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Assessing the Impact of Substrate and Shelter on Cannibalism in Blue Swimming Crab (*Portunus pelagicus*)

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Abstract

Blue swimming crab (*Portunus pelagicus*) is one of Indonesia's essential fishery commodities, but overfishing has depleted its population. Aquaculture, as an alternative to produce this crab, is hampered by high levels of cannibalism. Substrates or shelters can reduce cannibalism. This study compared the effectiveness of one substrate and two types of shelter for reducing cannibalism of *P. pelagicus* crablets. Crablets with average carapace length of 3.6 ± 2.2 cm and average weight of 6.4 ±1.5 g.ind^{-1} were stocked in 24 rectangular tarpaulin tanks with 1 m^2 surface and 50 cm water depth. The stocking density was 8 crablets in each tank. A split-plot experimental design was applied with four main plots and two subplots with 3 replications. The main plot consisted of A0 (without shelter), A1 (seaweed), A2 (pipes), and A3 (seaweed and pipes), and sub-plots had either no-substrate (B0) or sand-substrate (B1). Cannibalism (K), survival (S), and four water quality parameters were recorded for 42 days. The data were analyzed using an ANOVA for a split-plot design and post-hoc Tukey. The results show that the sand substrate significantly (P>0.05) reduced cannibalism and increased survival of the *P. pelagicus*. Treatment A0B0, without substrate or shelter, showed the highest cannibalism (54±7%) and lowest survival rates (33±7%). While treatment A1B1, with *Gracillaria sp*. as shelter and sand as bottom-substrate resulted in the lowest cannibalism (4±7%) and highest survival rates (88±13%).

Keywords: Blue swimming crab, cannibalism, shelter, substrate, survival

INTRODUCTION

The *Portunus pelagicus*, commonly known as the blue swimming crab and belonging to the Brachyura order within the Crustacea class (Portunidae family), is widely present in aquaculture and fisheries worldwide due to its extensive geographical range and economic importance (FAO, 2017; Yulianto *et al*, 2024). Blue swimming crab has become one of the commodities that has contributed to the development of the fishery business in Indonesia, in both fishing and aquaculture. Occupying the 4th position after shrimp, tuna, and squid, blue swimming crabs are usually exported to meet the world's crab consumption (Fahmi *et al*., 2015). The United States has been the largest market for Indonesian crab exports, consistently buying up to, 10 tousand tons of blue swimming crab from Indonesia, accounting for 43% of the total exports (Swainston, 2023). Some countries that have become crab export destinations include China (19%), Malaysia (1%) and Japan (0,5%).

The increasing demand of the world community for crab meat becomes a challenge and leads to concerns of the decreasing availability of crabs in nature. This happens because 70% of the fulfillment of consumption and export comes from natural catches, while the remaining 30% comes from cultivation activities. The destruction of the crab habitat exacerbates the decline of the nature population due to this overfishing, as environmental conditions impact growth and abundance of blue swimming crabs (Maryani, *et al*., 2023). Therefore, the development of aquaculture technology is a crucial effort to maintain and perhaps increase the production without disturbing their natural population (Hastuti *et al.,* 2020; Wang *et al.*, 2023).

Blue swimming crab culture in Indonesia has started since entering the 2000s, primarily as a response to the declining wild crab populations due to overfishing (Zamroni, 2023). The problem that

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often occurs in crab cultivation is the high level of cannibalism (Azra *et al*., 2018). Cannibalism in crabs lasts from megalopa to juvenile stages (Romano and Zeng., 2008). This cannibalism can cause high mortality in crab cultivation (Oniam *et al*., 2011; Xu *et al.*, 2020). Cannibalism during the molting stage results from the combination of heightened aggression among crabs before molting and their increased vulnerability and reduced defense capabilities during and after molting (Waiho *et al.,* 2021).

