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Effects of Stocking Density Fluctuations on Benthic Habitat Conditions in Freshwater Aquaculture Systems

[1*] Manuel Herrera, Seville Institute for Smart Agriculture Research, Seville, Spain.

[2] Alba Márquez, Seville Institute for Smart Agriculture Research, Seville, Spain.

Abstract: Stocking density is one of the major managerial tools in pond aquaculture; however, the actual density of organisms is not static throughout a crop due to death, harvesting, and restocking, and this variability could impact on the environment of the sediments inhabited by the benthic community. In this research project, it was determined how nominal stocking density and the actual mortality-related fluctuation in stocking density impact the sediment and bottom water in an earthen pond aquaculture system. The re-analyzed a publicly accessible experimental data set where green mud crabs (Scylla paramamosain) were stocked at 1, 1.5, 2 and 3 crab m^{-2} in replicated tanks over 101 days. Survival decreased exponentially with increasing density ($r^2 = 0.98$), total production dropped 31% with stocking rates tripling, and feed conversion efficiency went up from 5.2 to 21.7. Nitrite concentration of sediments increased 52% over the range of minimum and maximum density and correlated with feed loading ($r = 0.96$), while organic matter and sediment oxygen demand remained equal at different densities, and organic matter exceeded the normal range of 1–3% for all densities. Oxygen levels in the bottom water decreased from 4.2 to 2.8 mg/L throughout the culture period. These data suggest that loading with feed and dead fish due to density is what changes the nitrogen status in sediments, not organic matter.

Keywords: Stocking Density; Benthic Habitat; Sediment Quality; Aquaculture Ponds; Nitrite; Dissolved Oxygen; Mud Crab.

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I. Introduction

Currently, aquaculture provides much of the aquatic food consumed, with Asian inland aquaculture accounting for the greatest increase in world production during the past two decades (Naylor et al., 2021). Pond systems have been crucial for this growth trend. Their productivity depends on the input of feed and stocking density, while the nutrients that are not taken up remain in the pond and become an integral part of the nitrogen cycle as well as contribute to endogenous pollution and eutrophication (Zhang et al., 2022). Thus, sediment is not a mere storage medium but the living environment of benthos and microorganisms, whose properties determine aerobic or anaerobic decomposition of organic material (Bentzon-Tilia et al., 2016; Zhang et al., 2022).

The stocking density is often viewed as an exogenous design parameter; however, the density experienced by organisms varies all the time. The death rate, cannibalism, partial harvesting and stocking vary the animal density within the area, whereas feeding rates and excrements respond to this variability with some delay. The density dependence of cannibalism and mortality in decapod crustaceans causes that the actual density significantly deviates from the design one (Romano & Zeng, 2017). This deviation concerns twice the benthic organisms using the substrate both for protection and dwelling (Huang et al., 2018).

There is scanty evidence that shows the relationship between density and the benthic environment and this depends heavily on the context within which the study is carried out. Abundance and composition of bacteria are not the same in different shrimp ponds with varying stocking densities (Alfiansah et al., 2018). Fast environmental changes in crab ponds lead to changes in the water microbial community and are related to the survival of the crabs (Deng et al., 2020). Microbial community in water and sediment has been related to environmental parameters and the condition of the animal, and has been suggested as a means to evaluate the quality of aquaculture water (Alberts-Hubatsch et al., 2016) (Bentzon-Tilia et al., 2016). Benthic fauna

at various densities has been shown to alter the nitrogen form in the sediment, and the middle density is more effective in a freshwater experiment (Zhang et al., 2022).

Studies involving density, mortality, feed utilization, sediment composition and bottom-water oxygen in one experimental design are few and far between. This study was hence carried out to determine the effect of nominal stocking density and the mortality-influenced variability in realized density on benthic habitat, using the freely available data set from replicated ponds. In particular, this study seeks to find out (i) the effect of the density gradient on survival, yield and feed efficiency, (ii) what measures first respond in sediments and bottom-water, and (iii) if feed and mortality loading can explain sediment response.

