EFFECTS OF COLOURED FEED ON GROWTH AND SURVIVAL IN GIANT FRESHWATER PRAWN *Macrobrachium rosenbergii* LARVAE.

GWEE PEI XUAN

THIS DISSERTATION IS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR A DEGREE IN BACHELOR OF SCIENCE WITH HONOURS

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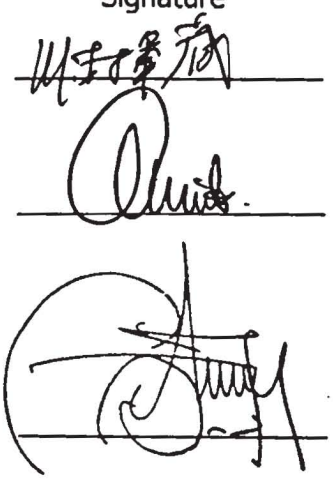

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ABSTRAK

Kajian ini menyelidik kesan-kesan kastard dengan pelbagai warna ke atas *Macrobrachium rosenbergii* semasa peringkat larva. Ujian telah dilakukan dalam dua fasa: fasa 1 iaitu respons kelakuan larva semasa peringkat VIII-IX dijalankan untuk mengenalpasti kecenderungan dan penerimaan kastard kuning dan biru serta fasa 2, iaitu kesan kastard kuning dan biru apabila diaplikasi dalam penternakan larva. Dalam fasa 1, ujian respons kelakuan, kastard kuning dan biru telah dijatuhkan ke tengah-tengah tangki 15-20cm dari tepi dinding tangki di mana larva berhimpun sewaktu ketiadaan pengudaraan. Nombor larva yang tertarik dan menerima kastard biru adalah lebih tinggi daripada kastard kuning. Dalam fasa 2, tangki bersaiz 100L telah diisi dengan 60L air payau iaitu 12ppt, dan kepadatan stok larva ialah 30/L. Untuk setiap rawatan karstard yang berwarna dijalankan dalam triplikat, kastard yang berwarna telah diperkenalkan bermula dari peringkat V hingga pasca larva (PL). Tiada kesan di antara kastard biru dan kuning ke atas perkembangan, metamorfosis dan kemandirian larva. Walau bagaimanapun, sewaktu metamorfosis, bagi larva yang makan kastard kuning, kehadiran PL hanya diperhatikan dalam satu daripada tiga tangki rawatan, manakala kehadiran PL diperhatikan dalam kesemua tangki rawatan yang diberi kastard biru semasa kultur hari ke 28. Pada akhir experimen, berat badan PL yang makan kastard biru adalah 28% lebih tinggi daripada larva yang makan kastard kuning. Tambahan pula, PL yang makan kastard biru badan adalah 5.56% lebih panjang daripada PL yang makan kastard kuning. Maksudnya, saiz badan PL yang makan kastard biru adalah lebih besar berbanding dengan PL yang makan kastard kuning. Oleh kerana kandungan nutrien dalam kastard berwarna adalah tetap sepanjang proses pembelaan, kastard biru hanya berupaya memperbaiki peningkatan berat badan yang disebabkan oleh PL dan sebaliknya tidak mempercepatkan perkembangan larva. Oleh itu, kajian ini menunjukkan daripada permerhatian, larva berupaya untuk mengesan, menangkap dan memakan lebih kastard biru daripada yang kuning.

