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Effect of Bivalves on Water Quality, Microbial Load and Growth Performance of *P. vannamei* and *M. cephalus* in Halophyte-based Integrated Multi-trophic Aquaculture Reared under Pond Conditions

Rathod Kumara[1], Karthireddy Syamala[2], P.S. Shyne Anand[3], N.K. Chadha[1], P.B. Sawant[1], M.S. Prasad[4], P.L. Doddamani[5], A.P. Muralidhar[2]

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ABSTRACT

Background: IMTA is an integrated and eco-friendly farming approach, where organisms from different trophic levels can be cultured in the same system to take advantage of the synergistic interaction between species.

Methods: The experiment was conducted in earthen ponds for four months to investigate the effect of bivalves (*M. casta*) in halophyte-based IMTA on water quality, growth performance and microbial load. Shrimp and fish were fed with commercial feed containing 35% and 30% crude protein, respectively.

Result: The total ammonia nitrogen, nitrate-nitrogen and nitrite were comparatively low in treatments, T1 (0.13±0.012, 0.013±0.003 and 0.45±0.01, respectively) and T2 (0.15±0.005, 0.017±0.003 and 0.50±0.01, respectively). Similarly, the better average body weight, specific growth rate and feed conversion ratio were found in treatments, T1 (Shrimp: 33.87±0.88 g, 6.20±0.01% 1.10±0.02 respectively, Fish: 94.18±0.08 g, 1.59±0.001% and 1.60±0.006 respectively) and T2 (Shrimp: 32.37±0.60 g, 6.16±0.02 % and 1.15±0.02 respectively fish: 92.91±0.2 g, 1.59±0.009 and 1.63±0.08 respectively). Therefore it can be concluded that the use of bivalves helps to create better water quality for the growth and survival of *P. vannamei* and *M. cephalus* in halophyte-based integrated multi-trophic aquaculture.

Key words: Extractive species, Feed conversion ratio, Halophyte-based IMTA, *M. cephalus, P. vannamei*.

INTRODUCTION

The extensive use of intensive aquaculture practices to meet the growing demand for aquatic products has often resulted in various environmental and sustainability issues. The discharge of aquaculture wastewater from the culture system is becoming a serious environmental concern. The irrational release of aquaculture wastewater leads to nutrient pollution in the receiving water body and adversely affects benthic biodiversity due to increased organic enrichment (Custodio *et al*., 2017). These issues require research into environmentally benign, economically viable and sustainable farming methods. In this context, Integrated Multi-Trophic Aquaculture (IMTA) is a potential solution to reduce the environmental impacts associated with aquaculture practices and enhance farmers' productivity.

IMTA is an integrated and eco-friendly farming approach that allows organisms from different trophic levels to be cultivated in the same system to exploit the advantage of the synergistic interaction between species (Chopin *et al*., 2013). IMTA differs from the conventional polyculture method in which fish species from the same trophic level are cultured together (Barrington, 2009). In IMTA models, nutrients unutilized by one trophic level are diverted to lower trophic levels, where they are recaptured and used by the organisms for their growth and development (Custodio *et al*., 2017). Thus, IMTA reduces waste, ensures environmental sustainability through biomitigation (Barrington, 2009), increases productivity through product diversification and improves the overall resilience of the aquaculture sector (Troell *et al*., 2014). IMTA involves the incorporation of fed aquaculture species

[1]Department of Aquaculture, ICAR-Central Institute of Fisheries Education, Mumbai-400 061, Maharashtra, India.
[2]ICAR-Central Institute of Fisheries Education, Kakinada-533 001, Andhra Pradesh, India.
[3]ICAR-Central Institute of Brackishwater Aquaculture, Chennai-600 028, Tamil Nadu, India.
[4]Department of Aquaculture, Fisheries Research Station, P.V. Narsimha Rao Telangana Veterinary University, Palair, Khammam-507 157, Telangana, India.
[5]College of Agriculture, Gangavati, University of Agricultural Sciences, Raichur-583 227, Karnataka, India.

