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Influence of different stocking densities of pearlspot (*Etroplus suratensis*) on plankton diversity indices of biofilm-based rearing system

S.R. Yadav¹, B.R. Chavan^{2*}, N.K. Chadha³, P.S. Bodake⁴, K.K. Krishnani⁵, P.B. Sawant³ and R. Bhosle¹¹Department of Aquaculture, College of Fishery Science, Latur-413 517, India²Department of Aquaculture, College of Fisheries, Ratnagiri-415 629, India³Aquaculture Division, ICAR-Central Institute of Fisheries Education, Mumbai-400 061, India⁴Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli-415 712, India⁵ICAR-Indian Institute of Agricultural Biotechnology (Deemed to be University), Ranchi-834 003, India

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*Corresponding Author Email : brc15672@gmail.com*ORCID: <https://orcid.org/0000-0003-1900-5066>

Abstract

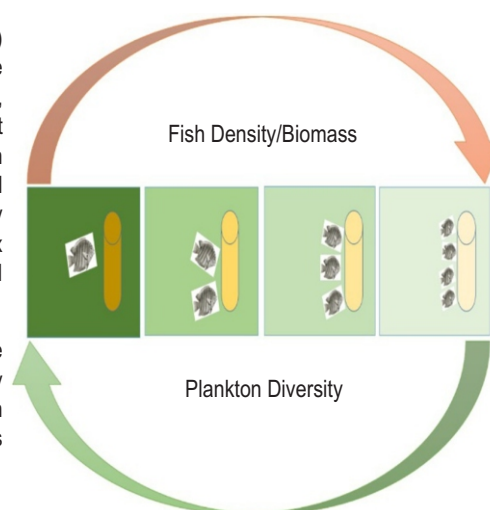
Aim: Biofilm-based aquaculture systems, proven cost-effective, reduce the need for expensive supplementary feed. The biofilm acts as a natural planktonic food source for reared organisms. Previous research highlighted the influence of fish stocking density and biomass on plankton diversity in different aquaculture settings, underscoring the significance of these factors in biofilm-based systems. Hence, the present study attempted to understand how varying stocking densities of pearlspot (*Etroplus suratensis*) influence plankton diversity indices in the biofilm-based aquaculture system.

Methodology: The present experiment was designed in 5 Practical Salinity Unit (PSU) brackishwater with soil-bottomed FRP circular (500 L) tanks for 60 days. The sugarcane bagasse was used as a substrate for biofilm production after fertilization with cow dung, urea, and lime. Fries of pearlspot (4.67 ± 0.04 mm 1.71 ± 0.03 g⁻¹) were stocked at four different stocking densities in triplicates viz T₁ (50 nos); T₂ (100 nos), T₃ (150 nos) and T₄ (200 nos) fish fry m⁻³ in the biofilm-based rearing system. Plankton samples were collected from water and biofilm deposited on the substrate and analyzed for diversity indices. The Shannon Diversity Index, Simpson Diversity Index, Simpson Dominance Index, and Margalef Richness Index were used to compute the plankton diversity indices in different treatments using standard equations.

Results: In the current investigation, lower stocking densities with lower fish biomass were associated with significantly higher ($P \leq 0.05$) Shannon Diversity Index, Simpson Diversity Index, and Margalef Richness Index in water. In contrast, the Simpson Dominance Index in water showed a significantly lower ($P \leq 0.05$) value for treatments with lower fish biomass than for treatments with higher biomass.

Interpretation: The values obtained for various diversity indices indicated that a biofilm-rearing system with lower *E. suratensis* biomass produced more planktonic abundance, evenness, and species richness.

