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# Growth performance of the sea cucumber Holothuria scabra and the seaweed Eucheuma denticulatum: integrated mariculture and effects on sediment organic characteristics

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ABSTRACT: Deposit-feeding sea cucumbers play a key role in marine ecosystems through bioturbation, burrowing and feeding on organic matter in marine sediments. Many deposit-feeding holothurians have therefore been recommended for integrated multitrophic aquaculture systems (IMTA). We set up an integrated mariculture system of sea cucumber Holothuria scabra and seaweed Eucheuma denticulatum in Bweleo, Unguja Island of Zanzibar, Tanzania, to investigate the effect of stocking density on the growth and survival of culture species, total organic matter (TOM) and total organic carbon (TOC) content in the sediment. Treatments that included a fixed stocking density (500 g, ca. 200 g  $m^{-2}$ ) of *E. denticulatum* and 4 sea cucumber stocking densities (monoculture, low, medium and high density; 0,  $150 \pm 5$ ,  $236 \pm 24$ ,  $345 \pm 48$  g m<sup>-2</sup>, mean  $\pm$  SD) of medium-sized H. scabra ( $114 \pm 37$  g) were established. Stocking density of H. scabra did not influence survival of either species. Seaweed cultured under high stocking density of *H. scabra* had a higher specific growth rate of 2.33 % d<sup>-1</sup> than that cultured at the medium or low densities or without sea cucumbers. Sea cucumbers cultured at low stocking density had a higher mean growth rate of 0.80 g d<sup>-1</sup> compared to those cultured at medium or high densities. TOM and TOC in sediments decreased over the experimental period at medium sea cucumber stocking density, while at low and high stocking densities, organic matter accumulated. The study demonstrates that the integration of *E. denticulatum* and *H. scabra* at 200 g  $m^{-2}$  enhances seaweed growth and can reduce organic matter content in the sediments.

KEY WORDS: Co-culture  $\cdot$  Stocking density  $\cdot$  IMTA  $\cdot$  Remediation  $\cdot$  Organic matter  $\cdot$  TOM  $\cdot$  TOC  $\cdot$  Zanzibar

## **INTRODUCTION**

Mariculture is an economic activity of increasing popularity among coastal communities around the world, particularly in developing countries. It presents

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diverse opportunities and benefits to coastal communities, such as food (protein), income and employment, and in some areas, people depend on it entirely as a source of livelihood (FAO 2014). For example, in the Zanzibar Archipelago of Tanzania,

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seaweed mariculture is among the main economic activities. It is the third largest foreign exchange earner after tourism and clove trade, and its major markets are in European countries and the USA. Commercial cultivation of seaweed, mainly *Eucheuma denticulatum* (commonly known as *Spinosum*) and *Kappaphycus alvarezii* (commonly known as *Cottonii*), started in 1989 in Zanzibar and later spread to mainland Tanzania around 1994 (Lirasan & Twide 1993, Msuya 2011a). Since then, the seaweed industry has expanded to 11 000 t of dry seaweed per year, employing more than 20 000 people, most of whom are women (Davis 2011, Msuya 2011a, 2012). Tanzania now ranks among the world's major producers of farmed aquatic plants (FAO 2014).

Similarly, Zanzibar is a hotspot for sea cucumber fisheries in the Western Indian Ocean. A variety of species are targeted, such as Holothuria scabra, H. nobilis (estimated to cost 15000 to 20000 TZS per individual, with 2000 Tanzania Shilling [TZS] = approx. 1 Euro), H. spinifera, Theleonota anax (1000 to 2000 TZS ind.<sup>-1</sup>), *H. leucospilota*, and *H. atra* (<500 TZS ind.<sup>-1</sup>), categorized as high-, medium- and low-value species, respectively (Mmbaga & Mgaya 2004, Eriksson et al. 2010, Mmbaga 2013). H. scabra is among the most exploited sea cucumber species in Zanzibar. As in Indo-Pacific areas, it is harvested and processed into 'beche-de-mer' (Trepang), which is exported to Asian markets (Mmbaga & Mgaya 2004, Conand 2008, Eriksson et al. 2010, Purcell 2010). Therefore, it is a source of income to the local people, particularly women, fishermen, village-based buyers, and the middlemen that are involved in the business. The sea cucumber fishery in Zanzibar is a source of income along the value chain to export. Fishing is mainly artisanal, carried out by women and children, who glean in the intertidal area during low tide, and men who SCUBA and skin dive to catch the sea cucumbers (Eriksson et al. 2010). While a fishery closure is in place on the mainland, the management of the sea cucumber fishery in Zanzibar is still underdeveloped. Key management instruments such as seasonal closures, restocking programs, detailed stock assessment of the resource and a concrete management plan are still lacking in Zanzibar (Muthiga et al. 2010). This has ultimately compromised the fisheries' self-replenishment and existence (Mmbaga & Mgaya 2004, Eriksson et al. 2010, Mmbaga 2013), leading to overexploitation of the high-valued species such as H. scabra and growing interest in sea cucumber mariculture (Eriksson et al. 2012).

