proposed as an IMTA candidate to reduce the nutrient discharge from aquaculture activity (Zamora et al. 2018, Chary et al. 2020). Sea cucumbers utilise biodeposits from aquaculture as food and reduce nutrient loading by their feeding activity, thereby reducing the environmental impact of its effluent (Slater & Carton 2007, Slater et al. 2009, Mahmoud 2011, Ren et al. 2012, Hannah et al. 2013, Qi et al. 2013, Yu et al. 2016, Israel et al. 2019, Grosso et al. 2021). An increasing number of studies on sea cucumber aquaculture are being conducted; however, most of these studies are directed at *Apostichopus japonicus* and *Holothuria scabra,* which are both commercially valuable sea cucumber species for aquaculture (Watanabe et al. 2012, Yokoyama 2013, Sun et al. 2015, Juinio-Meñez et al. 2017, Wang et al. 2017, Robinson et al. 2019).

The warty sea cucumber *Neostichopus grammatus* (Clark, 1923) is a stichopid deposit-feeding sea cucumber endemic to South Africa. It is found on sand substrate on the intertidal area of rocky shores from Cape Agulhas in Cape Town (34° 50′ 0″ S, 20° 00′ 5″ E) to Cape Vidal in Kwazulu Natal (28° 7′ 26″ S, 32° 33′ 26″ E) (Thandar 1987). *N. grammatus* is neither consumed nor fished in South Africa. Its gut contents consist of a large proportion of sand grains, algae, coralline algae, bacteria, crustacean appendages, sea urchin spines, other calcareous fragments, gastropods, and bivalve shells (Foster & Hodgson 1996). It has not been previously cultured nor explored in IMTA for waste bioremediation; as such, there is no information on its husbandry. Also, it is not known if this species undergoes seasonal aestivation or hibernation as reported for other species (Ji et al. 2008, Gao et al. 2011).

South Africa is the third-largest producer of abalone *Haliotis midae* worldwide, producing about 749 metric tons of abalone worth in 2019, estimated at R 491 million (DFFE 2022). In South Africa, land-based abalone farming is practised where abalone are fed intensively with commercial pelleted feed and seaweed. Hence, abalone farming is no exception in terms of high nutrient waste outputs. The waste (uneaten food and faeces) from intensive abalone culture is channelled into the sea, most times without treatment, causing potential environmental deterioration (Probyn et al. 2017).

This study aims to identify suitable diets for *N. grammatus* while simultaneously assessing the IMTA feasibility of *N. grammatus* and *H. midae*. The study assesses the survival, palatability, faecal production rate, and absorption efficiency of *N. grammatus* when fed on waste from abalone cultured under controlled conditions. It also analyses the relationships between the ingestion rate, faecal production rate, absorption efficiency, and the total organic matter (TOM) content of the feed. Thus, the study also assesses the ability of sea cucumbers to reduce the nutrient load of these wastes. This study aimed to provide the first data on the aforementioned parameters, which will serve as a baseline for further studies.

2. MATERIALS AND METHODS

2.1. Experimental animals

The experiment was conducted at Wild Coast Abalone (Pty) Ltd, located in the Eastern Cape province of South Africa (32° 45′ 06″ S, 28° 16′ 28″ E). Juvenile sea cucumbers (10–24 g) were collected from the wild by divers within the vicinity of the farm (2 km), placed in buckets containing seawater, and transported to the abalone farm, where they were acclimatised in holding tanks at a density of 7 sea cucumbers per tank for approximately 2 wk. During acclimatisation, sea cucumbers were fed with abalone waste (biodeposit). Before the commencement of the experiment, sea cucumbers were starved for 48 h to evacuate gut content fully and were weighed (Zamora & Jeffs 2012), after which they were allocated to experimental tanks. The water temperature and dissolved oxygen were measured weekly at 09:00 h using an Oxy-Guard Handy Polaris probe (OxyGuard International).

2.2. Experimental design

Sixteen flow-through tanks (L × W × H: 405 × 255 × 275 mm) without sand substrate were used for the experiment. Treatments were randomly distributed across the tanks. The tanks were fitted with an airline, and air was pumped in; tanks had a water volume of 24 l and a flow rate of approximately 1.6 l min^{-1}. The replacement rate was 6% new water min^{-1}, i.e. the total tank volume was replaced approximately every 17 min.

The experiment was made of 4 treatments and 4 replicates. Sea cucumbers were stocked at a density of 5 animals per tank with an average weight of 16.70 ± 0.55 g ind.$^{-1}$, resulting in a stocking density of 48 animals m^{-2} of tank floor and 801.6 g m^{-2} of tank floor. This density was chosen as information about appropriate stocking density is only available for juvenile sea cucumbers of commercial value, which differs for each species. This is the first study on *Neostichopus grammatus;* as such, there is no information on the appropriate stocking density for this species.

2.3. Experimental feeds

Four experimental feeds were used for the trial. The experimental feeds were abalone biodeposit (hereafter referred to as abalone waste), sand, commercially produced abalone feed (Abfeed^{TM} from Marifeed, hereafter referred to as pellet), and fermented algae (sea lettuce *Ulva lacinulata*) mixed with sand (U+S) (Table 1). Abalone waste and U+S mixed with sand were used in this study, as these diets can be used as effective feeds for juvenile sea cucumbers (Yuan et al. 2006, An et al. 2007). Sand was used in this study as a control treatment to mimic the diet of *N. grammatus* in the natural environment since sand makes up a high percentage of its gut content and other deposit-feeding sea cucumbers (Foster & Hodgson 1995, Mercier et al. 1999). Abalone pellet was also used as a diet in this study as cultured abalone are fed this, and uneaten abalone pellet forms part of the waste (faeces and pseudofaeces) derived from abalone farming.

The experiment was conducted for 6 wk. Abalone waste was sourced from Wild Coast Abalone Farm and collected twice throughout the experiment. The variability of abalone waste was extremely low due to the timeframe of the collection and the strictly adhered feeding regime of the farm where it was sourced. The abalone waste was hand-picked to remove large chunks of pellets, and the slurry was then passed through a 53 µm mesh sieve to reduce the water content, after which, it was stored at −18°C in a deep freeze. Sand used for U+S and sand treatments was collected from the top surface (about 2 mm) of the natural environment of the sea cucumbers. Sand was collected by scooping, after which the sand was sieved across a 2 mm mesh and frozen at −18°C. Fresh *U. lacinulata* (cultured in abalone effluent at Wild Coast Abalone) was added to water and then mashed with a hand blender. The slurry was mixed with sand from the natural environment of the sea cucumbers at an *Ulva*:sand ratio of ~960:700 (by g wet weight [wt]). The ratio of *U. lacinulata* and sand was used to achieve the desired consistency and palatability of the diet (Al-Harrasy 2016). The mixture was left to ferment at ambient temperature for 2 wk. Afterward, the mixture was sieved over a 53 µm mesh to reduce the water content and stored at −18°C.

Animals were fed ad libitum with 10 g dry wt of ground pellets (~1 mm crumbs) and 10 g wet wt of each wet feed (abalone waste, U+S, and sand) daily. These feeds were given to the sea cucumbers daily to mimic the daily deposition of waste from an abalone tank. Wet feeds were allowed to defrost for 2 h before provision to the animals. The airflow and seawater supply were interrupted before the introduction of the feeds. The wet feed was added as a slurry and left for 30 min to settle; thereafter, the airflow and seawater supply were restored. Tanks were cleaned once weekly using a sponge; the animals were gently moved to a clean tank before cleaning. Any dead animals were recorded, discarded, and not replaced during the experiment.