ABSTRACT

This work investigates the effects of different coloured custard on *Macrobrachium rosenbergii* during larvae stage. The trials were conducted in two phases: phase 1 behavioral response of larvae in stage VIII-IX larvae was done to determine the preference and acceptance on yellow and blue custards and phase 2, the effects of yellow and blue custard when applied in the larvae cycle. In phase 1, behavioral response tests, yellow and blue custards were dropped into the middle of the tank 15-20cm from the edge of the tank wall where the larvae gathered in the absence of aeration. The number of larvae attracted and accepted blue custard was higher than that of yellow custard. In phase 2, 100L size round tank filled with 60 liters of 12ppt brackish water, and the stocking density of the larvae was 30/L. For each coloured custard treatment was done in triplicate, coloured custards were introduced started from stage V until PL stage. There were no significant effects between blue and yellow custards on the larval development, metamorphosis and survival. However, on metamorphosis, larvae that fed with yellow custard, the appearance of PL only found in one out of the three treatment tanks, while the appearance of PL was found in all of the treatment tanks that fed with blue custard on 28th day of culture. At the end of the experiment, the body weight of the postlarvae (PL) fed on blue custard was 28% higher than the PL fed on yellow custard. Besides that, the body length of PL was 5.56% higher than the PL fed on yellow custard. Meaning that, the size of the PL fed on blue custard during the larvae cycle was larger than the PL fed on yellow custard. Due to the nutritional content in the coloured custard were constant throughout the rearing process, the blue custard was only able to improve the body weight gained of the PL instead to accelerate the larval development. Therefore, this study shows that by vision, larvae are able to detect, capture and ingest more blue custards than yellow custards.

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LIST OF ABBREVIATIONS

Abbreviation	
IPMB	Institusi Penyelidikan Marin Borneo
FAO	Food and Agriculture Organization of the United Nations
SPSS	Statistical Package for the Social Sciences
HUFA	Highly Unsaturated Fatty Acids
PL	postlarvae
ppt	Part Per Thousand
ppm	Part Per Million
cm	Centimetre
L	Litre
LSI	Larval stage index
mL	Millilitre
mm	Millimetre
nm	nanometre
µm	Micrometre
g	gram
mg	Milligram
µg	Microgram

CHAPTER 1

INTRODUCTION

1.1 Aquaculture

According to Stickney (2009), aquaculture can be defined as the breeding of aquatic organisms and caring for them under controlled or semi-controlled circumstances. More specifically, aquatic generally means various water environments. They include fresh water, brackish water, and seawater. Aquatic organisms refer to all the living entities that have adapted to living in water. For example, fish, crustaceans, molluscs, and a variety of aquatic plants. The global population keeps on increasing and is expected to reach 7.3-10.7 billion during 2050, aquaculture production is one of the main contributors that provide protein for the whole world. In addition, the aquaculture industry also provides plenty of job opportunities (Tacon, 2000).

1.2 Giant freshwater prawn, *Macrobrachium rosenbergii*

Macrobrachium rosenbergii is the largest *Macrobrachium* species. The biggest size for males and females, that were recorded, at 33 cm and 29 cm, respectively (New, 2000). Body colour of *M. rosenbergii* is green colour to brownish grey, or dark blue. *M. rosenbergii* is commonly found in freshwater environment and brackish water condition, which related to its life cycle (New & Ismael, 2000).

1.3 History and global production status of *Macrobrachium rosenbergii*

Commercial culture of *M. rosenbergii* started after 1980s, due to the fundamental knowledge discovered by Ling, continue with the successful Hawaiian hatchery and grow-out research (New, 2000). Ling & Merican (1961) reported the first successful larval culture of the giant freshwater prawn under laboratory conditions. Using the brackish water quickly achieved significant development in the larviculture of *M. rosenbergii*. The larvae were mainly fed with *Artemia* nauplii and strained egg custard. This first breakthrough boosted interest worldwide. Initiatives to conduct research on the rearing of this prawn were soon come out by research centres from many counties and, in most of the cases, broodstock from Malaysia was acquired and used.

The global production of *M. rosenbergii* has been rapidly increased since 1990s from 30,842,000 tonnes to 220,254,000 tonnes production per year with the increase of 6.14 times (Figure 1.1) (FAO, 2014). In year 2001 China was the largest producer of *M. rosenbergii* where its production reached to 128,300 tonnes, followed by Viet Nam with the production of 28,000 tonnes, there is a different of 100,000 tonnes different between China and Vietnam (New, 2005). To achieve the market demand, increase of *M. rosenbergii* production is expected in India, Thailand and Bangladesh, Indonesia and Taiwan, and currently China is still in the position of the largest producer (FAO, 2014).

