Improving bioremediation with extractive species in integrated aquaculture:

Towards application in tropical recirculating systems

A dissertation by

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Summary

Marine organisms are key to food security and to combat malnutrition, especially in the developing tropics. With the increase in the human population, so grows its appetite for seafood and with production from capture fisheries at its limit, aquaculture is left to fill the gap. Faced with resource limitations and environmental regulations, aquaculture operations have to augment their output while decreasing the relative use of resources, coastal space and effluent output. Integrated aquaculture is seen as a strategy to meet demands for more efficient and sustainable aquaculture production. Especially integrated multi-trophic aquaculture (IMTA) is often described as a solution, but proliferation of the industry has been slow, uneven and research is still lacking in regards to its application with tropical marine species.

This thesis provides an example of IMTA with edible tropical species at three different trophic levels. A proof-of-concept presents the viability of a low-technology recirculating aquaculture system (RAS) of milkfish *Chanos chanos*, integrating sea cucumber *Holothuria scabra* and halophyte *Sesuvium portulacastrum* as extractive species (**Chapter 2**). The species showed good growth and the system maintained good water quality and low concentrations of dissolved inorganic nitrogen even without an additional biofilter.

Removal, uptake and assimilation of feed-derived nutrients through the cultivation of the extractive species were further examined to determine strategies to optimize bioremediation. The high-value sea cucumber *H. scabra* was raised on solid waste from milkfish aquaculture (**Chapter 3**). Animals were supplied with the waste alone or waste amended with either microcrystalline cellulose or bagasse. Comparison with control systems without sea cucumbers showed that the animals decreased nitrogen and organic carbon in the sediment, albeit not statistically significant. For the first time, a context-specific trophic enrichment factor was applied in a stable isotope mixing-model for this species, demonstrating good assimilation of nutrients from aquaculture waste. Increasing the C/N ratio of the waste led to a higher biomass production and nutrient recovery into sea cucumber biomass and bagasse, an agricultural waste product, proved to be effective for carbon supplementation.

Removal and uptake of dissolved inorganic nutrients from milkfish effluents by systems planted with *S. portulacastrum* was investigated via labeling with ^{15}N nitrate or ammonium (**Chapter 4**). The halophyte was cultivated either hydroponically or with sand as substrate. Nitrogen recovery in edible plant biomass was significantly higher in hydroponic cultivation.

This thesis contributes to the knowledge needed to move towards sustainably intensifying multi-trophic aquaculture in tropical countries. The results hint at the intricate interplay between extractive species, their biotic environment and nutrient cycles and highlight the complexity, but also the opportunities of saline IMTA.

Zusammenfassung

Meeresorganismen spielen eine wichtige Rolle für die globale Ernährungssicherheit und in der Bekämpfung von Mangelernährung, besonders in tropischen Entwicklungsländern. Mit der wachsenden menschlichen Bevölkerung wächst auch die Nachfrage nach Meeresfrüchten und da die Fangfischerei an ihre Grenzen stößt, muss die Aquakultur die Lücke schließen. Angesichts von Ressourcenknappheit und Umweltvorschriften müssen Aquakulturbetriebe ihre Produktion steigern und gleichzeitig die Nutzung von Rohstoffen, Küstenraum und die Abwasserproduktion verringern. Integrierte Aquakultur gilt als Strategie, um die Anforderungen an eine effizientere und nachhaltigere Aquakulturproduktion zu erfüllen. Insbesondere die Integrierte Multitrophe Aquakultur (IMTA) wird häufig als Lösung beschrieben, aber die Verbreitung in der Industrie läuft langsam und ungleichmäßig. Auch mangelt es an Forschung hinsichtlich ihrer Anwendung bei tropischen marinen Arten.

Diese Arbeit liefert ein Beispiel für IMTA mit essbaren tropischen Arten auf drei verschiedenen trophischen Ebenen. Eine Proof of Concept-Studie zeigt die Durchführbarkeit eines rezirkulierenden Aquakultur-Systems (RAS) unter Einsatz von einfacher Technologie. Seegurken *Holothuria scabra* und Halophyten *Sesuvium portulacastrum* wurden als „Abfallverwerter“, sogenannte extraktive Arten, mit Milchfischen *Chanos chanos* in ein geschlossenes System integriert (**Kapitel 2**). Alle Arten zeigten dabei gutes Wachstum und das System hatte gute Wasserqualität mit niedriger Konzentration gelösten anorganischen Stickstoffs ohne zusätzlichen Biofilter.

Des Weiteren wurde die Entfernung, Aufnahme und Assimilation von Nährstoffen aus Futtermitteln durch die Kultivierung der extraktiven Arten untersucht, um Strategien zur Optimierung der Bioremediation herauszufinden. Die ökonomisch wertvolle Seegurke *H. scabra* wurde mit organischen Feststoffabfällen aus der Milchfisch-Aquakultur gefüttert (**Kapitel 3**). Die Tiere wurden entweder nur mit den Abfallprodukten gefüttert, oder unter Zugabe von mikrokristalliner Zellulose oder Bagasse. Ein Vergleich mit Kontrollsystemen ohne Seegurken zeigte, dass die Tiere die Anreicherung von Stickstoff und organischem Kohlenstoff im Sediment verringerten, wenn auch nicht statistisch signifikant. In einem stabilen Isotopenmodell wurde zum ersten Mal für diese Art ein kontextspezifischer trophischer Anreicherungsfaktor angewendet. Die Ergebnisse zeigten eine gute Aufnahme von Nährstoffen aus Aquakulturabfällen und dass eine Erhöhung des C/N Verhältnisses der Abfälle Wachstum und Nährstoffrückgewinnung in Seegurkenbiomasse verbessern. Bagasse, ein landwirtschaftliches Abfallprodukt, erwies sich als wirksam für die Supplementierung mit Kohlenstoff.

Die Entfernung und Aufnahme gelöster anorganischer Nährstoffe aus Milchfischabwässern in mit *S. portulacastrum* bepflanzten Systemen wurde durch Markierung mit ^{15}N Nitrat oder Ammonium untersucht (**Kapitel 4**). Der Halophyt wurde entweder hydroponisch oder mit Sand als Substrat kultiviert. Die Stickstoffrückgewinnung in essbarer Pflanzenbiomasse war im hydroponischen System signifikant höher.

Diese Arbeit trägt zum Wissen davon bei, wie multitrophe Aquakultur in tropischen Ländern nachhaltig intensiviert werden kann. Die Ergebnisse deuten auf das intrikate Zusammenspiel zwischen extraktiven Arten, ihrer biotischen Umwelt und den Nährstoffkreisläufen hin und unterstreichen die Komplexität, aber auch die Möglichkeiten der marinen IMTA.

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Table of Contents

Chapter 1

General Introduction

1. Aquaculture

1.1. Relevance and development

The aquatic realm, covering 71% percent of our planet, is full of life and productivity. Humans have long since benefitted from the resources it provides directly through gleaning or fishing (Richter et al. 2008). Just as early societies developed from hunting to farming, people in some locations progressed from fishing to cultivating aquatic organisms, and the earliest forms of aquaculture and mariculture emerged (Beveridge & Little 2002). Archaeological finds show that seabream were ranched in a Mediterranean lagoon during the Late Bronze Age and Hawaiian kings organized the construction of massive fishponds some 1700 years ago (Costa-Pierce 1987, Guy et al. 2018). Aquaculture is defined as the farming of aquatic animals or plants and implies ownership and a certain degree of intervention in the life cycle or growth process (Beveridge & Little 2002). Depending on the density of the cultivated organisms and the input of invested labor, feed or fertilizer, it is ranked from 'extensive', over 'semi-intensive' to 'intensive' aquaculture with increasing effort (Coche 1982).

Throughout the second half of the 20th century, the "Blue Revolution" has seen the widespread intensification of the sector as aquaculture is left to fill the gap between an increasing global demand for fish protein and static capture fishery production (MacKay 1983, Thia-Eng 1997, Costa-Pierce 2002, FAO 2018). Aquaculture now accounts for 46% of seafood production with a combined output of fish, crustaceans, and mollusks of more than 82 mio tonnes and 32 mio tonnes of farmed seaweed in 2018 (Edwards et al. 2019, FAO 2020). As intensive aquaculture is pushing towards maximized production, it has been met with numerous environmental challenges. Antibiotics and other pharmaceuticals are applied to high-density cultures, often leak into the adjacent ecosystem and accumulate around production sites (He et al. 2016). The expansion of land-based pond aquaculture has put pressure especially on coastal ecosystems and caused the loss of natural habitats as for example mangroves continue to be converted to shrimp ponds (Dorber et al. 2020). Considerable numbers of fish escape from marine farms, influence the genetic structure of wild populations and transfer diseases and pathogens (Jackson et al. 2015, Arechavala-Lopez et al. 2018). While overall aquaculture production increases, the contribution of non-fed organisms to aquaculture, such as filter-feeding carp and bivalves, has continuously declined from 44 % in 2000 to 31 % in 2018 (FAO 2020). The cultivation of animals on high trophic levels depends on a supply of feed rich in protein and fatty acids that are in turn sourced by fishing small pelagic fish or low value species (Naylor et al. 2000, Cao et al. 2015). Research into alternative feed ingredients has considerably reduced the dependence on marine ingredients and fed-aquaculture no longer consumes more fish than it produces (Kok et al. 2020), but the problem remains that regardless of type of feed or cultivated species, the majority of feed supplied nutrients is not retained by the fed organism and subsequently lost as particles or excreted in dissolved form (Piedrahita 2003 and references therein). When these excess nutrients are discharged into the natural environment, they can cause eutrophication and anoxic sediments, increased turbidity and lead to algae blooms (Holby & Hall 1991, Holmer et al. 2003, Herbeck et al. 2014, Jia et al. 2015, Ballester-Moltó et al. 2017).

In the process of intensification, aquaculture operations are increasingly moving into closed, land-based systems that provide greater control over environmental parameters, optimize the use of space and water and minimize discharge (Martins et al. 2010, Badiola et al. 2012, Verdegem 2013). In recirculating aquaculture systems (RAS) less than 10 % of the water is exchanged daily with "next-generation" technology moving toward closed systems with near-zero exchange (McCarthy & Gardner 2003, Badiola et al. 2012, Yogev et al. 2017). Waste discharge is thus reduced by a factor 500-1000, but the produced effluent and solid waste are highly concentrated (Timmons et al. 2001, Gutierrez-Wing & Malone 2006, Blancheton et al. 2007). RAS maintain water treatment by physically removing solid waste and employing biofilters for nitrification and denitrification (Neori et al. 2007a, Van Rijn 2013). Research is currently focused on integrating additional filtration based on denitrification, annamox and anerobic digestion (Gutierrez-Wing & Malone 2006, van Rijn et al. 2006, Yogev & Gross 2019).

1.2. Waste production in aquaculture

Due to their central role in metabolic processes and the synthesis of amino acids and proteins, formulated feeds include nitrogen (N) and phosphorous (P) as essential nutrients, but they are also limiting factors in aquatic ecosystems (Capone 2008, Conley et al. 2009). One of the biggest conundrums of aquaculture is that while supply needs to be plentiful to achieve good growth, assimilation of feed-supplied N and P by the target species is usually only around 30 % (Handy & Poxton 1993, Hargreaves 1998, Lefebvre et al. 2001, Chen et al. 2018). Half of the feed supplied in aquaculture remains uneaten and is directly lost as particulate waste (Ballester-Moltó et al. 2017). Around 75 % of N and P contained in fish feed is lost to surrounding waters (Holby & Hall 1991, Handy & Poxton 1993, Hargreaves 1998). While nitrate is the most important growth-limiting factor in marine ecosystems, excess supply can cause algae blooms and hyper-nutrification in the environment (Wu 1995, Casalduero et al. 1999, Troell et al. 1999, Chopin et al. 2001). Most P accumulates in sediments where it continuous to provide an otherwise limiting nutrient to short-lived primary producers and has a high potential to cause eutrophication (Holby & Hall 1991, Conley et al. 2009, Jia et al. 2015). Feed, feces and other organic material released from aquaculture elevate water turbidity, decrease photic depth and are finally deposited, modifying the biochemistry of the sediment (Holmer & Kristensen 1992, Price et al. 2014, Ballester-Moltó et al. 2017). Under high nutrient loads, microbial communities become overwhelmed and sediments turn increasingly anoxic (Holmer et al. 2003).

1.3. Nitrogen as a pollutant from aquaculture

N exists in eight different oxidation states as it moves through the nitrogen cycle, which is one of the most important and complex biogeochemical cycles on earth (Thamdrup & Dalsgaard 2008, Robinson 2017). Only a few microbial groups can close the nitrogen cycle and "fix" N from atmospheric N_2 (Capone 2000). Since the invention of the Haber-Bosch process, we have been able synthesize ammonia fertilizer from inert atmospheric N_2, and can used it luxuriously in our food production systems (Erisman et al. 2008). In anabolic processes, N is assimilated into biomass through the synthesis of amino acids, while it provides an energy source to microorganisms in catabolic reactions (Capone 2000, Thamdrup & Dalsgaard 2008, Fenchel et al. 2012). N is provided to cultivated organism in aquafeeds and released at mainly in the form of ammonium (NH_4^+) or un-ionized ammonia (NH_3), the end products of protein catabolism (Hargreaves 1998, Neori et al. 2007b). In aerobic environments such as the water column or oxic sediment, ammonium is converted to nitrite (NO_2^-) by autotrophic bacteria (usually *Nitrosomonas*), which is then further oxidized to nitrate (NO_3^-) usually by *Nitrobacter* (Blackburn & Blackburn 1992). In the absence of oxygen, NO_3^- can be transformed into nitrogen gas N_2 in the process of denitrification. More recently it was discovered that anaerobic oxidation of ammonia (annamox) also converts NH_4^+ to N_2 (Kuenen J. G. 2008). In the process of dissimilatory nitrate/nitrite reduction to ammonia (DNRA) NO_3^- and NO_2^- are reduced to NH_3/NH_4^+ (Thamdrup 2012, Song et al. 2014).

Aquaculture of fed species contributes with ~ 0.9 % of anthropogenic input to the global nitrogen cycle (Verdegem 2013). N is the most significant "pollutant" in aquaculture operations and its oxidation state is of importance due to differences in reactivity and toxicity (Handy & Poxton 1993, Hargreaves 1998). Un-ionized ammonia is toxic to marine life even at very low concentrations of 0.025 mg l^{-1} NH_3-N (Handy & Poxton 1993, Neori et al. 2004, Chen et al. 2006, Crab et al. 2007), but its concentration is influenced by the pH and temperature of water and NH_4^+-N is often the main form of dissolved inorganic nitrogen (DIN) and tolerable at higher levels of 1.5 mg l^{-1} NH_4^+-N (Lefebvre et al. 2001, Porrello et al. 2005, Colt 2006, Webb et al. 2012). Sensitivity to NO_2^--N varies with species and in fresh- or saltwater with tolerance limits around 1.5 mg l^{-1}, but has been found to impact fish growth and survival at concentrations as low as 0.55 mg l^{-1} (Svobodová et al. 2005, Arredondo-Figueroa et al. 2007, Crab et al. 2007, Ciji & Akhtar 2019). Tolerance to NO_3^--N varies between species, but it is usually only toxic at very high concentrations of 500 or even 1000 mg l^{-1} (Blancheton 2000, Colt

2006). Nitrogen forms and concentrations are influenced by the type of aquaculture system, the species reared, the diet and feeding regime (Schneider et al. 2005). A small proportion of N is also excreted in the form of urea and dissolved organic nitrogen (DON) such as trimethylamine or creatine (Handy & Poxton 1993, Hargreaves 1998, Lefebvre et al. 2001), but DON has been reported to make up more than 20% of the nitrogen load in aquaculture wastewater (Konnerup et al. 2011, Webb et al. 2012).

1.4. Waste treatment in aquaculture

As discharge limits are put in place by governments to minimize the environmental impact of aquaculture operations, effluents can no longer just be discarded and need to be treated (van Rijn 2013). In RAS, larger solid waste is collected through settlement and smaller particles via filtration to be removed from the system (Van Rijn 1996, Twarowska et al. 1997, Troell et al. 1999, Piedrahita 2003, Brambilla et al. 2008). For the treatment of inorganic nitrogen, closed systems commonly include bacterial biofilters (Van Rijn 1996, Troell et al. 2003, Summerfelt & Sharrer 2004, Chen et al. 2006, Lyssenko & Wheaton 2006, Malone & Pfeiffer 2006). Such filters provide a growth surface for nitrifying bacteria that maintain low concentration of NH_4^+ and NO_2^-, but a remaining problem is the accumulation of nitrate, reaching levels of 300 – 400 mg L^{-1} NO_3^--N (Van Rijn 1996). In order to effectively remove nitrogen from the system, denitrification of NO_3^- to N_2 gas is used in denitrification biofilters that provide growth conditions for facultative anaerobic bacteria (Van Rijn 1996, Menasveta et al. 2001, Gelfand et al. 2003, McCarthy & Gardner 2003, Neori et al. 2007a, Davidson et al. 2008, Sandu et al. 2008, Singer et al. 2008). However, such filters are expensive and difficult to maintain (Troell et al. 2003, Neori et al. 2004, van Rijn et al. 2006, Saliling et al. 2007, Singer et al. 2008). Alternative chemical filters on the basis of activated carbon and ion-exchange can quickly experience biofouling and become inactive (Troell et al. 2003).

Concentrations of inorganic nitrogen can also be controlled through biofloc technology. In this approach, microbial growth in the water column of fish tanks or ponds is stimulated through the addition of carbohydrates in the form of sugar, starch or cellulose (Avnimelech 2012). Heterotrophic bacteria, using these as a substrate to build proteins for growth, require nitrogen, which they take from the surrounding water. Dissolved nitrogen species in the water are thus transformed into bacterial biomass, aggregating into bioflocs that can be consumed again by shrimp or fish (Avnimelech 2012). The microbial aggregates can supplement or even replace feed. Biofloc technology in tilapia ponds can provide 50% of the protein consumed by the fish (Avnimelech 2007) and when used as a protein source in shrimp feed, microbial floc meal showed the same results or even outperformed soybean or fishmeal ingredients

(Kuhn & Flick 2009, Bauer et al. 2012). The successful application of the technology however requires knowledge of the system, close monitoring to maintain appropriate C:N ratios and microbial densities and upscaling from laboratory to economic application (Kuhn et al. 2010, Hende et al. 2014).

1.5. Addressing sustainability challenges in aquaculture

Although the aquaculture sector is still lagging behind food production from terrestrial systems, it is seen as an important component of future global food security (Edwards et al. 2019). The high nutritional qualities of seafood, its relatively efficient production and carbon footprint and contribution to health and income make it worthwhile to improve on current production processes (Béné et al. 2015). Optimizing feeding strategies and the composition of aquafeeds aim to minimize waste and the strain on natural resources (Luna et al. 2019, Kok et al. 2020). Diets enriched with plant supplements enhance growth, immunity and resistance to diseases, decreasing or even eliminating the need for chemotherapy (Reverter et al. 2014, 2020b). Numerous tools exist to aid practitioners and decision makers to minimize the impact on the ecosystem into which the aquaculture operation is placed through spatial planning and assessment of the ecological carrying capacity (Nunes et al. 2011, Theuerkauf et al. 2019, Weitzman & Filgueira 2019).

In an “ecosystem approach to aquaculture”, expectations for the growth of the industry are for it to use resources sustainably and promote comprehensive nutrient recycling within the culture system to reduce effluent streams and minimize feed input (FAO 2010). The disposal of uneaten feed, fecal matter and dissolved nutrients pose a challenge to the growth of the aquaculture sector, but in the past decades it has been increasingly called for approaching these as resources high in nutrients and essential fatty acids that should be recycled, not discarded (Bischoff et al. 2009).

The principle of reusing waste of one livestock for the production of another crop has a long history of application (Lu & Li 2006, Birhanu & Natarajan 2019). Evidence however suggests that the traditional integration of aquaculture with terrestrial livestock favors the emergence and dissemination of antibiotic resistance genes and may transfer antimicrobial-resistant bacteria from the terrestrial to the aquatic food production system (Petersen et al. 2002, Xu et al. 2020). This may jeopardize the sustainability of this type of integrated aquaculture as the emergence of antimicrobial resistance is a pressing concern for aquaculture development (Reverter et al. 2020a). The development of multi-trophic aquaculture systems that are suitable for the sustainable intensification of the sector is therefore of great research interest.

2. Integrated multi-trophic aquaculture

In integrated aquaculture, a fed species, such as fish or shrimp, is cultivated alongside an extractive species that utilizes its effluent sludge or water (Neori et al. 2004). This approach is arguably as old as aquaculture itself, with water from fishponds used to fertilize crops (Chimits 1957, Renkui et al. 1995). More recently, the concept of integrated multi trophic aquaculture (IMTA) was coined, striving to apply a simplified food web structure by combining species at different trophic levels for a balanced-ecosystem approach (Neori et al. 2004; Chopin et al. 2007). The IMTA concept is a direct application of the ecosystem approach at the farm level (Brugère et al. 2019). Nutrient load is reduced with the additional aim to produce higher yields than mono-species systems while satisfying rising consumer demands for environmental standards and improving the image of aquaculture (Neori et al. 2004, Barrington et al. 2010). In a fully optimized system, IMTA combines the feeding modes of deposit-feeders, filter-feeders and autotrophs that take up dissolved nutrients in addition to the fed organism (Buck et al. 2018). This co-culture approach finds application in a range of operations, from open-water mariculture to closed RAS with aquaponics (Gunning et al. 2016, Buck et al. 2018, Stenton-Dozey et al. 2020).

In open-water IMTA, hydrodynamic and seasonal conditions influence efficient and direct nutrient uptake (Kerrigan & Suckling 2018). Filter-feeding bivalves are for example cultivated close to fish farms to feed on suspended matter and reduce organic nutrient load in the water (Sarà et al. 2009, Troell et al. 2009, Reid et al. 2010). There can however be no extractive benefit at all if the shellfish fail to filter the right particles and in turn produce an additional organic load in the form of pseudofeces (Navarrete-Mier et al. 2010, Wang et al. 2012, Cubillo et al. 2016).

In more closed pond- or tank-based systems, concentrated dissolved effluents can be directly valorised by growing primary producers (Wei et al. 2017, Li et al. 2019). IMTA provides an additional opportunity in RAS, where effluents that are to be recycled are in controlled and accessible environments (Guerrero & Cremades 2012). Particulate organic waste collected by the filtration systems in RAS constitutes a readily available feed for deposit feeding invertebrates, for example polychaetes (Bischoff 2007, Brown et al. 2011, Palmer et al. 2014). In open-water IMTA, polychaetes can be introduced to a site or naturally occurring populations in sediments can be enhanced to process suspended particles and biodeposits of fish farms (Jansen et al. 2019). Valuable amino acid and lipid profiles as well as a protein content of 42 – 45 % make the worms a suitable ingredient for aquafeeds and provide a possibility to recycle nutrients back into the culture system (Wang et al. 2019).

3. Sea cucumbers in aquaculture

Deposit-feeding sea cucumbers are excellent candidates for integrated aquaculture with high market value and nutritional quality (Macdonald et al. 2013, Cubillo et al. 2016, Zamora et al. 2018). Their body wall is dried into bêche-de-mer, a Chinese delicacy and luxury food in the ranks of shark fin, fish maw and abalone (Robinson 2013, Purcell et al. 2014). Due to high demand, many wild populations have been decimated and aquaculture production is increasing (Lane & Limbong 2015, Purcell et al. 2018, Hair et al. 2019). Globally, the cultivation of *Apostichopus japonicus* dominates sea cucumber aquaculture production, but *Holothuria scabra* is a tropical species with fully developed aquaculture technology and increasing production (Purcell et al. 2012, Robinson 2013). Culture production of *H. scabra* is practiced in tropical countries throughout Asia, Africa, Australia and the Central Pacific (Ivy et al. 2006, Robinson & Pascal 2009, Hair 2012, Mills et al. 2012). *H. scabra* has been successfully cultivated in extensive mariculture in marine ponds, sea pens, and sea ranches (Fig. 1A) (Juinio-Meñez et al., 2017; Pitt, 2001; Purcell et al., 2012).

3.1. Feeding ecology of deposit-feeding sea cucumbers

H. scabra, commonly known as sandfish, is a deposit-feeding holothurian, usually found in habitats that are natural sinks of organic matter such as seagrass beds, mangrove areas, inner reef flats and estuaries (Fig. 1B) (Hamel et al. 2001). It selectively seeks out small-grained, organically enriched sediments, which are ingested using oral tentacles and transported through the intestine (Yingst 1976, Moriarty 1982, Baskar 1994, Mercier et al. 1999). Here, a portion of the ingested molecules is assimilated and the rest is excreted with sediment in the fecal pellet (Plotieau et al. 2014). Sea cucumbers are able to digest detritus, bacteria and microalgae (Plotieau et al. 2014). Furthermore, they assimilate nutrients made labile by microbial degradation in their environment and by the bacteria in their intestine (Yingst 1976, Plotieau et al. 2013, 2014). *H. scabra* may also be "nutritionally bipolar" as sea cucumbers with respiratory trees have the ability to take up macromolecules ingested with sea water through their anus (Jaeckle & Strathmann 2013).

By feeding on organisms multiple orders of magnitude smaller than them, sediment-feeders such as holothurians bridge multiple steps in the trophic cascade and influence the microbial loop (Uthicke 2001). Besides this direct extraction, they also have beneficial effects on sediments by re-working upper sediment layers and influencing the development of microbial communities (Moriarty & Pollard 1982, Moriarty et al. 1985, Uthicke 1999, MacTavish et al. 2012). Through their grazing activity, holothurians remineralize nutrients, which enhances the growth of primary producers and subsequently returns some organically bound nutrients to the sediment (Uthicke

2001). Throughout the day, *H. scabra* alternates between phases of active feeding and buried resting. This bioturbation oxygenates the sediment, enhances microbial activity and increases the rates of organic matter degradation and nutrient remineralization (Aller & Aller 1998, Kristensen 2000, Mermillod-Blondin & Rosenberg 2006). Through its feeding activity, *H. scabra* can remove high amounts of sediment organic matter, remediate hydrogen sulfide toxicity and one adult animal can re-work almost 200 g of sediment per day (Mercier et al. 1999, Roberts et al. 2000, Watanabe et al. 2012).

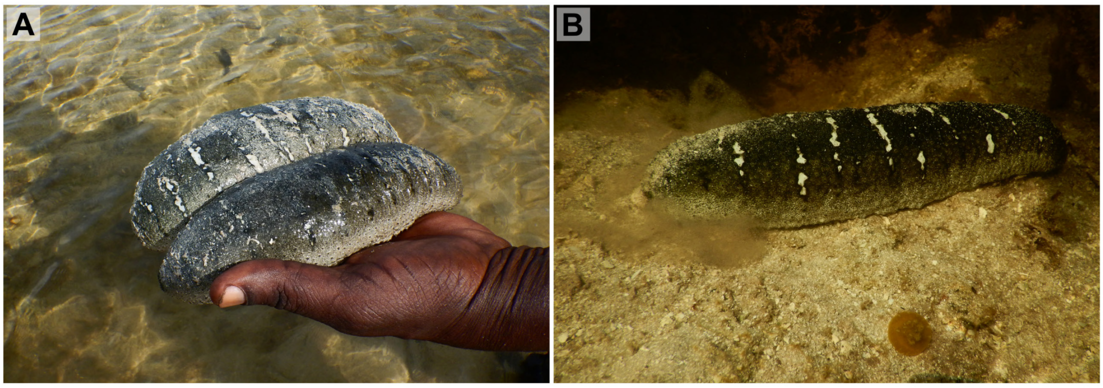

Fig. 1: Two adult *Holothuria scabra* sea cucumbers at a sea ranching site on Zanzibar, Tanzania and B) *H. scabra* feeding in a seagrass meadow on Zanzibar.

3.2. Sea cucumbers as extractive organisms

As detritivorous animals, sea cucumbers can feed on debris from fish or shellfish cultivation (Ahlgren 1998, Slater et al. 2011, Hannah et al. 2013). Co-cultivation of sea cucumbers with fish culture has been investigated with temperate and tropical species (review by Zamora et al. 2018). Experiments have shown that sea cucumbers *Parastichopus californicus* and *A. japonicus* can utilize organic waste from finfish aquaculture farms and even achieve higher growth rates than when reared in natural habitats (Ahlgren, 1998; Hannah et al., 2013; Yokoyama, 2013; Yu et al., 2013, 2014). Integration of *H. scabra* with net culture of red drum showed no significant remediative effect on the sediment, likely due to low sea cucumber biomass (Chary et al. 2020). It was postulated that 1 kg of finfish would require 1.3 kg of sea cucumber to consume 100% of fish feces (Chary et al. 2020). In tank experiments, *H. scabra* successfully fed on waste from shrimp farming (Pitt et al. 2004, Purcell et al. 2006, Watanabe et al. 2012). In direct co-cultivation, shrimps can however predate on the sea cucumbers (Pitt et al. 2004). A feasible possibility seems to be a rotational system in ponds where grow out of one harvest of sea cucumbers to market size rotates with one cycle of shrimp cultivation (Mills et al. 2012, Hochard et al. 2016).

H. scabra has been integrated with primary producers, e.g. in seaweed aquaculture. While co-cultivation with *Caulerpa lentillifera* was not successful, the animals showed good growth when cultivated underneath *Kappaphycus striatum* (Beltran-Gutierrez et al. 2014, Dobson et al. 2020). A sandfish stocking biomass of 200 g m^{-2} even enhanced the growth of *Eucheuma denticulata* and decreased organic matter content in the sediments (Namukose et al. 2016). Further research is necessary to optimize sea cucumber biomass production and/or bioremediation for practical integration into existing monoculture units and on sediments enriched by biodeposits from commercial marine fish farming (Dumalan et al. 2019, Chary et al. 2020).

The cultivation of deposit feeders on sediments adds further benefits for remediation of effluents by providing the oxic and anoxic conditions for nitrogen conversion and removal (Palmer 2010, Robinson et al. 2015). For good growth, *H. scabra* depends on sediments with an established microbial community (Lavitra et al. 2010, Robinson et al. 2015). Besides supporting food sources for the sea cucumber, sediments provide substrate for biogeochemical processes that play an important role in the cycling of elements including N and P through processes of immobilization, mineralization, ammonification, nitrification, and denitrification (Handy & Poxton 1993, Burford & Williams 2001, Kajimura et al. 2004). Through mineralization, the degradation of organic matter by microbial communities, inorganic nutrients are released (Kristensen 2000). In the water column or upper oxic sediments, aerobic processes occur as oxygen is the most thermodynamically efficient electron acceptor (Kristensen et al. 1995). Once oxygen is depleted and not replaced, as for example the case in stratified sediments, chemically bound forms of oxygen such as NO_3^- or SO_4^{2-} become the only available electron acceptors, giving an advantage to bacteria that can utilize them in anaerobic respiration (Middelburg et al. 1993, Kristensen 2000). Even short re-exposure of anoxic sediment layers to oxygen by bioturbating macrofauna improves decomposition, remineralization and stimulates bacterial growth (Aller 1994, Aller & Aller 1998, Kristensen 2000). *H. scabra* can biomitigate sediment eutrophication and anoxia (Wolkenhauer et al. 2010, Lee et al. 2018). Bioturbation and grazing link such deposit feeders and microbial communities through resource exploitation and nutrient cycling (Aller 1994, Uthicke 2001).

3.3. Deposit-feeder and microbial bioremediation

The interplay between deposit-feeding sea cucumbers and sedimentary microbial communities has great potential for the recycling of nutrients in aquaculture waste treatment (Robinson et al. 2016, Robinson 2017). Compared to higher organisms, bacteria are more efficient in the utilization of nutrients and immobilization into their biomass makes these nutrients available for transfer up the heterotrophic food-chain (Moriarty 1987, Avnimelech 2014). Adding nutrients that promote the growth of preferred bacteria is already an established method in controlling concentrations of DIN in aquaculture (Crab et al. 2007, Avnimelech 2014). Glucose supplementation improves water quality by decreasing concentrations of NH_4^+, NO_2^- and NO_3^- (Zheng et al. 2018).

Balancing dissolved C and N, e.g. in biofloc systems, further converts dissolved nitrogenous waste into bacterial biomass, providing a high-protein food source suspended in the water column (Schneider et al. 2005, Crab et al. 2012). Addition of carbohydrates in the form of sugar, starch or cellulose stimulates the growth of heterotrophic bacteria, which subsequently require nitrogen and will immobilize available forms into their biomass (Avnimelech 2012). The proteinaceous aggregates can directly act as a feed source for fish or shrimps in the water column or be harvested as an ingredient for aquafeeds (Avnimelech 2007, Kuhn & Flick 2009, Bauer et al. 2012). Successful application of the technology, especially at larger scale, requires detailed knowledge of system dynamics such as appropriate C:N ratios (Kuhn et al. 2010, Hende et al. 2014).

Modification of C:N ratios also improves performance of sediment-based bioremediation systems (Robinson et al. 2018, 2019). Carbon supplementation of solid wastes rich in nitrogen promotes dissimilatory nitrate reduction to ammonium (DNRA) and N_2 uptake over denitrification in microbial-deposit feeder systems (Robinson et al. 2018). Increasing the C:N of aquaculture sludge from 5:1 to 20:1 further supported higher sea cucumber biomass production and it was suggested that carbon from agricultural waste streams might be suitable sources of carbon to improve the performance of microbial-deposit-feeder bioremediation systems (Robinson et al. 2019).

4. DIN remediation through plants

Macroalgae are the extractive organism most commonly used in open-water IMTA to take up dissolved nutrients (Kerrigan & Suckling 2018). Algae have fast growth rates, are easy to cultivate and while producing valuable biomass, they increase concentrations of dissolved oxygen in the water (Gao & McKinley 1994, Chopin et al. 2001, Neori et al. 2003). Sales of seaweed grown in biofilters can cover the additional costs of implementing a co-culture system (Ditchburn and Carballeira 2019; Neori et al. 2004). Where the volatility of international markets has reduced the profitability of seaweed mariculture, integrating additional species is a strategy to increase income for farmers (Bryceson 2002, Beltran-Gutierrez et al. 2014, Namukose et al. 2016).

In freshwater aquaculture, aquaponics, the integration of hydroponic plant culture with aquaculture, makes use of the dissolved nutrients from the effluents of the fish (Diver 2006). Many plants grow well in hydroponics, with their roots submerged in soilless medium, and aquaponics have been explored with a wide array of fruits, vegetables and herbs as extractive species (Diver 2006, Danner et al. 2019, Ogah et al. 2020).

Salt-tolerant halophytes hold great potential as they can be irrigated with salt water, produce yields similar to conventional crops and can be integrated into saline aquaculture systems (Glenn et al. 1999, Shpigel et al. 2013). Several halophyte species are being investigated as vegetable crops, to produce biomass for biogas or livestock feed (Glenn et al. 1992, Turcios et al. 2016). Edible halophytes are popular on the gourmet market due to their salty taste and nutritional value including high α-Linolenic acid content (Ventura et al. 2011, Ventura & Sagi 2013). Many species contain high levels of antioxidants and up to 20 % protein (Glenn et al. 1999, Pinheiro et al. 2017). Some species are also promising oilseed crops, with 30 % protein and 28 % oil content in seeds (Glenn et al. 1991, 1999).

4.1. N metabolism in plants

Nitrogen is central to plant growth as part of proteins, nucleic acids, chlorophyll and enzymes (Haynes & Goh 1978, Dechorgnat et al. 2011). Although some plants can technically take up atmospheric nitrogen by harboring nitrogen fixing symbionts in their roots or stem, most higher plants derive the essential element from the soil or other medium around their roots (Gordon et al. 2001). N in the surrounding medium becomes available for plants by diffusion to the N depleted areas around the root (Näsholm & Persson 2001). Plants take up nitrogen over their roots, both in the inorganic and organic form (Warren 2009).

NH_4^+ is taken up by roots and converted in a two-step process to glutamine via the enzyme glutamine synthetase and further via glutamate synthetase to glutamate, which then provides building blocks for other amino acids and nitrogenous compounds (Guerrero et al. 1981, Tischner 2006). N taken up in the form of NO_3^- first undergoes reduction to NO_2^- via nitrate reductase and further to NH_4^+ via nitrite reductase (Guerrero et al. 1981, Siddiqi et al. 1990). Some plant species show fast growth, high uptake rates or even a preference for NH_4^+ over other nitrogen forms as it requires fewer steps in metabolic reactions (Tylova-Munzarova et al. 2005, Fang et al. 2007, Jampeetong & Brix 2009, Kudo & Fujiyama 2010, Jampeetong et al. 2012). Many plants however show signs of NH_4^+ toxicity, such as stunted growth or reduced photosynthetic activity and root uptake of water, when it is supplied as the sole N form or in high concentrations (Lasa et al. 2001, Britto & Kronzucker 2002, Guo et al. 2002). These negative effects can however be alleviated when NO_3^- is additionally available, preventing accumulation and promoting assimilation of NH_4^+-N (Houdusse et al. 2005, Garnica et al. 2009). NO_3^- is taken up by plant roots either via the high- or low-affinity transport systems depending on whether it is available in the surrounding medium at low or high concentrations, respectively (Dechorgnat et al. 2011).

Plant N uptake is influenced by various environmental factors such as temperature (Bassirirad 2000, Dong et al. 2001, Calatayud et al. 2008), pH (Falkengren-Grerup et al. 2000), CO_2 supply and light (Clement et al. 1978) and varies over diurnal and annual cycles (Haynes & Goh 1978, Lillo 1994, 2008, Macduff et al. 1997, Peuke & Jeschke 1998). Uptake of one form of nitrogen depends on the availability and concentration of other forms (Crawford & Glass 1998, Warren 2009) and on whether the plant has been nitrogen deficient or not (Tylova-Munzarova et al. 2005, Fang et al. 2007, Konnerup & Brix 2010, Mozdzer et al. 2010). While it was once believed that even at high availability plants do not take up more N than needed (Gallagher 1975), it has since been shown that N can be consumed in excess of what is limiting to growth (Smart & Barko 1980).

At low concentrations of around 10 µmol l^{-1}, N uptake seems to be dependent on substrate affinity, but at higher concentrations, uptake is determined by enzyme velocity, which in some species differs between N forms, thus for example favoring uptake of ammonium over nitrate (Kamminga-Van Wijk & Prins 1993, Warren 2009). N preference is depending on species and may give advantages in N acquisition (Miller & Bowman 2003, Miller et al. 2007, Harrison et al. 2008, Mozdzer et al. 2010). Some plants have preference for N forms that are more abundant in their habitat (Nordin et al. 2001, Bardgett et al. 2003, Kielland et al. 2006, Miller et al. 2007).

Besides inorganic N, plants can also take up organic N such as amino acids, peptides and even protein (Näsholm et al. 1998, Komarova et al. 2008, Paungfoo-Lonhienne et al. 2008, Ge et al. 2009, Mozdzer et al. 2010, Quintã et al. 2015a). Initially thought to be of little importance to plants and mostly used by competing microbes, it has been shown that plants directly absorb intact organic compounds, which can indeed constitute a significant source of nitrogen (Lipson & Näsholm 2001, Henry & Jefferies 2003a). Organic nitrogen can constitute a significant source as well as the preferred form of nitrogen (Falkengren-Grerup et al. 2000, Mozdzer et al. 2010).

Plants are in competition with microbes in the sediment, which might favor N in organic molecules, as it also provides a source of carbon (Owen & Jones 2001, Bardgett et al. 2003, Jones et al. 2005). Although in some situations plants still get organic N in the presence of microbes (Näsholm et al. 1998, 2000, Bardgett et al. 2003, Henry & Jefferies 2003b, Ge et al. 2009, Sauheitl et al. 2009), they can also be outcompeted (Hodge et al. 2000, Lipson & Näsholm 2001, Bardgett et al. 2003, Harrison et al. 2008).

4.2. Phyto-remediation with halophytes

The treatment of wastewater by artificial wetlands exploits natural bacterial and physical processes in these types of ecosystems to remove solids and dissolved nutrients (Porrello et al. 2003, Vymazal 2010). Widely used for the treatment of municipal waste, they are especially effective at removing nitrogen and storing phosphorus in the soil (Vymazal 2010, De Lange et al. 2013, Almuktar et al. 2018). The approach has been successfully applied to reduce nutrient concentrations and modify physico-chemical parameters of wastewater from fishponds, also integrating algae to assimilate N and P (Porrello et al. 2003, Bartoli et al. 2005). Various halophytes in different types of natural and constructed wetlands are already successfully used as down-stream filters of municipal, industrial or agricultural wastewater (Kadlec & Knight 1996, Verhoeven & Meuleman 1999, Vymazal 2010). Their use can have great economic benefits when compared to conventional water treatment (Cardoch et al. 2000). Edible halophyte species have been found to grow well when irrigated with aquaculture effluents, without the addition of nutrients, and be able to benefit from high ammonium availability (Glenn et al. 1991, Quintã et al. 2015b).

Nutrient removal from aquaculture wastewater in constructed wetlands planted with halophytes is highly variable (review by Custódio et al. 2017). Very high removal of up to 96 % of N and 99 % of P were achieved by *S. bigelovii* in saline Tilapia effluents (Brown et al. 1999). *Ipomea aquatica, Paspalim vaginatum,* and *Phragmites australis* removed 95 % N and 71 % P from milkfish wastewater (Lin et al. 2002). *Salicornia europaea* removed between 47% and 98% N and 36 – 89 % P (Webb et al. 2012, 2013). Removal can however be lower. *Aster tripolium*, *Bolboschoenus maritimus* and *Spatina anglica* removed only 11 % N and 35 % P (De Lange & Paulissen 2016).

The performance of planted constructed wetlands is influenced by the salinity tolerance of the plants (Brown et al. 1999) or nutrient concentrations of the effluent. Constructed wetlands with *Salicornia europaea*, removed only ~ 0.7 % N at concentrations of 14 mg N l^{-1}, but 59 % N at lower nutrient loads of 0.2 mg N l^{-1} (Shpigel et al. 2013). At lower removal rates, the size of the wetland required as the sole filter for intensive aquaculture operations can be prohibitively large (Robertson, 1995). Nitrogen removal in these types of systems occurs primarily via denitrification and ammonia volatilization and only a small fraction is converted into harvestable plant biomass (Kadlec & Knight 1996, Lin et al. 2002, Vymazal 2010). Additionally, if the produced biomass is not harvested, assimilated nutrients will ultimately be released through decomposition, reducing net removal (Porrello et al. 2003). Species that are edible, have pharmaceutical applications or potential as an oilseed crop recycle nutrients from effluents back into a valuable product (Buhmann & Papenbrock 2013, Turcios & Papenbrock 2014). It was demonstrated that halophytes *Salicornia ramosissima* and *Halimione portulacoides* cultured in effluent from a super-intensive marine RAS had higher *n*-3 fatty acids content than plants collected from the wild, making this cultivation mode attractive to produce high quality functional foods for human consumption (Maciel et al. 2020).

A few studies to date have successfully tested halophytes in an aquaponic or hydroponic set-up (Buhmann et al. 2015, Quintã et al. 2015b, Waller et al. 2015, Boxman et al. 2017). Uptake rates up to 1.11 mg N g^{-1} root dry weight (dw) h^{-1} were for example determined for edible *S. europaea* in nutrient solution, mimicking aquaculture effluents (Quintã et al. 2015b). Rates of N uptake depend on the species and nitrogen status of the plant as well as the cultivation mode (Jampeetong et al. 2012, Webb et al. 2012, Quintã et al. 2015a b). Compared to cultivation in soil, uptake of NH_4^+-N in hydroponics was 5 times higher for *Aster tripolium* and 3 times higher for *S. europaea* (Quintã et al. 2015a). Webb et al. (2012) found that even when planted in constructed wetlands, *S. europaea* assimilated 85 % of total N removed from wastewater, possibly because large pore spaces and daily draining of substrate prevented the development of anaerobic conditions.

Sesuvium portulacastrum or sea purslane is a perennial halophyte with a wide distribution in coastal habitats of the tropics and sub-tropics between 34° north and 42° south (Fig. 2) (Lonard & Judd 1997). It has potential as a valuable crop for saline soils with a number of applications (Lokhande et al. 2009). In several countries, *S. portulacastrum* is consumed fresh or used as a traditional medicinal plant and its essential oils exhibit antibacterial and antioxidant activities (Magwa et al. 2006, Lokhande et al. 2009, Ali et al. 2013, Anand et al. 2014, Desiyamani et al. 2017). *S. portulacastrum* has a good fatty acid profile with almost 50 % α-Linolenic acid (Nouairi et al. 2006). It also efficiently accumulates heavy metals and has been proposed as a candidate for phytoremediation of soils contaminated with heavy metals or high levels of salinity (Ayyappan et al. 2016).

