



Effects Of Different Diets On Digestive Enzyme Activities, Growth Performance, And Survival Rate Of Brandt's Rice Crab Juvenile (*Somanniathelphusa Germaini*)

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Abstract. Brandt's Rice crab (*Somanniathelphusa germaini*) is a potential species for aquaculture in the Mekong delta. This study was conducted to determine appropriate diets for growth and survival of rice crab at juvenile stage. The experiment consisted of 5 treatments, all treatments were designed and set up randomly in triplicate in 0.25 m² tanks. Five different types of feed were evaluated including (1) tubifex, (2) Artemia biomass, (3) tubifex + artificial feed, (4) Artemia biomass+ artificial feed and (5) artificial feed. Key digestive enzymes (trypsin, chymotrypsin, pepsin, lipase, and amylase) were measured. After 28 days of rearing, the results showed that water quality parameters were in appropriate range for crab performance. The digestive enzyme activities were fluctuated baseing on feed compositions, in which, trypsin, pepsin, lipase and amylase were important indicators. Crabs fed tubifex were recorded with the highest survival rate (80.0 %), growth performance (CW = 10 mm and W=0.3 g) and productivity (400 ind./m²), which were significantly higher than other treatments (p<0.05). Besides, the artificial feed was not suitable for crab rearing, leading to low growth and survival. The results suggested that tubifex could be applied for the husbandry practice of rice crab nursery.

Key Words: diet, digestive enzyme, growth, nursery, survival, rice crab.

Introduction. Brandt's rice crab (*Somanniathelphusa germaini*) is a native freshwater crab throughout the Mekong delta region, Vietnam. *S. germaini* crabs have high economic value due to their delicate flavor and high nutritional value. With the decline in natural resources, the aquaculture production of this species in Vietnam has recently increased leading to high demand for crablets (Khoa et al. 2011). The source of crablets is mainly from the wild and relies on the season, therefore, dependence on wild crablets makes sourcing unsustainable for long term growth. Khoa et al (2011) and Binh (2011) have succeeded in breeding and rearing rice crab. However, there is limited research on rice crab culture and nursery technology, especially on the optimum diet and technical parameters for effective rearing (Binh, 2011). Adequate diet for crablet rearing is crucial for the proliferation of crab species, given its significant impact on growth and survival during early developmental stages. However, due to lack of available data concerning feeding habits, dietary preferences, etc. of this species, more attention is needed through research for the nursing and farming. So the current investigation was undertaken to assess the the digestive enzyme activities, growth, and survival rate of *S. germaini* juvenile fed live feed and commercial feed.

Live feeds are considered as a convenient and essential food source for the larvae and fry of some cultivable species and widely accepted as living capsules of nutrition. Zooplankton is an important natural food for fish, and an excellent source of essential amino acids (EAAs) and polyunsaturated fatty acids (PUFAs) (Taipale et al. 2018; Barclay & Zeller, 1996). Live zooplanktons such as brine shrimps (*Artemia*), tubificids (*Tubifex*) are the most widely accepted live feeds globally and play a significant role in the feeding of cultivable species of fishes and crustaceans. Among the zooplankton, *Artemia* and *tubifex* are easily digestible due to the presence of digestive enzymes with high energetic caloric value (Morris & Mischke, 1999). The live feeds for this study were selected based on their high nutritive value, the capacity to grow in dense populations, easy to produce in mass scale under controlled conditions, short generation time and their beneficial use in previous studies.

Besides, the advantages of co-feeding of live feed and artificial feed has been reported on many fish and shrimp species (Kolkovski, 2001; Richard et al. 2015; Mata-Sotres et al. 2016). Feeding prey organisms for a short time to early stage larvae and gradually adding artificial feed is known to increase digestive activity and increase the larvae's ability to digest the dry diets (Kolkovski et al. 1997; Kolkovski, 2001) as live prey include certain neuro-hormonal factors which may stimulate digestive enzyme secretions in fish/shrimp larvae (Chan & Hale, 1992; Ruyet & Mugnier, 1993; Hamre et al. 2013). Diets containing high energy levels, supplied as neutral lipid and phospholipid mixtures could promote larval growth (Cahu & Infante, 2001). The use of improved diets would sustain the production of constant high quality fingerlings in the hatchery (Cahu & Infante, 2001; Kolkovski, 2013; Takeuchi, 2014). In this study, the experiment was designed to determine the effects of tubifex, artemia and artificial feed on the digestive enzymes, growth performance and survival rate of rice crab juveniles at the nursing phase.