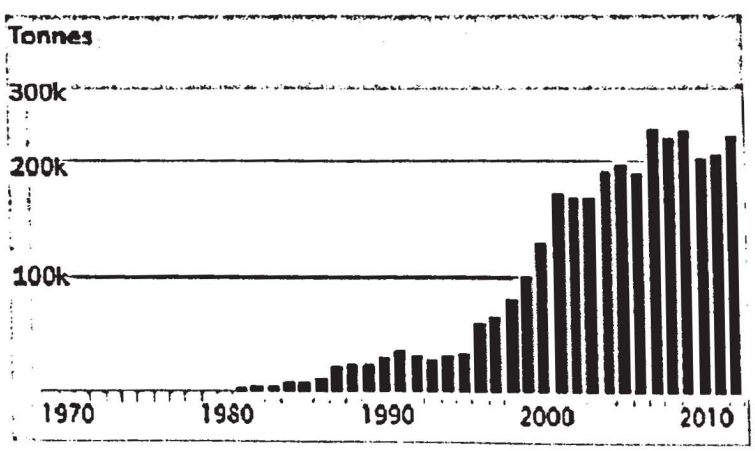


Figure 1.1: Global giant freshwater prawn production (FAO, 2014)

In Malaysia since 1970s , medium-size hatcheries were set up by the Fisheries Research Institute to boost juvenile production in order to meet the increasing demands (Ong, 2008). With government funding and technical supports from France

and Japan, the Fisheries Department successfully made a big step forward during the 1980s. The hatchery centre at Kampong Pulau Sayak, Kedah was upgraded and expanded in between 1985 and 1987, to be the National Prawn Fry Production and Research Centre (NAPFRE). However, compare to other Asian countries, Malaysia have a relative low production of *M. rosenbergii* with total 752 tonnes in year 2001 (New, 2005).

1.4 Behavioural response in colour vision and colour preference of *Macrobrachium rosenbergii* larvae

Kawamura *et al.*, (in press) reported that *M. rosenbergii* larvae have colour vision that they are able to discriminate colour. The larvae are most attracted to light blue, dark blue and white colour, whereas they give moderate response to yellow colour. On the other hand, red, black, and green are least attractive to the larvae. The eye development of grass shrimp *Palaemonetes pugio* larvae has been discovered is to be fully functional as similar *M. rosenbergii* Kawamura *et al.*, (In press). The behaviour showed on by the *M. rosenbergii* larvae on light blue colour is more on innate rather than by learn. With the contribution of this experiment, it may be applicable in the aquaculture industry with animal welfare.

M. rosenbergii larvae have 11 stages before metamorphosis into postlarvae. Before stage 5, larvae are feed on *Artemia* nauplii, in which live feed is highly digestible and provided with exogenous prey enzyme (Kamarudin & Jones, 1994). After that, larvae become more omnivorous and digestive enzyme production is sufficient (Lavens *et al.*, 2000). The feed usually provided in hatchery practices are in yellow colour, in which the colour shows less preference by the larvae. The response of larvae to food particles is important because it may bring well survival and development of the larvae (Moller, 1978). Hence, blue egg custard is suggested in this experiment in order to know the response of larvae through the larvae culture period, and this may encourage the application of blue egg custard in larvae culture of *M. ronsenbergii*.

1.5 Problem statement

In hatchery practice, egg custard with yellow colour is introduced for the freshwater prawn larvae. However, the previous study of Kawamura *et al.*, (In press) shows that the *M. rosenbergii* larvae prefer blue beads over yellow beads. Hence in this experiment yellow (control) and blue custards will be used to compare their influences on the growth and survival of the larvae.

1.6 Hypothesis

The larvae eat more blue custard than yellow custard therefore growth, development and survival are better.

1.7 Objective

To confirm that blue feed is better than yellow feed for growth, larval development and survival of *M. rosenbergii* larvae.

CHAPTER 2

LITERATURE REVIEW

2.1 Biology and taxonomy of *Macrobrachium rosenbergii*

Table 2.1 represent in detail the classification of *M. reosenbergii*. The cephalothorax of *M. rosenbergii* has five indistinct segments in the head and eight in the thoracic region. Its abdomen consists of six distinctly segmented, movable terga. Each abdominal segment is with a pair of biramous pleopods. There are two pairs of antennae, three pairs of jaws, three pairs of maxillipids and five pairs of walking legs attached to the cephalothorax. The second abdominal tergum, which is the largest, overlaps the first and the third laterally (Figure 2.1) (New & Ismael, 2000).