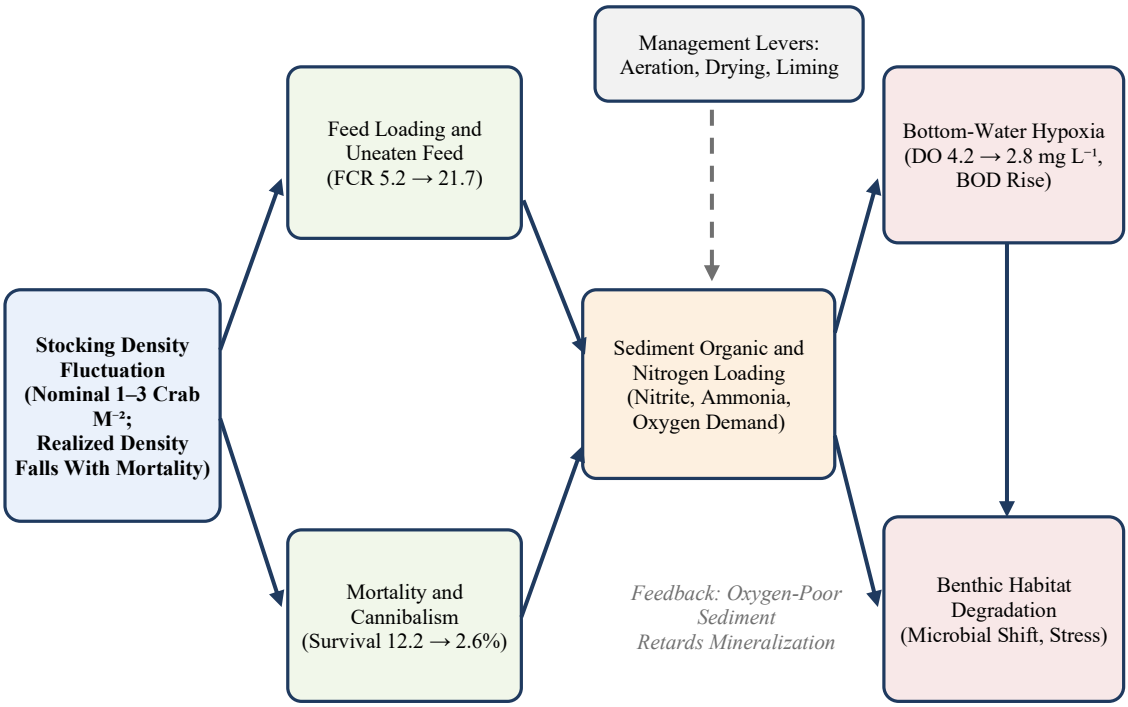


Figure 1: Conceptual Framework Effects of Stocking Density on Sediment Nutrient Loading and Aquatic Ecosystem Health

Figure 1 is a diagram that shows the interdependencies between stocking density, feed load, fish mortality, sediment nutrient load, and the health of aquatic ecosystems. Higher stocking density leads to higher feed intake and thus higher production of wastes, which lead to an increase in organic matter content and nutrient loads in sediments, including nitrite and ammonia loads. The model also indicates bottom-water hypoxia (DO 4.2 → 2.8 mg L^{-1}) and degradation of benthic habitats as possible outcomes associated with higher nutrient loads. Regulation of sediments through the use of aeration and drainage is shown in the model as management approaches.

II. Materials and Methods

Source Dataset

The experiment used the published data from Sawangphol et al., (2025) on the stocking of the green mud crab (Scylla paramamosain) in earthen ponds at four densities (1, 1.5, 2, and 3 crabs m^{-2}) with three replicates in the randomized complete block design in 101 days (May-August 2021). The ponds were split into 200 m^2 areas (200, 300, 400, and 600 crabs per area). Crabs were fed fish cut up at 10% of their body weight two times a day. The ponds were mechanically aerated, and the water was brackish (approximately 25 ppt). The growth, survival, feed conversion, sediment properties (organic matter, soil oxygen demand, pH, ammonia, nitrite, orthophosphate, particle size), and water quality were provided as the mean value

and standard deviation. These published values were used as the source data. The values of water quality were provided for the entire culture period and not by density; thus, they are used just to depict the temporal change in bottom water properties.

Derived Indicators

Four parameters were based on the values reported.