Boxman et al. (2017) evaluated biofiltration performance of *S. portulacastrum* integrated in a recirculation system with fish. Integration of the plant significantly lowered nitrogen concentrations and accounted for maximum removal of 75 % of aqueous nitrogen. Experiments testing plant filters in RAS in addition to other water cleaning mechanisms such as biofilters, do not give sufficient insight into the performance of the plants in unfiltered fish effluents (Webb et al. 2012, Waller et al. 2015), as they do not yield information on the exact filtration rates of the plant species and as water is continuously cycled in a closed system (Boxman et al. 2017). Specific uptake rates in aquaculture wastewater still remain to be determined for successful integration in aquaculture systems.

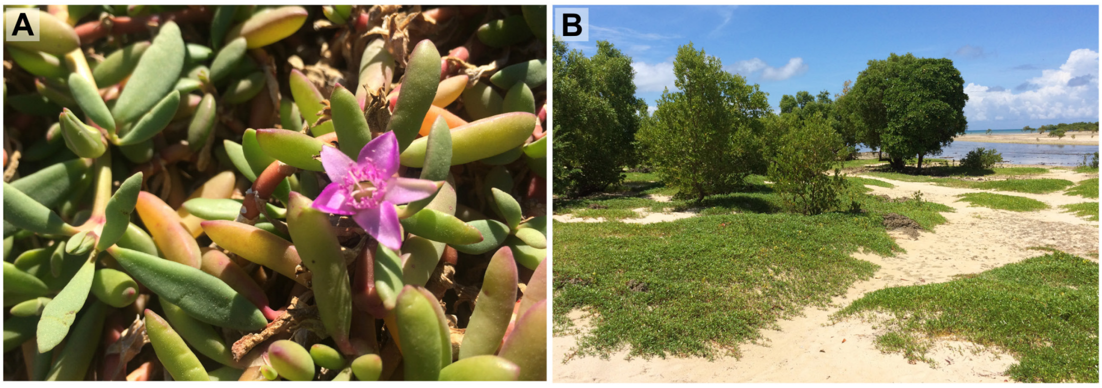

Fig. 2: A) Flowering *Sesuvium portulacastrum* and B) *S. portulacastrum* growing in a mangrove area on Zanzibar, Tanzania.

5. Stable isotope analysis in IMTA research

Stable isotope analysis lends itself well to reconstructing trophic relationships, diets and the assimilation of different nutrients and can be a powerful tool to gain insights into otherwise hidden processes (Gannes et al. 1998, Phillips et al. 2014). In aquaculture research, and particularly in IMTA research, it has been proven useful to examine feed use and nutrient dynamics (Focken 2008, Xu et al. 2017). Studying stable isotopes in ecosystems essentially gives 1) source information, where an isotope comes from, and 2) process information, the reactions that fractionated the isotope (Peterson & Fry 1987). In turn, reliable conclusions on the flow and transformation of matter used by a consumer depend on a knowledge base on both of these types of information - the possible sources available and the fractionation influencing final isotopic values. Accounting for all dietary sources available to a consumer in an ecosystem can be challenging if not impossible (Phillips et al. 2014). However, here, the often contained and controlled environments in aquaculture can provide a comparatively high degree of certainty.

Fractionation occurs as heavy and light isotopes react at different rates in physical, chemical or biochemical reactions, changing their relative concentrations (Peterson & Fry 1987). Whereas some chemical and geochemical reactions follow relatively straight-forward equilibrations, biological processes can be much more complex and influenced by numerous factors (Peterson & Fry 1987, Fry 2006). In an animal, fractionation occurs during assimilation and excretion and results in different (usually higher) isotopic values of tissue compared to the animal's diet (Olive et al. 2003). Uncertainty regarding the appropriate fractionation factor (or trophic enrichment factor, TEF, expressed as Δ) is considered a main weakness in the use of isotopic mixing models and there is a need for the increased use of experimentally determined, context- and species-specific values (Phillips & Koch 2002, Dalerum & Angerbjörn 2005, Phillips et al. 2014). Based on numerous controlled feeding experiments and field observations, a trophic enrichment factor of 1 ‰ $\delta^{13}C$ was determined and is often applied in trophic studies (DeNiro & Epstein 1978, Post 2002).

Stable isotope analysis has been applied to evaluate aquaculture diets for sea cucumbers, mainly focusing on the temperate species *Apostichopus japonicus* (Guo et al. 2016, Wen et al. 2017). Several feeding experiments with sea cucumbers, including *H. scabra,* have adjusted their values by 1 ‰ for use in isotope mixing models (Gao et al. 2011, Sun et al. 2013, Hou et al. 2017, Mondal & Kunzmann 2018, Song et al. 2018). The studies do so in reference to studies on bivalves (Gao et al. 2006, Sarà 2007), insects and fish (McCutchan et al. 2003) or literature that in turn references the work of Peterson and Fry (1987) (McClelland & Valiela 1998, Sun et al. 2013). Peterson and Fry (1987) however acknowledge, that there can be considerable variation in isotopic values of different tissues, with enrichments as high as 6 ‰ compared to the diet. Trophic enrichment has since been found to vary depending on an animal's environment (terrestrial, freshwater or marine), taxon, trophic level, sampled tissue, and physiological factors such as metabolic rate and nitrogenous excretion (Pinnegar & Polunin 1999, McCutchan et al. 2003, Vanderklift & Ponsard 2003, Dalerum & Angerbjörn 2005, Caut et al. 2009). A mono-feeding experiment for juvenile *H. scabra* resulted in trophic fractionation of up to 4.2 ‰ $\delta^{13}C$, suggesting that diet-tissue discrimination for this species may be much higher than previously assumed (Watanabe et al. 2013). Stable isotope analysis holds the potential to study feed use of *H. scabra* and can be used to evaluate nutrient assimilation in integrated aquaculture, but appropriate trophic fractionation factors are needed to produce reliable results.

6. Aquaculture in the tropics

Directly and indirectly, fisheries and aquaculture provide food and income to more than 10 % of the world population, especially in developing countries (Béné et al. 2015). Over the past 60 years, fish consumption has doubled in the least developed countries and by 2017 provided 50 % of animal protein consumed in some nations, for example Bangladesh, Ghana, Indonesia and Sri Lanka (FAO 2020). Small aquaculture operations directly improve nutrition and income of fish farmers and provide economic opportunities, especially for women (Irwin et al. 2020). They play a vital role in the health and livelihoods of communities in tropical coastal regions (Senff et al. 2018, Hanh & Boonstra 2019). As it is these regions that are projected to experience the strongest effects of climate change and population growth, the aquaculture sector needs to adapt to satisfy growth targets and environmental rules and regulations alike (Ahmed & Glaser 2016, Tran et al. 2017).

6.1. Research needs for tropical IMTA

The implementation of IMTA is seen as an important approach to meet current and future challenges, but the majority of IMTA research has been undertaken in temperate regions, with research in tropical countries lagging behind (Granada et al. 2015, Ahmed & Glaser 2016, Felaco et al. 2020, Putro et al. 2020). Research in the tropical context has focused on open-water systems, and there is still a lack of information on the use of tropical marine species in aquaponics (Boxman 2015, Chary et al. 2020). 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 are 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).

The economic and ecological potential of deposit-feeding sea cucumbers as extractive species in aquaculture is widely recognized, but their application in IMTA in tropical countries remains limited (Zamora et al. 2018). Although *H. scabra* has been demonstrated to grow well on a diet of particulate organic waste from fed aquaculture, attempts to move beyond laboratory-scale experiments have struggled to validate its feasibility (Dumalan et al. 2019, Chary et al. 2020). Further research is needed to optimize biomass production and bioremediation and enable the practical integration into existing monoculture units and on sediments enriched by biodeposits from commercial marine fish farming (Chary et al. 2020). A challenge to the integration of this species in intensive aquaculture operations is the growth-limiting effect of high stocking densities needed to achieve bioremediation of impacted sediments (Chary et al. 2020). Improving biomass density through carbon supplementations has been identified as a strategy to optimize sea cucumber carrying capacity in sediment-based bioremediation systems (Robinson et al. 2019, Chary et al. 2020). The viability of economical and practical types of carbon, ideally sourced from agricultural waste-streams, is thus of great research interest to enable the successful use of *H. scabra* as an extractive organism for tropical saline aquaculture (Dumalan et al. 2019, Mathieu-Resuge et al. 2020).

The research to date on the integration of edible halophytes into closed aquaculture systems has been limited, especially in the tropical context (Boxman 2015, Custódio et al. 2017). This diverse group of plants holds great potential for phytoremediation of nutrient-rich effluent waters and the simultaneous production of high-quality and valuable biomass (Pinheiro et al. 2017, Maciel et al. 2020). *S. portulacastrum,* with its wide geographical range and good nutritional properties, is a candidate for the treatment of aquaculture wastewater (Lokhande et al. 2013, Liu et al. 2019). Cultivated in RAS, it effectively decreases concentrations of dissolved inorganic nutrients, but further research is needed to optimize its use for the recovery of nutrients into biomass for human consumption (Boxman et al. 2017).

7. Aims and outline of this thesis

This thesis aims to meet the need for more information on the performance of tropical extractive species in saline IMTA systems. In a first proof-of-concept, a deposit-feeding sea cucumber species and an edible halophyte were combined for the first time as extractive species in a closed RAS of fed finfish (**Chapter 2**). Different methods of stable isotope analysis were then applied to examine bioremediation by the two extractive species in more detail (**Chapter 3 and 4**).

Chapter 2 examined the viability of a low-technology IMTA RAS of milkfish *Chanos chanos,* sea cucumber *H. scabra* and halophyte *S. portulacastrum.* Performance was evaluated based on biomass production and maintenance of adequate water quality without the use of a separate biofilter component. A nitrogen budget was constructed based on the input of feed-derived N. Disruption of water flow in the batch-operated system allowed to evaluate changes in water quality of each system component separately.

In **Chapter 3**, *H. scabra* was tested more closely as an extractive organism. Juvenile sea cucumbers were reared on solid waste from milkfish aquaculture. Comparison with control tanks without the animals provided insight into their bioremediation performance for impacted sediments. Furthermore, the aquaculture waste was fed either pure or supplemented with organically-bound carbon in the form of microcrystalline cellulose or bagasse. The different treatments gave insight into the possible improvement of modifying the C:N of organic matter through the addition of a readily available agricultural waste product. Natural abundance stable isotope analysis was used to reveal assimilation of waste-derived nutrients by applying a Bayesian mixing-model with a context-specific trophic enrichment factor.

Chapter 4 focused on nutrient uptake by the halophyte *S. portulacastrum* in milkfish effluents. Removal and assimilation of dissolved inorganic nitrogen was traced with the application of ^{15}N tracer and compared between the plants cultivated in sand substrate and hydroponics. Comparison with unplanted systems showed the contribution of the plant in effluent treatment.

8. Publication overview

Chapter 2 has been published and **Chapter 3** and **Chapter 4** have been submitted for publication in international peer-reviewed journals.

Chapter 2: Low-technology recirculating aquaculture system integrating milkfish *Chanos chanos*, sea cucumber *Holothuria scabra* and sea purslane *Sesuvium portulacastrum*

Paula Senff, Pierre-Philippe Blanc, Matthew Slater, Andreas Kunzmann

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Author contributions: PS, MS and AK designed the experiment. PS performed the experiment, analyzed the data and wrote the initial manuscript. PS, PB, MS and AK discussed the results and contributed to the final manuscript.

Chapter 3: Carbon supplementation promotes assimilation of aquaculture waste by the sea cucumber *Holothuria scabra*: evidence from stable isotope analysis

Paula Senff, Brendan Elba, Andreas Kunzmann, Lucy Gwen Gillis, Georgina Robinson

Manuscript has been submitted to *Aquaculture*.

Author contributions: PS, BE, AK and GR designed the experiment. PS and BE performed the experiment. PS analyzed the data and wrote the initial manuscript. PS, BE, AK, LGG and GR discussed the results and contributed to the final manuscript.

Chapter 4: Nutrient removal and nitrogen recovery from aquaculture effluents by the edible halophyte *Sesuvium portulacastrum* in hydroponics and in sand substrate

Paula Senff, Gaute Lavik, Mirta Teichberg, Andreas Kunzmann

Manuscript has been submitted to *Science of the Total Environment*

Author contributions: PS, MT and AK designed the experiment. PS performed the experiment. GL contributed analytical tools. PS and GL analyzed the data. PS wrote the initial manuscript. PS, GL, MT and AK discussed the results and contributed to the final manuscript.

Contribution of the candidate to the total workload (%)

	Chapter 2	**Chapter 3**	**Chapter 4**
Experimental design	90	90	90
Experimental work/ data collection	100	50	90
Data analysis and interpretation	100	95	80
Preparation of figures and tables	100	100	100
Drafting of the manuscript	100	100	95

References

Ahlgren MO (1998) Consumption and Assimilation of Salmon Net Pen Fouling Debris by the Red Sea Cucumber *Parastichopus californicus*: Implications for Polyculture. J World Aquac Soc 29:133–139.

Ahmed N, Glaser M (2016) Can "Integrated Multi-Trophic Aquaculture (IMTA)" adapt to climate change in coastal Bangladesh? Ocean Coast Manag 132:120–131.

Ali MYS, Anuradha V, Sirajudeen S, Vijaya P, Yogananth N, Rajan R, Parveen PMK (2013) Mosquito larvicidal properties of volatile oil from salt marsh mangrove plant of *Sesuvium portulacastrum* against *Anopheles stephensi* and *Aedes aegypti*. J Coast Life Med 1:66–71.

Aller RC (1994) Bioturbation and remineralization of sedimentary organic matter : effects of redox oscillationl. Chem Geol 114:331–345.

Aller RC, Aller JY (1998) The effect of biogenic irrigation intensity and solute exchange on diagenetic reaction rates in marine sediments. 905–936.

Almuktar SAAAN, Abed SN, Scholz M (2018) Wetlands for wastewater treatment and subsequent recycling of treated effluent: a review. Environ Sci Pollut Res 25:23595–23623.

Anand TP, Chellaram C, Praveen MM, Guru SG, Shanthini CF (2014) Herbicidal and growth promoter activity of three saltmarsh plants from pulikat coast. Int J ChemTech Res 6:1051–1054.

Arechavala-Lopez P, Toledo-Guedes K, Izquierdo-Gomez D, Šegvić-Bubić T, Sanchez-Jerez P (2018) Implications of Sea Bream and Sea Bass Escapes for Sustainable Aquaculture Management: A Review of Interactions, Risks and Consequences. Rev Fish Sci Aquac 26:214–234.

Arredondo-Figueroa JL, Ingle de la Mora G, Guerrero-Legarreta I, Ponce-Palafox JT, Barriga-Sosa A (2007) Ammonia and nitrite removal rates in a closed recirculating-water system, under three load rates of rainbow trout *Oncorhynchus mykiss*. Rev Mex Ing Química 6:301–308.

Avnimelech Y (2012) Biofloc Technology - A Practical Guide Book, 2nd ed. The World Aquaculture Society, Baton Rouge, Louisiana, United States.

Avnimelech Y (2014) Biofloc technology - a practical guidebook, 3rd ed. World Aquaculture Society.

Avnimelech Y (2007) Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture 264:140–147.

Ayyappan D, Sathiyaraj G, Ravindran KC (2016) Phytoextraction of heavy metals by *Sesuvium portulacastrum* l. a salt marsh halophyte from tannery effluent. Int J Phytoremediation 18:453–459.

Badiola M, Mendiola D, Bostock J (2012) Aquacultural Engineering Recirculating

Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. Aquac Eng 51:26–35.

Ballester-Moltó M, Sanchez-Jerez P, Cerezo-Valverde J, Aguado-Giménez F (2017) Particulate waste outflow from fish-farming cages. How much is uneaten feed? Mar Pollut Bull 119:23–30.

Bardgett RDB, Treeter TACS, Ol ROB (2003) Organic-Nitrogen Inputs To Temperate Grasslands. Ecology 84:1277–1287.

Barrington K, Ridler N, Chopin T, Robinson S, Robinson B (2010) Social aspects of the sustainability of integrated multi-trophic aquaculture. Aquac Int.

Bartoli M, Nizzoli D, Naldi M, Vezzulli L, Porrello S, Lenzi M, Viaroli P (2005) Inorganic nitrogen control in wastewater treatment ponds from a fish farm (Orbetello, Italy): Denitrification versus *Ulva* uptake. Mar Pollut Bull 50:1386–1397.

Baskar BK (1994) Some Observations on the Biology of the Holothurian *Holothuria (Metriatyla) scabra* (Jaeger). C (Central Mar Fish Res Institute) Bull 46:39–43.

Bassirirad H (2000) Kinetics of nutrient uptake by roots: Responses to global change. New Phytol 147:155–169.

Bauer W, Prentice-Hernandez C, Borges M, Wasielesky W, Poersch LHS (2012) Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp *Litopenaeus vannamei*. Aquaculture 342–343:112–116.

Beltran-Gutierrez M, Ferse SC, Kunzmann A, Stead SM, Msuya FE, Hoffmeister TS, Slater M (2014) Co-culture of sea cucumber *Holothuria scabra* and red seaweed *Kappaphycus striatum*. Aquac Res 47:1549–1559.

Béné C, Barange M, Subasinghe R, Pinstrup-Andersen P, Merino G, Hemre GI, Williams M (2015) Feeding 9 billion by 2050 – Putting fish back on the menu. Food Secur 7:261–274.

Beveridge MCM, Little DC (2002) The History of Aquaculture in Traditional Societies. In: *Ecological Aquaculture: The Evolution of the Blue Revolution*. Costa-Pierce BA (ed) Blackwell Science, Oxford

Birhanu SM, Natarajan P (2019) Status of integrated aquaculture-Agriculture systems in Africa. Int J Fish Aquat Stud 7:263–269.

Bischoff AA (2007) Solid waste reduction of closed recirculated aquaculture systems by secondary culture of detritivorous organisms

Blackburn TH, Blackburn ND (1992) Model of nitrification and denitrification in marine sediments. FEMS Microbiol Lett 100:517–521.

Blancheton J-P, Piedrahita R, Eding EH, d'Orbcastel ER, Lemarié G, Bergheim A, Fivelstad S (2007) Intensification of landbased aquaculture production in single pass and reuse systems. Aquac Eng Environ 0:21–47.

Blancheton JP (2000) Developments in recirculation systems for Mediterranean fish species. Aquac Eng 22:17–31.

Boxman S (2015) Resource Recovery Through Halophyte Production in Marine Aquaponics: An Evaluation of the Nutrient Cycling and the Environmental Sustainability of Aquaponics. University of Southern Florida

Boxman SE, Nystrom M, Capodice JC, Ergas SJ, Main KL, Trotz MA (2017) Effect of support medium, hydraulic loading rate and plant density on water quality and growth of halophytes in marine aquaponic systems. Aquac Res 48:2463–2477.

Brambilla F, Antonini M, Ceccuzzi P, Terova G, Saroglia M (2008) Foam fractionation efficiency in particulate matter and heterotrophic bacteria removal from a recirculating seabass (*Dicentrarchus labrax*) system. Aquac Eng 39:37–42.

Britto DT, Kronzucker HJ (2002) NH4+ toxicity in higher plants: a critical review. J Plant Physiol 584:567–584.

Brown JJ, Glenn EP, Fitzsimmons KM, Smith SE (1999) Halophytes for the treatment of saline aquaculture effluent. Aquaculture 175:255–268.

Brown N, Eddy S, Plaud S (2011) Utilization of waste from a marine recirculating fish culture system as a feed source for the polychaete worm, *Nereis virens*. Aquaculture 322–323:177–183.

Brugère C, Aguilar-Manjarrez J, Beveridge MCM, Soto D (2019) The ecosystem approach to aquaculture 10 years on – a critical review and consideration of its future role in blue growth. Rev Aquac 11:493–514.

Bryceson I (2002) Coastal Aquaculture Developments in Tanzania : Sustainable and Non-sustainable Experiences. West Indian Ocean J Mar Sci 1:1–10.

Buck BH, Troell MF, Krause G, Angel DL, Grote B, Chopin T (2018) State of the art and challenges for offshore Integrated multi-trophic aquaculture (IMTA). Front Mar Sci 5:1–21.

Buhmann A, Papenbrock J (2013) Biofiltering of aquaculture effluents by halophytic plants: Basic principles, current uses and future perspectives. Environ Exp Bot 92:122–133.

Buhmann A, Waller U, Wecker B, Papenbrock J (2015) Optimization of culturing conditions and selection of species for the use of halophytes as biofilter for nutrient-rich saline water. Agric Water Manag 149:102–114.

Burford MA, Williams KC (2001) The fate of nitrogenous waste from shrimp feeding. Aquaculture 198:79–93.

Calatayud Á, Gorbe E, Roca D, Martínez PF (2008) Effect of two nutrient solution temperatures on nitrate uptake, nitrate reductase activity, NH4+ concentration and chlorophyll a fluorescence in rose plants. Environ Exp Bot 64:65–74.

Cao L, Naylor R, Henriksson P, Leadbitter D, Metian M, Troell M, Zhang W (2015) China's aquaculture and the world's wild fisheries. Science (80-) 347:133–135.

Capone DG (2008) The marine nitrogen cycle. Microbe 3:186–192.

Capone DG (2000) The marine nitrogen cycle. In: *Microbial Ecology of the Oceans*, 1st ed. Kirchmann DL (ed) Wiley-Liss, p 455–493

Cardoch L, Day JW, Rybczyk JM, Kemp GP (2000) An economic analysis of using wetlands for treatment of shrimp processing wastewater - A case study in Dulac, LA. Ecol Econ 33:93–101.

Casalduero FG, Marina UDB, Ciencias D, Naturales R (1999) Integrated systems : " Environmentally clean " aquaculture. Self 66:139–145.

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Δ15N and Δ13C): The effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46:443–453.

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

Chen S, Ling J, Blancheton JP (2006) Nitrification kinetics of biofilm as affected by water quality factors. Aquac Eng 34:179–197.

Chen Z, Ge H, Chang Z, Song X, Zhao F, Li J (2018) Nitrogen Budget in Recirculating Aquaculture and Water Exchange Systems for Culturing *Litopenaeus vannamei*. J Ocean Univ China 17:905–912.

Chimits P (1957) Tilapia in ancient Egypt. FAO Fish Bull 10:1–5.

Chopin T (2017) Challenges of Moving Integrated Multi-Trophic Aquaculture along the R&D and Commercialization Continuum in the Western World. J Ocean Technol 12:34–47.

Chopin T, Buschmann AH, Halling C, Troell M, Kautsky N, Neori A, Kraemer GP, Zertuche-González JA, Yarish C, Neefus C (2001) Integrating seaweeds into marine aquaculture systems: A key toward sustainability. J Phycol 37:975–986.

Ciji A, Akhtar MS (2019) Nitrite implications and its management strategies in aquaculture: a review. Rev Aquac:1–31.

Clement CR, Hopper MJ, Jones LHP, Leafe EL (1978) The uptake of nitrate by *Lolium perenne* from flowing nutrient solution: II. Effect of light, defoliation, and relationship to co2 flux. J Exp Bot 29:1173–1183.

Coche AG (1982) Cage culture of tilapias. In: *The biology and culture of Tilapias*. International Center for Living Aquatic Resources Management, Manila, Philippines, p 205–246

Colt J (2006) Water quality requirements for reuse systems. Aquac Eng 34:143–156.

Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens GE (2009) Controlling Eutrophication: Nitrogen and Phosphorus. Science (80-) 323:1014 LP – 1015.

Costa-Pierce BA (1987) Aquaculture in Ancient Hawaii. Am Inst Biol Sci 37:320–331.

Costa-Pierce BA (2002) Ecological aquaculture: the evolution of the blue revolution. John Wiley and Sons Ltd.

Crab R, Avnimelech Y, Defoirdt T, Bossier P, Verstraete W (2007) Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270:1–14.

Crab R, Defoirdt T, Bossier P, Verstraete W (2012) Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture:351–356.

Crawford NM, Glass ADM (1998) Molecular and physiological aspects of nitrate uptake in plants. Trends Plant Sci 3:389–395.

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

Custódio M, Villasante S, Cremades J, Calado R, Lillebø AI (2017) Unravelling the potential of halophytes for marine integrated multi-trophic aquaculture (IMTA) - A perspective on performance, opportunities and challenges. Aquac Environ Interact 9:445–460.

Dalerum F, Angerbjörn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647–658.

Danner RI, Mankasingh U, Anamthawat-Jonsson K, Thorarinsdottir RI (2019) Designing aquaponic production systems towards integration into greenhouse farming. Water (Switzerland) 11.

Davidson J, Helwig N, Summerfelt ST (2008) Fluidized sand biofilters used to remove ammonia, biochemical oxygen demand, total coliform bacteria, and suspended solids from an intensive aquaculture effluent. Aquac Eng 39:6–15.

Dechorgnat J, Nguyen CT, Armengaud P, Jossier M, Diatloff E, Filleur S, Daniel-Vedele F (2011) From the soil to the seeds: the long journey of nitrate in plants. J Exp Bot 62:1349–1359.

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta ; Vol/Issue 425 42:Pages: 495-506.

Desiyamani P, Ali MS, Anuradha V, Yogananth N, Chitra J (2017) Mosquito Larvicidal Activity of Fractionated Methanolic Extractof *Rhizophora mucronata* Stilt Root and *Sesuvium portulacastrum* against Dengue Larvae. J Mosq Res 7:111–114.

Diver S (2006) Aquaponics — Integration of Hydroponics with Aquaculture.

ATTRA:1–28.

Dobson GT, Dinh N, Duy Q, C NAP, Southgate PC (2020) Assessing potential for integrating sea grape (*Caulerpa lentillifera*) culture with sandfish (*Holothuria scabra*) and Babylon snail (*Babylonia areolata*) co - culture. Aquaculture 522:735153.

Dong S, Scagel CF, Cheng L, Fuchigami LH, Rygiewicz PT (2001) Soil temperature and plant growth stage influence nitrogen uptake and amino acid concentration of apple during early spring growth. Tree Physiol 21:541–547.

Dorber M, Verones F, Nakaoka M, Sudo K (2020) Can we locate shrimp aquaculture areas from space? – A case study for Thailand. Remote Sens Appl Soc Environ 20.

Dumalan RJP, Bondoc KG V., Juinio-Meñez MA (2019) Grow-out culture trial of sandfish *Holothuria scabra* in pens near a mariculture-impacted area. Aquaculture 507:481–492.

Edwards P, Zhang W, Belton B, Little DC (2019) Misunderstandings, myths and mantras in aquaculture: Its contribution to world food supplies has been systematically over reported. Mar Policy 106:103547.

Erisman JW, Sutton MA, Galloway J, Klimont Z, Winiwarter W (2008) How a century of ammonia synthesis changed the world. Nat Geosci 1:636–639.

Eshchar M, Lahav O, Mozes N, Peduel A, Ron B (2006) Intensive fish culture at high ammonium and low pH. Aquaculture 255:301–313.

Falkengren-Grerup U, Månsson KF, Olsson MO (2000) Uptake capacity of amino acids by ten grasses and forbs in relation to soil acidity and nitrogen availability. Environ Exp Bot 44:207–219.

Fang YY, Babourina O, Rengel Z, Yang XE, Pu PM (2007) Ammonium and nitrate uptake by the floating plant *Landoltia punctata*. Ann Bot 99:365–370.

FAO (2010) Aquaculture development. 4. Ecosystem approach to aquaculture. In: *FAO Technical Guidelines for Responsible Fisheries*, No.5, Supp. Rome, Italy, p 53

FAO (2018) The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.

FAO (2020) The State of World Fisheries and Aquaculture 2020. Rome.

Felaco L, Olvera-Novoa MA, Robledo D (2020) Multitrophic integration of the tropical red seaweed *Solieria filiformis* with sea cucumbers and fish. Aquaculture 527.

Fenchel T, King GM, Blackburn TH (2012) Bacterial Biogeochemistry. The Ecophysiology of Mineral Cycling, 3rd ed. Academic Press, San Diego.

Focken U (2008) Application of Stable Isotopes in Aquaculture Nutrition Research. In: *Avances en Nutricíon Acuícola IX. Anais do IX Simposio Internacional de*

Nutricíon Acuícola. 24-27 Noviembre. Universidad Autónoma de Nueva León, Monterrey, Nueva León, México. p 258–270

Fry B (2006) Stable Isotope Ecology. Springer.

Gallagher JL (1975) Effect of an Ammonium Nitrate Pulse on the Growth and Elemental Composition of Natural Stands of *Spartina alterniflora* and *Juncus roemerianus*. Am J Bot 62:644.

Gannes LZ, Martınez del Rio C, Koch P (1998) Natural abundance variations in stable isotopes and their use in animal physiological ecology. Comp Biochem Physiol 119A:725–737.

Gao K, McKinley KR (1994) Use of macroalgae for marine biomass production and CO2 remediation: a review. J Appl Phycol 6:45–60.

Gao QF, Shin PKS, Lin GH, Chen SP, Siu GC (2006) Stable isotope and fatty acid evidence for uptake of organic waste by green-lipped mussels *Perna viridis* in a polyculture fish farm system. Mar Ecol Prog Ser 317:273–283.

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.

Garnica M, Houdusse F, Claude Yvin J, Garcia-Mina JM (2009) Nitrate supply induces changes in polyamine content and ethylene production in wheat plants grown with ammonium. J Plant Physiol 166:363–374.

Ge T, Song S, Roberts P, Jones DL, Huang D, Iwasaki K (2009) Amino acids as a nitrogen source for tomato seedlings: The use of dual-labeled (13C, 15N) glycine to test for direct uptake by tomato seedlings. Environ Exp Bot 66:357–361.

Gelfand I, Barak Y, Even-Chen Z, Cytryn E, Van Rijn J, Krom MD, Neori A (2003) A novel zero discharge intensive seawater recirculating system for the culture of marine fish. J World Aquac Soc 34:344–358.

Glenn E, O'LEARY J, Watson M (1991) *Salicornia bigelovii* Torr.: an oilseed halophyte for seawater irrigation.

Glenn EP, Brown JJ, Blumwald E (1999) Salt Tolerance and Crop Potential of Halophytes. CRC Crit Rev Plant Sci 18:227–255.

Glenn EP, Coates WE, Riley JJ, Kuehl RO, Swingle RS (1992) *Salicornia bigelovii* Torr.: a seawater-irrigated forage for goats. Anim Feed Sci Technol 40:21–30.

Gordon AJ, Lea PJ, Rosenberg C, Trinchant J-C (2001) Nodule Formation and Function. Plant Nitrogen:101–146.

Granada L, Sousa N, Lopes S, Lemos MFL (2015) Is integrated multitrophic aquaculture the solution to the sectors' major challenges? - a review. Rev Aquac 6:6–18.

Guerrero MG, Vega JM, Losada M (1981) The assimilatory nitrate-reducing system and its reglation. Annu Rev Plant Physiol 32:169–204.

Guerrero S, Cremades J (2012) Integrated Multi-trophic Aquaculture (IMTA): A sustainable, pioneering alternative for marine cultures in Galicia. 111:978–84.

Gunning D, Maguire J, Burnell G (2016) The Development of Sustainable Saltwater-Based Food Production Systems: A Review of Established and Novel Concepts. Water 8:598.

Guo K, Zhao W, Wang S, Liu B, Zhang P (2016) Food web structure and trophic levels in a saltwater pond sea cucumber and prawn polyculture system. Acta Oceanol Sin 35:58–62.

Guo S, Brück H, Sattelmacher B (2002) Effects of supplied nitrogen form on growth and water uptake of French bean (*Phaseolus vulgaris* L.) plants: Nitrogen form and water uptake. Plant Soil 239:267–275.

Gutierrez-Wing MT, Malone RF (2006) Biological filters in aquaculture: Trends and research directions for freshwater and marine applications. Aquac Eng 34:163–171.

Guy S-V, Thomas T, Irit Z, Andreas P, Dorit S, Omri L, Ayelet G, Guy B-O (2018) Tooth oxygen isotopes reveal Late Bronze Age origin of Mediterranean fish aquaculture and trade. Sci Rep 8.

Hair C (2012) Sandfish (Holothuria scabra) production and sea-ranching trial in Fiji. Asia–Pacific Trop sea cucumber Aquac:129.

Hair C, Foale S, Kinch J, Frijlink S, Lindsay D, Southgate PC (2019) Socioeconomic impacts of a sea cucumber fishery in Papua New Guinea: Is there an opportunity for mariculture? Ocean Coast Manag 179.

Hamel J-F, Conand C, Pawson D, Mercier A (2001) The Sea Cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): Its Biology and Exploitation as Beche-de-Mer. Adv Mar Biol 41:129–223.

Handy RD, Poxton MG (1993) Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. Rev Fish Biol Fish 3:205–241.

Hanh TTH, Boonstra WJ (2019) What prevents small-scale fishing and aquaculture households from engaging in alternative livelihoods? A case study in the Tam Giang lagoon, Viet Nam. Ocean Coast Manag 182.

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

Hargreaves JA (1998) Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166:181–212.

Harrison KA, Bol R, Bardgett RD (2008) Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen? Soil Biol Biochem 40:228–237.

Haynes RJ, Goh KM (1978) Ammonium and nitrate nutrition of plants. Biol Rev 53:465–510.

He Z, Cheng X, Kyzas GZ, Fu J (2016) Pharmaceuticals pollution of aquaculture and its management in China. J Mol Liq 223:781–789.

Hende S Van Den, Beelen V, Bore G, Boon N, Vervaeren H (2014) Bioresource Technology Up-scaling aquaculture wastewater treatment by microalgal bacterial flocs : From lab reactors to an outdoor raceway pond. Bioresour Technol 159:342–354.

Henry HAL, Jefferies RL (2003a) Interactions in the uptake of amino acids, ammonium and nitrate ions in the Arctic salt-marsh grass, *Puccinellia phryganodes*. Plant, Cell Environ 26:419–428.

Henry HAL, Jefferies RL (2003b) Plant amino acid uptake, soluble N turnover and microbial N capture in soils of a grazed Arctic salt marsh. J Ecol 91:627–636.

Herbeck LS, Sollich M, Unger D, Holmer M, Jennerjahn TC (2014) Impact of pond aquaculture effluents on seagrass performance in NE Hainan, tropical China. Mar Pollut Bull 85:190–203.

Heyman WD, Dobberteen RA, Urry LA, Heyman AM (1989) Pilot hatchery for the queen conch, *Strombus gigas*, shows potential for inexpensive and appropriate technology for larval aquaculture in the Bahamas. Aquaculture 77:277–285.

Hochard S, Ducroq M, Lemonnier H, Royer F, Hubert M, Michaut H, Vergé R, Letourneur Y, Lorrain A, Mathieu-Resuge M, Schaal G, Kraffe E, Legrand F, Burgy L, Kuhn J, Kuhn S (2016) Projet Hobical. Holothuries: Lien entre performances de production et de bioremédiation dans le contexte de la crevetticulture en Nouvelle-Calédonie. pp 163.

Hodge A, Stewart J, Robinson D, Griffiths BS, Fitter AH (2000) Competition between roots and soil micro-organisms for nutrients from nitrogen-rich patches of varying complexity. J Ecol 88:150–164.

Holby O, Hall POJ (1991) Chemical fluxes and mass balances in a marine fish cage farm. II Phosphorus. Mar Ecol Prog Ser 70:263–272.

Holmer M, Duarte CM, Heilskov A, Olesen B, Terrados J (2003) Biogeochemical conditions in sediments enriched by organic matter from net-pen fish farms in the Bolinao area, Philippines. Mar Pollut Bull 46:1470–1479.

Holmer M, Kristensen E (1992) Impact of marine fish cage farming on metabolism and sulfate reduction of underlying sediments. 80:191–201.

Hou Y, Sun Y, Gao Q, Dong S, Wen B, Yu H (2017) Optimal mud to sand ratios in sediment for sea cucumber aquaculture as revealed by carbon stable isotopes. Aquac Environ Interact 9:281–291.

Houdusse F, Zamarreño AM, Garnica M, García-Mina J (2005) The importance of nitrate in ameliorating the effects of ammonium and urea nutrition on plant development: The relationships with free polyamines and plant proline contents. Funct Plant Biol 32:1057–1067.

Irwin S, Flaherty MS, Carolsfeld J (2020) The contribution of small-scale, privately owned tropical aquaculture to food security and dietary diversity in Bolivia. Food Secur.

Ivy G, Azari D, Giraspy B (2006) Development of large-scale hatchery production techniques for the commercially important sea cucumber *Holothuria scabra* var . versicolor (Conand, 1986) in Queensland, Australia. 28–34.

Jackson D, Drumm A, McEvoy S, Jensen Ø, Mendiola D, Gabiña G, Borg JA, Papageorgiou N, Karakassis Y, Black KD (2015) A pan-European valuation of the extent, causes and cost of escape events from sea cage fish farming. Aquaculture 436:21–26.

Jaeckle WB, Strathmann RR (2013) The anus as a second mouth: Anal suspension feeding by an oral deposit-feeding sea cucumber. Invertebr Biol 132:62–68.

Jampeetong A, Brix H (2009) Nitrogen nutrition of *Salvinia natans*: Effects of inorganic nitrogen form on growth, morphology, nitrate reductase activity and uptake kinetics of ammonium and nitrate. Aquat Bot 90:67–73.

Jampeetong A, Brix H, Kantawanichkul S (2012) Effects of inorganic nitrogen forms on growth, morphology, nitrogen uptake capacity and nutrient allocation of four tropical aquatic macrophytes (*Salvinia cucullata*, *Ipomoea aquatica*, *Cyperus involucratus* and *Vetiveria zizanioides*). Aquat Bot 97:10–16.

Jansen HM, Hansen PK, Brennan N, Dahlgren TG, Fang J, Nederlof MAJ, Strohmeier T, Sveier H, Strand (2019) Enhancing opportunistic polychaete communities under fish farms: An alternative concept for integrated aquaculture. Aquac Environ Interact 11:331–336.

Jia B, Tang Y, Tian L, Franz L, Alewell C, Huang JH (2015) Impact of fish farming on phosphorus in reservoir sediments. Sci Rep 5:1–11.

Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A (2005) Dissolved organic nitrogen uptake by plants — an important N uptake pathway? Soil Biol Biochem 37:413–423.

Kadlec RH, Knight RL (1996) Treatment Wetlands. CRC Press, Boca Raton, Florida.

Kajimura M, Croke SJ, Glover CN, Wood CM (2004) Dogmas and controversies in the handling of nitrogenous wastes: the effect of feeding and fasting on the excretion of ammonia, urea and other nitrogenous waste products in rainbow trout. J Exp Biol 207:1993–2002.

Kamminga-Van Wijk C, Prins HBA (1993) The kinetics of NH4+ and NO3− uptake by Douglas fir from single N-solutions and from solutions containing both NH4+ and NO3−. Plant Soil An Int J Plant-Soil Relationships 151:91–96.

Kerrigan D, Suckling CC (2018) A meta-analysis of integrated multitrophic aquaculture: extractive species growth is most successful within close proximity to open-water fish farms. Rev Aquac 10:560–572.

Kielland K, McFarland J, Olson K (2006) Amino acid uptake in deciduous and coniferous taiga ecosystems. Plant Soil 288:297–307.

Kleitou P, Kletou D, David J (2018) Is Europe ready for integrated multi-trophic aquaculture? A survey on the perspectives of European farmers and scientists with IMTA experience. Aquaculture 490:136–148.

Kok B, Malcorps W, Tlusty MF, Eltholth MM, Auchterlonie NA, Little DC, Harmsen R, Newton RW, Davies SJ (2020) Fish as feed: Using economic allocation to quantify the Fish in - Fish-out ratio of major fed aquaculture species. Aquaculture 528:735474.

Komarova NY, Thor K, Gubler A, Meier S, Dietrich D, Weichert A, Grotemeyer MS, Tegeder M, Rentsch D (2008) AtPTR1 and AtPTR5 transport dipeptides in planta. Plant Physiol 148:856–869.

Konnerup D, Brix H (2010) Nitrogen nutrition of *Canna indica*: Effects of ammonium versus nitrate on growth, biomass allocation, photosynthesis, nitrate reductase activity and N uptake rates. Aquat Bot 92:142–148.

Konnerup D, Trang NTD, Brix H (2011) Treatment of fishpond water by recirculating horizontal and vertical flow constructed wetlands in the tropics. Aquaculture 313:57–64.

Kristensen E (2000) Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiologia 426:1–24.

Kristensen E, Ahmed SI, Devol AH (1995) Aerobic and anaerobic decomposition of organic matter in marine sediment: Which is fastest? Limnol Oceanogr 40:1430–1437.

Kudo N, Fujiyama H (2010) Responses of Halophyte *Salicornia bigelovii* to Different Forms of Nitrogen Source. Pedosphere 20:311–317.

Kuenen J. G. (2008) Anammox bacteria: from discovery to application. Nat Rev Microbiol 6:320–326.

Kuhn DD, Flick G (2009) Microbial floc meal as a reptacement ingredient for fish meal and soybean proein in shrimp feed shrimp feed. Aquaculture 296:51–57.

Kuhn DD, Lawrence AL, Boardman GD, Patnaik S, Marsh L, Flick GJ (2010) Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 303:28–33.

Lane DJW, Limbong D (2015) Catastrophic depletion of reef-associated sea cucumbers: Resource management/reef resilience issues for an Indonesian marine park and the wider Indo-Pacific. Aquat Conserv Mar Freshw Ecosyst 25:505–517.

De Lange HJ, Paulissen MPCP (2016) Efficiency of three halophyte species in removing nutrients from saline water: a pilot study. Wetl Ecol Manag 24:587–596.

De Lange HJ, Paulissen MPCP, Slim PA (2013) 'Halophyte Filters': the Potential of Constructed Wetlands for Application in Saline Aquaculture. Int J Phytoremediation 15:352–364.

Lasa B, Frechilla S, Lamsfus C, Aparicio-Tejo PM (2001) The sensitivity to ammonium nutrition is related to nitrogen accumulation. Sci Hortic (Amsterdam) 91:143–152.

Lavitra T, Rasolofonirina R, Eeckhaut I (2010) The Effect of Sediment Quality and Stocking Density on Survival and Growth of the Sea Cucumber *Holothuria scabra* Reared in Nursery Ponds and Sea Pens. West Indian Ocean J … 9:153–164.

Lee S, Ford AK, Mangubhai S, Wild C, Ferse SCA (2018) Effects of sandfish (*Holothuria scabra*) removal on shallow-water sediments in Fiji. PeerJ.

Lefebvre S, Bacher C, Meuret A, Hussenot J (2001) Modelling nitrogen cycling in a mariculture ecosystem as a tool to evaluate its outflow. Estuar Coast Shelf Sci 52:305–325.

Li M, Callier MD, Blancheton JP, Galès A, Nahon S, Triplet S, Geoffroy T, Menniti C, Fouilland E, Roque d'orbcastel E (2019) Bioremediation of fishpond effluent and production of microalgae for an oyster farm in an innovative recirculating integrated multi-trophic aquaculture system. Aquaculture 504:314–325.

Lillo C (1994) Light regulation of nitrate reductase in green leaves of higher plants. Physiol Plant 90:616–620.

Lillo C (2008) Signalling cascades integrating light-enhanced nitrate metabolism. Biochem J 415:11–19.

Lin Y-F, Jing S-R, Lee D-Y, Wang T-W (2002) Nutrient removal from aquaculture wastewater using a constructed wetlands system. Aquaculture 209:169–184.

Lipson D, Näsholm T (2001) The unexpected versatility of plants: Organic nitrogen use and availability in terrestrial ecosystems. Oecologia 128:305–316.

Liu X, Pu X, Luo D, Lu J, Liu Z (2019) Model assessment of nutrient removal via planting *Sesuvium portulacastrum* in floating beds in eutrophic marine waters: the case of aquaculture areas of Dongshan Bay. Acta Oceanol Sin 38:91–100.

Lokhande VH, Gor BK, Desai NS, Nikam TD, Suprasanna P (2013) *Sesuvium portulacastrum*, a plant for drought, salt stress, sand fixation, food and phytoremediation. A review. Agron Sustain Dev 33:329–348.

Lokhande VH, Nikam TD, Suprasanna P (2009) *Sesuvium portulacastrum* (L.) L. a promising halophyte: Cultivation, utilization and distribution in India. Genet Resour Crop Evol 56:741–747.

Lonard RI, Judd FW (1997) The biological flora of coastal dunes and wetlands. *Sesuvium portulacastrum* (L) L. J Coast Res 13:96–104.

Lu J, Li X (2006) Review of rice-fish-farming systems in China - One of the Globally Important Ingenious Agricultural Heritage Systems (GIAHS). Aquaculture 260:106–113.

Luna M, Llorente I, Cobo A (2019) Determination of feeding strategies in aquaculture farms using a multiple-criteria approach and genetic algorithms. Ann Oper Res.

Lyssenko C, Wheaton F (2006) Impact of positive ramp short-term operating disturbances on ammonia removal by trickling and submerged-upflow biofilters for intensive recirculating aquaculture. Aquac Eng 35:26–37.