Key words: Biofilm, Pearlspot, Plankton diversity, Stocking density



Introduction

Etroplus suratensis known as pearlspace or green chromide, holds a significant place in both natural ecosystems of the Indian subcontinent and aquaculture industry. This cichlid species is indigenous to the region, thriving in a variety of habitats ranging from freshwater bodies to brackish water environments like rivers, lakes, estuaries and coastal lagoons (Ward and Wyman, 1977). Its adaptability to diverse aquatic ecosystems underscores its ecological importance and resilience in fluctuating environmental conditions. One of the key factors contributing to the prominence of pearlspace in aquaculture is its culinary value and popularity as a food fish, particularly in South India (Balasubramaniam et al., 2022). The fish is widely relished for its mild flavor and tender flesh, making it a preferred choice among consumers. As a result, pearlspace is commonly farmed in ponds and various aquaculture systems, reflecting its economic significance and contribution to the food industry.

The terms 'biofilm' or 'periphyton' are used interchangeably in the literature to describe microbial attachment facilitated by an extracellular matrix, forming thin layers during a complex cyclic process involving initial, maturation and dispersal phases (Donlan, 2002; Khatoun et al., 2018), which adhere to surfaces such as rocks, plants, and submerged structures, serving as a natural food source for many aquatic organisms. In aquatic ecosystem studies, the term 'biofilm' includes different microbial organisms, including microalgae. Natural biofilms, composed of heterotrophic species like bacteria, fungi, and protists, as well as autotrophic species such as microalgae and cyanobacteria, are vital in aquatic ecosystems, forming a consortium of microorganisms (Xuemei et al., 2010; Yadav et al., 2022). Biofilms on submerged surfaces result from the selective attachment of microorganisms, facilitation, and interspecific competition in the microbial communities (Rummel et al., 2017). Biofilm systems offer an environmentally friendly approach to aquaculture by utilizing natural food sources and reducing reliance on commercial feeds (Pandey et al., 2014; Yadav et al., 2021).

Plankton, comprising diverse organisms including phytoplankton and zooplankton, serve as a primary food source for many cultured species during their early stages of development (Creswell, 2010). Plankton communities exhibit dynamic fluctuations in composition, abundance, diversity, and dominance within their habitat in response to environmental changes (Mathivanan et al., 2007). The physio-chemical parameters of water, such as temperature, pH, nutrient levels, and salinity, exert a significant influence on the quantity and diversity of planktonic organisms (Wetzel, 2001). The composition of plankton ecosystems serves as a crucial indicator of water quality, reflecting the overall health of aquatic environments (Kumar et al., 2020). Fluctuations in plankton diversity result from intricate interactions between biological and environmental factors, shaping aquatic ecosystems' complexity. Aquaculture practices integrating biofilms significantly influence plankton community composition, contingent upon stocked

species and culture environment characteristics (Gogoi et al., 2018; Kumar et al., 2009). Biofilms act as natural food and habitat sources for planktonic organisms, molding their abundance, distribution and diversity within aquaculture systems. This integration fosters planktonic organism growth, augmenting overall productivity and ecological sustainability.

Stocking density significantly impacts various aquaculture parameters in both clear water systems (Garr et al., 2011; Khatune-Jannat et al., 2012; M'balaka et al., 2012) and biofilm-based systems (Asaduzzaman et al., 2009; Richard et al., 2010; Uddin et al., 2007). However, its influence on plankton diversity in microbial-based culture systems like biofilm and biofloc is rarely documented. In biofloc systems, stocking density affects plankton composition, potentially leading to the dominance of specific groups and blooms at different densities (Silva et al., 2022). Research on Nile tilapia in biofloc systems has shown that biofloc enhances zooplankton composition, thereby improving growth and feed utilization at varying stocking densities (Eid et al., 2020). Despite these findings, the impact of stocking density on plankton diversity indices in biofilm-based rearing systems, particularly with species like pearlspace, remains understudied. Investigating this relationship could offer critical insights for optimizing aquaculture practices and fostering ecological balance in aquaculture environments.

Materials and Methods

The experiment was conducted over 60 days in the twelve circular fiber-reinforced plastic (FRP) tanks, each with an identical capacity of 500 l. All tanks were disinfected with KMnO_4 (10 mg l^{-1}) and rinsed with clean water. On the subsequent day, sun-dried mangrove soil was filled up to 9 to 10 cm thickness, and agricultural lime was applied @ 500 kg ha^{-1} . The required 5 Practical Salinity Unit (PSU) salinity was prepared by mixing 25 PSU brackish water with fresh water.