Material and Method

Experimental diets. The experiment consisted of 5 treatments were designed and set up randomly in triplicate. In which, five different types of feed were evaluated including (1) tubifex, (2) Artemia biomass, (3) tubifex + artificial feed, (4) Artemia biomass+ artificial feed and (5) artificial feed (Grobtest, 42% protein). The compositions (protein, lipid and carbohydrate) of tubifex and Artemia biomass were analyzed by upScience company (Can Tho city, Vietnam) (Table 1).

Table 1 Compositision of diets for crab juveniles

Composition	Protein (%)	Lipid (%)	Carbohydrate (%)
Tubifex	57,2 ± 1,4	13,3 ± 0,3	3,6 ± 0,5
Artemia biomass	52,7 ± 0,6	10,5 ± 0,3	7,8 ± 0,3
Artificial feed (Grobtest)	42	6	3

Crab juveniles (with initial carapace width (CW)=1.4mm and weight (W) =0.007g) were collected from the rice field, then acclimated in 4m³ tank for 2 days and used for the experiment. Crabs were stocked in 0.25m² plastic tanks at 500 ind./m² of density. The rearing tanks were set up with slight aeration and 15 cm of water column. Crabs were fed three meals a day to satiation (at 7 am, 13 pm and 16 pm). For the treatment fed combined diets with artificial feed, the artificial feed was fed at 13pm. Siphone and water exchange were done daily at 30% of water volume.

Sampling. Temperature, and pH were monitored twice daily (at 7:00 AM and 2:00 PM) using a digital meter (HI-98196 Multi-Parameter Waterproof Meter, HANNA Instruments, Ltd.). Total ammonium nitrogen (TAN) and nitrite/nitrate were measured every week by the Indophenol Blue colorimetric method and Griess-Ilosvay method, respectively. Alkalinity was weekly measured using HANA HI83303 multiparameter photometer.

Crab sampling was carried out every 7 days to determine the growth rate. Daily weight gain (DWG) and specific growth rate (SGR %) in weight of crab were determined using the following equations:

Daily weight gain (DWG)

$$DWG (g/day) = ((\text{Final weight} - \text{Initial weight}) / (\text{Day of culture}))$$

Specific growth rate (SGR %):

$$SGRW (\%/day) = (100 * (\ln \text{ final weight} - \ln \text{ initial weight}) / (\text{Day of culture}))$$

Daily length gain (mm/day):

$$DLG (mm/day) = ((\text{Final length} - \text{Initial length}) / (\text{Day of culture}))$$

Specific growth rate (%/day):

$$SGRL (\%/day) = [(\ln \text{ Final length} - \ln \text{ Initial length}) / \text{Day of culture}] * 100$$

Survival rate (SR): determined after harvesting

$$SR (\%) = 100 * (\text{Total number of crab harvested}) / (\text{Total number of crab stocked})$$

Enzyme assay. The crude enzyme extraction was made in triplicate, in which 10 individual crabs (collected intestine, liver, and stomach) were transferred to 100 µL of ice-chilled homogenization buffer (20 mM Tris-HCl, 1 mM EDTA, 10 mM CaCl₂, pH 7.5) (Bolasina et al. 2006), homogenized using pellet pestle cordless motor (Sigma-Aldrich, Saint Louis city, Missouri, USA). Thereafter the homogenate was mixed with 400 µL of homogenization buffer and coldly centrifuged for 30 min at 1700 x g. The supernatant was used for enzymatic assay analysis as crude enzyme. Bradford's method utilizing protein assay CBB solution (Nacalai tesque, Inc, Kyoto, Japan) was performed for protein content determination. **Trypsin.** The fluorometric assay for trypsin activity was performed according to Bolasina et al. (2006) using Z-L-arginine-7-amido-4-methylcoumarin hydrochloride (CBZ-LArg-MCA, C9521, Sigma-Aldrich) as the substrate. The substrate solution contained 50 mM Tris-HCl (pH 8.0), 10 mM CaCl₂, 0.2 mM CBZ-LArg-MCA. A total of 50 µL of crude enzyme was mixed with 500 µL substrate in a micro tube and incubated in a water bath at 30 °C for 30 min. The reaction was stopped by adding 100 µL of 30% acetic acid. For blank reading, the same pattern was prepared without the addition of acetic acid prior to mixing the substrate. Fluorescence was measured using a spectrofluorophotometer (F2000, Hitachi High-Technologies Corporation, Tokyo, Japan) and the difference in emissions at 440 nm (excitation 380 nm) was measured between the samples and the blanks. Trypsin activity was expressed in unit (U) in 30 min, as increase of emission per protein (U /µg protein).

