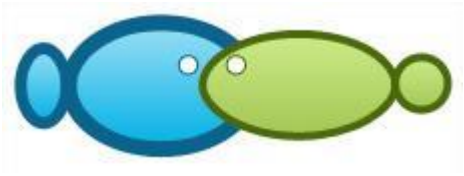

Nitrogen excretion rate of scalloped spiny lobster *Panulirus homarus* in recirculating aquaculture system

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Abstract. The scalloped spiny lobster *Panulirus homarus* is the most dominant commercialized spiny lobster species in Indonesia. The growing market demand for this spiny lobster has led to an increase in the growth of aquaculture efforts for this species. With the intensification and increasing magnitude of aquaculture activities, the increasing waste streams are becoming an increasingly relevant concern. Dissolved inorganic nitrogen (DIN) compounds such as ammonia, nitrite, and nitrate are dissolved wastes harmful to aquatic animals and the environment. We aimed to determine the excretion of dissolved inorganic nitrogen by *P. homarus* at different feeding rates, providing essential information for selecting and designing a more sustainable aquaculture system. Our study employed a completely randomized design (CRD) consisting of two treatments with three repetitions. The treatments consisted of restricted feeding (fed 5% FR) and starvation (unfed). Adult *P. homarus* were subjected to both treatments and were kept for seven days in six individual recirculating aquaculture systems (RAS). Under restrictive feeding, the estimated dissolved inorganic nitrogen excretion rate of *P. homarus* was 0.41 g kg^{-1} day^{-1} while the unfed lobsters excreted 0.06 g kg^{-1} day^{-1} of nitrogen. Our study indicated that both fed or unfed *P. homarus* excrete nitrogen, and the nitrogen excretion increases with higher feeding rates. Moreover, in functional RAS, nitrate emerged as the predominant dissolved inorganic nitrogen compound, constituting over 90% of N in the system.
Key Words: dissolved inorganic nitrogen, feeding rates, RAS, water quality.

Introduction. Spiny lobsters are a valuable seafood commodity with a substantial national and international market demand that continues to increase at an annual rate of 15% (Anissah et al 2015). Notably, in terms of production volume and value, lobster was ranked as the third-highest export commodity within crustacean group after shrimp and crab (Indonesia Ministry of Marine Affairs and Fisheries 2024). Furthermore, Indonesia is the world's largest spiny lobster seed-producing country with substantial natural lobster seed stock resources (Priyambodo et al 2020).

Several locations in Indonesia have been identified as spiny lobster natural seed capture areas, including the South Coast of Java, Bali, Lombok, and Sumbawa. The prevalent lobster species in these areas are the scalloped spiny lobster *Panulirus homarus* (63-87%) and the ornate lobster *Panulirus ornatus* (13-37%) (Priyambodo et al 2020). Lobster puerulus or early juveniles are captured with cost-effective traps made from recycled materials, such as cement bags that are deployed in areas where puerulus are known to settle (Priyambodo & Sarifin 2009; Priyambodo et al 2015). Indonesia's

abundant spiny lobster natural seed supply presents a promising opportunity for large-scale lobster production, offering alternative livelihoods for coastal communities. Moreover, no successful commercial breeding techniques exist to produce spiny lobster larvae in captivity (Jones 2010; Erlania et al 2017). Consequently, the spiny lobster grow-out industry depends entirely on harvesting lobster puerulus or juveniles (Jones 2010).

In addition to the abundance of natural seed, the viability of spiny lobster aquaculture in Indonesia is also supported by favorable sea farming conditions. According to statistical data from the ministry, at least 12 provinces in Indonesia have promising potential for spiny lobster production, with a collective output of 206.7 tons year^{-1} (Indonesian Ministry of Marine Affairs and Fisheries 2022) Above all, West Nusa Tenggara Province has the highest production share, contributing 68.01 tons annually. In the domestic market, *P. homarus* price ranges from IDR 250,000 to 400,000 kg^{-1}, while the export prices range from IDR 490,000 to 500,000 kg^{-1} (Wahyudin et al 2017). Floating net cages are the most predominant cultivation system for spiny lobster in Indonesia. It takes 8-10 months for *P. homarus* to grow from an initial weight of 25-35 g to the desired market size of 250–300 g. The primary source of feed in spiny lobster farming is bycatch fish because of its availability and relatively low cost. Bycatch fish includes diverse non-target small fish inadvertently caught during regular fishing activities (Junaidi 2018), including fish species like *Saurida* sp., *Priacanthus* sp., *Leiognathus* sp., *Engraulis* sp., and *Stolephorus* sp. (Mustafa 2013). To further elevate spiny lobster production in Indonesia, there is a need to advance cultivation technologies, such as innovation in cultivation systems and artificial feed, and provide high-added value to the available lobster seed resources (Junaidi 2018).

