Effect of feeding frequency on survival and growth of juvenile spiny lobster *Panulirus versicolor* in Indonesia

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Abstract. This research aimed to assess the effect of feeding frequency on survival and growth of juvenile bamboo spiny lobster *Panulirus versicolor* (Latreille, 1804). A completely randomized design of 4 treatments with 4 replicates was used with treatments consisting of: feeding once per day (FR1), feeding twice per day (FR2); feeding three times per day (FR3) and feeding four times per day (FR4). The experiment was performed in a tank system with a recirculating water supply. Sixteen 100 litre tanks were stocked with post-puerulus juveniles, mean weight 0.50±0.01 g, at a density of 89 m^{-2} and the experiment ran for 30 days. Diet consisted of a manufactured pellet diet formulated for tropical spiny lobster. Results indicated a statistically significant effect ($p < 0.05$) of feeding frequency on carapace length, although not for other variables. Carapace length for feeding two and four times per day was significantly greater than for feeding once per day. Mean survival rate across all treatments was 32% and mean final weight was 0.95 g. Notwithstanding the statistical non-significance for several of the variables, the significant effect on carapace length and trends evident for the other variables suggest feeding more than once per day may be beneficial.
Key Words: *Panulirus versicolor*, lobster, aquaculture, feeding frequency.

Introduction. Research in Indonesia indicates that an abundant resource of naturally settling seed lobsters (puerulus stage) exists, that may be exploited to support aquaculture (Jones 2018). This is based on the lobster aquaculture industry of Vietnam, which successfully and sustainably produces premium farmed spiny lobsters using a supply of naturally settling seed. Vietnam production of farmed lobsters in 2015 was 1500 tonnes valued at over $US120 million (Anh & Jones 2015).

In the period 2008 to 2014, fishing for seed lobsters in Indonesia developed, firstly in Lombok and then along the southern coasts of Java and Sumbawa Islands, that provided a supply of juveniles for local grow out. Due to limited knowledge, skills and technology, the growout industry (sea cage farms) developed slowly and reached a maximum production of no more than 100 tonnes (Priyambodo & Jones 2015). Many lobster seed fishers and farmers became aware of demand for the Indonesian lobster seed for sale to the Vietnamese, whose lobster farming industry was well established and successful. Consequently, growout farming diminished and export of lobster seed expanded. In 2015 the Indonesian Ministry of Marine Affairs and Fisheries introduced a new regulation (Indonesia M.K.D.P.R. 2015) specifying a minimum legal size for spiny lobster of 8 cm carapace length (200 g) that was then accompanied with a further regulation banning the export of lobsters smaller than 8 cm carapace length. These regulations made the catching and sale of seed lobsters to Vietnam, illegal. Despite the illegality, lobster seed fishing and export continued because the enterprise was lucrative, providing significant economic benefit to many impoverished coastal communities. As policing of these illegal activities has increased, more and more communities have suffered a negative impact. Research is therefore required to develop effective resource management of the lobster seed supply and to develop effective farming technology to enable Indonesians to on-grow the lobsters for maximum benefit to them and the Indonesian economy.

Growout of seed lobsters to exceed the minimum legal size (8 cm carapace length) provides a viable way for the seed fishery to continue and to increase the economic benefit to Indonesia. The strong aquaculture production credentials of several tropical spiny lobster species are well known, including *Panulirus argus* (Latreille, 1804), *Panulirus ornatus* (Fabricius, 1798) and *Panulirus homarus* (Linnaeus, 1758) (Jeffs & Davis 2003; Jones 2007, 2010). In Aceh province Indonesia, the tropical spiny lobster *Panulirus versicolor* (Latreille, 1804) is a common species, that may be a good candidate for aquaculture. Seed lobsters of this species are abundant in several areas of Aceh (Jones 2018).

A key component of successful aquaculture is cost-effective feeding, using formulated diets that provide the necessary nutrients at lowest cost and delivered to the farmed stock in the most efficient manner. Frequency of feeding is part of this, and the research reported here sought to assess feeding frequency of juvenile *P. versicolor* lobsters to determine the optimum, as per the report of Cox & Davis (2006).