Realized density at harvest was equal to nominal density multiplied by survival. The mortality rate was the number of dead animals per square meter and was equal to nominal density multiplied by (1 − survival).

Feed input implied per treatment was defined as the reported feed conversion ratio multiplied by total weight gain, and weight gain was the difference between reported total yield and stocked biomass (number stocked x initial weight of individual x three replicate cells), and was in units of per square meter of 600 m^2 treatment area. The deviation of the calculated total yield from the reported one based on the stocked number, survival, and final weight was −1.7% to −2.5%.

Statistical Treatment

Since means and SDs are provided for only four density treatments, the possibility of making statistical inferences is limited to just a few treatment means. Pearson/Spearman correlations and least squares regression slopes will be reported as exploratory descriptors, the survival rate as modeled using an exponential decay function (ln survival vs. density), and an effect size (Cohen's d) for the comparison of low versus high densities. The differences between significant treatments found in the source article (Duncan's test, $P < 0.05$) are indicated as such and are credited. The computations were done using Python (NumPy, SciPy, Matplotlib); code and resulting table are provided as supplementary material.

III. Results

Survival, Yield and Feed Efficiency Along the Density Gradient

The decline in production efficiency was consistent with the increase in nominal density (Table 1, figure 2 (a)(b)(c)). The survival rate fell from 12.2% for 1 crab m^{-2} to 2.6% for 3 crab m^{-2}, which is an exponential function (the rate of which is −0.76 crab m^{-2}, $r^2 = 0.98$) and each increase in crab density by one crab m^{-2} reduced the survival rate to 47%. The total yield decreased from 9.62 to 6.68 kg (a 31% reduction) despite tripling of the number of stocked animals, and yield per 100 stocked crabs decreased from 1.60 to 0.37 kg. Feed conversion ratio increased by 4.2 times, from 5.2 to 21.7 ($r = 0.99$). There were no differences in final individual weights between different densities (127-140 g).

Table 1: Growth performance, sediment quality and clay content by stocking density (mean ± SD where reported). Source: Sawangphol et al., (2025)

Variable	1 crab m^{-2}	1.5 crab m^{-2}	2 crab m^{-2}	3 crab m^{-2}
Survival (%)	12.17 ± 1.26	7.43 ± 1.12	4.75 ± 0.90	2.61 ± 0.19
Final weight (g)	128.8 ± 11.0	132.7 ± 5.8	126.8 ± 6.2	139.7 ± 6.9
Feed conversion ratio	5.2 ± 1.4	7.2 ± 1.4	13.7 ± 2.1	21.7 ± 1.8
Total yield (kg)	9.62	9.10	7.39	6.68
Sediment organic matter (%)	4.22 ± 0.35	4.00 ± 0.33	4.06 ± 0.23	4.03 ± 0.31
Soil oxygen demand (mg O_2 g^{-1} h^{-1})	2.28 ± 1.30	2.21 ± 1.45	2.27 ± 1.42	2.36 ± 1.57
Sediment total ammonia (mg L^{-1})	10.76 ± 4.45	14.80 ± 8.41	13.73 ± 7.24	13.12 ± 7.38
Sediment nitrite (mg L^{-1})	5.71 ± 2.22	6.27 ± 3.05	8.02 ± 3.61	8.68 ± 3.69
Sediment orthophosphate (mg L^{-1})	1.94 ± 0.67	2.56 ± 1.39	2.73 ± 1.50	2.20 ± 0.85
Clay fraction (%)	42.5 ± 4.5	42.7 ± 4.8	44.9 ± 4.2	43.3 ± 3.0