Deposit-feeding sea cucumbers are benthic detritus feeders that ingest muddy, sandy sediments and assimilate the organic matter contained within (Roberts 1979). Through their feeding activities, they recycle nutrients in the water column and hence increase primary production, for example in coral reefs (Uthicke 2001, Schneider et al. 2011). In addition, they also increase bacterial abundance in sediments, thereby providing a mechanism for enhanced organic matter decomposition (MacTavish et al. 2012). These effects make deposit-feeding sea cucumbers major components of tropical coastal ecosystems.

Although seaweed farming has proven to be economically beneficial to the people and the country at large, recent problems related to the impact of climate change and world market trends present a persistent challenge. Apparently, seaweed quality is declining, leading to abandonment of the activity by some farmers and switching to other livelihood options (Msuya & Porter 2014). Integrated farming of seaweed and other aquatic organisms in Zanzibar, Tanzania, and the Western Indian Ocean, rather than traditional monoculture of seaweed, has been investigated and recommended by several authors (Hayashi et al. 2010, Eklöf et al. 2012, Msuya 2013b). In an integrated system, seaweed can provide shading for sea cucumbers amidst intensive heat at low tide and could be a potential food source (Davis 2011). In addition, deposit-feeding sea cucumbers can reduce net nutrient inputs from mariculture to marine sediments (Slater & Carton 2009). It is against this background that intervention measures such as mariculture integration of sea cucumbers in seaweed farms can be promoted in Tanzania and Zanzibar in particular. If successful, it may reduce pressure on wild stocks of highly valued sea cucumbers and also lead to improved seaweed quality.

While mariculture is gaining popularity in many developing countries, its development comes with potential significant environmental impacts in coastal ecosystems, including threats to habitats such as mangroves, seagrass beds and coastal lagoons. Intensive mariculture is a potential source of pollution in terms of effluents or sediment eutrophication through biodeposition (Black 2001, Zhang et al. 2012). Chopin et al. (2001) showed in their extensive review that IMTA can be considered as a mitigation approach against excess nutrients and organic matter generated by intensive mariculture activities. The integration of sea cucumbers in seaweed farms can be seen as a mutually beneficial system for both cultured organisms. The burrowing activity of H. scabra re-suspends organic matter and nutrients that can be utilized and assimilated by seaweeds, which are primary producers in the system.

The optimization of seaweed-sea cucumber integrated systems requires establishment of stocking densities that can yield optimum growth of culture species and best utilise potential nutrient flow synergies between species (Agudo 2006, Li & Qi 2010). Following the design of a previous study that integrated K. striatum and H. scabra in Zanzibar (Beltran-Gutierrez et al. 2014), the current study extends research knowledge to the use of Eucheuma denticulatum, a much more widespread seaweed species in Zanzibar and apparently more resistant to environmental conditions than Kappaphycus (Hayashi et al. 2010, Msuya 2011b, Msuya & Porter 2014). A wide range of stocking densities (without sea cucumbers, low, medium, high) is applied in order to better understand trade-offs and limiting capacities with different densities and effects on organic matter content in sediments.

## MATERIALS AND METHODS

**Study area.** Experiments were conducted in Kiwani Bay (part of Menai Bay) in Bweleo, a coastal village in the south-western part of Unguja Island (Fig. 1), about 18 km from Zanzibar town, Tanzania, (06° 17' S, 39° 17' E). The bay is subjected to relatively strong currents at high tide, with a substrate comprised of coarse to medium-coarse sandy sediment and patches of seagrass. Hence the area was considered a potentially suitable site for the culture of sea cucumbers (Baskar 1994).

**Experimental set-up.** Twenty fully enclosed cages  $(1.5 \times 1.5 \times 0.5 \text{ m}, \text{L} \times \text{W} \times \text{H})$  were constructed using a 10 mm polyethylene oyster mesh (roof and walls), wood stakes, cable ties and 4 mm nylon ropes following the design described in Beltran-Gutierrez et al. (2014). The cages were installed 300 m from the shoreline, where the lowest water level at spring low tide ranged between 0.2 and 0.5 m, and pushed 0.25 m into the sediment.

