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Low-technology recirculating aquaculture system integrating milkfish *Chanos chanos*, sea cucumber *Holothuria scabra* and sea purslane *Sesuvium portulacastrum*

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ABSTRACT: Closed recirculation aquaculture systems (RAS) in combination with integrated multi-trophic aquaculture (IMTA) are considered best management practices, but high material costs and difficult maintenance still hinder their implementation, especially in developing countries and the tropics. Few case studies of such systems with tropical species exist. For the first time, an extremely low-budget system was tested combining the halophyte sea purslane *Sesuvium portulacastrum* and a detritivore, sandfish *Holothuria scabra*, with finfish milkfish *Chanos chanos* over 8 wk on Zanzibar, Tanzania. In a 2 m^3 RAS, milkfish and sea purslane showed good growth, producing an average (±SD) of 1147 ± 79 g fish and 1261 ± 95 g plant biomass, while sea cucumber growth was variable at 92 ± 68 g. The system operated without filter units and did not discharge any solid or dissolved waste. Water quality remained tolerable and ammonia levels were reliably decreased to <1 mg l^{-1}. A NO_2^- peak occurred within the first 30 d, indicating good biofilter performance of the different system compartments. Changes in dissolved inorganic nitrogen (DIN) species support the notion that the sea cucumber tank was the main site of nitrification, while the hydroponic halophyte tank acted as a net sink of NO_3^-. A nitrogen budget accounted for 63.7 ± 5.3% of the nitrogen added to the system as fish feed. Increasing the plant to fish biomass ratio to 5:1 would fully treat the DIN load. The experiment provides proof-of-concept of a simple pilot-scale RAS, integrating tropical species at 3 trophic levels.

KEY WORDS: IMTA · Appropriate technology · Halophyte · Biofilter · Nitrification · Denitrification · Nitrogen budget

1. INTRODUCTION

Aquaculture is expanding rapidly but with significant environmental impacts (Holmer et al. 2003, Primavera 2006, Herbeck et al. 2014). Closed recirculation aquaculture systems (RAS) in combination with integrated multi-trophic aquaculture (IMTA) offer an example of extremely low-impact aquaculture (Schneider et al. 2005, Chopin et al. 2007). However, high material costs, difficult maintenance and inadequate legislation still hinder their implementation, especially in the developing tropics (Chopin 2017, Kleitou et al. 2018, Stenton-Dozey et al. 2020). There is a dearth of research into low-cost and low-technology IMTA/RAS for tropical aquaculture-producing nations, and few case studies of such systems with tropical species exist (Largo et al. 2016, Felaco et al. 2020). Candidate species and, above all, local species

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combinations including valuable extractive species remain poorly identified (Ahmed & Glaser 2016, Zhang et al. 2019).

Halophytes are a possible extractive species for integrated aquaculture, but research focusing on them in this context has been limited (Custódio et al. 2017). As our agricultural food production is heavily reliant on glycophytes (plants that cannot tolerate salt), salt-tolerant halophytes are promising as vegetable crops or biomass for biofuel production (Buhmann & Papenbrock 2013). Different plant species and types of natural and constructed wetlands are already being successfully used for phytoremediation of municipal and industrial wastewater or contaminated soil (Verhoeven & Meuleman 1999, Vymazal 2010). Their use can have great economic benefits compared to conventional water treatment, as some species are nutritious and desired on the gourmet market (Cardoch et al. 2000, Ventura et al. 2011). *Sesuvium portulacastrum*, or sea purslane, is a perennial halophyte with potential as a valuable crop for saline soils (Lonard & Judd 1997, Lokhande et al. 2009). In several countries, *S. portulacastrum* is consumed fresh or raw and is used as a traditional medicinal plant (Magwa et al. 2006, Lokhande et al. 2009). *S. portulacastrum* is already used to remediate saline soils and extract toxic textile dyes from discharge water (Patil et al. 2012). Very little is known about its nutrient tolerance and biofiltration capacity, but previous studies suggest that it can also remediate aquaculture wastewater (Slama et al. 2006, Boxman et al. 2017).

Sea cucumbers are particularly promising for integrated aquaculture, as some species have been found to feed and grow on the debris of fish farms (review by Zamora et al. 2018). Furthermore, they can be of high economic value and have beneficial effects on sediments by re-working upper sediment layers and influencing the development of microbial communities (Moriarty et al. 1985, Wolkenhauer et al. 2010, MacTavish et al. 2012). The detritivorous sea cucumber *Holothuria scabra*, also known as sandfish, is among the most valuable species, with increasing aquaculture production as many wild populations have been decimated (Lane & Limbong 2015, Purcell et al. 2018, Hair et al. 2019). Global sandfish production in 2018 totaled 489 tons with a value of more than 2.5 million USD (FAO 2020). *H. scabra* has been studied as an IMTA species because it could prevent sediments from becoming anoxic under high loads of organic matter (Lee et al. 2017). In an experiment in the open sea, sandfish were not effective at remediating pollution from milkfish cultivation (Watanabe et al. 2017), but *H. scabra* can be integrated into sediment-based effluent treatment systems (Robinson et al. 2016) and be fed aquaculture waste to produce additional biomass (Robinson et al. 2019).