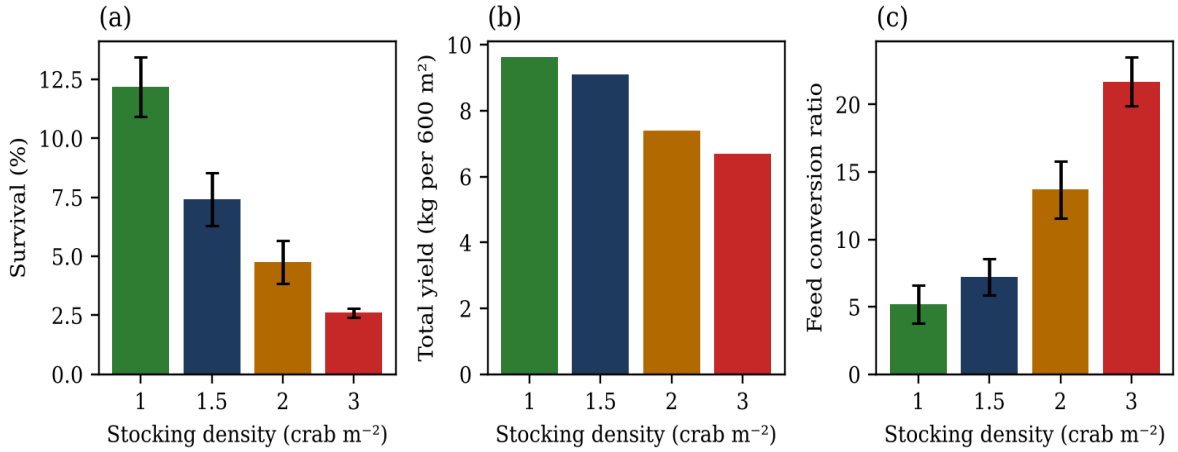


Figure 2: (a) Survival, (b) total yield and (c) feed conversion ratio at four stocking densities (mean ± SD; data from Sawangphol et al., (2025))

Sediment Response and The Role of Realized Density

The only parameter from sediment variables that exhibited changes depending on the crab population density was nitrite concentration (Table 1, figure 3a). The mean sediment nitrite concentration grew from 5.71 to 8.68 mg/L as the crab density shifted from 1 to 3 crab/m², which is 52% increase (the Cohen's d value between the extreme groups = 0.98; the slope is 1.56 mg/L per additional crab/m²; $r^2 = 0.90$; Spearman $\rho = 1.0$; n = 4 treatment means). The content of sediment organic matter, the soil oxygen consumption rate, as well as the concentration of ammonia and orthophosphates, did not vary at different crab population densities, whereas the clay content (42-45%) was consistent, eliminating texture as a factor. The sediment organic matter was within the range of 4.0-4.2%, which exceeds the optimal range of 1-3% recommended by Sawangphol et al., (2025).

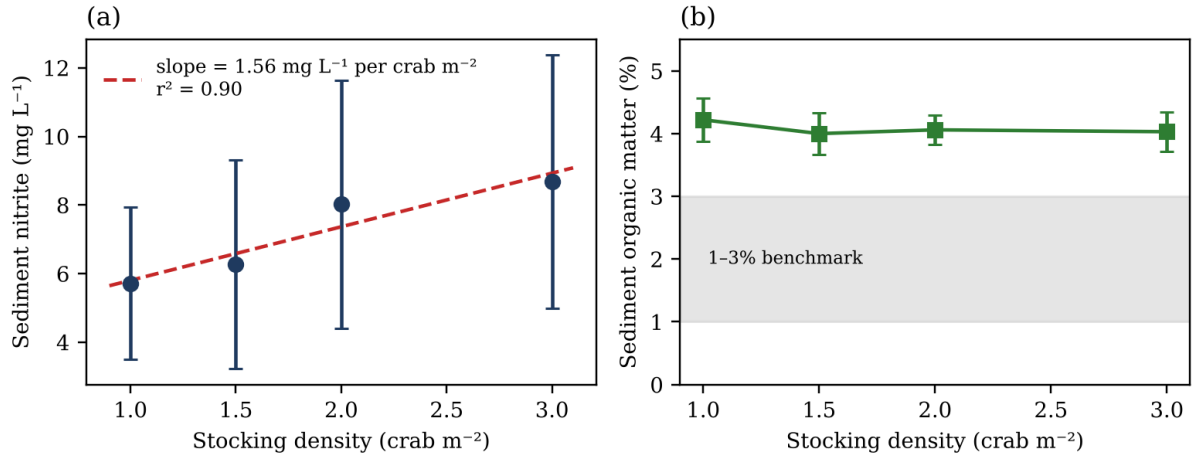


Figure 3: (a) Sediment Nitrite as A Function of Stocking Density with Least-Squares Trend; (b) Sediment Organic Matter Relative to the 1–3% Benchmark Cited in Sawangphol et al., (2025)