Chymotrypsin. Chymotrypsin activity was assayed by following a modified method of Murashita et al. (2018) using N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (SAPFNA, Sigma-Aldrich) as a substrate. Each assay contained 240 µL of 100 mM Tris buffer containing 20 mM CaCl₂ (pH 8.5), 100 µL of 2.4 mM SAPFNA and 50 µL of crude enzyme. Production of p-nitroanilide (pNA) was measured by monitoring the increase in absorbance at 405 nm per minute for 7 min at 37 °C using a spectrophotometer (U5100, Hitachi High-Technologies Corporation). One unit (U) of activity was defined as the amount of enzyme that caused an increase of 1 absorbance unit at 405 nm in 1 min.

Pepsin. Pepsin activity was evaluated using of 2 % haemoglobin in 0.06 N HCl as substrate according to Natalia et al. (2004). In the test tube, 100 µL of enzyme extract was incubated with 500 µL of substrate for 10 min. The reaction was

terminated using 1 mL of 5 % trichloroacetic acid and left for 5 min. The mixture was then centrifuged for 5 min at 12000 xg. Absorbance was recorded at 280 nm using a spectrophotometer (U5100, Hitachi High-Technologies Corporation). For blank reading, trichloroacetic acid was added to substrate prior to the addition of enzyme extract.

Lipase. Neutral lipase activity was determined based on protocol of Roberts (1985). The non-fluorescent substrate, 4-methylumbelliferyl butyrate (4 MUB) was used solubilizing in lecithin mixed micelles, is hydrolyzed to butyric acid and the highly fluorescent compound, 4-methylumbelliferone (4-MU). A 60 μ L substrate solution (0.5 mM 4 MUB, 5 mM egg lecithin, 10 mM sodium taurocholate and 150 mM NaCl) was placed in tested tube with 20 μ L of enzyme extract and 20 μ L of Tris buffer (pH 7.5). Each assay was conducted simultaneously at both 4 °C in an ice bath and at 37 °C in a water bath for 10 min. A 3 mL cold (4 °C) Tris buffer (1 M, pH 7.5) was added to quench the reaction. The change in fluorescence was recorded by a spectrofluorophotometer (F2000, Hitachi High-Technologies Corporation) utilizing emission setting at 450 nm (excitation 380 nm) and subtracting the reading of the 4 °C incubated samples against the 37 °C control samples for each assay. Fluorometer constants were calculated from standard stock solutions of 4-methylumbelliferone.

Amylase. Activity was assayed by following a modified protocol from Murashita et al. (2018) using 1 % starch solution as a substrate. The reaction mixture consisted of 50 μ L of enzyme extract, 25 μ L of the substrate solution and 25 μ L 20mM sodium phosphate buffer (pH 6.9, containing 6.0 mM NaCl) followed by 60 min at 37 °C of incubation. 50 μ L dinitrosalicylic acid reagent (1 % dinitrosalicylic acid and 30 % sodium potassium tartrate in 0.4 M NaOH) was added into the mixture and incubation in boiling water for 5 min. The absorbance was recorded at 540 nm using a Hitachi U5100 spectrophotometer and the amount of maltose released was determined by reference to a standard curve. The activity was expressed in U, which was defined as the amount (μ mol) of maltose released in 1 min.

Statistical analysis. Data were presented as mean \pm standard error of the mean (SEM) and were subjected to one-way ANOVA (SPSS 24.0 for Windows, IBM, Armonk, NY, USA) applied post-hoc DUNCAN test. All differences were considered at $\alpha = 0.05$.

Results and Discussion

Water quality. Although the temperatures in the rearing tanks were rather stable, there were minor fluctuations between mornings and afternoons. Nonetheless, the average temperature varied in the range 27.3-29.2°C. According to Hai et al (2017), the preferred temperature range for crustacean in larviculture is 28-30°C. The pH level varied in the range 8.09-8.16; this variation was negligible. Furthermore, this indicated the optimum level for rearing freshwater prawn; according to Phuong et al (2003), the appropriate pH range is 7-8.5. Table 2 also displays that alkalinity during larval rearing was in the range 71-83 mg CaCO₃ L⁻¹; this is considered a suitable alkalinity condition. There is still limited information about appropriate range of alkalinity for rice crab. Tao & Phu (2015) reported that the appropriate alkalinity for rearing the larvae and PL of freshwater prawn is in the range 100-120 mg CaCO₃ L⁻¹.