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

Macduff JH, Bakken AK, Dhanoa MS (1997) An analysis of the physiological basis of commonality between diurnal patterns of NH4+, NO3 and K+ uptake by *Phleum pratense* and *Festuca pratensis*. J Exp Bot 48:1691–1701.

Maciel E, Domingues P, Domingues MRM, Calado R, Lillebø AI (2020) Halophyte plants from sustainable marine aquaponics are a valuable source of omega-3 polar lipids. Food Chem 0:0–32.

MacKay KT (1983) Ecological Aquaculture, New Approaches To Aquaculture in North America. J World Maric Soc 14:704–713.

MacTavish T, Stenton-Dozey J, Vopel K, Savage C (2012) Deposit-Feeding Sea Cucumbers Enhance Mineralization and Nutrient Cycling in Organically-Enriched Coastal Sediments. PLoS One 7:e50031.

Magwa ML, Gundidza M, Gweru N, Humphrey G (2006) Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. J Ethnopharmacol 103:85–89.

Malone RF, Pfeiffer TJ (2006) Rating fixed film nitrifying biofilters used in recirculating aquaculture systems. Aquac Eng 34:389–402.

Martins CIM, Eding EH, Verdegem MCJ, Heinsbroek LTN, Schneider O, Blancheton JP, Roque E, Verreth JAJ (2010) New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquac Eng 43:83–93.

Mathieu-Resuge M, Grand F Le, Schaal G, Kraffe E, Lorrain A, Letourneur Y, Lemonnier H, Benoît J, Hochard S (2020) Living Resources Assimilation of shrimp farm sediment by *Holothuria scabra*: a coupled fatty acid and stable isotope approach. Aquat Living Resour 33:1–12.

Maucieri C, Nicoletto C, Zanin G, Birolo M, Trocino A, Sambo P, Borin M, Xiccato G (2019) Effect of stocking density of fish on water quality and growth performance of European Carp and leafy vegetables in a low-tech aquaponic system. PLoS One 14:1–15.

McCarthy MJ, Gardner WS (2003) An application of membrane inlet mass spectrometry to measure denitrification in a recirculating mariculture system. Aquaculture 218:341–355.

McClelland JW, Valiela I (1998) Changes in food web structure under the influence of increased anthropogenic nitrogen inputs to estuaries. Mar Ecol Prog Ser 168:259–271.

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390.

Menasveta P, Panritdam T, Sihanonth P, Powtongsook S, Chuntapa B, Lee P (2001) Design and function of a closed, recirculating seawater system with denitrification for the culture of black tiger shrimp broodstock. Aquac Eng 25:35–49.

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

Mermillod-Blondin F, Rosenberg R (2006) Ecosystem engineering: The impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. Aquat Sci 68:434–442.

Middelburg JJ, Vlug T, Jaco F, van der Nat WA (1993) Organic matter mineralization in marine systems. Glob Planet Change 8:47–58.

Miller A, Bowman WD, Suding KN (2007) Plant Uptake of Inorganic and Organic Nitrogen : Neighbor Identity Matters. Ecology 88:1832–1840.

Miller AE, Bowman WD (2003) Alpine plants show species-level differences in the uptake of organic and inorganic nitrogen A. Plant Soil 250:283–292.

Mills D, Duy NDQ, Juinio-Menez M a, Raison CM, Zarate JM (2012) Overview of sea cucumber aquaculture and sea-ranching research in the South-East Asian region. Proc an Int Symp held Noumea, New Caledonia, 15–17 Febr 2011 ACIAR Proc No 136 Aust Cent Int Agric Res Canberra 209 p:22–31.

Mondal K, Kunzmann A (2018) Inferior Assimilation of Algae-based Diets by Sea Cucumber *Holothuria scabra* under Laboratory Condition Expressed by Stable Isotope Mixing Model. Annu Res Rev Biol 28:1–8.

Moriarty DJ. W, Pollard PC, Hunt WG, Moriarty CM, Wassenberg TJ (1985) Productivity of bacteria and microalgae and the effect of grazing by holothurians in sediments on a coral reef flat. Mar Biol 85:293–300.

Moriarty DJW (1982) Feeding of *Holothuria atra* and *Stichopus chloronotus* on bacteria, organic carbon and organic nitrogen in sediments of the Great Barrier Reef. Mar Freshw Res 33:255–263.

Moriarty DJW (1987) Methodology for determining biomass and productivity of microorganisms in detrital food webs. In: *Detritus and microbial ecology in aquaculture*, 14th ed. Moriarty DJW, Pullin RS V (eds) International Center for Living Aqua, Bellagio, Como, Italy

Moriarty DJW, Pollard PC (1982) Diel variation of bacterial productivity in seagrass (*Zostera capricorni*) beds measured by rate of thymidine incorporation into DNA. Mar Biol 72:165–173.

Mozdzer TJ, Zieman JC, McGlathery KJ (2010) Nitrogen uptake by native and invasive temperate coastal macrophytes: Importance of dissolved organic nitrogen. Estuaries and Coasts 33:784–797.

Namukose M, Msuya FE, Ferse SC, Slater M, Kunzmann A (2016) Growth performance of the sea cucumber *Holothuria scabra* and the seaweed *Eucheuma denticulatum*: integrated mariculture and effects on sediment organic characteristics. Aquac Environ Interact 8:179–189.

Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M, Högberg P (1998) Boreal forest plants take up organic nitrogen. Nature 392:914–917.

Näsholm T, Huss-Danell K, Högberg P (2000) Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology 81:1155–1161.

Näsholm T, Persson J (2001) Plant acquisition of organic nitrogen in boreal forests. Physiol Plant 111:419–426.

Navarrete-Mier F, Sanz-Lázaro C, Marín A (2010) Does bivalve mollusc polyculture reduce marine fin fish farming environmental impact? Aquaculture 306:101–107.

Naylor RL, Goldburg RJ, Clay J, Troell M, Mooney H, Primavera JH, Folke C, Beveridge MCM, Kautsky N, Lubchenco J (2000) Effect of aquaculture on world fish supplies. Nature 405:1017–1024.

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004) Integrated aquaculture: Rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231:361–391.

Neori A, Krom MD, Rijn J van (2007a) Biogeochemical processes in intensive zero-effluent marine fish culture with recirculating aerobic and anaerobic biofilters. J Exp Mar Bio Ecol 349:235–247.

Neori A, Msuya FE, Shauli L, Schuenhoff A, Kopel F, Shpigel M (2003) A novel three-stage seaweed (*Ulva lactuca*) biofilter design for integrated mariculture. J Appl

Phycol 15:543–553.

Neori A, Troell M, Chopin T, Yarish C, Critchley A, Buschmann AH (2007b) The Need for a Balanced Ecosystem Approach to Blue Revolution Aquaculture. Environ Sci Policy Sustain Dev 49:36–43.

Nordin A, Högberg P, Näsholm T (2001) Soil nitrogen form and plant nitrogen uptake along a boreal forest productivity gradient. Oecologia 129:125–132.

Nouairi I, Ghnaya T, Ben Youssef N, Zarrouk M, Habib Ghorbel M (2006) Changes in content and fatty acid profiles of total lipids of two halophytes: *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum* under cadmium stress. J Plant Physiol 163:1198–1202.

Nunes JP, Ferreira JG, Bricker SB, O'Loan B, Dabrowski T, Dallaghan B, Hawkins AJS, O'Connor B, O'Carroll T (2011) Towards an ecosystem approach to aquaculture: Assessment of sustainable shellfish cultivation at different scales of space, time and complexity. Aquaculture.

Ogah SI, Kamarudin MS, Amin SMN, Edaroyati MWP (2020) Biological filtration properties of selected herbs in an aquaponic system. Aquac Res:1–9.

Olive PJW, Pinnegar JK, Polunin NVC, Richards G, Welch R (2003) Isotope trophic-step fractionation: a dynamic equilibrium. J Anim Ecol 72:608–617.

Owen AG, Jones DL (2001) Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant n acquisition. Soil Biol Biochem 33:651–657.

Palmer PJ (2010) Polychaete-assisted sand filters. Aquaculture 306:369–377.

Palmer PJ, Wang S, Houlihan A, Brock I (2014) Nutritional status of a nereidid polychaete cultured in sand filters of mariculture wastewater. Aquac Nutr 20:675–691.

Paungfoo-Lonhienne C, Lonhienne TGA, Rentsch D, Robinson N, Christie M, Webb RI, Gamage HK, Carroll BJ, Schenk PM, Schmidt S (2008) Plants can use protein as a nitrogen source without assistance from other organisms. Proc Natl Acad Sci U S A 105:4524–4529.

Petersen A, Andersen JS, Kaewmak T, Somsiri T, Dalsgaard A (2002) Impact of integrated fish farming on antimicrobial resistance in a pond environment. Appl Environ Microbiol 68:6036–6042.

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Sytematics 18:293–320.

Peuke AD, Jeschke WD (1998) The effects of light on induction, time courses, and kinetic patterns of net nitrate uptake in barley. Plant, Cell Environ 21:765–774.

Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ (2014) Best practices for use of stable isotope mixing models in food-web studies. Can J Zool 92:823–835.

Phillips DL, Koch PL (2002) Incorporating concentration dependence in stable isotope mixing models. Oecologia 130:114–125.

Piedrahita RH (2003) Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture 226:35–44.

Pinheiro I, Arantes R, do Espírito Santo CM, do Nascimento Vieira F, Lapa KR, Gonzaga LV, Fett R, Barcelos-Oliveira JL, Seiffert WQ (2017) Production of the halophyte *Sarcocornia ambigua* and Pacific white shrimp in an aquaponic system with biofloc technology. Ecol Eng 100:261–267.

Pinnegar JK, Polunin NVC (1999) Differential fractionation of δ 13 C and δ 15 N among fish tissues : implications for the study of trophic interactions. 225–231.

Pitt R, Duy N, Duy T, Long H (2004) Sandfish (*Holothuria scabra*) with shrimp (*Penaeus monodon*) co-culture tank trials. SPC beche-de-mer Inf Bull:12–22.

Plotieau T, Lavitra T, Gillan DC, Eeckhaut I (2013) Bacterial diversity of the sediments transiting through the gut of *Holothuria scabra* (Holothuroidea; Echinodermata). Mar Biol 160:3087–3101.

Plotieau T, Lepoint G, Lavitra T, Eeckhaut I (2014) Isotopic tracing of sediment components assimilated by epibiontic juveniles of *Holothuria scabra* (Holothuroidea). J Mar Biol Assoc United Kingdom.

Porrello S, Lenzi M, Ferrari G, Persia E, Savelli F, Tomassetti P (2005) Loading of nutrient from a land-based fish farm(Orbetello, Italy) at different times. Aquac Int 13:97–108.

Porrello S, Lenzi M, Persia E, Tomassetti P, Finoia MG (2003) Reduction of aquaculture wastewater eutrophication by phytotreatment ponds system I. Dissolved and particulate nitrogen and phosphorus. Aquaculture 219:515–529.

Post DM (2002) Using stable isotopes to estimate trophic position: models, methos, and assumptions. . Ecology 83:703–718.

Price C, Black KD, Hargrave BT, Morris JA (2014) Marine cage culture and the environment: Effects on water quality and primary production. Aquac Environ Interact 6:151–174.

Purcell SW, Hair CA, Mills DJ (2012) Sea cucumber culture, farming and sea ranching in the tropics: Progress, problems and opportunities. Aquaculture 368–369:68–81.

Purcell SW, Patrois J, Fraisse N (2006) Experimental evaluation of co-culture of juvenile sea cucumbers, *Holothuria scabra* (Jaeger), with juvenile blue shrimp, *Litopenaeus stylirostris* (Stimpson). Aquac Res 37:515–522.

Purcell SW, Polidoro B a, Hamel J-F, Gamboa RU, Mercier A (2014) The cost of being valuable: predictors of extinction risk in marine invertebrates exploited as luxury seafood. Proc R Soc B 281:20133296.

Purcell SW, Williamson DH, Ngaluafe P (2018) Chinese market prices of beche-de-mer: Implications for fisheries and aquaculture. Mar Policy 91:58–65.

Putro SP, Sharani J, Widowati, Adhy S, Suryono (2020) Biomonitoring of the application of monoculture and integrated multi-trophic aquaculture (IMTA) using macrobenthic structures at tembelas island, kepulauan riau province, indonesia. J Mar Sci Eng 8:1–13.

Quintã R, Hill PW, Jones DL, Santos R, Thomas DN, LeVay L (2015a) Uptake of an amino acid (alanine) and its peptide (trialanine) by the saltmarsh halophytes *Salicornia europaea* and *Aster tripolium* and its potential role in ecosystem N cycling and marine aquaculture wastewater treatment. Ecol Eng 75:145–154.

Quintã R, Santos R, Thomas DN, Le Vay L (2015b) Growth and nitrogen uptake by *Salicornia europaea* and *Aster tripolium* in nutrient conditions typical of aquaculture wastewater. Chemosphere 120:414–421.

Ranjan R, Megarajan S, Xavier B, Raju SS, Ghosh S, Gopalakrishnan A (2019) Design and performance of recirculating aquaculture system for marine finfish broodstock development. Aquac Eng 85:90–97.

Reid GK, Liutkus M, Bennett A, Robinson SMC, MacDonald B, Page F (2010) Absorption efficiency of blue mussels (*Mytilus edulis* and *M. trossulus*) feeding on Atlantic salmon (*Salmo salar*) feed and fecal particulates: Implications for integrated multi-trophic aquaculture. Aquaculture 299.

Renkui C, Dashu N, Jianguo W (1995) Rice-Fish Culture in China: The Past, Present, and Future. In: *Rice-Fish Culture in China*. MacKay KT (ed) IDRC, Ottowa, ON

Reverter M, Bontemps N, Lecchini D, Banaigs B, Sasal P (2014) Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture 433:50–61.

Reverter M, Sarter S, Caruso D, Avarre JC, Combe M, Pepey E, Pouyaud L, Vega-Heredía S, de Verdal H, Gozlan RE (2020a) Aquaculture at the crossroads of global warming and antimicrobial resistance. Nat Commun 11:1–8.

Reverter M, Tapissier-Bontemps N, Sarter S, Sasal P, Caruso D (2020b) Moving towards more sustainable aquaculture practices: a meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. Rev Aquac:1–19.

Richter C, Roa-Quiaoit H, Jantzen C, Al-Zibdah M, Kochzius M (2008) Collapse of a New Living Species of Giant Clam in the Red Sea. Curr Biol 18:1349–1354.

Van Rijn J (1996) The potential for integrated biological treatment systems in recirculating fish culture - A review. Aquaculture 139:181–201.

Van Rijn J (2013) Waste treatment in recirculating aquaculture systems. Aquac Eng 53:49–56.

van Rijn J, Tal Y, Schreier HJ (2006) Denitrification in recirculating systems: Theory and applications. Aquac Eng 34:364–376.

Roberts D, Gebruk A, Levin V, Manship BAD (2000) Feeding and digestive strategies in deposit-feeding holothurians. Oceanogr Mar Biol An Annu Rev 38:257.

Robinson G (2013) A Bright Future for Sandfish Aquaculture. World Aquac March:18–24.

Robinson G (2017) Microbial – deposit feeder aquaculture bioremediation systems. Newcastle University

Robinson G, Caldwell GS, Jones CLW, Slater M, Stead SM (2015) Redox stratification drives enhanced growth in a deposit-feeding invertebrate: Implications for aquaculture bioremediation. Aquac Environ Interact 8:1–13.

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.

Robinson G, Caldwell GS, Wade MJ, Free A, Jones CLW, Stead SM (2016) Profiling bacterial communities associated with sediment-based aquaculture bioremediation systems under contrasting redox regimes. Sci Rep.

Robinson G, MacTavish T, Savage C, Caldwell GS, Jones CLW, Probyn T, Eyre BD, Stead SM (2018) Carbon amendment stimulates benthic nitrogen cycling during the bioremediation of particulate aquaculture waste. Biogeosciences 15:1863–1878.

Robinson G, Pascal B (2009) From hatchery to community – Madagascar's first village-based holothurian mariculture programme. SPC Beche-de-mer Inf Bull 29:38–42.

Saliling WJB, Westerman PW, Losordo TM (2007) Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations. Aquac Eng 37:222–233.

Sandu S, Brazil B, Hallerman E (2008) Efficacy of a pilot-scale wastewater treatment plant upon a commercial aquaculture effluent. I. Solids and carbonaceous compounds. Aquac Eng 39:78–90.

Sarà G (2007) Sedimentary and particulate organic matter: Mixed sources for cockle *Cerastoderma glaucum* in a shallow pond, Western Mediterranean. Aquat Living Resour 20:271–277.

Sarà G, Zenone A, Tomasello A (2009) Growth of *Mytilus galloprovincialis* (mollusca, bivalvia) close to fish farms: a case of integrated multi-trophic aquaculture within the Tyrrhenian Sea. Hydrobiologia 636:129–136.

Sauheitl L, Glaser B, Weigelt A (2009) Uptake of intact amino acids by plants depends on soil amino acid concentrations. Environ Exp Bot 66:145–152.

Schneider O, Sereti V, Eding EH, Verreth JAJ (2005) Analysis of nutrient flows in integrated intensive aquaculture systems. Aquac Eng 32:379–401.

Senff P, Partelow S, Indriana LF, Buhari N, Kunzmann A (2018) Improving pond aquaculture production on Lombok, Indonesia. Aquaculture 497:64–73.

Shpigel M, Ben-Ezra D, Shauli L, Sagi M, Ventura Y, Samocha T, Lee JJ (2013) Constructed wetland with *Salicornia* as a biofilter for mariculture effluents. Aquaculture 413:52–63.

Siddiqi MY, Glass ADM, Ruth TJ, Rufty TW (1990) Studies of the uptake of nitrate in barley: I. Kinetics of 13NO3- influx. Plant Physiol 93:1426–1432.

Singer A, Parnes S, Gross A, Sagi A, Brenner A (2008) A novel approach to denitrification processes in a zero-discharge recirculating system for small-scale urban aquaculture. Aquac Eng 39:72–77.

Slater M, 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 Bio Ecol 409:315–323.

Smart RM, Barko JW (1980) Nitrogen Nutrition and Salinity Tolerance of Distichlis Spicata and Spartina Alterniflora. Ecology 61:630–638.

Song B, Lisa JA, Tobias CR (2014) Linking DNRA community structure and activity in a shallow lagoonal estuarine system. Front Microbiol 5:1–10.

Song X, Lin C, Zhou Y, Wang X, Gu R, Xu S, Xu Q, Chen K, Yang H (2018) Feeding preference of *Apostichopus japonicus*: Comparing carbon stable isotope analysis and carbon budget approach. Aquac Environ Interact 10:243–253.

Stenton-Dozey JME, Heath P, Ren JS, Zamora LN (2020) New Zealand aquaculture industry: research, opportunities and constraints for integrative multitrophic farming. New Zeal J Mar Freshw Res:1–21.

Summerfelt ST, Sharrer MJ (2004) Design implication of carbon dioxide production within biofilters contained in recirculating salmonid culture systems. Aquac Eng 32:171–182.

Sun Z, Gao Q, Dong S, Shin PKS, Wang F (2013) Seasonal changes in food uptake by the sea cucumber *Apostichopus japonicus* in a farm pond: Evidence from C and N stable isotopes. J Ocean Univ China 12:160–168.

Svobodová Z, Máchová J, Poleszczuk G, Hůda J, Hamáčková J, Kroupová H (2005) Nitrite poisoning of fish in aquaculture facilities with water-recirculating systems. Acta Vet Brno 74:129–137.

Thamdrup B (2012) New pathways and processes in the global nitrogen cycle. Annu Rev Ecol Evol Syst 43:407–428.

Thamdrup B, Dalsgaard T (2008) Nitrogen cycling in sediments. In: *Microbial Ecology of the Oceans*. John Wiley & Sons Ltd, p 527–568

Theuerkauf SJ, Morris JA, Waters TJ, Wickliffe LC, Alleway HK, Jones RC (2019) A global spatial analysis reveals where marine aquaculture can benefit nature and people. PLoS One 14:1–29.

Thia-Eng C (1997) Sustainable aquaculture and integrated coastal management. In: *Sustainable Aquaculture*. Bardach JE (ed) John Wiley and Sons Ltd, New York, p 177–200

Timmons MB, Ebeling JM, Wheaton F, Summerfelt S, Vinci BJ (2001) Recirculating Aquaculture Systems. Cayuga Aqua Ventures, Ithaka, NY.

Tischner R (2006) Nitrate uptake and reduction in plants. J Crop Improv 15:53–95.

Tran N, Rodriguez U-P, Chan CY, Phillips MJ, Mohan CV, Henriksson PJG, Koeshendrajana S, Suri S, Hall S (2017) Indonesian aquaculture futures : An analysis of fish supply and demand in Indonesia to 2030 and role of aquaculture using the AsiaFish model. Mar Policy 79:25–32.

Troell M, Halling C, Neori A, Chopin T, Buschmann AH, Kautsky N, Yarish C (2003) Integrated mariculture: Asking the right questions. Aquaculture 226:69–90.

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

Troell M, Rönnbäck P, Halling C, Kautsky N, Buschmann A (1999) Ecological engineering in aquaculture: Use of seaweeds for removing nutrients from intensive mariculture. J Appl Phycol 11:89–97.

Turcios AE, Papenbrock J (2014) Sustainable treatment of aquaculture effluents - What can we learn from the past for the future? Sustainability:836–856.

Turcios AE, Weichgrebe D, Papenbrock J (2016) Potential use of the facultative halophyte *Chenopodium quinoa* Willd. as substrate for biogas production cultivated with different concentrations of sodium chloride under hydroponic conditions. Bioresour Technol 203:272–279.

Twarowska JG, Westerman PW, Losordo TM (1997) Water treatment and waste characterization evaluation of an intensive recirculating fish production system. Aquac Eng 16:133–147.

Tylova-Munzarova E, Lorenzen B, Brix H, Votrubova O (2005) The effects of NH4+ and NO3- on growth, resource allocation and nitrogen uptake kinetics of *Phragmites australis* and *Glyceria maxima*. Aquat Bot 81:326–342.

Uthicke S (2001) Interactions between sediment-feeders and microalgae on coral reefs: Grazing losses versus production enhancement. Mar Ecol Prog Ser 210:125–138.

Uthicke S (1999) Sediment bioturbation and impact of feeding activity of Holothuria (Halodeima) atra and Stichopus chloronotus, two sediment feeding holothurians, at Lizard Island, great barrier reef. Bull Mar Sci 64:129–141.

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet δ15N enrichment: A meta-analysis. Oecologia 136:169–182.

Ventura Y, Sagi M (2013) Halophyte crop cultivation: The case for *Salicornia* and *Sarcocornia*. Environ Exp Bot 92:144–153.

Ventura Y, Wuddineh WA, Myrzabayeva M, Alikulov Z, Khozin-Goldberg I, Shpigel M, Samocha TM, Sagi M (2011) Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. Sci Hortic (Amsterdam) 128:189–196.

Verdegem MCJ (2013) Nutrient discharge from aquaculture operations in function of system design and production environment. Rev Aquac 5:158–171.

Verhoeven JTA, Meuleman AFM (1999) Wetlands for wastewater treatment : Opportunities and limitations. Ecol Eng 12:5–12.

Vymazal J (2010) Constructed wetlands for wastewater treatment. Water 2:530–549.

Waller U, Buhmann A, Ernst A, Hanke V, Kulakowski A, Wecker B, Orellana J, Papenbrock J (2015) Integrated multi-trophic aquaculture in a zero-exchange recirculation aquaculture system for marine fish and hydroponic halophyte production. Aquac Int 23:1473–1489.

Wang H, Hagemann A, Reitan KI, Ejlertsson J, Wollan H, Handå A, Malzahn AM (2019) Potential of the polychaete *Hediste diversicolor* fed on aquaculture and biogas side streams as an aquaculture food source. Aquac Environ Interact 11:551–562.

Wang X, Olsen LM, Reitan KI, Olsen Y (2012) Discharge of nutrient wastes from salmon farms: Environmental effects, and potential for integrated multi-trophic aquaculture. Aquac Environ Interact 2:267–283.

Warren CR (2009) Does nitrogen concentration affect relative uptake rates of nitrate, ammonium, and glycine? J Plant Nutr Soil Sci 172:224–229.

Watanabe S, Kodama M, Sumbing JG, Lebata-Ramos MJH (2013) Diet-tissue Stable Isotopic Fractionation of Tropical Sea Cucumber, *Holothuria scabra*. Japan Agric Res Q JARQ 47:127–134.

Watanabe S, Zarate JM, Lebata-Ramos MJH, Nievales MFJ, Kodama M (2012) Utilization of Organic Waste from Black Tiger Shrimp, *Penaeus monodon*, by Sandfish, *Holothuria scabra*. In: *Sustainable stock management and development of aquaculture technology suitable for Southeast Asia (JIRCAS Working Report No 75)*. Tanaka K, Morioka S, Watanabe S (eds) Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan, p 81–86

Webb JM, Quintã R, Papadimitriou S, Norman L, Rigby M, Thomas DN, Le Vay L (2012) Halophyte filter beds for treatment of saline wastewater from aquaculture. Water Res 46:5102–5114.

Webb JM, Quintã R, Papadimitriou S, Norman L, Rigby M, Thomas DN, Le Vay L (2013) The effect of halophyte planting density on the efficiency of constructed wetlands for the treatment of wastewater from marine aquaculture. Ecol Eng 61:145–153.

Wei Z, You J, Wu H, Yang F, Long L, Liu Q, Huo Y, He P (2017) Bioremediation using *Gracilaria lemaneiformis* to manage the nitrogen and phosphorous balance in an integrated multi-trophic aquaculture system in. Mar Pollut Bull 121:313–319.

Weitzman J, Filgueira R (2019) The evolution and application of carrying capacity in aquaculture: towards a research agenda. Rev Aquac:1–26.

Wen B, Sun YJ, Gao QF, Dong SL, Chen ZZ, Gao JZ (2017) Utilization of dietary carbohydrates by sea cucumber *Apostichopus japonicus* (Selenka) as indicated by carbon stable isotope analysis. Aquac Res 48:6001–6008.

Wolkenhauer S-M, Uthicke S, Burridge C, Skewes T, Pitcher R (2010) The ecological role of *Holothuria scabra* (Echinodermata: Holothuroidea) within subtropical seagrass beds. J Mar Biol Assoc United Kingdom 90:215.

Wu RSS (1995) The environmental impact of marine fish culture: Towards a sustainable future. Mar Pollut Bull 31:159–166.

Xu C, Lv Z, Shen Y, Liu D, Fu Y, Zhou L, Liu W, Chen K, Ye H, Xia X, Xia J, Wang Y, Ke Y, Shen J (2020) Metagenomic insights into differences in environmental resistome profiles between integrated and monoculture aquaculture farms in China. Environ Int 144:106005.

Xu Q, Zhang L, Zhang T, Zhang X, Yang H (2017) Functional groupings and food web of an artificial reef used for sea cucumber aquaculture in northern China. J Sea Res 119:1–7.

Yingst JY (1976) The utilization of organic matter in shallow marine sediments by an epibenthic deposit-feeding holothurian. J Exp Mar Bio Ecol 23:55–69.

Yogev U, Gross A (2019) Reducing environmental impact of recirculating aquaculture systems by introducing a novel microaerophilic assimilation reactor: Modeling and proof of concept. J Clean Prod 226:1042–1050.

Yogev U, Sowers KR, Mozes N, Gross A (2017) Nitrogen and carbon balance in a novel near-zero water exchange saline recirculating aquaculture system. Aquaculture 467:118–126.

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

Zheng X, Zhang D, Qin J, Wang Y (2018) The effect of C/N ratio on bacterial community and water quality in a mussel-fish integrated system. Aquac Res 49:1699–1708.

Chapter 2

A low-tech IMTA with tropical species at three trophic levels

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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 eight weeks on Zanzibar, Tanzania. In a 2 m^3 RAS system, 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 nitrite peak occurred within the first 30 days, 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 nitrate. 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 a proof-of-concept of a simple pilot-scale RAS, integrating tropical species at three trophic levels.

Keywords: 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 systems 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 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 successfully used for phytoremediation of municipal and industrial wastewater or contaminated soil (Verhoeven & Meuleman 1999, Vymazal 2010). Their use can have great economic benefits when 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 mio 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).

Recirculating aquaculture systems (RAS) are tank-based aquaculture facilities where 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 nitrite and further to nitrate, causing nitrite peaks followed by increases in nitrate (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 nitrate reduction, organisms such as plants, fungi or bacteria use nitrate as a source of nitrogen, with efficiencies depending on the availability of other, preferred, inorganic nitrogen species (ammonia or nitrate). Nitrate removal occurs under anaerobic conditions either as dissimilatory nitrate reduction to ammonium (DNRA) or as denitrification into nitrogen 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 are 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 its 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 set-up, 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 set-up

The experiment was conducted over a period of 70 days from 7th December 2018 to 14th 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 polyvinyl chloride (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 also 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 to 30.5 °C with an average ± SD of 28.1 ± 0.9°C Salinity ranged from 25.9 to 30.5, with an average of 30.3 ± 1.2. The fish tanks were stocked with milkfish (n=9) of 124 ± 29 g and ~20 cm length resulting in a stocking density 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.

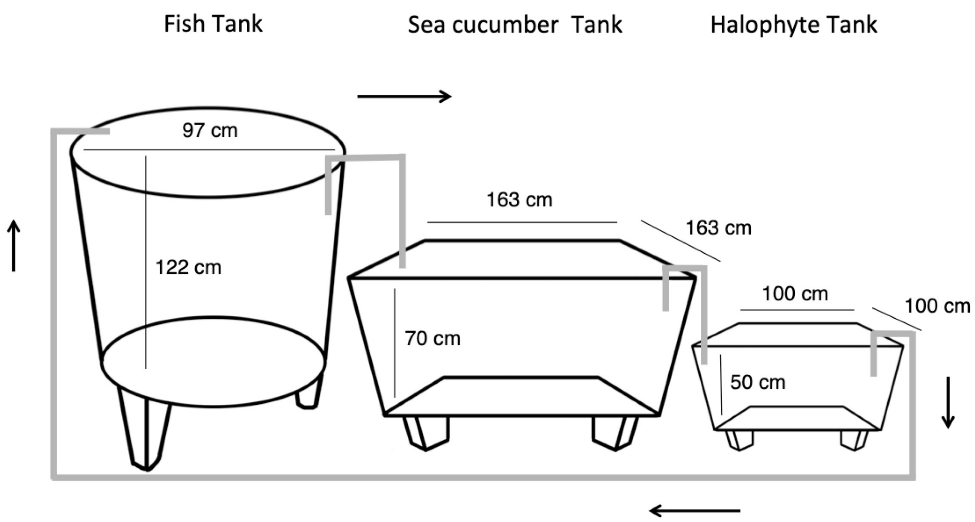

Fig.1: Schematic set-up of the experimental RAS. Grey lines represent water pipes, black arrows indicate direction of water flow.

A simplified airlift system was constructed to allow oxic conditions to be maintained by water circulation through the sediment (Robinson et al. 2015). It consisted of an outer frame of 1.25 cm high pressure pvc pipes with two 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 three days 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 x 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.

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

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

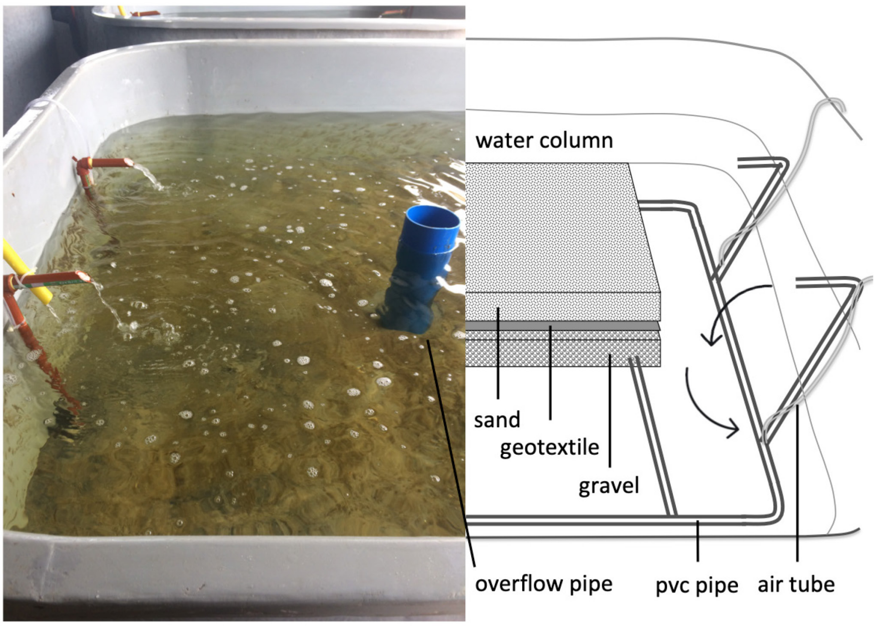

Fig. 2: Image (left) and schematic (right) of sea cucumber tank with airlift system. Arrows indicate the general direction of water flow down through the sediment and up through the airlift pipe.

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 Trading, China) back into the fish tank at a flow rate of 270 L h^{-1}. The water was circulated continuously for the first 16 days of the experiment. For the remainder of the experimental period, circulation was interrupted every Friday at 16:00 and resumed the following Monday at 10:00. This allowed 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 Ltd, Bagamoyo, Tanzania) at a rate of 4% body weight day^{-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 were 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, they were suspended in mesh bags for 24 hours to allow for the gut to be emptied, then placed on tissue paper for 1 minute, measured, photographed and weighed (Sewell & Bersquist 1990). Weight data were used to calculate absolute growth, specific growth rate (SGR) and food conversion ratio (FCR) for milkfish and for 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 GmbH, Germany).

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 nitrogen (TAN) and PO_4^--P. 20 ml water samples 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 and Parsons (1972) with an Infinite 200 PRO microplate reader (TECAN, Austria). Dissolved inorganic nitrogen (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, Germany).

Samples of the surface sediment, in the sea cucumber tanks, for the determination of nitrogen content were taken in two-week intervals. A 20 ml cut-off syringe was used as a corer (surface area of 3.14 cm^2), taking five 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 two 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 to 1500 ml were filtered over a glass microfiber filter with particle retention of 0.7 µm (VWR International, Leuven, Belgium). 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, Germany).

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 three systems increased in biomass at an SGR above 1 % per day for all species (Table 2) and survival was 100 %.

Table 2: Growth (mean ± SD) of the organisms in the RAS (n=3), - = not applicable.

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

3.2. Water quality

pH ranged from 7.7 to 8.2, with an average of 7.9 ± 0.1 and dissolved oxygen ranged from 5.5 to 8.9 mg l^{-1} averaging 7.3 ± 0.7. TAN concentrations in the systems 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 days, after which they remained low in all tanks. NO_3^- and PO_4^- concentrations increased over time in all tanks, in the case of $NO3^-$ 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 nitrogen 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 A1). An average of 3.5 ± 0.2 g of dissolved inorganic nitrogen (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 seven weeks of this sampling regime, DIN in the halophyte tanks was reduced by a total of 4.9 ± 1 g N.

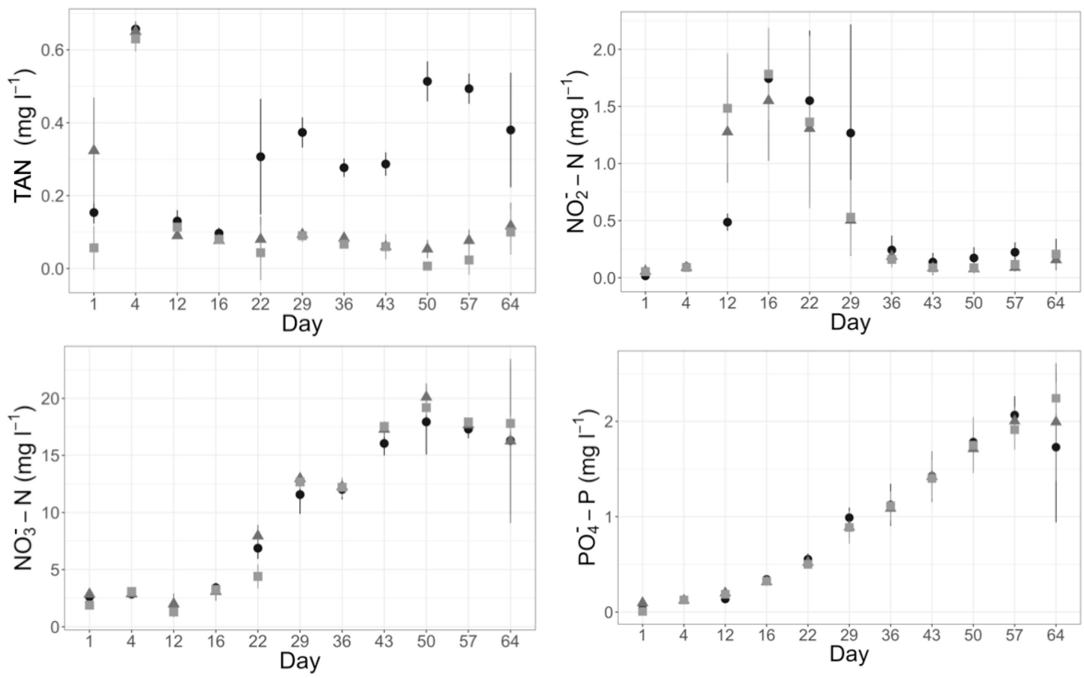

Fig. 3: Concentrations (mean mg l^{-1} ± SD) of dissolved nutrients measured weekly in the tanks of the RAS systems (n=3) over the course of the experiment. Black circles = Fish tanks, dark grey triangles = sea cucumber tanks, light grey squares = halophyte tanks.

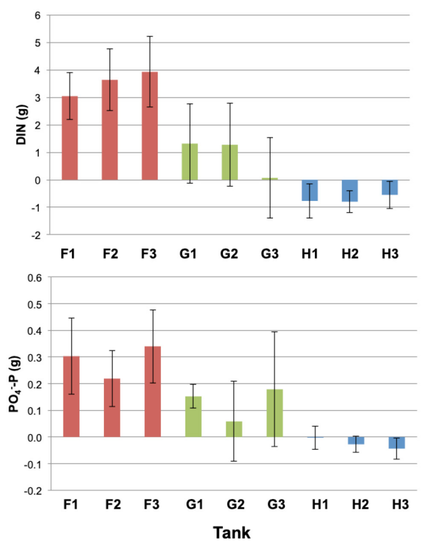

Fig. 4: Average change in total dissolved inorganic nitrogen (DIN) and PO_4-P in the individual tanks (F = fish, G = sea cucumber, H = halophyte) of the RAS (n=3), after periods of interrupted water circulation during the 70 day experiment. Error bars indicate standard deviation.

Concentrations of total suspended solids (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 by 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).

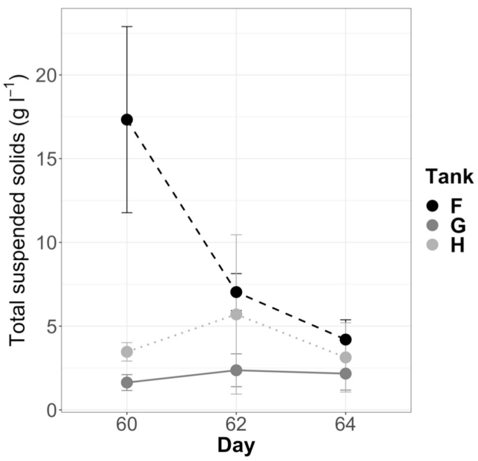

Figure 5: Total suspended solids (mean g l^{-1} ± SD) in the fish (F), sea cucumber (G) and halophyte (H) tanks of the RAS during the last week of the experiment.

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

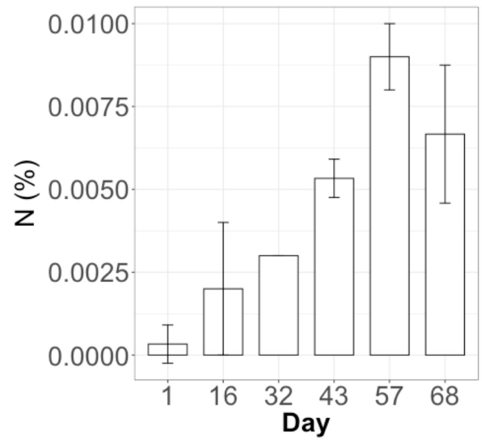

Fig. 6: Mean nitrogen (N) content (% of dry weight) in the surface sediment of the sea cucumber tanks in the RAS (n=3). Error bars indicate standard deviation

3.3. Nitrogen 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 nitrogen 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).

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-day 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

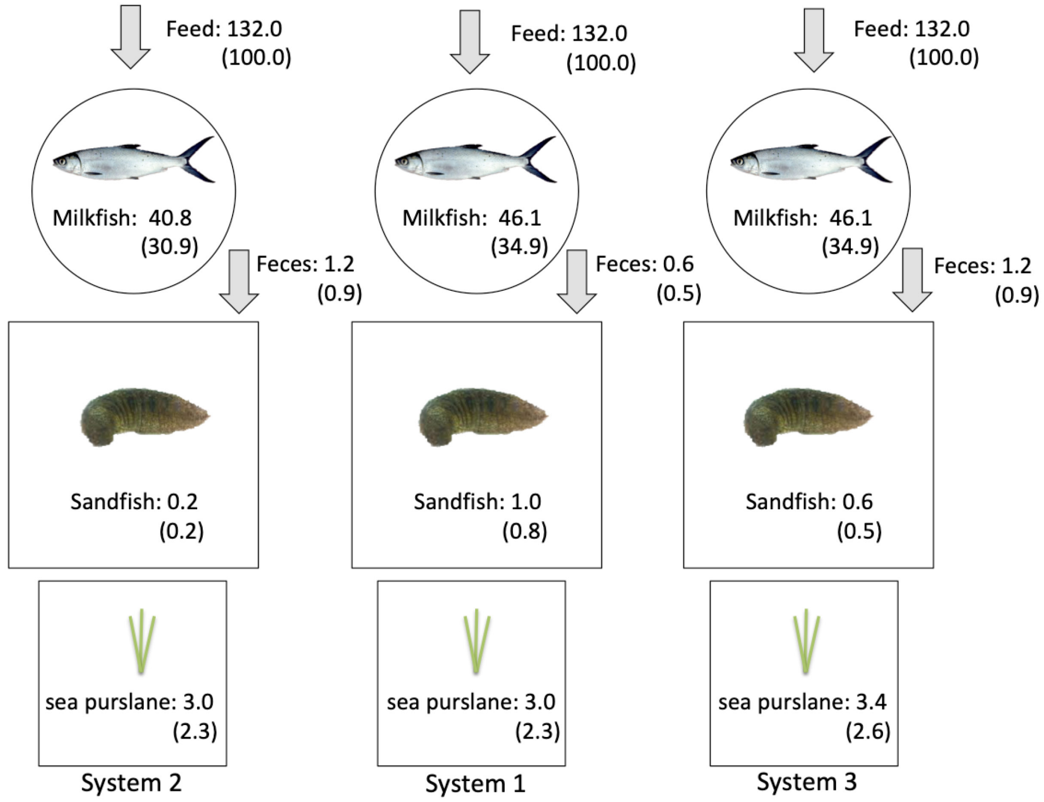

Fig. 7: Conceptual representation of the three experimental RAS, each including a milkfish tank (round), sea cucumber tank (large square) and hydroponic sea purslane tank (small square). Numbers indicate the nitrogen (N) content (g) of the different compartments, with the corresponding % N relative to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

4. Discussion

4.1. How does a RAS integrating milkfish, sandfish and sea purslane perform 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} day^{-1} and the average specific growth rate of 1.1 % day^{-1} was on the lower end, but comparable to other studies (Guanzon Jr et al. 2004; Jana et al. 2006). The average food conversion ratio 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 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 two 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 rate of the sandfish was 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 *H. 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 > 50g 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 three species of halophytes in hydroponic cultivation in the waste water of European seabass (*Dicentrarchus labrax*) (Waller et al. 2015). The authors 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. Is it feasible to run the RAS with sea cucumber and halophyte tanks instead of conventional biofilters and what is the individual filter performance?

Infrastructure restrictions did now 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 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 week-end, 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). Nitrite 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 NO_2^--N) (Almendras 1987). The highest nitrite concentrations were measured on day 16 and stayed low for the remainder of the experiment. Decreases in ammonia concentrations followed by a nitrite peak and subsequently increasing nitrate concentrations are indicative of the two-step aerobic nitrification process of biological oxidation of ammonia to nitrite and nitrite to nitrate 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 nitrate, 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 did apparently 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 phosphate uptake are unlikely to have occurred. NO_3 concentrations however decreased, indicating that the tanks acted as a net sink of nitrate, unlike the fish and sea cucumber tanks, suggesting that the hydroponic cultivation of *S. portulacastrum* did prevent higher nitrate 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 nitrogen 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. Nitrate concentrations in the halophyte tanks both increasing and decreasing 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 show a steady increase in the whole system. Phosphate 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 phosphate 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 days 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. Do the sea cucumber and halophyte tanks improve the use of feed derived N?