Recent studies have highlighted the importance of appropriate shelter and substrate in mitigating this behavior across various crab species. Longmire *et al.* (2021) demonstrated the potential of oyster reef habitats in supporting juvenile blue crab (*Callinectes sapidus*) populations by offering protection through structural complexity. Zeng *et al.* (2018) suggested that combining plant species serving different functions (food and shelter) could optimize both growth and survival rates in Chinese mitten crab (*Eriocheir sinensis*) aquaculture. Toi *et al.* (2023) found that red seaweed was an effective shelter material for high-density nursery rearing of juvenile mud crabs (*Scylla paramamosain*). Additionally, Zhou *et al.* (2023) found that fine sand and PVC pipes effectively reduce cannibalism and promote growth in mud crabs. These studies emphasize the need for shelter and substrate to reduce cannibalistic behavior in various crab species (Wahyuni *et al*., 2020). For blue swimming crabs (*P. pelagicus*) no studies combining shelter with substrate to reduce cannibalism were done. This study aims to assess the effect of adding sand substrate and different shelters on the survival rate and cannibalism of blue swimming crabs (*P. pelagicus*) in tanks using a split-plot design.

MATERIALS AND METHODS

We evaluated the effectiveness of using sand substrates and two difference types of shelter, *Gracilaria* sp. and artificial materials (PVC pipes), through the Survival Rate (SR) of juvenile *P. pelagicus*. The crab behavior and mortality from both cannibalism and other causes were observed for 42 days in microcosmos. The microcosmos were checked for dead crab twice a day and dead crab were removed immediately. This study distinguishes between crabs that died due to cannibalism or due to other causes. Crabs that died due to cannibalism were characterized by the loss part of the body, while crabs that died due to other causes had the opposite characteristics (Figure 1).

Figure 1. Dead crab caused by canibalism (below) and by other causes (above)

The experimental design used was a split-plot design with main plots and subplots, where the main plot consisted of 4 variables and the subplots consisted of 2 variables with three repetitions (order 4x2x3). The main plot was the provision of different shelters, consisting of A0 (without shelter), A1 (seaweed), A2 (pipes), and A3 (seaweed and pipes). Meanwhile, sub-plots had either no-substrate (B0) or sand-substrate (B1). This resulted in the following treatments: Treatment A0B0: no shelter and no sand substrate; Treatment A0B1: no shelter with sand substrate; Treatment A1B0: shelter (*Gracilaria* sp.) and no sand substrate; Treatment A1B1: shelter (200 gr *Gracilaria* sp.) with sand substrate; Treatment A2B0: shelter (4 PVC pipes) and no sand substrate; Treatment A2B1: shelter (4 PVC pipes) with sand substrate; Treatment A3B0: shelter (100 gr *Gracilaria* sp. and 2 PVC pipes) and no sand substrate; Treatment A3B1: shelter (100 gr *Gracilaria* sp. and 2 PVC pipes) with sand substrate.

A total of 192 crablets with an average carapace length of 3.6 ± 2.2 cm and an average weight of 6.4 ± 1.5 $g.ind^{-1}$ were stocked in 24 tanks at a stocking density of eight $individuals.m^{-2}$. The containers used in this study were square tarpaulin tanks with a length of 1 m, a width of 1 m, and a height of 1 m. Tanks were filled upto 0.5 m with sea water with a salinity of 25 ppt. The seawater was collected in the estuary of the study site, and before use rested for one week to let solids settle. The substrate used was sea sand at 15 cm thickness; thus, in these tanks the water column was 35 cm. This study used sand sourced from the natural habitat of Blue Swimming Crab. The sand used has been first filtered and then sun dried to minimize contamination of substances and foreign objects that may have a negative impact on the research. The shelter consisted of 2 types that were representing abiotic and biotic objects. Shelters in the form of PVC pipes with a length of 15 cm and a diameter of 2.5 inches. Each tank is provided with 4 PVC pipes for the 3rd treatment and two PVC pipes per tank for the 4th treatment. Another shelter used was *Glacilaria* sp., of 200 g in each tank in the 2nd treatment and 100 g in the 4th treatment.

The data collected in this study related to survival, cannibalism, and water quality during 42 days investigation. The water quality monitoring during the study included temperature, pH, salinity, dissolved oxygen (DO), and ammonia (NH_3). The survival rate (SR) was calculated based on the comparison between the number of crabs collected at sampling time (N_t) and the number of initial crabs stocked (N_0) using the method by Busacker *et al.* (1990).

$$S\ (\%) = \frac{Nt}{No} x\ 100$$

The rate of crab cannibalism was calculated using the formula of Suharyanto *et al.* (2008), by calculating the initial number of crabs (KA) minus the number of crabs in each test pond at the end of the study (KS) minus the number of crabs that died due to cannibalism (KBK) and divided by the initial number of crabs at the time of the study (KA).

$$K\ (\%) = \frac{KA - KS - KBK}{KA}\ x\ 100$$

The first step in the statistical analysis of the rates of cannibalism and survival was a Multifactorial Split-plot ANOVA. In case of non-significant interaction and indications for differences between shelter types this was followed by a Tukey HSD test to identify different groups.