Table 2 Water parameters

Parameters		Treatments				
		Tubifex	Artemia biomass	Tubifex + Artificial feed	Artemia + Artificial feed	Artificial feed
Temperature (°C)	AM	27.3 \pm 0.4	27.5 \pm 0.5	27.3 \pm 0.3	27.4 \pm 0.2	27.6 \pm 0.4
	PM	29.1 \pm 0.6	29.1 \pm 0.5	29.1 \pm 0.9	29.2 \pm 0.5	29.2 \pm 0.4
pH	AM	8.15 \pm 0.01	8.15 \pm 0.02	8.10 \pm 0.20	8.09 \pm 0.10	8.09 \pm 0.02
	PM	8.15 \pm 0.09	8.16 \pm 0.01	8.12 \pm 0.01	8.09 \pm 0.01	8.09 \pm 0.10
Alkalinity (mgCaCO ₃ /L)		83.5 \pm 10.3	83.5 \pm 10.3	71.6 \pm 17.9	77.6 \pm 20.7	77.6 \pm 20.7
TAN (mg/L)		0.4 \pm 0.25	0.5 \pm 0.41	0.5 \pm 0.41	0.5 \pm 0.41	0.7 \pm 0.48
NO ₂ ⁻ (mg/L)		0.3 \pm 0.29	0.4 \pm 0.25	0.5 \pm 0.41	0.5 \pm 0.41	0.6 \pm 0.48

This experiment's average TAN levels varied in the range 0.4-0.7 mg L⁻¹ and average NO₂⁻ varied in the range 0.3-0.5 mg L⁻¹. According to Mallasen & Valenti (2006), survival, growth, and larval stage indices of freshwater prawn do not produce significant differences when NO₂⁻ remains below 2 mg L⁻¹. Daily water exchange helped to maintain water quality in good condition. Thus, the parameters recorded by the current study (i.e., water temperature, pH, alkalinity, TAN, and NO₂⁻) were appropriate for the rearing of rice crab.

Digestive enzyme activities. the key digestive enzymes of crab (trypsin, chymotrypsin, pepsin, lipase and amylase) were detectable at low levels at stocking. After 28 days of nursery, the crabs fed tubifex showed a significant increase in digestive capacity, in which, trypsin, pepsin, lipase and amylase were significantly increased compared to others ($p < 0.05$). The treatments fed artificial feed or combined diets were recored with lower activity of enzymes. Especially, pepsin plays an important role in the digestion of formulated feed, however, the crab-fed only artificial feed showed low activity of pepsin, indicating inefficient digestive capacity.

Table 3 Digestive enzyme activities of crab juveniles

Digestive enzyme		Treatment				
		Initial activity	Tubifex	Artemia biomass	Tubifex + Artificial feed	Artemia + Artificial feed
Trypsin (U/mg protein)		1,88 ± 0,56 ^a	6,36 ± 1,20 ^c	3,55 ± 1,37 ^b	3,63 ± 1,27 ^b	3,37 ± 0,75 ^b
Chymotrypsin (U/mg protein)		0,83 ± 0,24 ^a	0,77 ± 0,35 ^a	0,86 ± 0,32 ^a	0,72 ± 0,36 ^a	0,61 ± 0,66 ^a
Pepsin (U/mg protein)		1,23 ± 0,52 ^a	9,36 ± 0,72 ^d	6,21 ± 0,46 ^c	6,52 ± 1,27 ^c	6,43 ± 1,02 ^c
Lipase (U/mg protein)		39,8 ± 12,7 ^{ab}	193,3 ± 11,5 ^c	87,5 ± 19,3 ^b	80,4 ± 27,7 ^b	113,7 ± 15,3 ^b
Amylase (U/mg protein)		3,52 ± 1,18 ^a	5,30 ± 1,09 ^{ab}	5,47 ± 1,30 ^{ab}	7,93 ± 0,63 ^b	7,65 ± 0,93 ^b

Values in the same row with different letters are significantly different ($p < 0.05$; $a < b < c$).