RAS are tank-based aquaculture facilities in which water from fish or shrimp cultivation is circulated to filtration units, requiring minimal water exchange, limiting the influence of environmental conditions on the cultivated species and potentially eliminating organic pollution. RAS technology is regarded as an important component of a sustainable future for aquaculture, moving towards closed cultivating systems (Martins et al. 2010, Yogev et al. 2017, Suantika et al. 2020). As closed aquaculture systems do not dispose of waste products of animal metabolism through water exchanges, they rely on bacteria to process elemental fluxes and particles (Blancheton et al. 2013). Nitrifying biofilters are a well-established component of RAS, where communities of nitrifying bacteria develop in RAS biofilters over time, oxidizing ammonia to NO_2^- and further to NO_3^-, causing NO_2^- peaks followed by increases in NO_3^- (Keuter et al. 2017, Brailo et al. 2019). Denitrification can be accomplished in RAS through a number of biological processes, summarized by van Rijn et al. (2006). In the process of assimilatory NO_3^- reduction, organisms such as plants, fungi or bacteria use NO_3^- as a source of nitrogen (N), with efficiencies depending on the availability of other, preferred, inorganic N species (e.g. ammonia). NO_3^- removal occurs under anaerobic conditions either as dissimilatory NO_3^- reduction to ammonium or as denitrification into N gas. Nitrifying biofilters can amount to 20% of the investment costs of a RAS (Eshchar et al. 2006), so reducing this cost or developing profitable alternatives is important on the way toward more economical systems. RAS technology is well advanced in Europe, already dominating production in a few countries (Badiola et al. 2012). In tropical countries, where the cost of land, energy and labor are lower, it is still in its infancy. However, with increasing scarcity of natural resources and coastal space and its application in broodstock development and larval rearing, its relevance is increasing in the developing tropics as well, and research is needed to meet future needs (Ranjan et al. 2019). Also, the implementation of IMTA is still hindered by various infrastructure, legislation and economic hurdles, and widespread adoption can only be achieved through site-specific targeted approaches and upscaling research and development beyond small tank or laboratory experiments (Chopin 2017, Kleitou et al. 2018). A focus on appropriate technology or 'low-tech' systems is an opportunity to support aquaculture adoption and success in the developing tropics (Heyman et al. 1989, Maucieri et al. 2019).

Tanzania is one of the main producers of seaweeds, and seaweed farming plays an important role in its economy (especially as an income opportunity for women) and has widely improved living standards (Eklöf et al. 2012, Msuya 2013). However, seaweed prices and associated income are volatile and strongly influenced by international markets (Bryceson 2002). Efforts have therefore been made by the government and a consortium of local and international partners to promote aquaculture activities and to diversify the aquaculture sector in terms of species produced (FAO 2017). *Chanos chanos*, commonly known as milkfish, are sufficiently tolerant to changing salinities and oxygen concentrations, making them a suitable candidate for the budding aquaculture sector of Zanzibar, but their cultivation is also prone to producing elevated nutrient levels and eutrophication (Mmochi et al. 2002, Holmer et al. 2003). The early stages of establishing milkfish production create an opportunity to investigate cultivation techniques with the aim to reduce environmental impacts (Rönnback et al. 2002, Troell et al. 2011).

This study provides a proof-of-concept of an IMTA RAS setup, integrating milkfish *C. chanos*, sea cucumber *H. scabra* and, for the first time, sea purslane *S. portulacastrum* in a tropical country. This experimental study aims to answer the following questions: (1) How does a RAS, integrating milkfish, sandfish and sea purslane, perform in terms of survival and biomass production? (2) Is it feasible to run the RAS with sea cucumber and halophyte tanks instead of conventional biofilters, and what is the individual filter performance? (3) Do the sea cucumber and halophyte tanks improve the use of feed-derived N?

2. MATERIALS AND METHODS

2.1. Experimental setup

The experiment was conducted over a period of 70 d, from 7 December 2018 to 14 February 2019, at the Zanzibar Mariculture hatchery, Tanzania. Three replicates of a closed recirculation system were constructed, each consisting of a fiberglass tank for fish, sea cucumber and halophyte cultivation (Fig. 1). In the fish tanks, 10 cm diameter PVC pipes were connected to an overflow hole, 20 cm from the tank top edge, as well as to a drainage hole in the middle of the tank bottom that could be drained using a ball valve. The fish tanks were covered with 5 mm mesh to reduce stress to the animals. The tanks were filled with saline well water. Water temperature in the tanks ranged from 25.9–30.5°C with an average (±SD) of 28.1 ± 0.9°C. Salinity ranged from 25.9–30.5, with an average (±SD) of 30.3 ± 1.2. The fish tanks were stocked with milkfish (n = 9) of 124 ± 29 g (±SD) and ~20 cm length, resulting in a stocking density (±SD) of 1657.3 ± 163.6 g m^{-3}. The sea cucumbers (n = 10) had an average weight of 8.5 ± 2.4 g and length of 5.6 ± 0.7 cm at stocking, resulting in a density of 32.1 ± 3.8 g m^{-2}. The halophytes (n = 70) weighed an average of 13.2 ± 4.0 g and were planted at a density of 2271.6 ± 30.8 g m^{-2}. An acclimatization phase was not deemed necessary, as all organisms had been kept in similar environmental conditions at the hatchery before the experiment.

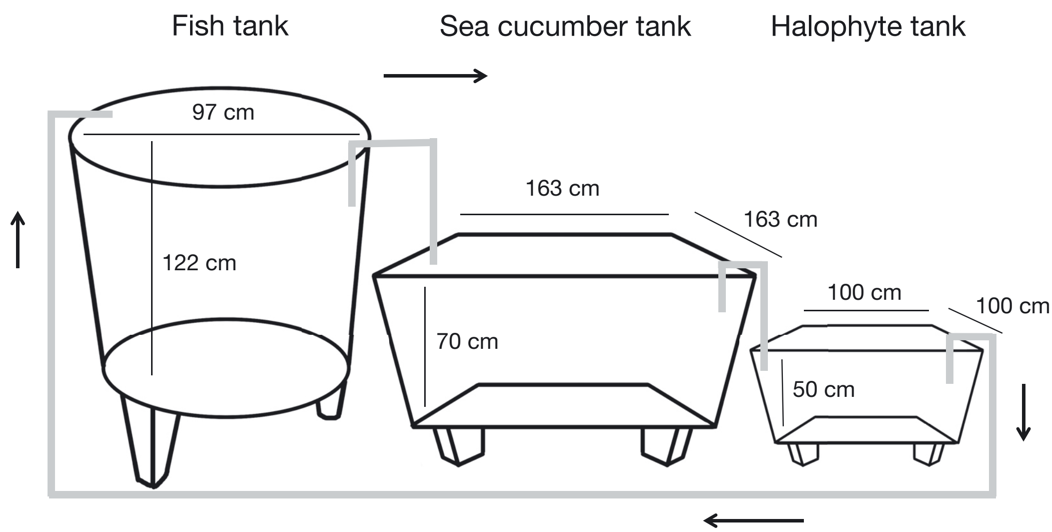

Fig. 1. Schematic set-up of the experimental recirculation aquaculture system (RAS). Grey lines: water pipes; black arrows: direction of water flow

