Salud, Ciencia y Tecnología. 2026; 6:2625 doi: 10.56294/saludcyt20262625

#### ORIGINAL



# Optimising Polyculture Systems for Stichopus horrens: Effects of Tilapia and Rabbitfish on Growth, Survival, and Benthic Dynamics

Optimización de los sistemas de policultivo para Stichopus horrens: efectos de la tilapia y el pez conejo sobre el crecimiento, la supervivencia y la dinámica bentónica

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Cite as: Maha setiawati K, Sembiring SBM, Hutapea JH, Gunawan G, Setiadi A, Giri INA, et al. Optimising Polyculture Systems for Stichopus horrens: Effects of Tilapia and Rabbitfish on Growth, Survival, and Benthic Dynamics. Salud, Ciencia y Tecnología. 2026; 6:2625. https://doi.org/10.56294/saludcyt20262625

Submitted: 01-08-2025 Revised: 06-10-2025 Accepted: 10-12-2025 Published: 01-01-2026

Editor: Prof. Dr. William Castillo-González

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#### **ABSTRACT**

**Introduction:** integrated multi-trophic aquaculture (IMTA) offers a sustainable alternative to monoculture by combining species with complementary trophic roles to improve nutrient recycling and environmental performance. Within this system, deposit-feeding sea cucumbers such as *Stichopus horrens* function as extractive organisms that convert organic wastes into biomass while contributing to sediment remediation. However, empirical evidence remains limited regarding how co-culturing *S. horrens* with tropical finfish influences growth, survival, and benthic conditions.

**Method:** a controlled tank experiment was conducted at the Kawasan Konservasi Ilmiah (KKI) Gondol, Indonesia, using twelve fibre-reinforced plastic tanks arranged in a completely randomised design. Four configurations were tested: *S. horrens* + tilapia (*Oreochromis niloticus*), *S. horrens* + rabbitfish (*Siganus* sp.), *S. horrens* monoculture, and a three-species assemblage. Each treatment consisted of three replicates with 15 sea cucumbers per tank. Growth performance, survival, faecal deposition, benthic community structure, and crustacean attachment were monitored throughout the culture period.

**Results:** survival of *S. horrens* remained high across treatments ( $\approx$ 90-98 %, interpreted as 90-98 %), indicating strong tolerance to polyculture conditions. Monoculture produced the highest final weight (84,7  $\pm$  34,8 g), followed by rabbitfish (75,3  $\pm$  26,3 g), tilapia (61,3  $\pm$  18,3 g), and the three-species system (54,9  $\pm$  14,6 g). Rabbitfish grazing suppressed algal proliferation and benthic fouling, whereas tilapia-induced sediment disturbance reduced feeding stability. Crustacean attachment was lowest in treatments containing fish, suggesting secondary biofouling control.

**Conclusions:** S. horrens can be effectively integrated into finfish polyculture, with ecological interactions strongly mediated by fish identity. Further research quantifying nutrient fluxes, stable-isotope assimilation, and benthic oxygen dynamics is recommended to refine stocking strategies and support system scalability.

**Keywords:** *Stichopus Horrens*; Integrated Multi-Trophic Aquaculture (IMTA); Rabbitfish; Tilapia; Detritivory; Nutrient Recycling; Polyculture; Sediment Bioremediation.

## **RESUMEN**

Introducción: la acuicultura multitrófica integrada (IMTA, por sus siglas en inglés) constituye una alternativa

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sostenible frente al monocultivo al combinar especies con funciones tróficas complementarias para mejorar el reciclaje de nutrientes y el rendimiento ambiental. Dentro de este sistema, los pepinos de mar detritívoros, como Stichopus horrens, actúan como organismos extractivos que convierten los desechos orgánicos en biomasa y contribuyen a la remediación del sedimento. No obstante, la evidencia empírica sigue siendo limitada en cuanto a cómo el co-cultivo de S. horrens con peces tropicales de aleta influye en el crecimiento, la supervivencia y las condiciones bentónicas.

Método: se llevó a cabo un experimento controlado en tanques en el Kawasan Konservasi Ilmiah (KKI) Gondol. Indonesia, utilizando doce tanques de plástico reforzado con fibra dispuestos en un diseño completamente aleatorizado. Se evaluaron cuatro configuraciones: S. horrens + tilapia (Oreochromis niloticus), S. horrens + pez conejo (Siganus sp.), monocultivo de S. horrens y un ensamble de tres especies. Cada tratamiento constó de tres réplicas con 15 pepinos de mar por tanque. Durante el periodo de cultivo se monitorearon el desempeño de crecimiento, la supervivencia, la acumulación de heces, la estructura de la comunidad bentónica y la adhesión de crustáceos.

Resultados: la supervivencia de S. horrens se mantuvo elevada en todos los tratamientos (≈90-98 %, interpretado como 90-98 %), lo que indica una alta tolerancia a las condiciones de policultivo. El monocultivo produjo el mayor peso final  $(84.7 \pm 34.8 \text{ g})$ , seguido del tratamiento con pez conejo  $(75.3 \pm 26.3 \text{ g})$ , tilapia  $(61.3 \pm 18.3 \pm 1$ g) y el sistema de tres especies (54,9 ± 14,6 g). El pastoreo del pez conejo redujo la proliferación algal y el ensuciamiento bentónico, mientras que la perturbación del sedimento inducida por la tilapia disminuyó la estabilidad de la alimentación. La adhesión de crustáceos fue menor en los tratamientos que incluían peces, lo que sugiere un control secundario del biofouling.