2.4. Sample collection

For the estimation of *N. grammatus* feeding rate, left-over feed and faeces were collected from each tank daily for the first 8 d of the study. It was easy to identify and cleanly collect the fresh faeces (string-

Table 1. The assay content of experimental feeds (abalone waste, *Ulva lacinulata* mixed with sand, pellet, and sand). TOM: total organic matter. Data are mean ± SE

Feed	Nitrogen (%)	Carbon (%)	Crude protein (%)	Crude lipid (%)	Crude fibre (%)	Carbohydrate (%)	TOM (%)
Abalone waste	2.69 ± 0.09	21.8 ± 2.00	16.25 ± 0.09	1.3	2.8	13.65	34.80 ± 0.24
Ulva lacinulata + sand	1.23 ± 0.15	11.1 ± 0.02	7.69	1	3.6	2.01	22.18 ± 0.95
Pellet	4.44 ± 0.14	41.2 ± 0.30	27.75	4.1	0.7	53.05	95.50 ± 0.16
Sand	0.03 ± 0.01	5.65 ± 0.35	0.19	0.3	0.5	0.21	5.43 ± 0.23

like shaped); this was siphoned each morning by 09:00 h, oven-dried at 60°C for 48 h to constant weight, weighed, and recorded daily. Uneaten feed was dried, weighed, and recorded daily as well.

Ingestion rate (IR), faecal production rate (FPR), and apparent assimilation efficiency (AAE) were calculated as per Yuan et al. (2006).

$$\text{IR (g ind.}^{-1}\text{ d}^{-1}) = c/n/t \quad (1)$$

$$\text{FPR (g ind.}^{-1}\text{ d}^{-1}) = Wf_a/n/t \quad (2)$$

$$\text{AAE (\%)} = 100 \times (\text{IR} - \text{FPR})/\text{IR} \quad (3)$$

$$\text{Percentage food uptake (body weight}^{-1}\text{ d}^{-1}) = (\text{IR/initial body weight}) \times 100 \quad (4)$$

- where c = dry wt of food consumed in a tank (dry wt of food given – dry wt of uneaten food)
- n = number of sea cucumbers in the tank
- t = time in d
- Wf_a = dry wt (g) of the faeces of the sea cucumbers in each tank.

2.5. Diet and faecal assays

The faeces collected from each tank during the 8 d of faecal collection were pooled together for faecal assay (4 replicates per treatment were used for assay). Samples of diet (4 replicates of freeze-dried samples of abalone waste, U+S, sand alone, and commercial abalone feed) were sent to an accredited laboratory (LUFA Nord-West), where they were analysed for lipid, ash, and fiber according to European standard (VO (EG) 152/2009 Annex III). The total nitrogen and carbon of diets and faeces were determined by the dry combustion method (DIN EN16168:2012-11). Carbohydrate was calculated by difference [100 − (moisture + ash + protein + fat + dietary fiber)]. The TOM was determined for diets and faeces, according to Byers et al. (1978). Samples were oven-dried at 60°C for 48 h and weighed. Oven-dried samples were then placed in a furnace at 500°C for 6 h for complete combustion, after which samples were weighed. Protein was calculated by multiplying the nitrogen content by 6.25.

2.6. Sea cucumber weight measurement

Sea cucumber was left on a sponge for 2 min to dry off surface water and expel water from the cloaca, and coelomic cavity, after which they were measured with a balance (g ± 0.01). The wet wt of sea cucumber was measured at the beginning, Week 4, and end of the experiment. Growth was calculated as a mean tank change in weight.

2.7. Statistical analyses

At the beginning of the experiment, a tank mean was calculated from the 5 sea cucumbers stocked in each tank. The mean weight was compared using a 1-way analysis of variance (ANOVA) to ensure there was no bias in the animal sizes in the various treatments. All data were tested for homogeneity (Levene) and normality of variances (Shapiro-Wilk). A Kruskal-Wallis 1-way ANOVA was used when the homogeneity of variance or the normality of distribution test failed. Where a significant result was found ($p < 0.05$), Tukey's and Dunn's post hoc tests were used for pairwise comparisons for ANOVA and Kruskal-Wallis 1-way ANOVA, respectively. A 1-way ANOVA (using IBM's SPSS Statistics v25) was used to test growth, IR, FPR, and AAE across treatments. Student's t-test was used to test the difference between feed and faeces' TOM, carbon and nitrogen content. The relationships between IR, FPR, AAE, and TOM was analysed using simple linear regression. All data are given as mean ± SE.

3. RESULTS

3.1. Temperature and dissolved oxygen

The water temperature during the study period was 20.3 ± 0.33°C (n = 6), and dissolved oxygen was 7.27 ± 0.07 mg l^{-1} (n = 96). These values were within abalone's normal and acceptable water quality parameter ranges.

3.2. Survival

Sea cucumber survival was high across all treatment groups; 100% in the abalone waste, U+S, and sand treatment groups and 95% in the pellet group. The single animal lost in the pellet treatment group was lost close to the end of the experiment, and the animal showed no signs of skin ulceration. Skin ulceration is a sign of poor health in sea cucumbers. Two animals were lost as escapes in the U+S treatment group through the tank's drainage system and were not recovered/replaced.

3.3. Ingestion rate (IR)

There was a significant difference in the IR (H_3 = 76.96, $p < 0.001$) among diets. The sea cucumbers fed on sand (1.35 ± 0.04 g ind.$^{-1}$ d^{-1}) exhibited a significantly higher ($p < 0.001$) IR than those fed other feeds (Fig. 1). The IR of those fed U+S (1.03 ± 0.02 g ind.$^{-1}$ d^{-1}) was significantly higher (Dunn, $p < 0.001$) than those fed abalone waste and pellet. Those fed pellets (0.67 ± 0.07 g ind.$^{-1}$ d^{-1}) and abalone waste (0.54 ± 0.02 g ind.$^{-1}$ d^{-1}) had similar IRs (Dunn, p = 0.138) and had the lowest IRs. The sea cucumber fed abalone waste, pellet, U+S, and sand consumed an average of 3.4, 3.9, 6.1, and 8% of their body weight d^{-1}, respectively. There was a significant inverse relationship ($p < 0.001$) between the IR and the TOM of the diets, such that the IR decreased with an increase in the TOM content of the diet (Table 2).

3.4. Faecal production rate (FPR)

The FPR of sea cucumbers differed significantly across different treatments (H_3 = 33, $p < 0.001$). Sea cucumbers fed sand produced significantly more (Dunn, $p < 0.001$) faecal waste (0.33 ± 0.04 g ind.$^{-1}$ d^{-1}) than those fed abalone waste (0.15 ± 0.02 g ind.$^{-1}$ d^{-1}) and pellet (0.12 ± 0.03 g ind.$^{-1}$ d^{-1}) but was not different from those fed U+S (0.22 ± 0.02 g ind.$^{-1}$ d^{-1}) (Fig. 2). Those fed U+S produced more (Dunn, $p < 0.05$) faecal waste than those fed pellets. The FPR of those fed abalone waste was not different (Dunn, p = 0.83) from those fed pellets. A significant inverse relationship ($p < 0.001$) was also observed between the FPR of the sea cucumbers and the TOM of the diet. The FPR decreased with increasing TOM content of the diet (Table 2).