A simplified airlift system was constructed to allow oxic conditions to be maintained by water circulation through the sediment (Robinson et al. 2015). The system consisted of an outer frame of 1.25 cm high-pressure PVC pipes with 2 pipes crossing the tank bottom, 30 cm from the tank wall, and with holes every 15 cm. The outer frame was connected to two 40 cm vertical pipes on each side, into which 2 mm diameter tubing pumped air in the bottom, causing water to be sucked up through the vertical tubes and creating water circulation through the sediment (Fig. 2). The bottom frame was covered in a 10 cm layer of gravel, covered with geotextile and a layer of carbonate sand sourced from the local beach. This type of tank design has been shown to prevent the formation of predominantly anoxic sediments (Robinson et al. 2015). The oxygenated sediment supports microbial communities that are more diverse, stable and have greater bioremediation potential of nitrogenous wastes than anoxic sediments (Robinson et al. 2016). The sea cucumber tank was filled with saline well water and treated with 100 ppm chlorine for 3 d before stocking. The tanks were stocked on Day 0 of the experiment (Table 1). The halophytes were planted into 2 cm diameter holes cut into a 50 × 80 cm piece of marine-grade plywood and held in place by 35 cm strips of geotextile wrapped around the stem right above the roots. Aeration in the fish and halophyte tanks was provided through tubing and airstones.

System design that is too costly and complex to maintain has been identified as a common reason for the failure of RAS operations (Badiola et al. 2012). The system studied here was built with this in mind and kept relatively simple. High-pressure PVC pipes (1.25 cm diameter) connected the tanks so that water flowed via syphon from the fish tank into the sea cucumber tanks and then into the halophyte tank. From the halophyte tank it was then pumped (Emperor 22000 submersible pump, Yi Hu Fish Farm

Table 1. Initial stocking (mean ± SD) of the tanks in the 3 RAS (n = 3)

Tank	Stocking (n)	Stocking density	Total biomass (g)	Water volume (l)
Fish	9 ± 1	1657.3 ± 163.6 g m^{-3}	1072 ± 106	647 ± 13
Sea cucumbers	10 ± 0	32.1 ± 3.8 g m^{-2}	85 ± 10	965 ± 41
Halophytes	70 ± 0	2271.6 ± 30.8 m^{-2}	909 ± 12	390 ± 44

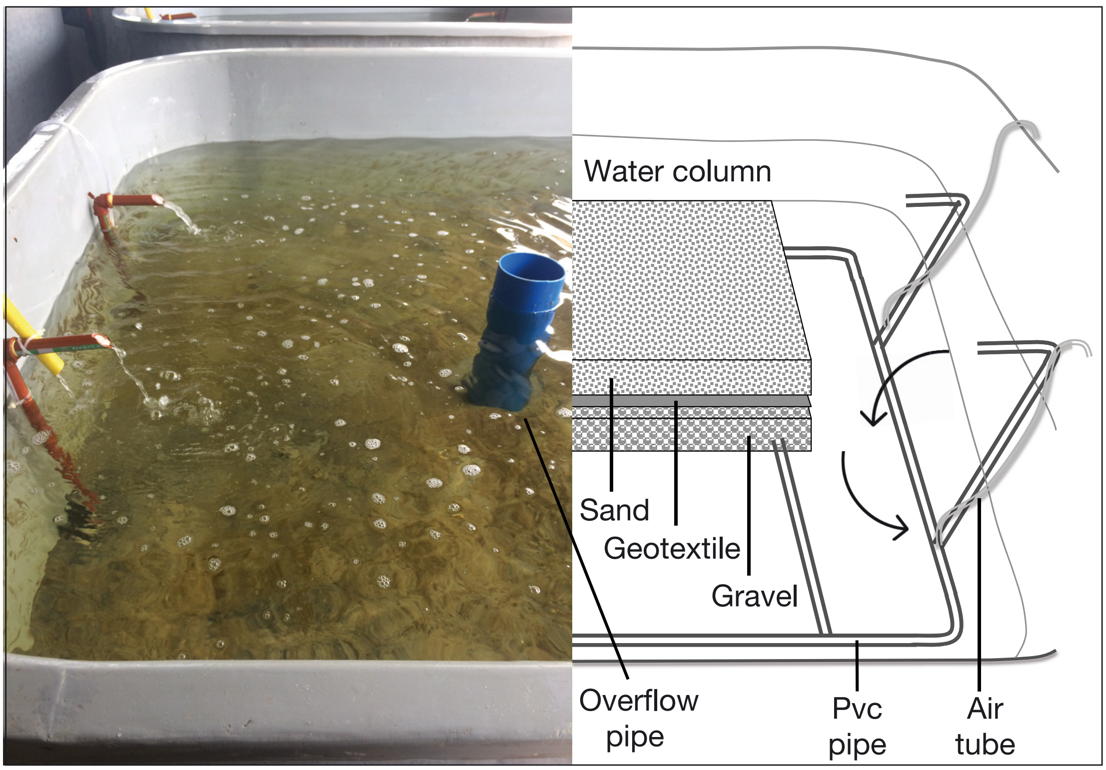

Fig. 2. Image and schematic of sea cucumber tank with airlift system. Arrows indicate the general direction of water flow down through the sediment and up through the airlift pipe

Trading) back into the fish tank at a flow rate of 270 l h^{-1}. The water was circulated continuously for the first 16 d of the experiment. For the remainder of the experimental period, circulation was interrupted every Friday at 16:00 h and resumed the following Monday at 10:00 h. This allowed us to determine the development of nutrient concentration in the fish, sea cucumber and halophyte tanks individually.

The fish were fed daily with commercial fish feed (Hill Pellet — Fish, Hill Animal Feeds & Agrovet Supplies) at a rate of 4% body weight d^{-1}. Once a week, saline well water was added to adjust for water losses from leakage and evaporation, and the salinity was adjusted by adding fresh water. Every Monday, Wednesday and Friday, settled sludge (fish feces and uneaten feed) from the bottom of the fish tank was flushed through the drainage pipe, filtered through a 80 µm sieve, weighed and distributed in the sea cucumber tank after re-suspension in 500 ml of tank water.

