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Use of crude salt in rearing of freshwater giant prawn, *Macrobrachium rosenbergii* (de Man) larvae

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Abstract
Three different types of culture media: (i) 100% brine (B_{100}), (ii) 75% brine and 25% crude salt ($B_{75}CS_{25}$), and 50% brine and 50% crude salt ($B_{50}CS_{50}$) were tested to evaluate the possible use of brackishwater reconstituted from the crude salt for the production of *M. rosenbergii* post-larvae. The production rate of 25.26±0.20 Pl/ℓ with a corresponding survival rate of 84.20±0.66% was significantly higher ($P<0.05$) for the larvae reared on B_{100} than that of 22.10±0.57 Pl/ℓ with a corresponding survival rate of 73.68±1.89% on $B_{50}CS_{50}$. Larvae cultured on $B_{75}CS_{25}$ did not show any significant difference ($P<0.05$) in production as well as in survival of post-larvae than that on B_{100}. The result shows that, for rearing of prawn larvae, use of brine can be replaced up to 25% without any undue reduction in production of post-larvae. However, the production as well as survival rate of post-larvae with 50% replacement ($B_{50}CS_{50}$) is also appreciable. It is assumed that the mineral constituents of natural seawater might have some triggering effects on prawn larvae in closing their larval cycle.

Key words: *M. rosenbergii,* Larvae, Crude salt

Introduction

The life cycle of giant freshwater prawn, *Macrobracium rosenbergii* is being completed both in fresh- and brackish-water. The prawn larvae require 12-14 ppt brackishwater for the development of different larval stages to post-larvae. Larvae developed in brackishwater areas migrate to freshwater, shortly after metamorphosis to the post-larval stage, where they grow and get matured.

Since the breakthrough in closing *M. rosenbergii* larval cycle in captivity, this is being practiced with the conventional techniques (Ling 1969), where brackishwater of about 12 ppt is prepared diluting the seawater. The requirement of seawater for production of *Macrobrachium* larvae restricts the establishment of prawn hatcheries along with the seashore and/or coastal belt, limiting sufficient and easy supply of post-larvae for stocking upper inland freshwater ponds. Though establishing prawn

hatcheries in inland areas may solve such problem, there is difficulty and added cost in trucking seawater from its source to the inland hatcheries.

Now a day, instead of seawater, trucking of concentrated brine solution from the coastal salt beds and its use in rearing of *M. rosenbergii* larvae either in large or in small backyard hatcheries have been found economical and effective (Yambot and Vera Cruz 1986, Pramanik and Halder1996). However, collection as well as transportation of brine solution from the salt pens to inland regions far away from of coastal areas is again a problem. This bottleneck may be minimized if the *M. rosenbergii* larval rearing medium can be instantly prepared using salt. Though Yambot and Vera Cruz (1986) made conjectures about the use of brackishwater reconstituted from sea salt in production of *M. rosenbergii* larvae, there has been no any thorough investigation on it so far. The purpose of the present experiment was to find out the effects of brackishwater reconstituted from crude salt and its different combinations with diluted brine solution in rearing of *M. rosenbergii* larvae in captive conditions.

Materials and methods

Three treatments having different compositions of brine and crude salt water (Table 1) were tested in rearing of *M. rosenbergii* larvae. Each treatment had three replications and assigned into a completely randomized design.

Table 1. Compositions of brine and crude salt solution used in rearing of *M. rosenbergii* larvae

Treatments	Notations	% composition	
		Brine solution	Crude salt solution
I	B_{100}	100	-
II	$B_{75}CS_{25}$	75	25
III	$B_{50}CS_{50}$	50	50

Concentrated brine (≈100 ppt) was collected from a salt pan of Cox's Bazar, the south-eastern coastal district of Bangladesh, while crude salt were collected form a salt refinery factory located in Narayangonj district and transported to the prawn hatchery at Freshwater Station (FS) of Bangladesh Fisheries Research Institute (BFRI), Mymensingh. The crude salt was diluted with underground freshwater and left for overnight to settle. The diluted clear supernatant crude salt solution was pumped into a fiberglass tank. The brine and crude salt water was then diluted separately to bring the salinity level at 12 ppt and kept under vigorous aeration a period of 24-h. Prior to subsequent use of prepared brackishwater media, the aeration was stopped and allowed the suspended materials to be settled down. The supernatant brine and crude salt water was then pumped into nine rectangular fiberglass tanks (100 cm x 75 cm x 65 cm) at the required volume for the preparation of 300ℓ larviculture medium of different test compositions (Table 1). The larval rearing tanks were placed under semi-transparent roofing and provided with constant aeration. The salinity of larviculture water was

maintained at 12 ppt throughout the experimental period. A fresh stock of both brine and crude salt-water medium of 12 ppt salinity was maintained for exchange of larviculture water during the course of experiment.