Corresponding Author: A.P. Muralidhar, ICAR-Central Institute of Fisheries Education, Kakinada-533 001, Andhra Pradesh, India. Email: Corresponding Email: murali@cife.edu.in

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(*e.g.*, fish/shrimp), inorganic extractive species (*e.g.*, seaweeds/algae) and organic extractive species (*e.g.*, suspension/deposit-feeders) at optimal ratio (Troell *et al.*, 2009). Proper selection and proportions of different species based on their economic value and ecosystem function are important to achieve a balance in the biological and chemical processes in IMTA (Barrington, 2009).

The integration of extractive species in IMTA involves the use of bivalves, the filter-feeding organism to remove the organic nutrients while seaweeds remove the inorganic waste nutrients to mitigate the negative environmental impact of fed aquaculture (Gallardi, 2014). Organic extractive species such as bivalves reduce the nutrient load in the culture system mainly particulate waste (fish/shrimp feed waste and feces) through suspension filter feeding (Jones and Iwama, 1991; Troell *et al.*, 2003). This in turn reduces the TSS or turbidity levels in post-harvest farm effluent, eventually reducing the eutrophication of the ecosystem (Sasikumar and Viji, 2015). So when the extractive species are incorporated into the fed aquaculture (*e.g.* fish/shrimp), they convert the waste into productive resources. In addition, they act as a carbon sink due to shell formation and are economically viable as they do not require pelleted feed (Sanz-Lazaro *et al.*, 2018). Alongside these ecological benefits, there is a growing market for cultured bivalves, which are considered high-value items that generate significant profits. The health benefits of bivalves are attributed mainly to their moderately high omega-3 fatty acids and lower fat content (Holmyard, 2008). Aquatic invertebrates, mostly marine bivalves, accounted for 16.2 million tonnes of the 24.3 million tonnes of non-fed species produced in 2020. Bivalve mollusc exports totaled USD 4.3 billion in 2020, accounting for about 2.8 percent of all aquatic product exports worldwide (FAO, 2022).

Bivalves affect the water column through filtering, grazing, altering nutrient cycles, direct excretion and microbially-assisted remineralization of their organic deposits in sediments (Petersen *et al.*, 2010). Phytoplankton numbers and blooms can be affected by massive bivalve assemblages. They can reduce phytoplankton concentration and decrease turbidity (Cranford *et al.*, 2003). The distribution of nitrogen is significantly altered by bivalves, particularly when they add nitrogen in the form of ammonium ($NH4^{+}$), remove phosphorus through deposition and recycle silicate by transporting it from the water column into the sediment. The ammonium excreted by bivalves is easily utilized for primary production, thereby increasing the nitrogen turnover in the water column. Furthermore, some bivalves, like mussels, can accumulate certain metals, such as copper in their pseudofeces (Cranford *et al.*, 2003).

Due to their environmental adaptability and marketability, it is necessary to consider native mussel species to design IMTA systems (Sasikumar and Viji, 2015). *Meretrix casta* was therefore used as an extractive species in this experiment due to its widespread availability in the experimental area.

MATERIALS AND METHODS

The experiment was conducted over 120 days from March to June 2022 at the brackishwater fish farm of the Central Institute of Fisheries Education (ICAR-CIFE), Kakinada regional centre, Kakinada, Andhra Pradesh, India (16.9400° N, 82.2576°E).

Experimental design and species used

Nine rectangular earthen ponds (200 m^{-2} each) were used for this study with two treatments, namely T1 (*A. officinalis* as an inorganic extractive and *M. casta* as organic extractive species) and T2 (*B. gymnorhiza* as an inorganic extractive and *M. casta* as organic extractive species and the control (without organic and inorganic extractive species). Each treatment has three replicates (n=3) randomly distributed between treatments. The fed species in this experiment were *Mugil cephalus* and *Penaeus vannamei*. The species combination and stocking density are shown in Table 1.

Pond preparation, stocking and post-stocking management

The experimental ponds were sundried, ploughed and prepared according to the method described by Biswas *et al*. (2019). Post-larvae -12 of *P. vannamei* were obtained from the commercial hatchery in the coastal region of Kakinada *and*hra Pradesh. The *grey mullet, M. cephalus* fingerlings collected from the wild were used for the study. *A. officinalis* and *B.gymnorhiza* saplings were obtained from the Coringa Mangrove Nursery of the Forest Department in Kakinada *and*hra Pradesh. *M. casta* was obtained from a nearby water-logged mangrove areas. *A. officinalis* and *B. gymnorhiza* were installed (Fig 1) in the pond using floating PVC frames, while *M. casta* was spread over the pond bottom. *P. vannamei* and *M. cephalus* were fed commercial diets containing 35% and 30% crude protein, respectively.