The RAS showed good recovery of feed derived N in the form of fish biomass, which accounted for the majority of N with approximately 34% (Zhong et al. 2011, Poli et al. 2019). The amount of nitrogen 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 recognize 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 nitrogen 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 nitrogen 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 nitrogen use by an additional 6.4% and produced 2 kg of halophytes for 1 kg of shrimp and 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 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 nitrogen transformation processes and functional groups of bacteria using qPCR and tracing nitrogen 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 three 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 nitrogen 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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References

Ahmed N, Glaser M (2016) Can "Integrated Multi-Trophic Aquaculture (IMTA)" adapt to climate change in coastal Bangladesh? Ocean Coast Manag 132:120–131.

Almendras J (1987) Acute Nitrite Toxicity and Methemoglobinemia in Juvenile Milkfish (*Chanos chanos* Forsskal). Aquaculture 61:33–40.

Badiola M, Mendiola D, Bostock J (2012) Aquacultural Engineering Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. Aquac Eng 51:26–35.

Blancheton JP, Attramadal KJK, Michaud L, d'Orbcastel ER, Vadstein O (2013) Insight into bacterial population in aquaculture systems and its implication. Aquac Eng 53:30–39.

Boxman SE, Nystrom M, Capodice JC, Ergas SJ, Main KL, Trotz MA (2017) Effect of support medium, hydraulic loading rate and plant density on water quality and growth of halophytes in marine aquaponic systems. Aquac Res 48:2463–2477.

Brailo M, Schreier HJ, McDonald R, Maršić-Lučić J, Gavrilović A, Pećarević M, Jug-Dujaković J (2019) Bacterial community analysis of marine recirculating aquaculture system bioreactors for complete nitrogen removal established from a commercial inoculum. Aquaculture 503:198–206.

Bryceson I (2002) Coastal Aquaculture Developments in Tanzania : Sustainable and Non-sustainable Experiences. West Indian Ocean J Mar Sci 1:1–10.

Buhmann A, Papenbrock J (2013) Biofiltering of aquaculture effluents by halophytic plants: Basic principles, current uses and future perspectives. Environ Exp Bot 92:122–133.

Cardoch L, Day JW, Rybczyk JM, Kemp GP (2000) An economic analysis of using wetlands for treatment of shrimp processing wastewater - A case study in Dulac, LA. Ecol Econ 33:93–101.

Carton-Kawagoshi RJ, Elle BJ, Corre V, Satoh S, Notoya M, Fujita D (2014) Low water exchange *Gracilariopsis bailiniae* Zhang & B.M. Xia culture in intensive milkfish culture effluents: Effects of seaweed density on seaweed production and effluent treatment. Aquac Int 22:573–584.

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

Chopin T (2017) Challenges of Moving Integrated Multi-Trophic Aquaculture along the R&D and Commercialization Continuum in the Western World. J Ocean Technol 12:34–47.

Chopin T, Yarish C, Sharp G (2007) Beyond the Monospecific Approach To Animal

Aquaculture — the Light of Integrated Multi-Trophic Aquaculture. Ecol Genet Implic Aquac Act Methods Technol Fish Biol Fish 6:447–458.

Custódio M, Villasante S, Cremades J, Calado R, Lillebø AI (2017) Unravelling the potential of halophytes for marine integrated multi-trophic aquaculture (IMTA) - A perspective on performance, opportunities and challenges. Aquac Environ Interact 9:445–460.

DeLong DP, Losordo TM (2012) How To Start A Biofilter. SRAC Publ No 4502 3:29–31.

Dumalan RJP, Bondoc KG V., Juinio-Meñez MA (2019) Grow-out culture trial of sandfish *Holothuria scabra* in pens near a mariculture-impacted area. Aquaculture 507:481–492.

Eklöf JS, Msuya FE, Lyimo TJ, Buriyo AS (2012) Seaweed Farming in Chwaka Bay: A Sustainable Alternative in Aquaculture? People, Nat Res Chwaka Bay:213–234.

Eshchar M, Lahav O, Mozes N, Peduel A, Ron B (2006) Intensive fish culture at high ammonium and low pH. Aquaculture 255:301–313.

FAO (2020) Global Aquaculture Production. http://www.fao.org/fishery/statistics/global-aquaculture-production/en (accessed 27 July 2020)

FAO (2017) Major boost for Zanzibar's aquaculture industry. http://www.fao.org/africa/news/detail-news/en/c/420823/ (accessed 3 August 2017)

Felaco L, Olvera-Novoa MA, Robledo D (2020) Multitrophic integration of the tropical red seaweed *Solieria filiformis* with sea cucumbers and fish. Aquaculture 527.

van der Gaag JJ, Paulissen MPCP, Slim PA (2010) Halophyte filters as saline treatment wetlands - Applications and constraints. Alterra-Report 2115:54 p.

Gelfand I, Barak Y, Even-Chen Z, Cytryn E, Van Rijn J, Krom MD, Neori A (2003) A novel zero discharge intensive seawater recirculating system for the culture of marine fish. J World Aquac Soc 34:344–358.

Gross A, Boyd CE, Wood CW (2000) Nitrogen transformations and balance in channel catfish ponds. Aquac Eng 24:1–14.

Guanzon Jr NG, De Castro-mallare TR, Lorque FM (2004) Polyculture of milkfish *Chanos chanos* (Forsskal) and the red seaweed *Gracilariopsis bailinae* (Zhang et Xia) in brackish water earthen ponds. Aquac Res 35:423–431.

Gutierrez-Wing MT, Malone RF (2006) Biological filters in aquaculture: Trends and research directions for freshwater and marine applications. Aquac Eng 34:163–171.

Hair C, Foale S, Kinch J, Frijlink S, Lindsay D, Southgate PC (2019) Socioeconomic impacts of a sea cucumber fishery in Papua New Guinea: Is there an opportunity for mariculture? Ocean Coast Manag 179.

Hargreaves JA (1998) Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166:181–212.

Herbeck LS, Sollich M, Unger D, Holmer M, Jennerjahn TC (2014) Impact of pond aquaculture effluents on seagrass performance in NE Hainan, tropical China. Mar Pollut Bull 85:190–203.

Heyman WD, Dobberteen RA, Urry LA, Heyman AM (1989) Pilot hatchery for the queen conch, *Strombus gigas*, shows potential for inexpensive and appropriate technology for larval aquaculture in the Bahamas. Aquaculture 77:277–285.

Hochard S, Ducroq M, Lemonnier H, Royer F, Hubert M, Michaut H, Vergé R, Letourneur Y, Lorrain A, Mathieu-Resuge M, Schaal G, Kraffe E, Legrand F, Burgy L, Kuhn J, Kuhn S (2016) Projet Hobical. Holothuries: Lien entre performances de production et de bioremédiation dans le contexte de la crevetticulture en Nouvelle-Calédonie.

Holmer M, Duarte CM, Heilskov A, Olesen B, Terrados J (2003) Biogeochemical conditions in sediments enriched by organic matter from net-pen fish farms in the Bolinao area, Philippines. Mar Pollut Bull 46:1470–1479.

Holmer M, Fortes MD (2002) Impacts of milkfish (*Chanos chanos*) aquaculture on carbon and nutrient fluxes in the Bolinao area, Philippines. Mar Pollut Bull 44:685–696.

Jana SN, Garg SK, Patra BC (2006) Effect of inland water salinity on growth performance and nutritional physiology in growing milkfish, *Chanos chanos* (Forsskal): field and laboratory studies. J Appl Ichthyol 22:25–34.

Keuter S, Beth S, Quantz G, Spieck E (2017) Long-term monitoring of nitrification and nitrifying communities during biofilter activation of two marine recirculation aquaculture systems (RAS). Int J Aquac Fish Sci.

Kim E, Yoo S, Ro H-Y, Han H-J, Baek Y-W, Eom I-C, Kim H-M, Kim P, Choi K (2013) Aquatic Toxicity Assessment of Phosphate Compounds. Environ Health Toxicol 28:7.

Klawonn I, Bonaglia S, Whitehouse MJ, Littmann S, Tienken D, Kuypers MMM, Brüchert V, Ploug H (2019) Untangling hidden nutrient dynamics: rapid ammonium cycling and single-cell ammonium assimilation in marine plankton communities. ISME J 13:1960–1974.

Kleitou P, Kletou D, David J (2018) Is Europe ready for integrated multi-trophic aquaculture? A survey on the perspectives of European farmers and scientists with IMTA experience. Aquaculture 490:136–148.

Lane DJW, Limbong D (2015) Catastrophic depletion of reef-associated sea cucumbers: Resource management/reef resilience issues for an Indonesian marine park and the wider Indo-Pacific. Aquat Conserv Mar Freshw Ecosyst 25:505–517.

Largo DB, Diola AG, Marababol MS (2016) Development of an integrated multi-trophic aquaculture (IMTA) system for tropical marine species in southern cebu, Central Philippines. Aquac Reports 3:67–76.

Lee S, Ferse S, Ford A, Wild C, Mangubhai S (2017) Effect of sea cucumber density on the health of reef-flat sediments. In: *Fiji's Sea Cucumber Fishery: Advances in Science for Improved Management*, Report No. Mangubhai S, Lalavanua W, Purcell SW (eds) Wildlife Conservation Society, Suva, Fiji, p 54–61

Lokhande VH, Nikam TD, Suprasanna P (2009) *Sesuvium portulacastrum* (L.) L. a promising halophyte: Cultivation, utilization and distribution in India. Genet Resour Crop Evol 56:741–747.

Lonard RI, Judd FW (1997) The biological flora of coastal dunes and wetlands. *Sesuvium portulacastrum* (L) L. J Coast Res 13:96–104.

MacTavish T, Stenton-Dozey J, Vopel K, Savage C (2012) Deposit-Feeding Sea Cucumbers Enhance Mineralization and Nutrient Cycling in Organically-Enriched Coastal Sediments. PLoS One 7:1–11.

Magwa ML, Gundidza M, Gweru N, Humphrey G (2006) Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. J Ethnopharmacol 103:85–89.

Marte CL (1988) Broodstock management and seed production of milkfish. In: *Perspectives in aquaculture development in Southeast Asia and Japan: Contributions of the SEAFDEC aquaculture department. Proceedings of the Seminar on Aquaculture Development in Southeast Asia*. Juario J V, Benitez L V (eds) SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, p 169–194

Martins CIM, Eding EH, Verdegem MCJ, Heinsbroek LTN, Schneider O, Blancheton JP, Roque E, Verreth JAJ (2010) New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. Aquac Eng 43:83–93.

Maucieri C, Nicoletto C, Zanin G, Birolo M, Trocino A, Sambo P, Borin M, Xiccato G (2019) Effect of stocking density of fish on water quality and growth performance of European Carp and leafy vegetables in a low-tech aquaponic system. PLoS One 14:1–15.

Mmochi AJ, Dubi AM, Mamboya FA, Mwandya AW (2002) Effects of Fish Culture on Water Quality of an Integrated Mariculture Pond System. West Indian Oceanogr J Mar Sci 1:53–63.

Moriarty DJ. W, Pollard PC, Hunt WG, Moriarty CM, Wassenberg TJ (1985) Productivity of bacteria and microalgae and the effect of grazing by holothurians in sediments on a coral reef flat. Mar Biol 85:293–300.

Msuya FE (2013) Social and economic dimensions of carrageenan seaweed farming in the United Republic of Tanzania. In: *Fisheries and Aquaculture Technical Paper*.

Valderrama D, Cai J, Hishamunda N, Ridler N (eds) p 115–146

Namukose M, Msuya FE, Ferse SC, Slater M, Kunzmann A (2016) Growth performance of the sea cucumber *Holothuria scabra* and the seaweed *Eucheuma denticulatum*: integrated mariculture and effects on sediment organic characteristics. Aquac Environ Interact 8:179–189.

Patil A V., Lokhande VH, Suprasanna P, Bapat VA, Jadhav JP (2012) *Sesuvium portulacastrum* (L.) L.: A potential halophyte for the degradation of toxic textile dye, Green HE4B. Planta 235:1051–1063.

Pinheiro I, Arantes R, do Espírito Santo CM, do Nascimento Vieira F, Lapa KR, Gonzaga LV, Fett R, Barcelos-Oliveira JL, Seiffert WQ (2017) Production of the halophyte *Sarcocornia ambigua* and Pacific white shrimp in an aquaponic system with biofloc technology. Ecol Eng 100:261–267.

Poli MA, Legarda EC, de Lorenzo MA, Pinheiro I, Martins MA, Seiffert WQ, do Nascimento Vieira F (2019) Integrated multitrophic aquaculture applied to shrimp rearing in a biofloc system. Aquaculture 511.

Priborsky J, Velisek J (2018) A Review of Three Commonly Used Fish Anesthetics. Rev Fish Sci Aquac 26:417–442.

Primavera JH (2006) Overcoming the impacts of aquaculture on the coastal zone. Ocean Coast Manag 49:531–545.

Purcell SW, Simutoga M (2008) Spatio-Temporal and Size-Dependent Variation in the Success of Releasing Cultured Sea Cucumbers in the Wild. Rev Fish Sci 16:204–214.

Purcell SW, Williamson DH, Ngaluafe P (2018) Chinese market prices of beche-de-mer: Implications for fisheries and aquaculture. Mar Policy 91:58–65.

Qiu T, Zhang L, Zhang T, Bai Y, Yang H (2014) Effect of culture methods on individual variation in the growth of sea cucumber. Chinese J Oceanol Limnol 32:737–742.

Quintã R, Santos R, Thomas DN, Le Vay L (2015) Growth and nitrogen uptake by *Salicornia europaea* and *Aster tripolium* in nutrient conditions typical of aquaculture wastewater. Chemosphere 120:414–421.

R-Core-Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Ranjan R, Megarajan S, Xavier B, Raju SS, Ghosh S, Gopalakrishnan A (2019) Design and performance of recirculating aquaculture system for marine finfish broodstock development. Aquac Eng 85:90–97.

van Rijn J, Tal Y, Schreier HJ (2006) Denitrification in recirculating systems: Theory and applications. Aquac Eng 34:364–376.

Robinson G, Caldwell GS, Jones CLW, Slater M, Stead SM (2015) Redox stratification drives enhanced growth in a deposit-feeding invertebrate: Implications for aquaculture bioremediation. Aquac Environ Interact 8:1–13.

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.

Robinson G, Caldwell GS, Wade MJ, Free A, Jones CLW, Stead SM (2016) Profiling bacterial communities associated with sediment-based aquaculture bioremediation systems under contrasting redox regimes. Sci Rep.

Rönnback P, Bryceson I, Kautsky N (2002) Coastal aquaculture development in eastern Africa and the Western Indian Ocean: prospects and problems for food security and local economies. Ambio 31:537–542.

Schneider O, Sereti V, Eding EH, Verreth JAJ (2005) Analysis of nutrient flows in integrated intensive aquaculture systems. Aquac Eng 32:379–401.

Sewell MA, Bergquist PR (1990) Variability in the reproductive cycle of *Stichopus mollis* (Echinodermata: Holothuroidea). Invertebr Reprod Dev 17:1–7.

Shpigel M, Ben-Ezra D, Shauli L, Sagi M, Ventura Y, Samocha T, Lee JJ (2013) Constructed wetland with *Salicornia* as a biofilter for mariculture effluents. Aquaculture 413:52–63.

Slama I, M’Rabet R, Ksouri R, Talbi O, Debez A, Abdelly C (2017) Effects of salt treatment on growth, lipid membrane peroxidation, polyphenol content, and antioxidant activities in leaves of *Sesuvium portulacastrum* L. Arid L Res Manag 0:1–14.

Slama I, Messedi D, Ghnaya T, Savoure A, Abdelly C (2006) Effects of water deficit on growth and proline metabolism in *Sesuvium portulacastrum*. Environ Exp Bot 56:231–238.

Stenton-Dozey JME, Heath P, Ren JS, Zamora LN (2020) New Zealand aquaculture industry: research, opportunities and constraints for integrative multitrophic farming. New Zeal J Mar Freshw Res:1–21.

Strickland J, Parsons T (1972) A practical Handbook of Seawater Analysis. Fisheries Research Board Canada, Ottawa.

Suantika G, Situmorang ML, Saputra FI, Putri SLE, Putri SP, Aditiawati P, Fukusaki E (2020) Metabolite profiling of whiteleg shrimp Litopenaeus vannamei from super-intensive culture in closed aquaculture systems: a recirculating aquaculture system and a hybrid zero water discharge–recirculating aquaculture system. Metabolomics 16:1–11.

Sumagaysay-Chavoso NS (2003) Nitrogen and phosphorus digestibility and excretion of different-sized groups of milkfish (*Chanos chanos* Forsskal) fed formulated and natural food-based diets. Aquac Res 34:407–418.

Sumagaysay-Chavoso NS, San Diego-McGlone ML (2003) Water quality and holding capacity of intensive and semi-intensive milkfish (*Chanos chanos*) ponds. Aquaculture 219:413–429.

Sumagaysay NS (1998) Milkfish (Chanos chanos) production and water quality in brackishwater ponds at different feeding levels and frequencies. J Appl Ichthyol 14:81–85.

Troell M, Hecht T, Beveridge M, Stead S, Bryceson I, Kautsky N, Mmochi A, Ollevier F. (2011) Mariculture in the WIO region - challenges and prospects. In: *WIOMSA Book Series*. p 59pp

Ventura Y, Wuddineh WA, Myrzabayeva M, Alikulov Z, Khozin-Goldberg I, Shpigel M, Samocha TM, Sagi M (2011) Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. Sci Hortic (Amsterdam) 128:189–196.

Verhoeven JTA, Meuleman AFM (1999) Wetlands for wastewater treatment : Opportunities and limitations. Ecol Eng 12:5–12.

Vymazal J (2010) Constructed wetlands for wastewater treatment. Water 2:530–549.

Waller U, Buhmann A, Ernst A, Hanke V, Kulakowski A, Wecker B, Orellana J, Papenbrock J (2015) Integrated multi-trophic aquaculture in a zero-exchange recirculation aquaculture system for marine fish and hydroponic halophyte production. Aquac Int 23:1473–1489.

Wang Y, Gu X, Zeng Q, Mao Z, Gu X, Li X (2015) Fate of 15N-enriched cyanobacteria feed for planktivorous fish in an enclosure experiment: a stable isotope tracer study. Fish Sci 81:821–830.

Watanabe S, Kodama M, Orozco ZGA, Sumbing JG, Novilla SRM, Lebata-Ramos MJH (2015) Estimation of energy budget of sea cucumber, *Holothuria scabra*, in integrated multi-trophic aquaculture. In: *Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia: Challenges in Responsible Production of Aquatic Species: Proceedings of the International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asi*. Romana-Eguia MRR, Parado-Estepa FD, Salayo ND, Lebata-Ramos MJH (eds) Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines, p 307–308

Watanabe S, Kodama M, Sumbing JG, Lebata-Ramos MJH (2017) Development of Integrated Multi-Trophic Aquaculture Using Sea Cucumber. In: *Genetics in Aquaculture: Proceedings of the 42nd U.S.-Japan Aqaculture Panel Symposium*. Ruenthal K, Olin P, Rust M, Trentacoste E (eds) U.S. Dept. Commerce, NOAA Tech. Memo, La Jolla, CA, p 87 p.

Watanabe S, Zarate JM, Lebata-Ramos MJH, Nievales MFJ, Kodama M (2012) Utilization of Organic Waste from Black Tiger Shrimp, *Penaeus monodon*, by Sandfish, *Holothuria scabra*. In: *Sustainable stock management and development* *of aquaculture technology suitable for Southeast Asia (JIRCAS Working Report No 75)*. Tanaka K, Morioka S, Watanabe S (eds) Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan, p 81–86

Wolkenhauer S-M, Uthicke S, Burridge C, Skewes T, Pitcher R (2010) The ecological role of *Holothuria scabra* (Echinodermata: Holothuroidea) within subtropical seagrass beds. J Mar Biol Assoc United Kingdom 90:215.

Wongkiew S, Hu Z, Chandran K, Lee JW, Khanal SK (2017) Nitrogen transformations in aquaponic systems: A review. Aquac Eng 76:9–19.

Yogev U, Gross A (2019) Reducing environmental impact of recirculating aquaculture systems by introducing a novel microaerophilic assimilation reactor: Modeling and proof of concept. J Clean Prod 226:1042–1050.

Yogev U, Sowers KR, Mozes N, Gross A (2017) Nitrogen and carbon balance in a novel near-zero water exchange saline recirculating aquaculture system. Aquaculture 467:118–126.

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

Zhang J, Zhang S, Kitazawa D, Zhou J, Park S, Gao S, Shen Y (2019) Bio-mitigation based on integrated multi-trophic aquaculture in temperate coastal waters: Practice, assessment, and challenges. Lat Am J Aquat Res 47:212–223.

Zhong F, Liang W, Yu T, Cheng SP, He F, Wu ZB (2011) Removal efficiency and balance of nitrogen in a recirculating aquaculture system integrated with constructed wetlands. J Environ Sci Heal - Part A 46:789–794.

Supplementary material

Table A1: Concentrations of dissolved inorganic nutrients (mean mg l^{-1} ± SD) in the fish, sea cucumber and halophyte tanks before and after time periods of interrupted water circulation.

	Before	**After**
Fish Tanks		
NO_2^--N	0.76 (± 0.78)	3.34 (± 1.19)
NO_3^--N	12.16 (± 5.32)	13.87 (± 6.38)
TAN	0.34 (± 0.15)	1.06 (± 0.64)
TN	13.26 (± 4.82)	18.28 (± 6.20)
PO_4^--P	1.12 (± 0.61)	1.64 (± 0.70)
Sea cucumber Tanks		
NO_2^--N	0.57 (± 0.67)	0.30 (± 0.72)
NO_3^--N	12.83 (± 5.78)	14.19 (± 5.48)
TAN	0.08 (± 0.08)	0.01 (± 0.02)
TN	13.47 (± 5.22)	14.50 (± 5.01)
PO_4^--P	1.12 (± 1.12)	1.26 (± 0.62)
Halophyte Tanks		
NO_2^--N	0.54 (± 0.69)	0.59 (± 0.72)
NO_3^--N	13.12 (± 6.02)	11.19 (± 5.48)
TAN	0.06 (± 0.05)	0.04 (± 0.02)
TN	13.72 (± 5.41)	11.81 (± 5.16)
PO_4^--P	1.27 (± 0.67)	1.12 (± 0.56)

Chapter 3

Bioremediation by the sea cucumber *Holothuria scabra*

This chapter has been submitted as:

Senff, P., Elba, B., Kunzmann, A., Gillis, L. G & Robinson, G. (XXXX). Carbon supplementation promotes assimilation of aquaculture waste by the sea cucumber *Holothuria scabra*: evidence from stable isotope analysis. (Submitted to *Aquaculture*)

Picture credit: Brendan Elba

Carbon supplementation promotes assimilation of aquaculture waste by the sea cucumber *Holothuria scabra*: evidence from stable isotope analysis

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Abstract

Cultivating high-value deposit feeders such as sea cucumbers on solid waste from intensive aquaculture is an innovative approach to turn potential nutrient pollution into a resource. Carbon supplementation to increase the carbon to nitrogen ratio (C:N) of the nitrogen-rich aquaculture waste has been demonstrated to support higher deposit feeder biomass, but commercially viable carbon sources remain to be tested. This experiment investigated the performance of laboratory grade cellulose and bagasse, an agricultural waste product, to improve growth and nutrient utilization from milkfish aquaculture waste in juveniles of the sea cucumber *Holothuria scabra*. Cellulose led to high initial growth, however supplementation with bagasse supported a significantly higher final biomass density (205.71 ± 11.08 g m^{-2}, mean ± SE) than waste without carbon supplementation ($p = 0.027$). For the first time, a species-specific trophic enrichment factor ($\Delta^{13}C_{org}$) was applied in stable isotope analysis of feed sources by *H. scabra* ($\Delta^{13}C_{org}$ of 2.56 ± 0.96 ‰ (mean ± SD) for the internal organs and 4.31 ± 0.96 ‰ for the body wall). Results indicated that sea cucumbers were able to assimilate carbon directly from the cellulose. Bagasse supported higher uptake of carbon from aquaculture waste, thus identifying it as a viable resource to improve bioremediation by deposit feeding sea cucumbers in integrated aquaculture. While illustrating the importance of nutrient balances for bioremediation, questions remain about the roles played by deposit feeders vs. microbial communities.

Key words: bioremediation, integrated aquaculture, C/N ratio, sandfish, MixSIAR

1. Introduction

One of the biggest challenges of intensive aquaculture is the production of organic waste. When released to natural water bodies, it can negatively influence water and sediment quality and impact trophic balances (Holmer et al. 2003, 2007). Waste produced in closed systems needs to be filtered and disposed of, posing an economic challenge. Identification of sustainable ways to mitigate effluents from traditional aquaculture by integrating the co-culture of additional bioremediative species, which can also provide a second production output, is an important avenue of research. Widely studied extractive organisms include algae (Neori et al. 2004, Samocha et al. 2015) that take up dissolved inorganic nutrients and deposit feeding polychaetes, crustaceans and mollusks that feed on particulate matter (MacDonald et al. 2011, Bergström et al. 2017, Guo et al. 2017).

Sea cucumbers are a good candidate bioremediator, as they feed on organic matter in sediment. Culture of the sea cucumber *Holothuria scabra* on organic waste from aquaculture can provide additional benefits to the system they are grown in, as well as to the surrounding environment by balancing disrupted nutrient budgets through bioturbation and bioremediation (Lee et al. 2017). Experiments have shown that fish feces can be an effective source of feed in the culture of *Holothuria scabra* (Watanabe et al. 2012). However, the feces are likely not the main feed for *H. scabra* directly, but provide a substrate for the growth of microorganisms (Plotieau et al. 2014, Robinson et al. 2016). The key for efficient growth seems to be the supplementation with carbon to improve the utilization of nitrogen provided by the aquaculture waste (Robinson et al. 2019). The concept is similar to bioflocs, used in recirculating aquaculture, where microbial growth in the water column of fish tanks or ponds is stimulated through the supplementation with carbohydrates such as sugar or starch (Avnimelech 2012). In addition to carbon, bacteria require nitrogen to build proteins for growth, which they take from the surrounding water, effectively transforming dissolved nitrogen species in the water into bacterial biomass. Biofloc technology is commonly used in the aquaculture of tilapia and shrimp (Avnimelech 2007, Kuhn et al. 2009, Bauer et al. 2012), but it requires knowledge and close monitoring of appropriate C:N and microbial densities (Kuhn et al. 2010, Hende et al. 2014).

How bacteria use major nutrients (carbon, nitrogen and phosphorus) for growth is dictated by their elemental composition and nitrogen use is thus stoichiometrically dependent on the availability of carbon (Herbert 1967). During mineralization of organic matter, a net N uptake occurs at C:N of > 20:1 as inorganic nitrogen is taken up to build bacterial biomass with a lower C:N (Goldman et al. 1987, Blackburn &

Blackburn 1992). Increasing the C:N (from 5:1 to 20:1) of waste from abalone aquaculture fed to *H. scabra* through the supplementation with starch significantly improved sea cucumber growth, possibly by improving the nutritional value of the feed mixture, while preventing build-up of organic carbon in the sediment (Robinson et al. 2019). The authors also recommend using complex carbon sources with low degradation rates, such as bagasse, available in agricultural waste streams. Bagasse is high in cellulose and lignin and has been used as a substrate for bacterial growth to control water quality in aquaculture ponds (Freeman et al. 1992, Krishnani et al. 2006, Gangadhar & Keshavanath 2012) and is readily available in many tropical countries as a by-product of sugar production. Robinson et al. (2019) also suggest that the C:N could be increased further to improve bacterial growth under reducing conditions. Microbial activity in sediment or water is closely linked to redox potential as cell energetic pathways oxidize organic substrates. Aerobic bacteria reduce dioxygen to water during respiration, decreasing the redox potential of their environmental medium (Prévost & Brillet-Viel 2014). Decomposition of organic matter and bacterial growth in marine sediments are generally considered to be more efficient under aerobic conditions (Andersen 1996, Hedges et al. 1999).

Further research is necessary to optimize sea cucumber biomass production and bioremediation for practical integration into existing monoculture units and on sediments enriched by biodeposits from commercial marine fish farming (Chary et al. 2020). As limits to stocking densities are a challenge to their integration into intensive aquaculture operations, improving biomass density through carbon supplementations is an avenue to enable the successful integration of *H. scabra* as an extractive organism for tropical saline aquaculture (Robinson et al. 2019, Chary et al. 2020). The viability of economical and environment-friendly types of carbon for optimizing sea cucumber carrying capacity remains to be tested (Dumalan et al. 2019).

In this context, the present study investigated the growth and bioremediation performance of the sea cucumber *H. scabra* on sediment impacted by aquaculture waste. Specifically, it aimed to determine if the integration of *H. scabra* prevents the build-up of nitrogen in the sediment and the water column and allows for good sea cucumber growth. Further, it tested if the supplementation with carbon provided as either lab grade (microcrystalline) cellulose or agricultural waste (bagasse) improves sea cucumber biomass production, bioremediation and assimilation of carbon and nitrogen from aquaculture sludge.

2. Materials and methods

2.1. Experimental design and setup

The experiment was conducted over 104 days (d) from 3rd December 2019 to 15th March 2020 at the Zanzibar Mariculture Project on Zanzibar, Tanzania (6°06'54"S 39°12'45"E). The tanks, 24 round plastic basins of 60 L volume, were filled with a 5 cm deep layer of sand with a final sand surface area of 0.44 m^2. The sand was sourced from the adjacent beach. Sediment particle size distribution was as follows: 0.7% > 2 mm, 4.2% >1 - 2 mm; 18.5% >500 µm – 1mm, 45.3% >250 – 500 µm, 30.6% >125 – 250 µm, 0.5% >63 – 125 µm. Before stocking, the sand was treated with 100 ppm chlorine solution to remove all organic matter and sterilize the sediment. It was then rinsed with saline well water and the basins were filled with saline well water. Both sterilization of sand and use of saline well water are common practice at the hatchery facility as coastal waters can be polluted by sewage that is released from surrounding infrastructure. Continuous aeration was provided through tubes with attached airstones placed in the center of each tank. Partial water exchanges were done twice a week by adding more well water and letting the tanks overflow for 20 minutes.

All experimental animals were hatchery-reared and no collections were made from wild populations. Before stocking, 42 juvenile *Holothuria scabra* (16.8 ± 0.6 g mean ± SE wet weight), sourced from the hatchery, were suspended in mesh bags for 24 hours to allow the intestine to empty. Animals were photographed individually to allow for identification based on natural marks. Their weight and length were recorded before distributing three animals into each tank. Any animals that developed lesions or died within the first month of the experiment were replaced with healthy individuals. Tanks in which animals died after this point were excluded from analysis.

Solid aquaculture waste, hereafter referred to as sludge, was sourced from the bottom of a 20 x 10 m concrete basin used to keep milkfish brood stock, fed daily to satiation with commercial feed. The sludge was pumped out, concentrated on 80 µm mesh gauze and stored frozen. Each tank received 7 g wet weight (1.3 g dry weight) of feces per day. The “Sludge” treatment received aquaculture waste only, while for the “Cellulose” and “Bagasse” treatments, the sludge was mixed with microcrystalline cellulose or finely ground bagasse, respectively (Table 1). Before each feeding, the frozen portions of sludge were thawed, mixed with the carbon source into slurry and distributed throughout the tank. Aeration was disrupted during administration of the organic matter and for 20 minutes after administration to allow it to settle.

Table 1: Experimental treatments receiving waste from milkfish aquaculture with or without the supplementation with different types of carbon sources and with or without the presence of sea cucumber *Holothuria scabra.*

Treatment	Feed type	C:N	n	mmol C m^{-2} d^{-1}	mmol N m^{-2} d^{-1}	Sea cucumbers (n)
A	Sludge	8.5	4	43.6	4.4	3
B	Sludge + bagasse	30	4	160.4	4.6	3
C	Sludge + cellulose	30	4	153.4	4.4	3
AC	Sludge	8.5	4	43.6	4.4	0
BC	Sludge + bagasse	30	4	160.4	4.6	0
CC	Sludge + cellulose	30	4	153.4	4.4	0

2.2. Sample collection and processing

2.2.1 Environmental samples

Water parameters (pH, dissolved oxygen, temperature, salinity) were measured weekly with a Multi 3430 probe (WTW). Every two weeks, water samples for the determination of dissolved inorganic nitrogen (DIN) species NH_4^+, NO_2^- and NO_3^- were taken through 0.45 µm filters and stored frozen in 20 ml polyethylene bottles until analysis. Spectrophotometric analysis of dissolved inorganic nutrients was carried out following the procedures of Strickland and Parsons (1972).

For elemental and stable isotope analysis, 3 sub-samples were taken of the sludge, cellulose, bagasse and the prepared feed mixtures, dried at 50°C and ground to a fine powder with a mortar and pestle. Sediment samples for the determination of nitrogen and organic carbon content as well as isotopic analysis were taken monthly with a cut-off syringe corer of 5 mm diameter and dried at 50°C. Sediment samples were ground in a Pulverisette 7 ball mill (Fritsch). Before stocking and on day 104, sediment cores of 5 mm diameter and 5 cm depth were also taken with a cut-off syringe to record the depth (mm) of the oxic-anoxic interface from the sediment surface. For sediment particle size distribution, the composite sample taken before stocking was sieved for 2 minutes on a AS 200 sieve tower (63 – 2000 µm) (Retsch) at an amplitude of 1.18.

2.2.2. Sea cucumber samples

Sea cucumber length and wet weight were recorded during stocking (day 0) and subsequently on days 53, 78 and 104 of the experiment, each time after suspension for 24 h in mesh bags to evacuate their guts. Length was measured to the nearest mm using a caliper and weight was determined to the nearest 0.01 g after leaving the animal out of the water for three minutes to expel water.

For the determination of isotopic baseline values, three sea cucumbers from the same cohort and tank as the experimental animals were sampled at the start. All experimental animals were sampled at the termination of the experiment. The sea cucumbers were weighed and dissected to verify that the intestine was empty. Internal organs and the body wall were dried at 50 °C and subsequently processed separately. Sea cucumber internal organs were homogenized with a mortar and pestle, while the body walls were pulverized with a ZM 100 centrifugal mill (Retsch).

2.3. Stable isotope analysis

All samples for the determination of $\delta^{13}C_{org}$ were pre-treated with 150 µl 1M HCl and dried for 48 hours at 40 °C. Analysis to determine the nitrogen and organic carbon (C_{org}) content was performed on a Euro EA3000 elemental analyzer (Eurovector S.p.a.). Stable isotope analysis was performed with a Delta Plus mass spectrometer (Thermo Fisher Scientific) at an instrument precision (SD) of 0.04 ‰ for $\delta^{15}N$ and 0.07 ‰ for $\delta^{13}C_{org}$. Results are expressed in delta notation (δ) parts per thousand (‰), which is the deviation from the standard reference material (atmospheric nitrogen for $\delta^{15}N$ and Pee Dee belemnite for $\delta^{13}C$).

The two-source stable isotope mixing model IsoError was applied to evaluate the proportional contribution of the two feed ingredients (sludge and bagasse or cellulose) to the feed mixtures applied to the sea cucumber tanks as follows:

$$(\delta^{13}C_{X} - \delta^{13}C_{M})\, f_{X,B} + (\delta^{13}C_{Y} - \delta^{13}C_{M})\, f_{Y,B} = 0$$

$$f_{X,B} + f_{Y,B} = 1$$

where $f_{X,B}$ and $f_{Y,B}$ represent the fractions of assimilated biomass (B) of the feed ingredients *X* and *Y*, respectively, in the feed mixture. *M* and *C* represent the C_{org} isotopic signatures of the ingredients and mixture (Phillips 2001).

The trophic enrichment factor (TEF, $\Delta^{13}C_{org}$) i.e. the change in $\delta^{13}C_{org}$ values from one trophic level to the next, was calculated as the isotopic difference between the sea cucumbers and their feed sources in treatment A, as isotopic values of the organic carbon in the sediment (-18.21 ± 1.47 ‰) and the aquaculture sludge (-19.57 ± 0.45 ‰) were very similar. A separate TEF was thus calculated for sediment and sludge as the difference between each and the sea cucumber tissue and averaged to obtain a mean TEF ± SD for internal organs and body wall. The dietary contribution of C_{org} from the feed ingredients to sampled sea cucumber tissue (internal organs or body wall) was determined via the Bayesian mixing model MixSIAR (Stock & Semmens 2016).

2.4. Statistical analysis

Data are reported as means ± standard error unless otherwise stated. Growth data and isotopic signatures of the three sea cucumbers were averaged per tank. Water parameters and sediment nitrogen and organic carbon content were averaged for each period of sea cucumber sampling, referred to as days 53, 78 and 104. Data on water temperature, salinity, DIN, sea cucumber growth rate and biomass density were tested for differences between treatments with a linear mixed effects model (lme) allowing for the inclusion of a random tank effect in the repeated measures design. Homogeneity of variance and normal distribution of the model residuals were tested using Levene's and Shapiro Wilk's tests, respectively and pairwise comparison was performed by estimated marginal means. Data that did not meet the test assumptions, dissolved oxygen, pH and sediment parameters, as well as sea cucumber stocking weight and final isotopic values were analyzed for each sampling period individually. Data were tested by analysis of variance (ANOVA) or Kruskal-Wallis ANOVA in case of non-parametric data, followed by Tukey Honest Significant Difference or Dunn's Rank Sum Post Hoc tests, respectively. Statistical analysis was done in *R* (R core team) and differences were considered significant at alpha < 0.05.

3. Results

3.1. Water parameters

Throughout the experiment, there was no significant difference between the treatments in water temperature (28.10 ± 0.03 °C, mean ± SE) or salinity (29.20 ± 0.10 PSU). The treatments did differ significantly in dissolved oxygen concentrations and pH (Table S1, Fig. S1).

Ammonia concentrations peaked during the first sampling period at 0.18 ± 0.01 mg L^{-1} and subsequently decreased without differences between treatments (lme; $\chi^2 = 3.84$, df = 5; p = 0.573). Nitrite concentrations reached maximum concentrations of 0.10 ± 0.01 mg L^{-1} by day 53 and by day 78 remained significantly higher in the sludge treatment compared to the bagasse (p = 0.015) and the cellulose (p = 0.044) treatments. Nitrate concentrations between treatments differed significantly between treatments throughout the experiment (lme; $\chi^2 = 132.93$, $p < 0.001$), with the highest concentrations in the sludge and sludge control treatments, reaching final values of 2.54 ± 0.34 and

2.73 ± 0.40 mg L^{-1}, while all other treatments decreased to close to the detection limit (Figure 1).

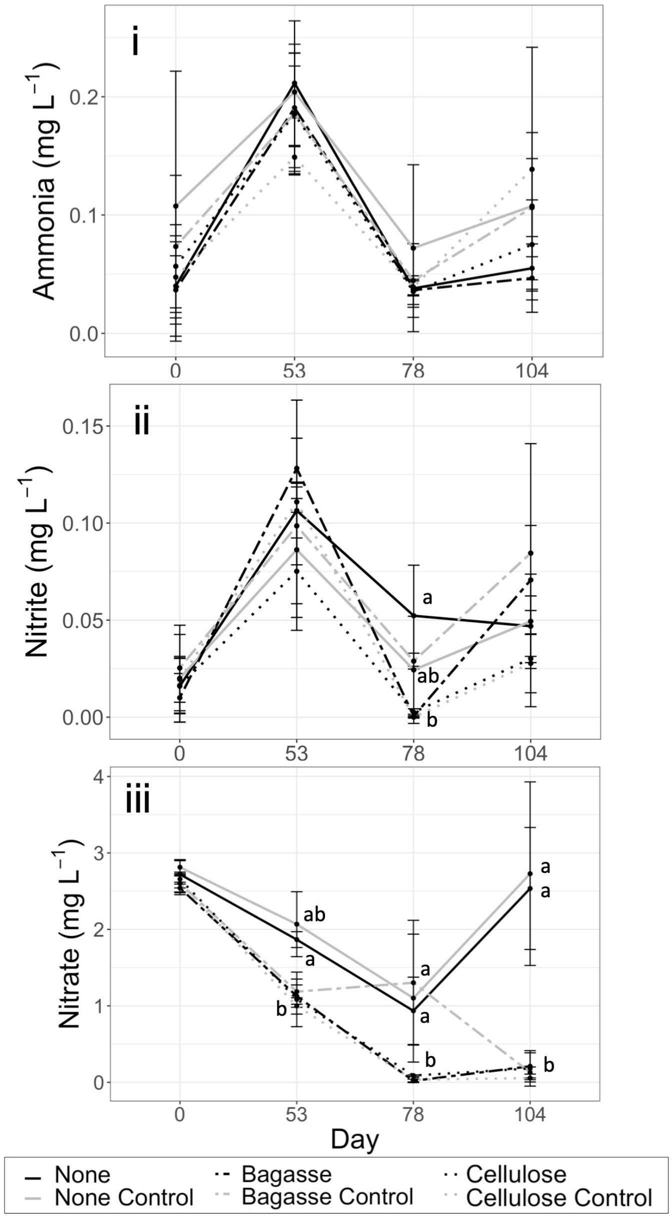

Fig.1. Concentrations (mean ± SE mg L^{-1}) of i) ammonia, ii) nitrite and iii) nitrate in the water of tanks with juvenile *Holothuria scabra* dosed with sludge from milkfish aquaculture only ("None") or with sludge amended with bagasse or microcrystalline cellulose and their respective control tanks without sea cucumbers. Different lowercase letters indicate significant differences; see text for detail on the treatments.

3.2. Sediment parameters

At the start of the experiment, sediment nitrogen content in all tanks was below the detection limit, then increased over the course of the experiment in all treatments. The bagasse control treatment had the highest increase in nitrogen content with final values of 0.05 ± 0.00 % significantly higher than the sludge (0.02 ± 0.00 %, $p = 0.013$) and sludge control (0.03 ± 0.00 %, $p = 0.040$) treatments. Content of organic carbon in the sediment also increased in all treatments and reached significantly higher values in the two treatments receiving bagasse (B = 0.76 ± 0.12 %, BC = 0.85 ± 0.09 %) than in the treatments receiving sludge only (0.25 ± 0.02 %, all $p < 0.05$). Sediment nitrogen content was lower in the tanks with sea cucumbers than in their respective control treatments, but this difference was not statistically significant (Fig. 2).

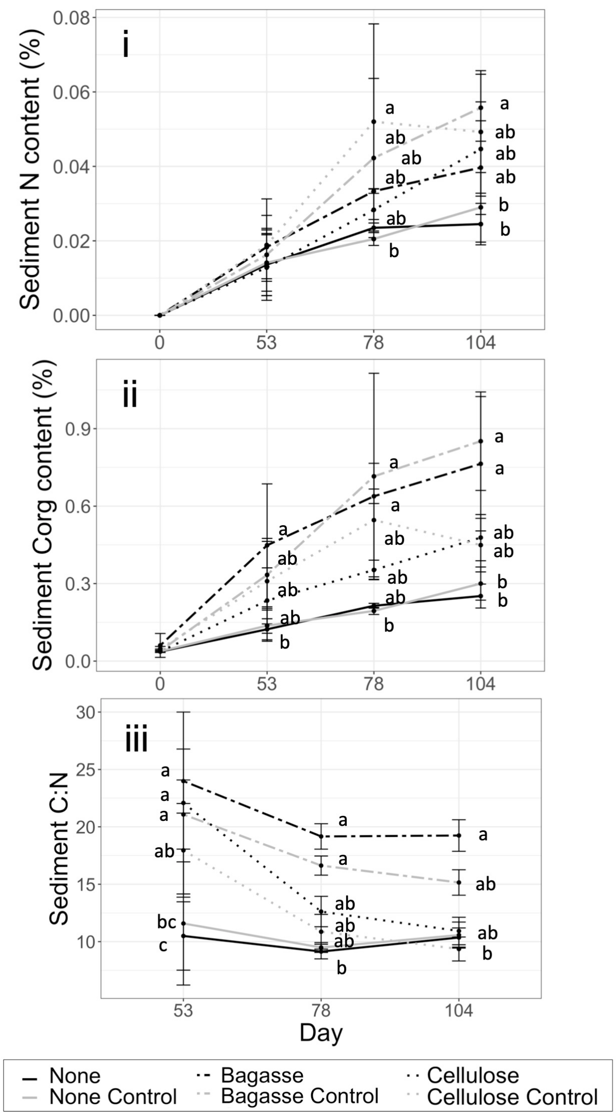

Fig. 2. The mean (± standard error) i) nitrogen content, ii) organic carbon content and iii) carbon to nitrogen ratio of sediment in tanks with juvenile *Holothuria scabra* dosed with sludge from milkfish aquaculture only ("None") or with sludge supplemented with bagasse or microcrystalline cellulose and their respective control tanks without sea cucumbers. Different lowercase letters indicate significant differences between treatments based on Kruskal-Wallis and Dunn Post Hoc Tests ($p < 0.05$).