Female prawn bearing gray coloured egg were collected from brood ponds of FS pond complex and kept in the fiberglass tank containing brackishwater of 6 ppt salinity. To obtain a larval batch with synchronized development, larvae from a single overnight spawning were stocked randomly into each larviculture tank at the density of 30 larvae/ℓ of culture medium.

Larvae in all the treatments were fed, up to the 4th day of rearing, with newly hatched *Artemia*, maintaining an approximate constant concentration of 3 nauplii per ml of culture medium. Afterwards larvae were fed with egg custard four times a day at 08.30, 11.30, 14.30 and 17.00 h followed by *Artemia* naupli at 18.00 h. Prior to every *Artemia* feeding, aeration was stopped and uneaten food particles were siphoned out. Egg custard was prepared according to the method given by Ang and Cheah (1986). Approximately 10 g powdered milk, 5 ml water and a whole chicken egg were blended. Ten drops of red food colouring (Bush Boaken Allen London E17 5 QP) were mixed and steamed for about 10-15 minutes. The prepared egg custard was stored in a refrigerator for not more than three days. Prior to daily feeding, the egg custard was passed through sieves (0.225- 0.600 μm Endecotts BS410) to obtain an appropriate particle size for the growing larvae. The particle size of egg custard used for feeding larvae at different stages is given in Table 2.

Table 2. Particle size of egg custard for feeding *M. rosenbergii* larvae

Stage of larvae	Size of food particle (mm)
II - IV	0.23
V - VIII	0.43
IX – post-larvae	0.60

About 25-50% of the total water volume of each larval rearing tank was siphoned out once in every 72 hrs and gradually replaced with fresh medium to maintain the volume of 300ℓ. Any fluctuation in salinity level and temperature of both fresh and larval rearing media was minimized at the time of each exchange. Ammonia-nitrogen (NH_3-N), pH, temperature and salinity of larviculture water were monitored periodically using LaMotte Ammonia Test Kit, pocket pH Meter (pH Scan 1™), maximum-minimum centigrade thermometer and ATAGO S/Mill Refractometer (8810), respectively.

The effectiveness of different test larviculture media was assessed on the basis of progressive development in larval stages and production of post-larvae at the termination of the experiment. Ten larvae from each tank were randomly sampled daily for the first week, at every alternate day for the second week and every third day for the rest of the experimental period. The larval stages were identified under a binocular microscope following the descriptions given by Uno and Kwon (1969). Progressive

development of larvae was determined by calculating the mean larval stage (MLS) at each sampling day using the following formula given by Lovett and Felder (1988):

$MLS = \Sigma (SxP_S)$

where, S is the larval stage number, and

P_S is the proportion of larvae at stage S.

The experiment was terminated when more than 95% of the larvae in all tanks metamorphosed to post-larvae. At the termination, total number of post-larvae was counted directly to determine the production rate. Post-larvae (n = 25) were randomly taken from each replicate tank to measure individual total length (from the tip of the rostrum to the end of the telson) and wet body weight.

Data were analyzed by one-way ANOVA using SAS linear model procedures (SAS, 1985). Differences among means were analyzed for significance using Duncan's Multiple Range Test (DMRT) at the 5% probability level. Percentage data were normalized by arcsine transformations (Zar 1984) prior to statistical analysis.

Results

Production of *M. rosenbergii* post-larvae (Pl) under various brine replacement treatments is presented in Table 3. The production rate of 25.26±0.20 Pl/ℓ with a corresponding survival of 84.20±0.66% obtained for larvae cultured on the 100% brine solution (B_{100}) was the highest, but not significantly different ($P<0.05$) to that of 24.49±0.71 Pl/ℓ with a corresponding survival rate of 81.68±2.36% for larvae cultured on 75% brine plus 25% crude salt solution ($B_{75}CS_{25}$). In contrast, the treatment 50% brine plus 50% crude salt solution ($B_{50}CS_{50}$) resulted in significantly lower ($P<0.05$) production of 22.10±0.57 Pl/ℓ. The results indicate that 25% of brine water could be replaced with crude salt solution without any undue reduction than in production rate of post-larvae with brine alone.