Table 2.1 Taxonomy of *Macrobrachium rosenbergii*

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Crustacea
Class	Malacostraca
Order	Decapoda
Family	Palaemonidae
Genus	*Macrobrachium*
Species	*rosenbergii*

M. rosenbergii have hepatic spines on the surface of carapace. Rostrum is curved upward and has 11 to 14 dorsal teeth, 8 to 10 ventral teeth. Mature male prawns are much larger than the females. Their second pair of chelipeds is considerably larger and thicker than that of female. The head of the male is proportionately larger but the abdomen is narrower. The left and right chellipeds of *M.rosenbergii* are equally big, unlike some other *Macrobrachium* species (Figure 2.2) (New & Ismael, 2000).

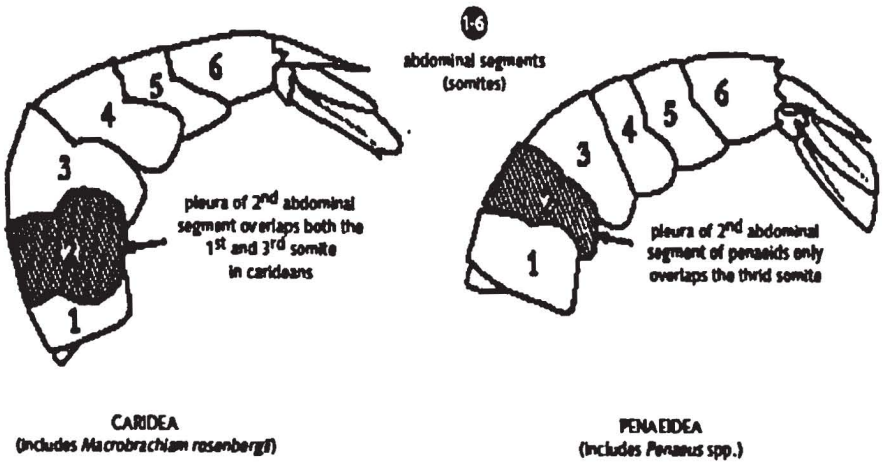


Figure 2.1 Distinguishing feature between carideans and penaeids. (Source: New, 2002)

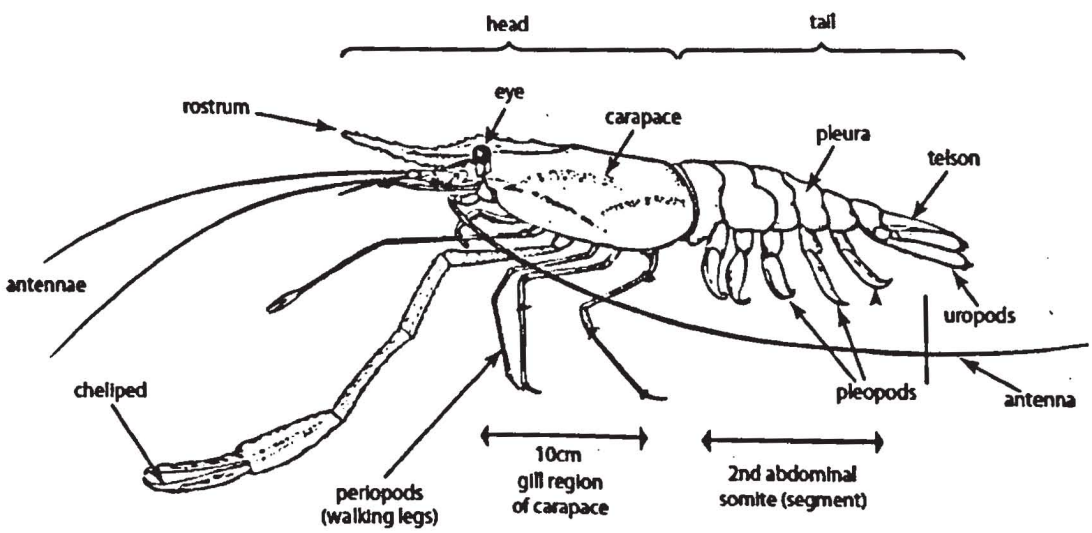


Figure 2.2 External morphology of *Macrobrachium rosenbergii* (source: Nandlal & Pickering, 2005)

