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Effects of different protein sources in the broodstock diet on reproductive performance of giant freshwater prawn (*Macrobrachium rosenbergii*)

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Abstract: The experiment was designed to study the effects of different protein sources on reproductive performance and offspring quality of *Macrobrachium rosenbergii* in a 180-day feeding trial. Each tank was stocked with four females and one male (wet weight: 21-35g). Five isonitrogeneous (40%) and isolipidic (10%) diets were formulated to contain either fishmeal (FM), shrimp meal (SHM), squid meal (SQM), bivalve meal (BVM) or a mixture of SHM, SQM and BVM (MIX). The prawns were fed at 3% of body weight, three times daily (0800h, 1200h and 1700h). Prawn fed with diet MIX attained the highest fecundity (1449.52±64.15 egg/g female) followed by BVM (1308.53±40.41 egg/g female), SQM (1085.73±62.92 egg/g female), FM (924.84±67.75 egg/g female) and SHM (875.74±28.14 egg/g female). The fecundity of prawn fed diets MIX and BVM was significantly higher (P<0.05) than those fed SQM, FM and SHM diets. The largest egg diameter was also found in MIX diet (0.567±0.005mm) and this value was significantly larger (P<0.05) than the FM diets. In general, survival rate of larvae in all diets is very high ranging from 95.06% to 99.71%. The present study revealed that formulated diet based on shrimp, squid and bivalve meal mixture can be successfully used in the breeding program of *M. rosenbergii*.

Key Word: protein, reproductive performance, *Macrobrachium rosenbergii*

Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) is an important aquaculture species in Asia because of its high commercial value, good meat quality and taste, adaptability in captive conditions and tolerance to wide range of salinity and temperature (Gupta *et al.,* 2007). The global production of giant freshwater prawn, M. rosenbergii was estimated at 229 419 tonnes in 2009 with China, Thailand and Bangladesh being the main producers. Production in Malaysia is relatively small compared to other Asian countries but expanded significantly since 2006 (New and Nair 2012).

Giant freshwater prawn culture is dependent on wild-caught broodstock, making it susceptible to a variable quality of seed. Broodstock diet plays a major role in influencing the success of hatchery production by affecting the reproductive performance (egg production, fertilization rate, egg quality, embryo development) and offspring quality (Millamena and Quinitio, 2000). In most cases, hatchery managers are dependent on fresh or frozen food to culture the broodstock with trash fish, squid, clam, muscle, shrimp and marine polychaete worms being commonly used. Unfortunately, fresh food are commonly associated with unreliable supply and fluctuated price, inconsistent nutritional value, need to be frozen, and can easily deteriorate water quality and increased risk for contamination (Wouters *et al.,* 2002). The use of formulated feeds in the broodstock stage has some advantages such as to provide reliable supply of feed with known nutrient content and minimize preparation time (Marsden *et al.,* 1997), ease of feeding management and reduce the risk of contamination (Braga *et al.,* 2010).

Little studies were done to evaluate the performance of formulated broodstock diets in *M. rosenbergii.* Fortunately, major nutrients requirement such as protein and lipid have been determined in this species (Cavalli *et al.,* 2000; Das *et al.,* 1996), making it possible to formulate a species-specific feed. In the present study, the effects of different protein sources in the formulated broodstock diets on fecundity performance and larvae quality of the prawn were evaluated.

Materials and methods

Experimental Broodstock

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The experiment was conducted at the Shrimp Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS) for 180 days. The giant freshwater prawns were purchased from a local freshwater prawn supplier with the size range of 21-35g. During this acclimatization period, prawns were acclimated to hatchery condition for 4 weeks prior to the start of the experiment. The prawns were fed commercial marine prawn feed (King Seahorse; 42% crude protein, 4% crude lipid) at 3% of body weight, three times daily (0800h, 1200h and 1700h).

Experimental diets

Fresh squid, shrimp and bivalve were purchased from the local fish market, Kota Kinabalu, Sabah. Preparation of meal was done at the Aquaculture Feed Lab of Borneo Marine Research Institute, UMS. The fresh samples were chopped into smaller portions and oven dried at 50 °C for 24 hours before they were ground into powder form using a mill grinder. The meal was packed in zip-lock plastic bag and stored in a freezer at -20°C until use. Fish meal protein was replaced with shrimp meal (SHM), squid meal (SQM), bivalve meal (BVM) and mixture of SHM, SQM and BVM (MIX) meal at 23% replacement level to contain 40% crude protein and 10% crude lipid. Fish-meal based diet was used as the control diet (FM) (Tab. 1).