(Mean ± Sd; Data From Sawangphol et al., (2025))

The calculated indices give the justification of the strong effect of nitrites and weak effect of organic matter on the process (see table 2, figure 4(a)(b)). The actual densities at harvesting were 0.12, 0.11, 0.10 and 0.08 crab m⁻² for four different treatments, i.e., there were less survivors in the end of the crop at the

higher densities, while the mortality rate was three times higher (from 0.88 to 2.92 crab m^{-2}). The implied feed supply increased from 0.073 to 0.111 kg m^{-2} and stabilized within the range of 2–3 crab m^{-2}, while feed for 1 kg of the harvested crabs increased more than twice (from 4.5 to 10.0 kg). The concentration of nitrites in the sediment was strongly correlated with the implied feed supply (r=0.96, n=4) and mortality (r=0.95), which means that the sediment responded to the waste produced by feeding and mortality, not to the density of stock.

Table 2: Derived Indicators of Realized Density, Mortality and Feed Loading and Their Association with Sediment Nitrite. Source: Calculated from Data in Sawangphol et al., (2025)

Indicator	1 crab m^{-2}	1.5 crab m^{-2}	2 crab m^{-2}	3 crab m^{-2}
Realized density at harvest (crab m^{-2})	0.122	0.111	0.095	0.078
Mortality load (crab m^{-2})	0.878	1.389	1.905	2.922
Implied feed input (kg per 600 m^2)	43.5	52.5	67.4	66.4
Implied feed loading (kg m^{-2})	0.073	0.088	0.112	0.111
Feed per kg harvested crab (kg)	4.5	5.8	9.1	10.0
Yield per 100 stocked crabs (kg)	1.60	1.01	0.62	0.37
Sediment nitrite vs. 1 crab m^{-2} (%)	0	+9.8	+40.5	+52.0
Association with nitrite (r, n = 4)	—	density 0.95	feed loading 0.96	mortality 0.95

Note. Implied feed input = FCR × (total yield − initial stocked biomass). Correlations are exploratory because they are based on four treatment means.

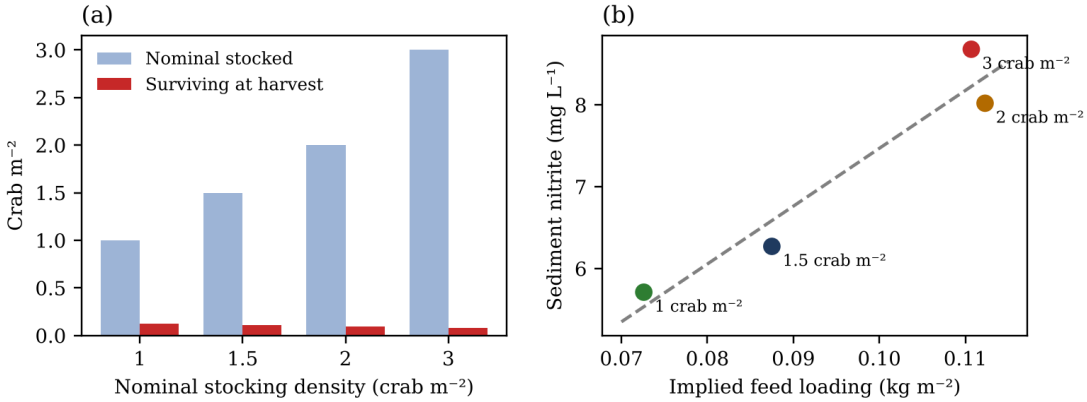


Figure 4: (a) Nominal Stocking Density Compared with Realized Density at Harvest, and (B) Sediment Nitrite as A Function of Implied Feed Loading (Calculated From Sawangphol et al., (2025))

Bottom-Water Conditions Through the Crop

The oxygen content in the bottom water showed a decline in the course of the culture period, starting from 4.19 mg L^{-1} before stocking and ending up at 3.53 and 3.17 mg L^{-1} in weeks 4 and 8 and finally at 2.76 mg L^{-1} at harvest, which is below the minimum value of 4 mg L^{-1} required by for crabs (Figure 5a). The biochemical oxygen demand increased from 4.2 to its maximum value of 10.5 mg L^{-1} at week 8 and was later reduced to 8.2 mg L^{-1}, and the total ammonia content was also increased rapidly up to 0.38 mg L^{-1} at harvest (Figure 5b). The above values were for the whole system since the water quality was not measured based on the treatment but the timeline is important because oxygen deficiency and ammonia increases occurred when the waste accumulation was in progress, which was at the same time as the mortality accumulation. Cyanobacteria made 46.7% of the water microorganisms, an organism known to cause night hypoxia by bloom.