Seaweed stocking. Eucheuma denticulatum was planted inside the cages using the off-bottom method, which is commonly used for seaweed farming in Zanzibar (Neish 2003, Fröcklin et al. 2012, Msuya & Salum 2012, Beltran-Gutierrez et al. 2014). Healthy fronds of seaweed weighing  $25 \pm 2$  g (mean  $\pm$  SD) were tied onto a 4 mm rope with 1 mm string (tie-tie), maintaining a distance of 20 cm between fronds. Three culture ropes each holding 7 fronds were suspended ~25 cm above the sea floor inside the cages with wooden stakes driven into the sediment. Two seaweed production cycles each lasting 6 wk (planting to harvest) were completed during the study period.

**Sea cucumber stocking.** Medium-sized *Holothuria scabra* were collected from intertidal areas of Bweleo and Unguja Ukuu (a village within Menai Bay) during spring low tide. The sea cucumbers were kept in open buckets filled with



Fig. 1. Location of the experimental site in the coastal area of Bweleo on Unguja Island, Zanzibar, Tanzania

seawater, ensuring constant water exchange to avoid stress prior to and during their transportation to the study site. The cucumbers were acclimatized for 14 d in a 9 m<sup>2</sup> fully enclosed cage, and were checked before the beginning of the experiment to ensure that they had not eviscerated. A total of 60 medium-sized *H. scabra* with average initial body weight of 114 ± 37 (mean ± SD) were allocated to experimental cages at low (3 ind. cage<sup>-1</sup>; 150 ± 5 g m<sup>-2</sup>), medium (5 ind. cage<sup>-1</sup>; 236 ± 24 g m<sup>-2</sup>) and high (7 ind. cage<sup>-1</sup>; 345 ± 48 g m<sup>-2</sup>) stocking densities.

Experimental design. Five treatments were set up in this experiment, each with 4 replicates. Densities of sea cucumbers varied among the treatments to reflect monoculture of seaweed (i.e. without sea cucumbers), and co-culture in a range of stocking densities. Optimal growth was expected to occur at medium density, while growth limitation was expected at high stocking density, as observed in other studies, such as Battaglene et al. (1999). Cages without seaweed and sea cucumbers were added as procedural control for the experiment. A fixed seaweed stocking density of 500 g was maintained for seaweed control, low, medium and high sea cucumber density treatments. Replicates for each treatment were randomly allocated to 4 blocks, and a distance of 3 m was kept between cages in the same block, while the blocks were 20 m apart in order to minimize hydrodynamic disturbances (Wolkenhauer et al. 2010). Sea cucumbers were cultured for 84 d in the cages, while the seaweed was cultivated in 2 harvest cycles lasting 42 d each.

**Collection of data.** Growth and survival of both seaweed and sea cucumbers were measured at 2 wk intervals. For the seaweed, percentage survival was calculated by dividing the number of surviving fronds at the end of the harvest cycle by the initial number of fronds planted. To do this, seaweed lines were untied, removed from the cages, and the number of surviving fronds was counted and recorded. After every 14 d, all the seaweed was taken out and from the surviving fronds, 7 were randomly picked, shaken to remove excess water (Fujita 1985, Msuya 2013a) and weighed individually (to the nearest ±1 g).

An average weight of these was obtained and multiplied by the total number of surviving fronds to obtain  $W_t$  (see Eq. 1). In order to estimate the seaweed biomass loss due to breakage, a formula [(Total number of fronds lost during the cycle/Number of fronds planted) × 100] was used to calculate the lost biomass and this was incorporated in the calculation of seaweed growth. For each harvest cycle, specific growth rate (SGR,  $\% d^{-1}$ ) was calculated as follows:

SGR = 
$$[\ln(W_t/W_0)]/t \times 100$$
 (1)

where  $W_0$  is the initial weight at t = 0, and  $W_t$  is the weight at t cultivation days.

For the sea cucumbers, survival was recorded as presence or absence of individuals in the assigned experimental cages (Slater & Carton 2007, Beltran-Gutierrez et al. 2014, Li et al. 2014). Individuals were taken out of cages, photo-identified and weighed using a digital weighing scale (Camry, EK 8150 with  $\pm 1$  g precision). Prior to weighing, individual animals were held for about 2 min to allow the release of water from their respiratory trees.

Daily growth rate (DGR, g  $d^{-1}$ ) for individual sea cucumbers per sampling period was calculated as:

$$DGR = (W_2 - W_1)/d$$
 (2)

where  $W_1$  and  $W_2$  are initial and final weights at the beginning and end of the period, respectively, and *d* is number of days between sampling periods.