Tab.1: Proximate composition of ingredients and diets (dry matter basis); and ingredient composition of experimental diets (g/100g dry weight).

Ingredients	Protein (%)	Lipid (%)	Fibre (%)	Ash (%)	Moisture (%)
FM	72.58	7.99	ND*	15.34	8.65
SHM	69.46	3.95	ND*	17.16	11.81
SQM	78.32	6.44	ND*	9.11	8.61
BVM	73.39	11.14	ND*	11.13	13.12
Ingredients	**FM**	**SHM**	**SQM**	**BVM**	**MIX**
Fish meal [1]	48.22	16.53	16.53	16.53	-
Soybean meal[2]	9.81	9.81	9.81	9.81	9.81
Shrimp meal[3]	-	33.11	-	-	16.80
Squid meal[4]	-	-	29.37	-	14.90
Bivalve meal[5]	-	-	-	31.34	15.90
Tapioca starch[6]	24.97	22.32	26.65	26.28	25.13
Fish oil[7]	6.0	7.2	6.6	5.0	6.5
Vitamin premix[8]	3.0	3.0	3.0	3.0	3.0
Mineral premix[9]	2.0	2.0	2.0	2.0	2.0
CMC[10]	6.0	6.0	6.0	6.0	6.0
Diets	**FM**	**SHM**	**SQM**	**BVM**	**MIX**
Protein	41.88±0.02	41.66±0.00	40.94±0.05	40.65±0.01	41.77±0.02
Lipid	8.84±0.09	8.36±0.05	9.31±0.01	8.79±0.00	8.61±0.01
moisture	8.74±0.03	10.91±0.00	8.95±0.02	8.21±0.04	10.58±0.01
Ash	13.95±0.02	12.85±0.04	10.15±0.01	10.46±0.01	9.47±0.05
Fibre	1.59±0.12	3.66±0.05	0.92±0.13	1.65±0.10	2.99±0.05
NFE	33.74	33.47	38.68	38.45	37.16
Gross energy (kcal/100g)	371.94	368.65	380.68	377.43	377.44

*NFE= 100- (protein+lipid+fibre+ash)

*FM= Fish meal, SHM= shrimp meal, SQM=squid meal, BVM=bivalve meal, MIX= squid meal, shrimp meal and bivalve meal, *ND=Not determined

[1] TripleNine fish meal, Denmark

[2]Defatted soybean, China

[3, 4, 5] Laboratory made

[6]AAA Brand, Bake with Me Sdn. Bhd., Malaysia

[7]Tuna oil, commercial oil

[8]Vitamin premix. Contained (as g/kg): ascorbic acid, 300; inositol, 125; niacin, 50; riboflavin, 15;pyridoxine. 12; thiamin mononitrite, 15; retinyl acetate, 1.72; cholecalciferol, 0.025; menadione sodium bisulphite, 5;biotin, 0.5; folic acid, 2.5; DL-α-tocopheryl acetate, 50; vitamin B12, 0.025; calpan, 25. Dexchem Industries Sdn. Bhd, Malaysia

[9]Mineral premix. Contained (as g/kg): calcium phosphate.H2O (MDCP), 397.65; calcium lactate, 327; ferrous sulphate.H2O, 25; magnesium sulphate.7H2O, 137; potassium chloride, 50; sodium chloride 60; potassium iodide, 0.15; copper sulphate.5H_2O, 0.785; manganese oxide, 0.8; cobalt carbonate, 0.1; zinc oxide, 1.5; sodium selenite.5H_20, 0.02. Dexchem Industries Sdn. Bhd, Malaysia

[10] Carboxymethyl cellulose (Calbiochem, USA)

Raw materials with size of more than 500µ were ground and sieved. Then all the dry ingredients were mixed with fish oil for 10 minutes. Tapioca starch was cooked with water until transparent colour was obtained and added into the mixture to form moist dough. Finally the dough was placed in a pellet machine with a die size of 2 mm. The strand of pellet was dried in an oven for 4 hours at 45°C and later was broken into smaller particle sizes (2 cm). Feed were packed and labelled inside zip-lock plastic bags and kept in a freezer (-20°C) until use. Proximate composition of diets was determined according to AOAC (1997) in triplicates.