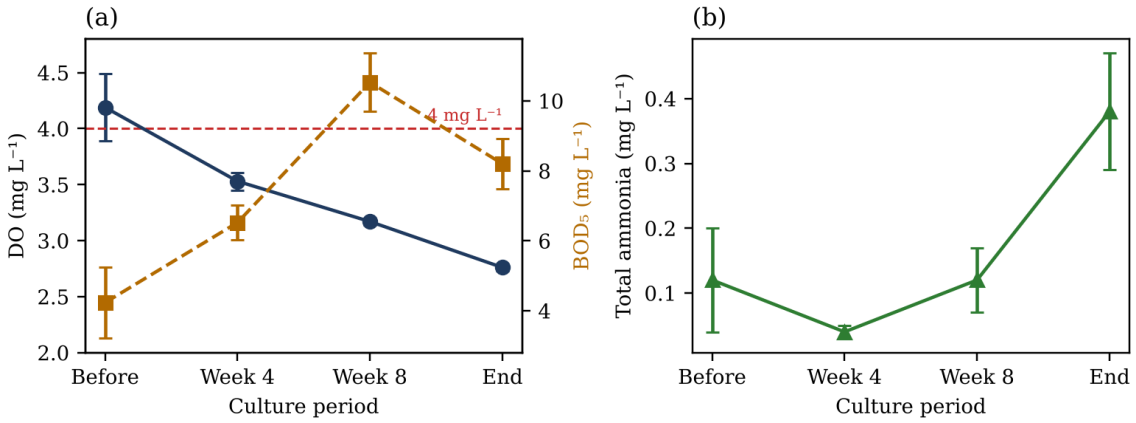


Figure 5: (a) Dissolved Oxygen and Biochemical Oxygen Demand and (B) Total Ammonia Through the Culture Period (Mean ± SD; Pooled Across Densities; Data from Sawangphol et al., (2025))

IV. Discussion

Density Acts Through Waste Loading, Not Nominal Numbers

The key point is that the nitrogen load in the benthos mirrored the amount of wastes created by a density regime, not the number of animals stocked. At high density, mortality was extremely high; thus, the additional animals did not result in an increase in biomass or uneaten food, but rather to additional dead biomass and feed needed to produce a kilogram of crab grew more than two times (Table 2). This is supported by the knowledge about density dependent cannibalism and mortality in decapods as well as by the occupation of bottom by mud crabs. The management meaning is that the important density is the actual one and the feed supplied in accordance with it. Scheduling the feed in terms of the nominal stocking number would mean overfeeding a dying population, which is exactly what happened in the experiment.

Nitrite as an Early Indicator and The Absence of an Organic-Matter Signal

Separation of sediments according to nitrite content, but not to organic matter and oxygen demand, was observed among density treatments. Two explanations are plausible in accordance with the data. Firstly, the content of organic matter reached 4.0–4.2%, which exceeded the values of 1-3% that were given as a benchmark for sediment in. Consequently, a short growing period of 101 days might be insufficient for the separation of bulk organic matter, whereas intermediates of nitrogen are more responsive to environmental fluctuations. Secondly, aerating helped to raise oxygen content on the surface, but it was not sufficient to keep oxygen at the bottom of ponds; thus, partial transformation of nitrogen would take place on the surface of the sediment. Environmental fluctuations affect microbial communities very rapidly. Rapid environmental changes were related to alterations in microbial structure of the ponds and crabs' survival. Different bacterial communities were found depending on the stocking density in shrimp ponds and microbial assemblages were associated with environment. Measurement of sediment nitrite is relatively inexpensive; consequently, it may supplement microbial monitoring.