Table 3. Production of *M. rosenbergii* post-larvae reared on different combinations of brine and crude salt solution for a period of 33 days

Treat-ments	Production of post-larvae (mean±SD)[1]						
	Larvae stocked		Total post-larvae	Post-larvae/ℓ	% Survival	Growth of post-larvae	
	Total	Per liter				Length (mm)	Weight (mg)
B_{100}	9000	30	7578±59.02[a]	25.26±0.20[a]	84.20±0.66[a]	9.39±0.52[a]	8.84±0.49[a]
$B_{75}CS_{25}$	9000	30	7351±212.78[a]	24.49±0.71[a]	81.68±2.36[a]	9.32±0.42[a]	8.40±0.39[a]
$B_{50}CS_{50}$	9000	30	6630±170.23[b]	22.10±0.57[b]	73.68±1.89[b]	8.84±0.48[a]	8.20±0.43[a]
			F=26.19	*F=26.26*	*F=26.16*	*F=0.25*	*F=7.29*

[1]Values not sharing common superscript letter are significantly different ($P<0.05$).

The development of the larvae, expressed as the mean larval stage (MLS) is presented in Table 4. The MLSs were similar ($P<0.05$) with B_{100}, $B_{75}CS_{25}$, $B_{50}CS_{50}$ up to the day 6 of the rearing period, showing mean larval stages of 3.37±0.03, 3.23±0.15 and

Table 4. Mean larval stages of *M. rosenbergii* larvae under different combinations of brine and crude salt solutions[1]

Treatments		Mean larval stages													
		Elapsed days													
		2	3	4	5	6	7	9	11	13	16	19	22	25	28
B_{100}	Mean	1.57^{b}	2.13^{a}	2.48^{a}	2.97^{a}	3.37^{a}	3.93^{c}	4.60^{b}	5.42^{b}	6.33^{b}	7.41^{b}	9.00^{b}	10.17^{a}	11.22^{a}	11.77^{a}
	SD	0.06	0.06	0.10	0.03	0.03	0.15	0.20	0.19	0.15	0.09	0.10	0.21	0.04	0.06
$B_{75}CS_{25}$	Mean	1.50^{ab}	2.08^{a}	2.47^{a}	2.83^{a}	3.23^{a}	3.70^{b}	4.40^{b}	5.33^{b}	6.33^{b}	7.37^{b}	8.97^{b}	10.07^{a}	11.07^{a}	11.70^{a}
	SD	0.05	0.10	0.12	0.12	0.15	0.10	0.10	0.06	0.15	0.25	0.15	0.12	0.15	0.10
$B_{50}CS_{50}$	Mean	1.40^{a}	2.05^{a}	2.40^{a}	2.87^{a}	3.30^{a}	3.47^{a}	4.20^{a}	5.00^{a}	6.03^{a}	7.03^{a}	8.57^{a}	9.93^{a}	10.93^{a}	11.63^{a}
	SD	0.10	0.13	0.07	0.25	0.17	0.02	0.10	0.17	0.06	0.06	0.15	0.12	0.15	0.12

[1]Mean values in each column not sharing a common superscript letter are significantly different ($P<0.05$).

3.30±0.17, respectively. From the day 7 to day 19, the MLS values were significantly lower with the treatment $B_{50}CS_{50}$, but afterwards those were similar ($P<0.05$) ranging from 10.17 to 11.77, 10.07 to 11.70, 9.93 to 11.63 for the treatments B_{100}, $B_{75}CS_{25}$, and $B_{50}CS_{50}$, respectively. The overall MLS data indicate that the development of larvae reared in 100% of brine (B_{100}) and in 75% of brine and 25% of crude salt ($B_{75}CS_{25}$) was apparently faster throughout the experimental period than that of larvae reared in 50:50 parts of brine and crude salt ($B_{50}CS_{50}$). On the 7th day of hatching, the treatment $B_{50}CS_{50}$ resulted in the highest of 60% larvae of stage III and the lowest of 5% of stage V (Fig. 1). Similarly, on the 16th day, the treatment $B_{50}CS_{50}$ also resulted in the highest percent composition of larvae at stages VI and VII, but the lowest of that at stage VIII. However, on that 25th day of hatching, the percent composition of stages in larvae reared in 50:50 brine and crude salt solution was quite comparable with that of larvae reared in other two test media (Fig. 1).