All tanks were provided with uniform continuous aeration and were fertilized with cow dung and urea @ 3000 kg and 150 kg ha^{-1} , respectively (Ramesh et al., 1999). Sugarcane bagasse was selected as a substrate since it was found more suitable in the pearlspace biofilm-based culture system (Shilta et al., 2016). Fresh bagasse was procured from local sugarcane juice vending shops and soaked in water for two days to eliminate the residual sugar (Gangadhar et al., 2015). Subsequently, sugarcane bagasse was dried under bright sunshine. The small cylindrical bundles of $30 \pm 5 \text{ cm}$ were made using nylon threads. Six bundles covering approximately 4000 cm^2 (800 g) surface areas were suspended vertically at a regular distance using ropes tied to the walls in each biofilm tank (Shilta et al., 2016).

Etroplus suratensis fry was procured from a private hatchery located at Kakadwip, Kolkata, and transported in polythene bags containing 5 PSU saline water via airways and road transportation. After transportation, the fish were acclimatized to a salinity of 5 PSU. Fries were fed a fixed ration


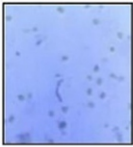

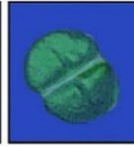
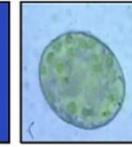
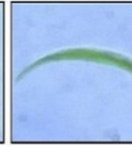
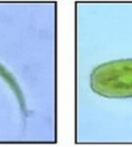
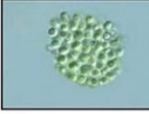
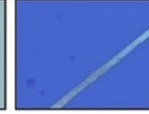
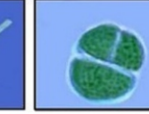


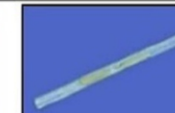


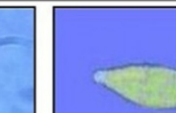

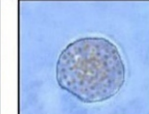

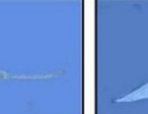

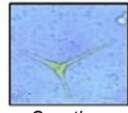
Group	Genera
Green algae	       <i>Microspora</i> <i>Chlorella</i> <i>Scenedesmus</i> <i>Cosmarium</i> <i>Volvox</i> <i>Closterium</i> <i>Chlamydomonas</i>
Blue-green algae	   <i>Microcystis</i> <i>Oscillatoria</i> <i>Chroococcus</i>
Diatom	      <i>Cyclotella</i> <i>Navicula</i> <i>Synedra</i> <i>Craticula</i> <i>Amphipleura</i> <i>Diatoma</i>      <i>Planktoniella</i> <i>Concinodiscus</i> <i>Nitzschia</i> <i>Gyrosigma</i> <i>Pleurosigma</i>
Dinoflagellate	 <i>Ceratum</i>

Fig. 1: Phytoplankton recorded in the water and substrate in experimental biofilm tanks.

equivalent to 20% of their biomass per day of a commercial diet (FeedWale) consisting sinking pellets of size 0.5 mm, containing 38% protein and 6% fat, with two meals provided daily over a 15-day period in indoor tanks. To ensure water quality, fifty percent of brackish water (5 PSU) in the experimental tanks was replaced every two days with freshly treated water. The experimental design involved four stocking density levels (T_1 : 50, T_2 : 100, T_3 : 150, and T_4 : 200 fish m^{-3}) in biofilm tanks, each filled with 300 l of water. Uniform-sized fry (4.67 ± 0.04 mm 1.71 ± 0.03 g $^{-1}$) of *E. suratensis* were stocked randomly at selected stocking density levels in triplicate fifteen days after adding fertilizers and substrates.