Material and Method. The experiment was performed in a wet laboratory at the Brackishwater Aquaculture Development Centre, Ujung Batee (BADC Ujung Batee) in Aceh, Indonesia (5°37'33"N, 95°36'54"E) in April 2017. Treatments for the study represented different frequencies of feeding, based on the approach of Cox & Davis (2006), with an initial ration of 100% of biomass per day, reduced progressively to 50% of biomass at day 6, 30% at day 14 and 25% at day 21 to approximate feeding to satiation. The feeding frequencies were once per day with time of feeding at 1700 (FR1), twice per day with time of feed at 0900 and 1700 (FR2), three times per day at 0900, 1300 and 1700 (FR3), and four times per day at 0900, 1130, 1400 and 1700 (FR4). There were 4 replicates of each treatment allocated randomly to 16 tanks.

The food provided was a manufactured noodle shaped pellet (2.2 mm diameter), made by the Institute for Mariculture Research and Development (IMRAD) located at Gondol, Bali, Indonesia. The formulation was based on a diet developed through an associated research project (Anon 2015) with composition as follows: fish meal (65.30%), wheat flour (6.00%), wheat gluten (6.00%), BioMos™ (0.50%), fresh fish flesh (6.00%), fresh mussel flesh (6.00%), fresh squid flesh (1.00%), fish oil (2.60%), astaxanthin (1.00%), cholesterol (0.50%), lecithin (1.70%), mineral premix (0.60%), vitamin premix (1.10%), Stay-C™ (0.40%), binder (1.30%). The proximate composition was: dry matter 83%, crude protein 47.9%, and lipid 10.55%.

The lobsters used in this experiment were post puerulus stage, bamboo spiny lobsters (*P. versicolor*) collected from fishers in Pulo Raya Aceh Jaya District, Aceh Province. Initially, approximately 1,200 seed lobsters were obtained from fishers using traditional lift nets (bagan ikan) over one week. These lobsters were then transferred to BADC Ujung Batee for two weeks acclimation (1 to 14 March 2017), fed fresh fish flesh. Those surviving represented several species, and for this study 352 *P. versicolor* were chosen, with mean weight of 0.50±0.01 g.

Spiny lobsters were reared in cylindrical plastic tanks with diameter of 56 cm and height of 46 cm (water height 37 cm, water volume 91 L), provided with aeration and water supplied from a recirculation system comprising biological filter, UV sterilisation and protein skimmer. Water was supplied to each tank at a rate equivalent to 100% replacement per hour. Light was ambient natural light. All lobsters were weighed and their carapace length measured before stocking to the tanks. The number of lobsters in each tank was 22 representing a density of lobsters of 89 m^{-2}. Shelter was provided in the form of a 1 m x 0.2 m piece of netting material, bunched loosely and weighed down with a stone. Experimental lobsters were allowed one week to acclimate to the tank environment, fed fresh fish, and were starved for one day before the start of the experiment. The treatment feeding frequencies were then applied using the pellet diet for 30 days. At the completion of the experiment all lobsters were counted to determine survival and their individual carapace length and weight were measured to determine growth. Moulted exuviae were removed from each tank and counted to record number of moults per tank.

Measurement of variables followed the procedures of Rodríguez-Viera & Perera (2012) and included initial individual weight (Wi) and final individual weight (Wf) in grams, initial carapace length (CLi) and final carapace length (CLf) in mm, number of moults, mean specific growth rate (SGR) ((ln TWf – ln TWi)/T*100), where TWf is the mean weight of lobsters per tank at harvest and Twi is the mean weight of lobsters per tank at stocking, survival rate (final count per tank / number stocked (22)) x 100 as a percentage and biomass calculated from the mean final weight x the number of lobsters at harvest. Water quality variables were monitored, including dissolved oxygen, salinity, water temperature, pH, total ammonia nitrogen (TAN), nitrite and nitrate, approximately every second day (14 times) over the 30 day experiment period.

Data were analysed by ANOVA followed by Duncan's multiple-range test using SPSS version 23, at significance level of 0.05.

Results and Discussion. Weight and carapace length of all 352 lobsters after allocation to their respective tanks was recorded and ANOVA performed, confirming there was no significant difference at the start of the experiment (Table 1).

Table 1

Initial mean weight and carapace length data for juvenile *Panulirus versicolor* in feeding frequency experiment

Treatment	*Initial weight*	*Initial CL*	*Range weight*	*Range CL*
FR1	0.50 ± 0.01^{a}	10.36 ± 0.07^{a}	0.31-0.79	8.93-11.85
FR2	0.50 ± 0.01^{a}	9.97 ± 0.06^{a}	0.34-0.79	8.43-11.66
FR3	0.51 ± 0.01^{a}	10.19 ± 0.08^{a}	0.28-1.17	8.40-12.42
FR4	0.48 ± 0.01^{a}	10.04 ± 0.07^{a}	0.31-1.06	8.57-12.45

Means with same superscript are not significantly different ($p < 0.05$); CL = carapace legth.