The reproductive organ in female, in dorsal part of near hepatopancreas there are a pair ovaries, and the oviduct extended to the gonospores located at third peropods. For male, a pair of testes located at mid-dorsal part of cephalothorax extended through vas deferens to the gonospores which located at fifth pair of pereopods (New, 2000) (Figure 2.3).

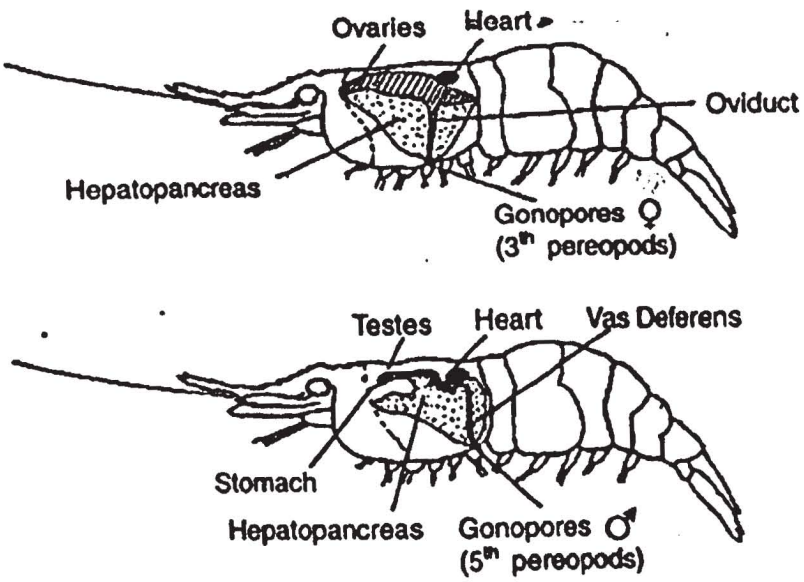


Figure 2.3 Reproductive organs between male and female of *Macrobrachium rosenbergii* (Source: New, 2000)

2.2 Habitat and life cycle

Areas in freshwater environment and brackish water condition can be the habitat of *M. rosenbergii*, this is due to the different stages of *M. rosenbergii* through its life cycle. Brackish water (8-12ppt) is important for the larvae development. Larvae will metamorphosis into the postlarvae. They exhibit as benthic organism and they will slowly migrate to the upstream. Growth out of juvenile into mature adult will take place in the in the river region. Mating will occur in the river region and only pregnant females will migrate downstream into estuaries in which the eggs hatch as free swimming larvae refer to Figure 2.4 (New & Ismael, 2000). *M. rosenbergii* is active during night time (Ling & Merican 1961), while during day time they get themselves half buried in sediments (Karplus & Harpaz 1990). They usually like shallow, detritus rich and vegetated areas (New & Ismael, 2000).

The spawning season of *M. rosenbergii* lasts for a longer time and varies in different distributional areas in the Indo-west pacific region. In temperate regions, the breeding season always occurs in the summer. In tropical regions, it usually coincides with the beginning of the rainy season. In each season, spawning happens two or more times (Ling & Merican, 1961). Successful mating of *M. rosenbergii* happens between hard shelled males and soft shelled females.

Fecundity of *M. rosenbergii* varies greatly for different age, different size and different stage of maturity. The eggs of *M. rosenbergii* are slightly elliptical with a long axis of 0.6-0.7 mm, and are bright orange in colour. They become grey-black in two or three days before hatching (Ling, 1969; Manush *et al.*, 2006).