Plankton Analysis: Plankton samples were collected by filtering 10 l of water using a plankton net (mesh size 40 μ) at an interval of 15 days (Mridula, 2003; Ramesh *et al.*, 1999). Biofilm deposited on a 4 cm 2 area of the substrate was scraped and suspended in 10 ml distilled water at an interval of 15 days. The plankton samples were preserved using a 5% Lugols iodine solution for further analysis. The counting of plankton was carried out using a Sedgewick Rafter Cell counter and identified at the genus level by using standard literature with the help of a binocular microscope at 10x, 40x and 100x magnification (APHA, 2012; Newell and Newell, 1965; Ward and Whipple, 1959).

Plankton diversity indices: Shannon Diversity Index (H), Simpson Diversity Index-Reciprocal (D), Simpson Dominance Index (D'), and Margalef Richness Index (d) were used to compute the plankton diversity indices in different treatments using equations given by Magurran (2004).

Statistical Analyses: The data were statistically analyzed using SPSS (Statistical Package for Social Science) version 20.0 (SPSS Inc., Chicago, IL, USA). The treatment means were compared by One-way ANOVA followed by Duncan's Multiple rRange Test (DMRT) at 5% probability level. The relationships between different variables were established using regression models (Kleinbaum *et al.*, 1988).

Results and Discussion

A total number of 26 genera of the planktonic community were identified, which comprised of diatoms (11 genera), green algae (7), blue-green algae (3), dinoflagellate (1), protozoa (2), rotifer (1) and copepod (1) (Fig. 1, 2). The diatom was the dominant plankton in water and substrate in all biofilm treatment tanks. The earlier research reported different types of dominant algae in the biofilm-based culture system. Green algae

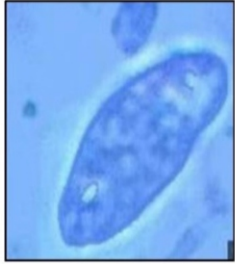
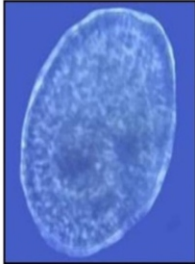

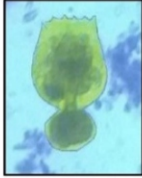


Group	Genera
Protozoa	   <p><i>Paramecium</i> <i>Stylonychia</i></p>
Rotifer	  <p><i>Brachionus</i></p>
Copepod	 <p><i>Cyclops</i></p>

Fig. 2: Zooplanktons recorded in the water and substrate in experimental biofilm tanks.

(Chlorophyceae) was a dominant alga in the periphyton composition of the carp polyculture system (Gogoi *et al.*, 2018); phytoplankton (Chlorophyceae and Bacillariophyceae) and two groups of zooplankton (rotifer and copepod) as a principal community during *E. suratensis* rearing in the inland saline groundwater ponds provided with the substrate (Kumar *et al.*, 2009); diatom (Baccilariophyta) as a dominant community in substrates added brackish water ponds (Khatoon *et al.*, 2007) and pennate diatoms as the main alga in biofilm during intensive shrimp culture (Thompson *et al.*, 2002). In microbial-based aquaculture systems such as biofloc, Khanjani *et al.* (2022) found that environmental factors, including salinity, light intensity, carbon sources, carbon-to-nitrogen ratio, aeration, total suspended solids, and stocking density, significantly influence the quality, diversity, and density of microbial communities. These findings highlight the ecological adaptability and specificity of algal communities within diverse aquaculture environments.

The diversity of plankton communities within aquaculture systems plays a pivotal role in maintaining the ecosystem balance and productivity. Understanding the intricate relationships between fish biomass, stocking density and