Physico-chemical parameters of water

A mercury thermometer was used to measure the water temperature. pH meter (Eutech PC 450, Thermo Scientific, Singapore) was used for measuring water pH. The Winkler's method was used to determine dissolved oxygen (DO), total ammonical nitrogen (TAN.), nitrite-nitrogen ($NO_{2-}N$), nitrate-nitrogen ($NO_{3-}N$), total phosphorous, total alkalinity, hardness, total suspended solids, biological Oxygen Demand (BOD), chemical oxygen Demand (COD), were measured at 15-day intervals using standard methods (APHA, 2005).

Estimation of microbial population in pond water

The total vibrio count (TVC) and total heterotrophic bacteria (THB) were observed fortnightly by following the method of Kumar *et al*. (2014).

Growth parameters

The growth performance of the shrimp and fish is calculated by usingthe following formulas:

Average body weight (ABW) =

$$\frac{\text{Total weight (g)}}{\text{Total number of shrimp or shrimp taken}}$$

Specific growth rate (SGR) =

$$\frac{\text{In final weight - In inital weight}}{\text{Duration of feeding}} \times 100$$

Feed conversion ratio (FCR) =

$$\frac{\text{Feed given (g in dry weight)}}{\text{Body weight gain (g in wet weight)}}$$

Protein efficiency ratio (PER) =

$$\frac{\text{Body weight gain (Wet weight)}}{\text{Total protien fed}}$$

Survival rate (%) =

$$\frac{\text{Total number of animals harvested}}{\text{Total number stocked}} \times 100$$

RESULTS AND DISCUSSION

Physico-chemical parameters of water

The physic-chemical characteristics of all the experimental pond water are shown in Table 2. All water quality criteria for the culture of brackishwater fish and shrimp were within the ideal range throughout the experiment; (Biswas *et al*., 2012; Biswas *et al*., 2017). There was no difference (p>0.05) in the mean values of pH, temperature, alkalinity, salinity, or hardness between the treatments. Other measures including DO, TAN, NO_2 and NO_3 showed a statistically significant difference (p<0.05) between treatments. In contrast to the polyculture system, a significantly (p<0.05) lower DO level was observed in the IMTA-based treatments (T1 and T2). Compared to the polyculture system, the IMTA-based system showed significantly (p<0.05) lower values for the nutritional parameters TAN, NO_2, NO_3 and phosphate-phosphorus. DO is essential for aquaculture production (Rahman *et al*., 2020; Mahmudi *et al*., 2022). All treatment ponds recorded a DO level greater than 5 mg L^{-1} (MoEF, 2005; Boyd, 1992). Shrimp, fish and microbes all consume the DO produced by photosynthesis during the day (Boyd, 1992). In all treatments, the DO concentration was higher than 8 mg L^{-1} during the day, with a decrease in oxygen levels at night. The mangrove treatments had a low oxygen concentration; This could be because treatments with mangrove-based vegetation resulted in higher respiratory rates. Oxygen depletion at night was not a serious problem due to the presence of aerators (Moroyoqui-Rojo *et al*., 2012). Total ammonia levels (TAN) increased concomitantly with the higher stocking density of Nile tilapia, which negatively impacted growth, immunity, dissolved oxygen levels and digestive enzyme function (Dawood *et al*., 2019). The bacterial breakdown of organic materials such as feed waste, feces and other organic waste is the main source of ammonia in any aquatic system. Nitrite is a by-product of nitrification and its concentration is inversely correlated with that of ammonia (Bhatnagar and Devi, 2013). Therefore, the IMTA