With the intensification and increasing magnitude of spiny lobster aquaculture activities, the waste streams increasingly become a relevant concern, including the dissolved inorganic nitrogenous compounds, such as ammonia, nitrite, and nitrate that increase eutrophication risk in marine environments. A partial solution to mitigate eutrophication is the biological utilization of nitrogenous waste streams in marine environments. There are several strategies for sustainable aquaculture implementations by minimizing the waste in marine environments, such as biofloc technology, recirculation aquaculture systems (RAS), bio-RAS, partitioned aquaculture systems, and integrated multi-trophic aquaculture (Zimmermann et al 2023).

In designing a sustainable aquaculture system, the load of nutrients produced by the cultivated species should be determined (Verdegem 2013). The quantification of nitrogenous compound excretion by specific species becomes crucial in balancing the released nutrients with the nutrient removal capacity of the aquaculture systems. Aquatic animals typically excrete excess nitrogen, primarily in the form of ammonia, through the gills and urine as a byproduct of food protein breakdown. If not managed, this nitrogen excretion can pose toxicity risks to aquatic life (Ebeling et al 2006; Wahyuningsih & Gitarama 2020). In decapod crustaceans, the deleterious impact of low concentrations of ammonia, nitrite, and nitrate can disrupt vital physiological processes, including osmoregulation, immunology, acid/base balance, and gas exchange. Furthermore, these substances can intensify oxidative stress, heighten susceptibility to pathogens, induce histopathological damage, and lead to mortality at elevated concentrations (Romano & Zeng 2013).

The information regarding nitrogen excretion by some lobster species is already available, such as the European lobster *Homarus gammarus* (Goncalves et al 2020), green rock lobster *Sagmariasus verreauxi* (Jensen et al 2013), the southern rock lobster *Jasus edwardsii*, the western rock lobster *Panulirus cygnus* (Crear & Forteath 2002), as well as for east coast rock lobster *Panulirus homarus rubellus,* a subspecies of the scalloped spiny lobster *Panulirus homarus* that inhabits the south-western Indian Ocean along the south-east coasts of Madagascar and Southern Africa (Kemp & Britz 2008; Kemp et al 2009; Lavery et al 2014). However, the same information for *Panulirus homarus* from Indonesia is not yet available. Obtaining this information is crucial, particularly as a foundational step in developing sustainable aquaculture systems like recirculation aquaculture systems (RAS) and integrated multi-trophic aquaculture (IMTA)

for *P. homarus* cultivation. The current study aims to discern dissolved inorganic nitrogen excretion under restricted feeding (5% FR) and starvation (unfed), providing insights into the relationship between feeding rate and nitrogen excretion in *P. homarus*. Further, the trial lasted for seven days in closed pilot scale RAS with zero water exchange to provide insight into prolonged inorganic nitrogen excretion and accumulation in RAS.

Material and Method

Experimental animals and system design. The lobsters (*Panulirus homarus*) weighing 200-250 g were procured from lobster farmers in Telong Elong, East Lombok Regency. These lobsters were cultivated in floating cages and had a diet consisting of bycatch fish. After a 24-hour fasting period, they were caught and directly transported to the Marine Bioindustry Laboratory at the Kurnaen Sumadiharga Science Complex–BRIN, located in Teluk Kodek, Pemenang District, North Lombok Regency. Prior to the beginning of the experiment, all lobsters underwent a seven-day acclimatization period within a 4.5 m^3 recirculation aquaculture system (RAS). Following this acclimatization, they were transferred to six individual pilot RAS units (Figure 1). Each system was stocked with three individuals, and the lobsters were acclimatized to the experiment setting environments for three days. Throughout this second acclimatization period, lobsters in each RAS unit were fed a daily ration of 50 g of bycatch fish at 4:00 p.m.