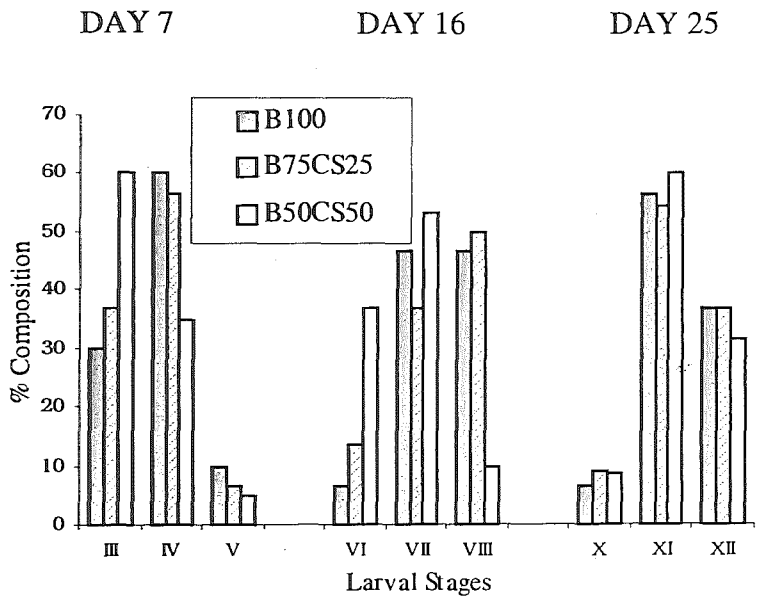

Fig. 1. Percent composition of *M. rosenbergii* larval stages on day 7, 16 and 25 post-hatch under different combinations of brine and salt solutions.

Discussion

A preliminary experiment was conducted to evaluate the possibilities of using (i) 100% crude salt (CS_{100}), (ii) 75% crude salt and 25% brine ($CS_{75}B_{25}$), and (iii) 50% crude salt and 50% brine ($CS_{50}B_{50}$) in rearing of *M. rosenbergii* larvae. The larval rearing technique was similar, as followed in the present experiment. The percentage survival rates of larvae to post-larvae under different treatments used in the preliminary study are given in Table 5. No larvae in 100% crude salt metamorphosed to post-larvae. They started to die from the day 5, the mortality rate increased gradually and all were died at

day 12. The larvae were found to stop taking food, become whitish, and to aggregate at the bottom of the tank from the early days of rearing period. With the culture medium of 75% crude salt and 25% brine, only 7.05% of stocked larvae metamorphosed to post-larvae. However, 50% crude salt and 50% brine resulted in a quite significant rate of survival (57.85%) of larvae to post-larvae. The result of the preliminary study indicated that neither the 100% crude salt water nor its 25% of replacement with brine could be used in hatchery production of *M. rosenbergii* post-larvae. A similar opinion was made by Yambot and Vera Cruz (1986), though they reported the survival rate of *M. rosenbergii* post-larvae ranging from 2.88% to 11.48% with an average of 6.71%, while they used a combination of sea salt, deionized freshwater and *Chlorella* culture (greenwater). Probably this percentage of survival rate was obtained due to an advantageous use of greenwater in larviculture (Cheah and Ang 1979). Based on the results of the preliminary study, the present experiment was designed, keeping in mind that 50% of brine could be replaced with crude salt solution in *M. rosenbergii* hatchery operation.

Table 5. Percentage survival of *M. rosenbergii* from larvae to post-larvae in the preliminary trial under different combinations of crude salt and brine solution

Treatments	Survival (%)			Treatment mean
	Replication-1	Replication-2	Replication-3	(%)
CS_{100}	-	-	-	-
$CS_{75}B_{25}$	4.37	10.65	6.14	7.05
$CS_{50}B_{50}$	43.58	71.13	58.83	57.85