The larvae of *M.rosenbegii* undergo 11 stages of zoea, before they turn into postlarvae stage (Uno & Kwon, 1969). A small morphological changes and size increase slightly when moulting occurred at each stage of the larvae (Table2.2). During moulting period, larvae may either die or proceed to continuous nature moulting until they complete the development process and turn into postlarvae (New & Ismael, 2000).

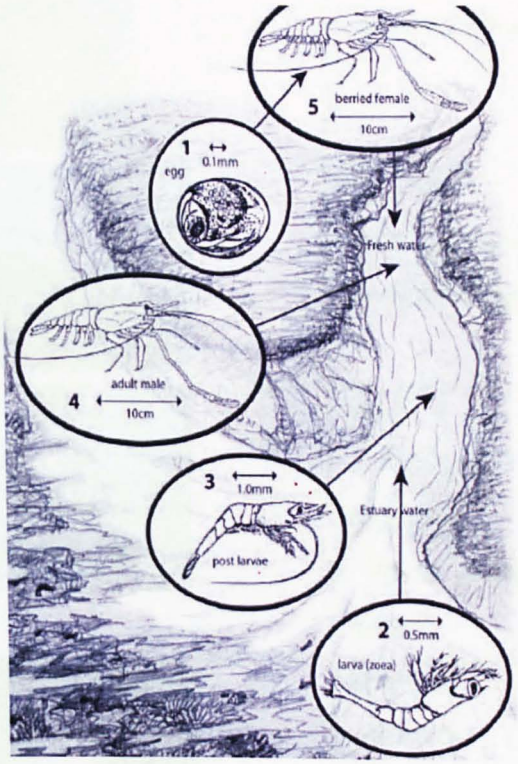
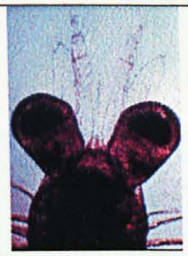
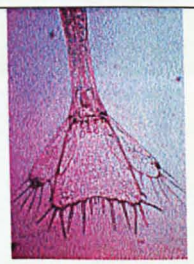
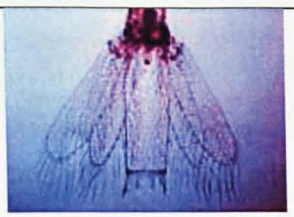
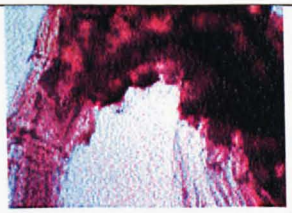
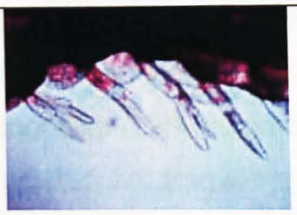
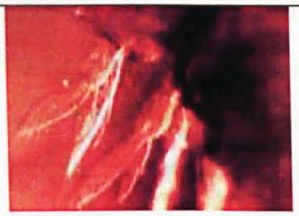
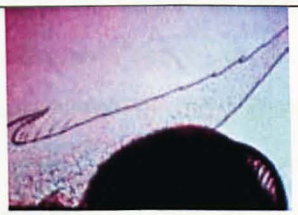
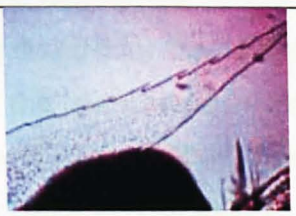


Figure 2.4 Life cycle of *Macrobrachium rosenbergii* in different areas. (Nandlal, & Pickering, 2005)

Table 2.2 Morphological characteristics during the development of freshwater giant prawn larvae from stage I to stage XI, and initial stage of postlarvae. (Source: Uno & Kwon, 1969).