Experimental design

The experiment was conducted in a completely randomized design. The female and male broodstock were stoked in triplicate groups at density of 5 prawns (1 male and 4 females) per tank (105x105 x36cm;400L). The prawns were fed at a rate of 3% of body weight, three times daily (0800h, 1200h and 1700h) during the feeding trial. Uneaten feed and exoskeleton were removed from the tank before a new feeding being introduced. Each tank was provided with white PVC pipes (diameter =8 cm length =23 cm) as a shelter to minimize cannibalism during molting process.

Water was exchanged at a rate of 40% in the morning. At the same time, mortality and number of molting prawn were recorded. Water quality (water temperature, pH and dissolved oxygen) was monitored daily during the experimental period.

Fecundity

Maturation stage was identified according to the size of the ovary and its colour was observed through the carapace. Egg was removed manually from abdomen of each female using forceps. Egg clutch and female weight were determined separately. Egg clutch was weighed to the nearest 0.01g after excess water had been removed by repeated blotting using filter paper. The total number and size of eggs (n=30) was measured under a stereo microscope (Olympus, Japan). Fecundity was estimated as the number of eggs per female weight (No of egg/g female).

Larval quality

At 24 hours after hatching, prawns were removed from the tank and larvae were collected and transferred into larval rearing tank (49x49x31cm). The total length of thirty larvae was measured under a stereo microscope at the beginning and end of the trial. Larvae were randomly transferred to larval tank at stocking density of 50 larvae/ litre with a total density of 3000 larvae/tank. Water salinity in the larval rearing tank was prepared at 12 ppt. Feeding commenced from day 2 by providing Artemia nauplii twice daily (0800 and 1600hr). Larvae development in each treatment was estimated using larval stage index (LSI) according to Uno and Kwon (1969). The survival rate of larvae was determined at day 8.

Statistical analysis

The data were analyzed using SPSS v 18.0 with one way analysis of variance (ANOVA) and Tukey post-hoc test ($p<0.05$) to compare fecundity, eggs diameter, survival rate and larvae size. The results are presented as mean±S.E of the triplicate experimental diets.

Result

Table 1 shows the proximate composition of ingredients and diet composition. Squid meal contained the highest level of protein (78.32%), followed by bivalve meal (73.39%), fish meal (72.58%) and shrimp meal (69.46%), while bivalve meal contained the highest amount of lipid (11.14%), followed by fish meal (7.99%), squid meal (6.44%) and shrimp meal (3.95%). Higher ash levels were observed in the shrimp meal (17.16%) and fish meal (15.34%), mostly contributed by the exoskeleton of the shrimp and fish scales. The analysed crude protein values (40.65-41.88%) for all experimental diets correspond to the calculated value (Tab. 1). Meanwhile, the lipid contents were slightly lower than the calculated value (8.36 to 9.31%). The crude fibre and ash values were in the range of 0.92-3.66% and 9.47-13.95%, respectively. The calculated energy value of experimental diets ranged from 368.65 to 380.68 kcal/100g.

Table 2 shows the number of spawning for all treatments. The highest occurrence (%) of spawning was found in MIX (100±0.00%) diet while the lowest percentage of spawning was found in SQM (66.67±8.33%) diet.

Prawn broodstock fed with diet MIX attained the highest fecundity of 1449.52±64.15 egg per gram body weight followed by diet BVM (1308.53±40.41) egg per gram body weight), SQM (1085.73±62.92 egg per gram body weight), FM (924.84±67.75 egg per gram body weight) and SHM (875.74±28.14 egg per

Tab.2: Effect of diet containing different source of protein on number of spawning.

Treatments	% of spawning occurrence
FM	91.67±8.33
SHM	75.00±14.43
SQM	66.67±8.33
BVM	83.33±8.33
MIX	100.00±0.00

gram body weight). The largest egg diameter was found in MIX (0.567±0.005mm) diet but with no significant difference from BVM (0.564±0.005mm), SQM (0.555±0.004mm) and SHM (0.554±0.008mm) diets. The smallest egg diameter was found in FM (0.522±0.012mm) diet (Tab. 3). Figure 1 shows relationship between body weight of female with mass of egg. In this trial, there was no relationship between spawner size and production of egg.