2.2. Sampling and analysis

The milkfish were weighed at the beginning and at the end of the experiment. The animals were sedated with clove oil (Priborsky & Velisek 2018), wrapped in a wet cloth and weighed individually. Sea cucumber weight was assessed at stocking, on Day 28 and at the end of the experiment. Before weighing, the sea cucumbers were suspended in mesh bags for 24 h to allow for the gut to be emptied, then placed on tissue paper for 1 min, measured, photographed and weighed (Sewell & Bergquist 1990). Weight data were used to calculate absolute growth, specific growth rate (SGR) and food conversion ratio (FCR) for milkfish and absolute growth and SGR for sea cucumbers.

Before stocking the halophytes, each plant was weighed individually. At the end of the experiment, entanglements of the grown plants made harvesting of individuals difficult and only 10 plants from each tank were sampled to determine the individual biomass as well as above ground (stem and leaves) and below ground (roots) biomass. The rest of the plants were weighed cumulatively. Weight data were used to calculate absolute growth and SGR. Three plants from each tank were dried at 60°C, homogenized and ground for C:N content. All organisms were weighed on a Kern PCB 6000-1 Precision Scale (Kern & Sohn).

Every Monday before the system was started and every Friday after stopping the water circulation, water was sampled for analysis of NO_2^--N, NO_3^--N, total ammonia N (TAN) and PO_4^--P. Water samples (20 ml) were taken with a syringe, filtered through 0.45 µm syringe filters into acid-washed 20 ml plastic bottles and stored frozen. Spectrophotometric analysis of dissolved inorganic nutrients was carried out following the procedures of Strickland & Parsons (1972) with an Infinite 200 PRO microplate reader (TECAN). Dissolved inorganic N (DIN) was determined as the sum of NO_2^--N, NO_3^--N and TAN. On sampling occasions, we also determined the volume of water in each tank by measuring water depth with a 100 cm ruler. Every Monday, Wednesday and Friday, we measured pH, salinity, temperature and dissolved oxygen with a multiparameter probe (WTW).

Samples of the surface sediment in the sea cucumber tanks for the determination of N content were taken in 2 wk intervals. A 20 ml cut-off syringe was used as a corer (surface area of 3.14 cm^2), taking 5 samples, 2 cm deep into the sediment, which were then pooled for each tank, dried and analyzed.

During the last week of the experiment, water from all tanks was sampled on 2 consecutive Mondays before starting water circulation, as well as on the Wednesday and Friday in between, and filtered for total suspended solids (TSS). From each tank, 1500 ml of water were sampled and 250–1500 ml were filtered over a glass microfiber filter with particle retention of 0.7 µm (VWR International). Filters were then dried and weighed. At the end of the experiment, organic matter and algae on the tank walls and bottom were collected, weighed and measured for C:N content. All samples for C:N content were analyzed using a Euro EA-CHNSO Elemental Analyzer (HEKAtech).

Basic data analysis and visualization was performed in Microsoft Excel and R version 3.6.2 (R Core Team 2019). Data are presented as mean ± SD or as individual data points.

3. RESULTS

3.1. Growth and survival

The organisms in all 3 systems increased in biomass at a SGR above 1% d^{-1} for all species (Table 2). Survival was 100%.

3.2. Water quality

The pH ranged from 7.7–8.2, with an average of 7.9 ± 0.1; dissolved oxygen ranged from 5.5–8.9 mg l^{-1}, averaging 7.3 ± 0.7. TAN concentrations in the sys-

Table 2. Growth (mean ± SD) of the organisms in the RAS (n = 3). SGR: specific growth rate; FCR: food conversion ratio; (–) not applicable

Tank	Absolute growth ($g\ d^{-1}$)	SGR ($\%\ d^{-1}$)	FCR	Final density ($g\ m^{-2}$)
Fish	1.9 ± 0.2	1.1 ± 0.1	2.3 ± 0.2	–
Sea cucumbers	1.2 ± 1.1	1.1 ± 0.7	–	67 ± 25
Halophytes	0.3 ± 0.02	1.3 ± 0.1	–	5425 ± 216

tems peaked on Day 4 in all tanks and subsequently decreased. They remained low in the sea cucumber and halophyte tanks, while concentrations in the fish tanks were higher. NO_2^- concentrations were highest within the first 29 d, after which they remained low in all tanks. NO_3^- and PO_4^- concentrations increased over time in all tanks, in the case of NO_3^- up to a plateau around 17 mg N l^{-1} (Fig. 3).

During the time periods when water circulation was turned off and water remained in the same tank, concentrations of all inorganic N species as well as PO_4^- increased in the fish tanks. In the sea cucumber tanks, concentrations of NO_3^- and PO_4^- increased as well, while NO_2^- and TAN decreased. In the water that remained in the halophyte tanks, concentrations of NO_2^-, TAN and PO_4^- did not change, but NO_3^- concentrations decreased (Table S1 in the Supplement at www.int-res.com/articles/suppl/q012p471_supp.pdf). An average of 3.5 ± 0.2 g of DIN developed in the fish tanks (Fig. 4). During this time, the total DIN in the sea cucumber tanks increased by 0.9 ± 0.7 g. Only the halophyte tanks showed a net decrease of 0.7 ± 0.1 g DIN. During the 7 wk of this sampling regime, DIN in the halophyte tanks was reduced by a total of 4.9 ± 1 g N.

Concentrations of TSS visibly increased in the fish tanks when the system was turned off and decreased when the water was circulated. Sampling during the last week of the experiment revealed an average decrease of 75.7 ± 0.8% in the fish tanks. During the same time period, overall concentrations in the sea cucumber and halophyte tanks were much lower and more variable, decreasing by 33.3 ± 39.9% in the sea cucumber tanks and increasing by 12.3 ± 45.1% in the halophyte tanks (Fig. 5).

N content in the surface sediments of the sea cucumber tanks was very low overall, but increased over the course of the experiment (Fig. 6).