3.5. Assimilation/nutrient uptake

The TOM of the diets was 96.30 ± 0.33% for pellet, 34.80 ± 0.24% for abalone waste, 22.18 ± 0.33% for U+S, and 5.43 ± 0.23 for sand (Fig. 3). Tukey post hoc test showed that the TOM of pellets was significantly higher ($p < 0.001$) than those of all other treatments (abalone waste, U+S, and sand). Also, the TOM of abalone waste was significantly higher ($p < 0.001$) compared to that of U+S and sand. Similarly, the TOM of U+S was significantly higher ($p < 0.001$) than sands. The TOM of the sea cucumber faeces was 65.46 ± 0.61, 21.74 ± 1.44, 11.35 ± 3.3, and 6.51 ± 1.51% for pellet, abalone waste, U+S, and sand, respectively (Fig. 3). The faecal TOM of sea cucumbers fed pellet was significantly higher than the faecal TOM of those fed abalone waste, U+S and sand ($p < 0.001$). Similarly, the faecal TOM of sea cucumber fed abalone waste was significantly higher compared to the faecal TOM of sea cucumber fed U+S

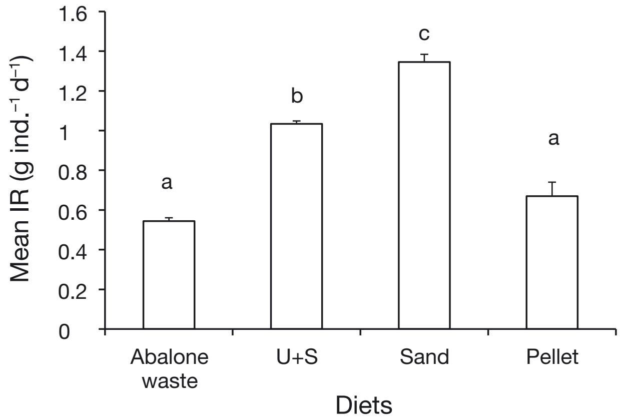

Fig. 1. Mean (+SE) ingestion rate (IR) of *Neostichopus grammatus* fed 4 experimental diets n = 32). Different letters above each bar indicate a significant difference between treatments (Dunn, $p < 0.05$). U+S: *Ulva lacinulata* mixed with sand

Table 2. Regression results for the relationships between ingestion rate (IR), faecal production rate (FPR), apparent assimilation efficiency (AAE) and the total organic matter content of the diets (n = 128)

Parameter	Unstandardized coefficients		Standardized coefficients		
	β	SE	β	*t*	p
IR (g ind.$^{-1}$ d^{-1})	1.119	0.045	−0.008	−6.498	<0.010
FPR (g ind.$^{-1}$ d^{-1})	0.276	0.023	−0.003	−4.193	<0.001
AAE (%)	71.374	2.972	0.179	2.118	0.036

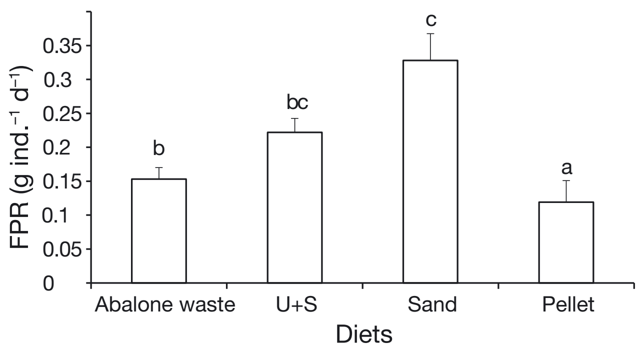

Fig. 2. Mean (+SE) faecal production rate (FPR) of *Neostichopus grammatus* fed 4 experimental diets (n = 32). Different letters above each bar indicates a significant difference between treatments (Dunn, $p < 0.05$). U+S: *Ulva lacinulata* mixed with sand

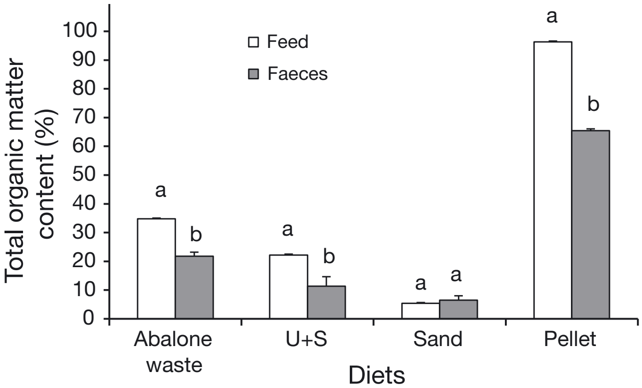

Fig. 3. Mean (+SE) total organic matter of feed and resultant faeces of *Neostichopus grammatus* fed 4 experimental diets (n = 4). Different letters above each bar within a diet indicate a significant difference between feed and faeces (*t*-test, $p < 0.05$). U+S: *Ulva lacinulata* mixed with sand

($p = 0.019$) and sand ($p = 0.002$). The faecal TOM of sea cucumber fed pellet, abalone waste, and U+S differed significantly from the TOM of the diet by 31.39% ($t = 47.63$, $p < 0.001$); 37.53% ($t = 17.13$, $p < 0.001$) and 48.83% ($t = 3.15$, $p = 0.03$), respectively; whereas the faeces TOM of sea cucumbers fed sand was not different from the TOM of the feed ($t = -0.71$, $p = 0.52$) (Fig. 3).

The carbon content of the diets was 41.20 ± 0.30, 21.80 ± 2.00, 11.30 ± 0.20, and 5.65 ± 0.35% for pellet, abalone waste, U+S, and sand, respectively (Fig. 4). Tukey's post hoc test showed that the carbon content of pellet was significantly higher ($p < 0.001$) than those of other treatments (abalone waste, U+S, and sand). Similarly, the carbon content of abalone waste was significantly higher ($p = 0.007$) than that of U+S and sand. However, no significant difference ($p = 0.059$) was observed between the carbon of U+S and sand (Fig. 4)

The percentage carbon content of sea cucumber faeces was 25.65 ± 0.05, 26.25 ± 0.65, 10.05 ± 0.83, and 6.48 ± 0.19 for sea cucumbers fed pellet, abalone waste, U+S, and sand, respectively. The faecal carbon of sea cucumber fed pellet was similar to those of sea cucumber fed abalone waste ($p = 0.331$) but was significantly higher than those fed sand and U+S ($p < 0.001$). Moreso, the faecal carbon of sea cucumber fed abalone waste was significantly higher than those fed U+S and sand ($p < 0.001$). The faecal carbon of sea cucumber fed U+S was also higher than those fed sand ($p = 0.004$). There was a 37.74% decrease ($t = 51.13$, $p < 0.001$) in the percentage carbon content of faeces of sea cucumber fed pellets. However, the faecal carbon (%) of sea cucumber fed abalone waste ($t = -2.31$, $p = 0.15$) was similar to the feed. The carbon content (%) of sea cucumber faeces fed U+S decreased by 9.46% but was not significantly different from the feed ($t = 2.79$, $p = 0.11$). However, the carbon content of sea cucumber faeces fed sand was similar ($t = -1.85$, $p = 0.21$) to that of its feed.