RESULT AND DISCUSSION

The lowest rate of cannibalism (4%) was found in the treatment with sand and Gracillaria only (A1B1) and the highest (54%) in the treatment A0B0, i.e. without substrate or shelter (Figure 2). In the treatment without substrate but using seaweed shelter (A1B0), the rate of cannibalism was 33%, which was comparable to that found for A2B1 (29%), a treatment with substrate and pipe shelter (A2B1). The addition of sand significantly reduced the rate of cannibalism of blue swimming crabs (Table 1). Both, the overall effect of shelter type and substrate type was significant (P=0.007 and P=0,000 respectively). Likewise, the interaction substrate*shelter was non-significant (p=0.16).

The highest cannibalism rate (54±7%) was found in the treatment without sand and without shelter (A0B0). The cannibalism rates of A2B0, pipes only or A3B0, pipes plus seaweed, were not significantly different (Figure 2). Intermediate cannibalism rates (33 to 20%) were found in the treatments A1B0, A2B1, A0B1 and A3B1. However, compared to all other treatments, the rate of cannibalism was significantly lower in treatment A1B1, 200g *Gracilaria* sp. plus sandy substrate: 4±7%. The post-hoc test on shelter type confirmed that the addition of 200 gr *Gracilaria* as shelter (A1) is significantly more effective in reducing cannibalism than addition of pipes or of pipes plus 100g *Gracillaria* (Table 2). The rates of cannibalism with and without substrate found in this study are in the same range as those found by Oniam *et al*. (2011) and Longmire *et al*. (2021). The effect of shelter type on reducing cannibalism were in line with those found by Mirera and Moksnes (2013).

Table 1. Multifactorial Split-plot ANOVA on The Effects of Shelter and Substrate Type on Blue Swimming Crab's on Cannibalism and Survival Rates, and on the Ammonia levels

		Cannibalism Rate			Survival Rate			Ammonia		
Source of variation	Df	MS	F	Sig.	MS	F	Sig.	MS	F	Sig
Intercept	1	24225	195.8	0.005	92194	1089	0.001	10310	433.6	0.002
Shelter type	3	527.3	11.6	0.07	440.5	4.3	0.061	0.053	1.45	0.319
Repetition	3									
Substrate type	1	3444	132.3	0.000	6257	137.3	0.000	0.019	0.49	0.502
Shelter * substrate	3	58.6	2.25	0.16	58.6	1.29	0.344	0.001	0.023	0.995

Note: Bold values indicate significant differences

Table 2. Tukey HSD Matrix of pair-wise comparison probabilities for different treatments on Cannibalism and Survival rates, and on the Ammonia levels

		Cannibalism		Survival Rate	Ammonia
		Subset		Subset	Subset
Shelter	N	1	2	1	1
A1 (Gracilaria)	6	18.7500		72.9167	0.5933
A3 (Gracilaria and PVC)	6		31.2500	64.5833	0.6017
A2 (PVC)	6		37.5000	56.2500	0.63330
No shelter (B0)	6		39.5833	54.1667	0.7933
Sig.		1.000	.085	0.106	0.348