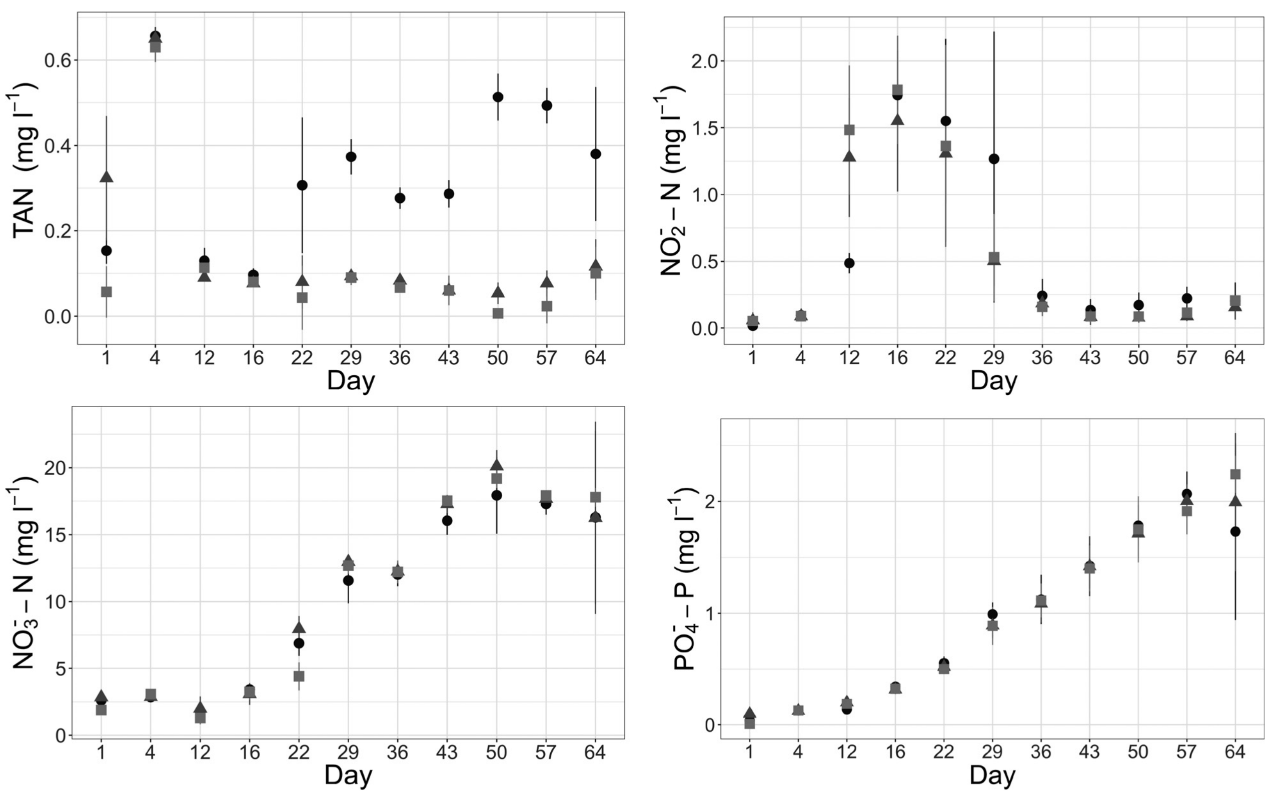

Fig. 3. Mean (±SD) concentrations of dissolved nutrients measured weekly in the tanks of the RAS systems (n = 3) over the course of the experiment. Black circles: Fish tanks; dark grey triangles: sea cucumber tanks; light grey squares: halophyte tanks; TAN: total ammonia N

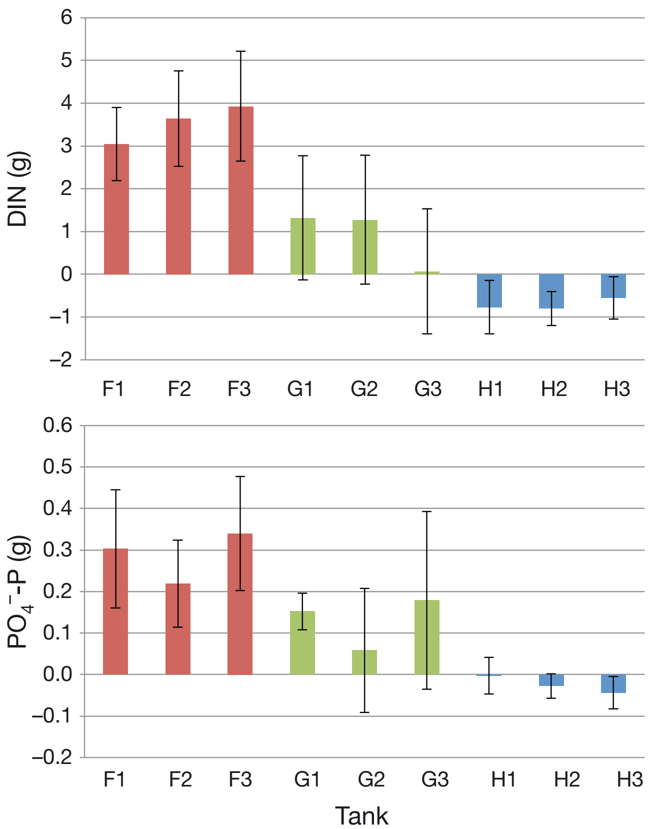

Fig. 4. Average (±SD) change in total dissolved inorganic nitrogen (DIN) and PO_4-P in the individual tanks (F: fish; G: sea cucumber; H: halophyte) of the RAS (n = 3), after periods of interrupted water circulation during the 70 d experiment

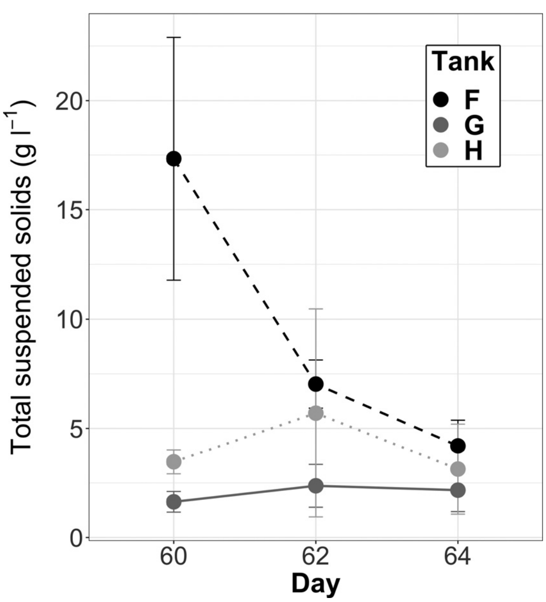

Fig. 5. Mean (±SD) total suspended solids in the fish (F), sea cucumber (G) and halophyte (H) tanks of the RAS during the last week of the experiment