Tab.3: Effects of diet containing different source of protein on fecundity and egg diameter of *M. rosenbergii*.

Protein sources	Fecundity (egg/g female)	Egg diameter (mm)
FM	924.84±67.75^{a}	0.522±0.012^{a}
SHM	875.74±28.14^{a}	0.554±0.008^{b}
SQM	1085.73±62.92^{a}	0.555±0.004^{b}
BVM	1308.53±40.41^{b}	0.564±0.005^{b}
MIX	1449.52±64.15^{b}	0.567±0.005^{b}

(FM= Fish meal, SHM= shrimp meal, SQM=squid meal, BVM=bivalve meal, MIX= squid meal, shrimp meal and bivalve meal)

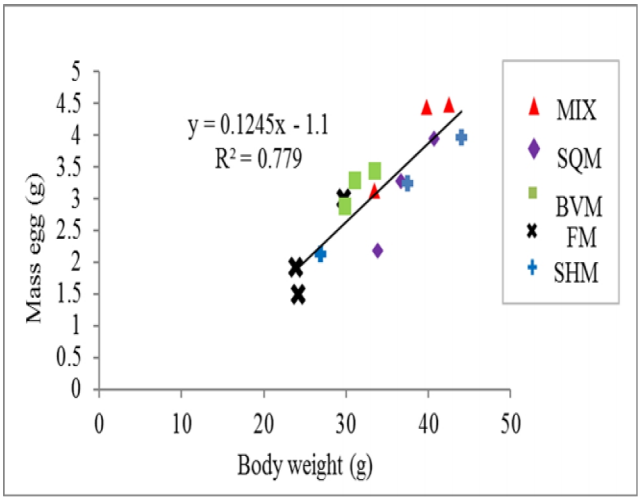

Fig.1: Relationship between female body weight and mass of egg.

The larval stage indices (LSI) of *M. rosenbergii* larvae from different treatments are presented in Table 4. Larvae development from stage 1 to stage 5 took 8 days to complete. There exists not significant difference in larvae developmental stage ($p>0.05$) from Day 1 to Day 8 in all dietary treatments (Tab. 5). Table 6 shows that the water quality parameters were relatively constant during the experimental period. In this trial, water temperature, salinity, dissolved oxygen and pH of larval culture were maintained at 28.81-29.19°C, 11.43-11.55 ppt, >5mg/L and 7.74-7.91, respectively.

Tab.4: Effect of different diet containing different source of protein on larval development of *M. rosenbergii*.

Diet	Initial larvae size (mm)	Larvae size at Day 8 (mm)	Survival rate (%)
FM	2.31±0.04^{a}	3.00±0.15^{a}	97.29±1.20^{ab}
SHM	2.36±0.07^{ab}	3.08±0.05^{a}	97.39±0.97^{ab}
SQM	2.47±0.04^{ab}	3.24±0.11^{a}	95.06±1.07^{a}
BVM	2.52±0.03^{b}	3.25±0.06^{a}	99.52±0.19^{b}
MIX	2.52±0.03^{b}	3.32±0.03^{a}	99.71±0.06^{b}

Tab.5: Larvae stage index from Day 1 to Day 8 of *M. rosenbergii* from different treatments.

Day	FM	SHM	SQM	BVM	MIX
1	1.0±0.00	1.0±0.00	1.0±0.00	1.0±0.00	1.0±0.00
2	1.9±0.00	1.9±0.00	1.9±0.00	1.9±0.00	1.9±0.00
3	2.8±0.1	2.9±0.00	3.0±0.00	2.9±0.00	3.0±0.00
4	3.7±0.1	3.8±0.00	3.8±0.1	3.8±0.00	3.9±0.00
5	4.7±0.00	4.7±0.00	4.7±0.00	4.8±0.00	4.8±0.00
6	4.8±0.00	4.8±0.00	4.8±0.00	4.8±0.00	4.8±0.00
7	5.0±0.00	4.9±0.00	4.9±0.00	5.0±0.00	5.0±0.00
8	5.0±0.00	5.0±0.00	5.0±0.00	5.0±0.00	5.0±0.00