The nitrogen content (%) of the diets was 4.40 ± 0.14, 2.69 ± 0.09, 1.37 ± 0.14, and 0.03 ± 0.01 for pellet, abalone waste, U+S, and sand, respectively. The pellet's nitrogen content (%) was significantly higher ($p = 0.001$) than those of all other diets. Similarly, the nitrogen content (%) of abalone waste was significantly higher than those of U+S ($p < 0.001$) and sand ($p = 0.003$), and that of U+S was also significantly higher than those of sand ($p = 0.003$). The percentage nitrogen content of sea cucumber faeces was 2.86 ± 0.45, 3.29 ± 0.09, 0.51 ± 0.11, and 0.31 ± 0.04 for sea cucumbers fed pellet, abalone waste, U+S, and sand, respectively (Fig. 5). The faecal nitrogen content (%) of sea cucumber fed pellet was similar to that of abalone waste ($p = 0.611$) but was significantly higher ($p = 0.01$) than U+S ($p = 0.004$) and sand ($p = 0.002$). Also, the faecal nitrogen content (%) of sea cucumber fed abalone waste was significantly higher than those fed U+S ($p = 0.004$) and sand ($p = 0.002$). Sea cucumbers fed pellet had a 35.59% decrease ($t = 3.33$, $p = 0.08$) in faecal nitrogen when compared to the feed (Fig. 5). The faecal nitrogen content of sea cucumber fed U+S significantly decreased by 58.54% ($t = 6.11$, $p = 0.03$) from that of the feed (Fig. 4). The faecal nitrogen content (%) of sea cucumber fed abalone waste and sand

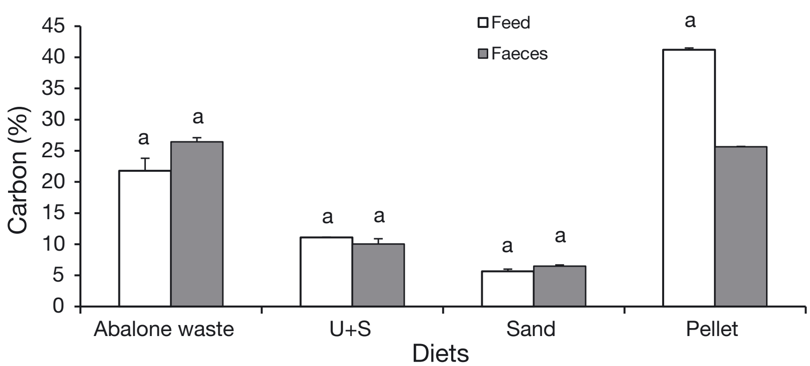

Fig. 4. Carbon content (%) of feed and faeces of *Neostichopus grammatus* fed 4 experimental diets. Data are mean ± SE (n = 4). Different letters above each bar within a column indicate a significant difference between feed and faeces (*t*-test, $p < 0.05$). U+S: *Ulva lacinulata* mixed with sand

were similar to those of the feed ($t = 3$, p = 0.33; $t = -1.992$, p = 0.19, respectively) (Fig. 5).

3.6. Apparent assimilation efficiency (AAE)

The mean AAE was significantly different among treatments ($H_3 = 12.47$, p = 0.006). Sea cucumbers fed abalone waste had the lowest AAE (68.5 ± 4.86). In comparison, those fed pellets had the highest AAE (84.35 ± 2.88) (Dunn, p < 0.05) (Fig. 6). However, the AAE of sea cucumbers fed pellets was not significantly different (Dunn, p > 0.05) from those fed either U+S (78.53 ± 1.96) or sand (72.61 ± 5.0) (Fig. 6). Similarly, the AAE of sea cucumbers fed abalone waste was not significantly different (p > 0.05) from those fed U+S or sand. Also, a significant positive relationship (p = 0.036) was observed between the AAE and the TOM of the diet. AAE increased with an increase in the TOM of the diet (Table 2).

3.7. Growth

At the beginning of the experiment, the animals' mean weight was compared to ensure there was no bias in the animal sizes in the various treatments ($F_{3,76} = 0.136$, p = 0.94). At Week 4, a significant difference ($F_{3,74} = 3.19$, p = 0.028) in growth was observed. The weight of sea cucumbers in the U+S, pellet and sand treatment groups showed a slight decrease in weight, while sea cucumbers fed the abalone waste treatment showed a slight increase in weight (10.02%) and was only significantly greater than those fed pellets (Tukey, p = 0.05) (Fig. 7). However, by Week 6, there was an overall reduction in the weight of sea cucumbers across all treatments. Sea cucumber weight was not different across treatments by Week 6 ($F_{3,72} = 1.34$, p = 0.27). The average specific growth rates of animals in the different diet treatments were −0.31, −0.37, −0.58, and −0.63 for abalone waste, U+S mixed with sand, pellet, and sand, respectively.

4. DISCUSSION

This study aimed to assess the survival, ingestion rate, FPR, absorption efficiency, and bioremediation ability of *Neostichopus grammatus* when fed on waste from abalone *Haliotis midae* culture. The survival of sea cucumbers in this study is high (ca. 98%)

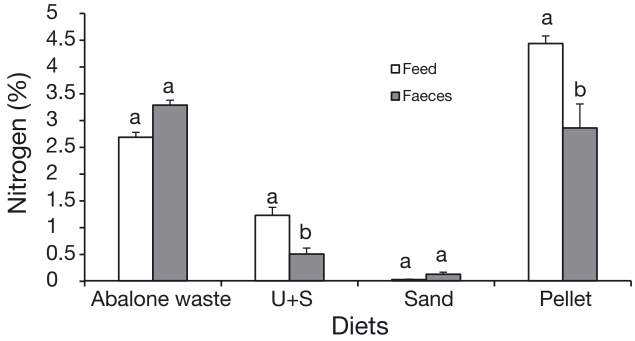

Fig. 5. Nitrogen content (%) of feed and faeces of *Neostichopus grammatus* fed 4 experimental diets. Data are mean ± SE (n = 4). Different letters above each bar within a column indicate a significant difference between feed and faeces (*t*-test, p < 0.05). U+S: *Ulva lacinulata* mixed with sand

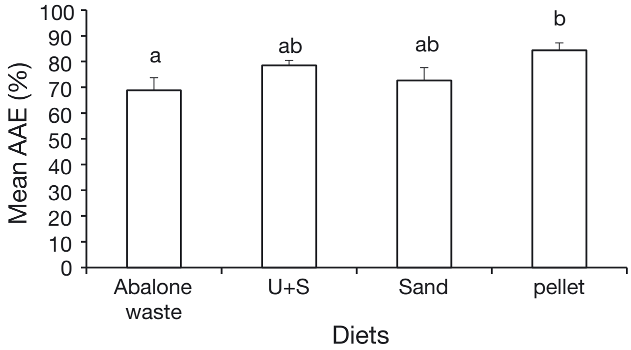

Fig. 6. Mean (+SE) apparent assimilation efficiency (AAE) of *Neostichopus grammatus* fed 4 experimental diets (n = 32). Different letters above each bar indicate a significant difference between treatments (Tukey, p < 0.05). U+S: *Ulva lacinulata* mixed with sand

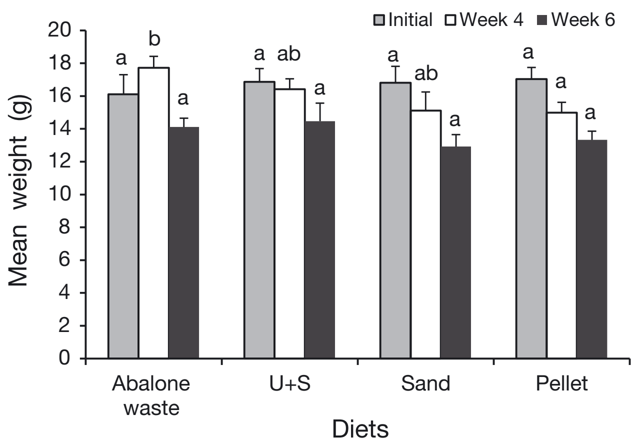

Fig. 7. Mean (+SE) weight of *Neostichopus grammatus* fed 4 experimental diets over a period of 6 wk (n = 20). Different letters above each bar indicate a significant difference between diet treatments for each time period (Tukey, p < 0.05). U+S: *Ulva lacinulata* mixed with sand

and illustrates *N. grammatus* as a potential aquaculture and IMTA species.