Digestive enzyme activity may have an important function on feed utilization and growth performance, especially protease, amylase, and lipase, which are important for digestive processes (Zhao et al. 2016). The study of digestive enzymes may be helpful for understanding the digestive capacity and nutrient utilization in crab. Crustacean digestive enzyme activity indicated the digestive and absorptive capacity is positively correlated with nutrient content. Digestive enzymes activities fluctuate according to feed type and feed composition, whereby live feed play a role in stimulating the secretion of digestive enzymes while artificial feeds with high energy content can help improve growth, and molting (Cahu & Infante, 2001; Takeuchi, 2014). Tubifex and artemia biomass have high nutritional contents and are rich in n-3, which plays a large role in stimulating digestive enzyme secretion at the early stages of juvenile crabs. This result is similar to the study of Khoa et al. (2011) and Binh (2011) when using tubifex for rearing rice crabs, crabs fed tubifex achieved the highest survival rate compared to formulated feed. Besides, artemia biomass was also reported to be a very suitable feed for the postlarval stage of the white leg shrimp *L. vannamei* (Naegel & Rodriguez Astudillo, 2004), the giant freshwater prawn *M. rosenbergii* (Anh et al. 2009) and mud crabs (Anh et al. 2011).

Feeding with formulated feeds has been reported to be ineffective, low enzyme activities indicated low digestive capacity which could lead to slow growth, cannibalism, and high mortality. Feeding treatments that combined live feed with artificial feed from the beginning also showed low enzyme activity, which could be explained by the inappropriate time of weaning, crabs need more time to complete their digestive functions. Therefore, in order to improve the digestive performance of crabs, it is very important to develop co-feeding regimes with appropriate timing of weaning (Hassan et al. 2011), it could help to increase molting ability, survival rate and reduce production costs (Wickins & Lee, 2002; Ut et al. 2007).

Growth performance of crab juveniles

After 28 days of rearing,

the results showed that the average carapace width of rice crabs ranged from 6.6-10.8 mm, and were significantly different among treatments ($p < 0.05$) (Table 4). In which, the crabs fed tubifex were observed with the largest carapace width (10.8 mm). Similarly, DLG and SGR_L of crab juveniles among treatments ranged from 0.18-0.33 mm/day and 5.5 - 7.27%/day, respectively. In which, crab fed tubifex showed the highest growth rate at 0.33 mm/day (7.27%/day) which was statistically higher than other treatments ($p < 0.05$). Crab fed with artificial feed completely resulted in the lowest carapace width (6.6mm; SGR_L = 5.5% day⁻¹). According to Khoa et al. (2011), crabs fed tubifex had the highest growth rate of carapace width at 5.69%/day. This shows that tubifex is suitable feed for crabs due to its small size, alive but catchable, and high nutrition. Early addition of artificial feed or completed replacement with artificial food will result in slow growth of crabs, high cannibalism, and low survival rate (Khoa et al., 2011).

Table 4 Growth in carapace width

Parameters	Treatment				
	Tubifex	Artemia biomass	Tubifex + Artificial feed	Artemia + Artificial feed	Artificial feed
CW _i (mm)	1.4±0.3	1.4±0.3	1.4±0.3	1.4±0.3	1.4±0.3
CW _f (mm)	10.8±1.3 ^b	8.1±0.6 ^b	8.2±0.9 ^{ab}	7.8±1.1 ^{ab}	6.6±0.2 ^a
DLG (mm day ⁻¹)	0.33±0.06 ^c	0.24±0.02 ^{ab}	0.24±0.03 ^{ab}	0.22±0.04 ^{ab}	0.18±0.02 ^a
SGR _L (% day ⁻¹)	7.27±0.23 ^c	6.27±0.29 ^{ab}	6.30±0.32 ^{ab}	6.13±0.38 ^{ab}	5.5±0.46 ^a

Values in the same row with different letters are significantly different ($p < 0.05$; $a < b < c$).