Conclusiones: S. horrens puede integrarse eficazmente en sistemas de policultivo con peces, con interacciones ecológicas fuertemente mediadas por la identidad de la especie íctica. Se recomienda realizar investigaciones adicionales que cuantifiquen los flujos de nutrientes, la asimilación mediante isótopos estables y la dinámica de oxígeno bentónico, con el fin de optimizar las estrategias de siembra y favorecer la escalabilidad del sistema.

Palabras clave: Stichopus Horrens; Acuicultura Multitrófica Integrada (IMTA); Pez Conejo; Tilapia; Detritivoría; Reciclaje de Nutrientes; Policultivo; Biorremediación de Sedimentos.

#### INTRODUCTION

Aquaculture is increasingly shifting from single-species production to integrated multi-trophic aquaculture (IMTA), in which complementary trophic groups are co-cultured to valorise wastes, stabilise outputs, and improve benthic habitat quality. (1,2,3) Within this framework, deposit-feeding sea cucumbers function as extractive organisms that assimilate particulate and sedimentary organic matter from finfish effluents, thereby converting residues into harvestable biomass while re-working sediments and contributing to bioremediation. (4,5,6) Empirical demonstrations span recirculating and open-water settings and show that holothurians can be operationalised at farm scale with measurable environmental benefits and tractable carrying-capacity considerations. (7,8)

A growing evidence base indicates that sea cucumbers can incorporate farm-derived organic inputs, with stable-isotope and fatty-acid tracers confirming the transfer of finfish waste into holothurian tissues and, thus, direct trophic coupling in IMTA. (4,9) Related polycultures further demonstrate extractive performance and waste uptake by sea cucumbers co-cultured with other fed species, such as abalone, reinforcing the generality of detrital pathways supporting holothurian growth. (10,11) Tank- and field-based work with Holothuria scabra and Apostichopus japonicus shows growth and survival under fish-farm conditions while altering sediment organic characteristics and benthic community dynamics, underscoring their system-level regulatory roles. (12,13) Complementary studies in semi-intensive and recirculating systems highlight benthic habitat recovery and closed-loop nutrient use when holothurians are integrated with finfish and macroalgae. (2,14)

Against this backdrop, Stichopus horrens—a tropical holothurian with commercial value in Southeast Asia presents a strong candidate for integration with finfish owing to its detritivorous feeding mode and tolerance of organically enriched conditions, yet species-specific performance data in polyculture remain limited. (4) The extent to which the identity of co-cultured fish (e.g., omnivorous tilapia Oreochromis niloticus versus herbivorous rabbitfish Siganus spp.) modulates the trophic landscape for S. horrens, and thereby growth, survival, and fouling dynamics, has not been robustly tested despite IMTA studies showing that fish behaviour, excretion profiles, and bioturbation can restructure nutrient fluxes and benthic communities. (1,3) Addressing this gap is pertinent to the eco-engineering of species-complementary units that minimise disturbance while maximising waste assimilation and periphyton control in tropical production contexts. (15)

The present study therefore evaluates S. horrens under four tank-based configurations-monoculture and

polycultures with tilapia, rabbitfish, and their combination—to isolate how finfish identity and assemblage shape the feeding substrate, benthic community composition, and organismal performance of the extractive component. By coupling growth and survival metrics with measurements of faecal deposition, wall-attached benthos, and fouling crustaceans, the work seeks to test the expectations that finfish integration reduces benthic fouling via grazing/disturbance, that rabbitfish create a more favourable rearing environment for *S. horrens* than tilapia owing to herbivory, and that *S. horrens* utilises fish-derived particulates as supplementary nutrition in line with documented IMTA nutrient pathways.

#### **METHOD**

#### Study Site and Experimental Organisms

The study was conducted at the Kawasan Konservasi Ilmiah (KKI, Scientific Conservation Area) in Gondol, Indonesia. The facility provides controlled environmental conditions suitable for experimental aquaculture. The test species was the sea cucumber *Stichopus horrens*, sourced from hatchery production at KKI Gondol. The initial average body weight of the experimental animals was 33,3 g.

# **Experimental Design**

The experiment utilised twelve fibre-reinforced plastic tanks measuring  $2 \times 1 \times 0.7$  m. Each tank was equipped with a PVC pipe shelter, halved longitudinally to provide attachment and refuge structures for the sea cucumbers. A green waring net  $(1 \times 0.7 \times 0.3 \text{ m})$  was positioned on the water surface, with approximately 20 cm submerged to promote the growth of benthic and periphytic organisms serving as natural feed sources. Four culture treatments were established in a completely randomised design, each with three replicates, as follows:

- Treatment A: S. horrens (15 ind.) + tilapia (Oreochromis niloticus, 8 ind.)
- Treatment B: S. horrens (15 ind.) + rabbitfish (Siganus sp., 8 ind.)
- Treatment C: S. horrens (15 ind.) monoculture
- Treatment D: S. horrens (15 ind.) + tilapia (4 ind.) + rabbitfish (4 ind.)

The stocking density of *S. horrens* was maintained at 15 individuals per tank, consistent with semi-intensive culture conditions.