4.1. Ingestion rate (IR)

IR is used for assessing feed quality as it shows how palatable and acceptable a feed is. The IR of *N. grammatus* fed the experimental diets was in the order of sand > U+S > pellets = abalone waste. The IRs recorded in the present study were low compared to Zamora & Jeffs (2012), who reported an IR ranging from 3–9 g ind.$^{-1}$ d^{-1} when *Australostichopus mollis* was fed mussel *Perna canaliculus* biodeposits containing 1, 4, 12 and 20% TOM. The IR reported in the present study is also low compared to that achieved by Zhou et al. (2006) (1.82 ± 0.13 g ind.$^{-1}$ d^{-1}) when *Stichopus japonicus* was fed sediments from Zhikong scallop culture. The lower IR recorded in this study compared to these studies could be due to the consumption ability of the sea cucumber species used in this study (*N. grammatus*), unlike Zamora & Jeffs (2012) and Zhou et al. (2006), who reported on *A. mollis* and *S. japonicus*, respectively. The present study results indicate that circa 14–15 sea cucumbers (about 17 g each) will process all solid waste of abalone produced per square meter in a commercial abalone farm per day if the appropriate deposition and temperature rates are maintained (Magill et al. 2006).

The TOM of the diets influenced the IR in this study, the lower the TOM, the higher the IR, while the higher the TOM, the lower the IR. A high IR characterised by low TOM of feed and vice versa is considered due to sea cucumbers' compensatory feeding and has been observed in recent studies on sea cucumber IMTA (Hudson et al. 2004, Zamora & Jeffs 2011). In the natural environment, sea cucumbers that ingest sediment of low nutritional quality tend to consume more sediment to meet dietary and energy demands (Hudson et al. 2004, Azad et al. 2011). However, when the sediment's nutrient content is high, sea cucumber reduces its appetite and tends to consume less.

The result obtained from this study supports other studies on the IR of sea cucumbers which report that sea cucumbers show a negative relationship with the nutrient content of food (Hudson et al. 2004, Yuan et al. 2006, Liu et al. 2009, 2010, Zamora & Jeffs 2011, Orozco et al. 2014, Chen et al. 2015b). For example, in an experiment conducted by Zamora & Jeffs (2012), mussel *P. canaliculus* waste with varying amounts of TOM (1, 4, 12, and 20%) was used as feed for the sea cucumber *A. mollis*. Sea cucumbers fed a diet containing 4% TOM had the highest IR, followed by those fed 12% TOM, while those fed the 20% TOM diet had the lowest. The exception was those fed with 1% TOM, whose IR was lower than the 4% TOM group and similar to the 12% TOM. The low IR observed for sea cucumber fed 1% TOM compared to that of 4% TOM was reportedly due to reduced nutrients in the feed leading to low palatability (Zamora & Jeffs 2012). Similarly, Xia et al. (2012) found that sea cucumbers *A. japonicus* fed *Laminaria japonica* and *Ulva lactuca* had higher IRs than those fed *Sargassum thunbergii* or *S. polycystum* even though *Sargassum* used in their study had higher protein and lipid content than *L. japonica* and *U. lactuca*. Similarly, when Yuan et al. (2006) fed the sea cucumber *A. japonicus* with dried mussel faeces (Diet A) and diets containing varying amount of dried mussel faeces and powdered algae (*L. japonica*, *S. thunbergii,* and *Sargassum* sp.) (Diet B — 75% dried faeces and 25% powdered algae; Diet C — 50% dried faeces and 50% powdered algae; Diet D — 75% dried faeces and 25% powdered algae; Diet E — powdered algae), the feed quality affected the IR, with lower quality of feed (Diet A) ingested more than those with higher quality.

However, Maxwell et al. (2009) showed that IR had a positive relationship between organic matter content when *Stichopus mollis* was fed with *L. japonica* (TOM = 76.40%), dried abalone faeces (TOM = 54.50%), and dried abalone faeces (TOM = 37%) contrary to the results obtained in this study, i.e. the higher the TOM, the higher the IR, and vice versa. Maxwell et al. (2009) proposed that the positive relationship between ingestion rate and organic matter content, which was contrary to studies available, was because faecal diet and macroalgal diet were used in the study, whereas other studies available as of when theirs was conducted used natural sediment (Hauksson 1979, Yingst 1982, Hudson et al. 2004). However, studies on IR years after the study of Maxwell et al. (2009) show an inverse relationship even when sea cucumbers are fed with faecal waste (Yuan et al. 2006, Liu et al. 2010, Zamora & Jeffs 2011, Orozco et al. 2014, Chen et al. 2015b).

4.2. Faecal production rate (FPR)

The FPR of sea cucumbers in this study followed the same trend as the IR. FPR decreased with an increase in the TOM of the feed and vice versa. This depicts that as TOM increases, sea cucumber consumes less food and, as a result, produces less faeces.

This result corresponds with Zamora & Jeffs (2012) and Yuan et al. (2006), who reported that FPR decreased with increased food quality in *A. mollis* and *A. japonicus*, respectively. Also, when Chen et al. (2015a) fed the sea cucumber *Stichopus monotuberculatus* with fresh shrimp *Litopenaeus vannamei* waste (Diet A = 46.53 % TOM) and various mixtures of sea mud mash and shrimp waste (Diet B = 36.7 % TOM; Diet C = 26.94 % TOM; Diet D = 16.61 % TOM; Diet E = 7.38 % TOM), it was reported that those fed Diet D had a higher FPR than those fed Diets A and B. Those fed Diet A had the lowest FPR.

However, this study's result contradicts those of Zamora & Jeffs (2011), who showed that FPR decreases with an increase in organic matter content in *A. mollis* though without a significant difference, and with Yu et al. (2020), who showed no difference in the FPR of *Cucumaria frondosa* fed with microalgal diets (*Ascophyllum nodosum*, TOM = 78.91 %; *Saccharina latissimi*, TOM = 62.55 %), shellfish diet (TOM = 78.72 %) and natural seston (TOM = 39.46 %). The result of Zamora & Jeffs (2011) could be due to the method used for estimating FPR, as FPR was done for day and night without both being pooled together — unlike this study, which did not differentiate the FPR of the day from night. Also, the result of Yu et al. (2020), which showed no difference in the FPR of *A. mollis* fed microalgal diets and natural seston of varying organic matter, could be because of the very little difference in the organic matter content of the microalgal diets. The natural seston may be of better quality as it may be richer in fatty acids and amino acids, which may compensate for the lower organic matter content and, as such, have the same FPR with microalgae of higher organic matter content.