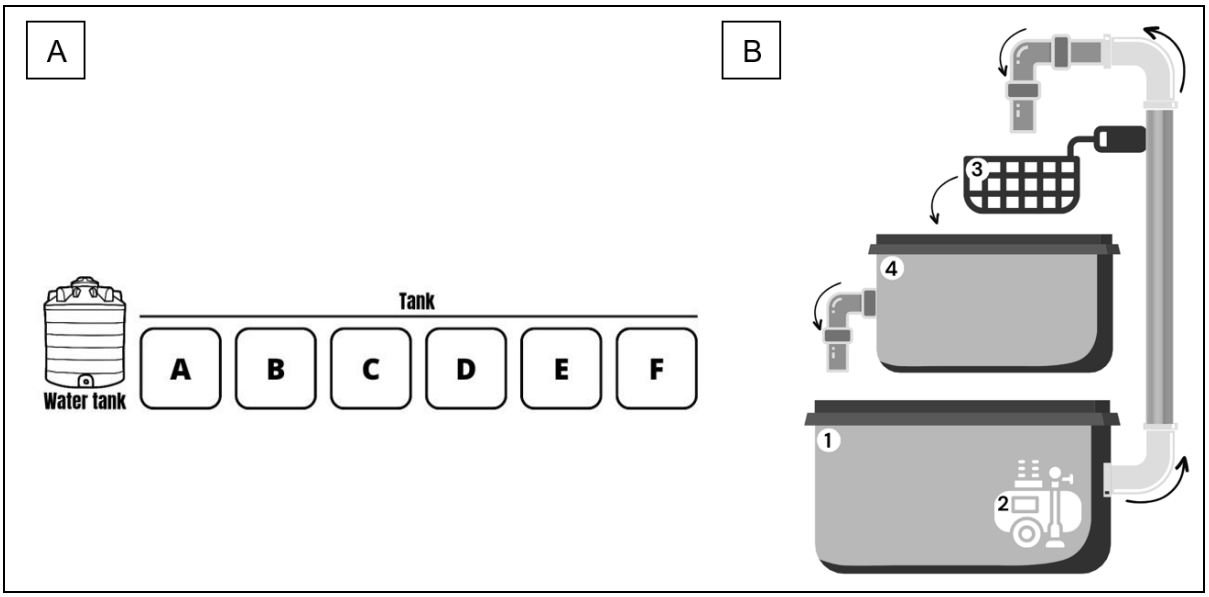

Figure 1. Graphical scheme of pilot recirculating aquaculture system (RAS). A) row of tanks; B) main RAS components: 1) main tank (V=130 L), 2) submersible pump, 3) physical filter (Dacron cloth), 4) biofiltration tank (V=95 L) filled with Kaldnes K3 bio-media (V=32 L).

Each pilot RAS featured a main tank dedicated to housing lobsters. The main tank was designed with a 130 liter plastic container enveloped in black plastic to diminish light exposure and replicate the natural habitat conditions conducive to lobsters. The upper section of the main tanks was enclosed with nets to prevent escape, while three PVC pipes provided shelter for the lobsters. Seawater from the main tank underwent a structured filtration process. Seawater from the main tank was pumped with a submersible pump (Resun SP-3800) to the physical filter made of Dacron cloth. The physical filter removed solid wastes such as feces, uneaten feed, and sloughed biofilm (Figure 1). The Dacron cloth of each system was replaced daily before feeding. Following the initial filtration, the water entered a 95 liter moving bed biofilter tank filled with 32 liters of mature bio-media (Kaldness K3). Before the experiment, the bio-media underwent a two-month conditioning process to facilitate the growth of nitrifying bacteria crucial for converting ammonia into nitrate. The conditioning process was done in a functional RAS designed for pompano (*Trachinotus blochii*) with a total volume of 4.5 m^3.

After passing the biofilter, the treated water circulates back into the main tank, creating a continuous cycle. Each recirculation system utilized a total water volume of 185 liters. The main and biofiltration tanks were aerated continuously with a blower system to ensure dissolved oxygen was sufficient. The functionality of the recirculation system was thoroughly confirmed before introducing lobsters. No seawater exchange was conducted during the whole experiment.