Relevance for Freshwater Aquaculture Systems

The reason why there is a need for transferring fresh water to such a pond has to be based on process reasoning because it is an earthen-pond ecosystem that contains a high concentration of salts. The processes involved are not specific to the presence of salt. An increase in the level of nitrogen and organic matter occurs on the surface of the sediment, the oxygen requirement rises, and nitrogen cycling becomes the process carried out by the microbes and benthos. In the case of fresh water, in one study, the density of the benthic snails has influenced the sediment nitrogen forms and bacterial communities, and the highest

influence was at the intermediate density; this implies that the reactions of the benthos to the density may not be monotonic. The issue of nitrogen excess in freshwater ponds also arises Zhu et al., (2019), as well as nitrogen export in the effluents to the streams (Herbeck et al., 2021).

Management Implications

These are four implications of the findings.

First, control nominal density within the range with the best yields and feed efficiencies, in this case, 1-1.5 crab m^{-2} (Sawangphol et al., 2025). Second, connect feeding amounts with the actual rather than nominal population through regular measurements, and eliminate dead individuals immediately. Third, apply bottom aeration and mixing because only surface aeration is not enough to maintain bottom water oxygen levels above 4 mg L^{-1}.Fourth, monitor sediment nitrite levels, along with dissolved oxygen, and remove organic materials and dry the pond after harvest, as suggested by Sawangphol et al., (2025). These steps lower the risk of degradation and nitrogen content in effluents Herbeck et al., (2021) and promote the general sustainability strategy for the industry (Naylor et al., 2021) (table 3).

Table 3: Evidence Base Linking Density-Related Processes to Benthic Habitat Conditions

Process	Evidence used in this study	Source
Density-dependent mortality and cannibalism	Prevalence and management of disease in decapod aquaculture industry; survival rate decreased exponentially with increasing density in the database	Romano & Zeng, (2017); Sawangphol et al., (2025)
Feed and waste loading to sediment	Extra feed leads to nitrogen change in pond nitrogen cycle and causes endogenous pollution	Zhang et al., (2022)
Microbial response to density and environment	Distinct microbial populations in different concentrations of shrimps; crab farm microorganisms and their relationship with survival	Alfiansah et al., (2018); Deng et al., (2020); Alberts-Hubatsch et al., (2016)
Benthic fauna and sediment nitrogen	Density of snails modified nitrogen status and bacterial community in freshwater sediments	Zhang et al., (2022)
Pond microbial ecology and nitrogen monitoring and management	Water quality monitoring through microbes; Microbial nitrogen management in pond water	Bentzon-Tilia et al., (2016); Zhu et al., (2019)
Hypoxia and cyanobacteria	Cyanobacteria in food production water; cyanobacteria dominance in crab pond microbes	Mutoti et al., (2022); Sawangphol et al., (2025)
Effluent export and sector context	Pond discharge creates a nitrogen cycle in the estuary while inland farming is the major contributor to world production	Herbeck et al., (2021) Naylor et al., (2021)

Limitations

It is a secondary analysis using statistics published for a single 101-day crop. There are only four densities provided; hence correlation can only be made based on four means of treatment and must be considered descriptive. Feed load, assuming feed conversion ratio and each treatment as three 200m2 cells. Water quality was not analyzed per density level, no sampling of benthic macrofauna was made, and since it is brackish water, direct application to freshwater benthics is not possible. Furthermore, rainfall from July to September resulted in variation of salinity and suspended solids in all treatments. Results can only be verified by freshwater experiments with varying densities per crop.

V. Conclusion

Survival showed exponential decline over a gradient of 1 to 3 crab m^{-2}, there was a 31% reduction in yield, the feed conversion ratio increased more than four times and sediment nitrite concentration increased by 52% and corresponded to the feed and mortality load produced in each density. There were no

differences for bulk sediment organic matter and oxygen demand between densities and bottom water oxygen concentrations were under 4 mg L^{-1} at the end of the crop. This suggests that variation of density as a result of mortality, as well as feed provided to compensate mortality, influence development of nitrogen conditions in sediments. Regulation of density, density-based feeding, aeration of bottom waters and monitoring of nitrite levels are the management options. Further research will involve the same analysis in freshwater ponds and manipulation of density during the crop period.

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