The body weight of rice crabs was significantly increased after 28 days of rearing (Table 5), ranging from 0.09 to 0.34 g/crab. Which, crabs fed tubifex had the highest weight (0.34g) and was a statistically significant difference compared to

other treatments ($p < 0.05$). Similarly, DLGw and SGRw of crabs juvenile were different between treatments, ranging from 0.003 to 0.012 g/day (9.10 to 13.65 %/day). Especially, the growth rates were highest in the treatment-fed tubifex (0.0125g/day; 13.65%/day), while the treatment fed artificial feed gave the lowest results (0.003 g/day; 9.10 %/day) ($p < 0.05$). Tubifex (*Tubifex tubifex*, Annelida) is a living bait which is widely used in the production of ornamental fish due to its suitable size and availability. It is stable in nutrient components and rich in n-3 (C18: 3n-3 and C20: 5n-3) and n-6 (C18:2n-6 and C20:4n-6) fatty acids (Yanar et al. 2003). This live feed has been proven to enhance growth performance of fish/crabs. Our current study has been shown that feeding tubifex to the rice crab juveniles can in fact significantly increase growth. The reason for this may be due to rich nutritional contents (high protein, lipid, and some fatty acids), palatability, and some other unknown factors that may be present in tubifex worms (Tamaru et al. 1997; Roshada et al. 1993; Mohideen et al. 2014).

Table 5 Growth in body weight of crab juveniles

Parameters	Treatments				
	Tubifex	Artemia biomass	Tubifex + Artificial feed	Artemia + Artificial feed	Artificial feed
CW _i (g)	0.007±0.001	0.007±0.001	0.007±0.001	0.007±0.001	0.007±0.001
CW _f (g)	0.34±0.13 ^c	0.21±0.01 ^b	0.21±0.01 ^b	0.18±0.1 ^b	0.09±0.01 ^a
DLG _w (g day ⁻¹)	0.0125±0.002 ^c	0.0073±0.001 ^b	0.0073±0.001 ^b	0.0063±0.002 ^b	0.003±0.001 ^a
SGR _w (% day ⁻¹)	13.65±0.23 ^c	12.09±0.19 ^b	12.06±0.21 ^b	11.90±0.38 ^b	9.10±0.18 ^a

Values in the same row with different letters are significantly different ($p < 0.05$; $a < b < c$).

Molt cycle in crustaceans is under the control of several regulatory hormones, internal and external factors including diet nutrition (Kleinholz, 1985). Thereby, it shows that the nutritional content of the feed directly affects the growth of rice crabs. Studies on crustaceans at the nursing stage show that the combined diets will contribute to nutritional balance and reduce production costs (Jee et al., 2007), however, in this experiment, crab fed artificial feed or combined feed (ratio 2:1) are not effective, digestive enzyme activities are low, this may be due to the early stage of juvenile crab not being able to digest formulated feed, further researches are required to optimize weaning time and feeding ratio.

Survival rate and productivity of crab juveniles

Table 6 shows that the average survival rate of rice crabs among treatments ranged from 52.0 to 80.0%. In which, the highest survival rate was in the treatment fed tubifex (80.0%) and statistically higher than other treatments ($p < 0.05$). The treatment fed Artemia biomass reached 62.0% of survival, followed by combined diet treatments (67.3 -66.3%) with no difference between these treatments. According to Khoa et al. (2011) when rearing rice crabs with different diets, the results showed that crabs fed tubifex were recorded with the highest survival rate (75.6%), and the lowest survival was reared by steam fish (36.2%). Similarly, the productivity of crabs obtained in the treatments ranged from 260 -400 ind./m², the highest value in the treatment fed tubifex (400±20 ind./m²) which was significantly different ($p < 0.05$) compared to other treatments (Table 6). The reason for this difference was due to the nutritional composition of the feed in different treatments leading to different digestive enzymes, growth, molting and survival rate of crabs.

Table 6 Survival rate and productivity of crab juveniles

Parameters	Treatment				
	Tubifex	Artemia biomass	Tubifex + Artificial feed	Artemia + Artificial feed	Artificial feed
Survival (%)	80.0±4.1 ^c	62.0±2.0 ^b	66.7±1.5 ^b	67.3±4.5 ^b	52.0±3.0 ^a
Productivity (ind./m ²)	400±20 ^c	311±10 ^b	333±6 ^b	335±23 ^b	260±16 ^a

Values in the same row with different letters are significantly different ($p < 0.05$; $a < b < c$).

Conclusions. Rearing rice crab juveniles using tubifex displayed advantages in terms of digestive capacity and growth performance. In which, digestive enzymes (trypsin, pepsin, lipase, and amylase) were significantly improved. Artificial feed was not suitable for rearing crabs at early stages. Therefore, it required further studies to optimize the feeding regimes, focusing in co-feeding and weaning time for rice crab juveniles, which could help to enhance growth, survival and reduce production costs.

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