4.3. Apparent assimilation efficiency (AAE)

The AAE of sea cucumbers in this study increased with an increase in the TOM of the feed except for those fed abalone waste which showed a reduction in AAE. A high AAE is associated with sea cucumbers which feed on sediment containing a low organic matter content (Paltzat et al. 2008). The results of the present study are in accordance with Zamora & Jeffs (2012) and Yuan et al. (2006), who concluded that the AAE of *A. mollis* and *A. japonicus* increased as the TOM content of the feed increased. Maxwell et al. (2009) also reported that the AAE of *S. mollis* was higher when fed Diet A (TOM 76.40 %) and Diet B (TOM 54.50 %), unlike Diet C (TOM 37.00 %). The AAE of *N. grammatus* fed the various diets compared well with other studies (Maxwell et al. 2009) and outperformed others (Yuan et al. 2006, Zamora & Jeffs 2012).

4.4. Evidence of assimilation/nutrient uptake by sea cucumber

The main aim of IMTA, apart from diversification, is bioremediation, which involves the uptake and utilisation of waste into biomass by the extractive species (Troell et al. 2009, Chopin et al. 2012). Sea cucumbers (deposit feeders) have been identified as potential organisms to absorb and reduce excessive organic nutrients from aquaculture waste (Cubillo et al. 2016, Zamora et al. 2018). In the current study, *N. grammatus* was also able to reduce the TOM, carbon, and nitrogen content of pellet and U+S, though there was no significant reduction in nitrogen for sea cucumbers fed pellet and carbon for those fed U+S. This result is consistent with studies that showed the ability of sea cucumber species to reduce TOM, carbon, and nitrogen content of aquaculture biodeposit by their feeding activity (Michio et al. 2003, Zhou et al. 2006, Slater et al. 2009, Mahmoud 2011, Ren et al. 2012, Hannah et al. 2013).

However, for abalone waste, the sea cucumber could only reduce the TOM; faecal carbon and nitrogen content remained similar to that of the feed. The reduction of TOM reported in this study for abalone waste is similar to that of Neofitou et al. (2019). They reported that the sea cucumber *Holothuria tubulosa* reduced the TOM of sediment impacted by sea bream *Sparus aurata* and sea bass *Dicentrarchus labrax* by 30.48 and 30.97 % in the laboratory and field, respectively. Contrarily, Dobson et al. (2020) stated that sandfish *H. scabra* could not reduce the TOM content of Babylonia pond substrate. They did, however, note that this could be due to the input of TOM from the exchange water. Similarly, Plotieau et al. (2013) reported that the organic matter content of sediments within and outside *H. scabra* pens was the same (i.e. no difference). Also, de la Torre-de la Cruz et al. (2018) reported an increase in the sediment organic matter of *H. scabra* cultured in sea pens.

The carbon and nitrogen content of *N. grammatus* faeces fed abalone waste was similar to that of the feed and corresponds with Mathieu-Resuge et al. (2020), who reported that sandfish cultured on shrimp farm sediments were unable to alter the isotopic carbon and nitrogen composition of the culture substrate. Likewise, MacDonald et al. (2013) reported no difference in the total organic carbon of the European sea bass biodeposit *D. labrax* due to the feeding activity of the sea cucumber *H. forskali* after 5 d of deposition and non-deposition period of *D. labrax* waste. This study is, however, in disagreement with studies on sea cucumbers depressing carbon and nitrogen accumulation through their grazing activity (Michio et al. 2003, Zhou et al. 2006, Slater et al. 2009, Mahmoud 2011, Ren et al. 2012, Hannah et al. 2013, Neofitou et al. 2019). It is not well understood why *N. grammatus* could not suppress the carbon and nitrogen content when fed abalone faeces, but it could be due to selective feeding. Selective feeding is a phenomenon where sea cucumber selectively picks particles richer in nutrients to meet its nutrient requirement (Slater et al. 2011). This is evident in situations where the nutrient content of the sea cucumber gut is higher than that of its feed/sediment and, as a result, excretes faeces higher in nutrients than the initial feed or sediment ingested. For example, The TOM of faeces of sea cucumber *A. mollis* fed feeds containing 1 and 4% TOM were 110 and 26% higher than their respective feed (Zamora & Jeffs 2011). Also, the TOM of the foregut *of Parastichopus californicus* was about 4 times richer than the TOM of the sediment ingested (sediment beneath oyster farm) (Paltzat et al. 2008).

4.5. Growth

Several factors influence growth in sea cucumbers, including food quality and quantity, spawning, temperature, and stocking density (Yang et al. 2006, Slater et al. 2009, Zamora & Jeffs 2012, Xia et al. 2017, Bauer et al. 2019). Contrary to our expectations, the weight of sea cucumber in this study was reduced after the 6 wk trial, resulting in negative growth. We are of the opinion that the weight reduction may be due to the stocking density used in this study (801.6 g m^{-2}). The reduction in weight observed in this study is in line with other studies, which reported a reduction in the weight of various species of sea cucumbers when stocking density exceeds a particular threshold (Ramofafia et al. 1997, Battaglene et al. 1999, Slater et al. 2009). For example, *A. mauritiana* reared in tanks at a stocking density of 26 g m^{-2} had a specific growth rate of 0.6 g d^{-1}; however, when the biomass grew to 260 g m^{-2}, *A. mauritiana* experienced a reduction in growth (Ramofafia et al. 1997). Similarly, a decrease in *A. mollis* growth was observed when wet wt increased from 557 to 801.6 g m^{-2} (Slater et al. 2009). However, it should be noted that the sea cucumber in the present study was reared at an initial stocking density of 801.6 g m^{-2}, which far exceeds the initial stocking density used by both Ramofafia et al. (1997) and Slater et al. (2009). Moreso, this study's initial stocking density (801.6 g m^{-2}) was the density at which weight reduction was experienced by *A. mollis* as reported by Slater et al. (2009), which could be the reason no substantial growth was experienced in this study.

5. CONCLUSION

This study shows the acceptance, high survival rate, and nutrient suppression of *Neostichopus grammatus* when fed on aquaculture waste from commercial abalone, *Haliotis midae*, aquaculture. The study depicts *N. grammatus* as a potential candidate for coculture with abalone. It has also shown that at a stocking density of 801.6 g m^{-2}, *N. grammatus* exhibits negative growth regardless of the food quality.

Temperature affects the growth of sea cucumbers, and at extreme thresholds sea cucumbers undergo aestivation. However, for *N. grammatus*, there is no information on suitable temperature and husbandry techniques for optimal growth. Also, it is unknown whether *N. grammatus* species undergo seasonal aestivation as recorded for other commercial species, nor whether it requires a rearing tank which better simulates its natural environment, e.g. by the addition of a substrate layer rather than a bare floor. The experimental period of this study was quite short; it will therefore be worthwhile for further studies to assess the growth of *N. grammatus* over a more extended period. It is recommended that further studies be carried out on various stocking densities to ascertain the density favourable for *N. grammatus* growth. Also, the optimal temperature requirement of substrate for growth should be investigated. The feasibility of integrated multitrophic aquaculture of *N. grammatus* and abalone should be investigated to assess whether both animals can survive and grow well.

Data availabilty. The data that support the findings of this study are openly available in Zenodo (doi:10.5281/Zenodo.7602785).