# **Feeding Regime**

All treatments received a daily feed consisting of fresh macroalgae (*Ulva* and *Sargassum* spp.) equivalent to approximately 3 % of total biomass, supplemented with 10 g of compacted fresh benthos. Feeding was conducted twice daily at 08:00 and 16:00 h. In treatments containing fish (A, B, and D), a commercial pellet feed was provided at 3 % of total fish biomass. Residual feed was monitored to ensure stable water quality.

#### **Observed Parameters**

The following parameters were recorded during the culture period:

- 1. Growth performance of S. horrens, O. niloticus, and Siganus sp.;
- 2. Density of crustaceans attached to S. horrens surfaces;
- 3. Density and composition of benthic organisms on tank walls;
- 4. Quantity of faeces accumulated; and
- 5. Survival rate of S. horrens at the end of the study.

# **Growth and Survival Measurements**

The body weight of *S. horrens* was recorded at 14-day intervals, while the length and body weight of finfish were measured at both the beginning and end of the experiment. Weights were determined using a digital balance  $(\pm 0,01~g)$  and lengths with a digital caliper  $(\pm 0,1~mm)$ . Survival rate was calculated as the percentage of individuals remaining alive at the end of the culture period.

## Collection and Quantification of Faeces

Faecal material was collected by siphoning the tank bottom using a 1 cm-diameter hose. Samples were transferred into 20 L containers, filtered through a 60  $\mu$ m plankton net, and oven-dried at 70 °C until constant weight. The resulting dry faeces were weighed using an analytical balance. Faecal samples from replicate tanks within each treatment were pooled prior to analysis.

#### Sampling of Benthic Organisms

Benthic samples were collected by scraping a 5 cm-wide section of the inner tank wall. Samples were preserved in 4% buffered formalin and analysed at the Fish Health and Environmental Laboratory, BPBAP

Situbondo. Identification of planktonic and benthic taxa followed standard taxonomic keys, and densities were expressed as individuals cm<sup>-2</sup>.

## **Enumeration of Crustaceans Attached to Sea Cucumbers**

Attached crustaceans were removed by immersing the sea cucumbers in freshwater for approximately three minutes, facilitating detachment. Detached individuals were counted under a stereo microscope (40× magnification) and identified morphologically.

# **Ethical Aspects**

The Ethics Commission for Animal Care and Use of the Indonesian National Research and Innovation Agency decided that this study does not require Ethics Clearance, and the animals in this study are invertebrates that are exempt from Ethics Clearance (Reference Number: 215/KE.02/SK/12/2023).

## **Data Processing and Presentation**

All data were expressed as mean ± standard deviation (SD). No inferential statistical tests were applied. Treatment comparisons were based on descriptive statistics and visual trends illustrated through graphical representations (line and bar charts). Graphs were constructed using Microsoft Excel 2021.

#### **RESULTS**

## Growth Performance of Stichopus horrens

The growth performance of Stichopus horrens varied among the four culture treatments (A-D). The final mean body weights recorded were 61,3 ± 18,3 g in treatment A (sea cucumber-tilapia), 75,3 ± 26,3 g in treatment B (sea cucumber-rabbitfish),  $84.7 \pm 34.8$  g in treatment C (monoculture), and  $54.9 \pm 14.6$  g in treatment D (sea cucumber-tilapia-rabbitfish). The highest growth occurred in the monoculture (C), whereas the lowest was found in the three-species polyculture (D). The sea cucumber-rabbitfish polyculture (B) produced better growth than the sea cucumber-tilapia polyculture (A). These results suggest that rabbitfish exerted a more favourable ecological influence on S. horrens growth than tilapia, likely due to differences in foraging behaviour and habitat disturbance.

## **Growth of Associated Fish Species**

Growth performance of the associated finfish is summarised in Table 1. Tilapia (Oreochromis niloticus) exhibited consistently greater growth than rabbitfish (Siganus sp.). In treatment A, tilapia attained an average total length of 12,84  $\pm$  1,06 cm and body weight of 36,13  $\pm$  8,75 g, while in treatment D these values increased slightly to 13,78  $\pm$  0,77 cm and 39,39  $\pm$  2,19 g, respectively.

Rabbitfish displayed comparatively slower growth, with  $8.77 \pm 0.63$  cm total length and  $14.10 \pm 2.15$  g body weight in treatment B, and  $7.61 \pm 0.66$  cm and  $9.50 \pm 0.94$  g in treatment D. The difference reflects the species' herbivorous diet and lower assimilation of commercial feed. Despite this, rabbitfish contributed ecologically through their grazing activity, which controlled algal proliferation on tank surfaces.

<b>Table 1.</b> Total length and body weight (mean $\pm$ SD) of tilapia (Oreochromis niloticus) and rabbitfish (Siganus sp.) at the end of the experiment								
Treatment	Tilapia		Rabbitfish					
	Total length (cm)	Body weight (g)	Total length (cm)	Body weight (g)				
Α	12,84± 1,06	36,13±8,75	-	-				
В	-	-	8,77± 0,63	14,1± 2,15				
D	$13,78 \pm 0,77$	39,39±2,19	7,61± 0,66	9,5± 0,94				

# **Faeces Production and Sediment Accumulation**

Faeces production increased progressively throughout the culture period across all treatments (Figure 1). In early observations (14-28 June), faecal accumulation ranged from 25 g to 45 g, with only minor differences between treatments. A marked rise occurred from mid-July onwards, reaching 60-70 g on 14 July, 70-80 g on 28 July, and peaking at 80-85 g on 12 August.

