

Study on Morphological Changes and First Feeding of Peacock Eel (*Macragnathus siamensis*)

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Abstract: This research was carried out to examine morphological changes and the feeding behavior of peacock eel (*Macragnathus siamensis*) at the larval stage. Fish was stocked in nylon lining tank 10 m² at a density of 300 fish/m². Water supply from natural pond was fertilized to create natural food. Fish samples were collected daily for analyzing of food composition and quantity beside the observation of morphological changes. The results showed that mouth size of peacock eel larvae was 0.44 ± 0.05 mm in the third day after hatch (DAH). Peacock eel larvae had carnivorous feeding habit ($Li/Lt < 1$). From 20th day onwards, external morphology of the peacock eel was nearly the same as adult fish. Peacock eel from 3th day to 5th day chose nauplius to feed on. From the 6th day to the 8th day, fish chose rotifera. Peacock eel chose cladocera and copepoda from the 9th day to the 23th day. From the 24th day onwards, fish can feed on blood worms. There was no selected phytoplankton for feeding

Keywords: Peacock eel, morphological change, feeding behavior, digestive tract, food composition, food selectivity

1. Introduction

Over the past few decades, freshwater fish plays an important role in the national aquaculture because of the economic value. For a long time, Vietnamese people use freshwater fish as daily meal because this is a nutritious, inexpensive food and very good for health. Nowadays, along with the population growth, demand for fish has been increasing (Thanh, 2014).

At the present time, Peacock eel is considered as one of the freshwater species has high economic value in domestic market besides some main species in Mekong delta such as striped catfish, basa catfish, red tilapia... Peacock eel (*Macragnathus siamensis*) is a tropical fish belonging to the family Mastacembelidae, this species is mainly found in the rivers of Southeast Asia, including the Mekong, Chao Phraya, and Mae Klong.

In Vietnam, there are many researches on aquatic species. However, the species which has high economic value in export is often prioritized to research while some species that people often use in daily meal was not concerned. Peacock eel (*Macragnathus siamensis*) is a good example (Trang, 2006)

Peacock eel has good taste and nutritious, therefore it is very popular in the local market and daily meal of the people in Mekong delta. However, the sources of peacock eel supplied in local market daily are become very limited. In fact, there are no hatchery for nursing this species in Mekong delta so the sources of peacock eel supplied for markets depend mostly on the wild, but climate change and many different factors in recent years make their habitat change and their population decreased significantly. Some peacock eel farmers reported that peacock eel juveniles from the wild can never be weaned to formulated feed because they have passed a certain critical age when weaning could occur, so they have to feed them with blood worm and other fresh feed. However, this source of feed become more and more scarce and the price of them also high. Therefore, many farmers do not choose peacock eel for farming. In addition,

researches on this species, especially nursing are very restricted in Viet Nam as well as in the world, due to this reason, the breeder do not have enough basic information for nursing and feeding this species from larval stage, so that the peacock eel hatchery was not operated.

The purpose of our practical study was to provide more information about morphological change and the feeding behavior of this species from larval stage as a basis to improve the efficiency of nursing this species by making the observation on the change in morphology at the time after hatching until the end of larval stage beside analyzing digestive tract to observe the food component in the digestive tract.

2. Materials and Methods

2.1 Experimental design

Larvae were obtained from reproduction of brood stock after conditioning in freshwater fish laboratory in Can Tho University. Larvae were stock in 10 m² nylon lining tank with the density of 300 fish/ m². Water was pumped from natural pond into nylon lining tank through filter net. To create the natural environment for nursing, this tank was fertilized to induce water color and the bottom is covered by a thin layer of mud to create the good condition for zooplankton such as moina, rotifer, copepod... Aeration was used continuously in the nylon lining tank. The experiment began when the larvae were three days after hatch.

After stocking (3rd day), fish was not fed with any food to observe the selection of fish for natural food in nylon lining tank. Water in the tank was fertilized with NPK fertilizer two time per week to supply nutrients for plankton and algae. Water was supplied from natural pond into nylon lining tank through filter net when water in the tank was less

2.2 Sample collecting

Fish sample: Fish were collected randomly 30 fish/ observation by using a fine scoop net at different points in

nylon lining tank on the day: 3, 4, 5, 6, 7, 8, 9, 10, 13, 16, 19, 22, 25, and 30. Fish sample after collected was stored in Formalin solution to determine morphological change (color, length, mouth size, intestinal length). To observe the food components in digestive tract, 30 fresh samples of fish were collected and analyzed daily.