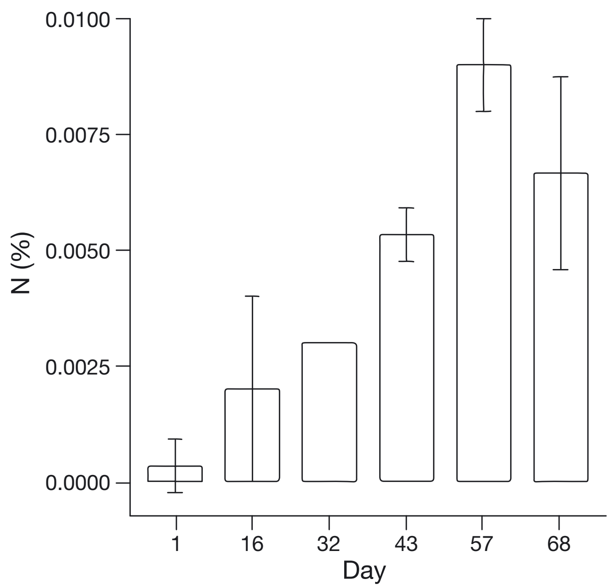

Fig. 6. Mean (±SD) nitrogen (N) content (% of dry weight) in the surface sediment of the sea cucumber tanks in the RAS (n = 3)

3.3. N budget

Over the course of the experiment, each RAS received an input of 132 g N via the fish feed. The constructed budget accounted for an average of 89.1 ± 8.7 g and 69.4 ± 5.1% of the N input (Table 3); 48.1 ± 3.5 g and 36.4 ± 2.6% of N were in the form of usable biomass of fish, sea cucumbers or halophytes (Fig. 7).

4. DISCUSSION

4.1. Performance of the RAS in terms of biomass production

Overall, the IMTA RAS performed well, as survival was 100% of all organisms and growth was recorded across all system components. All milkfish increased in weight and were apparently healthy. The observed average individual growth of 1.9 g fish^{-1} d^{-1} and the average SGR of 1.1% d^{-1} was on the lower end, but comparable to other studies (Guanzon et al. 2004, Jana et al. 2006). The average FCR of 2.3 was high compared to milkfish in pond aquaculture (Sumagaysay-Chavoso & San Diego-McGlone 2003). At 1.7 kg m^{-3}, the initial stocking density was much lower than in intensive aquaculture and more comparable to the maintenance of broodstock (Marte 1988, Ranjan et al. 2019). The study had, however,

Table 3. Average (±SD) total input of feed (into fish tanks) and sludge (into sea cucumber tanks) and biomass production as wet weight (dry weight for algae) (g), nitrogen (g N) and % of feed input (% N) of the RAS (n = 3) at the end of the 70 d experiment. (–) not applicable

Tank		Input (feed/sludge)	Biomass produced	Dissolved N	Sediment N	Algae	Organic matter	Total N accounted for
Fish	g	2620.0 ± 0.0	1147.3 ± 78.2	11.4 ± 4.6	–	–	–	1147.4 ± 78.2
	g N	132.1 ± 0.0	44.3 ± 3.0	–	–	–	–	55.8 ± 7.6
	% N	100 ± 0.0	33.6 ± 2.3	8.66 ± 3.5	–	–	–	42.2 ± 5.7
Sea cucumber	g	814.3 ± 1.0	92.3 ± 68.3	16.24 ± 6.9	–	–	28.8 ± 20.3	935.4 ± 170.4
	g N	1.0 ± 0.3	0.6 ± 0.4	–	6.5 ± 2.0	–	0.1 ± 0.1	108.9 ± 53.2
	% N	0.8 ± 0.2	0.5 ± 0.3	12.3 ± 5.2	4.9 ± 1.5	–	0.1 ± 0.1	18.5 ± 3.9
Halophyte	g	–	1261.0 ± 95.1	6.2 ± 0.9	–	51.7 ± 39.7	226.0 ± 144.6	–
	g N	–	3.1 ± 0.2	–	–	1.6 ± 1.2	0.5 ± 0.2	11.4 ± 1.8
	% N	–	2.4 ± 0.2	4.7 ± 0.6	–	1.2 ± 0.9	0.4 ± 0.1	8.6 ± 1.3