plankton diversity indices is essential for optimizing aquaculture practices and promoting ecological sustainability. Different plankton diversity indices of all biofilm treatments in water are presented in Table 1. The significantly higher ($P \leq 0.05$) Shannon Diversity Index (H) index was recorded in T_1 than in T_3 and T_4 , whereas it was insignificant ($P \leq 0.05$) with T_2 . The significantly higher ($P \leq 0.05$) Simpson Diversity Index - Reciprocal (D) and Margalef Richness Index (d) were recorded in T_1 than in other groups of the experiment. On the contrary, there was a significantly higher ($P \leq 0.05$) Simpson Dominance Index (D') recorded in T_3 than in T_1 , whereas it was insignificant ($P \geq 0.05$) with T_2 and T_4 . A correlation analysis was carried out to investigate the relationship between plankton diversity indices in the water and the final fish biomass (Tables 1, 2). The results demonstrated a significant ($P \leq 0.05$) strong negative linear relationship ($R^2=0.942$) between the Shannon Diversity Index (H) of planktons in the water and the final fish biomass. According to Keshava *et al.* (1988), *E. suratensis* is a bottom-feeding scavenger with an herbivorous tendency, preferring micro-vegetation and decayed organic matter in younger stages. Thus, in the present rearing experiment, micro-vegetation may serve as the primary food for

Table 1: Diversity indices of plankton in water and substrate at different stocking densities in the biofilm-based rearing system

Treatments	Plankton diversity indices								Final fish biomass (g)
	Water				Substrate				
	H	D	D'	d	H	D	D'	d	
T ₁	1.47 ^b ± 0.04	3.38 ^b ± 0.05	0.30 ^a ± 0.01	2.18 ^a ± 0.25	1.46 ^a ± 0.11	5.60 ^a ± 0.93	0.19 ^a ± 0.04	2.76 ^a ± 0.24	81.6 ^a ± 3.74
T ₂	1.32 ^{ab} ± 0.06	2.75 ^a ± 0.15	0.37 ^{ab} ± 0.01	1.52 ^a ± 0.11	1.55 ^a ± 0.05	4.58 ^a ± 0.20	0.22 ^a ± 0.01	2.10 ^a ± 0.18	130.9 ^b ± 5.20
T ₃	1.16 ^a ± 0.07	2.33 ^a ± 0.25	0.44 ^b ± 0.05	1.34 ^a ± 0.11	1.33 ^a ± 0.02	4.68 ^a ± 0.58	0.22 ^a ± 0.03	2.17 ^a ± 0.30	151.3 ^b ± 5.04
T ₄	1.23 ^a ± 0.06	2.84 ^a ± 0.16	0.36 ^{ab} ± 0.02	1.15 ^a ± 0.08	1.43 ^a ± 0.06	4.22 ^a ± 0.40	0.24 ^a ± 0.02	2.11 ^a ± 0.15	139.2 ^b ± 0.91

H- Shannon Diversity Index; D- Simpson Diversity Index; D'- Simpson Dominance Index and d- Margalef Richness Index. T₁, T₂, T₃ and T₄ treatments with a stocking density of 50, 100, 150 and 200 fish m⁻³ in the biofilm rearing system.

Table 2: Correlation of diversity indices of planktons with final biomass of *E. suratensis* reared in the biofilm-based system

Plankton diversity index	Relationship analysis of plankton diversity index (y) with final fish biomass (x)							
	Water				Substrate			
	Type of relationship	Equation	R ²	Significance (p-value)	Type of relationship	Equation	R ²	Significance (p-value)
H	Linear	y = -0.0042x + 1.829	0.942	0.029*	Linear	y = -0.0012x + 1.589	0.1536	0.608
D	Linear	y = -0.0133x + 4.497	0.888	0.058	Linear	y = -0.0168x + 6.888	0.7693	0.123
D'	Linear	y = 0.0017x + 0.155	0.8117	0.099	Linear	y = 0.0006x + 0.146	0.7085	0.158
d	Linear	y = -0.0138x + 3.280	0.8861	0.059	Logarithmic	y = -1.093ln(x) + 7.542	0.9068	0.048*

H- Shannon Diversity Index; D- Simpson Diversity Index; D'- Simpson Dominance Index and d- Margalef Richness Index. R²- Coefficient of determination. *Significance at P ≤ 0.05; NS-Non-significance at P > 0.05

pearlspace fry. Lazzaro *et al.* (1992) concluded that fish biomass plays a more significant role in regulating plankton structure than the kind of fish species. Increased fish density and biomass could lead to higher feeding pressure on plankton, thereby impacting plankton diversity indices in aquaculture systems (Attayde and Menezes, 2008).