Photo	Characteristics
	Stage I -Sessile Eyes
	Stage II -Stalked eyes
	Stage III -Rostrum have one dorsal tooth -Appearance of uropods
	Stage IV -Rostrum consist of 2 dorsal teeth -Uropod is biramous with setae
	Stage V - Antennal flagellum with 2 or 3 segments - More elongated and narrower of telson
	Stage VI -Antennal flagellum with 4 segments -Telson more narrow and first pleopod appearance of buds

	Stage VII -Antennal flagellum with 5 segments -Biramous appearance with bare
	Stage VIII -Antennal flagellum with 7 segments -Biramous with setae
	Stage IX -Antennal flagellum with 9 segments -Endopods with appendices internae
	Stage X - 3 to 4 dorsal teeth -Antennal flagellum with 12 segments
	Stage XI: -Many dorsal teeth -Antennal flagellum with 15 segments -first and second pereiopods are fully chelated
	PL -Rostrum with dorsal and ventral teeth -Behaviour predominantly benthonic like adults

2.3 Feed and feeding behaviour of *Macrobrachium rosenbergii* larvae

2.3.1 Live feed

In natural habitat, larvae are feed on wide range of materials present in the water column in which the size are suitable for the larvae to capture and consume (New, 1980), New & Singholka (1985) stated that, they are mainly consume of zooplankton such as minute crustacean, thin worms, and the larvae of the other species (as cited in New & Valenti, 2000). When larvae are cultured in captivity, the live feed provided usually is *Artemia* nauplii, while *Miona* may also use as feed for freshwater prawn larvae but it is less preferred, due to low ingestion rate of *Moina* compared with *Artemia* nauplii (Alam *et al.*,1995), and *Miona* is unable to survive more than 10 minutes with salinity of 12ppt condition hence *Miona* is easily collapsed and causing water quality problem (Tattanon & Ruangpanit, 1978). Larvae are usually start feeding with newly hatched *Artemia* after 1 day hatching. Then after 7 to 10 days of larvae rearing, they are provided prepared diet combinated with *Artemia*. The common prepared diet for the freshwater prawn larvae is egg custard. The purpose of providing egg custard for the larvae is to supply the nutritional requirement for the larvae, however the quantitative information on the nutritional requirement is hard to obtain (Abramo & New, 2000). Some experiments have been done in *Artemia* enrichments by HUFA and vitamin C, resulted with improvement of the growth and survival of the shrimp larvae (Romdhane *et al*, 1995; Merchie, 1997).

2.3.2 Formulated feed for *Macrobrachium rosenbergii*

The physical characteristic of prepared feed is also an important consideration to ensure that it is suitable for larvae consumptions. Size of the feed should be in 200 to 400µm for the larvae up to the stage IV, 400 to 600µm can applied for the larvae in the stage of V-VIII, and size with 600 to 1000µm for the larvae in the stage of IX-XI (Abramo & New, 2000). Hence the cooked custard is sieved into the desirable size according to the larvae stage, besides that, softer feed is preferred by the larvae. Buoyancy and water stability of the egg custard is important because the larvae catch the feed mostly depending on the chances of encounter (Kamarudin *et al.*, 1994).

2.3.3 Feeding strategy

Proper feeding strategy is important on order to prevent the larvae underfed or overfed. When larvae are underfed the larvae do not have sufficient nutrient and thus of larvae rearing period may be extended, weaken the larvae, and cannibalism may occur (Correia *et al.*, 2000). While, when the larvae are overfed, it leads to will poor water quality problem. In order to reduce the cost of feed, the use of prepared feed are applied in most of the commercial hatcheries, in the condition without affecting the growth and survival of the larvae. Aquacop (1983), and Daniels *et al* (1992), recommend that prepared feed should be applied when after 10-12 days of larvae rearing period. A guideline of feeding strategy on larvae rearing of giant freshwater prawn provided by Aquacop (1983) (Table 2.3).

Table 2.3 Feed amount applied for per larva per day during larval rearing. (Aqaucop,1983).

Day	Number of *Artemia* nauplii	Pellets (µg DW)
3	5	0
4	10	0
5-6	15	0
7	20	0
8	25	0
9	30	0
10-11	35	0
12	40	70
13-14	45	80-90
15-24	50	100-180
25-30	45	200
30+	40	200

The timing of feeding, procedure and feed recipes applied in hatcheries are differing among each other. However, the feeding regime and types of feed are important to optimize the feeding efficiency. Aquacop (1983) suggest that feeding of *Artemia* nauplii only can be applied once during evening. Besides that, for larvae stage I, feeding is not required (Ling, 1969; Aquacop, 1983).

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