At the final sampling, treatment C (monoculture) recorded the highest mean faeces output (≈ 85 g), followed by treatment B ( $\approx$  80 g), treatment A ( $\approx$  70 g), and treatment D ( $\approx$  60 g). These trends suggest that rabbitfish and sea cucumber monoculture promoted greater organic deposition, whereas the combined activity of tilapia and rabbitfish in treatment D caused sediment resuspension and reduced visible accumulation.

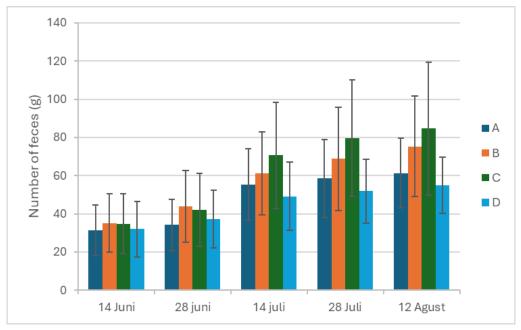


Figure 1. Monthly faeces production (mean ± SD) in each treatment

# **Average Faeces Production across Treatments**

When averaged over the entire experimental period (figure 2), the sea cucumber-rabbitfish polyculture (B) produced the highest mean faecal accumulation ( $\approx$  170 g), followed by treatments A and C ( $\approx$  140 g), while the lowest was observed in the combined polyculture (D,  $\approx$  100 g). The enhanced organic output in treatment B indicates that rabbitfish generated more nutrient-rich particulate waste, supporting the detritivorous feeding of S. horrens. Conversely, the reduced faecal retention in treatment D may have been caused by increased particle suspension due to dual fish activity.

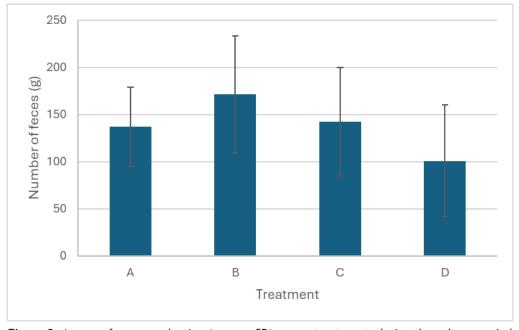


Figure 2. Average faeces production (mean  $\pm$  SD) across treatments during the culture period

# Survival Rate of Stichopus horrens

Survival of S. horrens remained high across all treatments (figure 3), ranging from approximately 90 % to 98 %. The highest survival occurred in treatment B (sea cucumber-rabbitfish polyculture), followed by treatments C (monoculture), D (combined), and A (tilapia). Statistical analysis showed no significant differences (p > 0,05) among treatments. These findings demonstrate that S. horrens is robust and tolerant to polyculture conditions. The slightly higher survival in treatment B may be attributed to a cleaner substrate and improved water quality resulting from rabbitfish grazing.

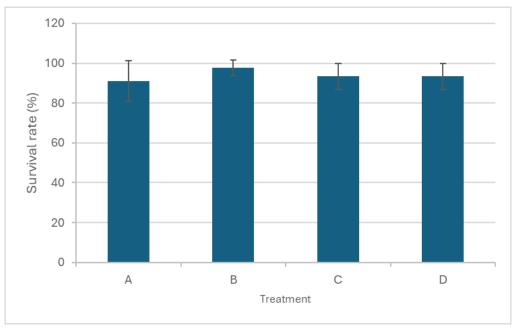


Figure 3. Survival rate (mean ± SD) of Stichopus horrens under different culture treatments

# Crustacean Attachment to the Body Surface

The number of crustaceans attached to sea cucumber surfaces differed among treatments and between sampling intervals (figure 4). On day 14, treatment B (sea cucumber-rabbitfish) showed the highest attachment (~ 17 individuals per specimen), followed by A (~ 14), C (~ 13), and D (~ 7). By day 28, crustacean numbers declined sharply in treatments A, B, and D but increased substantially in the monoculture (C), reaching ~ 23 individuals per specimen. This pattern suggests that fish presence, particularly tilapia and rabbitfish, suppressed crustacean colonisation, likely through direct predation or habitat disturbance. Tilapia were observed consuming small crustaceans detached from sea cucumber bodies. In contrast, the absence of fish in treatment C allowed crustacean populations to proliferate, leading to the highest infestation levels.

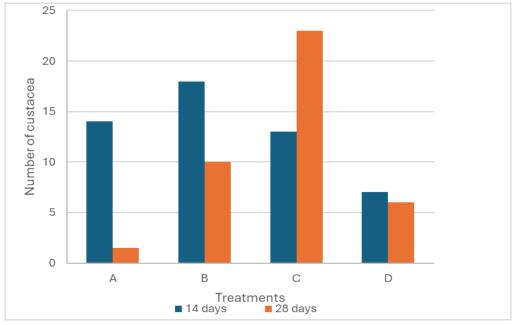


Figure 4. Number of crustaceans attached to the body surface of Stichopus horrens recorded on days 14 and 28 under different culture treatments

## Benthic Community Composition on Tank Walls

Benthic organism density and composition varied markedly among treatments (Table 2). Across all experimental groups, twelve planktonic and benthic taxa were identified, dominated by diatoms-Navicula, Nitzschia, Coscinodiscus, Synedra, and Melosira-along with filamentous algae, protozoa, and small invertebrates. The

monoculture treatment (C) exhibited the highest cumulative density, reaching 311 467 individuals cm<sup>-2</sup>, followed by treatments B (81 220 individuals cm<sup>-2</sup>) and A (79 996 individuals cm<sup>-2</sup>). The lowest density was recorded in the three-species polyculture (D), with only 9 506 individuals cm<sup>-2</sup>. These results indicate that the introduction of fish species into *Stichopus horrens* culture systems substantially reduced benthic biomass on tank surfaces.