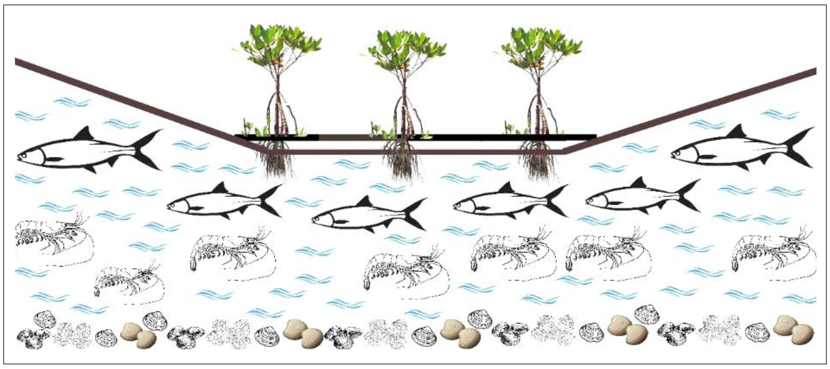


Fig 1: Schematic representation of Integration of mangrove plants.

Table 1: Species combination and stocking density in different treatment groups.

Species	C	T1	T2
Penaeus vannamei (number ha^{-1})	300000	300000	300000
Mugil cephalus (number ha^{-1})	10000	10000	10000
Meritrix casta (kg ha^{-1})	-	1350	1350
Avicennia officinalis (number ha^{-1})	-	2000	-
Bruguiera gymnorhiza (number ha^{-1})	-	-	2000

C, Control; T1 and T2, Halophyte based IMTA treatments.

system is successful in reducing the inorganic nitrogenous compounds (NO2-N, NO3-N and TAN) and phosphate-phosphorus (PO_4-P) concentration when *M. casta*, *A. officinalis* and *B. gymnorhiza* were used as extractive species (Moroyoqui-Rojo *et al*., 2012; Oliveira *et al*., 2021). BOD and COD were found to differ significantly between treatments (p<0.05); the T1 treatment had the lowest values, followed by the T2 treatment and control ponds had the highest values, which may be related to an increase in the concentration of decaying materials (Nandan and Azis, 1990). The presence of filter feeders such as clams, which reduce TSS (p<0.05), may account for the lower values in the halophyte-based system (Cloern, 1982). In an integrated cage aquaculture system, Kaspar *et al*. (1985) found that bivalves can reduce phytoplankton bloom intensity and turbidity and oysters can decrease eutrophication (Viji *et al*., 2014). Studies by Cloern (1982) and Officer *et al*. (1982) in the San Francisco Bay area further demonstrated that the abundance of bivalve suspension feeders directly decreased the amount of suspended solids available for remineralization by pelagic consumers and bacterioplankton.

Table 2: Effect of extractive species on water quality parameters among different treatments during 120-day experiment period.

Treatments	C	T1	T2
DO (mg L^{-1})	6.3±0.5^b	5.8±0.6^a	6±0.6^a
PH	8.02±0.15	8.12±0.21	8.05±0.2
Temperature	31.5±0.6	31.6±0.5	32±0.62
salinity (g L^{-1})	7.5±0.35	8±0.6	7.66±0.9
Alkalinity (mg L^{-1})	159±1.5	165±2.4	163±1.2
Hardness (mg L^{-1})	1928±92	1949±132	2012±189
TAN (mg L^{-1})	0.45±0.005^b	0.13±0.012^a	0.15±0.005^a
NO_2 (mg L^{-1})	0.160±0.006^b	0.013±0.003^a	0.017±0.003^a
NO_3 (mg L^{-1})	0.88±0.02^c	0.45±0.01^a	0.50±0.01^a
PO_4 (mg L^{-1})	0.54±0.01^b	0.11±0.02^a	0.13±0.01^a
BOD (mg L^{-1})	7.3±0.09^b	3.9±0.09^a	4.1±0.12^a
COD (mg L^{-1})	108.00±1.53^b	40.07±0.30^a	41.43±0.52^a
TSS (mg L^{-1})	123.00±1.53^b	23.43±0.52^a	24.00±0.58^a

The different superscripts in the column indicate statistical differences among treatments (P<0.05). The results are expressed as mean SE.

Monitoring of microbial population in pond water

In this work, it was discovered that the presence of bivalves is crucial in reducing both THB and TVC in culture water. The total vibrio count and total heterotrophic bacteria are shown in Table 3, Fig 2 and 3. Significantly (p<0.05) lower

Table 3: Effect of extractive species on microbial population (mean ± S.E.) from different treatments at 15-day intervals.