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LITERATURE CITED

Al-Harrasy BKA (2016) Enhancing growth of sea cucumbers *Apostichopus japonicus* by feeding fermented seaweed. PhD dissertation, Pukyong National University

An Z, Dong Y, Dong S (2007) Temperature effects on growth-ration relationships of juvenile sea cucumber *Apostichopus japonicus* (Selenka). Aquaculture 272:644–648

Azad AK, Pearce CM, McKinley RS (2011) Effects of diet and temperature on ingestion, absorption, assimilation, gonad yield, and gonad quality of the purple sea urchin (*Strongylocentrotus purpuratus*). Aquaculture 317:187–196

Battaglene SC, Seymour JE, Ramofafia C (1999) Survival and growth of cultured juvenile sea cucumbers, *Holothuria scabra.* Aquaculture 178:293–322

Bauer J, Vázquez-Vera L, Espinoza-Montes A, Lorda J, Beas-Luna R, Vela-Gallo C, Reyes-Bonilla H (2019) Multitrophic aquaculture of the green abalone *Haliotis fulgens* and the warty sea cucumber *Apostichopus parvimensis* enhances production. J Shellfish Res 38:455

Byers SC, Mills EL, Stewart PL (1978) A comparison of methods of determining organic carbon in marine sediments, with suggestions for a standard method. Hydrobiologia 58:43–47

Cai N, Luo W, Yao L, Li X and others (2020) Activation of murine RAW264.7 macrophages by oligopeptides from sea cucumber (*Apostichopus japonicus*) and its molecular mechanisms. J Funct Foods 75:104229

Chary K, Aubin J, Sadoul B, Fiandrino A, Covès D, Callier MD (2020) Integrated multitrophic aquaculture of red drum (*Sciaenops ocellatus*) and sea cucumber (*Holothuria scabra*): assessing bioremediation and life-cycle impacts. Aquaculture 516:734621

Chen Y, Hu C, Ren C (2015a) Application of wet waste from shrimp (*Litopenaeus vannamei*) with or without sea mud to feeding sea cucumber (*Stichopus monotuberculatus*). J Ocean Univ China 14:114–120

Chen Y, Luo P, Hu C, Ren C (2015b) Effect of shrimp (*Litopenaeus vannamei*) farming waste on the growth, digestion, ammonium-nitrogen excretion of sea cucumber (*Stichopus monotuberculatus*). J Ocean Univ China 14:484–490

Chopin T, Cooper JA, Reid G, Cross S, Moore C (2012) Open-water integrated multitrophic aquaculture: environmental biomitigation and economic diversification of fed aquaculture by extractive aquaculture. Rev Aquacult 4:209–220

Cubillo AM, Ferreira JG, Robinson SMC, Pearce CM, Corner RA, Johansen J (2016) Role of deposit feeders in integrated multitrophic aquaculture — a model analysis. Aquaculture 453:54–66

de la Torre-de la Cruz M, Villamor JL, Diodoco RJP (2018) Effect of *Holothuria scabra* (Jaeger) on particle size composition and components of sediments. Environ Asia 11: 148–160

DFFE (2022) Aquaculture yearbook 2020: status of the sector. Department of Forestry, Fisheries and the Environment, Cape Town

Dobson GT, Duy NDQ, Paul NA, Southgate PC (2020) Assessing potential for integrating sea grape (*Caulerpa lentillifera*) culture with sandfish (*Holothuria scabra*) and Babylon snail (*Babylonia areolata*) coculture. Aquaculture 522:735153

Foster GG, Hodgson AN (1995) Annual reproductive cycles of three sympatric species of intertidal holothurians (Echinodermata) from the coast of the Eastern Cape Province of South Africa. Invertebr Reprod Dev 27:49–59

Foster GG, Hodgson AN (1996) Feeding, tentacle and gut morphology in five species of southern African intertidal holothuroids (Echinodermata). S Afr J Zool 31:70–79

Gao QF, Wang Y, Dong S, Sun Z, Wang F (2011) Absorption of different food sources by sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea): evidence from carbon stable isotope. Aquaculture 319: 272–276

González-Wangüemert M, Domínguez-Godino JA, Cánovas F (2018) The fast development of sea cucumber fisheries in the Mediterranean and NE Atlantic waters: from a new marine resource to its over-exploitation. Ocean Coast Manage 151:165–177

Grosso L, Rakaj A, Fianchini A, Morroni L, Cataudella S, Scardi M (2021) Integrated multi-trophic aquaculture (IMTA) system combining the sea urchin *Paracentrotus lividus*, as primary species, and the sea cucumber *Holothuria tubulosa* as extractive species. Aquaculture 534:736268

Han Q, Keesing JK, Liu D (2016) A review of sea cucumber aquaculture, ranching, and stock enhancement in China. Rev Fish Sci Aquacult 24:326–341

Hannah L, Pearce CM, Cross SF (2013) Growth and survival of California sea cucumbers (*Parastichopus californicus*) cultivated with sablefish (*Anoplopoma fimbria*) at an integrated multitrophic aquaculture site. Aquaculture 406–407:34–42

Hauksson E (1979) Feeding biology of *Stichopus tremulus*, a deposit-feeding holothurian. Sarsia 64:155–160

Hudson IR, Wigham BD, Tyler PA (2004) The feeding behaviour of a deep-sea holothurian, *Stichopus tremulus* (Gunnerus) based on in situ observations and experiments using a remotely operated vehicle. J Exp Mar Biol Ecol 301:75–91

Israel D, Lupatsch I, Angel DL (2019) Testing the digestibility of seabream wastes in three candidates for integrated multitrophic aquaculture: grey mullet, sea urchin and sea cucumber. Aquaculture 510:364–370

Ji T, Dong Y, Dong S (2008) Growth and physiological responses in the sea cucumber, *Apostichopus japonicus* Selenka: aestivation and temperature. Aquaculture 283:180–187

Juinio-Meñez MA, Tech ED, Ticao IP, Gorospe JRC, Edullantes CMA, Rioja RAV (2017) Adaptive and integrated culture production systems for the tropical sea cucumber *Holothuria scabra.* Fish Res 186:502–513

Liu Y, Dong S, Tian X, Wang F, Gao Q (2009) Effects of dietary sea mud and yellow soil on growth and energy budget of the sea cucumber *Apostichopus japonicus* (Selenka). Aquaculture 286:266–270

Liu Y, Dong S, Tian X, Wang F, Gao Q (2010) The effect of different macroalgae on the growth of sea cucumbers (*Apostichopus japonicus* Selenka). Aquacult Res 41: e881–e885

López-Rocha JA, Velázquez-Abunader I (2019) Fast decline of the sea cucumber *Isostichopus badionotus* as a consequence of high exploitation in Yucatan, Mexico. Reg Stud Mar Sci 27:100547

MacDonald CLE, Stead SM, Slater MJ (2013) Consumption and remediation of European Seabass (*Dicentrarchus labrax*) waste by the sea cucumber *Holothuria forskali.* Aquacult Int 21:1279–1290

Magill SH, Thetmeyer H, Cromey CJ (2006) Settling velocity of faecal pellets of gilthead sea bream (*Sparus aurata* L.) and sea bass (*Dicentrarchus labrax* L.) and sensitivity analysis using measured data in a deposition model. Aquaculture 251:295–305

Mahmoud H (2011) A study on the effect of the sea cucumber *Actinopyga mauritiana* (Echinodermata: Holothuroidea) on the sediment characteristics at El-Gemsha Bay, Red Sea coast, Egypt. Int J Environ Sci Eng (Ijese) 2: 35–44

Mathieu-Resuge M, Grand FL, Schaal G, Kraffe E and others (2020) Assimilation of shrimp farm sediment by *Holothuria scabra*: a coupled fatty acid and stable isotope approach. Aquat Living Resour 33:3

Maxwell KH, Gardner JPA, Heath PL (2009) The effect of diet on the energy budget of the brown sea cucumber, *Stichopus mollis* (Hutton). J World Aquacult Soc 40: 157–170