The temperature, pH and NH_3-N varied from 26 to 33°C, 7.8 to 8.6 and 0.06 to 0.12 mg/ℓ, respectively and were similar in all larval rearing tank throughout the experimental period. Water quality parameter levels were within the optimum range for rearing of *M. rosenbergii* larvae in captivity (New and Singholka 1985, Ang and Cheah 1986). The production data of *M. rosenbergii* larvae obtained under different combinations of brine and crude salt (Table 3) reveal that 25% of brine solution could be replaced with crude salt solution without any undue reduction in per unit yield of *M. rosenbergii* post-larvae. Though the average production of 22.10±0.57 Pl/ℓ with 50% replacement of brine (T-III) was significantly lower, but higher than that of 11.93 Pl/ℓ (Islam and Khan 1990), 10.22 Pl/ℓ (Adisukresno *et al.* 1982), 9.5 – 18.9 Pl/ℓ (Lee, 1982), 7.56 Pl/ℓ (Yambot and Vera Cruz 1986), while the authors used seawater either in static or in closed recirculatory system. Pramanik and Halder (1996), who used 100% of brine solution in a closed recirculatory backyard hatchery system, reported a production rate of 25 Pl/ℓ at a stocking density of 40 larvae/ℓ. This production rate is comparable with 22.10±0.57 – 25.26±0.20 Pl/ℓ obtained in the present experiment with either 100% brine or with different combinations of brine and crude salt solution (Table 3). It indicates that the either brine alone or up to 50% replacement of brine with crude salt solution can be used as suitable as of using seawater in rearing of *M. rosenbergii* larvae.

The results of our preliminary study and that of Yambot and Vera Cruz (1986) indicate that the sea salt alone cannot be used in rearing of *M. rosenbergii* larvae up to post-larvae. Similar to that observed in the preliminary study, Yambot and Vera Cruz (1986) recorded a substantial mortality of larvae within five days after stocking. Though the authors suspected improper acclimatization of larvae prior to stocking as one cause of larval mortalities with sea salt alone, but this might not be so. It is presumed that as *M. rosenbergii* is apparently evolving "out of the sea" (Johnson 1960), as it requires a salinity of 10-14‰ in closing the larval cycle (Ling 1962) . Therefore, it is plausible that the complex nutrient and mineral constituents of the natural seawater regulate the physiological growth and survival of larvae. The ions that make up the salt content of natural seawater are shown in Table 6.

Table 6. Constituents of seawater (after Ingmanson and Wallace 1985)

Substrate	Symbol	‰ seawater	% total weight of salt
Chloride	Cl^-	18.980	55.04
Sodium	Na^+	10.556	30.61
Sulphate	SO_4^{-2}	2.649	7.68
Magnesium	Mg^{+2}	1.272	3.69
Calcium	Ca^{+2}	0.400	1.16
Potassium	K^+	0.380	1.10
Bicarbonate	HCO_3^-	0.140	0.41
Bromide	Br^-,	0.065	0.19
Boric acid	H_3BO_3	0.026	0.07
Stornium	Sr^{+2}	0.013	0.04
Fluride	F^-	0.001	0.00
Total		34.482‰	99.99%

The salt in seawater is not the same as in regular table salt. When table salt (NaCl) dissolved in water, it breaks up into Na^+ and Cl^- ions with an equal amounts of positive and negative ions (Ingmanson and Wallace 1985). Therefore, most of the ions except Cl^- and Na^+ are lost in the process of preparation salt from seawater. This non-presence of a number trace minerals in brackishwater reconstituted by salt might be a cause of not supporting the normal survival and growth of *M. rosenbergii* larvae. The production data of prawn post-larvae in the present experiment of supplementing salt made culture media with 50% of brine (naturally concentrated seawater) further prove that the ionic constituents of natural seawater might have significant triggering effects on growth and survival of *M. rosenbergii* larvae.

It is interesting to note that in the early rearing period, with a mean larval stage of 3.23 – 3.37 at day 6, the growth of larvae were not significantly affected by 50% supplement of salt media with brine (Table 4). However, from day 7 to 19, the larvae reared on $CS_{50}B_{50}$ showed significantly lower development in terms of MLSs (Table 4). Fig. 1 shows that on day 7 and 16, the highest number of larvae were at stage III and VII, respectively. The progressive larval development (Table 3) and occurrence of larvae at

different stages (Fig. 1) towards the end of the rearing period for the treatment $CS_{50}B_{50}$ are more or less similar to that for treatments B_{100} and $B_{75}CS_{25}$. These results amply demonstrate that the minerals of natural seawater not only might have some effect on growth of larvae but are also required particularly at the mid-stages of larval development.