The abundance patterns among diatom genera support this trend. In the monoculture, *Bacillaria paradoxa*, *Melosira*, and *Synedra* were especially prolific, demonstrating unrestricted algal colonisation in the absence of grazing fish. Treatments A and B retained moderate diatom and filamentous algal densities, suggesting partial control through grazing activity. In contrast, treatment D, which included both tilapia and rabbitfish, exhibited the lowest benthic colonisation levels. This pattern reflects the combined grazing and substrate-disturbing behaviours of these species, which effectively limit algal settlement and biofilm accumulation.

The markedly higher densities in the monoculture indicate that without fish-mediated regulation, diatoms and filamentous algae can rapidly proliferate, increasing benthic density and potentially altering substrate oxygen dynamics. Conversely, the presence of herbivorous and omnivorous fish maintained cleaner tank surfaces and clearer water by suppressing algal and epiphytic communities through direct consumption and physical disturbance. These findings confirm the ecological role of fish as biological regulators in polyculture environments, aligning with established principles of ecological balance and nutrient control in integrated multi-trophic aquaculture systems.

Table 2. Composition of Phytoplankton and Zooplankton in Each Treatment (Density per cm²)							
Taxonomic Group	Species / Parameter	Α	В	С	D		
Diatoms (Diatomae)	Coscinodiscus	181	393	-	-		
	Achnanthes	324	707	809	556		
	Bacillaria paradoxa	14 223	3 854	8 815	935		
	Synedra	24 015	370	2 130	389		
	Nitzschia	4 379	2 970	6 952	1 963		
	Navicula	5 346	2 883	1 148	-		
	Thalassiotrix	9 061	1 333	1 083	285		
	Pseudo-nitzschia	3 444	122	11 333	-		
	Melosira	16 569	2 974	7 124	4 437		
	Asterionella	2 037	-	5 539	519		
	Amphiprora	51	61	506	37		
	Licmophora	306	341	196	333		
	Diploneis	61	311	1 976	52		
Chlorophyceae (cells mL <sup>-1</sup> )		-	422	250	-		
Cyanophyceae (BGA, units mL <sup>-1</sup> )		-	64 352	256 463	-		
Dinoflagellates		-	-	-	-		
Euglenophyceae		-	519	6 750	-		
Detritus / Clamp / Flog		-	-	-	-		
Cryptophyta		-	-	-	-		
Zooplankton		-	-	-	-		
Cumulative Density (Total)		79 996	81 220	311 467	9 506		

Visual observations of the PVC shelter pipes confirmed these quantitative findings (figure 5). Shelters from the monoculture treatment (C) exhibited the heaviest algal colonisation, with thick biofilm and filamentous algal mats covering most surfaces. In contrast, shelters from treatment D appeared visibly clean, with minimal algal growth. Moderate attachment was observed in treatments A (tilapia) and B (rabbitfish), where thin biofilms and scattered algal patches were present.

The reduced fouling in treatments involving rabbitfish can be attributed to the herbivorous grazing activity of *Siganus* sp., which effectively limited the accumulation of filamentous algae on both tank walls and shelters. Tilapia also contributed to substrate disturbance through their active foraging behaviour, although their effect was less pronounced. The lack of grazing pressure in the monoculture treatment allowed benthic microalgae to proliferate freely, resulting in dense colonisation of the PVC surfaces.



Figure 5. Benthic organisms attached to PVC shelter pipes at the bottom of rearing tanks for each treatment (from left to right: A, B, C, D)

# **DISCUSSION**

# Feasibility of Polyculture Systems with Stichopus horrens

The present study demonstrates the feasibility of culturing Stichopus horrens in polyculture systems with finfish species such as tilapia (Oreochromis niloticus) and rabbitfish (Siganus sp.), as indicated by its capacity to survive and grow under conditions characterised by diverse biological interactions and organic inputs. These findings align with the broader body of literature establishing the potential of holothurians as effective detritivores within integrated multi-trophic aquaculture (IMTA) systems. (3,4,5,12,16) Collectively, these studies substantiate the proposition that sea cucumbers can utilise organic waste derived from finfish farming and contribute significantly to nutrient recycling and sediment bioremediation.

The demonstrated adaptability of S. horrens in polyculture with tilapia and rabbitfish corroborates observations made for other holothurian species in IMTA systems. For instance, Holothuria polii successfully co-cultured with gilthead seabream (Sparus aurata) and European seabass (Dicentrarchus labrax) exhibited sustained growth and survival, underscoring the compatibility of sea cucumbers with fed finfish species in multi-trophic environments. (3,4) Similarly, Holothuria scabra has demonstrated substantial bioremediation potential in co-culture with red drum (Sciaenops ocellatus), effectively reducing nutrient accumulation and enhancing environmental performance within aquaculture systems. (5) These outcomes collectively reinforce the conceptual and operational feasibility of employing S. horrens as a functional detritivore within finfish-based IMTA frameworks.