Sediment total organic matter (TOM) and total organic carbon (TOC). Five samples of sediment were taken randomly from the surface (up to 3 cm depth) inside each cage before stocking sea cucumbers and thereafter every 2 wk, on the same days as the abiotic parameters. Sediment samples were immediately transported to the laboratory of the Institute of Marine Science (IMS) in Zanzibar town, where they were dried in an oven at 60°C for 72 h. TOM analysis was done using the 'ash method' (Slater & Carton 2009). For each sample, 12 g were taken and burned at 450°C for 5 h in a combustion chamber. Samples were then cooled in a desiccator and the final weight was recorded. TOM content was then calculated as loss in weight after combustion. Approx. 12 g of unsorted sediment samples were homogenized by grinding (Kennedy et al. 2005) for TOC analysis.

Sediment samples collected before stocking the cages, after 42 d, and at the end of the experiment were used for TOC analysis. Three homogenized samples (~25–35 g) per cage were weighed using a Mettler Teledo weighing scale (precision: 0.001 g) in pre-combusted (500°C, 3 h) silver cups. Inorganic carbon removal from the samples prior to TOC analysis followed a protocol similar to that in Kennedy et al. (2005), and the elemental determination for carbon was carried out using a CN Analyzer (Eurovector EA3000).

**Abiotic parameters.** Over the experimental period of 84 d, surface seawater temperature, pH, salinity

and dissolved oxygen concentration were measured on Days 1, 14, 28, 42, 56, 70 and 84 of the experiment at low spring tide between 08:00 and 11:00 h (local time) by using a HQ40D Multimeter (Hach Lange).

**Analysis of data.** To analyze the effect of sea cucumber stocking density on sediment organic matter, TOC, growth and survival of seaweed and sea cucumbers in the different treatments, generalized linear mixed models (GLMMs) were applied using the 'lme4' package (Bates et al. 2012) of the statistical software R version 2.15.3 (R Core Team 2013).

The effect of stocking density on daily growth rate of *H. scabra* in the treatments low, medium and high was analyzed in a single model, with treatment and period as fixed terms, while the initial weight of individuals nested in cages was added to the model as a random term. The effect of stocking density of H. scabra on the survival and growth of E. denticulatum in the treatments monoculture, low, medium and high was analyzed in 2 separate models for the 2 harvest cycles, since harvest cycle 2 was characterized by different weather conditions in the form of severe storms and severe 'ice-ice' disease (stress reaction of seaweed in resonse to e.g. abrupt changes in temperature and/ or salinity). Treatment and number of culture days were included as fixed terms. SGRs of seaweed for each harvest cycle were at first analyzed in 2 separate

models, then later in a single model with treatment and harvest cycle as fixed terms, while blocks were added as random terms to the models. For the second harvest cycle, negative values were interpreted as massive breakage loss due to the storms that occurred, and hence not considered in the analysis. All interaction terms that were not significant were eliminated from the models. Where significant effects were detected, pairwise comparisons using Tukey's honest significant difference (HSD) post-hoc test was employed.

## RESULTS

#### Study site abiotic factors

Sea surface water temperature during the experimental period ranged between 28.3 and 30.7°C, with an average of  $29.3 \pm 1.3$  °C (mean ± SD). The pH ranged between 8.02 and 8.36, averaging  $8.15 \pm 0.01$ , salinity ranged between 35.3 and 36.3 ‰, with an average of  $36.1 \pm 0.43$  ‰, and dissolved oxygen ranged between 5.97 and 9.85 mg l<sup>-1</sup>, with an average of  $8.15 \pm 1.87$  mg l<sup>-1</sup>.

### TOM and TOC in the sediment

TOM content differed significantly among treatments (GLMM,  $F_{4,1} = 3.22$ , p = 0.01) along sampling periods. The interaction between treatment and culture period was marginally above the significance level ( $F_{4,1} = 2.29$ , p = 0.06). Generally, TOM in sediments increased in all treatments over the sampling periods, with the exception of the medium treatment. The highest increase in TOM was observed in the treatment without sea cucumbers (seaweed control), while TOM remained almost the same in the procedural control treatment. TOC content of the sediment differed significantly among treatments ( $F_{4,1} = 2.79$ , p = 0.03) along sampling periods. The highest decrease in TOC was observed in the medium treatment, with the lowest overall mean value of  $0.26 \pm 0.02\%$  dry weight (mean  $\pm$  SD) in the last sampling period. TOM content in the guts was higher than that in the sediment (comparison made with TOM in sediments at 84 d of cultivation, the last sampling period, Table 1).