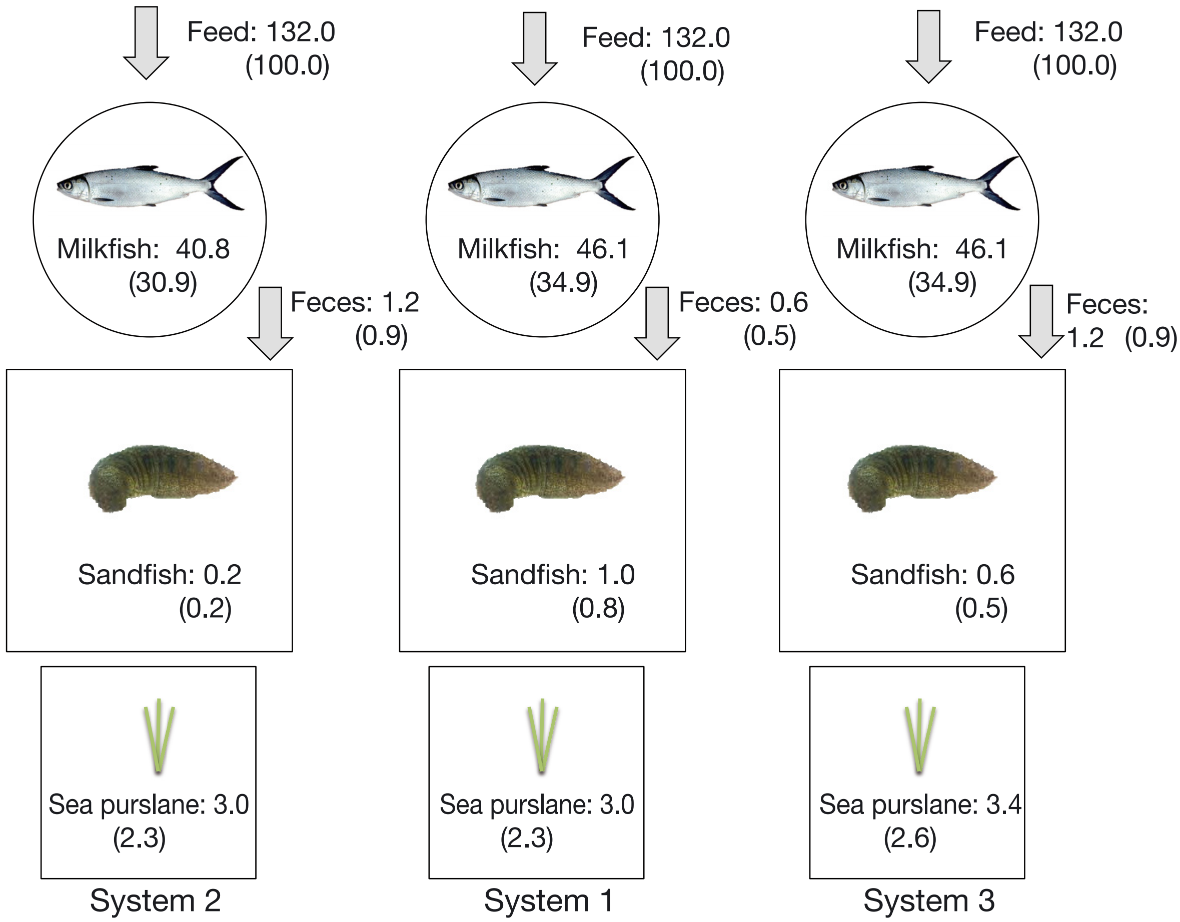

Fig. 7. Conceptual representation of the 3 experimental RAS, each including a milkfish tank, sea cucumber tank and hydroponic sea purslane tank. Numbers indicate the N content (g) of the different compartments, with the corresponding % N relative to the input (feed) in parentheses

been designed according to semi-intensive aquaculture practices on Zanzibar with conservative stocking densities and low-performance equipment. Given that water quality remained acceptable throughout the experiment, stocking density could be increased and a higher feeding rate could be divided between 2 or more daily feedings in order to achieve better growth and allow for the results to act as better reference values for aquaculture production (Sumagaysay 1998).

Despite similar initial size, individual growth rates of the sandfish were highly variable. This is to be expected as sea cucumber growth often varies and is determined by a number of different factors, including individual genetics (Qiu et al. 2014, Dumalan et al. 2019). Absolute growth and SGR observed in this experiment were comparable to that of *Holothuria scabra* during sea ranching studies (Namukose et al. 2016), feeding experiments on organic waste from shrimp farming (Watanabe et al. 2012, Hochard et al. 2016) and grow-out trials underneath milkfish net pens (Dumalan et al. 2019). As mortality can be high when small sandfish are transferred to grow-out pens, keeping juveniles at hatcheries until a size of >50 g improves survival (Purcell & Simutoga 2008, Dumalan et al. 2019).

The yield of sea purslane in the RAS was lower than the biomass production of 0.53 kg m^{-2} found in aquaponics integrated with platy fish *Xiphophorus* sp. (Boxman et al. 2017), and the relative growth rate was comparatively low (Slama et al. 2017). Concentrations of NO_3^- and PO_4^- in the halophyte tanks were similar to a RAS experiment testing 3 species of halophytes in hydroponic cultivation in the waste water of European seabass *Dicentrarchus labrax* (Waller et al. 2015). The authors of that study measured concentrations of 19–21 mg N l^{-1} and 3 mg P l^{-1}, considering low nutrient availability, compared to typical nutrient solution in hydroponic culture. Higher nutrient concentration in the halophyte tanks would therefore be favorable for the production of sea purslane biomass.

4.2. Feasibility to run the RAS without conventional biofilters

Infrastructure restrictions did not allow testing the filter performance of the sea cucumber and halophyte tanks compared to control treatments. While single systems are sometimes evaluated in aquaculture research to study feasibility (Waller et al. 2015, Ranjan et al. 2019, Yogev & Gross 2019), running the experiment in triplicate allowed us to also show performance variability.

The concentrations of dissolved inorganic nutrients remained acceptable for aquaculture production and organism health throughout the duration of the experiment. After an initial increase during the first week, TAN levels in the sea cucumber and sea purslane tanks remained low. During continuous water circulation, concentrations in the fish tanks were below 1 mg l^{-1} TAN-N, and even after increases over the weekend, concentrations were comparatively low (Carton-Kawagoshi et al. 2014). While some ammonia oxidizing microbes might have been present in the sediment of the sea cucumber tank despite the initial chlorine treatment, it usually takes weeks to months for a bacterial community to establish (Gutierrez-Wing & Malone 2006, Keuter et al. 2017). The quick decreases in TAN concentrations may also be attributed to direct assimilation of ammonium by halophytes and microbes (Quintã et al. 2015, Klawonn et al. 2019). NO_2^- can be toxic to fish and is thus of great concern in aquaculture, but even during peak concentrations, levels in this experiment remained far below lethal levels (around 675 mg l^{-1} NO_2^--N) (Almendras 1987). The highest NO_2^- concentrations were measured on Day 16 and stayed low for the remainder of the experiment. Decreases in ammonia concentrations followed by a NO_2^- peak and subsequently increasing NO_3^- concentrations are indicative of the 2-step aerobic nitrification process of biological oxidation of ammonia to NO_2^-, and NO_2^- to NO_3^-, and provide evidence of nitrification activity (Keuter et al. 2017, Brailo et al. 2019). This is likely to have occurred in the oxygenated sand substrate of the sea cucumber tanks, which would have provided an ideal habitat for a diverse and stable bacterial community of facultative aerobes with good bioremediation potential (Robinson et al. 2016)