Discussion

Squid, bivalves (mussels, oyster and clams) and polychaetes are the most common feeds used in the maturation diet of cultured prawn. Different combinations of natural marine organism generally produce high reproductive performance in wild *Panaeus monodon* broodstock (Coman *et al.,* 2007). In captivity, optimal and stable environmental conditions and a more balanced and constant nutrition in culture conditions may also play a significant role (Andriantahina *et al.,* 2012). In addition, maturation diets fed to the broodstock immediately prior to spawning has been shown to greatly affect fertilization and hatching of the egg (Coman *et al.,* 2007). Similarly, the present study demonstrated the significant influence of maturation diets can be having on egg fertilization and hatching in *M. rosenbergii.* Partial and complete replacement of fresh food with

formulated diets has been reported in *Penaeus monodon*, *Litopenaeus vannamei* and *Scylla serrata* (Millamena *et al.,* 1986; Marsden *et al.,* 1997; Wouters *et al.,* 2002; Millamena and Quinitio, 2000).

Tab.6: Water quality parameters in the broodstock and larval culture tanks.

Water quality parameter in broodstock tanks			
Diets	Temp (°C)	pH	DO (mg/L)
FM	28.34±0.34	7.77±0.31	5.75±0.06
SHM	28.23±0.16	7.88±0.28	5.72±0.05
SQM	28.39±0.09	7.69±0.29	5.74±0.17
BVM	28.15±0.09	7.39±0.06	6.14±0.12
MIX	28.59±0.18	7.82±0.28	5.94±0.10

Water quality parameter in larval tanks				
Diets	Temp (°C)	pH	DO (mg/L)	Salinity (ppt)
FM	28.95±0.10	7.74±0.13	5.89±0.10	11.55±0.07
SHM	29.05±0.24	7.91±0.14	5.94±0.02	11.43±0.02
SQM	29.19±0.11	7.84±0.05	5.89±0.13	11.43±0.02
BVM	29.12±0.08	7.91±0.05	5.89±0.09	11.43±0.08
MIX	28.81±0.31	7.89±0.09	5.90±0.08	11.55±0.08

Shrimp, squid, bivalve meal and the mixture of these meal used in the broodstock diets demonstrated that they can support the maturation of *M. rosenbergii* in captivity. Nevertheless, fecundity of prawn fed the control diet (fish meal-based) and diet SHM was slightly lower than in other treatments, maybe due to the differences in nutritional composition of the meal. In the present study, the fish and shrimp meal were characterized by high ash content derived from the fish scales and shrimp exoskeleton. High chitin level in shrimp meal may not be well digested by the target animal (Jaime-Ceballos *et al.,* 2009). On the positive note, shrimp meal consisting of the heads, appendages and exoskeleton is particularly rich in lysine (Fanimo *et al.,* 2000). In the previous studies, prawn head were good sources of fatty acids and pigments for M. malcolmsonii and acts as a chemoattractant (Izquierdo *et al*., 2001; Miniadis-meimaroglou and Sinanoglou, 2012; Samuel *et al.,* 2001). It was also reported that the inclusion of SHM in diets for juvenile *P. monodon* had significantly improved palatability compared with the fish meal-based diet (Jaime-Ceballos *et al.,* 2009).

Squid meal was reported to have high protein digestibility, enhancing effect on the absorption of nutrients, stimulating effect on digestive enzymes, beneficial effects on gonadal development, egg quality, larval survival and growth rate (Rodriguez-viera and Perera, 2012). Squid is an excellent source of cholesterol which is an essential dietary nutrient for shrimp growth and successful reproductive performance (Chimsung, 2014). In the previous study, squid meal inclusion in the diet had improved the larval growth of *P. vannamei* and also enhanced the trypsin activity in adult shrimp (Cordova-murueta and Garcia-Carreno, 2002). In a study by Hoa *et al.,* (2009), a combination of oyster, pork liver, squid and marine worm in the formulated diets for *P. monodon* (diet was formulated to have the similar ARA/EPA and DHA/EPA ratios to that of mature *P. monodon* ovaries) was able to enhance the growth and fecundity of the shrimp. The hard clam was reported to contain high amount of amino acid, MUFA and PUFA (n-3) as reported by Karnjanapratum *et al.,* (2013). In addition, molluscs were also reported to contain chemo-attractant properties (Chimsung, 2014; Shyla *et al.,* 2009). Among the free amino acid, alanine, glycine and tourine are most abundant in marine mollusc, polychaetes, crustacean and other marine invertebrate (Kube *et al.,* 2007).