Table 1. Total organic matter (TOM) and total organic carbon (TOC) contents in integrated mariculture. TOM in the sediment and the gut of the co-cultured sea cucumber *Holothuria scabra* during the cultivation period of seaweed *Eucheuma denticulatum* at different stocking densities of *H. scabra* (see Table 2). Control: procedural control without seaweed and sea cucumbers. TOC in sediments across treatments before stocking the cages, after 42 d and at the end of the cultivation period. Mean ± SD (% dry weight)

Cultivation period (d)/gut	Control	Mono- culture	Low density	Medium density	High density
том					
0	$2.55 \pm 0.19$	$2.32 \pm 0.09$	$2.42 \pm 0.11$	$2.48 \pm 0.04$	$2.31 \pm 0.06$
14	$2.29 \pm 0.05$	$2.38 \pm 0.06$	$2.60 \pm 0.08$	$2.58 \pm 0.09$	$2.58 \pm 0.08$
28	$2.39 \pm 0.08$	$2.46\pm0.04$	$2.55 \pm 0.06$	$2.37 \pm 0.06$	$2.47 \pm 0.09$
42	$2.39\pm0.06$	$2.54\pm0.06$	$2.42\pm0.06$	$2.39\pm0.04$	$2.41\pm0.06$
56	$2.33 \pm 0.07$	$2.54 \pm 0.07$	$2.55\pm0.04$	$2.50\pm0.05$	$2.56 \pm 0.06$
70	$2.37 \pm 0.05$	$2.51\pm0.09$	$2.48 \pm 0.05$	$2.35\pm0.06$	$2.51\pm0.05$
84	$2.42\pm0.05$	$2.66 \pm 0.12$	$2.62 \pm 0.07$	$2.35\pm0.06$	$2.45\pm0.07$
Gut of			$5.57 \pm 0.68$	$5.63 \pm 0.95$	$4.60\pm0.95$
H. scabra					
тос					
0	$0.36 \pm 0.08$	$0.37 \pm 0.07$	$0.39 \pm 0.09$	$0.37 \pm 0.08$	$0.34 \pm 0.08$
42	$0.37 \pm 0.06$	$0.36 \pm 0.09$	$0.34 \pm 0.07$	$0.34 \pm 0.06$	$0.33 \pm 0.07$
84	$0.32 \pm 0.07$	$0.38\pm0.06$	$0.37\pm0.09$	$0.26\pm0.02$	$0.30\pm0.07$



Fig. 2. Specific growth rate (SGR, mean  $\pm$  SD) of *Eucheuma* denticulatum grown without sea cucumbers (monoculture) and at low, medium and high stocking densities of sea cucumbers *Holothuria* scabra during December 2013 and March 2014 in both harvest cycle 1 (SGR-HC1) and harvest cycle 2 (SGR-HC2) of the study. Letters indicate results of Tukey's HSD post-hoc test. Values with different letters are significantly different from each other at p < 0.05

## Seaweed survival and growth

In both harvest cycles, survival rates of *Eucheuma* denticulatum were not significantly different across treatments (GLMM,  $F_{3,1} = 0.55$ , p = 0.65 for harvest cycle 1, and  $F_{3,1} = 0.53$ , p = 0.67 for harvest cycle 2) along the sampling period. Average survival rates at the end of the harvest period ranged between 54 and 64% in harvest cycle 1 and 43 and 64% for harvest cycle 2. Highest loss of seaweed fronds was observed

in the low-density treatment (46%) in harvest cycle 1, and in the monoculture treatment (57%) in harvest cycle 2. There was a significant effect of cultivation days on the survival rates of seaweed in the treatments for both harvest cycles ( $F_{1,3} = 143.04$ , p < 0.0001 for harvest cycle 1, and  $F_{1,3} = 151.04$ , p < 0.0001 for harvest cycle 2). Survival rates were often high in the first 2 wk of the harvest time.

SGRs in the first seaweed harvest cycle differed significantly among treatments ( $F_{3,1}$  = 3.20, p = 0.03; Fig. 2). The highest SGRs growth rate of 2.33 ± 0.67 % d<sup>-1</sup> (mean ± SD) was observed in the high-density treatment, while the lowest value of 0.79 ± 0.36 % d<sup>-1</sup> was observed in the treatment without sea cucumbers. Post-hoc tests revealed that for harvest cycle 1, mean growth rates in the medium and high culture density differed significantly from growth rates in monoculture (p < 0.05).

In the second harvest cycle (storms), differences in SGRs among treatments were not significant ( $F_{3,1} = 0.90$ , p = 0.46), and mean growth rates ranged between 0.43  $\pm$  0.39% d<sup>-1</sup> and 1.01  $\pm$  0.32% d<sup>-1</sup>. SGRs between the 2 harvest cycles differed significantly ( $F_{1,3} = 7.39$ , p = 0.008) among treatments for the low-, medium-, and high-density treatments (Table 2).

#### Sea cucumber survival and growth

Overall survival of *Holothuria scabra* was 83% in the low-density treatment (2 animals lost) and 75% in the medium and high-density treatments (5 and 7 animals lost, respectively). The loss in the medium and high-density treatments was attributed to damage of the cages caused by crabs leading to escape of the sea cucumbers, while in the low-density treatment it is suspected that the 2 missing individuals could have escaped through the bottom of the cage.