Throughout the experiment, the bagasse treatment consistently showed the highest sediment C:N of 20.80 ± 0.87, while its control treatment had slightly lower values with 17.62 ± 0.91. The treatments receiving cellulose had overall lower values with 15.21 ± 1.96 in the tanks with sea cucumbers and 12.73 ± 1.20 in the control tanks. These tanks however showed an overall decrease from day 53 to day 104. The lowest values were in the treatments receiving sludge only with C:N of 10.00 ± 0.33 in the sea cucumber and 10.56 ± 0.61 in the sludge control tank. On days 53 and 78, both bagasse treatments had significantly higher C:N in the sediment than in the sludge treatment with sea cucumbers (all $p < 0.05$) (Fig. 2 iii).

Visual assessment of the depth of the oxic-anoxic interface revealed fully oxic sediments at the start of the experiment in all tanks. By the end of the experiment, the cellulose and cellulose control tanks had 45.00 ± 20.60 % and 55.00 ± 20.60 % anoxic sediment, respectively. The sludge control tank had 85.00 ± 15.00 % and the bagasse control tank had 32.50 ± 21.40 % anoxic sediment. Final values were significantly different between treatments (Kruskal-Wallis; χ^2=13.213, df=5, p=0.021) with the sludge treatment having fully oxic sediment (0.00 ± 0.00 %), while the sediment in the bagasse treatment was found to be 100.00 ± 0.00 % anoxic (Dunn Test, p= 0.036).

3.3. Sea cucumber survival and growth

Of the 36 sea cucumbers stocked at the start, 28 survived until completion of the experiment. Survival was 100.00 ± 0.00 % in the sludge treatment, 91.60 ± 8.38 % in the bagasse treatment and 75.00 ± 16.40 % in the cellulose treatment. All animals that died showed similar symptoms: reduced or no feeding activity/movement and development of white lesions. Within the first weeks of the experiment, all three sea cucumbers in a tank of the cellulose treatment died and were replaced with three healthy individuals from the same cohort on day 26. The animals then showed good health and feeding activity and the tank was included in subsequent analysis, adjusting the daily growth rate to account for the later starting date. Three sea cucumbers in another tank of the cellulose treatment and two in a tank of the bagasse treatment died as well. The replacement animals also showed low feeding activity and bad health. No irregularities in environmental factors could be determined and those replicate tanks were therefore excluded from analysis.

There were no significant differences between individual weight at the start of the experiment (ANOVA; $F_{(2,7)} = 2.049$, $p = 0.199$). The sea cucumbers grew in all treatments over the course of the experiment, but the growth rate in the cellulose treatment (0.30 ± 0.01 g d^{-1}) was significantly higher than that in the sludge treatment (0.05 ± 0.01 g d^{-1}) by day 53 ($p = 0.006$). However, the sea cucumbers in the cellulose treatment subsequently showed negative growth rates for the remainder of the experiment, while growth in the sludge and bagasse treatments was relatively stable. The resulting biomass densities were significantly different between treatments (lme, $\chi^2=8.17$, $p=0.017$). By the end of the experiment, the sea cucumbers in the bagasse treatment had the highest final biomass density of 205.71 ± 11.08 g m^{-2} and values were significantly higher than for the sea cucumbers in the sludge treatment at 134.76 ± 14.09 g m^{-2} ($p = 0.027$) (Figure 3). There was no significant difference in the final nitrogen content of the sea cucumber body wall between treatments (ANOVA; $F_{(2,7)}=1.48$, $p=0.290$).

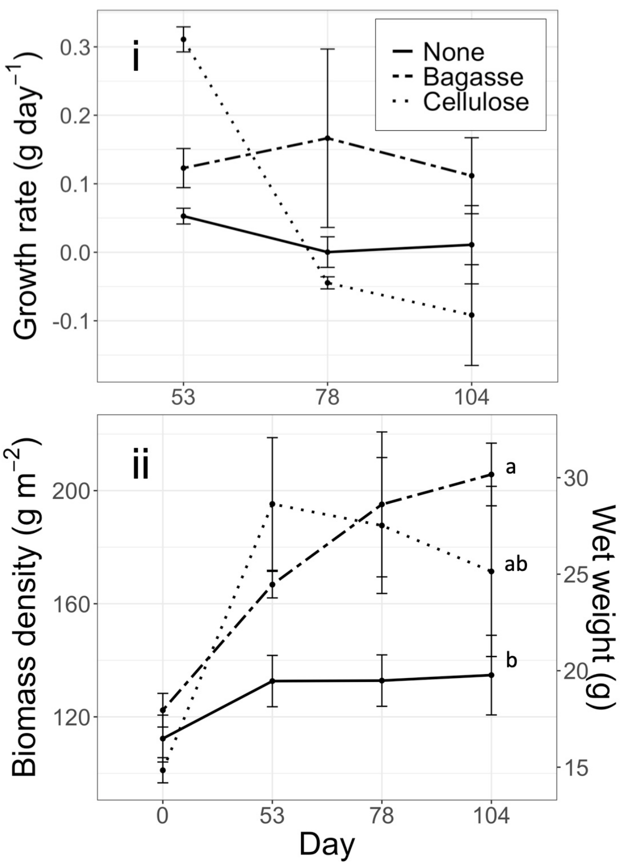

Fig. 3. The mean (± SE) i) growth rate, ii) biomass density and wet weight of juvenile *Holothuria scabra* in tanks dosed with sludge from milkfish aquaculture only ("None") or with

sludge amended with bagasse or microcrystalline cellulose. Different lowercase letters indicate significant differences between treatments.

3.4. Stable isotopic values

3.4.1. Carbon isotopic values

The organic carbon isotopic signature of the feeds was significantly different between all treatments (Tukey HSD, $p < 0.05$): sludge was -19.36 ± 0.06 ‰, the mixture including bagasse was -14.86 ± 0.08 ‰ and the mixture with cellulose was -24.23 ± 0.02 ‰. Compared to the baseline samples, the sea cucumber organ values in the cellulose treatment had become significantly different by the end of the experiment (Dunn Test, $p = 0.039$), while the values of the body wall were significantly different both in the sludge and bagasse treatments (both Tukey HSD, $p = 0.001$).

The final sea cucumber organs differed significantly in their $\delta^{13}C_{org}$ values between all treatments (ANOVA; $F_{(2,7)} = 28.71$, $p < 0.001$). The sea cucumbers in the bagasse treatment showed the most enriched values (-14.61 ± 0.25 ‰), while the sludge treatment had lower values (-16.33 ± 0.27 %) and the cellulose treatment had the most depleted values (-20.59 ± 0.12 ‰). $\delta^{13}C_{org}$ values of the body wall showed a similar trend and statistical differences (Kruskal-Wallis; $\chi^2 = 6.3$, df = 2, $p = 0.04$), with the body wall of the cellulose treatment having lower values than the bagasse treatment (Dunn Test, $p = 0.046$).

At the beginning of the experiment, the surface sediment had an average $\delta^{13}C_{org}$ of -18.21 ± 1.47 ‰ and there were no significant differences between treatments (ANOVA; $F_{(5,18)} = 0.733$, $p = 0.61$). Final $\delta^{13}C_{org}$ values differed significantly between treatments (ANOVA; $F_{(5,16)} = 27.99$, $p < 0.001$; Fig. 6). Sediments in tanks with sea cucumbers were not significantly different from their control tanks without sea cucumbers, while sediment $\delta^{13}C_{org}$ values differed significantly between the cellulose, bagasse and sludge treatments (Tukey HSD, all $p < 0.05$).

3.4.2. Nitrogen isotopic values

$\delta^{15}N$ values between the feeds differed significantly (Kruskal-Wallis; χ^2=6.4889, df = 2, p=0.039). The bagasse mixture at 10.65 ± 0.27 ‰ was significantly different compared to the cellulose mixture at 11.71 ± 0.10 ‰ (p=0.004), whilst the sludge alone had a $\delta^{15}N$ value of 11.06 ± 0.06 ‰. Over the course of the experiment, $\delta^{15}N$ values of the organs and body wall had become more enriched in all treatments. The isotopic values of the organs of the sea cucumbers in the sludge treatment had become significantly different compared to the baseline values (Dunn Test, $p = 0.011$). Also the final isotopic body wall values were significantly different in the cellulose treatment

(Dunn Test, p=0.020). Comparison between the final $\delta^{15}N$ values of the organs of the sea cucumbers indicated significant differences between treatments (ANOVA; $F_{(2,7)}=6.26$, p=0.028) with the sludge treatment having higher values than the cellulose treatment (Tukey HSD, p=0.020). There was no significant difference between the $\delta^{15}N$ values of the body wall of the sea cucumbers at the end of the experiment (ANOVA; $F_{(2,7)}=4.63$, p=0.052).

At the start of the experiment, the sediment did not contain sufficient amounts of nitrogen to determine the $\delta^{15}N$ value, however at the end of the experiment, $\delta^{15}N$ values in the surface sediment differed significantly between treatments (Kruskal-Wallis; χ^2 = 15.189, df = 5, p=0.010) with cellulose treatments showing the most depleted and the bagasse treatments the most enriched $\delta^{15}N$ values (Table 2, Fig. 4).

Table 2: Final mean (±SE) stable isotope values of sediment, feeds and tissues of juvenile sea cucumber *Holothuria scabra* fed sludge from milkfish aquaculture (A) or with sludge amended with bagasse (B) or microcrystalline cellulose (C) and their respective control tanks without sea cucumbers (AC, BC and CC).

Tissue/Sample	**Treatment**	**$\delta^{13}C_{org}$ (‰)**	**$\delta^{15}N$ (‰)**
Organs	Baseline	-14.12 ± 0.86	8.33 ± 1.05
	A	-16.33 ± 0.16	15.16 ± 0.24
	B	-14.61 ± 0.16	14.46 ± 0.14
	C	-20.59 ± 0.13	13.61 ± 0.22
Body wall	Baseline	-18.29 ± 0.82	5.83 ± 0.67
	A	-14.58 ± 0.26	10.47 ± 0.38
	B	-14.13 ± 0.21	11.34 ± 0.32
	C	-18.88 ± 0.31	11.71 ± 0.29
Sediment	A	-21.51 ± 0.28	11.26 ± 0.16
	AC	-21.90 ± 0.35	11.2 1± 0.17
	B	-17.29 ± 0.22	10.56 ± 0.14
	BC	-18.79 ± 0.39	10.81 ± 0.14
	C	-24.52 ± 0.84	9.94 ± 0.41

CC	-24.64 ± 0.84	11.29 ± 0.29

3.5. Trophic enrichment factor (TEF)

Calculations based on the $\delta^{13}C_{org}$ of sea cucumbers in the sludge treatment, the isotopic values of the aquaculture waste and the organic carbon in the sediment at the start of the experiment resulted in a TEF of 2.56 ± 0.96 ‰ (mean ± SD) for the internal organs and 4.31 ± 0.96 ‰ for the body wall. The isotopic difference for nitrogen ($\Delta\ ^{15}N$) was 4.10 ‰ for organs and -0.59 ‰ for body wall in the sludge treatment. In the bagasse treatment, the isotopic difference between the values of organs and of the feed mixture was slightly lower, but higher for the body wall tissue. The cellulose treatment had a much lower $\Delta^{15}N$ in the organ tissue, and low but positive $\Delta\ ^{15}N$ in the body wall (Table 3).

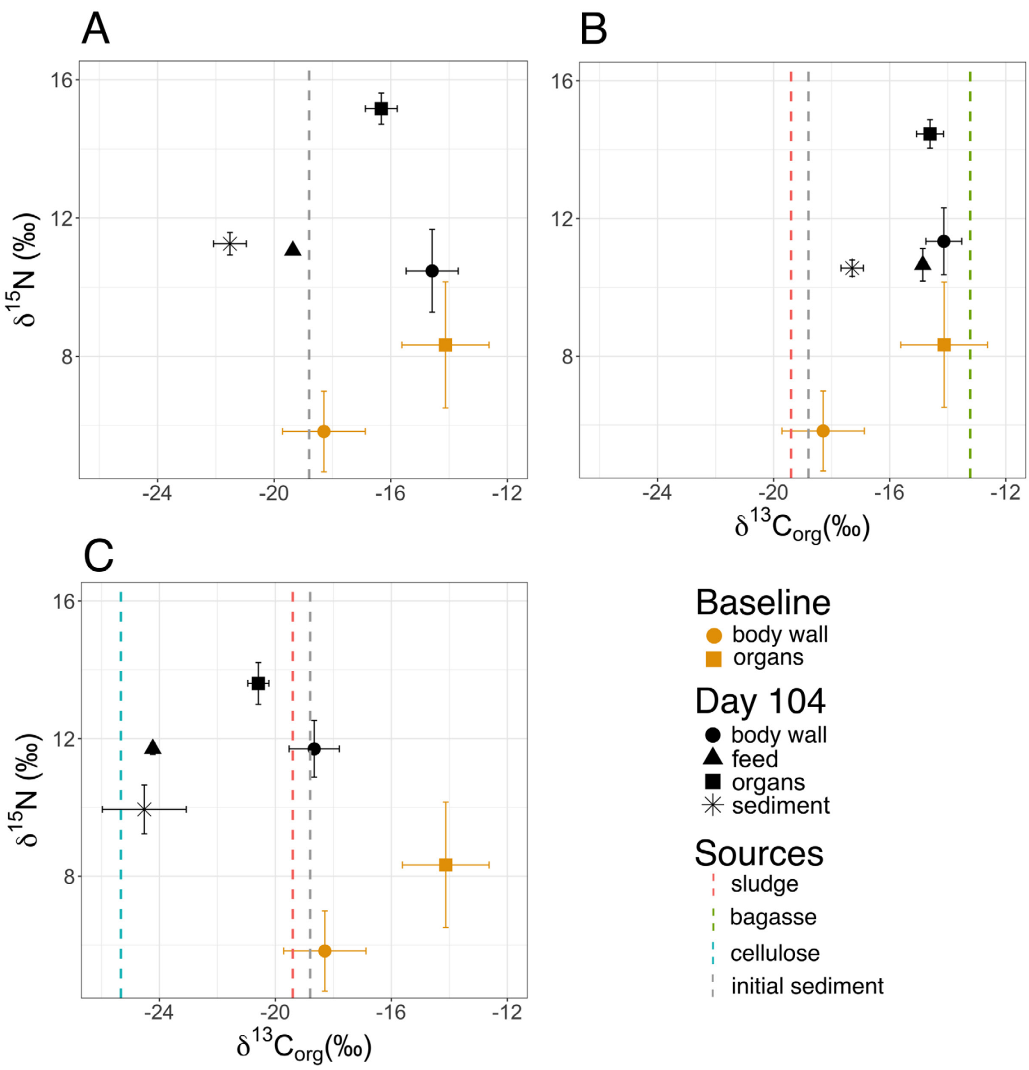

Fig. 4: Mean (± SD) stable isotope values of sediment, and tissues of juvenile sea cucumber *Holothuria scabra* fed sludge from milkfish aquaculture (A) or with sludge supplemented with bagasse (B) or microcrystalline cellulose (C). Dashed lines indicate pure endmembers of sources: sludge (red) and bagasse (green), cellulose (blue) and initial sediment (grey) for which no $\delta^{15}N$ values are available.

3.6. Assimilation of feed

The actual contributions of the two feed ingredients to carbon in the feed mixture of the bagasse treatment were very close to the planned contributions. The feed mixture in the cellulose treatment showed a slightly higher contribution of cellulose (Table 4).

The mixing model for the body wall indicated that in the sludge treatment, the aquaculture waste contributed the vast majority of carbon compared to the organic carbon initially present in the sediment. The two sources were strongly negatively correlated (Fig. S2). Samples of sea cucumbers in the bagasse treatment indicated a larger contribution from sludge than bagasse, while in the cellulose treatment, cellulose contributed more carbon to sea cucumber tissue than sludge (Fig. 5). As cellulose does not contain any nitrogen and it was not possible to obtain $\delta^{15}N$ values for the bagasse, the contribution of nitrogen from feed ingredients could not be determined by the isotopic mixing model using $\delta^{15}N$.

Table 3: Mean (± SE) difference in $\delta^{15}N$ between the feed mixtures and tissues of juvenile sea cucumber *Holothuria scabra* fed sludge from milkfish aquaculture (A) supplemented with bagasse (B) or microcrystalline cellulose (C).

	$\Delta^{15}N$	
	Organs	**Body wall**
Sludge (A)	4.10	-0.59
Bagasse (B)	3.81	0.68
Cellulose (C)	2.00	0.06

Table 4: Mean (± SE) proportional contribution of the feed ingredients to the organic carbon in feed mixtures containing sludge from milkfish aquaculture amended with bagasse (B) or microcrystalline cellulose (C) fed to juvenile sea cucumber *Holothuria scabra.* The contributions were derived by IsoError mixing model.

Treatment	**Ingredient**	**Theoretical contribution**	**Actual contribution ± SE**	**95% Confidence interval**
Bagasse (B)	Sludge	0.27	0.26 ± 0.02	0.21 – 0.31
	Bagasse	0.73	0.74 ± 0.02	0.69 – 0.79
Cellulose (C)	Sludge	0.28	0.19 ± 0.01	0.15 – 0.23
	Cellulose	0.72	0.81 ± 0.01	0.77 – 0.85

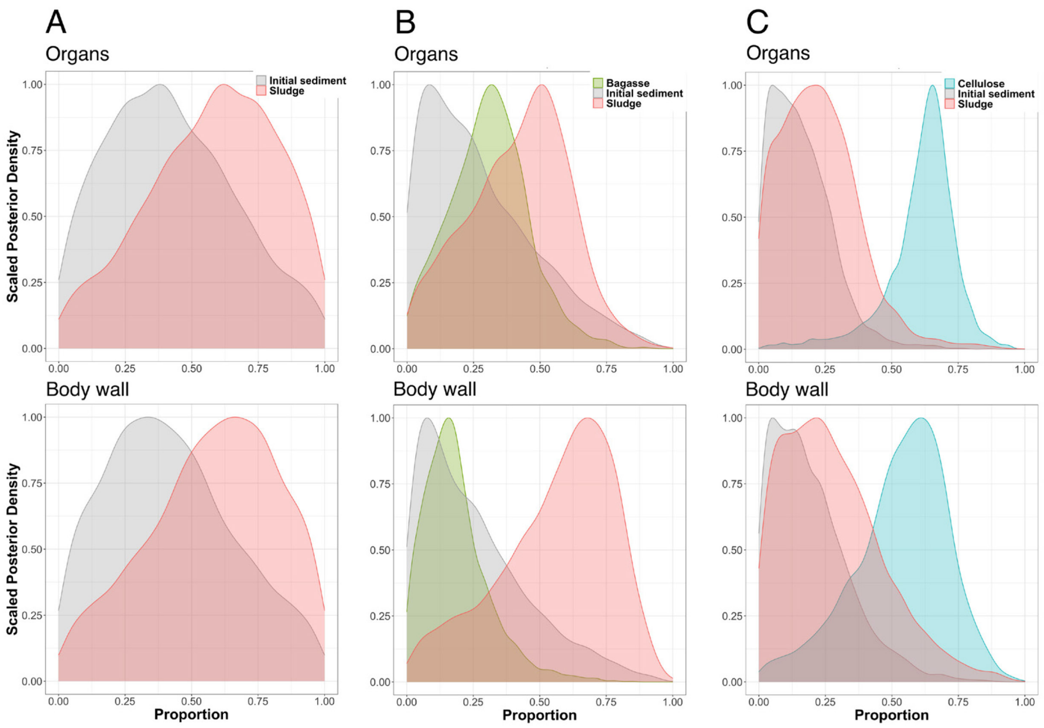

Fig. 5: Proportional contribution of the feed ingredients to the tissues (internal organs or body wall) of juvenile sea cucumber *Holothuria scabra* fed sludge from milkfish aquaculture (A) amended with bagasse (B) or microcrystalline cellulose (C). Contributions are derived by Bayesian mixing model MixSIAR.

4. Discussion

4.1. Effectiveness of carbon supplementations

The results of this study confirm that carbon supplementation to aquaculture sludge improves the growth of juvenile *Holothuria scabra*. Bagasse led to the highest biomass production over the course of the experiment and cellulose initially improved growth as well. This supports the findings of similar experiments (Robinson et al. 2015, 2019) and indicates that carbon of low digestibility sourced from agricultural waste streams is a suitable supplementation to nitrogenous waste.

Mixing-model results for the sludge and bagasse treatments showed a high assimilation of organic carbon from aquaculture sludge, identifying it as a good feed source for the sea cucumbers. Assimilation of the sludge may even be underestimated due to the strong negative correlation between the isotopic values of the aquaculture waste and the organic carbon in initial sediment, of which only a very small amount was available. The results further indicate that bagasse and cellulose functioned differently as feed supplementations. Organic carbon from cellulose was assimilated into sea cucumber tissue, while the bagasse contributed only to a small proportion of organic carbon in the sea cucumber tissue and most carbon from the sludge was assimilated. Combined with the significantly higher final sea cucumber biomass in the bagasse treatment compared to the sludge treatment, these results demonstrate that bagasse supplementation improved the assimilation of aquaculture waste. While cellulose supplementation also improved sea cucumber growth, it likely contributed directly as feed and did not improve the recovery of sludge-derived nutrients into secondary high-value biomass.

The different effects of the carbon supplementation could be due to their difference in digestibility. Feeds high in cellulose are not generally digestible for deposit feeding holothurians, as they lack cellulase, the enzyme to digest it directly, however the presence of cellobiase in their digestive tract suggests that they use the breakdown products of cellulose, made available to them by symbiotic gut bacteria (Yingst 1976, Roberts et al. 2000). Bagasse also contains high amounts of cellulose (42%), but is further composed of about 25 % lignin (Triana et al. 1990, Rocha et al. 2012, 2015). Lignin content is a limiting factor to microbial decomposition, especially under anaerobic conditions, slowing down decay (Benner et al. 1984, Harrison 1989), which would delay its availability to sea cucumbers. Bagasse could play a similar role to seagrass detritus in the sea cucumbers' natural habitat, by providing a slowly digestible substrate for microbes, which combined with detritus, forms a food source for the detritivores and transports carbon and nitrogen into higher trophic levels (Harrison 1989, Schneider et al. 2006, Siders et al. 2018).

The C:N of the milkfish sludge was 8.5:1, which is below the threshold of 10:1 to change from net nitrogen regeneration to assimilation (Goldman et al. 1987). Through supplementation with bagasse and cellulose, the C:N was increased considerably to 30:1. A functional shift in the bacterial community in aquaculture ponds has been found at a threshold of 10:1 in the water column and 12:1 in the sediment and heterotrophic pathways became superior when the ratio exceeded 15:1 (Zheng et al. 2018). In this experiment, only supplementation with bagasse was able to maintain C/N ratios above this threshold, increasing it to around 20:1. The supplementation with cellulose increased the C:N in the sediment during the first half of the experiment, but the tanks did not maintain this high value. During the same sampling period, high growth rates in the cellulose treatments were also seen which then decreased simultaneously with a decrease in sediment C:N values, providing further evidence that the growth of deposit feeding sea cucumbers is linked to the C:N of sedimentary organic matter. While the isotopic signatures and C:N values of the sediment samples could suggest that little cellulose actually reached the sediment, the sea cucumber tissue samples indicate that the cellulose was available to the sea cucumber and incorporated from the feed and did not remain in the sediment. Bagasse would provide a favorable carbon supplementation compared to cellulose and it was confirmed that a C:N of 15 is sufficiently high to benefit nitrogen transformation activity (Lovett et al. 2004, Toi et al. 2013, Zhao et al. 2019).

4.2. *H. scabra* as an extractive species

Increases in sediment carbon and nitrogen content as well as changes in the $\delta^{13}C_{org}$ and $\delta^{15}N$ of the sediment towards the values of the feed indicate that the feed mixtures did accumulate in the tanks. The presence of sea cucumbers only led to a slight reduction in the accumulation of nitrogen and organic carbon in the sediment and the animals were not able to fully prevent the build-up of solid aquaculture waste. In experiments on sediment from Babylon snail culture, *H. scabra* juveniles were able to significantly reduce sediment total nitrogen, identifying them as a better bioremediator in the absence of a constant flux of particles from aquaculture (Dobson et al. 2020).

The presence of the sea cucumbers also had no effect on concentrations of dissolved nitrogen. Nitrate concentrations were however lower in the systems receiving carbon supplementation and a nitrite peak occurred earlier, as would be expected if environmental conditions favored immobilization or denitrification by microbes (Zheng et al. 2018). Dissolved nitrogen accumulated in the sludge treatments and led to significantly higher nitrate concentrations. Significantly higher sedimentary nitrogen concentrations compared to the sludge treatment could be caused by dissolved nitrogen assimilated into microbial biomass. This resource would then be taken up by the grazing sea cucumbers. Further experiments applying pulse chase with dissolved nitrogen labeling with high ^{15}N could shed further light onto these pathways.

4.3. Sea cucumber biomass production

Small weight gain was measured in the animals fed sludge alone. One reason could be that milkfish waste did not provide enough nutrients. Sea cucumbers require a certain level of protein in their diet (>15%) (Zacarias-Soto & Olvera-Novoa 2015). While the 43.6 mmol C m^{-2} d^{-1} that were added through sludge could be considered high under natural conditions, the 61 mg N m^{-2} d^{-1} that it supplied may have been too low for good deposit feeder growth (Alongi & Hanson 1985, Tenore & Chesney 1985, Lehtoranta et al. 2009, Robinson et al. 2019). The average 156.9 mmol C m^{-2} d^{-1} supplied through carbon supplementation improved sea cucumber biomass production. Not only higher C:N, but also increased supplies of total organic carbon lead to higher abundance of deposit feeders along with meio- and macrofauna (Campanyà-Llovet et al. 2017).

$\delta^{15}N$ isotopic values of sea cucumber tissues changed compared to the signatures of the animals sampled for the determination of baseline values, showing assimilation of nitrogen from the aquaculture sludge. In the sea cucumber organs, a higher isotopic fractionation in the bagasse treatment compared to the cellulose treatment would further support that sludge derived nitrogen was assimilated better if mixed with bagasse than with cellulose. Final $\delta^{15}N$ values of the body wall in the sludge treatment were below the value of the feed. Previous findings show that internal organs have faster turnover rates than the body wall, but the length of the experiment provided ample time for full tissue turnover in sea cucumbers of this size (Sun et al. 2012, Xia et al. 2015). Presumably, not enough nitrogen was available to reach isotopic equilibrium as maintenance rather than growth metabolism is the main use of energy in sea cucumbers (Sun et al. 2012). $\delta^{15}N$ in the treatments with added carbon was higher, with the bagasse treatment supporting the highest assimilation. The overall greater availability of nutrients may have supported increased transport of nitrogen into the body wall - a site of nutrient storage.

Observed growth rates generally decrease over time, as animals' size and biomass density increase. Density limitations to growth around 220 g m^{-2} are common (Battaglene et al. 1999, Mathieu-Resuge et al. 2020). Some experiments have also been able to achieve much higher biomass densities in the range of 600 to 900 g m^{-2} (Watanabe et al. 2014, Robinson et al. 2015, 2019) and Mathieu-Resuge and colleagues (2020) hypothesize that reasons for these different outcomes are initial conditions, controlled water and sediment inputs and manipulated C/N ratios. Robinson et al. (2015) found high growth rates of sea cucumbers reared in oxic-anoxic treatments, while we only observed fully anoxic sediments in the tanks receiving bagasse. The use of unfiltered saline well water may have caused sufficiently different environmental conditions for the observed outcome. Yang et al. (2019) recommended the use of underground seawater instead of coastal water due to lower dissolved nutrient concentrations, fewer microorganisms and pathogens as well as more denitrifying bacteria. High nitrate concentrations in the well water used in this experiment suggests that this water source was not pristine. It is possible that using unfiltered well water as opposed to coastal seawater introduced different microbes than usually found in *H. scabra* habitats that may not readily break down organic material, provide a food source and leave the systems unable to cope with the influx of high amounts of organic matter.

Besides influencing the microbial community in the water, the source water can also determine the gut microbes of cultured animals (Moss et al. 2000). The role of the sediment microbial community is still an important research subject in sea cucumber aquaculture (Gao et al. 2014, Yamazaki et al. 2020). Administering probiotics with aquaculture feeds is considered as an approach to support sea cucumber growth and immune response (Wang et al. 2015, 2020, Ma et al. 2019), but it is questionable if treating the entire culture system can prevent the establishment of pathogenic communities (Zheng et al. 2019). Given the environmental circumstances (under "realistic" non-sterile conditions), desirable microbial bioremediators can be easily outcompeted by undesired microorganisms (Tal et al. 2001). However, increasing C:N could benefit both potential probiotic bacteria and opportunistic pathogens at the same time (Zheng et al. 2018).

4.4. Species-specific trophic enrichment factor

This is the first study that uses a species-specific TEF in the stable isotope analysis of feed use in *H. scabra*. Several feeding experiments with sea cucumbers have adjusted their values by 1 ‰ for use in different mixing models, in reference to literature unrelated to holothurians (Gao et al. 2011, Sun et al. 2013, Hou et al. 2017, Mondal & Kunzmann 2018, Song et al. 2018). Trophic enrichment can however vary greatly depending on species, feed, tissue and sample treatment (Bodin et al. 2007, Caut et al. 2009, Watanabe et al. 2013). Higher $\Delta^{13}C$ close to the values found in this study have been experimentally determined for sea cucumbers (Sun et al. 2012, 2020, Watanabe et al. 2013), calling into question the appropriateness of using a TEF of 1 ‰ to adjust isotopic values of holothurians. As the assumptions on trophic enrichment greatly influence the outcome of experiments and conclusions based on isotopic mixing-models, this study further underlines the need to determine and apply appropriate TEF for stable isotope studies of sea cucumbers (Phillips et al. 2014).

5. Conclusions

This study verified the supplementation with bagasse as a suitable carbon source to improve the cultivation of juvenile sea cucumber *Holothuria scabra* on sediment impacted by milkfish aquaculture. Carbon supplementation also prevented the accumulation of dissolved inorganic nitrogen and the sea cucumbers slightly reduced the build-up of organic matter from aquaculture waste in the sediment. Our findings support the benefit of slowly degrading carbon sources to sea cucumbers cultivated in detritivore-bioremediation systems, as stable isotope analysis indicated improved nutrient assimilation from aquaculture waste. The results however also hint at the complexity of the interplay between microbes and detritivores. A well-established bacterial community in the sediment seems to be a key factor for successful bioremediation, and the respective role that these components play still remains to be investigated further. Stable isotope analysis is a valuable tool to investigate nutrient assimilation by deposit feeding holothurians, but species- or context-specific trophic enrichment factors, as presented in this study for two different tissues of *H. scabra*, are needed to produce reliable results.

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References

Alongi DM, Hanson RB (1985) Effect of detritus supply on trophic relationships within experimental benthic food webs. II. Microbial responses, fate and composition of decomposing detritus. J Exp Mar Bio Ecol 88:167–182.

Andersen FØ (1996) Fate of organic carbon added as diatom cells to oxic and anoxic marine sediment microcosms. Mar Ecol Prog Ser 134:225–233.

Avnimelech Y (2012) Biofloc Technology - A Practical Guide Book, 2nd ed. The World Aquaculture Society, Baton Rouge, Louisiana, United States.

Avnimelech Y (2007) Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture 264:140–147.

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

Bauer W, Prentice-Hernandez C, Borges M, Wasielesky W, Poersch LHS (2012) Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp *Litopenaeus vannamei*. Aquaculture 342–343:112–116.

Benner R, Maccubbin AE, Hodson RE (1984) Anaerobic Biodegradation of the Lignin and Polysaccharide Components of Lignocellulose and Synthetic Lignin by Sediment Microflora. Appl Environ Microbiol 47:998–1004.

Bergström P, Carlsson MS, Lindegarth M, Petersen JK, Lindegarth S, Holmer M (2017) Testing the potential for improving quality of sediments impacted by mussel farms using bioturbating polychaete worms. Aquac Res 48:161–176.

Blackburn TH, Blackburn ND (1992) Model of nitrification and denitrification in marine sediments. FEMS Microbiol Lett 100:517–522.

Bodin N, Le Loc'h F, Hily C (2007) Effect of lipid removal on carbon and nitrogen stable isotope ratios in crustacean tissues. J Exp Mar Bio Ecol 341:168–175.

Campanyà-Llovet N, Snelgrove PVR, Parrish CC (2017) Rethinking the importance of food quality in marine benthic food webs. Prog Oceanogr 156:240–251.

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Δ15N and Δ13C): The effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46:443–453.

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

Dobson GT, Dinh N, Duy Q, Southgate PC (2020) Utilisation of organic matter from Babylon snail (*Babylonia areolata*) culture sediments by cultured juvenile sandfish (*Holothuria scabra*). Aquac Reports 18:100532.

Dumalan RJP, Bondoc KG V., Juinio-Meñez MA (2019) Grow-out culture trial of sandfish *Holothuria scabra* in pens near a mariculture-impacted area. Aquaculture 507:481–492.

Freeman DW, Duerr EO, Leber KM (1992) Use of Bagasse as a Feed Input to Semi-Intensive Shrimp Growout Ponds. J World Aquac Soc 23:23–30.

Gangadhar B, Keshavanath P (2012) Growth performance of rohu, *Labeo rohita* (Hamilton) in tanks provided with different levels of sugarcane bagasse as periphyton substrate. Indian J Fish 59:77–82.

Gao F, Li F, Tan J, Yan J, Sun H (2014) Bacterial Community Composition in the Gut Content and Ambient Sediment of Sea Cucumber *Apostichopus japonicus* Revealed by 16S rRNA Gene Pyrosequencing. PLoS One 9:1–10.

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.

Goldman JC, Caron DA, Dennett MR (1987) Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C: N ratio. Limnol Oceanogr 32:1239–1252.

Guo K, Zhao W, Jiang Z, Dong S (2017) A study of organic carbon, nitrogen and phosphorus budget in jellyfish – shellfish – fish – prawn polyculture ponds. Aquac Res 48:68–76.

Harrison PG (1989) Detrital processing in seagrass systems: A review of factors affecting decay rates, remineralization and detritivory. Aquat Bot 23:263–288.

Hedges JI, Hu FS, Devol AH, Hartnett HE, Tsamakis E, Keil RG (1999) Sedimentary organic matter preservation: A test for selective degradation under oxic conditions. Am J Sci 299:529–555.

Hende S Van Den, Beelen V, Bore G, Boon N, Vervaeren H (2014) Bioresource Technology Up-scaling aquaculture wastewater treatment by microalgal bacterial flocs : From lab reactors to an outdoor raceway pond. Bioresour Technol 159:342–354.

Herbert D (1967) Stoichiometric aspects of microbial growth. In: *Continuous culture 6: Applications and New Fields*. Dean ACR, Ellwood DC, Evans CGT, Melling J (eds) Horwood, Chichester, UK, p 1–27

Holmer M, Duarte CM, Heilskov A, Olesen B, Terrados J (2003) Biogeochemical conditions in sediments enriched by organic matter from net-pen fish farms in the Bolinao area, Philippines. Mar Pollut Bull 46:1470–1479.

Holmer M, Marba N, Diaz-Almela E, Duarte CM, Tsapakis M, Danovaro R (2007) Sedimentation of organic matter from fish farms in oligotrophic Mediterranean assessed through bulk and stable isotope (∂13C and ∂15N) analyses. Aquaculture 262:268–280.

Hou Y, Sun Y, Gao Q, Dong S, Wen B, Yu H (2017) Optimal mud to sand ratios in sediment for sea cucumber aquaculture as revealed by carbon stable isotopes. Aquac Environ Interact 9:281–291.

Krishnani KK, Parimala V, Gupta BP, Azad IS, Meng X, Abraham M (2006) Bagasse-Assisted Bioremediation of Ammonia from Shrimp Farm Wastewater. Water Environ Res 78:938–950.

Kuhn DD, Boardman GD, Lawrence AL, Marsh L, Jr GJF (2009) Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture 296:51–57.

Kuhn DD, Lawrence AL, Boardman GD, Patnaik S, Marsh L, Flick GJ (2010) Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 303:28–33.

Lee S, Ferse S, Ford A, Wild C, Mangubhai S (2017) Effect of sea cucumber density on the health of reef-flat sediments. In: *Fiji's Sea Cucumber Fishery: Advances in Science for Improved Management*, Report No. Mangubhai S, Lalavanua W, Purcell SW (eds) Wildlife Conservation Society, Suva, Fiji, p 54–61

Lehtoranta J, Ekholm P, Pitkänen H (2009) Coastal eutrophication thresholds: A matter of sediment microbial processes. Ambio 38:303–308.

Lovett GM, Weathers KC, Arthur MA, Schultz JC (2004) Nitrogen cycling in a northern hardwood forest: Do species matter? Biogeochemistry 67:289–308.

Ma Y xin, Li L yao, Li M, Chen W, Bao P yun, Yu Z chao, Chang Y qing (2019) Effects of dietary probiotic yeast on growth parameters in juvenile sea cucumber, *Apostichopus japonicus*. Aquaculture 499:203–211.

MacDonald BA, Robinson SMC, Barrington KA (2011) Feeding activity of mussels (*Mytilus edulis*) held in the field at an integrated multi-trophic aquaculture (IMTA) site (*Salmo salar*) and exposed to fish food in the laboratory. Aquaculture 314:244–251.

Mathieu-Resuge M, Grand F Le, Schaal G, Kraffe E, Lorrain A, Letourneur Y, Lemonnier H, Benoît J, Hochard S (2020) Living Resources Assimilation of shrimp farm sediment by *Holothuria scabra*: a coupled fatty acid and stable isotope approach. Aquat Living Resour 33:1–12.

Mondal K, Kunzmann A (2018) Inferior Assimilation of Algae-based Diets by Sea Cucumber *Holothuria scabra* under Laboratory Condition Expressed by Stable Isotope Mixing Model. Annu Res Rev Biol 28:1–8.

Moss SM, LeaMaster BR, Sweeney JN (2000) Relative abundance and species composition of gram-negative, aerobic bacteria associated with the gut of juvenile white shrimp *Litopenaeus vannamei* reared in oligotrophic well water and eutrophic pond water. J World Aquac Soc 31:255–263.

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004) Integrated aquaculture: Rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231:361–391.

Phillips DL (2001) Mixing models in analyses of diet using multiple stable isotopes: A critique. Oecologia 127:166–170.

Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ (2014) Best practices for use of stable isotope mixing models in food-web studies. Can J Zool 92:823–835.

Plotieau T, Lepoint G, Lavitra T, Eeckhaut I (2014) Isotopic tracing of sediment components assimilated by epibiontic juveniles of *Holothuria scabra* (Holothuroidea). J Mar Biol Assoc United Kingdom.

Prévost H, Brillet-Viel A (2014) Ecology of Bacteria and Fungi in Foods: Influence of

Redox Potential. Encycl Food Microbiol Second Ed 1:595–601.

Roberts D, Gebruk A, Levin V, Manship BAD (2000) Feeding and digestive strategies in deposit-feeding holothurians. Oceanogr Mar Biol An Annu Rev 38:257.

Robinson G, Caldwell GS, Jones CLW, Slater M, Stead SM (2015) Redox stratification drives enhanced growth in a deposit-feeding invertebrate: Implications for aquaculture bioremediation. Aquac Environ Interact 8:1–13.

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.

Robinson G, Caldwell GS, Wade MJ, Free A, Jones CLW, Stead SM (2016) Profiling bacterial communities associated with sediment-based aquaculture bioremediation systems under contrasting redox regimes. Sci Rep.

Rocha GJ de M, Nascimento VM, Gonçalves AR, Silva VFN, Martín C (2015) Influence of mixed sugarcane bagasse samples evaluated by elemental and physical-chemical composition. Ind Crops Prod 64:52–58.

Rocha GJM, Martín C, da Silva VFN, Gómez EO, Gonçalves AR (2012) Mass balance of pilot-scale pretreatment of sugarcane bagasse by steam explosion followed by alkaline delignification. Bioresour Technol 111:447–452.

Samocha TM, Fricker J, Ali AM, Shpigel M, Neori A (2015) Growth and nutrient uptake of the macroalga Gracilaria tikvahiae cultured with the shrimp *Litopenaeus vannamei* in an Integrated Multi-Trophic Aquaculture (IMTA) system. Aquaculture 446:263–271.

Schneider O, Sereti V, Eding EH, Verreth JAJ (2006) Molasses as C source for heterotrophic bacteria production on solid fish waste. Aquaculture 261:1239–1248.

Siders AC, Compson ZG, Hungate BA, Dijkstra P, Koch GW, Wymore AS, Grandy AS, Marks JC (2018) Litter identity affects assimilation of carbon and nitrogen by a shredding caddisfly. Ecosphere 9.

Song X, Lin C, Zhou Y, Wang X, Gu R, Xu S, Xu Q, Chen K, Yang H (2018) Feeding preference of *Apostichopus japonicus*: Comparing carbon stable isotope analysis and carbon budget approach. Aquac Environ Interact 10:243–253.

Stock BC, Semmens BX (2016) MixSIAR GUI User Manual. Version 3.1.

Strickland J, Parsons T (1972) A practical Handbook of Seawater Analysis. Fisheries Research Board Canada, Ottawa.

Sun J, Hamel J, Gianasi B, Graham M, Mercier A (2020) Growth, health and biochemical composition of the sea cucumber *Cucumaria frondosa* after multi-year holding in effluent waters of land-based salmon culture. Aquac Environ Interact 12:139–151.

Sun Z, Gao Q, Dong S, Shin PKS, Wang F (2013) Seasonal changes in food uptake by the sea cucumber *Apostichopus japonicus* in a farm pond: Evidence from C and N stable isotopes. J Ocean Univ China 12:160–168.

Sun ZL, Gao QF, Dong SL, Shin PKS, Wang F (2012) Estimates of carbon turnover rates in the sea cucumber *Apostichopus japonicus* (Selenka) using stable isotope analysis: The role of metabolism and growth. Mar Ecol Prog Ser 457:101–112.

Tal Y, Schwartsburd B, Nussinovitch A, Van Rijn J (2001) Enumeration and factors influencing the relative abundance of a denitrifier, Pseudomonas sp. JR12, entrapped in alginate beads. Environ Pollut 112:99–106.

Tenore KR, Chesney EJ (1985) The effects of interaction of rate of food supply and population density on the bioenergetics of the opportunistic polychaete, *Capitella capitata* (type 1). Limnol Oceanogr 30:1188–1195.

Toi HT, Boeckx P, Sorgeloos P, Bossier P, Van Stappen G (2013) Co-feeding of microalgae and bacteria may result in increased N assimilation in *Artemia* as compared to mono-diets, as demonstrated by a 15N isotope uptake laboratory study. Aquaculture 422–423:109–114.

Triana O, Leonard M, Saavedra F, Fernández N, Gálvez G (1990) Atlas del Bagazo de la Caña de Azucar. GEPLACEA/ICIDCA, Mex.

Wang G, Meng Z, Chen L, Jiang J, Feng Y, Zhang B (2020) Effects of kelp residues fermented with probiotics on the culture of sea cucumber, *Apostichopus japonicus*. Aquac Res 51:1133–1142.

Wang J hui, Zhao L qun, Liu J feng, Wang H, Xiao S (2015) Effect of potential probiotic Rhodotorula benthica D30 on the growth performance, digestive enzyme activity and immunity in juvenile sea cucumber *Apostichopus japonicus*. Fish Shellfish Immunol 43:330–336.

Watanabe S, Kodama M, Sumbing JG, Lebata-Ramos MJH (2013) Diet-tissue Stable Isotopic Fractionation of Tropical Sea Cucumber, *Holothuria scabra*. Japan Agric Res Q JARQ 47:127–134.

Watanabe S, Sumbing JG, Lebata-Ramos MJH (2014) Growth pattern of the tropical sea cucumber, *Holothuria scabra*, under captivity. Japan Agric Res Q 48:457–464.

Watanabe S, Zarate JM, Lebata-Ramos MJH, Nievales MFJ, Kodama M (2012) Utilization of Organic Waste from Black Tiger Shrimp, *Penaeus monodon*, by Sandfish, *Holothuria scabra*. In: *Sustainable stock management and development of aquaculture technology suitable for Southeast Asia (JIRCAS Working Report No 75)*. Tanaka K, Morioka S, Watanabe S (eds) Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan, p 81–86

Xia B, Wang J, Gao QF, Sun Y, Zhang L, Ma J, Liu X (2015) The nutritional contributions of dietary protein sources to tissue growth and metabolism of sea cucumber *Apostichopus japonicus* (Selenka): Evidence from nitrogen stable isotope analysis. Aquaculture 435:237–244.