Mercier A, Battaglene SC, Hamel JF (1999) Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. J Exp Mar Biol Ecol 239:125–156

Michio K, Kengo K, Yasunori K, Hitoshi M, Takayuki Y, Hideaki Y, Hiroshi S (2003) Effects of deposit feeder *Stichopus japonicus* on algal bloom and organic matter contents of bottom sediments of the enclosed sea. Mar Pollut Bull 47:118–125

Neofitou N, Lolas A, Ballios I, Skordas K, Tziantziou L, Vafidis D (2019) Contribution of sea cucumber *Holothuria tubulosa* on organic load reduction from fish farming operation. Aquaculture 501:97–103

Orozco ZGA, Sumbing JG, Lebata-Ramos Ma JH, Watanabe S (2014) Apparent digestibility coefficient of nutrients from shrimp, mussel, diatom and seaweed by juvenile *Holothuria scabra* Jaeger. Aquacult Res 45:1153–1163

Paltzat DL, Pearce CM, Barnes PA, McKinley RS (2008) Growth and production of California sea cucumbers (*Parastichopus californicus* Stimpson) cocultured with suspended Pacific oysters (*Crassostrea gigas* Thunberg). Aquaculture 275:124–137

Plotieau TA, Baele JM, Vaucher R, Hasler CA, Koudad D, Eeckhaut I (2013) Analysis of the impact of *Holothuria scabra* intensive farming on sediment. Cah Biol Mar 54: 703–711

Probyn TA, Pretorius M, Seanego K, Bernatzeder A (2017) Characterisation of water quality in effluents of land-based abalone farms in the Western Cape, South Africa. Aquacult Environ Interact 9:87–102

Qi Z, Wang J, Mao Y, Liu H, Fang J (2013) Feasibility of offshore co-culture of abalone, *Haliotis discus hannai Ino*, and sea cucumber, *Apostichopus japonicus*, in a temperate xzone. J World Aquacult Soc 44:565–573

Ramofafia C, Foyle TP, Bell JD (1997) Growth of juvenile *Actinopyga mauritiana* (Holothuroidea) in captivity. Aquaculture 152:119–128

Ren Y, Dong S, Qin C, Wang F, Tian X, Gao Q (2012) Ecological effects of coculturing sea cucumber *Apostichopus japonicus* (Selenka) with scallop *Chlamys farreri* in earthen ponds. Chin J Oceanology Limnol 30:71–79

Robinson G, Caldwell GS, Jones CLW, Stead SM (2019) The effect of resource quality on the growth of *Holothuria scabra* during aquaculture waste bioremediation. Aquaculture 499:101–108

Slater MJ, Carton AG (2007) Survivorship and growth of the sea cucumber *Australostichopus* (Stichopus) *mollis* (Hutton 1872) in polyculture trials with green-lipped mussel farms. Aquaculture 272:389–398

Slater MJ, Jeffs AG, Carton AG (2009) The use of the waste from green-lipped mussels as a food source for juvenile sea cucumber, *Australostichopus mollis.* Aquaculture 292:219–224

Slater MJ, Jeffs AG, Sewell MA (2011) Organically selective movement and deposit-feeding in juvenile sea cucumber, *Australostichopus mollis*, determined *in situ* and in the laboratory. J Exp Mar Biol Ecol 409:315–323

Sun J, Zhang L, Pan Y, Lin C, Wang F, Kan R, Yang H (2015) Feeding behavior and digestive physiology in sea cucumber *Apostichopus japonicus.* Physiol Behav 139:336–343

Thandar AS (1987) The southern African stichopodid holothurians, with notes on the changes in spicule composition with age in the endemic *Neostichopus grammatus* (HL Clark). Afr Zool 22:278–286

Troell M, Joyce A, Chopin T, Neori A, Buschmann AH, Fang JG (2009) Ecological engineering in aquaculture — potential for integrated multitrophic aquaculture (IMTA) in marine offshore systems. Aquaculture 297:1–9

Wang T, Yang Z, Zhou N, Sun L, Lv Z, Wu C (2017) Identification and functional characterisation of 5-HT4 receptor in sea cucumber *Apostichopus japonicus* (Selenka). Sci Rep 7:40247

Watanabe S, Kodama M, Zarate M, Lebata-Ramos MJH, Nievales MFJ (2012) Ability of sandfish (*Holothuria scabra*) to utilise organic matter in black tiger shrimp ponds. In: Hair CA, Pickering TD, Mills DJ (eds) Asia–Pacific tropical sea cucumber aquaculture. ACIAR Proceedings, Noumea. Australian Centre for International Agricultural Research (ACIAR), Canberra, p 113–120

Xia S, Yang H, Li Y, Liu S, Zhou Y, Zhang L (2012) Effects of different seaweed diets on growth, digestibility, and ammonia-nitrogen production of the sea cucumber *Apostichopus japonicus* (Selenka). Aquaculture 338–341: 304–308

Xia B, Sun Z, Gao Q, Dong S, Li L, Wen H, Feng J (2017) Uptake of farming wastes by sea cucumber *Apostichopus japonicus* in polyculture systems of abalone *Haliotis discus* hannai: evidence from C and N stable isotopes. Aquacult Environ Interact 9:223–230

Yang H, Zhou Y, Zhang T, Yuan X, Li X, Liu Y, Zhang F (2006) Metabolic characteristics of sea cucumber *Apostichopus japonicus* (Selenka) during aestivation. J Exp Mar Biol Ecol 330:505–510

Yingst JY (1982) Factors influencing rates of sediment ingestion by *Parastichopus parvimensis* (Clark), an epibenthic deposit-feeding holothurian. Estuar Coast Shelf Sci 14: 119–134

Yokoyama H (2013) Growth and food source of the sea cucumber *Apostichopus japonicus* cultured below fish cages — potential for integrated multitrophic aquaculture. Aquaculture 372–375:28–38

Yu H, Qinfeng G, Dong S, Yongjun S, Fang W (2016) Effect of coculture of Chinese shrimp (*Fenneropenaeus chinensis*) and sea cucumber (*Apostichopus japonicus* Selenka) on pond environment. J Ocean Univ China 15:905–910

Yu Z, Robinson S, MacDonald B, Lander T, Smith C (2020) Effect of diets on the feeding behavior and physiological properties of suspension-feeding sea cucumber *Cucumaria frondosa.* J Oceanol Limnol 38:883–893

Yuan X, Yang H, Zhou Y, Mao Y, Zhang T, Liu Y (2006) The influence of diets containing dried bivalve feces and/or powdered algae on growth and energy distribution in sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea). Aquaculture 256:457–467

Zamora LN, Jeffs AG (2011) Feeding, selection, digestion and absorption of the organic matter from mussel waste by juveniles of the deposit-feeding sea cucumber, *Australostichopus mollis.* Aquaculture 317:223–228

Zamora LN, Jeffs AG (2012) The ability of the deposit-feeding sea cucumber *Australostichopus mollis* to use natural variation in the biodeposits beneath mussel farms. Aquaculture 326–329:116–122

Zamora LN, Yuan X, Carton AG, Slater MJ (2018) Role of deposit-feeding sea cucumbers in integrated multitrophic aquaculture: progress, problems, potential and future challenges. Rev Aquacult 10:57–74

Zhou Y, Yang H, Liu S, Yuan X and others (2006) Feeding and growth on bivalve biodeposits by the deposit feeder *Stichopus japonicus* Selenka (Echinodermata: Holothuroidea) cocultured in lantern nets. Aquaculture 256: 510–520

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