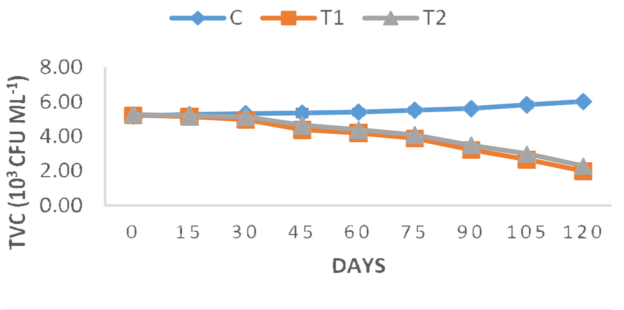
Days	TVC (10^3 CFU mL^{-1})			THB (10^5 CFU mL^{-1})		
	C	T1	T2	C	T1	T2
0	5.20±0.06^a	5.23±0.03^a	5.27±0.02^a	5.19±0.19^a	5.16±0.29^a	5.20±0.14^a
15	5.27±0.09^a	5.15±0.08^a	5.20±0.06^a	5.52±0.09^a	5.32 ±0.17^a	5.34±0.06^a
30	5.32±0.07^b	4.95±0.09^a	5.13±0.03^{ab}	6.37±0.24^b	5.72±0.18^a	5.76±0.06^{ab}
45	5.36±0.23^b	4.40±0.06^a	4.67±0.09^a	7.69±0.09^b	5.30±0.1^a	5.31±0.08^a
60	5.42±0.17^b	4.20±0.06^a	4.40±0.06^a	8.52±0.21^b	4.69±0.06^a	4.73±0.15^a
75	5.52±0.17^b	3.90±0.1^a	4.10±0.06^a	9.37±0.21^b	4.12±0.06^a	4.16±0.03^a
90	5.62±0.11^c	3.23±0.03^a	3.50±0.06^b	9.77±0.09^b	3.73±0.07^a	3.83±0.06^a
105	5.83±0.18^b	2.67±0.09^a	3±0.06^a	10.82±0.15^b	3.41±0.09^a	3.46±0.17^a
120	6.03±0.09^c	2±0.10^a	2.3±0.06^b	11.22±0.06^b	2.93±0.09^a	2.94±0.05^a

Different superscripts in the column indicate statistical differences among treatments (P<0.05). The results are expressed as mean SE.

Table 4: Effect of extractive species on growth performance of shrimp (*Penaeus vannamei*), mullet (*Mugil cephalus*).

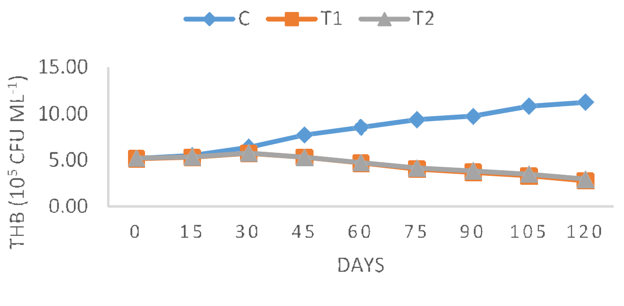
Treatments	Initial weight (g)	Final weight (g)	ADG (g)	SGR (%)	Survival rate (%)	FCR	PER	Total production
P. vannamei								
C	0.02±0^a	31.47±0.63^a	0.26±0.01^a	6.13±0.02^a	86±1^a	1.29±0.05^b	2.15±0.1^a	8125.4±115.7^a
T1	0.02±0^a	33.87±0.88^b	0.28±0.01^b	6.20±0.01^b	91±1.5^c	1.10±0.02^a	2.54±0.03^b	9268.3±72.9^b
T2	0.02±0^a	32.37±0.60^{ab}	0.27±0.01^{ab}	6.16±0.02^{ab}	89±1^b	1.15±0.02^a	2.41±0.05^b	8619±121.5^a
M. cephalus								
C	4.67±0.03^a	85.67±0.66^a	0.68±0.01^a	2.43±0.01^a	96±0.5^a	1.52±0.02^b	2.70±0.02^a	790.59±11.2^a
T1	4.70±0.05^a	90.60±0.98^b	0.72±0.01^b	2.47±0.01^b	97±0.60^a	1.43±0.02^a	2.86±0.03^b	834.99±5.90^b
T2	4.70±0.06^a	89.73±0.77^b	0.71±0.01^b	2.46±0.01^{ab}	97±1^a	1.44±0.04^a	2.83±0.06^b	816.07±9.77^b
M. casta								
T1	17.27±0.08	21.64±0.03	0.04±0.00		52.91±0.90			894.90±17.42
T2	17.52±0.14	21.69±0.15	0.03±0.00		52.89±0.84			883.73±7.31