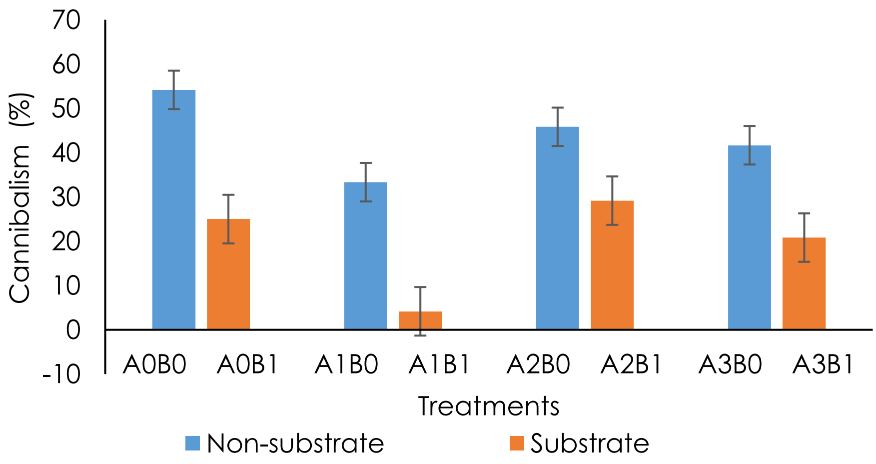

Figure 2. The rates of cannibalism of Blue swimming crabs (*P. pelagicus*) during the study

During the study we observed that the pipe shelter of 2.5-inch diameter was used only by crab smaller than 5 cm (Figure 3). Crabs exceeding 5 cm in length no longer used the inside of the pipe as a hiding place, but preferred staying outside the pipe. This might have contributed to the low effectiveness as shelter. Another factor might be that the crabs are not yet used to this material in contrast to sand and seaweed.

The survival rates of the blue swimming crab were calculated based on all crabs that died, both from cannibalism and those that died from other causes. The highest survival rate (88%) was in treatment A1B1, using substrate as well as seaweed while those of the other treatments with sand and other shelters had intermediate levels (71 to 79%). The lowest survival rates were found in all treatments without the substrate sand (33% to 58%). The survival rates with and without substrate found are in the same range as those found by Ravi and Manisseri (2012) and Kohinoor *et al*. 2018. The effect of shelter type on improving survival were in line with those found by He *et al*., 2017.

The addition of substrate had a significant effect on the survival of blue swimming crabs (Table 1). The effect of shelter type was non-significant (P=0.06), and neither was the interaction substrate*shelter (P=0.344). The post-hoc test confirmed that types of shelter did not impact the survival rate of juvenile blue swimming crabs (*P. pelagicus*) significantly (Table 2). This indicates that combining the sand substrate with seaweed provides the best rearing environment for juvenile blue swimming crabs (*P. pelagicus*).

Figure 3. A small blue swimming crab is hiding inside a PVC pipe

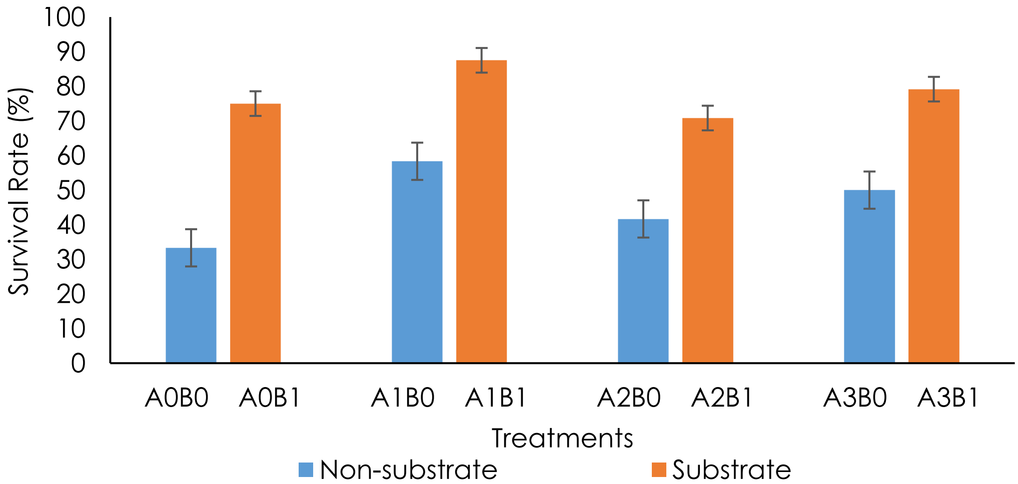

Figure 4. Survival Rate of crabs (*P. pelagicus*) during the study

During the study, the water temperature in the rearing tanks ranged from 26.7°C to 30.2°C, which was within the recommended range for blue swimming crab (Table 3). The study was conducted during the wet season and those temperatures were to be expected; during the dry season temperatures of pond water can reach 36°C. Dissolved oxygen values, which are related to temperature, varied between 4 and 5.3 mg L^{-1} and remained within the recommended range. The salinity values during the study ranged from 25.1 ppm to 26.7 ppm, which was below the recommended value for crab cultivation. The study was done during a wet season; heavy rains had impacted the salinity value in the estuary when the water for the study was sourced. However, crab is a euryhaline organism having a wide tolerance of salinity. Moreover, at this stage the crabs live in estuaries and later migrate to waters with higher salinity (Rinaldi *et al.,* 2019). We did our experiments with juveniles.