Water quality remained acceptable through the study, although temperature (mean 32.83°C) was at the high end of optimal range for this and similar tropical species (Jones 2009) and nitrite briefly exceeded 38 ppm in some tanks. Despite the brief spike in nitrite, total ammonia nitrogen (TAN) remained very low and within desirable range (Table 2).

Table 2

Water quality data in rearing tanks for juvenile *Panulirus versicolor*, for 4 feeding frequencies over 30 days

	DO	*Salinity*	*Temperature*	*pH*	*TAN*	*NO_2*	*NO_3*
FR1	4.85±0.08	32.98±0.03	32.88±0.02	8.28±0.02	0.02±0.00	1.48±0.31	16.55±1.53
FR2	4.75±0.09	32.86±0.05	32.79±0.03	8.30±0.01	0.02±0.00	1.69±0.38	18.16±1.55
FR3	4.80±0.08	32.89±0.04	32.85±0.03	8.29±0.02	0.02±0.00	1.35±0.25	19.04±1.52
FR4	4.84±0.08	32.96±0.03	32.80±0.03	8.28±0.02	0.03±0.00	1.58±0.33	18.37±1.47

Statistics and their units include: DO – dissolved oxygen (ppm), salinity (ppt), temperature (°C), pH, TAN – total ammonia nitrogen (ppm), NO_2 – nitrite (ppm) and NO_3 – nitrate (ppm).

Analysis of survival, final weight, final carapace length, mean specific growth rate (SGR), harvest biomass and total moults per tank revealed there was no significant difference for any variable ($p > 0.05$), with the exception of final carapace length ($p = 0.018$). Mean values for these variables for each treatment are presented in Table 3. Duncan's multiple range test revealed that the final carapace length for treatment FR1 was significantly less than that for FR2 and FR4, but not significantly different to FR3. Mean values of the variables measured for each feeding frequency treatment are illustrated for comparative purposes in Figures 1 to 3.

Table 3

Summary statistics (means±S.E.) for juvenile *Panulirus versicolor* in feeding frequency experiment

	Survival (%)	*Weight (g)*	*CL (mm)*	*SGR*	*Biomass*	*Moults*
FR1	28.4±2.2[a]	0.87±0.07[a]	11.27±0.32[a]	1.81±0.19[a]	5.44±0.68[a]	21.5±1.6[a]
FR2	30.7±2.2[a]	0.98±0.05[a]	11.95±0.15[b]	2.23±0.21[a]	6.54±0.39[a]	20.3±1.9[a]
FR3	36.4±1.9[a]	0.91±0.04[a]	11.57±0.30[ab]	1.95±0.10[a]	7.28±0.44[a]	19.5±0.9[a]
FR4	31.8±3.2[a]	0.96±0.03[a]	11.78±0.15[b]	2.28±0.17[a]	6.77±0.85[a]	20.8±0.5[a]

Survival represents mean per tank, weight is final mean weight, CL is final mean carapace length, SGR is mean specific growth rate, biomass is the final mean tank biomass of lobsters and moults is the mean number of moults per tank. Means with different superscripts are significantly different ($p < 0.05$).