Yamazaki Y, Sakai Y, Mino S, Sawabe T (2020) An annual faecal 16S amplicon sequencing of individual sea cucumber (*Apostichopus japonicus*) demonstrates the feeding behaviours against eukaryotes in natural environments. Aquac Res:1–7.

Yang L, Tang L, Li H, Wang L, Zhang Y (2019) Unique Microbial Communities Inhabiting Underground Seawater in an Intertidal Area Utilized for Industrialized Aquaculture, as Compared with the Coastal Water. Geomicrobiol J 36:483–491.

Yingst JY (1976) The utilization of organic matter in shallow marine sediments by an epibenthic deposit-feeding holothurian. J Exp Mar Bio Ecol 23:55–69.

Zacarias-Soto M, Olvera-Novoa MÁ (2015) Effect of Different Diets on Body Biochemical Composition of the Four-sided Sea Cucumber, *Isostichopus badionotus*, Under Culture Conditions. J World Aquac Soc 46:45–52.

Zhao K, Tian X, Li H, Dong S, Jiang W (2019) Characterization of a novel marine origin aerobic nitrifying–denitrifying bacterium isolated from shrimp culture ponds. Aquac Res 50:1770–1781.

Zheng X, Wang Y, Zhang D (2019) Effects of microbial products and extra carbon on water quality and bacterial community in a fish polyculture system. Aquac Res 50:1220–1229.

Zheng X, Zhang D, Qin J, Wang Y (2018) The effect of C/N ratio on bacterial community and water quality in a mussel-fish integrated system. Aquac Res 49:1699–1708.

Supplementary material

Table S1: Results of statistical analysis of water parameters between treatments.

Parameter	Test statistics
Temperature	(lme; χ^2=3.12, df=5, p=0.68)
Salinity	(lme; χ^2=1.72, df=5, p=0.89)
DO	Day 53 (ANOVA; $F_{(5,16)}$=5.52, p=0.004) Day 78 (Kruskal-Wallis; χ^2=14.21, df=5, p=0.014)
pH	Day 53 (ANOVA; $F_{(5, 16)}$=5.63, p=0.004) Day 78 (ANOVA; $F_{(5, 16)}$=4.16, p= 0.013) Day 104 (Kruskal-Wallis, χ^2=11.43, p=0.043)

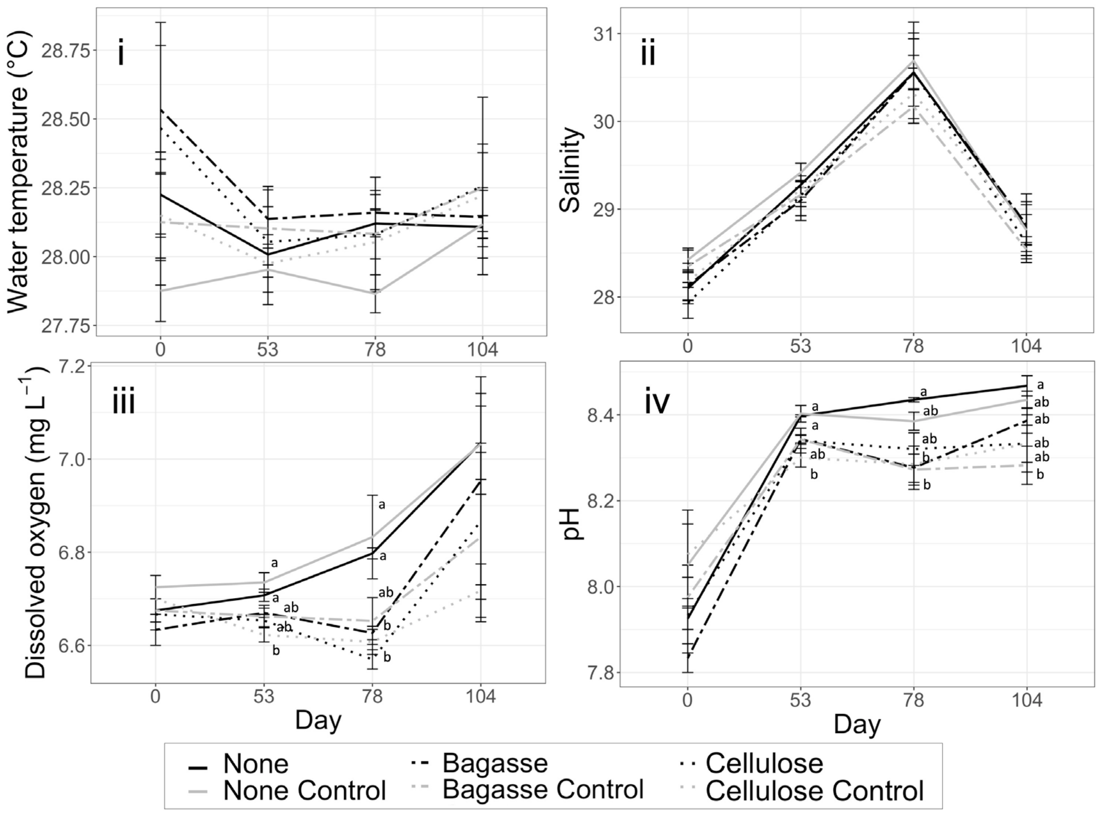

Fig. S1: The mean (± standard error) i) water temperature, ii) salinity, iii) dissolved oxygen and iv) pH in the water column of tanks containing sea cucumbers or control treatments without sea cucumbers dosed with sludge from milkfish aquaculture only ("None") or with sludge amended with bagasse or microcrystalline cellulose. Different lowercase letters indicate significant differences between treatments.

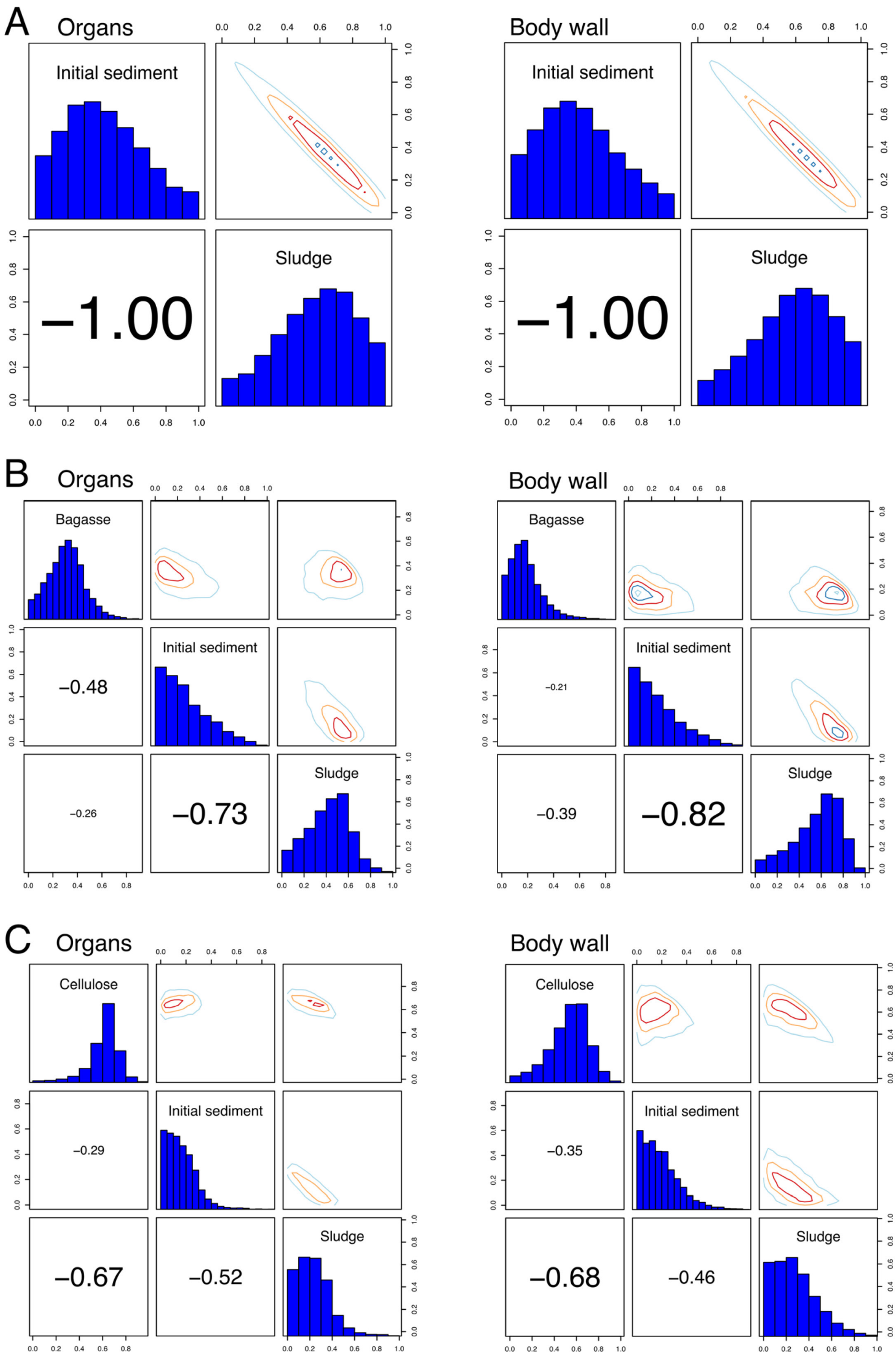

Fig. S2: Matrix plot of food sources for juvenile sea cucumbers *Holothuria scabra* fed sludge from milkfish aquaculture (A) or sludge amended with bagasse (B) or microcrystalline cellulose (C) based on samples of internal organs or body wall. The diagonal cells show graphs of the posterior probability distribution for each food source. The cells below the diagonal show the correlations between contributions for pairs of food sources. More negative values in larger print indicate a strong negative correlation. The cells above the diagonal show contours of the joint posterior probability distribution for contributions for pairs of food sources.

Chapter 4

Cultivating halophyte *Sesuvium portulacastrum* as an extractive species

This chapter has been submitted as:

Senff, P., Lavik G., Teichberg, M. & Kunzmann, A. (XXXX). Nutrient removal and nitrogen recovery from aquaculture effluents by the edible halophyte *Sesuvium portulacastrum* in hydroponics and in sand substrate. (Submitted to *Science of the Total Environment*).

Nutrient removal and nitrogen recovery from aquaculture effluents by the edible halophyte *Sesuvium portulacastrum* in hydroponics and in sand substrate

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Abstract

Aquaculture is of growing importance for food security and livelihoods, especially in the developing tropics. Nutrients supplied by feed are largely lost to the water, wasting resources and degrading water quality. Whereas fixed nitrogen can be microbially converted into dinitrogen gas via denitrification and anammox, the removal of P can only occur via assimilation into new biomass or sedimentation. Salt tolerant halophytic plants have the potential to remove and recover nutrients from saline aquaculture. Most studies have focused on temperate species, but there is a need for extractive species suitable for integration in tropical saline aquaculture. Sea purslane *Sesuvium portulacastrum* is a promising candidate that can provide biomass for human consumption combined with potential health and pharmaceutical applications, but to date, nutrient uptake has not been studied in this species. This study investigated nutrient removal from milkfish wastewater by systems planted with *S. portulacastrum* in hydroponics, with the roots in water, *versus* planted in sand, to determine the mode of cultivation which most efficiently assimilates nutrients from wastewater into new valuable biomass. The assimilation of fixed N by *S. portulacastrum* was traced by adding a defined amount of $^{15}NH_4$ or $^{15}NO_3$ in experiments with planted or unplanted (control) systems. Most of the nutrients were removed from the wastewater within 48h. The N removal was most efficient in treatments with sand (> 99% of N) and on average lower in the hydroponic incubations (55 – 99%). However, in hydroponic systems, plants assimilated on average 50.17 % of the added labeled N with no significant difference in recovery after 48 h when $^{15}NH_4$ or $^{15}NO_3$ was added. Overall, the recovery of wastewater N into new plant biomass was several-fold higher in hydroponics than in sand substrate. These results provide useful details for the successful integration of *S. portulacastrum* into aquaculture systems with the hydroponic cultivation identified as a far more efficient way to turn aquaculture effluents into a valuable resource.

Key words: Bioremediation, phytoremediation, integrated aquaculture, tropics

1. Introduction

Aquaculture currently accounts for 46 % of fish production and plays a key-role in increasing food security (FAO 2020). Over the past 60 years, fish consumption in the least developed countries has doubled, providing 50 % of animal protein consumed in many developing nations of the tropics by 2017 (FAO 2020). Here, small aquaculture operations play a vital role for the health and livelihoods of coastal communities (Senff et al. 2018, Hanh & Boonstra 2019, Irwin et al. 2020). Nitrogen (N) and phosphorous (P) are essential nutrients for cultured organisms and supplied via feed or fertilizer (Verdegem 2013). Up to 50% of feed supplied can go uneaten and be lost as particulate waste and of the feed consumed by the target species, around 25 % of N and 30 % of P are retained with the rest excreted in feces or dissolved form (Piedrahita 2003, Ballester-Moltó et al. 2017). N that is not assimilated by the cultivated organism is released mainly in the form of ammonium (NH_4^+) (Lefebvre et al. 2001, Neori et al. 2007). In aerobic environments, NH_4^+ is converted to nitrite (NO_2^-) and further oxidized to nitrate (NO_3^-) by bacteria and archaea (Blackburn & Blackburn 1992, Martens-Habbena et al. 2009). Closed recirculating aquaculture systems (RAS) apply this microbial activity in nitrifying biofilters to maintain low concentrations of NH_4^+, NH_3^- and NO_2^-, which become harmful to marine fish at concentrations around 2, 0.1 and 80 mg l^{-1} respectively (Person-Le Ruyet et al. 1995, Van Rijn 1996, Kir & Sunar 2018). To prevent the build-up of N, some RAS further apply denitrifying biofilters where NO_3^- is ultimately released as N_2 gas (Van Rijn 1996). An intermediate product of denitrification is however nitrous oxide (N_2O), which is a greenhouse gas with 296 times the global warming potential of CO_2 and a further challenge to sustainable N treatment in aquaculture (IPCC 2007, Hu et al. 2013, 2015).

If discharged, nutrients in aquaculture effluents cause water bodies to become eutrophic and increase organic matter deposits to the sediments (Luo et al. 2018). Under high nutrient loads, microbial communities consume all the available free oxygen after which anaerobic respiration prevails and eventually produces environmentally harmful compounds such as hydrogen sulfide, which is toxic to fish and most plants, and methane, a strong greenhouse gas (Jorgensen 1982, Capone & Kiene 1988). In coastal environments, excess dissolved inorganic N and P favors the growth of micro- and macroalgae, leading to eutrophication and imbalances in natural ecosystems (Diaz & Rosenberg 2008, Herbeck et al. 2014, Thomsen et al. 2020). Sandy marine sediments have been shown to very efficiently remove inorganic nitrate from eutrophic coastal systems (Rao et al. 2008, Gao et al. 2012), but due to the characteristic variable oxygen conditions in permeable sands, the ratio of N_2O vs. N_2 production is very high (Marchant et al. 2018). Unlike fixed N, P can only be removed by assimilation into new biomass or retained in the sediment due to adsorption, leading to blooms of dinitrogen fixing algae regaining the fixed nitrogen, or other harmful algal blooms (Conley et al.

2009). Consequently, both fixed N as well as the P need to be removed from effluents to prevent eutrophication of receiving waterbodies.

An important step towards sustainable aquaculture is to consider excess nutrients not as a waste product, but as a resource that should be utilized and not discarded (Bischoff et al. 2009). With clear limits to global P deposits and the high energy costs and CO_2 emissions of artificial N-fertilizer production, we need to increase our nutrient use efficiency for food production systems, including aquaculture (Erisman et al. 2008, Cordell et al. 2009, Verdegem 2013, Huang et al. 2020). The concept of integrated aquaculture is based on the idea of combining the protection of the surrounding environment and efficient use of resources with the production of additional valuable crops. This applies a simplified food web structure to a farming system of fed-species (fish and shrimp) combined with extractive organisms, such as bivalves or seaweeds that take up particulate or dissolved nutrients from the water (Neori et al. 2004). By reusing feed-derived N, integrated aquaculture produces higher yields than mono-species systems in addition to satisfying rising consumer demands for environmental standards (Neori et al. 2004, Barrington et al. 2010).

Constructed wetlands (CW) are widely used to treat wastewater and can be applied to filter discharge from aquaculture operations as well (Shpigel et al. 2013, Almuktar et al. 2018). Planted with salt-tolerant halophytes, they can further produce valuable crops for human consumption, pharmaceutical application or biomass for biogas production (Glenn et al. 1999, Buhmann & Papenbrock 2013b, Custódio et al. 2017). Edible halophytes from both temperate and tropical regions have been successfully integrated in CWs for the treatment of saline aquaculture effluents (Webb et al. 2012, Shpigel et al. 2013). Removal of nutrients from wastewater in these systems is highly variable, but can achieve > 99 % (Brown et al. 1999, Webb et al. 2012, 2013, Shpigel et al. 2013, De Lange & Paulissen 2016). N is, however, primarily removed through denitrification and ammonia volatilization and only a small fraction is converted into harvestable plant biomass (Kadlec & Knight 1996, Lin et al. 2002, Vymazal 2010). In terrestrial soils, microbes can outcompete plants for inorganic N (Hodge et al. 2000, Lipson & Näsholm 2001, Bardgett et al. 2003, Harrison et al. 2008). In freshwater RAS, aquaponic systems are already well established, where plants are integrated in hydroponic (without soil) culture (Diver 2006, Hu et al. 2015, Ogah et al. 2020). Plants in hydroponic systems can suffer nutrient deficiencies, but the method has been recommended for some halophyte species for practical reasons and higher biomass production compared to plants in sand culture (Singh et al. 2014, Buhmann et al. 2015).

The selection of edible plants that can grow in a saline environment is far smaller than for glycophytes that depend on freshwater. However, the edible halophyte *Sarcocornia ambigua* has been successfully integrated in tropical RAS with fish and /or shrimp and increased system yield and nitrogen recovery without adverse effects on the fed species

(Pinheiro et al. 2017, Poli et al. 2019). Liu et al. (2019) found good growth of the tropical halophyte *Sesuvium portulacastrum* planted in floating structures in coastal waters and suggest that it can be cultivated to reduce N and P loads around net cages. *S. portulacastrum* significantly lowered N concentrations, accounting for maximum removal of 75 % of aqueous N in a RAS with fish (Boxman et al. 2017) and acted as a net sink of N in a RAS with milkfish and sea cucumbers (Senff et al. 2020). *S. portulacastrum* or sea purslane is a perennial halophyte with a wide distribution in coastal habitats of the tropics and sub-tropics between 34° north and 42° south (Lonard & Judd 1997). It has potential as a valuable crop for saline soils and is consumed fresh or used as a traditional medicinal plant with antibacterial and antioxidant activities (Magwa et al. 2006, Lokhande et al. 2009). *S. portulacastrum* has a good fatty acid profile with almost 50 % α-Linolenic acid (Nouairi et al. 2006).

Experiments testing plant filters in RAS in addition to other water cleaning mechanisms, such as biofilters, do not give insight into the performance of the plants in unfiltered fish effluents (Webb et al. 2012, Waller et al. 2015) or yield information on nutrient uptake and assimilation by the plants, as water is continuously cycled in a closed system (Boxman et al. 2017). This information is however needed to determine the effectiveness of edible halophytes as extractive species, where a lack of research exists especially for tropical species (Custódio et al. 2017). This study thus investigates *S. portulacastrum* as an edible extractive species for saline aquaculture in the tropics by evaluating its potential for nutrient removal and optimized assimilation of nutrients into new biomass from milkfish effluents. It aims to provide new insights into its application in integrated aquaculture and optimize utilization of resources by 1) determining the removal efficiency of dissolved inorganic nitrogen (DIN) and phosphorous (DIP) from aquaculture wastewater and 2) comparing how plants cultivated hydroponically *versus* in sand perform in terms of N recovery into new valuable biomass.

2. Materials and methods

2.1. Experimental setup

Experimental plants were propagated from plants provided by the Institute of Botany at the Leibniz University Hannover, planted in silicate sand (Bauhaus, Germany) and irrigated with tap water (Buhmann et al. 2015). At a height of approximately 10 cm, the plants were transferred to boxes, each planted with six plants.

Two different types of plant boxes of different sizes were prepared for the hydroponic (Fig. 1A) and sand (Fig. 1B) treatments to allow for the same volume of water to be applied for the two treatments. The boxes for sand culture were 17.5 cm wide and 32 cm long. The bottom of the box was covered by a 3 cm layer of glass marbles, covered by a layer of pond fleece, on which 8 kg of sand was placed. The hydroponic boxes were of 15 cm width and 25 cm length with a 3 cm layer of glass marbles on the bottom. For each treatment, eight replicates were used, resulting in a total of 32 boxes. For the hydroponic treatment, the plants were held on a styrofoam raft into which six holes were cut. Foam strips held the plants in place in the holes, and the walls of the boxes were covered in black, opaque foil. Before planting, the plants were weighed. Aeration was provided by air stones placed into the water of the hydroponic treatments and into the water layer on top of the sand. Light was supplied with rows of 3W LED ("Reef", JMB Aqua Light) lamps at an average of 240 µmol m^{-2} s^{-1} and a light:dark period of 12:12 h.

Treatments:	Plants cultivated hydroponically	(HP)
	Hydroponic control without plants	(HC)
	Plants cultivated in sand	(SP)
	Sand control without plants	(SC)

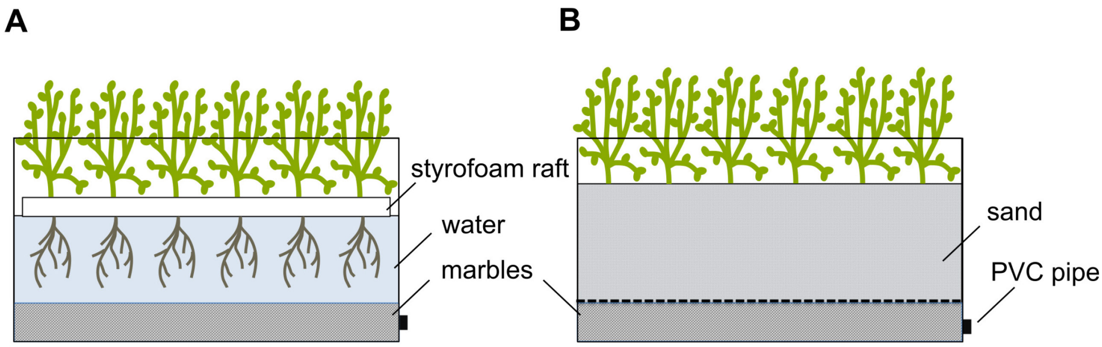

Fig. 1: Schematic drawing of the two different boxes planted with *Sesuvium portulacastrum* grown A) in hydroponics or B) in sand substrate in the experiments.

2.2. Pre-experimental period

All boxes were filled with 2500 ml of water sourced from a milkfish recirculating system and left to establish for a period of about two months. The water was exchanged weekly. Average nutrient concentrations (mg l^{-1} ± SD) in the milkfish water were 0.00 ± 0.00 NH_4^+-N, 0.02 ± 0.01 NO_2^--N, 2.78 ± 0.32 $NO3^-$-N and 1.18 ± 0.56 PO_4^+-P. Salinity was 34 ± 0 PSU.

2.3. Experiments

Two separate experiments were performed to evaluate bioremediation using different types of wastewater from cultivating a total biomass of 125 g milkfish: direct effluent (Fig. 2A) and RAS water (Fig. 2B). In both systems, the fish were fed at 5% body weight day^{-}1. Prior to the experiments, the water was filtered through a 300 µm gauze to remove larger solids. Before each experiment, the sand boxes were left to completely drain over night while the hydroponic boxes were drained on the day of the experiment.

Dissolved inorganic nutrients	Direct effluent	RAS water
NH_4^+-N (mg L^{-1})	2.32 ± 0.03	2.37 (added as $^{15}NH_4Cl$-N)
NO_2^--N (mg L^{-1})	0.14 ± 0.00	0.02 ± 0.00
NO_3^--N (mg L^{-1})	2.18 (added as $Na^{15}NO_3$-N)	14.64 ± 0.07
PO_4^+-P (mg L^{-1})	0.55 ± 0.01	2.05 ± 0.03
N:P	8.30	8.31

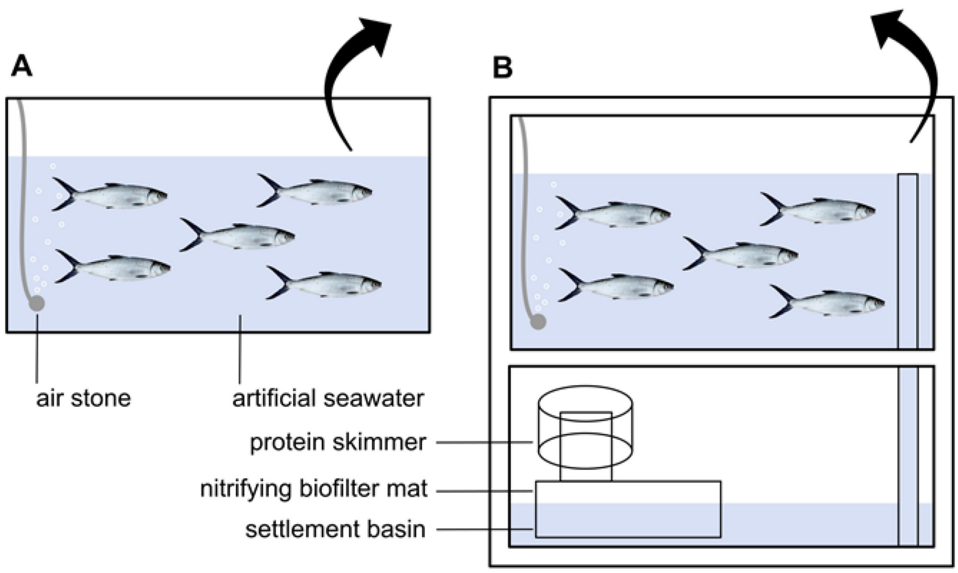

Fig. 2: Concentrations (mean mg L^{-1} ± SD) of dissolved inorganic nutrients, ^{15}N tracer and N:P in the aquaculture wastewater supplied to the boxes planted with *Sesuvium portulacastrum* during the two experiments and conceptual set-up of sources of milkfish wastewater used: A) milkfish kept in a 300 l aquarium without a filtration system for 48 hours (= direct effluent) and B) milkfish kept in an established 300 l recirculating system (= RAS water).

Pucher et al. (2014) recommended adding between 0.1 and 1% of total N present in the system (in inorganic and organic form) as ^{15}N tracer. Based on a rough budget estimate, stock solutions of 150mg ^{15}N L^{-1}were prepared by dissolving 860 mg ^{15}N-$NaNO_3$ and 544.86 mg $^{15}NH_4Cl$ in 100 ml of Milli-Q water. To each box from both experiments (direct effluent or RAS water), 2.345 ml of this stock solution were pipetted into a glass beaker of 1500 ml of water from the fish tank, well mixed and poured into the plant boxes. In total, each replicate of the direct effluent or RAS water received 3.518 mg of ^{15}N.

2.4. Sample collection and analysis

Before the start of the experiment (at 0 h) and after 3, 6, 12, 24, and 48 h, one plant from each box was sampled, weighed as a whole, divided into above ground ("shoots") and below ground ("roots") biomass and weighed again. The plant relative growth rate (RGR) was calculated as

$$RGR = (\ln WW_2 - \ln WW_1) / (t_2 - t_1),$$

where WW = wet weight of the entire plants (g), t = time (d) and subscripts 1 and 2 indicate at planting (1) and at the end of the experiment (2).

The plant parts were placed in plastic bags and stored at -20°C until lyophilization and homogenization for isotope analysis. Plant parts were weighed again to determine dry weight (DW) before homogenizing roots with a mortar and pestle and shoots in an IKA Benchtop Tube Mill (Cole-Parmer, Vernon Hills, USA). Water volume, pH, temperature and salinity of the drainage water were measured using a 1500 ml beaker and a multiparameter probe (WTW, Germany). Water samples were taken from the milkfish wastewater before and after the addition of the isotope tracer and after 48 hours, when the plant boxes were drained. All water samples were taken with 10 ml syringes through a 4.5 µm filter and immediately stored at -20°C. Concentrations of NH_4^+-N, NO_x-N, NO_2^--N and PO_4^+-P (DIP) were analyzed following the protocol of Strickland and Parsons (1972) and photometrically determined using an Infinite 200 PRO microplate reader (TECAN, Austria). NO_3^--N concentrations were subsequently determined as NO_x - NO_2^- and DIN was calculated as NH_4^+-N + NO_3^- -N + NO_2^- -N.

Stable isotope analysis was done by combustion on a Thermo Flash EA 1112 elemental analyzer coupled to an isotopic ratio mass spectrometer (Thermo Delta Plus XP, Thermo Fisher Scientific, Germany) at the Max Planck Institute for Marine Microbiology in Bremen (Halm et al. 2011). The nitrogen isotopes are measured as dinitrogen gas ($^{29}N_2$/total N_2) and expressed as atomic % ^{15}N, Based on this, the excess ^{15}N in the sample ($^{15}N_{Excess}$) was determined as

$$^{15}N_{excess} = (\text{atom \% } ^{15}N \text{ sample- } 0.37) / 100$$

where 0.37 = mean atomic % of ^{15}N of baseline plants sampled before label addition. The $^{15}N_{excess}$ was then quantified (into g or mol) using a known standard (caffeine) and used to calculate the uptake rate of the isotopic label in mg ^{15}N g dw^{-1} h^{-1}. These were calculated separately for the shoots and roots. ^{15}N label measured in shoots and roots were combined to determine the % mg ^{15}N added that was recovered cumulatively in plant biomass at the different sampling times.

2.5. Statistical analysis

All statistical analyses were performed in R (R Core team, 2019). The data were evaluated for normality and homoscedasticity by Shapiro-Wilk and Levene's test, respectively. Water parameters (pH, temperature and Salinity) at the end of the experiments were compared between treatments by one-way analysis of variance (ANOVA) or non-parametric Kruskal-Wallis in case assumptions of normality and homoscedasticity were not met. Weights of the plants at stocking and relative growth rate were compared between the hydroponic and sand treatments with two-sample t-test. Percent removal of total N and P was compared between all treatments by Kruskal-Wallis test. Concentrations of phosphate and inorganic N species in the water were compared between the treatments with plants and their corresponding controls by Wilcoxon signed-rank test.

N uptake in the shoots and roots were compared between the hydroponic and sand treatment by linear mixed effect model (lme) with treatment and time of sampling as independent factors and the boxes taken into account as random factor in the repeated measures design. Box-Cox transformation was applied to the data on mg ^{15}N g^{-1} dw h^{-1} and data on atom % ^{15}N cumulative ^{15}N uptake were log transformed. Normality and homoscedasticity of model residuals were tested by Shapiro-Wilk and Levene's test. Post-hoc comparison between treatments was performed by pairwise comparison of estimated marginal means. Statistical significance was evaluated at $p < 0.05$.

3. Results

3.1. Plant growth

The plants in the experiment with direct effluent weighed 10 ± 3 g (mean ± SD) at stocking and grew at 0.01 day^{-1} (RGR). There was no significant difference in the size of plants at stocking (df = 14, p = 0.23) or in the relative growth rate (t= -2.156, df=14, p=0.05) between the hydroponic and sand treatment. Average biomass density at the start of the experiment was 2862 g m^{-2} and biomass subsequently decreased over the course of the experiment, as plants were sampled (Table 2).

Table 2: Plant biomass (mean wet weight (g) ± SD) at the six sampling times of *Sesuvium portulacastrum* grown in hydroponics or sand substrate and irrigated with direct effluent from milkfish. Growth over the course of the 48 h experiment was not taken into account.

Treatment	**0 h**	**3 h**	**6 h**	**12 h**	**24 h**	**48 h**
Hydroponic (HP)	119 (± 13)	100 (± 13)	81 (± 11)	59 (± 11)	40 (± 9)	20 (± 5)
Sand (SP)	144 (± 22)	120 (± 20)	95 (± 20)	71 (± 18)	47 (± 9)	25 (± 7)

The plants receiving RAS water weighed 16 ± 5 g at stocking and grew at 0.01 d^{-1} (RGR). There was no significant difference in weight at stocking (df=14, p=0.27) or relative growth rate (p=0.73, df=14) between hydroponic and sand cultivation. Average biomass density at the start of the experiment was 4676 g m^{-2} and the biomass subsequently decreased over the course of the experiment as plants were sampled (Table 3).

Table 3: Plant biomass (mean wet weight (g) ± SD) at the six sampling times of *Sesuvium portulacastrum* grown in hydroponics or sand substrate and irrigated with RAS water. Growth over the course of the 48 h experiment was not taken into account.

Treatment	**0 h**	**3 h**	**6 h**	**12 h**	**24 h**	**48 h**
Hydroponic (HP)	214 (±26)	180 (±17)	147 (±14)	106 (±11)	70 (±12)	40 (±8)
Sand (SP)	203 (±33)	167 (±26)	133 (±21)	95 (±17)	65 (±17)	36 (±14)

3.2. Water parameters

At the final sampling of the 48-hour experiment, there were small differences between treatments in pH and temperature. Salinity increased in the hydroponic boxes compared to the boxes with sand (Table S1, S2). Changes in the concentrations of DIN species and PO_4-P in the systems varied across treatments and time (Tables S3, S4).

DIN removal from the direct effluent experiment was not significantly different for plants in hydroponics or in sand, but the planted systems removed more than the unplanted controls (Fig. 3A). Final P removal was lowest in the treatments with plants in hydroponics (Fig. 3B). In the experiment with RAS water, removal of DIN was significantly higher in the treatments with plants cultivated in sand than in the plants cultivated hydroponically after 24 h, but neither of the treatments with plants performed significantly better than their control treatments without plants. N removal in the hydroponic control treatments however showed high variability, and after 48 h overall concentrations increased relative to initial concentrations of the milkfish wastewater (Fig. 4A). The planted and unplanted systems removed the same P. The sand treatment however removed more than the hydroponic plant treatment, both after 24 and 48 hours (Fig. 4B).

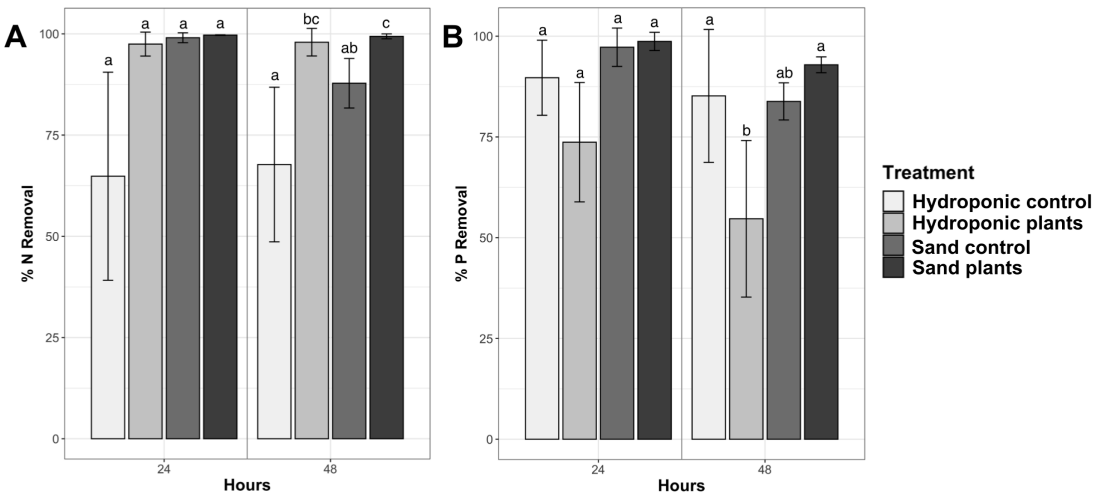

Fig. 3: Change in concentrations (% of initial) of dissolved inorganic A) N and B) P in direct effluent from milkfish after 24 hours in boxes with *Sesuvium portulacastrum* or unplanted control boxes and in the drainage water after 48 hours. Different letters indicate statistically significant differences based on multiple comparison test after Kruskal-Wallis test.

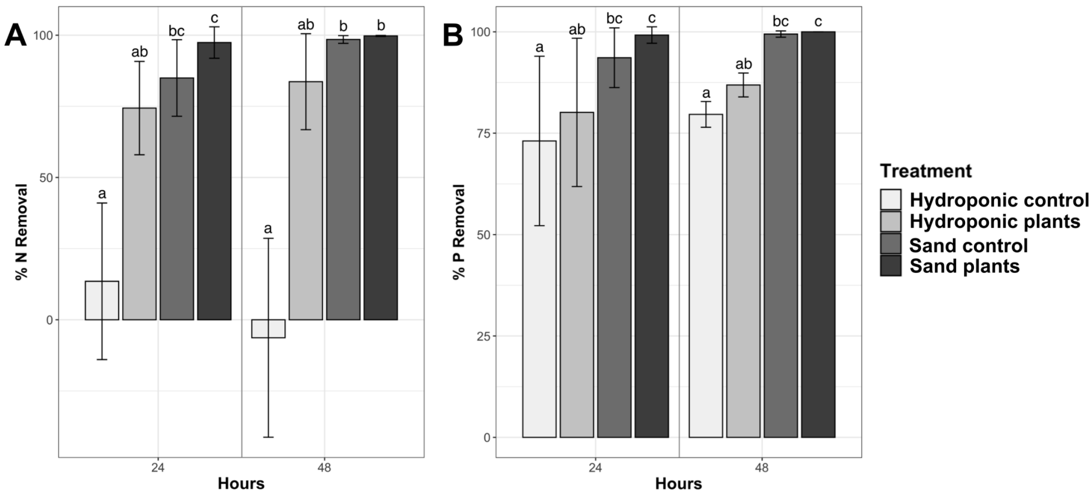

Fig. 4: Change in concentrations (% of initial) of dissolved inorganic A) N and B) P in RAS water after 24 hours in boxes with *Sesuvium portulacastrum* or unplanted control boxes and in the drainage water after 48 hours. Different letters indicate statistically significant differences based on multiple comparison test after Kruskal-Wallis test.

3.3. ^{15}N tracer uptake

Atomic % ^{15}N increased over time in the plants grown hydroponically in both experiments. In the plants supplied with Na^{15}NO$_3$ in the direct effluent experiment, isotopic label concentrations were significantly higher in hydroponics than in sand after 24 and 48 h (Fig. 5A). The rate of ^{15}N label uptake did not show a clear trend, but was generally higher in the hydroponic treatment (Fig 5B).

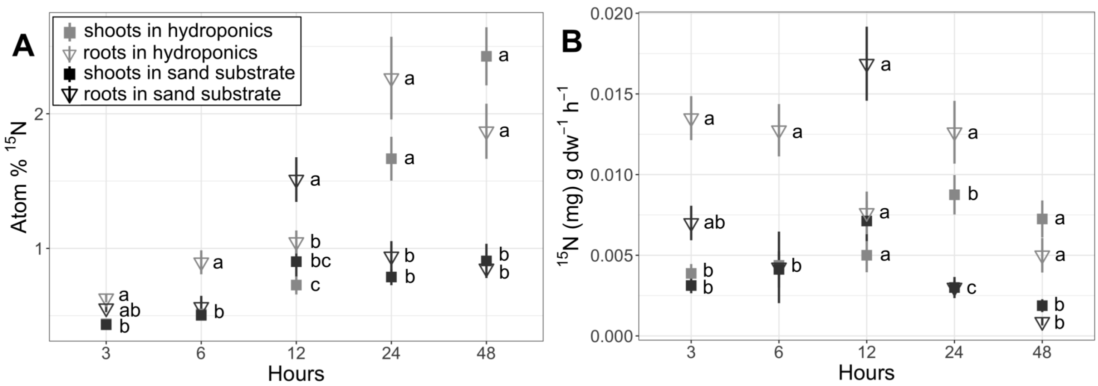

Fig. 5: Mean (± SE) ^{15}N assimilated in the stem and leaves (shoots) and roots of *Sesuvium portulacastrum* grown in hydroponics or sand substrate and irrigated with direct effluent from milkfish labeled with Na^{15}NO$_3$. Lower case letters indicate statistically significant differences at the same time point identified by pairwise comparison between estimated marginal means at $p < 0.05$.

In the plants supplied with $^{15}NH_4Cl$ in RAS water, atomic % ^{15}N was significantly higher in the plants grown hydroponically than in sand substrate. In hydroponics, the concentration was consistently high in the roots and increased throughout the experiment in the leaves and stem (Fig. 6A). Also, the uptake rate of the ^{15}N tracer was significantly higher in hydroponics than in sand. The highest uptake rate was measured at the 3 h sampling point and it decreased thereafter (Fig. 6B).

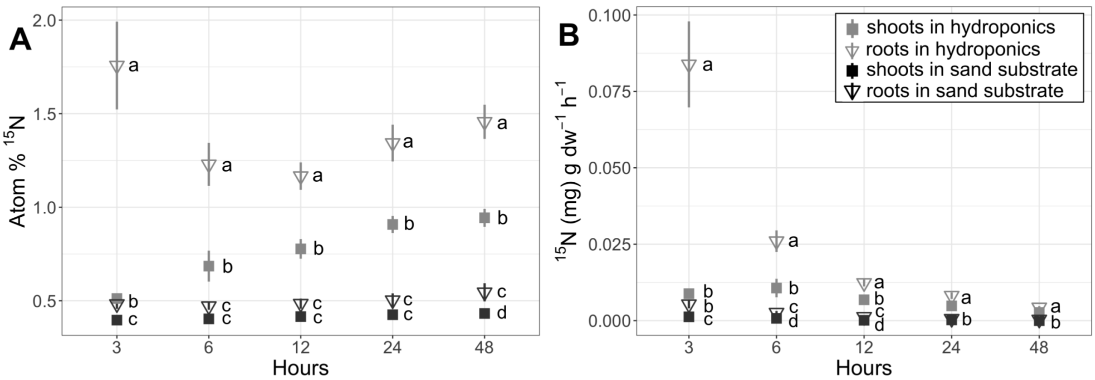

Fig. 6: Mean (± SE) ^{15}N assimilated in the stem and leaves (shoots) and roots of *Sesuvium portulacastrum* grown in hydroponics or sand substrate and irrigated with RAS water labeled with $^{15}NH_4Cl$. Lower case letters indicate statistically significant differences at the same time point identified by pairwise comparison between estimated marginal means at $p < 0.05$.

In both experiments, a significantly higher proportion of ^{15}N was recovered in total plant biomass in the hydroponic treatment than the sand substrate treatment. From 3.518 mg ^{15}N supplied as $Na^{15}NO_3$ in direct effluent, the plants in hydroponics assimilated 47.50 ± 8.14 % (mean ± SD) and plants in sand assimilated 18.77 ± 6.53 % (Fig. 7A). From 3.35 mg ^{15}N supplied as $^{15}NH_4Cl$ in RAS water, plants in hydroponics assimilated 52.84 ± 8.27 % while the plants in sand substrate assimilated 3.26 ± 1.25 % (Fig. 7B).

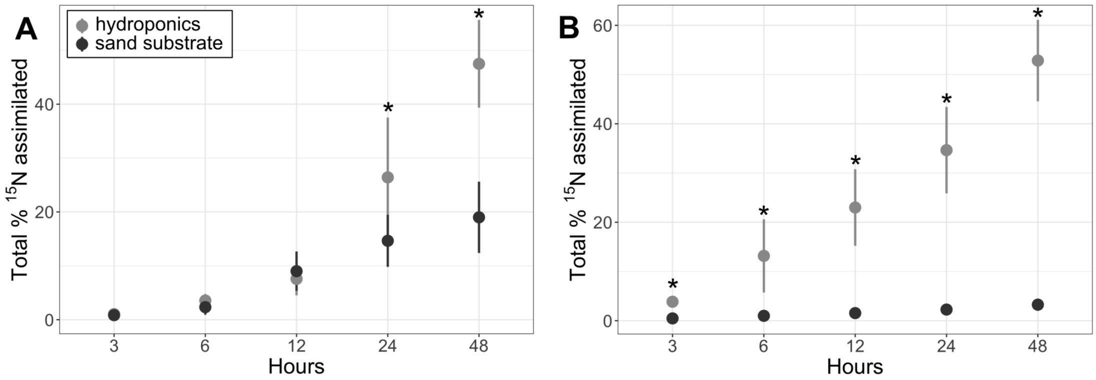

Fig. 7: Mean (± SD) % assimilated cumulatively to whole plants of *Sesuvium portulacastrum* grown in hydroponics or sand substrate. ^{15}N label was supplied as A) $Na^{15}NO_3$ in direct effluent or B) $^{15}NH_4Cl$ added to RAS water. * = statistically significant differences at the same time point identified by pairwise comparison between estimated marginal means at $p < 0.05$.