Experimental design. The trial was conducted in May 2023 and lasted seven days using a completely randomized design (CRD) involving two treatments and three repetitions. The treatments included: 1) restricted feeding with bycatch fish and 2) starvation, simulated to mimic basal metabolism (Table 1). For a comprehensive understanding of the nutritional composition of the feed (bycatch fish), a proximate analysis was undertaken in June 2023 at the Analytical Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram. The nutritional contents of bycatch fish used are detailed in Table 2.

Before the trial, the seawater during the acclimatization phase was replaced with new filtered seawater. In the beginning, the lobster weight and biomass were measured using a digital scale. The initial average weight of lobsters was 222 ± 18.6 g individuals^{-1}, and the average biomass in each tank was 665 ± 6.4 g. The initial biomass of the lobsters was used as a reference to determine the feed amount. The feed amount was 35 g per tank per day based on the feeding rate of 5% lobster biomass (Doddy et al 2019). The feed used was whole-body bycatch fish, and the proximate composition of the bycatch fish is presented in Table 2. Feeding was performed every day at 03:30 p.m. The uneaten feed from each tank was collected and weighed every following day at 09:00 a.m.

Table 1

Experimental design

Repetition	*Treatments*	
	Fed	*Unfed*
1	B	A
2	C	D
3	E	F

Table 2

Proximate composition of bycatch fish

Parameters	*Composition (%)*
Moisture	67.74 ± 0.481
Ash	11.79 ± 0.332
Crude protein	14.90 ± 0.049
Crude lipid	0.35 ± 0.007
Crude carbohydrate	5.23 ± 0.184
Crude fiber	0.19 ± 0.014

Note: Values are presented as mean ± SD (n = 2 repetitions).

Data collection and calculation. Lobsters were weighed at both the beginning and the end of the experiment, and the number of lobsters for each tank was also recorded. Water quality parameters, including nitrogen ammonia (N-NH_3), nitrogen nitrite (N-NO_2), nitrogen nitrate (N-NO_3), and orthophosphate (PO_4^{3-}) concentrations, were measured at the beginning and the end of the experiment. Measurements were carried out using a portable spectrophotometer (DR1900, Hach) following respective methods: N-NH_3 (Method 8155), N-NO_2 (Method 8507), N-NO_3 (Method 8192), and $PO4^{3-}$ (Method 8048). In addition, daily measurements of temperature, salinity, pH, and total dissolved solids (TDS) were carried out every morning at 08.00 a.m. using a multiparameter water quality meter (HI98194, Hanna Instruments). Water quality analysis was conducted at the Marine Bioindustry Laboratory, Kurnaen Sumadiharga Science Complex – BRIN.

Average weight, biomass, and number of lobsters were used to calculate survival rate (SR), specific growth rate (SGR), and biomass gain (Ridwanudin et al 2022; Firdaus 2022). The dissolved inorganic nitrogen excretion was estimated based on the measured concentrations of $N\text{-}NH_3$, $N\text{-}NO_2$, and $N\text{-}NO_3$ (Firdaus 2022). Total dissolved inorganic nitrogen concentration (TN, mg L^{-1}) was estimated as the sum of $N\text{-}NH_3$, $N\text{-}NO_2$, and $N\text{-}NO_3$ concentration in the system, assuming no denitrification occurred. Next, by multiplying the difference between the initial and final TN concentration with the total system volume, the total nitrogen excreted to the system (NS, g) was estimated. Total dissolved inorganic nitrogen excretion per kg lobster biomass (NB, g kg^{-1}) was calculated by dividing NS by the total final lobster biomass. The estimated dissolved inorganic nitrogen excretion rate (NER, g kg^{-1} day^{-1}) was calculated by dividing NB by the total duration of the experiment.

Statistical analysis. The performance parameters of *P. homarus*, the daily water quality of RAS, and estimated nitrogen excretion were analyzed using one-way ANOVA with Tukey's test at a 95% confidence interval and $p<0.05$. All statistical analyses were performed using statistical analysis software (SPSS Statistics 25, IBM).