DIN was mostly present in the form of NO_3^-, which increased over the course of the experiment. Such a build-up is typical for zero-exchange RAS without denitrifying filters (DeLong & Losordo 2012, Keuter et al. 2017). Although fluctuating nutrient concentrations can impact bacterial communities (Blancheton et al. 2013), interrupted water circulation apparently did not prevent an adequate biofilter performance in the sea cucumber and halophyte tanks in this experiment. Furthermore, the time periods without water exchange provided an opportunity to examine the development of nutrient concentrations in the individual tanks. The increase in concentration of all measured dissolved inorganic nutrients plausibly identified the fish tanks as the main source of N and P in the system. The decrease of TAN and NO_2^- with an increase of NO_3^- in the sea cucumber tanks further identifies it as the main site of nitrification. When the water remained in the halophyte tanks, concentrations of NO_2^-, TAN and PO_4^- remained the same, so nitrification or PO_4^- uptake are unlikely to have occurred. NO_3^- concentrations, however, decreased, indicating that the tanks acted as a net sink of NO_3^-, unlike the fish and sea cucumber tanks, suggesting that the hydroponic cultivation of *Sesuvium portulacastrum* did prevent higher NO_3^-concentrations. The mean decrease of 4.9 g N was well accounted for by the N measured in the biomass of sea purslane, as well as filamentous green algae and other organic matter recovered from the halophyte tanks, which averaged a total of 5.2 g N. Considering that some N was taken up during the first weeks of the experiment as well as the constant oxic conditions in the water column of the halophyte tanks, it can be assumed that the mechanism of N removal was due to assimilation, not denitrification (van Rijn et al. 2006). Denitrification can be achieved in settling basins of RAS (Gelfand et al. 2003), and a longer experimental period is recommended to investigate if it can be observed in this system as well. NO_3^- concentrations in the halophyte tanks both increased and decreased at times. Such fluctuations have been observed in hydroponic cultivation of halophytes (Waller et al. 2015, Boxman et al. 2017) and can be caused by mineralization of particulate organic matter (Hargreaves 1998).

PO_4^- decreased in both the halophyte tanks, as well as in the sea cucumber tanks to some extent, but concentrations showed a steady increase in the whole system. PO_4^- is not directly toxic to fish, but because it is usually limited in the environment, higher concentrations can cause harmful algal blooms (Kim et al. 2013). Improved removal of feed-derived PO_4^- would therefore be desirable and could be achieved through the use of steel slag or limestone (Naylor et al. 2003).

TSS removal by constructed wetlands has been summarized by van der Gaag et al. (2010), who found a decrease of 67%, leaving the performance of this IMTA system relatively high with an average decrease of 76% of TSS in the fish tanks over the course of the 5 d sampled. As suspended solids can cause clogging of the system, their removal is a requirement in integrated (aquaponic) systems (Wongkiew et al. 2017). This experiment suggests that the sea cucumber tanks serve well as settling basins without the need for further solids removal.

4.3. Use of feed-derived N in the sea cucumber and halophyte tanks

The RAS showed good recovery of feed-derived N in the form of fish biomass, which accounted for the majority of N at approximately 34% (Zhong et al. 2011, Poli et al. 2019). The amount of N that was collected in the form of sludge from the fish tanks was small considering that the sedimentation rate in milkfish aquaculture has been estimated to be as high as 60% of feed-derived N (Holmer & Fortes 2002, Sumagaysay-Chavoso 2003). Taking into account the decrease in suspended solids from the fish to the sea cucumber tanks as well as the increase in sediment N content over time, it is likely that a considerable fraction of particulate matter was not collected manually, but remained suspended in the water column and settled into the sea cucumber tank. The percentage of N that was subsequently recovered as sea cucumber biomass was higher than the decrease of particle load by 0.73% previously determined for this species (Chary et al. 2020). A box model on the integration of *H. scabra* under milkfish cages estimated that 6.4% of particulate N could be recovered by the sea cucumbers, but the authors recognized that such recovery would require farming the sea cucumbers at densities much higher than has been found supportive of their growth (Watanabe et al. 2015). Good growth of *H. scabra* has been found up to densities of 250 g m^{-2}, suggesting that stocking in this experiment could have been increased threefold to facilitate better N recovery in sea cucumber biomass (Purcell & Simutoga 2008, Hochard et al. 2016). Biomass production in this study remained small, but the role of sea cucumbers in this IMTA RAS was rather as a valuable aquaculture organism to be cultivated on sand substrate, which in turn provides a medium for nitrifying bacterial communities (Zamora et al. 2018).

The constructed N budget accounted for approximately 69% of the feed-derived N, which is high compared to other studies (Gross et al. 2000, Wang et al. 2015). After fish biomass, the second largest portion of N was found as DIN in the water of the different tanks. Recovery of N as halophyte biomass in this study was similar to the recovery of N in *Salicornia* in a wetland filter (Shpigel et al. 2013) and *Sarcocornia ambigua* in an IMTA with fish and shrimp (Poli et al. 2019). Higher rates have been achieved with the hydroponic cultivation of *S. ambigua* in hydroponic cultivation with shrimp (Pinheiro et al. 2017), which improved N use by an additional 6.4% and produced 2 kg of halophytes for 1 kg of shrimp, resulting in the recommendation to further increase the plant biomass relative to the biomass of fed animals. This study had a plant to fish biomass ratio of approximately 1:1. Given the changes in DIN concentrations in the fish and halophyte tanks, it would be recommended to increase this ratio to 5:1.

Future experiments studying the predominant N transformation processes and functional groups of bacteria using quantitative PCR and tracing N assimilation in detritivores and plants through stable isotope or fatty acid analysis could shed more light on the performance of low-tech integrated RAS.

5. CONCLUSIONS

This study showed the viability of a simple and low-cost RAS system, integrating valuable tropical species of 3 different trophic levels. For the first time, sea cucumbers and halophytes were combined, with these units replacing a separate biofilter. The system produced additional biomass while maintaining good water quality, and the overall low levels of dissolved inorganic nutrients and accumulated solid waste suggest that higher fish biomass could have been applied and maintained for a longer time period. Furthermore, higher sea cucumber stocking density and increased plant biomass would be recommended to improve recovery of both particulate and dissolved N and prevent accumulation in the system. The system operated without producing solid waste or discharging polluted water and optimized the use of water and space. This kind of sustainable aquaculture model has the potential to provide food security and income, and the simple system used here also allows for application in developing coastal communities.

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