3.4. System observations

Upon removal of the wall cover from the boxes of the sand treatment, it was noted that the substrate showed clear differences in its microbial and /or algal community. While the sand surface in the boxes planted with halophytes was overgrown with green and filamentous algae, the control treatments only showed small patches of brown or red microalgae and/or cyanobacteria. The control treatments also showed a clear anoxic layer at an approximate depth of 3 cm, while the boxes with plants did not (Fig. 8).

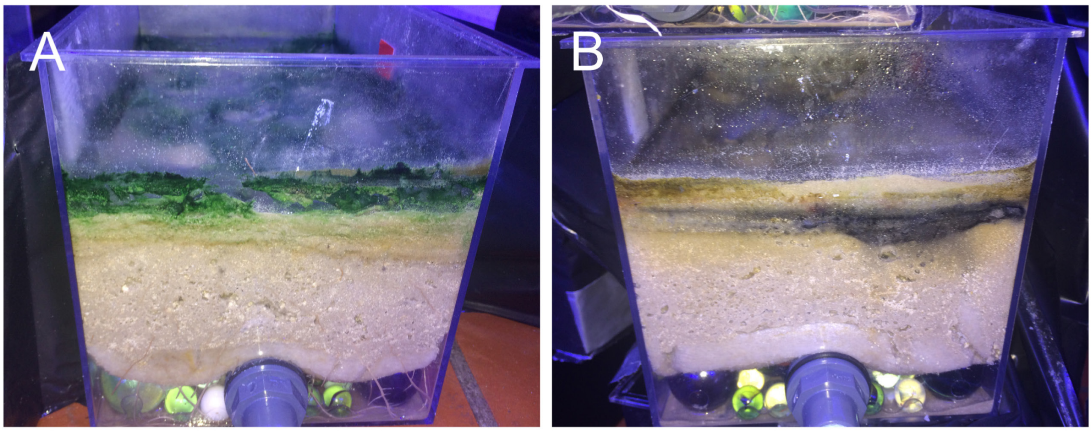

Fig. 8: Example of the sand in the boxes that had received RAS water A) planted with *S. portulacastrum* and B) unplanted control treatment after black cover was removed upon termination of the experiment.

4. Discussion

This study investigated nutrient removal from milkfish wastewater by systems planted with *S. portulacastrum* in hydroponics, with the roots in water, *versus* planted in sand. The two experiments allowed comparing nutrient removal and nitrogen recovery by the plants in the two systems when exposed to wastewater from fish cultivation.

4.1. Nutrient removal

The results of both experiments presented here show efficient removal of N and P from milkfish wastewater, irrespective of the system being hydroponic or with sand substrate, unplanted or planted with *Sesuvium portulacastrum*. N removal was fastest in the systems with sand, which comes at no surprise as sands have been shown to be extremely efficient in terms of N-loss by denitrification (Rao et al. 2008, Gao et al. 2012). In the systems without plants, N removal would have to be driven by assimilation into un-harvestable biomass growing on the sediment and tank walls and/or anaerobic processes like anammox and denitrification in anaerobic environments that developed in the tanks (Fig. 8). As P cannot be decayed into a gaseous form like N, P would have to have been removed from the wastewaters by adsorption to the sediment and assimilation into un-harvestable biomass and thus remained in the tank in a different form (Barak et al. 2003).

Removal of up to 99 % DIN and 100 % DIP from initial concentrations of 16.73 and 2.05 mg l^{-1}, respectively is comparatively high (Brown et al. 1999, Lymbery et al. 2006, Webb et al. 2012, 2013, Shpigel et al. 2013, De Lange & Paulissen 2016). High removal occurred despite NH_4^+ and NO_3^- concentrations in the milkfish RAS water being similar or even higher than effluents in other studies (Carton-Kawagoshi et al. 2014, Senff et al. 2020), showing that *S. portulacastrum* can remediate wastewater with concentrations higher than what has been shown previously (Boxman et al. 2017). This differs from performances of other halophytes such as *Salicornia europaea*, which, in constructed wetlands, removed only about 0.7 % N at concentrations of 14 mg N l^{-1}, but 59 % N at low nutrient loads of 0.2 mg N l^{-1} (Shpigel et al. 2013). The wastewater in this experiment remained in the systems for a total of 48 h, comparable with filter systems receiving effluents in batches or operated at low water flow (Webb et al. 2012, Shpigel et al. 2013). A similar removal efficiency of 36 – 98 % P and N was achieved with constructed wetlands planted with *S. europaea* receiving aquaculture discharge water once a day in a flood and drain system, but concentrations were lower at 1.5 to 5.4 mg N l^{-1} and 1.05 to 2.79 mg P l^{-1} (Webb et al. 2012).

4.2. Nitrogen recovery

The focus of this study was not on the overall removal of nutrients from wastewaters, but to determine the most efficient way of using these nutrients and assimilating them into valuable biomass. As P does not have stable isotopes that can be easily traced (Schoffelen et al. 2018), we focused on adding a specific amount of 3.518 mg labeled NO_3-N or NH_4-N to milkfish waste water, allowing to identify which condition would result in the highest N recovery into halophyte biomass. In the systems with sand substrate, the plants assimilated only a low percentage of the added ^{15}N label, whereas in the hydroponic systems around 50% could be recovered. This trend was clear and not affected by the form in which ^{15}N was supplied or overall DIN concentration. Recovery of 50% DIN is comparatively high especially when considering the relatively small plant biomass that was effectively maintained throughout the experiment while plants were being harvested (Buhmann et al. 2015, Waller et al. 2015).

High ^{15}N concentrations in root biomass showed fast N uptake. $^{15}N/^{14}N$ ratios were higher in the roots where it is taken up, but due to the far higher biomass, most of the assimilated ^{15}N was retrieved in the leaves and stems and thus converted into a harvestable resource. As only a portion of the DIN available to the plants in this experiment was in the form of ^{15}N, resulting uptake rates are not necessarily representative of overall N uptake. Where label was added as $^{15}NH_4Cl$, $^{15}N/^{14}N$ ratios continued to increase even after NH_4^+ was removed from the water, showing that *S. portulacastrum* was able to take up the tracer in different nitrogen forms. This is further supported by the fact that equally large proportions of ^{15}N were taken up by hydroponic plants in both experiments and may indicate that *S. portulacastrum* can take up different forms of dissolved nitrogen equally and that overall rates of DIN uptake are similar to what has been determined for temperate species of edible halophytes (Quintã et al. 2015a b). The fact that the 15N/14N ratio in plants decreased after a certain point in the roots of the hydroponic treatments indicates that N uptake continued and *S. portulacastrum* was able to take up other available forms of N. While the focus of this study was on the phytoremediation performance in effluents from fish, further labeling studies with dissolved inorganic and organic N tracers are needed to reveal specific uptake rates and possible preferences for N species.

Recovery of ^{15}N label in plant biomass was up to 17 times higher in hydroponics compared to sand substrate. Similar differences have been found with other species of halophytes (Quintã et al. 2015a). This shows the better recovery of dissolved N in hydroponics and the suitability to integrate *S. portulacastrum* into RAS to improve N-use efficiency (Pinheiro et al. 2017). N uptake by plants in hydroponic cultivation is supported by the large root surface area developed in the absence of substrate, facilitating nutrient uptake and, to some extent, nutrient storage (Wallace and Pate 1967; Hu et al. 2015). When subtracting the effect of the unplanted controls, the planted hydroponic systems removed 407 mg N m^{-2} in the first 24 hours. This is higher than the 117 to 303 mg N m^{-2} d^{-1} found in cultivation experiments in floating structures (Xiaojie et al. 2011, Liu et al. 2019), indicating that *S. portulacastrum* can be used to remediate waters impacted by more concentrated effluents.

The removal determined here is however a rough estimate and does not take into account possible mineralization of dissolved and particulate organic N into the DIN pool. It further does not account for the differences in N-loss due to the presence or absence of plants. In addition to N assimilation, halophytes can contribute to faster nitrification and better N removal by supplying oxygen to the rhizosphere and maintaining an oxic layer in otherwise anoxic, flooded substrates, supporting environmental conditions favorable to microbial activity (summarized by Reddy and Patrick, 1984). The roots of salt marsh *Salicornia* sp., were for example found to possess aerenchyma, which allow the exchange of gases between the shoot and the root and indirectly aerate the surrounding soil zone, resulting in increased nitrification/denitrification efficiency (Brix 1994, Haberl et al. 1995, Faulwetter et al. 2009). While dark anoxic layers were observed in the unplanted sand treatment, the absence of such a layer in the planted systems indicates that *S. portulacastrum* was able to increase oxygen availability in the water below the plants. Furthermore, aquaponic systems have been found to harbor more nitrite oxidizing bacteria than conventional RAS and the roots of halophytes grown hydroponically in aquaculture effluents were enriched in bacterial taxa involved in nutrient cycling (Bartelme et al. 2019, Oliveira et al. 2020).

4.3. Potential for efficient resource-use

While continuous exposure to excess DIN from aquaculture effluents has clear adverse environmental impacts, microbial processes have the ability to quickly remove excess N from eutrophic systems (Gao et al. 2012, Sokoll et al. 2016, Thomsen et al. 2020). P however remains and is mostly removed from the water column by sorption to sediment and other particles (Krom et al. 1991, Jia et al. 2015). Weakly bound in sediments, it can be mobilized easily, fueling the growth of primary producers with the potential to cause eutrophication (Jia et al. 2015). Harvesting organically-bound P is thus the only strategy to remove it from the aquaculture system and effluents. The significantly higher DIP removal observed in the sand treatments, suggests that this was achieved was partly due to mechanisms supported by the sediment (Kadlec & Knight 1996, Barak et al. 2003, Cronk & Fennessy 2016). In hydroponics, P removal was also high, possibly driven by immobilization and uptake (Rubio et al. 1997, Barak et al. 2003). As phosphate uptake in halophytes has been found to increase with high N availability, the N:P ratio of 8.31 in the aquaculture effluent of this experiment may have supported good uptake of DIP (Webb et al. 2005). The productivity of modern aquaculture relies on our limited global P reservoirs that are projected to become depleted within a century (Cordell et al. 2009). Between fisheries and aquaculture, we are currently using more P to produce fish than we are harvesting in fish biomass, and aquaculture is thus contributing to the anthropogenic flux of this valuable nutrient from the terrestrial to the aquatic realm (Huang et al. 2020). Therefore, recapture of P from food production waste streams is imperative and the integration of halophytes is an appropriate strategy to do so in saline aquaculture.

Since we have been able to produce fertilizer from inert atmospheric N_2, we are able to use N luxuriously in our food production systems, but at the cost of high greenhouse gas emissions and discharge to natural ecosystems (Erisman et al. 2008). Unlike N, P is of finite supply and we are becoming increasingly aware of the urgency to reduce its waste (Cordell et al. 2009). As aquaculture production is ever increasing, the contribution of fertilizer use in the sector is increasing as well (Huang et al. 2020). Minimizing the waste of nutrient resources is seen as a necessary step to turn aquaculture into a sustainable food production sector contributing to food security and recovery of nutrients from effluents into valuable biomass is an approach to significantly contribute to this goal (Huang et al. 2020). *S. portulacastrum* and other edible halophytes can be considered functional foods due to high antioxidant activity, protein content and levels of n-3 fatty acids (Boestfleisch et al. 2014, Barreira et al. 2017, Pinheiro et al. 2017). They can also be cultivated for the commercial production of secondary metabolites (Magwa et al. 2006) and it has recently been shown that marine aquaponics produced halophytes with improved n-3 fatty acid content compared to wild populations (Maciel et al. 2020). Rising interest and demand provide an option to produce for local and/or international niche markets (Buhmann & Papenbrock 2013a). The ability to produce high quality health food with consistent nutritional conditions can be a strategy to profitably include *S. portulacastrum* in aquaculture systems and valorize nutrients from aquaculture effluents (Custódio et al. 2020, Maciel et al. 2020).

5. Conclusions

Integrating extractive species in aquaculture to treat wastewater and increase nutrient recovery into usable biomass is an important step towards sustainability. Cultivating the edible halophyte *Sesuvium portulacastrum* in hydroponics or sand substrate effectively treated milkfish effluents and removed dissolved inorganic nutrients. Hydroponic cultivation recovered a high proportion of nitrogen into an edible product and would be the recommended cultivation system to prevent losses through denitrification or discharge. The contribution of plants towards effluent treatment appears to go beyond uptake and assimilation by possibly enabling conditions that support microbial nitrification, favoring immobilization over denitrification and potentially reducing nitrous oxide emissions. Our direct result of recovering half of the added ^{15}N nutrient back into new biomass is very high compared to previous studies. Determining uptake rates for different N forms could aid in further optimizing the hydroponic growth method applied in this study and move towards more complete resource recovery in closed recirculation systems and integrated aquaculture.

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References

Almuktar SAAAN, Abed SN, Scholz M (2018) Wetlands for wastewater treatment and subsequent recycling of treated effluent: a review. Environ Sci Pollut Res 25:23595–23623.

Ballester-Moltó M, Sanchez-Jerez P, Cerezo-Valverde J, Aguado-Giménez F (2017) Particulate waste outflow from fish-farming cages. How much is uneaten feed? Mar Pollut Bull 119:23–30.

Barak Y, Cytryn E, Gelfand I, Krom M, Van Rijn J (2003) Phosphorus removal in a marine prototype, recirculating aquaculture system. Aquaculture 220:313–326.

Bardgett RD, Streeter TC, Boi R (2003) Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. Ecology 84:1277–1287.

Barreira L, Resek E, João M, Isabel M, Pereira H, Bandarra N, Moreira M, Varela J, Custódio L (2017) Halophytes: Gourmet food with nutritional health benefits? J Food Compos Anal 59:35–42.

Barrington K, Ridler N, Chopin T, Robinson S, Robinson B (2010) Social aspects of the sustainability of integrated multi-trophic aquaculture. Aquac Int.

Bartelme RP, Smith MC, Sepulveda-Villet OJ, Newton RJ (2019) Component Microenvironments and System Biogeography Structure Microorganism Distributions in Recirculating Aquaculture and Aquaponic Systems. mSphere 4:1–15.

Bischoff AA, Fink P, Waller U (2009) The fatty acid composition of *Nereis diversicolor* cultured in an integrated recirculated system: Possible implications for aquaculture. Aquaculture 296:271–276.

Blackburn TH, Blackburn ND (1992) Model of nitrification and denitrification in marine sediments. FEMS Microbiol Lett 100:517–521.

Boestfleisch C, B.Wagenseil N, Buhmann A, Seal CE, MerrettWade E, Muscolo A, Papenbrock J (2014) Manipulating the antioxidant capacity of halophytes to increase their cultural and economic value through saline cultivation. AoB Plants 6:1–16.

Boxman SE, Nystrom M, Capodice JC, Ergas SJ, Main KL, Trotz MA (2017) Effect of support medium, hydraulic loading rate and plant density on water quality and growth of halophytes in marine aquaponic systems. Aquac Res 48:2463–2477.

Brix H (1994) Functions of macrophytes in constructed wetlands. Water Sci Technol 29:71–78.

Brown JJ, Glenn EP, Fitzsimmons KM, Smith SE (1999) Halophytes for the treatment of saline aquaculture effluent. Aquaculture 175:255–268.

Buhmann A, Papenbrock J (2013a) An economic point of view of secondary compounds in halophytes. Funct Plant Biol 40:952–967.

Buhmann A, Papenbrock J (2013b) Biofiltering of aquaculture effluents by halophytic plants: Basic principles, current uses and future perspectives. Environ Exp Bot 92:122–133.

Buhmann A, Waller U, Wecker B, Papenbrock J (2015) Optimization of culturing conditions and selection of species for the use of halophytes as biofilter for nutrient-rich saline water. Agric Water Manag 149:102–114.

Capone DG, Kiene RP (1988) Comparison of microbial dynamics in marine and freshwater sediments: Contrasts in anaerobic carbon catabolism. Limnol Oceanogr 33:725–749.

Carton-Kawagoshi RJ, Elle BJ, Corre V, Satoh S, Notoya M, Fujita D (2014) Low water exchange *Gracilariopsis bailiniae* Zhang & B.M. Xia culture in intensive milkfish culture effluents: Effects of seaweed density on seaweed production and effluent treatment. Aquac Int 22:573–584.

Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens GE (2009) Controlling Eutrophication: Nitrogen and Phosphorus. Science (80-) 323:1014 LP – 1015.

Cordell D, Drangert JO, White S (2009) The story of phosphorus: Global food security and food for thought. Glob Environ Chang 19:292–305.

Cronk JK, Fennessy MS (2016) Wetland plants:biology and ecology. CRC PRESS.

Custódio M, Maciel E, Domingues MR, Lillebø AI, Calado R (2020) Nutrient availability affects the polar lipidome of *Halimione portulacoides* leaves cultured in hydroponics. Sci Rep 10:1–13.

Custódio M, Villasante S, Cremades J, Calado R, Lillebø AI (2017) Unravelling the potential of halophytes for marine integrated multi-trophic aquaculture (IMTA) - A perspective on performance, opportunities and challenges. Aquac Environ Interact 9:445–460.

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. Science (80-) 321:926–929.

Diver S (2006) Aquaponics — Integration of Hydroponics with Aquaculture. ATTRA:1–28.

Erisman JW, Sutton MA, Galloway J, Klimont Z, Winiwarter W (2008) How a century of ammonia synthesis changed the world. Nat Geosci 1:636–639.

FAO (2020) The State of World Fisheries and Aquaculture 2020. Rome.

Faulwetter JL, Gagnon V, Sundberg C, Chazarenc F, Burr MD, Brisson J, Camper AK, Stein OR (2009) Microbial processes influencing performance of treatment wetlands: A review. Ecol Eng 35:987–1004.

Gao H, Matyka M, Liu B, Khalili A, Kostka JE, Collins G, Jansen S, Holtappels M, Jensen MM, Badewien TH, Beck M, Grunwal M, de Beer D, Lavik G, Kuypers

MMM (2012) Intensive and extensive nitrogen loss from intertidal permeable sediments of the Wadden Sea. Limnol Oceanogr 57:185–198.

Glenn EP, Brown JJ, Blumwald E (1999) Salt Tolerance and Crop Potential of Halophytes. CRC Crit Rev Plant Sci 18:227–255.

Haberl R, Perfler R, Mayer H (1995) Constructed Wetlands in Europe. Water Sci Technol 32:305–315.

Halm H, Lam P, Ferdelman TG, Lavik G, Dittmar T, Laroche J, D'Hondt S, Kuypers MMM (2011) Heterotrophic organisms dominate nitrogen fixation in the south pacific gyre. ISME J 6:1238–1249.

Hanh TTH, Boonstra WJ (2019) What prevents small-scale fishing and aquaculture households from engaging in alternative livelihoods? A case study in the Tam Giang lagoon, Viet Nam. Ocean Coast Manag 182.

Harrison KA, Bol R, Bardgett RD (2008) Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen? Soil Biol Biochem 40:228–237.

Herbeck LS, Sollich M, Unger D, Holmer M, Jennerjahn TC (2014) Impact of pond aquaculture effluents on seagrass performance in NE Hainan, tropical China. Mar Pollut Bull 85:190–203.

Hodge A, Stewart J, Robinson D, Griffiths BS, Fitter AH (2000) Competition between roots and soil micro-organisms for nutrients from nitrogen-rich patches of varying complexity. J Ecol 88:150–164.

Hu Z, Lee JW, Chandran K, Kim S, Sharma K, Brotto AC, Khanal SK (2013) Nitrogen transformations in intensive aquaculture system and its implication to climate change through nitrous oxide emission. Bioresour Technol 130:314–320.

Hu Z, Woo J, Chandran K, Kim S, Coelho A, Kumar S (2015) Effect of plant species on nitrogen recovery in aquaponics. Bioresour Technol 188:92–98.

Huang Y, Ciais P, Goll DS, Cresto-aleina F, Zhang H (2020) The shift of phosphorous transfers in global fisheries and aquaculture. Nat Commun:1–10.

IPCC (2007) Changes in atmospheric constituents and in radioactive forcing. In: *Climate change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Solomon S et al. (Eds. . (ed) Cambridge University Press, Cambridge, p 114–143

Irwin S, Flaherty MS, Carolsfeld J (2020) The contribution of small-scale, privately owned tropical aquaculture to food security and dietary diversity in Bolivia. Food Secur.

Jia B, Tang Y, Tian L, Franz L, Alewell C, Huang JH (2015) Impact of fish farming on phosphorus in reservoir sediments. Sci Rep 5:1–11.

Jorgensen BB (1982) Ecology of the Bacteria of the Sulphur Cycle with Special Reference to Anoxic-Oxic Interface Environments. Philos Trans R Soc B Biol Sci 298:543–561.

Kadlec RH, Knight RL (1996) Treatment Wetlands. CRC Press, Boca Raton, Florida.

Kir M, Sunar MC (2018) Acute Toxicity of Ammonia and Nitrite to Sea Bream, *Sparus aurata* (Linnaeus, 1758), in Relation to Salinity. J World Aquac Soc 49:516–522.

Krom MD, Kress N, Brenner S, Gordon LI (1991) Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. Limnol Oceanogr 36:424–432.

De Lange HJ, Paulissen MPCP (2016) Efficiency of three halophyte species in removing nutrients from saline water: a pilot study. Wetl Ecol Manag 24:587–596.

Lefebvre S, Bacher C, Meuret A, Hussenot J (2001) Modelling nitrogen cycling in a mariculture ecosystem as a tool to evaluate its outflow. Estuar Coast Shelf Sci 52:305–325.

Lin Y-F, Jing S-R, Lee D-Y, Wang T-W (2002) Nutrient removal from aquaculture wastewater using a constructed wetlands system. Aquaculture 209:169–184.

Lipson D, Näsholm T (2001) The unexpected versatility of plants: Organic nitrogen use and availability in terrestrial ecosystems. Oecologia 128:305–316.

Liu X, Pu X, Luo D, Lu J, Liu Z (2019) Model assessment of nutrient removal via planting *Sesuvium portulacastrum* in floating beds in eutrophic marine waters: the case of aquaculture areas of Dongshan Bay. Acta Oceanol Sin 38:91–100.

Lokhande VH, Nikam TD, Suprasanna P (2009) *Sesuvium portulacastrum* (L.) L. a promising halophyte: Cultivation, utilization and distribution in India. Genet Resour Crop Evol 56:741–747.

Lonard RI, Judd FW (1997) The biological flora of coastal dunes and wetlands. *Sesuvium portulacastrum* (L) L. J Coast Res 13:96–104.

Luo Z, Hu S, Chen D (2018) The trends of aquacultural nitrogen budget and its environmental implications in China. Sci Rep 8:1–9.

Lymbery AJ, Doupe RG, Bennett T, Starcevich MR (2006) Efficacy of a subsurface-flow wetland using the estuarine sedge *Juncus kraussii* to treat effluent from inland saline aquaculture. Aquac Eng 34:1–7.

Maciel E, Domingues P, Domingues MRM, Calado R, Lillebø AI (2020) Halophyte plants from sustainable marine aquaponics are a valuable source of omega-3 polar lipids. Food Chem 0:0–32.

Magwa ML, Gundidza M, Gweru N, Humphrey G (2006) Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. J Ethnopharmacol 103:85–89.

Marchant HK, Tegetmeyer HE, Ahmerkamp S, Holtappels M, Lavik G, Graf J, Schreiber F, Mussmann M, Strous M, Kuypers MMM (2018) Metabolic specialization of denitrifiers in permeable sediments controls N2O emissions. Environ Microbiol 20:4486–4502.

Martens-Habbena W, Berube PM, Urakawa H, De La Torre JR, Stahl DA (2009) Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461:976–979.

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004) Integrated aquaculture: Rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231:361–391.

Neori A, Krom MD, Rijn J van (2007) Biogeochemical processes in intensive zero-effluent marine fish culture with recirculating aerobic and anaerobic biofilters. J Exp Mar Bio Ecol 349:235–247.

Nouairi I, Ghnaya T, Ben Youssef N, Zarrouk M, Habib Ghorbel M (2006) Changes in content and fatty acid profiles of total lipids of two halophytes: *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum* under cadmium stress. J Plant Physiol 163:1198–1202.

Ogah SI, Kamarudin MS, Amin SMN, Edaroyati MWP (2020) Biological filtration properties of selected herbs in an aquaponic system. Aquac Res:1–9.

Oliveira V, Martins P, Marques B, Cleary DFR, Lillebø AI, Calado R (2020) Aquaponics using a fish farm effluent shifts bacterial communities profile in halophytes rhizosphere and endosphere. Sci Rep 10:1–11.

Person-Le Ruyet J, Chartois H, Quemener L (1995) Comparative acute ammonia toxicity in marine fish and plasma ammonia response. Aquaculture 136:181–194.

Piedrahita RH (2003) Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture 226:35–44.

Pinheiro I, Arantes R, do Espírito Santo CM, do Nascimento Vieira F, Lapa KR, Gonzaga LV, Fett R, Barcelos-Oliveira JL, Seiffert WQ (2017) Production of the halophyte *Sarcocornia ambigua* and Pacific white shrimp in an aquaponic system with biofloc technology. Ecol Eng 100:261–267.

Poli MA, Legarda EC, de Lorenzo MA, Pinheiro I, Martins MA, Seiffert WQ, do Nascimento Vieira F (2019) Integrated multitrophic aquaculture applied to shrimp rearing in a biofloc system. Aquaculture 511.

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2014) 15N tracer application to evaluate nitrogen dynamics of food webs in two subtropical small-scale aquaculture ponds under different managements. Isotopes Environ Health Stud 50:428–441.

Quintã R, Hill PW, Jones DL, Santos R, Thomas DN, LeVay L (2015a) Uptake of an amino acid (alanine) and its peptide (trialanine) by the saltmarsh halophytes

Salicornia europaea and *Aster tripolium* and its potential role in ecosystem N cycling and marine aquaculture wastewater treatment. Ecol Eng 75:145–154.

Quintã R, Santos R, Thomas DN, Le Vay L (2015b) Growth and nitrogen uptake by *Salicornia europaea* and *Aster tripolium* in nutrient conditions typical of aquaculture wastewater. Chemosphere 120:414–421.

Rao AMF, McCarthy MJ, Gardner WS, Jahnke RA (2008) Respiration and denitrification in permeable continental shelf deposits on the South Atlantic Bight: N2:Ar and isotope pairing measurements in sediment column experiments. Cont Shelf Res 28:602–613.

Reddy KR, Patrick WH (1984) Nitrogen transformations and loss in flooded soils and sediments. C R C Crit Rev Environ Control 13:273–309.

Van Rijn J (1996) The potential for integrated biological treatment systems in recirculating fish culture - A review. Aquaculture 139:181–201.

Rubio G, Oesterheld M, Alvarez CR, Lavado RS (1997) Mechanisms for the increase in phosphorus uptake of waterlogged plants: Soil phosphorus availability, root morphology and uptake kinetics. Oecologia 112:150–155.

Schoffelen NJ, Mohr W, Ferdelman TG, Littmann S, Duerschlag J, Zubkov M V., Ploug H, Kuypers MMM (2018) Single-cell imaging of phosphorus uptake shows that key harmful algae rely on different phosphorus sources for growth. Sci Rep 8:1–13.

Senff P, Blanc P, Slater M, Kunzmann A (2020) Low-technology recirculating aquaculture system integrating milkfish *Chanos chanos*, sea cucumber *Holothuria scabra* and sea purslane *Sesuvium portulacastrum*. Aquac Environ Interact 12:471–484.

Senff P, Partelow S, Indriana LF, Buhari N, Kunzmann A (2018) Improving pond aquaculture production on Lombok, Indonesia. Aquaculture 497:64–73.

Shpigel M, Ben-Ezra D, Shauli L, Sagi M, Ventura Y, Samocha T, Lee JJ (2013) Constructed wetland with *Salicornia* as a biofilter for mariculture effluents. Aquaculture 413:52–63.

Singh D, Buhmann A, Flowers TJ, Seal CE, Papenbrock J (2014) *Salicornia* as a crop plant in temperate regions: Selection of genetically characterized ecotypes and optimization of their cultivation conditions. AoB Plants 6:1–20.

Sokoll S, Lavik G, Sommer S, Goldhammer T, Kuypers MMM, Holtappels M (2016) Extensive nitrogen loss from permeable sediments off North-West Africa. J Geophys Res Biogeosciences 121:1144–1157.

Strickland J, Parsons T (1972) A practical Handbook of Seawater Analysis. Fisheries Research Board Canada, Ottawa.

Thomsen E, Herbeck LS, Jennerjahn TC (2020) The end of resilience: Surpassed nitrogen thresholds in coastal waters led to severe seagrass loss after decades of exposure to aquaculture effluents. Mar Environ Res 160:104986.

Verdegem MCJ (2013) Nutrient discharge from aquaculture operations in function of system design and production environment. Rev Aquac 5:158–171.

Vymazal J (2010) Constructed wetlands for wastewater treatment. Water 2:530–549.

Wallace W, Pate JS (1967) Nitrate Assimilation in Higher Plants with Special Reference to the Cocklebur (*Xanthium pennsylvanicum* Wallr .). Ann Bot 31:213–228.

Waller U, Buhmann A, Ernst A, Hanke V, Kulakowski A, Wecker B, Orellana J, Papenbrock J (2015) Integrated multi-trophic aquaculture in a zero-exchange recirculation aquaculture system for marine fish and hydroponic halophyte production. Aquac Int 23:1473–1489.

Webb JM, Quintã R, Papadimitriou S, Norman L, Rigby M, Thomas DN, Le Vay L (2012) Halophyte filter beds for treatment of saline wastewater from aquaculture. Water Res 46:5102–5114.

Webb JM, Quintã R, Papadimitriou S, Norman L, Rigby M, Thomas DN, Le Vay L (2013) The effect of halophyte planting density on the efficiency of constructed wetlands for the treatment of wastewater from marine aquaculture. Ecol Eng 61:145–153.

Xiaojie W, Yuchun S, Ning Y, Zaohe W, Xiaodong D (2011) The preliminary study of *Sesuvium portulacastrum* on the nitrogen and phosphorus absorption rate. Chinese Agric Bull 27:92–96.

Supplementary material

Table S1: Water parameters (mean ± SD) of drainage water from systems planted with *S. portulacastrum* hydroponically (HP) or in sand substrate (SP) and the unplanted control systems (HC and SC, respectively) after the 48-hour experiment receiving direct effluent. Different letters indicate statistical significance determined by Dunn's post hoc rank sum test.

	HC	HP	SP	SC	
pH	7.4 ± 0.4 a	7.2 ± 0.3 a	7.6 ± 0.3 a	7.2 ± 0.3 a	$\chi^2 = 6.15$ df = 3 p = 0.10
Salinity (PSU)	36.9 ± 1.5 a	38.6 ± 3.0 a	32.3 ± 2.9 b	32.5 ± 3.4 b	f = 10.52 df = 3 p < 0.001
Temp- (°C)	24.1 ± 0.2 a	23.7 ± 0.7 a	24.0 ± 0.0 a	23.9 ± 0.2 a	$\chi^2 = 6.82$ df = 3 p = 0.07

Table S2: Water parameters (mean ± SD) of drainage water from systems planted with *S. portulacastrum* hydroponically (HP) or in sand substrate (SP) and the unplanted control systems (HC and SC, respectively) after the 48-hour experiment receiving RAS water. Different letters indicate statistical significance determined by Dunn's post hoc rank sum test.

	HC	HP	SP	SC	
pH	7.4 ± 0.4 a	7.2 ± 0.3 ab	7.6 ± 0.3 c	7.2 ± 0.3 bc	$\chi^2 = 27.2$ df = 3 p < 0.001
Salinity (PSU)	40.1 ± 2.6 a	40.8 ± 2.6 a	27.9 ± 2.9 b	36.6 ± 1.8 ab	$\chi^2 = 24.9$ df = 3 p < 0.001
Temperature (°C)	26.4 ± 0.3 a	26.4 ± 0.6 ab	26.4 ± 0.3 ab	26.8 ± 0.2 b	$\chi^2 = 8.8$ df = 3 p = 0.032

Table S3: Concentrations (mean mg L^{-1} ± SD) of dissolved inorganic nutrients in the systems planted with *S. portulacastrum* hydroponically (HP) or in sand substrate (SP) and the unplanted control systems (HC and SC, respectively) that received direct effluent, sampled after 24 and 48 hours. Significant differences between pairs of treatments are based on p-values produced by Wilcoxon rank-sum test (n.s. ≥ 0.05, * < 0.05, ** < 0.01, *** < 0.001). Experiment 1.

		HC	HP	SP	SC
NH_4-N	24 h	0.47 (± 0.47)	0.00 (± 0.00)	0.00 (± 0.00)	0.00 (± 0.00)
		n.s.	n.s.	n.s.	

	48 h	0.14 (± 0.14)	0.00 (± 0.00)	0.00 (± 0.00)	0.00 (± 0.00)
		**	n.s.	n.s.	
NO_2-N	24 h	0.05 (± 0.02)	0.01 (± 0.01)	0.01 (± 0.01)	0.01 (± 0.00)
		n.s.	n.s.	n.s.	
	48 h	0.45 (± 0.55)	0.06 (± 0.07)	0.01 (± 0.01)	0.04 (± 0.03)
		*	*	n.s.	
NO_3-N	24 h	1.12 (± 0.72)	0.10 (± 0.14)	0.00 (± 0.00)	0.03 (± 0.05)
		n.s.	n.s.	n.s.	
	48 h	0.90 (± 0.59)	0.04 (± 0.10)	0.02 (± 0.03)	0.53 (± 0.23)
		**	n.s.	***	
PO_4-P	24 h	0.06 (± 0.05)	0.15 (± 0.08)	0.01 (± 0.01)	0.02 (± 0.03)
		n.s.	n.s.	n.s.	
	48 h	0.08 (± 0.09)	0.25 (± 0.10)	0.04 (± 0.01)	0.09 (± 0.03)
		*	**	**	

Table S4: Concentrations (mean mg L^{-1} ± SD) of dissolved inorganic nutrients in the systems planted with *S. portulacastrum* hydroponically (HP) or in sand substrate (SP) and the unplanted control systems (HC and SC, respectively) that received RAS water, sampled after 24 and 48 hours. Significant differences between pairs of treatments are based on p-values produced by Wilcoxon rank-sum test (n.s. ≥ 0.05, * < 0.05, ** < 0.01, *** < 0.001). Experiment 2.

		HC	HP	SP	SC
NH_4^+-N	24 h	0.59 (± 0.24)	0.00 (± 0.00)	0.00 (± 0.00)	0.00 (± 0.00)
		***	n.s.	n.s.	
	48 h	0.03 (± 0.09)	0.00 (± 0.00)	0.00 (± 0.00)	0.04 (± 0.06)
		n.s.	n.s.	n.s.	
NO_2^--N	24 h	0.07 (± 0.07)	0.03 (± 0.02)	0.12 (± 0.09)	0.08 (± 0.10)
		n.s.	n.s.	n.s.	

	48 h	0.02 (± 0.02)	0.03 (± 0.02)	0.01 (± 0.01)	0.02 (± 0.01)
		n.s.	n.s.	**	
NO_3^--N	24 h	13.81 (± 4.52)	4.25 (± 2.75)	0.31 (± 0.88)	2.43 (± 2.18)
		**	**	**	
	48 h	17.74 (± 5.85)	2.70 (± 2.82)	0.04 (± 0.03)	0.20 (± 0.21)
		***	**	*	
PO_4-P	24 h	0.55 (± 0.43)	0.41 (± 0.38)	0.02 (± 0.04)	0.13 (± 0.15)
		**	***	*	
	48 h	0.42 (± 0.07)	0.27 (± 0.06)	0.00 (± 0.00)	0.01 (± 0.01)
		***	***	n.s.	

Chapter 5

General Discussion

1. Relevance and key findings

Marine organisms provide nutritious and proteinaceous resources and play a critical role for global food security (Béné et al. 2015; Edwards et al. 2019). As the appetite for seafood grows alongside the human population and production from capture fisheries has reached its limit, aquaculture is left to fill the gap (FAO 2018). In the developing tropics, aquaculture plays an important role for livelihoods, nutrition and food security (Béné et al. 2015; FAO 2020; Irwin et al. 2020). Here, the sector is dominated by small-scale and often rural operations (Brugere et al. 2021).

In response to environmental concerns and regulations, space limitations and economic optimization, aquaculture in Western countries is projected to move from coastal waters to large off-shore operations or to closed, contained and highly intensive production systems on land (Martins et al. 2010; Badiola et al. 2012; Gentry et al. 2017; Barillé et al. 2020). Both production modes are suitable for the implementation of multi-trophic systems, which are seen as a key component for growing aquaculture production without an increased environmental footprint (Knowler et al. 2020). Large-scale integrated multi-trophic aquaculture (IMTA) operations can be integrated in multi-use offshore areas with wind farms to avoid competition for coastal space (Buck et al. 2018). In RAS, research to date has focused on freshwater aquaponics and been lagging behind for tropical marine species that can be cultivated in inland or coastal areas of developing countries (Rakocy 2012; Boxman 2015; Lamprianidou et al. 2015; Chary et al. 2020). This area needs more research to develop aquaculture sustainably and equitably (Knowler et al. 2020; Brugere et al. 2021).

Integrated aquaculture is widely practiced as freshwater aquaculture combined with agricultural crops or livestock (Lu and Li 2006; Birhanu and Natarajan 2019) or as extensively managed inland or coastal multi-trophic ponds (Pucher et al. 2014b; Wang and Lu 2015; Partelow et al. 2018). Under pressure from population growth and climate change, such systems will however need to adapt further to use space and resources efficiently while improving production output (Ahmed and Glaser 2016; Henriksson et al. 2017; Tran et al. 2017). At the same time, traditional integrated agriculture-aquaculture systems may favor the emergence of antimicrobial resistance, especially under rising temperatures (Reverter et al. 2020a). As an optimized version of such integrated aquaculture systems, IMTA has the potential to meet the sector's challenges.

This thesis provides an example of IMTA with edible tropical species at three different trophic levels. Chapter 2 shows how a popular fish (milkfish) can be cultivated with minimal water exchange in a low-technology RAS alongside a high-value deposit-feeder and an edible halophytic plant to allow the efficient reuse of water and dissolved nutrients under controlled conditions.

The research presented here further investigated how the use of extractive species can be modified and adjusted to improve the recovery of nutrients from aquaculture effluents (**Chapter 3 and 4**). It provides an example of rearing the sea cucumber *Holothuria scabra* on sludge from milkfish aquaculture and shows that final biomass production and nutrient uptake are improved by adding bagasse, a readily available agricultural waste product (**Chapter 3**). For the first time, nitrogen uptake from aquaculture effluents was traced by *Sesuvium portulacastrum*, showing superior nitrogen recovery in hydroponic cultivation compared to sand substrate (**Chapter 4**).

2. Synthesis of key findings

The research presented in this thesis demonstrates how a high value deposit-feeding sea cucumber species and an edible halophyte can be integrated as extractive species with fed finfish in a RAS (**Chapter 2**). The closed RAS operated without water exchange and without investing into maintenance or space for a nitrifying/denitrifying biofilter. Concentrations of ammonia, nitrite and nitrate were kept consistently low and below harmful levels and good biomass production was achieved. The halophyte *S. portulacastrum* removed nitrogen from the system and assimilated it into harvestable biomass. Juvenile sea cucumbers *H. scabra* were cultivated on sediment and their tanks double-functioned as a settlement basin for solid waste. The sand also likely provided substrate for bacterial nitrification-denitrification activity.

The juvenile sea cucumbers however showed little biomass gain and their contribution to nitrogen recovery in the system was therefore minor, although they may have improved sediment-based nutrient cycling processes. It was further investigated if the growth and assimilation of sludge-derived nitrogen could be improved through the addition of carbon (**Chapter 3**). Carbon amendments have previously been shown to improve the growth of sea cucumbers in microbial-deposit-feeder remediation systems (Robinson et al. 2019). The effect of adding lab-grade microcrystalline cellulose was compared to bagasse, an agricultural waste product. Bagasse significantly improved sea cucumber final biomass, which cellulose failed to do. Stable isotope analysis of feed components and sea cucumber tissues indicated that this improved growth was not due to direct assimilation of nutrients from bagasse, but a higher assimilation of sludge-derived nutrients. The carbon amendments also decreased concentrations of dissolved nitrate in the systems, indicating that they had the desired effect of stimulating microbial growth leading to immobilization of DIN into microbial biomass.

In integrated aquaculture or aquaponics, a high biomass ratio of extractive vs. fed species is required to ensure complete nutrient uptake (Rakocy 2012). If space for plant cultivation is limited, the approach may need to find a balance between nitrogen removal and recycling into usable biomass. The biomass of *S. portulacastrum* in

Chapter 2 was about 20 % of what would be required for complete nitrogen assimilation. The bioremediation potential of the halophytes was further investigated by comparing nitrogen removal and uptake by the plants in hydroponics and sand substrate (**Chapter 4**). Removal of dissolved nutrients from the water was higher in sand substrate. After 48 h, nutrient removal in hydroponics was however not significantly lower while uptake of DIN by the plants was 17 times higher. This highlights the ability of *S. portulacastrum* in this cultivation mode to both remove nitrogen from effluent while also minimizing loss of the essential nutrient.

In the framework of this thesis, we provide two examples of the sea cucumber *H. scabra* as a tropical extractive species for IMTA (**Chapter 2 and 3**). As an extractive species integrated in a low-technology IMTA RAS, around 0.5 % of the nitrogen supplied in fish feed was recovered as sea cucumber biomass (**Chapter 2**). By supplementing aquaculture sludge with bagasse or cellulose, the feed-derived nitrogen converted into sea cucumber biomass was 3.2 to 3.8 times higher than when sea cucumbers were fed sludge alone (**Chapter 3**). Presumably, carbon amendments could thus have been applied in the closed-recirculation system of **Chapter 2** to increase nitrogen recovery to 1.9 % (if considering a 3.8-fold increase), 3.5 % (if considering the highest recovery observed) or even higher if better growth at the lower initial densities is assumed. Even though nitrogen content in surface sediments in the RAS was low, the maximum growth rate of the sea cucumbers was comparatively high, adding to the body of evidence on biomass density as a limiting factor (Battaglene et al. 1999; Robinson et al. 2015; Mathieu-Resuge et al. 2020). In a few cases, much higher biomass densities have however been reached without adverse effects on sea cucumber health (Robinson et al. 2015, 2019). It remains to be tested what density of sea cucumbers could be reached at higher levels of fish production, when more solid waste can accumulate in the sediment. The higher nitrogen accumulation in the sediment and lower dissolved nitrogen concentrations in treatments amended with bagasse or cellulose could further control water quality and improve sea cucumber growth.

Final NO_3^- concentrations in the IMTA RAS reached a plateau around 17 mg N l^{-1} (**Chapter 2**). Comparable DIN concentrations were measured in the milkfish RAS to which systems planted with *S. portulacastrum* were exposed (**Chapter 4**). The observation that proportional N uptake by the halophyte in hydroponic cultivation was consistent in both experiments, regardless of the type of effluent, tracer or DIN concentration (**Chapter 4**), could indicate that ^{15}N uptake is representative of overall DIN uptake rates. Overall DIN uptake (N_{uptake}), can be estimated based on the uptake of ^{15}N tracer, ($^{15}N_{excess}$, see **Chapter 4** for details), as

$$N_{uptake} = (^{15}N_{excess} \text{ x } N_{Plant}) / f$$

where N_{Plant} = Total N measured in plant tissue (µg)

f = concentration of ^{15}N added as label (µmol L^{-1}) / concentration of total N in the irrigation water (µmol L^{-1}).

This would result in an hourly uptake rate of approximately 0.075 mg N g^{-1} dw in the shoots of *S. portulacastrum*. Considering a daily feeding of 4 % of fish body weight and the retention of 34 % feed-derived N observed (**Chapter 2**), this results in a total halophyte dry weight of 511 g needed to take up the DIN produced by 1 kg of milkfish. At a ratio of wet to dry weight of 9:1 (data not presented), this is equivalent to 4.6 kg plant wet weight, and would thus confirm the estimated ratio of 5:1 plant to fish biomass based on the proof-of-concept (**Chapter 2**). The assumptions on which this calculation is based need to be confirmed to prevent overestimation of DIN recovery by *S. portulacastrum*. Further isotope labeling studies comparing between different forms of dissolved N can provide valuable information in the form of specific uptake rates. It is however clear that potential sites of denitrification, such as biofilm, should be limited in hydroponic tanks if nitrogen recovery is to be maximized, The plant biomass that can be produced hydroponically may be limited by the available space, so a system with multiple modes of plant cultivation, both in substrate and in hydroponics, could be used to prevent the accumulation of dissolved nitrogen in the water of the RAS.