Results and Discussion. The performance parameters of fed and unfed *Panulirus homarus* groups are presented in Table 3. At the end of the experiment, the survival rate of lobsters was 100% for all experimental units. After seven days, the two treatments did not exhibit a noticeable difference in lobster biomass ($p>0.05$). However, a significant difference ($p<0.05$) was observed in the biomass gains and specific growth rate. The lobster biomass in the fed group exhibited an increase in biomass by 6 g with positive SGR (0.14 % day^{-1}), whereas in the unfed group, average biomass decreased by 8 g with negative SGR (-0.17 % day^{-1}). The feed provided to the fed group seemed to adequately supply the necessary nutrition for the biosynthesis of lobster biomass. Conversely, the unfed lobsters relied on the breakdown of existing biomass to maintain basic metabolic functions. Despite observing positive growth at the 5% feeding rate, SGR for the fed group was lower than the value reported by Kemp and Britz (2008), that *P. homarus rubellus* kept at 24°C and 28°C and fed excessively with mussels had an SGR of 0.26 % day^{-1}. The lower SGR in the current study suggests that the amount of feed supplied may be inadequate to facilitate optimal growth. Previous research on *P. homarus* reported a feeding rate of 10% of lobster biomass using green mussel *Perna viridis* tissue (Verghese et al 2008). Similarly, in a previous study by Arumugam et al (2020), *P. homarus* was fed at a 10–15% feeding rate with various fresh and pelleted feeds. Hence, considering higher feeding rates is advisable to achieve more optimal lobster growth.

Table 3

Performance of fed and unfed *Panulirus homarus* in RAS throughout the study period

Parameters	*Treatments*	
	Fed	*Unfed*
Survival rate (%)	100[a]	100[a]
Initial body weight (g)	220 ± 2.3[a]	223 ± 0.6[a]
Final body weight (g)	222 ± 2.2[a]	220 ± 0.3[a]
Initial biomass (g)	661 ± 6.8[a]	669 ± 1.7[a]
Final biomass (g)	667 ± 6.6[a]	661 ± 1.0[a]
Biomass gain (g)	6 ± 1.2[b]	-8 ± 2.6[a]
Specific growth rate (% day^{-1})	0.14 ± 0.025[b]	-0.17 ± 0.057[a]

Note: Values are presented as mean ± SD (n = 3 tanks). Different superscript letters in each row show significant differences ($p<0.05$) by Tukey's test.

The results of the daily seawater quality measurements throughout the seven-day experimental period are presented in Table 4. All parameters, which included TDS, salinity, temperature, pH, and DO, remained within the expected values. The two groups had no significant differences ($p>0.05$) in TDS, salinity, and temperature values. Typically, the system subjected to fed treatment should exhibit a higher TDS value due to

leached feed and fecal degradation (Rafiee & Saad 2005). However, in the present study, the physical filter of RAS efficiently removed the dissolved particles, resulting in a similar TDS value.

In contrast to TDS, salinity, and temperature, the pH and DO in the fed treatment were significantly lower than those in the unfed treatment ($p<0.05$). These differences were attributed to the nitrification process in the system. Nitrification is a two-step process carried out by nitrifying bacteria that involves the oxidation of ammonia (NH_3) to nitrite (NO_2^-) and then to the less harmful nitrate (NO_3^-). Nitrification consumes approximately 4.57 g of O_2 and 7.14 g of alkalinity (as $CaCO_3$) for each 1 g of ammonia-nitrogen (Ebeling & Timmons 2012). Therefore, the lower pH and DO values in the fed treatment indicate a higher nitrification reaction rate than in the unfed group. Additionally, the lower DO value in the fed group was influenced by the higher need for oxygen during the digestion of the feed (Perera & Simon 2015).

Table 4

Daily seawater quality of RAS stocked with fed and unfed *Panulirus homarus* throughout the study period

Water quality parameters	*Measured values (average; range)*		*Expected value*
	Fed	*Unfed*	
TDS (g L^{-1})	24.61 ± 0.062[a]; 24.22 – 25.00	24.56 ± 0.100[a]; 24.03 – 25.03	<34 (Erlania et al 2014)
Salinity (psu)	32.07 ± 0.087[a]; 31.50 – 32.66	32.00 ± 0.144[a]; 31.23 – 32.70	29-35 (Vidya & Joseph 2012; Cahya et al 2023)
Temperature (˚C)	28.16 ± 0.071[a]; 27.80 – 28.52	28.04 ± 0.023[a]; 27.74 – 28.53	24-31 (Kemp & Britz 2008; Jones 2009)
pH	7.87 ± 0.006[a]; 7.60 – 8.09	8.08 ± 0.035[b]; 8.00 – 8.13	7.07-8.30 (Thesiana & Pamungkas 2015; Aji et al 2019)
DO (g L^{-1})	7.49 ± 0.134[a]; 6.87 – 7.92	7.75 ± 0.098[b]; 7.42 – 7.91	>6 (Indonesian Government Regulation No. 22 2021)

Note: Values are presented as mean ± SD (n = 3 tanks). Different superscript letters in each row show significant differences ($p<0.05$) by Tukey's test.