During the study the value of the pH ranged from 8.3 to 8.5, which is above the recommended range for aquaculture (Table 3). These alkaline pH values influence the aquatic nitrogen cycle. More specifically, alkaline levels of the pH stimulate the transformation of ammonium (NH_4^+) to ammonia (NH_3) that can evaporate from the water (Edwards *et al.*, 2024).

Table 3. Water Quality in the tanks with Blue Swimming Crabs (*P. pelagicus*) during the study

Variables	Treatments								Recom-mended values
	A0B0	A0B1	A1B0	A1B1	A2B0	A2B1	A3B0	A3B1	
Temp (°C)	29-30	29-30	29-30	29-30	29-30	29-30	28-29	27-28	23,6 – 31,8 [b]
DO (mg.L^{-1})	4,0-4,7	4,1-4,9	4,4-4,9	4,5-5,3	4,3-4,9	4,3-4,8	4,1-4,8	4,3-4,5	4 - 6 [a]
pH	8,4-8,5	8,4-8,4	8,4-8,5	8,4-8,4	8,3-8,4	8,4-8,5	8,4-8,5	8,4-8,5	6,78 – 8 [a]
Salinity (ppt)	25-26	25-26	26-27	26-27	25-26	25-26	25-26	25-26	25 [c]
Ammonia (mg.L^{-1})	0,7-1,0	0,7-0,8	0,3-1.1	0,4-0,8	0,5-0,8	0,5-0,6	0,5-0,7	0,6-0,7	<1 [d]

Notes: a)Ihsan *et al.,* 2020; b) Hamid, 2019; c) Hilmi & Ikhwanuddin, 2020; d) Azra *et al.,* 2018 & Yusneri *et al.,* 2021

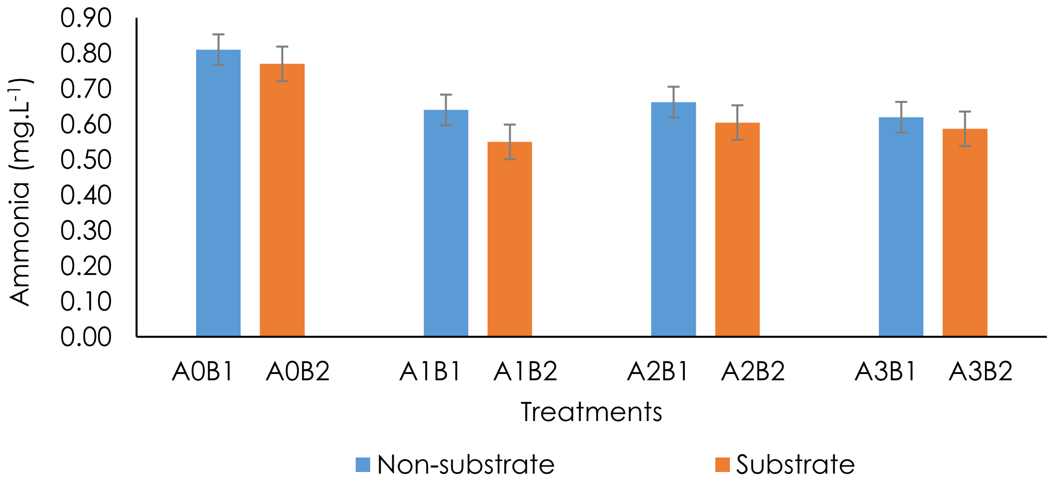

Figure 5. Average ammonia content in the rearing containers during the study

The ammonia levels ranged from 0.28 mg L^{-1} to 0.83 mg L^{-1} (Table 2 and Figure 5). Those values remained well below the recommended level of 1 mg L^{-1} acceptable for blue swimming crab (*Portunus pelagicus*) aquaculture and were in line with those found in previous studies on crustacean (Azra *et al.,* 2018). The treatments with sand substrate plus any shelter showed slightly lower ammonia levels than those without sand (B0) or sand only (Figure 4). The treatments had a significant effect on the level of ammonia (Table 1). However, the effect on the ammonia levels of shelter, that of substrate and that of their interaction were non-significant. The type of shelter had neither any significant impact (Table 2). As the pH was not different between the treatments also the balance ammonium-ammonia as well as the evaporation potential of ammonia (Edwards *et al.*, 2023) will not be different between the treatments.