In the present study, the relationship between spawner size and production of egg was not observed. Similarly, no relationship in spawner size and production of egg in *L. vannamei* (43-56g) were observed although some biochemical variables in egg and nauplii were negatively correlated to spawner size (Racotta *et al.,* 2003). In contrast, bigger size of wild female of *P. paulensis* produced lower spawning frequency, fecundity, fertilization and hatching rate compare to the smaller females (Cavalli *et al.,* 1997).

Fecundity refers to the number of egg produced by a female animal. Generally, larger female produce more egg than do smaller females. However, brooders are chosen not on the basis of size, but on the availability and readiness to spawn (Balamurugan *et al.,* 2015). In a hatchery condition, the production of smaller female was more efficient in term of egg production per body weight because the production of egg per unit time is crucial (Habashy, 2013). Fecundity of *M. rosenbergii* varies considerably with the different conditions of female maintenance in laboratory, physiological condition, season, age, size, and stage maturity (Alam and Alam, 2014; Habashy, 2013; Oniam *et al.,* 2012). Size is closely related to the age of the spawners, but the size of same-age population can vary in relation to grow out or site conditions. Age has also been reported to influence

reproductive performance and offspring quality (Racotta *et al.,* 2003). In *P. monodon*, male with relatively young age seem likely to have caused the poor sperm quality and consequently the hatching rate (Menasveta *et al.,* 1993).

The egg production in the present study was not significantly affected by the size of female broodstock. According to Cavalli *et al.* (2001), some wild population of *M. rosenbergii* from Sri Lanka and India have produced a higher number of eggs per unit body weight of smaller females. A similar trend has also been demonstrated in *P. monodon* (Villegas *et al.,* 1986). In contrast, some researchers found that the number of eggs per body weight increased with female size. Egg size appears to be species-specific among decapods. The size of egg correlates with stage of development and serves as an indicator of energy content. Generally species with larger size egg contain more yolk nutrient and their embryonic development time is longer (Habashy *et al.,* 2012; Jun-jie *et al.,* 2006). Smaller eggs tend to have poorer survival rate (Das *et al.,* 1996).

The egg of *M. rosenbergii* are slightly elliptical in shape and initially bright orange to yellow in colour, then the colour is gradually changed to deep brown in a few days before hatching. Environmental temperature was the main factor affecting the development of M. rosenbergii embryos. In this study, the egg of broodstock hatched out after 18 until 20 days of incubation at 28-29 °C. In other study by Habashy *et al.* (2012), the egg of *M. rosenbergii* hatched out after 25 days at 26 °C, 20 days at 28 °C and 17 days at 32 °C which clearly showed the influence of temperature in hatching process.

The experimental diets used in the present study not only had some effect on fecundity but also showing some influence on the survival rate of the early larvae stage. The larval survival rate of prawn fed MIX and BVM diets increased significantly compared with other diets. Nevertheless, larval development and survival may also be affected by many other factors such as broodstock quality, the experimental system used and weather condition during the experiment (Shailender *et al.,* 2012). The water quality parameters in the present study were within the acceptable range for the culture of giant freshwater prawn. In addition, adult prawns were also reported to be tolerance to wide range temperature of 18-34°C (Ratnayake *et al.,* 2011). In captivity, crustacean broodstock are generally fed with chopped fresh food, which has high nutritional value and is generally regarded as superior to compound diets. However the foods decay rapidly and deteriorate the water quality (Oniam *et al.,* 2012). Therefore, use of formulated diets is highly recommended to maintain the good water quality and support the productive performance (Azra and Ikhwanuddin, 2015).

The findings from the present study demonstrated that formulated broodstock diets based on fishery resources, especially using bivalve and the mixture of squid, shrimp and bivalve, can be successfully used to support good fecundity performance and early offspring quality of *M. rosenbergii* in captivity. This information is very useful to ease the dependency of the industry on raw or frozen feed.

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