Phytoplankton and zooplankton:

Phytoplankton: 10 liters of water at 5 different points in Nylon lining tank was collected and then condensed by plankton net (mesh size is 25µm).

Zooplankton: Collect 60 liters of water in the nylon lining tank at 5 different points, condensed the water sample by plankton net (mesh size 60µm.) After filtering the water, the sample was collected in a bottle of 100 ml and fixed with Formalin 2-4% for qualitative and quantitative analysis

2.3 Method for analyzing sample

Length of fish: the change in length of fish was measured by using objective of magnifying glass with the accuracy is 0.1mm (small fish) and straight ruler with the accuracy is 1mm (big fish)

Ratio between intestinal length and body length RLG (relative length of the gut): After measured total length of fish, fish was dissected to take out the intestine. After that, intestine was measured by using magnifying glass, then correlation index between intestinal length and body length RLG was calculated by Al-Hussainy (1949) formula:

$$RLG = Li / Lt$$

In which:

Li: Length of intestine

Lt: Length of fish body

Size of mouth:

The size of mouth is determined by this formula according to Shirota (1970):

$$MH (90^\circ) = AB \times \sqrt{2}$$

When:

AB: length of upper jaw (mm)

MH: width of mouth (mm) when fish open mouth by 90°

Frequency of Occurrence: frequency occurrence of a certain type of food is the ratio between the stomach contain and total number of stomachs was observed (Hynes, 1950). This method including 2 steps:

Step 1: All type of food present in the observed sample was listed, then, the presence or absence of each kind of food in each stomach was recorded.

Step 2: the number of stomach in which the presence of each kind of food was added together and the calculation was the same for other remain food sample then converted into percentage of total sample.

Frequency of Occurrence followed by this formula:

$$O_i = \frac{J_i}{P}$$

Where,

Ji is number of stomach containing prey *i*

P is the total number of stomach.

The food selection index is calculated by this formula, according to Ivlev (1961):

$$E_i = (ri - pi) / (ri + pi)$$

Where:

ri: The ratio of feed type (i) to the total number of feed in fish intestine,

pi: Ratio of type of feed (i) in the total number of feed in the water.

The value of E ranges from (-1) to (+1). Positive index indicates the choice, and the negative index indicates the avoidance to that feed. Value 0 indicates the type of feed eaten by fish in a random way.

2.4 Phytoplankton and zooplankton

Qualitative sample:

Water sample was condensed by plankton net and shaken evenly, then using a dropper to remove the sediment of collected samples and drop into lame 1-2 drops. After that, lamella was used to cover the lame. Finally, the sample was observed under a microscope. The name of family and genus of plankton was identified according to Shirota (1966), Boltovskoy (1999), DangNgoc Thanh and ctv. (1980)

Quantitative sample:

Water sample was condensed and shaken evenly. After that, the condensed sample was dropped into the counting chamber Sedgwick- Rafter. Finally, all zooplankton and phytoplankton in the counting chamber were counted under microscope.

Formula to calculate plankton density:

$$P = \frac{T \times 1000 \times Vcd}{A \times N \times Vm} \times 1000$$

In which:

P: Plankton density (individual / L),

T / *N*: Number of cells of one cell on the counting chamber,

A: Volume of 1 counting cell (1mm³)

N: Number of counting cells,

Vcd: volume of condensed sample (mL)

Vm: Volume of collected water sample (mL).

The presence of each kind of food in digestive tract was calculated by ratio between stomach contain and total number of stomachs. After that, the food selection index formula, according to Ivlev (1961) was applied to calculate the food selection of fish.