The pH and dissolved oxygen (DO) measurements corresponded with the data of N-NH_3, N-NO_2, and N-NO_3 (Figure 2). At the beginning of the experiment, the concentration of N-NH_3, N-NO_2, and N-NO_3 in both group systems did not differ significantly ($p>0.05$). However, after seven days, the fed group exhibited notably higher concentrations ($p<0.05$) in these nitrogenous compounds, indicating an intensified nitrification process that subsequently decreased the systems' pH and DO. Interestingly, the final N-NH_3 concentration in the unfed group was not detected. It implied that all NH_3 were converted to NO_2 or NO_3. Based on the Indonesian Government Regulation No. 22 (2021) regarding fishery water quality criteria, the limit for N-ammonia concentration is 0.5 mg L^{-1}. Accordingly, the concentration of N-NH_3 observed in this experiment falls within the "very good" category.

The initial N-NO_2 concentration in both treatment groups exhibited no statistically significant difference ($p>0.05$), measuring at 0.005 mg L^{-1} (fed) and 0.003 mg L^{-1} (unfed). After seven days, nitrite concentration in both systems was increased. This rise in nitrite concentration is attributed to the nitrification process facilitated by ammonium oxidizing bacteria (AOB) bacteria, which oxidize ammonia to nitrite (Aji et al 2019). However, since there were higher NH_3 concentrations in the fed groups, the final N-NO_2 concentration in the fed group systems showed a significant increase ($p<0.05$), surpassing four times that of the unfed group. According to Drengstig and Bergheim (2013), the good nitrite concentration for the growth of lobster *Homarus gammarus* is <5 mg L^{-1} or 1.52 mg L^{-1} of nitrite-N. Based on this baseline, the nitrite concentration in the current experiment remained within the acceptable range for promoting lobster growth.

The final $N\text{-}NO_3$ concentration significantly differed between the two groups ($p<0.05$), in which systems subjected to the restrictive feeding treatment exhibited nearly seven times higher $N\text{-}NO_3$ concentration. According to Drengstig and Bergheim (2013), the good nitrate concentration for the growth of *Homarus gammarus* is <100 mg L^{-1} or 22.6 mg L^{-1} nitrate-N. Therefore, when referring to this concentration, the nitrate concentration in this experiment was in the tolerable concentration range for lobster cultivation. The elevated nitrate concentration in both treatments also proved the existence of the nitrification process.

A similar trend was also observed for soluble phosphorus as PO_4^{3-}, with a significantly higher final PO_4^{3-} concentration in the systems subjected to fed treatment. We suggest that the higher PO_4^{3-} in the fed treatment systems originated from the accumulation of feces and feed crumbs, particularly smaller particles not filtered by Dacron cloth. While phosphorus is generally not toxic to cultured fish, high concentrations can lead to eutrophication (Amirkolaie 2011; Dauda et al 2019). In a closed aquaculture system, phosphorus can accumulate up to 20 mg L^{-1} (Martins et al 2009). In the current study, PO_4^{3-} concentration in systems subjected to fed and unfed treatment was below that value. Thus, the concentration was still acceptable for the lobsters.