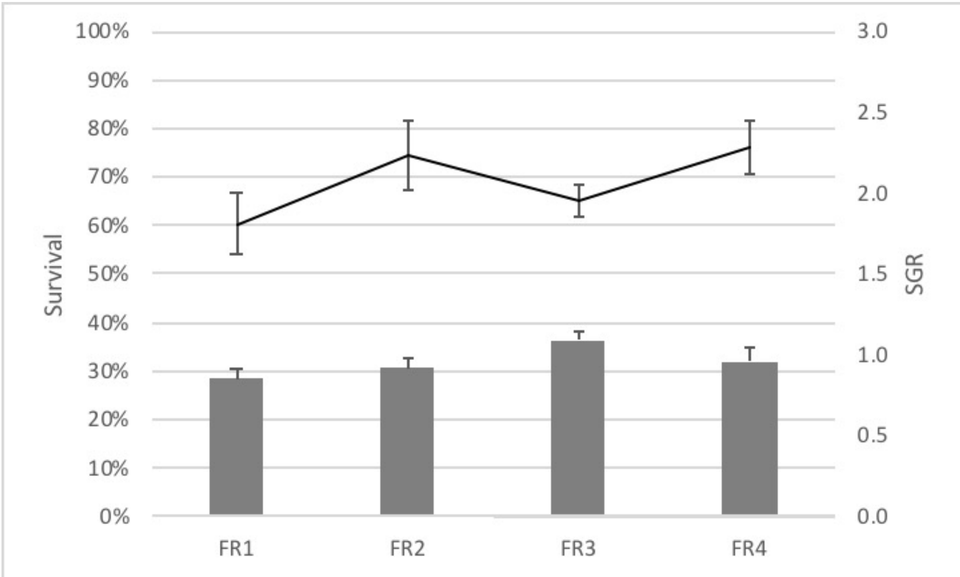

Figure 1. Mean (±SE) survival (columns) and specific growth rate (line) of juvenile *Panulirus versicolor* for the 4 feeding frequencies at completion of experiment (30 days).

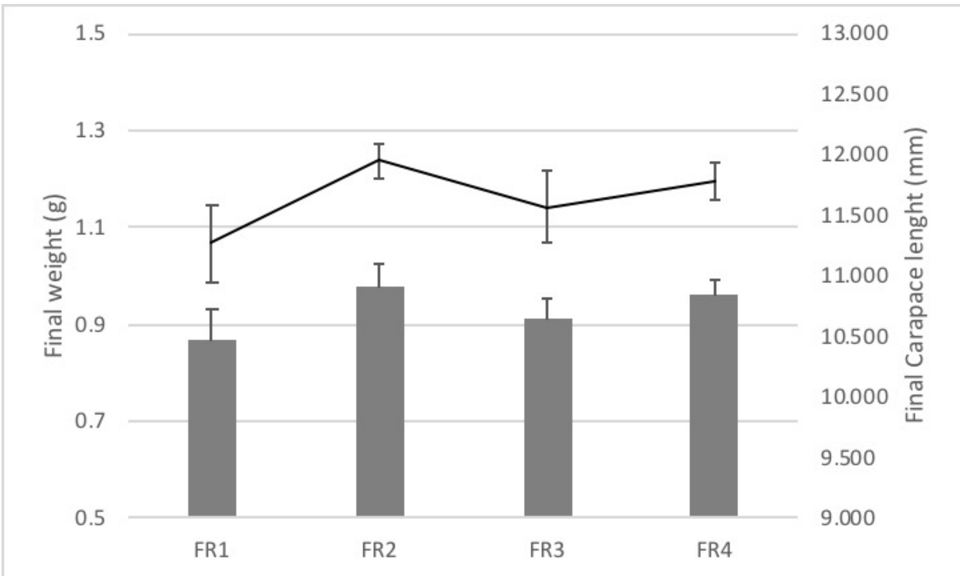

Figure 2. Mean (±SE) final weight (columns) and final carapace length (line) of juvenile *Panulirus versicolor* for the 4 feeding frequencies at completion of experiment (30 days).

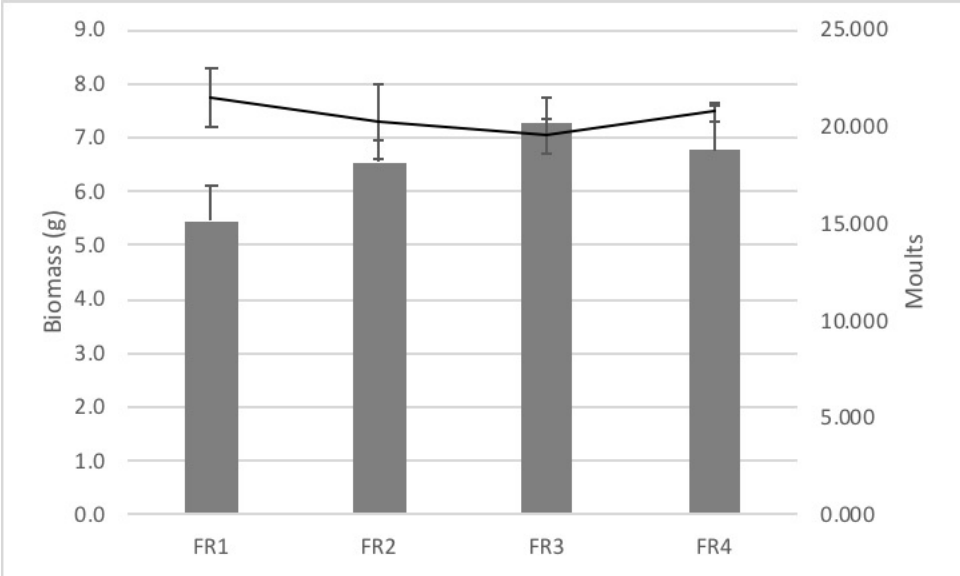

Figure 3. Mean (±SE) final biomass (columns) and number of moults (line) of juvenile *Panulirus versicolor* for the 4 feeding frequencies at completion of experiment (30 days).