Daily growth rates of *H. scabra* differed significantly among treatments (low, medium and high;  $F_{2,5} = 5.15$ , p = 0.0095) over the sampling periods. Sea cucumbers cultured at a low stocking density had the highest average growth rate of  $0.80 \pm 0.3$  g d<sup>-1</sup> (mean  $\pm$  SD), whereas those cultured at a high

Table 2. Summary of integrated mariculture set up and growth results of seaweed *Eucheuma denticulatum* in monoculture and co-culture with sea cucumber *Holothuria scabra* over 84 d at different stocking densities of *H. scabra*. SGR: specific growth rate. Mean  $\pm$  SD

Parameter	Mono- culture	Low density	Medium density	High density
Holothuria scabra				
Initial individual wet weight (g)	_	$110 \pm 27$	$106 \pm 26$	$115 \pm 39$
Initial stocking density (g m <sup>-2</sup> )	_	$150 \pm 5$	$236 \pm 24$	$345 \pm 48$
Individual daily growth rate (g $d^{-1}$ )	_	$0.80 \pm 0.3$	$0.43 \pm 0.2$	$0.14 \pm 0.4$
Individual wet wt at harvest (g)	_	$163 \pm 34$	$141 \pm 33$	$118 \pm 39$
Biomass at harvest (g m <sup>-2</sup> )	_	$211 \pm 16$	$297 \pm 34$	$367 \pm 25$
Eucheuma denticulatum				
Stocking density (g)	$500 \pm 23$	$500 \pm 23$	$500 \pm 20$	$500 \pm 26$
SGR harvest cycle 1 (% d <sup>-1</sup> )	$0.79 \pm 0.36$	$1.59 \pm 0.46$	$1.87 \pm 0.54$	$2.33 \pm 0.67$
SGR harvest cycle 2 (% d <sup>-1</sup> )	$0.75\pm0.36$	$0.45 \pm 0.39$	$1.01\pm0.33$	$1.01\pm0.32$

stocking density had the lowest average growth rate of  $0.14 \pm 0.2$  g d<sup>-1</sup>. The interaction between cultivation days and treatments were not significant (Table 2).

Post-hoc tests revealed that mean growth rates in low- and high-density treatments differed significantly from each other (p = 0.002), but mean growth rates in the low- and medium-, and high- and medium-density treatments did not differ significantly (p > 0.05).

Sea cucumbers cultured at low stocking density had the highest average weight on the last day of the experiment  $(163 \pm 34 \text{ g})$ , while those cultured at high stocking density had the lowest average weight  $(118 \pm 39 \text{ g})$ .

## DISCUSSION

There is scant information related to the growth performance of culture species in such seaweed–sea cucumber integrated culture. In this study, the aim was to measure growth and survival rates of sea cucumbers and seaweed in response to varying densities of sea cucumbers in Zanzibar, Tanzania.

## TOM and TOC in sediment

Deposit-feeding sea cucumbers tend to modify their foraging behavior and digestive capabilities to optimize the intake of nutrients from the organic component in sediments (Roberts et al. 2000, Zamora & Jeffs 2011). In the present study, an uptake of TOM through feeding by the sea cucumbers was evident in the medium-density treatment, which showed a significant decrease in TOM and TOC over the experimental period. On the other hand, there seems to have been an accumulation of TOM in the treatment without sea cucumbers. This could have been due to break-offs of seaweed thalli sedimenting inside the cages. In low- and high-density treatments, TOM release exceeded uptake. Studies on TOC utilization by Holothuria scabra for comparison are limited in the literature; however, in other deposit-feeding holothurians, Michio et al. (2003) observed a decrease in TOC in surface sediments with sea cucumbers Apostichopus japonicus compared to sediments without sea cucumbers. Slater & Carton (2009) made a similar observation with Australostichopus mollis in coastal sediments impacted by mussel farm deposits. Furthermore, in an integrated system of sea cucumber A. japonicus with scallop Chlamys farreri, Ren et al.

(2012) observed a decline in TOC as a result of sea cucumber feeding, but there was an increase in TOC in sediments during periods of sea cucumber dormancy. Sediment reworking and organic matter uptake by holothurians could depend on individual numbers and sizes, food availability and local conditions (Dar & Ahmad 2006). In the present study, it is evident that the number of sea cucumbers (stocking density) was a key factor that controlled TOM and TOC utilization in the system, but it does not rule out other possible factors that were beyond the scope of this study. These may include the sediment type ingested, and evisceration events (when sea cucumbers release their respiratory trees, intestines and gonads) that have been observed to cause cessation in feeding in the sea cucumber Parastichopus parvimensis (Yingst 1982).