The overall results of the study supports the findings of Yamboot and Vera Cruz (1986) and Pramanik and Halder (1996) that brine can effectively be used in rearing of *M. rosenbergii* larvae. There is also the possibility of using brackishwater reconstituted from crude salt for more economically rearing of prawn larvae. However, the crude salt media need to be replaced or supplemented by at least 50% with brine for any undue reduction in production of freshwater prawn post-larvae. The stocking density of prawn larvae in the present culture condition is also a factor to be taken into consideration. With an increasing stocking density of 40 - 60 larvae/ℓ, Pramanik and Halder (1996) reported a decreased survival rate of 63% to 47%. As a stocking density of 30/ℓ in the present experiment resulted in the survival rate of 74% to 84%, this stocking rate can be used for better production of post-larvae.

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References

Adisukresno, S., G.L. Escritor and K. Mintardio, 1982. Mass production of *Macrobrachium* post larvae in the Brackishwater Aquaculture Development Centre, Japra, Indonesia. *In*: Giant Prawn Farming (ed. M.B. New). Elsvier, New York. pp. 143-156.

Alam, M.J., S.H. Cheah and K.J. Ang, 1991. Use of coloured egg custard for larval rearing of the giant freshwater prawn *Macrobrachium rosenbergii* (de Man). *Bangladesh J. Fish.*, 13(1&2): 63-72.

Ang, K.J. and S.H. Cheah, 1986. Juvenile production of the Malaysian giant freshwater prawn (*Macrobrachium rosenbergii* de Man) using modified static modified greenwater system. Proc. Int. Conf. Dev. Manage. Trop. Living. Aquat. Resources, Serdang, Malaysia, 2-5 Aug. 1983. UPM Publ., Serdang, pp. 141-144.

Cheah, S.H. and K.J. Ang, 1979. Preliminary trials on juvenile *Macrobrachium rosenbergii* production under modified static "green water" conditions. *Pertinika*, 36: 69-71.

Ingmanson, E. and W.J. Wallace, 1985. *Oceanography: An Introduction.* Wordsworth Publishing Company, California.

Islam, K.S. and Y.S.A. Khan, 1990. Mass production of post larvae of *Macrobrachium rosenbergii* (de Man) at the Prawn Hatchery and Research Centre, Cox's Bazar, Bangladesh. *Bangladesh J. Zool.*, 18(1): 53-59.

Johnson, D.S., 1960. Some aspects of the distribution of freshwater organisms in the Indo-Pacific area and their relevance to the validity of the concept of an oriental region in zoogeography. *Proc. Cent. Bicent. Congr. Biol.*, Singapore, 2-9 Dec., 1958. Singapore, pp. 170-181.

Lee, C.L., 1982. Progress in developing standardized system for producing of juvenile *Macrobrachium rosenbergii* (de Man) at Mardi, Malacca. *In*: Giant Prawn Farming (ed. M.B. New). Elsvier, New York, NY, pp. 129-142.

Ling, S.W., 1962. Studies on the rearing of larvae and juveniles and culturing adults of *Macrobrachium rosenbergii* (de Man). *FAO/IPFC Curr. Aff. Bull.*, 35: 1-11.

Ling, S.W., 1969. Methods of rearing and culturing *Macrobrachium rosenbergii* (de Man). *FAO Fish. Rep.*, 57(3): 607-619.

Lovett, D.L. and D.L. Felder, 1988. Evaluation of the rotofer *Brachionus plicatilis* as a substitute for *Artemia* in feeding of larvae of *Macrobrachium rosenbergii* (de Man). *Aquaculture*, 71: 331-338.

New, M.B. and S. Shingholka, 1985. Freshwater Prawn Farming. A manual for the culture of *Macrobrachium rosenbergii*. *FAO Fish. Tech. Pap.*, 1/FIRI/T225, 118pp.

Pramanik, M.W.A. and G.C. Halder, 1996. Development of backyard *Macrobrachium* hatchery system and larval rearing technique in different stocking density. *J. Inland Fish. Soc. India*, 28(1): 21-27.

SAS, 1985. SAS/STAT Guide to Personal Computers Version 6, SAS Inc., Cary, NC, USA.

Uno, Y and C.S. Kwon, 1969. Larval development of *Macrobrachium rosenbergii* (de Man) reared in the laboratory. *J. Tokyo Univ. Fish.*, 55(2): 179-190.

Yambot, A.V. and E.M. Vera Cruz, 1986. Larval rearing of *Macrobrachium rosenbergii* (de Man) in brine solution and sea salt. *In*: The First Asian Fish. Forum (eds. J.L. Maclean, L.B. Dizon and L.V. Hosillos). Asian Fisheries Society, Manila, Philippines. pp, 185-188.

Zar, J.H., 1984. Biostatistical Analysis, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ, 717 pp.

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