From an ecological and physiological standpoint, previous research on Holothuria leucospilota and Apostichopus japonicus provides additional evidence supporting the adaptability of holothurians to aquaculture environments with elevated organic inputs. (12) These studies have shown that sea cucumbers can withstand a range of thermal and environmental conditions, efficiently assimilating organic matter derived from fish farms. Such findings support the inference that S. horrens, native to tropical and subtropical regions, possesses similar physiological resilience, enabling it to thrive in the nutrient-enriched sediments typical of tilapia-rabbitfish culture systems. However, environmental parameters such as temperature, substrate type, and organic load remain critical determinants of performance and must be optimised to ensure sustained growth and ecosystem stability. (17,18)

The integration of S. horrens within tilapia-rabbitfish systems could thus offer dual ecological and economic benefits. Ecologically, the species' detritivorous feeding habits may facilitate the recycling of organic waste and the maintenance of sediment quality, consistent with IMTA objectives of waste mitigation and resource efficiency. (3,5) Economically, the co-culture of sea cucumbers as a high-value by-product can enhance system profitability, providing additional revenue streams while contributing to the sustainability of aquaculture operations. These outcomes mirror those observed in large-scale co-culture trials involving H. scabra and Babylonia areolata, which demonstrated scalable production potential and positive interspecific interactions under well-managed conditions. (16,19)

Despite these promising indications, certain limitations warrant consideration. The present findings offer the

first experimental insight into *S. horrens* performance in tilapia-rabbitfish systems, but the absence of extensive empirical data on this specific species combination constrains the ability to generalise results. Previous studies caution that co-culture success can be influenced by site-specific variables such as water exchange, stocking density, and substrate management. (16,19) Moreover, issues such as ammonia accumulation, disease transmission, and competition for organic resources may pose operational challenges if system parameters are not carefully regulated. Future research should therefore focus on controlled trials examining nutrient flux dynamics, growth efficiency, and physiological responses of *S. horrens* across different trophic and environmental configurations to refine management protocols.

## Comparative Growth Performance under Different Polyculture Combinations

The results indicate that *Stichopus horrens* exhibited comparatively higher growth performance when cocultured with rabbitfish (*Siganus* sp.) than in polycultures involving tilapia (*Oreochromis niloticus*) alone or in combination with both finfish species. This outcome suggests that the rearing environment shaped by rabbitfish may be more conducive to the detritivorous feeding ecology of *S. horrens*. One plausible explanation is that rabbitfish produce organic waste that enhances the availability of fine particulate organic matter in sediments without causing excessive disturbance. In contrast, the vigorous foraging and sediment-churning behaviour of tilapia may disrupt the sediment layer, diminishing both habitat stability and feeding efficiency for the sea cucumbers. Such species-specific effects on sediment quality and nutrient distribution have been recognised as important determinants of holothurian performance within integrated multi-trophic aquaculture (IMTA) systems. (3,20)

The differential growth observed under various polyculture configurations aligns with established IMTA principles that emphasise the influence of co-cultured species identity on system dynamics and nutrient cycling. In their study of IMTA farms in Greece, Chatzivasileiou et al.<sup>(3)</sup> demonstrated that the integration of fish, bivalves, and holothurians across multiple sites led to variable growth responses in sea cucumbers, driven by differences in organic input and bioturbation processes. This finding supports the interpretation that changes in nutrient flow and sediment composition—mediated by specific fish assemblages—can significantly modulate sea cucumber growth and ecological performance. Thus, the superior performance of *S. horrens* in rabbitfish polyculture likely reflects a more balanced nutrient environment and reduced physical interference relative to systems dominated by tilapia activity. <sup>(3,21)</sup>

The observed differences in *Stichopus horrens* growth across polyculture combinations can be attributed to species-specific influences on sediment quality and nutrient dynamics. As continuous deposit feeders, sea cucumbers rely on the organic matter content of surface sediments, which varies according to fish behaviour and associated disturbance. Evidence from *Holothuria leucospilota* indicates that higher sedimentary organic matter enhances growth, suggesting that rabbitfish excreta may improve nutrient availability while maintaining a stable benthic layer, unlike the more disruptive foraging of tilapia. Onsequently, the behavioural and trophic traits of co-cultured fish likely govern the feeding efficiency and growth of *S. horrens*. However, as direct empirical comparisons between rabbitfish and tilapia in such systems remain unreported, these interpretations should be viewed as plausible rather than definitive. Future controlled studies quantifying sediment enrichment, nutrient fluxes, and disturbance dynamics are needed to validate these mechanisms and optimise IMTA configurations that balance organic inputs and habitat stability to enhance holothurian productivity.