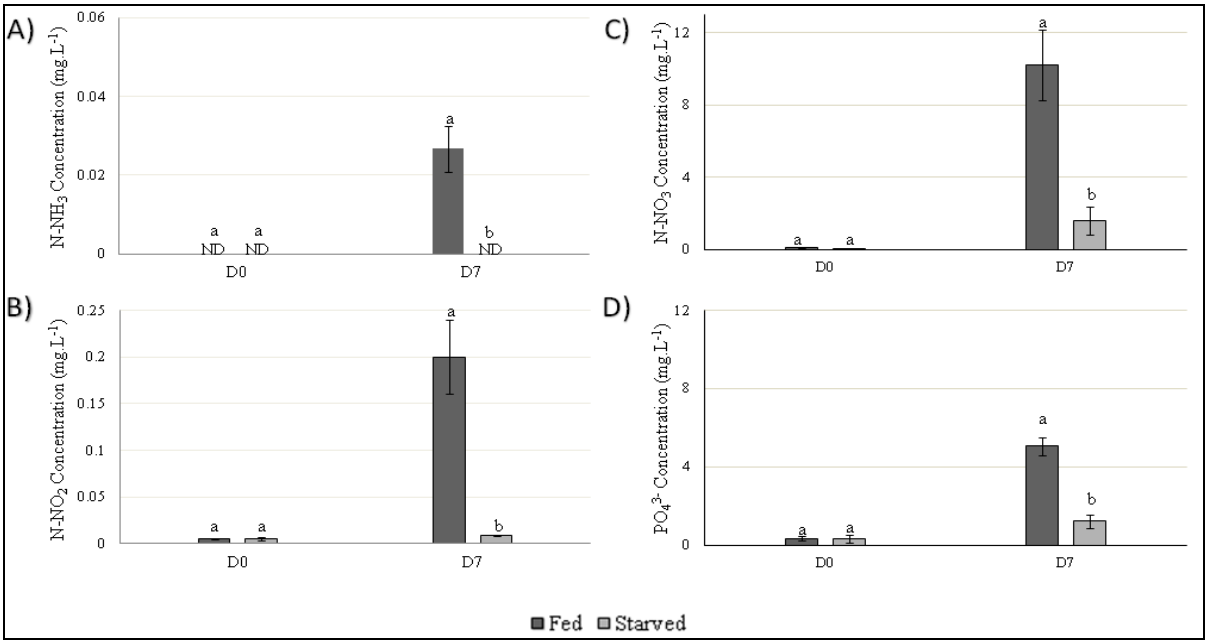

Figure 2. Measurement results of water quality parameters of RAS stocked with fed and unfed *Panulirus homarus* during seven days trial: A) $N\text{-}NH_3$; B) $N\text{-}NO_2$; C) $N\text{-}NO_3$; D) PO_4^{3-}. Different superscript letters in graphs show significant differences ($p<0.05$) by Tukey's test.

Observations on the estimation of the total dissolved inorganic nitrogen excreted by *P. homarus* between the fed and unfed treatments are presented in Table 5. The total dissolved nitrogen concentration was estimated from the $N\text{-}NH_3$, $N\text{-}NO_2$, and $N\text{-}NO_3$ concentrations in the seawater. The initial dissolved inorganic nitrogen concentration between the two groups was not significantly different ($p>0.05$). After seven days of culture, the concentration of dissolved inorganic nitrogen in both groups increased, and there was a significant difference in the final value ($p<0.05$) between the groups. The estimated total dissolved inorganic nitrogen excreted by *P. homarus* by feeding 5% bycatch fish for seven days was 2.88 g nitrogen per kg of lobster, while the total dissolved inorganic nitrogen excreted by *P. homarus* in the unfed group was 0.43 g nitrogen per kg of lobster biomass. It could be estimated that in one day, every 1 kg of lobster fed with 5% bycatch fish feed will excrete 0.41 g of nitrogen, while the unfed lobsters excrete 0.06 g of nitrogen. Based on the results above, the present study revealed that, with or without feeding, lobsters excrete dissolved inorganic nitrogen. However, the excretion of dissolved inorganic nitrogen tends to increase with increased feeding rates.

Table 5

Estimated dissolved inorganic nitrogen excretion of fed and unfed *Panulirus homarus*

Parameters	*Treatments*	
	Fed	*Unfed*
Estimated initial concentration of total dissolved inorganic nitrogen (mg L^{-1})	0.08 ± 0.011[a]	0.06 ± 0.015[a]
Estimated final concentration of total dissolved inorganic nitrogen (mg L^{-1})	10.43 ± 1.929[b]	1.61 ± 0.781[a]
Estimated total dissolved inorganic nitrogen excreted to the system (g)	1.91 ± 0.356[b]	0.29 ± 0.145[a]
Estimated total dissolved inorganic nitrogen excretion per kg of lobster biomass (g kg^{-1})	2.88 ± 0.536[b]	0.43 ± 0.217[a]
Estimated daily dissolved inorganic nitrogen excretion per kg of lobster biomass (g kg^{-1} day^{-1})	0.41 ± 0.077[b]	0.06 ± 0.031[a]

Note: Values are presented as mean ± SD (n = 3 tanks). Different superscript letters in each row show significant differences ($p<0.05$) by Tukey's test.