The hypothesis stated that using no shelter has a higher cannibalism compared to the use of other shelters in all conditions. The treatments providing shelter with both sand and 200g *Gracillaria*, had the lowest rate of cannibalism. That treatment, as well as those with sand and either pipes only or *Gracillaria* plus pipes, had the highest survival rates. The rates of survival in the treatment with only seaweed was higher than in those with other shelters without sand but was lower than all treatment with sand. The effectiveness of sandy substrate on reducing cannibalism is probably due to mimicking the natural habitat of blue swimming crab (*P. pelagicus*). Blue swimming crabs inhabit shallow waters, typically found at depths of up to 5 meters, where the substrate consists of silt, sand and muddy sand (Tribamrung *et al*. (2023). There, by digging themselves in, the sandy substrate provides the blue swimming crab a shelter from predators and an ambush for preying. In addition, seaweeds provide also natural food sources and nursery grounds to marine organisms such as crab (Anh *et al.,* 2019a; Ahn *et al*., 2019b; Mantri, *et al*., 2020).

Seaweed provides better protection than the pipes because seaweed can be spread all over the bottom of the water, therefore, it is safe for crabs to take shelter at any time (Zhang *et al*., 2021). *Gracillaria* sp. as a shelter being more effective than the pipes might also be related to aquatic plants having a similar role in nature (Beisiegel *et al.*, 2019). Adding pipe shelters did not significantly change the cannibalism compared to providing sand only. Thus, the pipes did not narrow the crab's space for movement and hiding in the sand, however larger crabs could not use the pipes with a diameter 2,5cm.

Adding shelters thus proves to be an effective method to lower cannibalism in confined swimming blue crabs. This is in agreement with Zhang *et al.* (2021) and Wahyuni *et al.* (2020). However, our findings do not support Wahyuni *et al.* (2020) stating that seaweed shelters are better crab protectors than other shelters when kept in containers, because sand only gives lower cannibalism than providing the other shelters only.

Our findings on ammonia are in contrast with the findings of Rejeki *et al.* (2020), who observed that sandy substrates can serve as a natural biofilter. Neither were we able to confirm that Gracilaria seaweed kept ammonia levels low, as found by Cahill *et al*. (2010) and Wahyuni *et al.* (2020). The observed variations in ammonia levels across treatments underscore the importance of habitat complexity in managing water quality for *P. pelagicus* aquaculture (Josileen and Menon, 2018). All shelter types might have been colonised by a microbial community that transforms and contributes to fixation of nitrogen compounds generated from fecal deposits and unconsumed feed and thus contribute to reducing ammonia levels, just like in aquaculture systems using biofloc technology (Monroy *et al*., 2015). This organic material probably did not grown on sand or tanks. This suggestion warrants further research.

In this study we did not vary the stocking density (SD), while lower SD also reduces the risk of cannibalism (He, *et al*., 2017). One limitation of this study might be the lower water column in the treatments with substrate. However, as even blue swimming crab are mostly bottom dwellers (Romano and Zeng, 2006) we expect that the lower water columns did not impact the results of our study.

CONCLUSION

Using sand substrate significantly reduced cannibalism, but the lowest rate of cannibalisme (4%) and the highest of survival (88%) were in the treatment with sand and Gracilaria sp.. The highest rate of cannibalism and the lowest rate of survival during rearing blue swimming crab (*P. pelagicus*) were in the treatment with no substrate and no shelter (A0B0). Overall, adding shelters reduced cannibalism while the sandy substrate was the most effective. Using *Gracilaria* sp., a seaweed, provided the second-best shelter. More research is needed regarding the effect of shelters on ammonia levels through the growth of organisms having a biofloc function.

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