## Ecological Role of Rabbitfish in Algae Regulation

The suppression of algal growth observed in the presence of rabbitfish underscores their potential ecological role as biological regulators within polyculture and integrated multi-trophic aquaculture (IMTA) systems. As herbivorous grazers, rabbitfish likely exert direct grazing pressure on filamentous algae colonising tank walls, substrates, and shelters, thereby limiting excessive algal proliferation and improving overall water clarity. Such ecological regulation is consistent with IMTA principles that highlight how the identity and trophic behaviour of co-cultured fish can shape nutrient dynamics, biofouling patterns, and habitat quality within culture systems. (3) In comparable IMTA settings, species composition and functional traits have been shown to influence waste recycling and benthic-pelagic coupling, suggesting that rabbitfish-mediated grazing can contribute to maintaining ecological balance and water quality in semi-closed aquaculture environments. (4,23)

The observed algae-suppressive effect can be interpreted within the broader framework of grazer-mediated regulation documented in IMTA literature. Sea cucumbers (*Apostichopus californicus*) reduced biofouling accumulation in salmon IMTA systems through direct grazing and organic matter removal, providing empirical support for the regulatory role of grazers in controlling surface-associated primary producers. (1) Low-technology recirculating systems integrating fish, sea cucumbers, and halophytes achieved ecological stability through multi-trophic nutrient utilisation and biofouling management. (2) Although rabbitfish-specific data are lacking, their herbivorous feeding behaviour positions them functionally parallel to other grazers known to suppress

algal buildup and stabilise aquatic substrates. (1,2)

Beyond direct grazing, nutrient fluxes and fish-driven alterations in sediment organic matter can also influence algal dynamics. Variations in culture configuration affect sedimentary organic matter content, thereby shaping microbial and algal productivity. (17) This supports the inference that rabbitfish, through controlled nutrient excretion and minimal sediment disturbance, may indirectly regulate algal proliferation by influencing substrate enrichment and oxygen availability. IMTA studies further emphasise that waste dispersion, species interactions, and system design determine the extent of benthic algal growth and biofilm formation, with spatial configuration affecting both benthic oxygen dynamics and carbon cycling. (4) Therefore, rabbitfish presence in polyculture tanks may enhance system efficiency by contributing both direct grazing and indirect environmental modulation that limit algal overgrowth. (4,12)

While the ecological role of rabbitfish as algal regulators is supported conceptually by IMTA principles, empirical validation under controlled conditions remains necessary. Future studies should quantify rabbitfish grazing rates, nutrient excretion profiles, and their effects on algal biomass and water-quality indicators in closed and semi-closed systems. (24) Such experiments would enable clearer attribution of observed algal suppression to biological grazing rather than secondary environmental effects. Integrating these findings into system design could support the development of sustainable, self-regulating aquaculture frameworks where rabbitfish function as natural agents of algal control alongside detritivorous species such as sea cucumbers, thereby enhancing both ecological performance and production stability. (2,7)

## Utilisation of Fish Faeces by Stichopus horrens

Observations that Stichopus horrens ingests detritus and fish faecal particulates are congruent with Integrated Multi-Trophic Aquaculture (IMTA) principles, whereby wastes from fed trophic levels are revalorised as resources for extractive organisms to enhance nutrient recycling and system sustainability. (5,7) Stable-isotope and fatty-acid tracers have demonstrated that holothurians assimilate fish-farm waste into tissue, providing a direct trophic linkage from finfish residues to sea cucumber biomass and substantiating the plausibility of S. horrens utilising fish-derived organic matter as a supplementary food source in polyculture. (4) Farm- and field-scale demonstrations of bottom-cultured holothurians within fish farms further evidence the practical integration of deposit feeders into finfish systems, reinforcing the capacity of sea cucumbers to process residual organics while growing to harvestable sizes. (12)

The mechanistic basis rests on the deposit-feeding ecology of holothurians, which continuously ingest sediment-associated detritus, microalgae and faecal particulates, thereby mediating the downward transfer of organic matter and converting it into benthic consumer biomass. (8) Cross-system results from integrated mariculture and biofloc settings show that sea cucumbers can exploit farm-generated particulates and organic residues, consistent with the proposed supplementary nutrition for S. horrens from fish faeces in IMTA configurations. (14,25) Isotopic evidence explicitly tracking the assimilation of farm wastes by holothurians provides rigorous support for this pathway, confirming that detrital ingestion translates into tissue accretion and energy storage in extractive deposit feeders. (4)

At the system level, positioning S. horrens as a benthic detritivore contributes to the attenuation of organic build-up, improves benthic oxygen dynamics through reduced sedimentary loading, and supports circular nutrient flows that align with IMTA design objectives. (5) Real-world dispersion modelling underscores how farm layout and benthic-pelagic coupling influence the fate of particulates and the efficacy of extractive components, highlighting the value of sea cucumbers in stabilising nutrient fluxes and mitigating localised waste accumulation. (7) In combination with documented bottom-culture successes, these insights indicate that incorporating S. horrens can improve waste-to-biomass conversion efficiency while adding a marketable product stream in polyculture systems. (12)

Notwithstanding this strong mechanistic and empirical foundation, species-specific validation for S. horrens remains a priority; future trials should quantify assimilation of fish-derived organics via stable isotopes and fatty-acid profiling, alongside growth, survival and benthic biogeochemical indicators under controlled detrital inputs. (4) Experimental designs comparing treatments with and without S. horrens should monitor sediment organic content, benthic oxygen demand and nutrient budgets to resolve process contributions and optimise stocking and substrate management for maximal bioremediation gains. (7,14) Such targeted evaluations would refine IMTA protocols and substantiate the role of S. horrens in closing nutrient loops and reducing environmental waste accumulation while producing valuable biomass. (5)