The survival in this study was low across all treatments, although this is not uncommon in studies with juvenile spiny lobster (Smith et al 2003; Dubber et al 2004; Cox & Davis 2006; Jones 2007; Simon & James 2007). Very few dead or moribund lobsters were observed and the mortality that occurred is attributed primarily to cannibalism. Although it may be reasonably hypothesised that more frequent feeding would mediate cannibalism, the data from the experiment did not support this contention. This may be due to inadequacies in the manufactured diet such that recently moulted, soft-shelled lobsters provided an attractive alternative to the pelleted feed. Another factor potentially influencing survival is shelter. Although shelter was provided in the form of folded netting, no specific assessment of shelter for this species has been performed. Dennis et al (1997) have suggested that *P. ornatus* juveniles are gregarious, and are often found in tight groupings. It is also documented that the adults of *P. ornatus* are social (Dennis et al 2004), occupying dens together at high density. For *P. versicolor* however, there is no published data on their social behaviour, and it may be that they are more solitary and territorial than other tropical species. This may explain the low survival in the reported experiment as density may have been too high and shelter inadequate. Further research of density, shelter and social behaviour for this species is warranted.

The SGR of the lobsters in this study was significantly higher than that reported for other similar species under artificial culture conditions. For studies on several rock lobster species (temperate and tropical), typical SGR values were between 0.5 and 1.3% d^{-1} for juvenile lobsters (Crear et al 2000; Smith et al 2003; Smith et al 2005; Jones 2007; Simon & James 2007). The mean SGR of 2.28% d^{-1} for FR4 in this study suggests *P. versicolor* has a relatively fast growth rate.

The study provides the first reported data on moult frequency for juveniles of *P. versicolor*. Although individual moult data was not recorded, the number of moults per tank per day was counted and averaged for each treatment (Table 2). Based on the number surviving to day 30, it was apparent that most lobsters moulted at least twice.

The diet used was formulated for *P. homarus* through a parallel study to develop aquaculture technology for this species (Anon 2015). Although the formulation provides adequate nutrition for *P. homarus* (Irvin & Shanks 2015) it may be deficient for *P. versicolor*, based on different feeding habits. There is no published data on *P. versicolor* natural diet or nutrient requirements. Nevertheless, the SGR achieved across all treatments was relatively high, suggesting nutrition was adequate.

The significant difference in final carapace length, but not the other variables is likely to be an artefact of the short duration of the experiment. Figures 1 to 3 suggest that multiple feeds per day provide some advantage compared with once per day, and that a continuation of the treatments may have resulted in statistically greater values for the multiple feed treatments for final weight and other variables, over a longer time period. It would be worthwhile repeating this experiment with a longer culture period to confirm this suggestion.

Cox & Davis (2006) performed a similar study to the current experiment, with juveniles of the tropical species *P. argus*. The results were in contrast, as feeding once per day was found to support greater growth (in both weight and carapace length) than feeding twice per day. An equivalent study for *Jasus edwardsii* (Hutton, 1987) juveniles (Thomas et al 2003) also concluded that once per day feeding after dusk was recommended, even though feeding 4 times per day decreased feed completion and agonistic behaviour. There are studies of other crustacean species (Sedgwick 1979; Cortes-Jacinto et al 2003; Chandrasekaran et al 2005) that demonstrate the benefits of multiple feeds per day, however it appears that there is great variability among species and indeed that optimal feeding protocols may be species specific. For lobsters such as *P. versicolor*, that are known to be nocturnal, it would be worthwhile to examine feeding frequency through the night rather than through the day.

Conclusions. The results of this research suggest that multiple feeds per day could have a beneficial effect for rearing of juvenile *P. versicolor*, but that further study is required to confirm. Carapace length was significantly greater in lobsters receiving more than one feed per day, and trends of increased survival and other growth variables with increased feeding frequency were evident, that may have been statistically significant over a longer culture period. Improving survival in the nursery culture of *P. versicolor* is a priority, and optimising feeding frequency along with improved diet formulation and other husbandry factors is likely to be part of the solution.

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