Our finding is comparable with previous studies on tropical spiny lobster *Panulirus ornatus* reported dissolved inorganic nitrogen output in the lobster group fed at 3 % of the lobster body weight with fresh seafood and fed artificial feed was 0.30 g.kg^{-1} day^{-1} and 0.27 g kg^{-1} day^{-1}, respectively (Lee et al 2015). The dissolved inorganic nitrogen excretion in both fed groups was 54 and 49 % higher than the unfed lobsters (0.14 g kg^{-1} day^{-1}). Another study on unfed 500 g *Jasus edwardsii,* revealed a total ammonia nitrogen (TAN) excretion rate of 0.024 g kg^{-1} day^{-1} at 13°C (Crear & Forteath 2002). Higher nitrogen excretion rates found in our study might be attributed to the fact that *P. homarus* was kept at a higher temperature than *J. edwardsii.* Nevertheless, it should be noted that the nitrogen excretion might vary between lobster size. As reported in previous studies on *Jasus edwardsii* (Crear & Forteath 2002), *Panulirus homarus rubellus* (Kemp et al 2009), and *Sagmariasus verreauxi* (Jensen et al 2013), ammonia excretion tends to decrease with an increase in body weight. The current study represents adult-size *P. homarus* with a size larger than 200 g. Therefore, we predict that a smaller size *P. homarus*, might have a higher nitrogen excretion rate than the results of the current study.

Furthermore, based on dissolved inorganic nitrogenous compound concentration in RAS (Figure 2), it can be inferred that the proportion of nitrate-N concentration relative to the total dissolved inorganic nitrogen in the system was 97.73 $\pm$ 0.759 % (fed) and 99.39 $\pm$ 0.217 % (unfed). In contrast to our finding, a prior study on *P. ornatus* reported that ammonia excretion constituted the predominant portion, accounting for 81.5 ± 7.0% of the total dissolved inorganic nitrogen output, while nitrate and nitrite collectively contributed 12.5 ± 5.5% and 6.0 ± 2.5%, respectively (Lee et al 2015). Different results observed in the current study were due to the fact that the experiment was done in RAS and lasted for a more extended period of seven days compared to the flow-through system and 24-hour experimental period, as reported in the previous study by Lee et al 2015. While we agree with the consensus that ammonia is the main type of dissolved inorganic nitrogen compound excreted by crustacean decapods (Kemp et al 2009; Lee et al 2015; Romano & Zeng 2013), the results of the current study indicated that the biofilter set-up in the pilot RAS worked effectively. The lower proportion of ammonia-N and nitrite-N indicated that both ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) co-existed in the culture system and simultaneously converted ammonia into nitrite and nitrite into nitrate (Ruiz et al 2020). Consequently, after seven days, the concentration of ammonia-N and nitrite-N in all RAS was maintained below the limit while nitrate-N concentration elevated significantly.

Conclusions. The total dissolved inorganic nitrogen excreted by *Panulirus homarus* fed restrictively at a 5% feeding rate for seven days was 1.91 g or about 2.88 g per kg of lobster biomass. The unfed group excreted a significantly lower total dissolved inorganic nitrogen, with only 0.29 g of N or roughly 0.43 g per kg of lobster biomass. Daily

excretion of dissolved inorganic nitrogen in the fed group lobster was 0.41 g kg^{-1} day^{-1}, while the unfed lobsters excreted only 0.06 g kg^{-1} day^{-1}. Our results suggested that *P. homarus* excretes dissolved inorganic nitrogen into the systems even without feeding and that the excretion of dissolved inorganic nitrogen tends to increase with increased feeding rates. Moreover, in functional RAS, nitrate was the predominant dissolved inorganic nitrogen compound (>90% of N) that accumulated in the system as the product of the ammonia and nitrite nitrification process.

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Conflict of interest. The authors declare that there is no conflict of interest.

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