# Implications for Sustainable Aquaculture Development

Integrating sea cucumbers with rabbitfish and tilapia aligns with Integrated Multi-Trophic Aquaculture (IMTA) principles that harness species complementarity to enhance nutrient use efficiency, recycle wastes, and mitigate environmental impacts while sustaining production. (5,26) Evidence across holothurian-finfish polycultures indicates that deposit-feeding sea cucumbers function as extractive organisms that assimilate

organic residues and contribute to sediment remediation, supporting their inclusion in mixed finfish systems for environmentally sustainable development. (2,4,25) Although direct trials with *Stichopus horrens* alongside rabbitfish and tilapia are not reported in the cited literature, the convergent IMTA evidence base supports the expectation of coexistence and functional complementarity under appropriate system design and management. (4,5) The proposition that pairing *S. horrens* with rabbitfish yields particularly balanced ecological and productive outcomes should be considered a plausible, testable hypothesis grounded in IMTA mechanisms rather than a confirmed generality at this stage. (2,4)

Species complementarity emerges as a central design principle: finfish supply organic inputs, while holothurians convert these residues into biomass, thereby supporting nutrient recycling and benthic quality. Stable-isotope and fatty-acid tracers demonstrate that sea cucumbers assimilate fish-farm wastes, confirming a direct nutrient pathway from fed species to holothurian tissue and validating the extractive role envisioned in IMTA. (4,10) Diverse implementations—from low-technology recirculating systems to open-water farms—show that holothurians can be integrated across settings, reinforcing scalability for sustainable polyculture development. (2,4) Complementary co-cultures with seaweeds and other invertebrates further enhance space use and resilience, underscoring the value of multi-trophic linkages for production stability and ecological balance. (13,25)

To translate these principles into commercial practice, future work should quantify nutrient fluxes and partitioning in *S. horrens*-rabbitfish-tilapia systems using stable-isotope and fatty-acid analyses alongside whole-farm nutrient budgets to calibrate stocking ratios and feeding strategies. (4,7) Farm-scale modelling of waste dispersion and benthic impacts should be incorporated into design tools to optimise cage layout, carrying capacity, and risk mitigation for benthic enrichment and oxygen demand. (7,26) Such integrative assessments will support evidence-based decisions on operational parameters that balance production targets with environmental safeguards. (5,26)

In parallel, research should assess microbial community responses and longer-term production cycles, given the role of holothurian bioturbation and detrital processing in shaping sediment microbiomes and biogeochemical functioning. (2,12) Multi-season trials comparing rabbitfish- versus tilapia-dominated pairings with *S. horrens* should monitor growth, survival, nutrient trajectories, sediment chemistry, and productivity to resolve interaction pathways and validate species-specific complementarities for sustainable scale-up. (3,5) Establishing these mechanistic and operational benchmarks will enable robust commercialisation pathways for sea cucumber-finfish polycultures that deliver ecological balance and reliable yields. (4,7)

#### **CONCLUSION**

This study demonstrates that *Stichopus horrens* can be maintained successfully in polyculture with tilapia (*Oreochromis niloticus*) and rabbitfish (*Siganus* sp.) under controlled conditions, with consistently high survival ( $\approx$ 90-98 %) across all treatments. Growth responses, however, were assemblage-dependent: sea cucumber monoculture produced the greatest final body mass, while the *S. horrens*-rabbitfish pairing outperformed the *S. horrens*-tilapia pairing, indicating that species identity and associated behaviours mediate culture performance. Fish presence strongly reduced benthic biomass on tank walls—most markedly in the three-species treatment—supporting a regulatory role of grazing and disturbance on algal and epiphytic communities and contributing to cleaner substrates and clearer water.

Functional interactions underpin these outcomes. Visual and quantitative evidence indicates that rabbitfish suppressed filamentous algae and diatom colonisation, while *S. horrens* utilised fish-derived detritus and faecal particulates as supplemental nutrition, reinforcing waste-to-biomass conversion. Faeces dynamics further reflected trophic coupling: cumulative and mean faecal accumulation were highest in monoculture and in the *S. horrens*-rabbitfish system, and lowest when both fish species were co-present—consistent with greater sediment resuspension under dual fish activity. Fish inclusion also coincided with reduced crustacean attachment to sea cucumber surfaces, suggesting ancillary biofouling control through predation or disturbance.

Operationally, these findings highlight species complementarity as a core design principle for sustainable polyculture: rabbitfish provide algal regulation and contribute organic inputs suitable for detritivore assimilation, while *S. horrens* enhances nutrient recycling and sediment remediation. For near-term practice, rabbitfish emerge as the more compatible finfish partner for *S. horrens* than tilapia when the objective is to balance growth with biofouling control and benthic hygiene.

Future work should move beyond descriptive comparisons to incorporate inferential statistics and process-based measurements. Priorities include quantifying nutrient fluxes and partitioning (e.g., stable isotopes, fatty acids), characterising microbial community responses and benthic oxygen dynamics, and testing longer production cycles to optimise stocking ratios, feeding regimes and shelter/substrate configurations for commercial adoption. Validation across seasons and sites will be essential to refine carrying capacity, minimise resuspension in mixed-fish settings, and translate the demonstrated ecological benefits into robust, scalable integrated multi-trophic aquaculture systems.

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#### **FINANCING**

This research was funded by the National Research and Innovation Agency (BRIN), Indonesia and CV. Inti Resources Aquatic, Indonesia.

# **CONFLICT OF INTEREST**

The author declares that there are no conflicts of interest.

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