### Survival and growth of Eucheuma denticulatum

Similar to the present study, the presence or absence of sea cucumbers has been reported to have no or minimal effect on the survival of seaweed in integrated systems (Davies et al. 2011, Beltran-Gutierrez et al. 2014). Stocking density and environmental conditions are among the factors that influence growth of seaweed (Msuya 2013a). SGRs of farmed Eucheuma spp. usually range between 2 and  $10\% d^{-1}$  (Doty 1986). The observed growth rates of seaweed in the present study (0.45-2.33%  $d^{-1}$ ) are lower compared to previous studies on E. denticulatum in Zanzibar and other areas. Such higher growth rates reported are, notably,  $3.50\% d^{-1}$ in Hawaii (Glenn & Doty 1990), 4.7 % d<sup>-1</sup> in Kenya (Wakibia et al. 2006), and 3-11% d<sup>-1</sup> in Tanzania (Msuya & Salum 2012). Recently Msuya et al. (2012) reported a growth rate of  $5.1\% d^{-1}$  for unfertilized E. denticulatum in Uroa, Zanzibar. Nevertheless, the SGRs in this study compare acceptably with other studies of E. denticulatum. Lower SGRs in E. denticulatum  $(1.4\% d^{-1})$  were reported from the southwest coast of Madagascar (Mollion & Braud 1993), and recently Msuya et al. (2012) encountered minimum values of less than 1 % d<sup>-1</sup> in Uroa, Zanzibar. As in the present study, lower seaweed growth rates have been attributed to the 'ice-ice' disease and severe storm events. These, in addition to epiphytism and fouling, are some of the major production problems faced by seaweed farmers in Zanzibar and the Western Indian Ocean region in general (Hayashi et al. 2010, Msuya 2012, 2013a,b, Msuya & Porter 2014, Msuya et al. 2014). The results of the

current study show that presence of sea cucumbers enhances seaweed growth. Similar results were obtained by Uthicke (2001) and Wolkenhauer et al. (2010), who observed that sea cucumbers enhance productivity of primary producers through their burrowing and nutrient recycling activities. In contrast to Beltran-Gutierrez et al. (2014), where a low stocking density (154 g m<sup>-2</sup>) of *H. scabra* yielded higher growth rates of *Kappaphycus striatum* than either a density of 200 g m<sup>-2</sup> or an absence of sea cucumbers, in the current study higher stocking densities of *H. scabra* (200 and 300 g m<sup>-2</sup>) resulted in the highest growth grates of *E. denticulatum*. Potential reasons are discussed in the section on the effect of stocking density below.

## Survival, growth and the effect of stocking density on *H. scabra*

Survival of sea cucumbers did not depend on the stocking density, and losses likely caused by escape were the only kind of losses observed during the study. Crustaceans, notably crabs, are potential predators of sea cucumbers, and juvenile holothurians are more vulnerable compared to medium-sized individuals (Pitt & Duy 2004, Pitt et al. 2004). Sea pens certainly attract other marine organisms, and this can compromise the survival of sea cucumbers. To this note, potential farmers need to be aware of potential predators such as crabs, carnivorous fishes, birds, sea stars, gastropods and other invertebrates (Francour 1997, Purcell et al. 2012) during site selection and also ensure constant monitoring of sea pens. Important with regard to integrated mariculture operations is that survival of sea cucumbers in such systems does not only depend on the abovementioned factors but could also be influenced by the co-culture species in question. For example, co-culture of H. scabra with shrimp Litopenaues stylirostris is not viable and survival of H. scabra in such a system has been observed to be very low (Bell et al. 2007). On the other hand, in an integrated system of the seaweed K. striatum and H. scabra, seaweed did not appear to have a negative effect on sea cucumbers (Beltran-Gutierrez et al. 2014).

Stocking density is one of the major factors that influence sea cucumber growth rates both in ponds and sea pens (Battaglene et al. 1999, Pitt & Duy 2004, Lavitra et al. 2010, Li & Qi 2010, Hannah et al. 2013). At medium size, *H. scabra* has been observed to grow at an average rate of 0.5 cm mo<sup>-1</sup>, which corre-

sponds to 14 g mo<sup>-1</sup> (i.e. 0.46 g d<sup>-1</sup>, Agudo 2006). Growth rates of sea cucumbers in the present study were density-dependent, with the highest growth rates being observed at the low-density treatment and the lowest growth rates observed at the highdensity treatment. The effect of stocking density on sea cucumber growth rates could be attributed to competition for resources, such as food and space. High growth rates at low stocking density could be due to reduced intraspecific competition among individual sea cucumbers, while low growth rates at high stocking densities could be attributed to increased competition for the available resources (Battaglene et al. 1999, Slater & Carton 2007, Davies et al. 2011). Increased growth rates at low stocking densities of sea cucumbers as observed in the present study have been observed in other studies on H. scabra (Battaglene et al. 1999, Beltran-Gutierrez et al. 2014) and in other deposit-feeding sea cucumbers such as A. mollis (Slater & Carton 2007), A. japonicus (Yokoyama 2013) and Parastichopus californicus (Hannah et al. 2013).