Different plankton diversity indices on the substrate of all biofilm treatments are presented in Table 1. No significant (P ≥ 0.05) difference was observed for all measured indices among different treatments. However, relationship analysis between the Margalef Richness Index (d) of plankton present on the substrate and the final fish biomass revealed a significant (P ≤ 0.05) strong negative logarithmic relationship (R² = 0.9068) between these two variables. Shannon Diversity Index (H) and Simpson's Diversity Index (represented by "D") are commonly used metrics to assess the richness and evenness of species in a community (Hossain *et al.*, 2017). The present study in a biofilm-based aquaculture system revealed that lower stocking densities and reduced fish biomass were associated with significantly higher H and D indices in water, indicative of more diverse and evenly distributed plankton communities. This aligns with previous research suggesting that reduced competition for resources and lower predation pressure at lower stocking densities foster conditions

conducive to higher plankton diversity (Attayde and Menezes, 2008). Moreover, Simpson's Dominance Index (D) provides insights into the dominance of specific plankton species within an aquatic system (Magurran, 2004). In contrast to H index, the present study observed significantly lower D' indices in treatments with lower stocking densities and fish biomass, indicating less dominance by specific plankton species and promoting a more evenly distributed community structure. This supports the opinion that lower stocking densities result in reduced competition, allowing for a more balanced distribution of species (Soedibya *et al.*, 2022).

The observed higher values of the Margalef Richness Index (d) in water for lower stocking densities with lower fish biomass further reinforce the association between these factors and plankton community diversity and stability. This suggests that conditions favoring longer food chains and complex food webs, often associated with lower stocking densities, support a more diverse and balanced plankton community, ultimately contributing to a stable ecosystem (Margalef, 1956). Furthermore, the multifaceted influence of fish biomass on plankton diversity indices underscores the complexity of aquatic ecosystems (Williams and Moss, 2003). Greater fish biomass exerts increased feeding pressure, potentially leading to a decline in

plankton abundance and diversity. Selective feeding by different fish species can also alter the composition of plankton communities (Feniova et al., 2019). When fish biomass exceeds a certain threshold, the interactions between fish and plankton biomass become nonlinear and reach equilibrium (Lazzaro et al., 1992; Attayde and Menezes, 2008). This might explain why most plankton diversity indices on the substrate remain affected, except for the Margalef Richness Index, which is influenced by the increased fish biomass due to intraspecific competition. Therefore, optimizing fish biomass and stocking density in aquaculture systems is crucial for maintaining plankton diversity and overall ecosystem health. The presented research underscores the importance of considering fish biomass and stocking density in aquaculture management practices to maintain ecological balance and productivity. Lower stocking densities and reduced fish biomass contribute to higher plankton diversity, promoting a more stable ecosystem. Further studies exploring the long-term effects of different stocking densities on plankton communities will provide valuable insights into the sustainable management of aquaculture systems and the preservation of aquatic ecosystems.

The present study sheds light on the profound impact of stocking density on planktonic abundance, evenness, and species richness within biofilm-based pearlspace rearing systems. The observed enhancement in these key metrics underscores the critical role that stocking density plays in shaping the composition and dynamics of planktonic communities. Lower stocking densities create conditions conducive to greater species diversity and balanced community structures by reducing competition for limited resources. Optimizing stocking density not only enhances ecological sustainability but also boosts economic viability in pearlspace aquaculture. Sustainable management practices prioritizing biodiversity conservation are crucial for ecosystem resilience and stability. Further research is needed to understand the long-term effects on plankton dynamics and ecosystem functioning, emphasizing the integration of ecological principles into aquaculture strategies.

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Authors' contribution: S.R. Yadav: Performed the work as part of his Ph. D. research work; B.R. Chavan: Provided technical support, drafted the manuscript and provided overall guidance; N.K. Chadha: Conceptualized and designed the experiment; P.S. Bodake: Supported with experimental facilities and assisted in laboratory analysis; K.K. Krishnani: Assisted in laboratory analysis; P.B. Sawant: Extended help with statistical analysis

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