The RAS IMTA tested in **Chapter 2** had three trophic levels. Decrease of suspended particles when water was not circulated showed that the sea cucumber tank double-functioned as a settling basin. Alternatively, and to achieve improved efficiency with four trophic levels, the integration of filter-feeders, such as bivalves or ascidians can transfer filtered organic matter in the water as faeces or pseudoefaeces to the sediment, where it becomes available to bacteria and/or deposit-feeders (Slater and Carton 2009; Lin et al. 2016).

3. Role of microbes

In the framework of this thesis, methodological- and time constraints did not allow for microbial analysis. All conclusions drawn on the role of microbiota thus remain speculative and are based on indirect information or research of similar systems by others. Target species in aquaculture, even more so than terrestrial livestock, are permanently in close contact with their surrounding medium and its microbes, directly shaping their internal microbiome, which is an important driver of animal health (Huang et al. 2018). Microbial communities in aquaculture systems thus influence a range of factors from water quality over biomass production to disease outbreaks (Zhou et al. 2009; Huang et al. 2018). In this study, the role of microbial processes was likely evidenced in the form of a) nitrification activity in sea cucumber tanks when water circulation was interrupted (**Chapter 2**), b) lower nitrogen concentrations in treatments receiving carbon amendments to aquaculture sludge (**Chapter 3**) and c) considerable nitrogen removal, even in unplanted systems receiving milkfish effluents (**Chapter 4**). In future research, the role of microbes needs to be examined for a more complete understanding of system dynamics and to determine how probiotics could further improve nutrient recovery by extractive species.

3.1. Probiotics

Under the pressure of stressful and disease-prone intensive cultivation, the increased emergence of antibiotic resistance and the need for more sustainable and environmentally friendly aquaculture, the use of bioactive compounds and probiotics provides alternatives to prophylactic antibiotics and other chemotherapy (De et al. 2014; Reverter et al. 2020b). Probiotics are whole or parts of microorganisms such as bacteria, microalgae and yeast which, when administered in adequate amounts, confer health benefit to the host, including improved disease resistance, growth, feed utilization and adequate stress response (Marrifield et al. 2010; Morelli and Capurso 2012). Often administered via feed to the gastrointestinal tract, they can improve the intestinal microbial balance (Fuller 1989; Gatesoupe 1999). Especially in aquatic animals, whose internal microbiota constantly interacts with the surrounding medium, their application can also aim at improving the quality of the ambient environment to support the health of the target species (Verschuere et al. 2000; Hai 2015). In the environment, beneficial microorganisms fulfill important functions of preventing disease outbreaks, improving water quality and producing bioactive compounds that improve growth and feed use efficiency in aquaculture (Zhou et al. 2009). In its most basic form, this is practiced widely in traditional “green water aquaculture”, where fertilization of pond water promotes the growth of microalgae, bacteria, fungi, protozoa and zooplankton that provide feed for filter-feeding fish and shrimp while at the same time inhibiting the growth of pathogens (Neori 2011; Yarnold et al. 2019). Optimized in the so-called “bio-floc technology”, the concept is applied to promote the assimilation of inorganic nitrogen by heterotrophic bacteria, which remove nitrogen from the water and convert it into microbial protein (Schneider et al. 2005; De Schryver and Verstraete 2009).

3.2. Enabling the prevalence of probiotics

Although applied as a strategy in aquaculture for decades, effectiveness of probiotics is not guaranteed and depends on the appropriate microorganisms, correct dosage and duration of treatment and the successful colonization of either the gastrointestinal tract and/or the culturing environment (Hai 2015). Simply adding commercial microbial products to the water may fail to have any positive effect if the environmental conditions do not enable the probiotics to prevail over the native bacterial community (Zhou et al. 2017; Zheng et al. 2019). Researchers and aquaculture practitioners are becoming increasingly aware of how water parameters drive microbial community compositions and how sometimes modifying even a single factor such as pH or salinity, can control their community composition or prevent a disease outbreak (Hou et al. 2017a; Alfiansah et al. 2020). C:N ratios can for example be modified by dissolving glucose as a carbon source and alter the bacterial community composition to boost their positive effect on water quality (Zheng et al. 2018). Deng et al. (2020) found that feeding formulated feed instead of trash fish to crabs in an IMTA system with bivalves and shrimp had a similar effect on water quality, improved animal growth and achieved higher cost efficiency. The higher carbohydrate content of the formulated feed influenced the microbial community in the pond water toward pathways of carbon and lipid metabolism. This mechanism may well have contributed to the lowered NO_3^- concentrations in the treatments receiving bagasse or cellulose of the present thesis (**Chapter 3**).

3.3. An ecosystem approach to microbes in IMTA

Since scientific methods have been giving us insight into the bacterial world, we are able to see plants and animals not only as individual organisms, but also as the holobiont, the system that they constitute with their microbial communities (McFall-Ngai et al. 2013). It is then only a logical next step to consider aquaculture not only as cultivating one or more aquatic organisms, but also as “cultivating ecosystems” (Bartelme 2018). Further growing our knowledge on the drivers behind the microbiome in aquaculture systems and if and how they can be manipulated brings us closer to managing disease resistance and productivity based on ecological principles (De Schryver and Vadstein 2014). Besides abiotic factors, the biotic components of IMTA systems, namely the cultivation of extractive species, could provide a strategy to harness not only the direct function of those species, but also the benefits of their associated microbiome or their influence on the microbial community in water or sediment. IMTA systems can inhibit the growth of potential pathogens and increase beneficial bacteria (Lin et al. 2016). Compared to monoculture systems, IMTA systems with shellfish and seaweed were found to prevent the development of drug-resistance and maintain the integrity of the microbial community in the seawater (Ying et al. 2018). The role played by extractive species in controlling the circulating gene flow between aquaculture systems, the environment and human consumers still needs to be investigated in more depth (Ying et al. 2018).

Bacterial community imbalances threaten and limit sea cucumber aquaculture and the complex factors driving the pond microbiota are of great research interest, as is the search for dietary probiotics (Deng et al. 2009; Chi et al. 2014; Xu et al. 2019). Sediment feeding sea cucumbers are in constant exchange with the sediment and the overlying water column, depend on detritus and microbial biomass and in turn influence the microphytobenthos and bacterial abundance (Yingst 1976; Moriarty 1982; Uthicke 2001; Yamazaki et al. 2019). Via their fecal pellets, sea cucumbers enrich sediments with their heterotrophic gut microbiota and thus support decomposition of organic matter not only with their own digestion, but also by supporting fermentation and mineralization in the sediment (MacTavish et al. 2012; Plotieau et al. 2013; Yamazaki et al. 2019).

In the cultivation of plants, growth-promoting rhizobacteria have positive effects on plants both in soil and hydroponic culture through nitrogen fixation, regulating plant hormones, reducing stress and outcompeting pathogens (Lee and Lee 2015). Plants in sediments as well as in hydroponics also influence the microbial community. The roots of halophytes grown in aquaponics were recently found to harbor a rich community of nutrient cycling bacteria involved in nitrogen fixation, denitrification and mineralization. The diversity of the bacterial community differs depending on plant species, thus influencing the potential to treat aquaculture effluents (Oliveira et al. 2020).

Our knowledge base about microbial processes in aquaculture in general and RAS in particular is growing. For example, while *Nitrosomonas* and *Nitrobacter* are commonly considered the main nitrifiers, this essential role has also been found to be fulfilled by different taxa (Hovanec and Delong 1996; Bartelme et al. 2019). The fact that the IMTA sector is still largely dominated by natural scientists has been cited as a reason why economic evaluations are lacking and market proliferation in Western countries has been slow (Chopin 2017; Knowler et al. 2020). However, this continuous research effort is also an opportunity to fully understand the complex processes that influence integrated aquaculture and reach a point where a system can be managed holistically to reach its most efficient and sustainable potential before its widespread application.

4. Stable isotope analysis in IMTA research

The research for this thesis applied two different approaches in stable isotope analysis to examine the assimilation of aquaculture-derived nutrients by extractive species:

analyzing natural abundance stable isotope variations (**Chapter 3**) and isotopic labeling (**Chapter 4**). Stable isotope analysis lends itself well to reconstructing diets and evaluating feed use in aquaculture (Focken 2014; Xu et al. 2017). While in an ecosystem context, accounting for all dietary sources available to a consumer can be challenging if not impossible (Phillips et al. 2014), the limited number of feed sources available to the sea cucumbers in this thesis (**Chapter 3**), gave the opportunity to examine the resource use of an animal, which exploits a diverse and complex mixture of materials in its natural habitat (Uthicke 2001; Plotieau et al. 2014). Furthermore, to trace food sources being metabolized into a consumer's tissue, the isotopic signatures of different sources have to be sufficiently distinct from each other and the large differences in $\delta^{13}C_{org}$ between aquaculture sludge, bagasse and cellulose in this thesis allowed to distinguish between the feed components (**Chapter 3**). Not considering a single source can introduce substantial bias into the output of an isotopic mixing model (Phillips et al. 2014) and therefore the organic carbon that was introduced to the experimental tanks via the sediment, albeit at very low concentrations, was also considered.

4.1. Sea cucumber-specific trophic fractionation factor

Stable isotope analysis has been used to evaluate feed ingredients and feeding ecology of sea cucumbers in an aquaculture context (Guo et al. 2016; Wen et al. 2017). In the majority of these feeding experiments, isotopic values were adjusted by the commonly used value of 1 ‰ (Gao et al. 2011; Sun et al. 2013; Hou et al. 2017b; Mondal and Kunzmann 2018; Song et al. 2018). Isotopic fractionation can, however, vary widely between different tissues, with enrichments as high as 6 ‰ compared to the diet (Peterson and Fry (1987). Trophic enrichment also varies depending on an animal's environment (terrestrial, freshwater or marine), taxon, trophic level, sampled tissue, and physiological factors such as metabolic rate and nitrogenous excretion (Pinnegar and Polunin 1999; McCutchan et al. 2003; Vanderklift and Ponsard 2003; Dalerum and Angerbjörn 2005; Caut et al. 2009). Using appropriate discrimination factors, ideally by experimentally determining specific values for the study context and target species, is thus considered best practice in the use of isotopic mixing models in trophic studies (Phillips et al. 2014). The small isotopic difference between organic carbon in aquaculture sludge and the initial sediment reported in this thesis made it possible to calculate a trophic discrimination factor based on the treatment where the two were the only possible feed sources for *H. scabra* (**Chapter 3**).

To our knowledge, this is the first time, that a context-specific TEF has been applied to evaluate feeding in *H. scabra* (**Chapter 3**). Specific TEF have indeed been determined for other sea cucumbers and support the notion that isotopic values in sea cucumber tissue would be enriched by more than 1 ‰. *Apostichopus japonicus* fed formulated algae-based feed had $\Delta^{13}C$ ranging from 1.2 to 3.6 ‰ for the whole body wall and from 0.9 to 2.2 ‰ for the intestine with enrichment positively related to the animal's size (Sun et al. 2012). A different feeding experiment determined a $\Delta^{13}C$ of 2.6 ‰ in acid treated body wall of *A. japonicus* (Yokoyama 2013). Watanabe et al. (2013) reared juvenile *H. scabra* solely on diatoms and found $\Delta^{13}C$ of 4.2 ‰ for whole and 3.2 ‰ for acid treated body wall and 2.2 ‰ for the intestine. Most recently, *Cucumaria frondosa* kept for 45 months in an IMTA tank system receiving effluents from salmon tanks were found to have $\delta^{13}C$ signatures enriched by approximately 5.0 ‰ in muscle and 2.7 ‰ in intestine compared to the salmon waste (Sun et al. 2020). A comparison between the output of the mixing model with values corrected using the specific TEF (**Chapter 3**) or the literature value of 1 ‰ illustrates how adjusting the data with a value from the literature without considering its suitability would lead to entirely different results and conclusions (Fig. 1)

As trophic enrichment further varies with the diet quality and protein content, the most accurate approach to feeding studies would require determining individual TEF for each feed ingredient and diet, which is often not realizable (Webb et al. 1998; Gaye-Siessegger et al. 2004a). Even then, some variability due to an individual's metabolism and feed intake, is unavoidable, highlighting the usefulness of Bayesian mixing models that allow incorporating uncertainty around the TEF (Gaye-Siessegger et al. 2004b; Phillips et al. 2014; Stock and Semmens 2016). The TEF used in the mixing-model of *H. scabra* tissue in this thesis (**Chapter 3**) appears to be the most accurate available at this time to determine dietary incorporation by *H. scabra*, and through the use of mixing model MixSIAR, a standard deviation could be incorporated. As the aquaculture production of sea cucumbers increases, so does the effort to study appropriate aquafeeds (FAO 2020). If the potential of stable isotope analysis is to be harnessed in this context, the determination of class- or species-specific TEF is a necessary step.

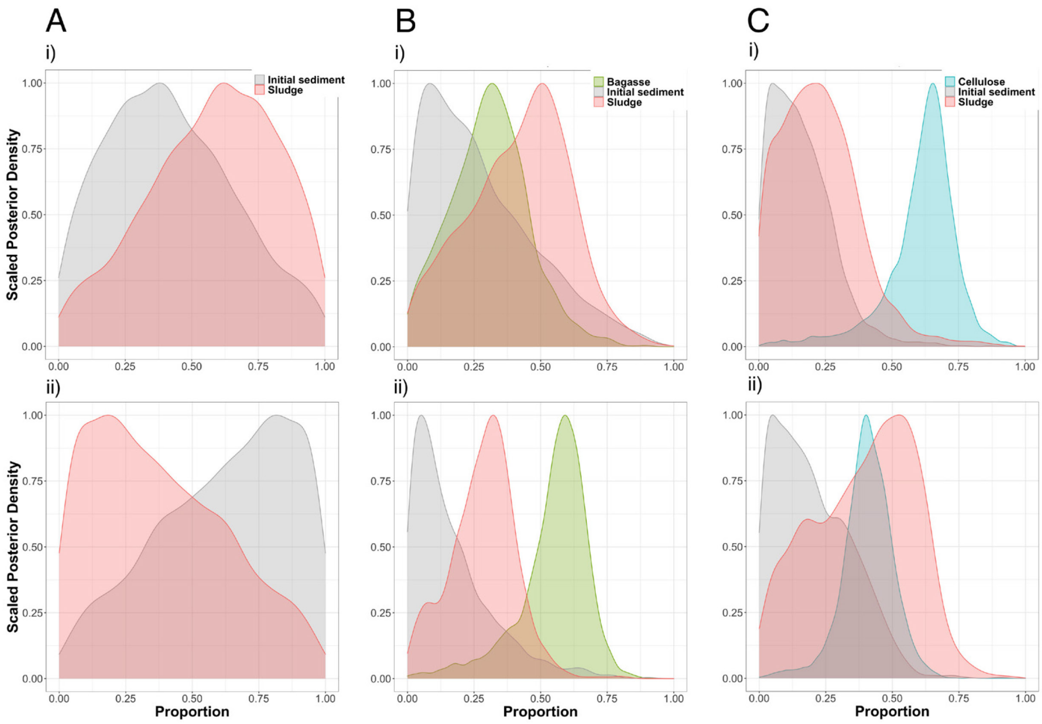

Fig. 1: Output of the MixSIAR mixing model based on $\delta^{13}C_{org}$ of sea cucumber internal organs (**Chapter 3**). Data were corrected using trophic fractionation factor i) 2.56 ± 0.96 ‰ and ii) 1.00 ± 0.00 ‰. See Chapter 3 for details on treatments A, B and C.

4.2. Advanced nutrient tracing through stable isotope labeling

Investigating sea cucumber nutrition through stable isotope analysis is complicated due to the potential influence of microbial processes on stable isotope signatures. Bacterial biomass can directly constitute a major food source for sediment-feeding sea cucumbers such as *H. scabra* and organic matter provided as feed may act as a substrate for heterotrophic bacteria (Plotieau et al. 2014). Generally, the isotopic signature of heterotrophic microorganisms is very close to the ratios of their food sources (Blair et al. 1985; Coffin et al. 1990; Boschker et al. 1999). Strong depletion in $\delta^{13}C$ in cells has however been observed during the exponential growth phase as long as C was not limiting and resulted in fractionation by as much as -6.12 ‰ in detritivores relative to detritus (McGoldrick et al. 2008). While potentially significant for studies on carbon supplementation (**Chapter 3**), where C should not be the limiting nutrient, it is unlikely that a strong influence by bacterial discrimination against heavier isotopes on the isotopic signature of the sea cucumber tissue occurred, because 1) no strong depletion in the sea cucumbers relative to the feed was seen and 2) nutrient recycling as occurring between organic matter, microbes and deposit-feeders would reduce the effect of isotopic separation (Coffin et al. 1990; Uthicke 2001; McGoldrick et al. 2008).

Stable isotope labeling can disentangle nutrient flows and avoid some of the challenges of natural abundance stable isotope studies. It is unlikely that natural abundance stable isotopes would create detectable differences over the short time period used to measure nitrogen uptake in this thesis (**Chapter 4**) and since *Sesuvium portulacastrum* had been watered with milkfish effluents for weeks, it was probably already in isotopic equilibrium. Adding the ^{15}N ammonium nitrate tracer thus allowed artificially creating significant changes in the isotopic signature. It would also allow incorporating the role of bacteria and the identification and quantification of nitrogen transformation pathways in water or sediments (Christensen et al. 2000; Boschker and Middelburg 2002; Robertson et al. 2019). Applied in a closed RAS (**Chapter 2**) it could provide increased certainty over the fate of nitrogen removed from the water. In experiments with deposit-feeders in integrated aquaculture, it could be used to trace dissolved inorganic nitrogen and investigate if the lower nitrate concentrations observed (**Chapter 3**) are indeed due to better immobilization in the treatments with cellulose and bagasse. Uptake of tracer into sea cucumber tissue would also enable a more thorough comparison of the assimilation of nitrogen from aquaculture sludge where natural abundance values are inconclusive.

The method would however only give insights into the fate of dissolved nutrients. To apply the isotope label in a way that more closely mimics N and/or C derived from aquafeeds in intensive systems is more challenging. Applied as ^{15}N ammonium sulphate to mimic fertilization in greenwater ponds, stable isotope labeling has been used to study efficiencies in semi-intensive polyculture (Pucher et al. 2014a; Pucher and Focken 2017). By enriching *Microcystis*, ^{15}N was incorporated in organic form into fish feed and showed assimilation into fish tissue and sedimentary detritus, but conclusions are specific to the digestibility and assimilation of the cyanobacteria (Wang et al. 2015).

During the research for this thesis, an attempt was made to produce labeled fish feces by feeding ^{15}N-*Spirulina* incorporated in feed to juvenile milkfish (data not presented) as previously applied to determine protein use in fish (Beltràn et al. 2009; Felip et al. 2015). While it increased the $\delta^{15}N$ of the milkfish from 10.8 ± 0.0 ‰ to 76.0 ± 20.5 ‰ the isotopic signal of the feces remained at 12.0 ± 0.1 ‰, showing good incorporation of *Spirulina,* but not providing an enriched isotopic signal to facilitate tracing of feed-derived N into extractive species. Felsing et al. (2006) produced ^{15}N-labeled fishmeal by feeding *Ulva* cultivated in enriched water to trout and subsequently producing labeled fishmeal from the trout. The resulting feed with a $\delta^{15}N$ of almost 300 ‰ was fed to red snapper in sea cages and the signal could be picked up in suspended particles and primary producers as well as deposit-feeders under the cages within 14 days. The considerable effort necessary to produce isotopically labeled aquafeeds seem to be a hurdle to their potential widespread use, but they would produce valuable insights into the fate of solid as well as dissolved fractions of effluents in the environment or culture system.

5. Economics of IMTA

An economic assessment or profitability study of an aquaculture operation with the investigated species was not in the scope of the research presented in this thesis. Not mentioning this point at all would however be delusive. In the case of freshwater aquaponics, decades of research and experiences from small-scale practice have shown theoretically promising results, but have still not resulted in widespread commercial success and the majority of studies does not evaluate economic feasibility (Greenfeld et al. 2018). In the case of saline aquaculture, it has also been challenging to translate IMTA experiments into profitable enterprises as small operations are often not competitive and vulnerable to changes in the market prices of the products (Martínez-Espineira et al. 2015; Greenfeld et al. 2018; Knowler et al. 2020). Cases of successful ventures do however exist, showing that research in this field is worthwhile (Nobre et al. 2010; Chopin et al. 2012; Xiang 2015; Greenfeld et al. 2018).

The IMTA RAS approach presented here (**Chapter 2**) and the extractive species in focus (**Chapter 3 and 4**) are examples and may not be appropriate in every tropical context. The inherent diversity of the aquaculture sector provides vast scope for innovations and manifold solutions (Estim et al. 2020; Houston et al. 2020). The success of IMTA will thus not be a one-size-fits-all approach and needs to take into account substantial differences between affluent and poor regions (Knowler et al. 2020). Whereas in Western countries, where the market is dominated by large operations, the small size of an aquaculture farm can be the reason why an IMTA design is not profitable, it is especially these small-scale ventures that account for much of the valuable contribution of aquaculture to nutrition in the developing tropics (Greenfeld et al. 2018; Knowler et al. 2020; Brugere et al. 2021). This in turn provides an opportunity for IMTA to benefit smallholders directly with waste reduction and/or increased food security (Knowler et al. 2020). The diversification of crops in these systems and their multiple harvest cycles lead to increased and/or more steady income and improve household nutrition and resilience (Haque et al. 2015; Tran et al. 2020). They can have further positive effects of social learning in the community and women participation (Haque et al. 2015; Tran et al. 2020). Integrating halophytes is an opportunity to produce an additional edible crop or animal feed in areas of freshwater shortage (Glenn et al. 1999; Lokhande et al. 2009; Ksouri et al. 2011).

Adopting new, more sustainable aquaculture practices comes with starting investments and a need for new knowledge and skills (Greenfeld et al. 2018; Henriksson et al. 2019). Governments and policy makers can support proliferation of the industry through rules and regulations that not only allow IMTA, but also provide a competitive advantage to operations that use it to prevent pollution and use space more efficiently over aquaculture that externalizes its negative impacts (Kleitou et al. 2018; Ellis and Tiller 2019). There is also an opportunity for governments, international organizations and research institutions to provide financial support, capacity building and benefit sharing (Knowler et al. 2020; Brugere et al. 2021). Extension services, loans and subsidized seed and feed, as already provided by governments in some places to improve aquaculture output, can be linked to helping IMTA operations lift off (Senff et al. 2018). A possible application at a larger scale can be in IMTA parks or bays, supported by government-backed credits, that provide space and technical help for multiple practitioners contributing to one large multi-trophic system (Xiang 2015; Knowler et al. 2020). Here sea cucumbers as extractive species can be integrated for the grow-out in pens, similar to sea-ranching already practiced in countries such as Madagascar, China and the Philippines (Purcell et al. 2012; Robinson and Pascal 2012; Robinson and Lovatelli 2015).

Political will and administrative agendas alone are not enough to change the behavior of aquaculture practitioners and shape the sustainable development of the sector (Brugère et al. 2019; Milewski and Smith 2019). Distributing information about IMTA is an important step to educate practitioners and other stakeholders, address the public perception of re-using fish feces for food production and raise awareness about its role in sustainable aquaculture (Fig. 2) (Alexander et al. 2016; Gunning et al. 2016; Correia et al. 2020; Knowler et al. 2020).

The economic viability of IMTA RAS could be improved through value-added products. Edible halophytes are suitable extractive plants for integration into multi-trophic RAS (Pinheiro et al. 2020; Senff et al. 2020). Although demand is not as high as for many freshwater vegetables, their comparatively high price as a gourmet food can make their production in RAS profitable (Barreira et al. 2017; Castilho-Barros et al. 2018). While in some places, demand is satisfied by the collection of plants from the wild, it has been shown recently that the fatty acid and lipid profile of edible coastal halophytes from integrated aquaculture are superior to wild harvests (Maciel et al. 2020). Such arguments further underline the value of biomass production in IMTA, especially in terms of recycling fatty acids and amino acids that are specific to and especially valuable in aquafeeds.

Fig. 2: Showcasing the IMTA RAS of milkfish, sea cucumbers and halophytes at the hatchery of the Zanzibar Mariculture Project (**Chapter 2**) as part of a university course on sustainable aquaculture. Picture credit: Holger Kühnhold.

Additional value of halophytic plants could further be an application as feed extracts in aquafeeds to achieve full recycling of valuable nutrients. The use of bioactive compounds and extracts from plants has various beneficial effects in aquaculture production (Reverter et al. 2020b). Research indicates that species of halophytes are suitable as fodder for ruminant livestock and can reduce the production of greenhouse gases by the animals by suppressing fermentation in the rumen (Elahi et al. 2019). Future studies should explore this as a possible avenue for their application.

6. Conclusions and implications for future research

This thesis contributes to the necessary advancement of intensified multi-trophic aquaculture in tropical countries by providing the first proof-of-concept of a closed RAS in which sea cucumbers and halophytes are integrated as extractive species with fed finfish (**Chapter 2**). Through different methodologies of stable isotope analysis, waste nutrient assimilation by sea cucumbers and halophytes was further investigated and it was examined how nitrogen recycling can be improved to increase system efficiency and decrease nutrient concentrations in effluent. For the first time, a context-specific trophic enrichment factor was applied in a mixing-model to evaluate resource use by *Holothuria scabra.* Natural abundance stable isotope analysis showed good assimilation of carbon and nitrogen from milkfish sludge by *Holothuria scabra*. Biomass production, nitrogen recovery and concentrations of dissolved nitrogen were further improved through the addition of carbon (**Chapter 3**). As an agricultural waste product, bagasse was identified as a suitable carbon supplement to improve cultivating this high-value sea cucumber species on solid aquaculture waste. Stable isotope labeling demonstrated nitrogen uptake efficiency by *Sesuvium portulacastrum* and allowed differentiation between nitrogen assimilated in the plant and nitrogen removal by the cultivation system (**Chapter 4**). Hydroponic halophyte production, as opposed to cultivation in sand, was clearly identified as the mode in which more nitrogen is recovered and harvested as edible plant biomass. With the information and results obtained in this thesis, the experimental RAS can be sustainably and efficiently intensified. In addition, background is provided that allows for the integration of extractive organisms into other types of aquaculture systems and in different settings. Although not specifically investigated, the importance of microbial processes and their role in nutrient cycling was evident, as well as the connectivity of cultivated target species and their associated microbiota.

The findings of this thesis hint at the complexity of the interplay between detritivores and plants and associated microbial communities. Future studies should investigate the respective role of these components in integrated bioremediation systems. In this context, the potential of species at different trophic levels to influence RAS performance as holobionts requires further investigation as does the possibility to improve nutrient recovery by extractive species through probiotics. Isotopic tracing of carbon and nitrogen – dissolved and particulate, organic and inorganic – in aquaculture effluents, as might be achieved through isotopically labeled aquafeeds, would give valuable insights into the fate of these essential nutrients in integrated aquaculture and help identify avenues to optimize their recovery. Finally, to move towards application of IMTA RAS, research on their commercial viability is crucial and needs to incorporate considerations for equitable knowledge transfer and sustainable use in the developing tropics.

References

Almuktar SAAAN, Abed SN, Scholz M (2018) Wetlands for wastewater treatment and subsequent recycling of treated effluent: a review. Environ Sci Pollut Res 25:23595–23623.

Ballester-Moltó M, Sanchez-Jerez P, Cerezo-Valverde J, Aguado-Giménez F (2017) Particulate waste outflow from fish-farming cages. How much is uneaten feed? Mar Pollut Bull 119:23–30.

Barak Y, Cytryn E, Gelfand I, Krom M, Van Rijn J (2003) Phosphorus removal in a marine prototype, recirculating aquaculture system. Aquaculture 220:313–326.

Bardgett RD, Streeter TC, Boi R (2003) Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. Ecology 84:1277–1287.

Barreira L, Resek E, João M, Isabel M, Pereira H, Bandarra N, Moreira M, Varela J, Custódio L (2017) Halophytes: Gourmet food with nutritional health benefits? J Food Compos Anal 59:35–42.

Barrington K, Ridler N, Chopin T, Robinson S, Robinson B (2010) Social aspects of the sustainability of integrated multi-trophic aquaculture. Aquac Int.

Bartelme RP, Smith MC, Sepulveda-Villet OJ, Newton RJ (2019) Component Microenvironments and System Biogeography Structure Microorganism Distributions in Recirculating Aquaculture and Aquaponic Systems. mSphere 4:1–15.

Bischoff AA, Fink P, Waller U (2009) The fatty acid composition of *Nereis diversicolor* cultured in an integrated recirculated system: Possible implications for aquaculture. Aquaculture 296:271–276.

Blackburn TH, Blackburn ND (1992) Model of nitrification and denitrification in marine sediments. FEMS Microbiol Lett 100:517–521.

Boestfleisch C, B.Wagenseil N, Buhmann A, Seal CE, MerrettWade E, Muscolo A, Papenbrock J (2014) Manipulating the antioxidant capacity of halophytes to increase their cultural and economic value through saline cultivation. AoB Plants 6:1–16.

Boxman SE, Nystrom M, Capodice JC, Ergas SJ, Main KL, Trotz MA (2017) Effect of support medium, hydraulic loading rate and plant density on water quality and growth of halophytes in marine aquaponic systems. Aquac Res 48:2463–2477.

Brix H (1994) Functions of macrophytes in constructed wetlands. Water Sci Technol 29:71–78.

Brown JJ, Glenn EP, Fitzsimmons KM, Smith SE (1999) Halophytes for the treatment of saline aquaculture effluent. Aquaculture 175:255–268.

Buhmann A, Papenbrock J (2013a) An economic point of view of secondary compounds in halophytes. Funct Plant Biol 40:952–967.

Buhmann A, Papenbrock J (2013b) Biofiltering of aquaculture effluents by halophytic plants: Basic principles, current uses and future perspectives. Environ Exp Bot 92:122–133.

Buhmann A, Waller U, Wecker B, Papenbrock J (2015) Optimization of culturing conditions and selection of species for the use of halophytes as biofilter for nutrient-rich saline water. Agric Water Manag 149:102–114.

Capone DG, Kiene RP (1988) Comparison of microbial dynamics in marine and freshwater sediments: Contrasts in anaerobic carbon catabolism. Limnol Oceanogr 33:725–749.

Carton-Kawagoshi RJ, Elle BJ, Corre V, Satoh S, Notoya M, Fujita D (2014) Low water exchange *Gracilariopsis bailiniae* Zhang & B.M. Xia culture in intensive milkfish culture effluents: Effects of seaweed density on seaweed production and effluent treatment. Aquac Int 22:573–584.

Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens GE (2009) Controlling Eutrophication: Nitrogen and Phosphorus. Science (80-) 323:1014 LP – 1015.

Cordell D, Drangert JO, White S (2009) The story of phosphorus: Global food security and food for thought. Glob Environ Chang 19:292–305.

Cronk JK, Fennessy MS (2016) Wetland plants:biology and ecology. CRC PRESS.

Custódio M, Maciel E, Domingues MR, Lillebø AI, Calado R (2020) Nutrient availability affects the polar lipidome of *Halimione portulacoides* leaves cultured in hydroponics. Sci Rep 10:1–13.

Custódio M, Villasante S, Cremades J, Calado R, Lillebø AI (2017) Unravelling the potential of halophytes for marine integrated multi-trophic aquaculture (IMTA) - A perspective on performance, opportunities and challenges. Aquac Environ Interact 9:445–460.

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. Science (80-) 321:926–929.

Diver S (2006) Aquaponics — Integration of Hydroponics with Aquaculture. ATTRA:1–28.

Erisman JW, Sutton MA, Galloway J, Klimont Z, Winiwarter W (2008) How a century of ammonia synthesis changed the world. Nat Geosci 1:636–639.

FAO (2020) The State of World Fisheries and Aquaculture 2020. Rome.

Faulwetter JL, Gagnon V, Sundberg C, Chazarenc F, Burr MD, Brisson J, Camper AK, Stein OR (2009) Microbial processes influencing performance of treatment wetlands: A review. Ecol Eng 35:987–1004.

Focken U (2008) Application of Stable Isotopes in Aquaculture Nutrition Research. In: *Avances en Nutricíon Acuícola IX. Anais do IX Simposio Internacional de*

Nutricíon Acuícola. 24-27 Noviembre. Universidad Autónoma de Nueva León, Monterrey, Nueva León, México. p 258–270.

Gao H, Matyka M, Liu B, Khalili A, Kostka JE, Collins G, Jansen S, Holtappels M, Jensen MM, Badewien TH, Beck M, Grunwal M, de Beer D, Lavik G, Kuypers MMM (2012) Intensive and extensive nitrogen loss from intertidal permeable sediments of the Wadden Sea. Limnol Oceanogr 57:185–198.

Glenn EP, Brown JJ, Blumwald E (1999) Salt Tolerance and Crop Potential of Halophytes. CRC Crit Rev Plant Sci 18:227–255.

Haberl R, Perfler R, Mayer H (1995) Constructed Wetlands in Europe. Water Sci Technol 32:305–315.

Halm H, Lam P, Ferdelman TG, Lavik G, Dittmar T, Laroche J, D'Hondt S, Kuypers MMM (2011) Heterotrophic organisms dominate nitrogen fixation in the south pacific gyre. ISME J 6:1238–1249.

Hanh TTH, Boonstra WJ (2019) What prevents small-scale fishing and aquaculture households from engaging in alternative livelihoods? A case study in the Tam Giang lagoon, Viet Nam. Ocean Coast Manag 182.

Harrison KA, Bol R, Bardgett RD (2008) Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen? Soil Biol Biochem 40:228–237.

Herbeck LS, Sollich M, Unger D, Holmer M, Jennerjahn TC (2014) Impact of pond aquaculture effluents on seagrass performance in NE Hainan, tropical China. Mar Pollut Bull 85:190–203.

Hodge A, Stewart J, Robinson D, Griffiths BS, Fitter AH (2000) Competition between roots and soil micro-organisms for nutrients from nitrogen-rich patches of varying complexity. J Ecol 88:150–164.

Hu Z, Lee JW, Chandran K, Kim S, Sharma K, Brotto AC, Khanal SK (2013) Nitrogen transformations in intensive aquaculture system and its implication to climate change through nitrous oxide emission. Bioresour Technol 130:314–320.

Hu Z, Woo J, Chandran K, Kim S, Coelho A, Kumar S (2015) Effect of plant species on nitrogen recovery in aquaponics. Bioresour Technol 188:92–98.

Huang Y, Ciais P, Goll DS, Cresto-aleina F, Zhang H (2020) The shift of phosphorous transfers in global fisheries and aquaculture. Nat Commun:1–10.

IPCC (2007) Changes in atmospheric constituents and in radioactive forcing. In: *Climate change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Solomon S et al. (Eds. . (ed) Cambridge University Press, Cambridge, p 114–143

Irwin S, Flaherty MS, Carolsfeld J (2020) The contribution of small-scale, privately owned tropical aquaculture to food security and dietary diversity in Bolivia. Food Secur.

Jia B, Tang Y, Tian L, Franz L, Alewell C, Huang JH (2015) Impact of fish farming on phosphorus in reservoir sediments. Sci Rep 5:1–11.

Jorgensen BB (1982) Ecology of the Bacteria of the Sulphur Cycle with Special Reference to Anoxic-Oxic Interface Environments. Philos Trans R Soc B Biol Sci 298:543–561.

Kadlec RH, Knight RL (1996) Treatment Wetlands. CRC Press, Boca Raton, Florida.

Kir M, Sunar MC (2018) Acute Toxicity of Ammonia and Nitrite to Sea Bream, *Sparus aurata* (Linnaeus, 1758), in Relation to Salinity. J World Aquac Soc 49:516–522.

Krom MD, Kress N, Brenner S, Gordon LI (1991) Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. Limnol Oceanogr 36:424–432.

De Lange HJ, Paulissen MPCP (2016) Efficiency of three halophyte species in removing nutrients from saline water: a pilot study. Wetl Ecol Manag 24:587–596.

Lefebvre S, Bacher C, Meuret A, Hussenot J (2001) Modelling nitrogen cycling in a mariculture ecosystem as a tool to evaluate its outflow. Estuar Coast Shelf Sci 52:305–325.

Lin Y-F, Jing S-R, Lee D-Y, Wang T-W (2002) Nutrient removal from aquaculture wastewater using a constructed wetlands system. Aquaculture 209:169–184.

Lipson D, Näsholm T (2001) The unexpected versatility of plants: Organic nitrogen use and availability in terrestrial ecosystems. Oecologia 128:305–316.

Liu X, Pu X, Luo D, Lu J, Liu Z (2019) Model assessment of nutrient removal via planting *Sesuvium portulacastrum* in floating beds in eutrophic marine waters: the case of aquaculture areas of Dongshan Bay. Acta Oceanol Sin 38:91–100.

Lokhande VH, Nikam TD, Suprasanna P (2009) *Sesuvium portulacastrum* (L.) L. a promising halophyte: Cultivation, utilization and distribution in India. Genet Resour Crop Evol 56:741–747.

Lonard RI, Judd FW (1997) The biological flora of coastal dunes and wetlands. *Sesuvium portulacastrum* (L) L. J Coast Res 13:96–104.

Luo Z, Hu S, Chen D (2018) The trends of aquacultural nitrogen budget and its environmental implications in China. Sci Rep 8:1–9.

Lymbery AJ, Doupe RG, Bennett T, Starcevich MR (2006) Efficacy of a subsurface-flow wetland using the estuarine sedge *Juncus kraussii* to treat effluent from inland saline aquaculture. Aquac Eng 34:1–7.

Maciel E, Domingues P, Domingues MRM, Calado R, Lillebø AI (2020) Halophyte plants from sustainable marine aquaponics are a valuable source of omega-3 polar lipids. Food Chem 0:0–32.

Magwa ML, Gundidza M, Gweru N, Humphrey G (2006) Chemical composition and biological activities of essential oil from the leaves of *Sesuvium portulacastrum*. J Ethnopharmacol 103:85–89.

Marchant HK, Tegetmeyer HE, Ahmerkamp S, Holtappels M, Lavik G, Graf J, Schreiber F, Mussmann M, Strous M, Kuypers MMM (2018) Metabolic specialization of denitrifiers in permeable sediments controls N2O emissions. Environ Microbiol 20:4486–4502.

Martens-Habbena W, Berube PM, Urakawa H, De La Torre JR, Stahl DA (2009) Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461:976–979.

Neori A, Chopin T, Troell M, Buschmann AH, Kraemer GP, Halling C, Shpigel M, Yarish C (2004) Integrated aquaculture: Rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231:361–391.

Neori A, Krom MD, Rijn J van (2007) Biogeochemical processes in intensive zero-effluent marine fish culture with recirculating aerobic and anaerobic biofilters. J Exp Mar Bio Ecol 349:235–247.

Nouairi I, Ghnaya T, Ben Youssef N, Zarrouk M, Habib Ghorbel M (2006) Changes in content and fatty acid profiles of total lipids of two halophytes: *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum* under cadmium stress. J Plant Physiol 163:1198–1202.

Ogah SI, Kamarudin MS, Amin SMN, Edaroyati MWP (2020) Biological filtration properties of selected herbs in an aquaponic system. Aquac Res:1–9.

Oliveira V, Martins P, Marques B, Cleary DFR, Lillebø AI, Calado R (2020) Aquaponics using a fish farm effluent shifts bacterial communities profile in halophytes rhizosphere and endosphere. Sci Rep 10:1–11.

Person-Le Ruyet J, Chartois H, Quemener L (1995) Comparative acute ammonia toxicity in marine fish and plasma ammonia response. Aquaculture 136:181–194.

Piedrahita RH (2003) Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture 226:35–44.

Pinheiro I, Arantes R, do Espírito Santo CM, do Nascimento Vieira F, Lapa KR, Gonzaga LV, Fett R, Barcelos-Oliveira JL, Seiffert WQ (2017) Production of the halophyte *Sarcocornia ambigua* and Pacific white shrimp in an aquaponic system with biofloc technology. Ecol Eng 100:261–267.

Poli MA, Legarda EC, de Lorenzo MA, Pinheiro I, Martins MA, Seiffert WQ, do Nascimento Vieira F (2019) Integrated multitrophic aquaculture applied to shrimp rearing in a biofloc system. Aquaculture 511.

Pucher J, Focken U (2017) Uptake of nitrogen from natural food into fish in differently managed polyculture ponds using15N as tracer. Aquac Int 25:87–105.

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2014) 15N tracer application to evaluate nitrogen dynamics of food webs in two subtropical small-scale aquaculture ponds under different managements. Isotopes Environ Health Stud 50:428–441.

Quintã R, Hill PW, Jones DL, Santos R, Thomas DN, LeVay L (2015a) Uptake of an amino acid (alanine) and its peptide (trialanine) by the saltmarsh halophytes *Salicornia europaea* and *Aster tripolium* and its potential role in ecosystem N cycling and marine aquaculture wastewater treatment. Ecol Eng 75:145–154.

Quintã R, Santos R, Thomas DN, Le Vay L (2015b) Growth and nitrogen uptake by *Salicornia europaea* and *Aster tripolium* in nutrient conditions typical of aquaculture wastewater. Chemosphere 120:414–421.

Rao AMF, McCarthy MJ, Gardner WS, Jahnke RA (2008) Respiration and denitrification in permeable continental shelf deposits on the South Atlantic Bight: N2:Ar and isotope pairing measurements in sediment column experiments. Cont Shelf Res 28:602–613.

Reddy KR, Patrick WH (1984) Nitrogen transformations and loss in flooded soils and sediments. C R C Crit Rev Environ Control 13:273–309.

Van Rijn J (1996) The potential for integrated biological treatment systems in recirculating fish culture - A review. Aquaculture 139:181–201.

Rubio G, Oesterheld M, Alvarez CR, Lavado RS (1997) Mechanisms for the increase in phosphorus uptake of waterlogged plants: Soil phosphorus availability, root morphology and uptake kinetics. Oecologia 112:150–155.

Schoffelen NJ, Mohr W, Ferdelman TG, Littmann S, Duerschlag J, Zubkov M V., Ploug H, Kuypers MMM (2018) Single-cell imaging of phosphorus uptake shows that key harmful algae rely on different phosphorus sources for growth. Sci Rep 8:1–13.

Senff P, Blanc P, Slater M, Kunzmann A (2020) Low-technology recirculating aquaculture system integrating milkfish *Chanos chanos*, sea cucumber *Holothuria scabra* and sea purslane *Sesuvium portulacastrum*. Aquac Environ Interact 12:471–484.

Senff P, Partelow S, Indriana LF, Buhari N, Kunzmann A (2018) Improving pond aquaculture production on Lombok, Indonesia. Aquaculture 497:64–73.

Shpigel M, Ben-Ezra D, Shauli L, Sagi M, Ventura Y, Samocha T, Lee JJ (2013) Constructed wetland with *Salicornia* as a biofilter for mariculture effluents. Aquaculture 413:52–63.

Singh D, Buhmann A, Flowers TJ, Seal CE, Papenbrock J (2014) *Salicornia* as a crop plant in temperate regions: Selection of genetically characterized ecotypes and optimization of their cultivation conditions. AoB Plants 6:1–20.

Sokoll S, Lavik G, Sommer S, Goldhammer T, Kuypers MMM, Holtappels M (2016)

Extensive nitrogen loss from permeable sediments off North-West Africa. J Geophys Res Biogeosciences 121:1144–1157.

Strickland J, Parsons T (1972) A practical Handbook of Seawater Analysis. Fisheries Research Board Canada, Ottawa.

Thomsen E, Herbeck LS, Jennerjahn TC (2020) The end of resilience: Surpassed nitrogen thresholds in coastal waters led to severe seagrass loss after decades of exposure to aquaculture effluents. Mar Environ Res 160:104986.

Verdegem MCJ (2013) Nutrient discharge from aquaculture operations in function of system design and production environment. Rev Aquac 5:158–171.

Vymazal J (2010) Constructed wetlands for wastewater treatment. Water 2:530–549.

Wallace W, Pate JS (1967) Nitrate Assimilation in Higher Plants with Special Reference to the Cocklebur (*Xanthium pennsylvanicum* Wallr .). Ann Bot 31:213–228.

Waller U, Buhmann A, Ernst A, Hanke V, Kulakowski A, Wecker B, Orellana J, Papenbrock J (2015) Integrated multi-trophic aquaculture in a zero-exchange recirculation aquaculture system for marine fish and hydroponic halophyte production. Aquac Int 23:1473–1489.

Webb JM, Quintã R, Papadimitriou S, Norman L, Rigby M, Thomas DN, Le Vay L (2012) Halophyte filter beds for treatment of saline wastewater from aquaculture. Water Res 46:5102–5114.

Webb JM, Quintã R, Papadimitriou S, Norman L, Rigby M, Thomas DN, Le Vay L (2013) The effect of halophyte planting density on the efficiency of constructed wetlands for the treatment of wastewater from marine aquaculture. Ecol Eng 61:145–153.

Xiaojie W, Yuchun S, Ning Y, Zaohe W, Xiaodong D (2011) The preliminary study of *Sesuvium portulacastrum* on the nitrogen and phosphorus absorption rate. Chinese Agric Bull 27:92–96.

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