The average growth rates in this study (0.14- $0.80 \text{ g d}^{-1}$ ) are in the same range of what previous studies observed for H. scabra (medium size) in the same study area in Zanzibar. Davies et al. (2011) reported growth rates of  $0.06-1.39 \text{ g d}^{-1}$  in the Fumba lagoon of Menai Bay, Zanzibar. In the same bay, Beltran-Gutierrez et al. (2014) observed higher mean growth rates of *H. scabra* when integrated with K. striatum (1.6 and 0.9 g  $d^{-1}$  at 100 and 200 g m<sup>-2</sup> of *H. scabra*, respectively) than in the present study (0.80 and 0.43 g  $d^{-1}$  at 100 and 200 g m<sup>-2</sup> of *H. scabra*, respectively) for the same total production area. This could be attributed to differences in the initial weight of animals used (i.e. 96 ± 31 g in Beltran-Gutierrez et al. 2014 vs.  $114 \pm 37$  g in the present study). However, it could also be that H. scabra grows better when integrated with *K. striatum* than with E. denticulatum. This aspect requires further studies using direct comparisons of both seaweed species as co-cultivars. Lower growth rates of H. scabra due to high stocking densities are not only evident in medium-sized individuals, but have also been observed in juvenile sea cucumbers. Pitt et al. (2004) observed a growth rate of 0.24 g  $d^{-1}$  at a high stocking density (227 g m<sup>-2</sup>) for juveniles of <1 g wet weight. Battaglene et al. (1999) reported an average growth rate of 0.2 g d<sup>-1</sup> for newly settled juveniles (<5 mm) and observed declining growth rates as the stocking densities exceeded  $225 \text{ g m}^{-2}$ .

#### Practical and economic implications of the study

Overall, environmental conditions were within the boundary range for the survival and growth of the culture species. The optimal stocking density of sea cucumbers can be influenced by factors such as organic matter content in the food and the strategy of the farmer, which could be bioremediation of sediments, or maximizing growth to optimize profits. In any case, it is important to note that exceeding recommended stocking densities compromises the growth performance of sea cucumbers and may have implications on TOM and TOC budgets in the system.

The study provides useful information for future mariculture development in Zanzibar and Tanzania as a whole; however, information gaps concerning mariculture of sea cucumber resources in Zanzibar still remain. Aspects like the species' absorption efficiency need to be investigated for its potential use in IMTA systems. Mariculture integration of seaweed and sea cucumbers is a potential livelihood alternative, which if adopted can diversify livelihood portfolios of Zanzibar's coastal communities, especially of women involved in seaweed farming. While the coculture of K. stratiatum and H. scabra results in higher growth rates of cultured species (Beltran-Gutierrez et al. 2014) than the co-culture with *E. denticulatum* in the current study, E. denticulatum continues to dominate the seaweed market in Zanzibar, because it has been observed to be more resistant to environmental changes than K. stratiatum. Therefore, integrating sea cucumbers in E. denticulatum farms should be promoted in Zanzibar to boost seaweed production.

## CONCLUSION

This study clearly showed that integrating sea cucumbers *Holothuria scabra* and seaweed *Eucheuma denticulatum* mutually benefits both species and is a better farming option compared to monoculture of seaweed. Presence of sea cucumbers enhanced seaweed growth, and a good survival rate of sea cucumbers in the presence of seaweed indicates that seaweed has no negative effect on sea cucumber performance.

The optimum stocking density of sea cucumbers under natural conditions that yields best growth and that could be economically viable is 200 g m<sup>-2</sup>. A positive effect of sea cucumber culture on sediment organic matter content was found. Sea cucumbers modify the sediment through their extractive feeding, thereby facilitating the nutrient remineralisation process in such a system. Acknowledgements. The authors thank The German Academic Exchange Service (DAAD) and the Center for Marine Tropical Ecology (ZMT) Bremen for the financial support. Thanks to Dr. Narriman Jiddawi of the Institute of Marine Sciences (IMS) for facilitation of contacts within Zanzibar and Mr. Mtumwa Mwadini of IMS for his help in laboratory analysis of organic matter content. The people of Bweleo, especially those that assisted in the preparation of the experiment and fieldwork, are heartily thanked. Lastly, we are grateful to the Open Access Fund of the Leibniz Association for funding the publication of this article.

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