The different superscripts in the same column indicate the significant difference among the treatment (p<0.05). The results are expressed as meanSE.



The results are expressed as mean±S.E with a significant difference (P<0.05).

Fig 2: Variation in total vibrio count (TVC) among different treatments at 15-day intervals.



The results are expressed as mean±S.E with a significant difference (P<0.05).

Fig 3: Variation in total heterotrophic bacteria (THB) among different treatments at 15-day intervals.

TVC and THB were found in T1 and T2 than in control. The presence of bivalves such as *M. casta* in T1 and T2 treatments helps to reduce THB and TVC. Bivalves have effective filtration abilities that can remove excess nitrogenous waste from feed and feces, reducing the nutrients available for bacterial growth. Unlike nitrate, a higher concentration of ammonia or organic nitrogen provides a better environment for microbial growth (Zhuang *et al*., 2020). Many species of the genus Vibrio are known to transmit various diseases to farmed shrimp and finfish (Alavandi *et al*., 2004; Austin and Zhang, 2006). The co-culture of *P. vannamei* and *C. gigas* appeared to improve the physiological circumstances that reduced the prevalence of Vibrio in the intestine, which was vulnerable to environmental factors (Xing *et al*., 2013). Bivalves raised in a co-culture system had gut bacterial populations that were more diverse than the ones raised in a monoculture system (Omont *et al*., 2020). The eastern oyster (*C. virginica*) may have the ability to control microbial populations in the tidal creek of South Carolina's North Inlet (Wetz *et al*., 2002). One of the main causes of the disappearance of micro-sized protists is believed to have been the grazing activity of *C. gigas*, which efficiently retains particle size >5 µm (Dupuy *et al*., 2000). As a result, the decline in Vibrio populations in the presence of bivalves can be used as a sign that aquatic species are in good health (Xing *et al*. 2013).

Growth performance

The growth parameters of *P. vannamei* and *M. cephalus* are presented in Table 4. There were significant ($p<0.05$) changes in final body weight, SGR (%), FCR, PER and total production in *P. vannamei* and *M. cephalus*. The higher final weight, SGR, PER and total production in the T1 and T2 group of fed species due to better water quality in IMTA resulted in better growth performances of shrimp and fish (Rejeki*et al*., 2016). On the other hand, when seabass was exposed to high concentrations of ammonia, Lemarie *et al*. (2004) observed weight loss and growth retardation. In the present study, the FCR was lower in the halophyte-based IMTA system than in the control due to better feed utilization of fed species in the IMTA systems, Biswas *et al*. (2020) reported that in IMTA there was 22% reduction in FCR compared to the polyculture system. In the present study, FCR was reduced by 14% in T1 and 7% in T2 treatment compared to control. The survival rate of *P. vannamei* is significantly higher in the halophyte-based IMTA than in the control, this could be attributed to the better water quality created by the extractive species in IMTA, the same result was reported by Biswas *et al*., 2019 and Naskar *et al*., 2022. While in the case of *M. cephalus,* no significant difference was observed among the treatments.

CONCLUSION

This study concludes that the use of extractive species creates favorable water quality conditions for better growth performance of *P. vannamei* and *M. cephalus.* The shrimp and fish of T1 and T2 groups showed better ABW (g), SGR, FCR, PER, survival rate and lower microbial loads were found in the T1 and T2 groups. In view of all this, the present study concludes that the use of bivalve species *M. casta* in T1 and T2 treatments contributes to better water quality, good growth performance and low microbial load for rearing *P.vannamei* and *M.cephalus*.

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