3. Results

Artificial reproduction of peacock eel

Broodstock selection

Female was chosen with big and soft belly, genital papilla has pink colour and protruded. Male was chosen with small and thin belly, small and concave genital papilla

Hormone stimulation

Female was injected two times. In the first injection, female was injected by HCG with the dose of 3000-4000 IU/ 1kg of female. In the second injection, female was injected by 200µg LHRH-a + 10mg DOM/ 2kg of female. Male was injected one time by using LHRH-a + DOM, at the same time with the second injection of female. Dose for male injection was half of female injection. Male and female was injected at muscle. After 12 hours of injection, egg and sperm were stripped then mixed together. The eggs after stripping was treated in solution of 4g salt+ 3g urea+ 2L water. Embryo development process of peacock eel lasted from 32-42 hours at 29-30°C

Table 1: Morphological changes of peacock eel

Hours after hatch	Morphological characteristics
06-12 hrs	Inactive larvae with large yolk sac
36-48 hrs	Yolk sac partially reduced and elongated. The eyes had the pigment and mouth cleft became prominent.
80-90 hrs	Yolk sac was diminished and the eyes was fully pigmented and the notochord became clearly visible
120 hrs	Body laterally elongated. Eyes placed near each other.
240 hrs	Pectoral fin, airbladder and gut were distinct. Mouth of fish was elongated.
384 hrs	Development of caudal and dorsal fin with soft rays
480 hrs	Green colour on the back and five black spots on the caudal fin. The spiny of notochord and most fin rays were distinct.

Table 1 shown the changes in morphology of peacock eel (*Macroglyphus siamensis*) from 6- 12 hours (after hatching) until 480 hours (20 days). From 20 DAH, the morphology of fish was nearly the same as adult fish.

Ratio between intestinal length and body length RLG

Intestinal length of peacock eel increased gradually from 2.2±0.1mm in the 3rd day to 16.5±0.5mm in the 30th day. The length of intestine was directly proportional to the length of fish. Ratio between intestinal length and body length was fluctuated from 0.44±0.01 (3rd day old) to 0.78±0.01 (30th day old) (Table 2).

Table 2: Ratio between intestinal length and body length

Day	Li (mm)	Lt (mm)	Li/Lt
3	2.21±0.13	5.06±0.30	0.44±0.01
4	2.60±0.22	5.69±0.29	0.46±0.02
5	3.16±0.24	6.17±0.24	0.51±0.03
6	4.23±0.20	7.14±0.33	0.59±0.01
7	5.15±0.23	7.94±0.34	0.65±0.01
8	6.14±0.20	9.04±0.22	0.68±0.01
9	6.73±0.15	9.86±0.22	0.68±0.01
10	7.17±0.19	10.3±0.33	0.70±0.02
13	7.52±0.28	10.7±0.39	0.70±0.01
16	8.40±0.29	11.9±0.59	0.71±0.02
19	11.1±0.55	15.0±1.06	0.74±0.02
22	12.7±0.35	16.8±0.54	0.75±0.01
25	14.2±0.40	18.3±0.53	0.78±0.01
30	16.5±0.50	21.2±0.64	0.78±0.01

Mean ± standard deviation, Li: intestinal length, Lt: standard length

Table 2 shown ratio of intestinal length and standard length of peacock eel. This ratio increased gradually with the

development of fish during nursing and intestinal length was always shorter than body length. According to Nikolsky (1963), carnivorous fish has the ratio $Li/Lt \leq 1$, omnivorous fish has $Li/Lt = 1-3$ and herbivorous fish has $Li/Lt \geq 3$. The ratio of intestinal length and standard length of peacock eel was always smaller than 1, indicated that peacock eel was carnivorous species. Some other carnivorous species have correlation index Li/Lt smaller than 1 such as flower spiny eel (Thanh, 2014), clown featherback (Tran Thi Thanh Hien and ctv., 2007).

The changes in mouth size

Size of fish mouth determine the size of food that fish is able to feed. The change in fish mouth was shown in Table 3.

Table 3: Mouth size variation of peacock eel by age

Day	Lt (mm)	AB (mm)	MH (mm)
3	5.06±0.30	0.31±0.03	0.44±0.05
4	5.69±0.29	0.38±0.03	0.54±0.04
5	6.17±0.24	0.42±0.02	0.60±0.03
6	7.14±0.33	0.52±0.03	0.73±0.04
7	7.94±0.34	0.59±0.03	0.83±0.05
8	9.04±0.22	0.66±0.02	0.93±0.02
9	9.86±0.22	0.72±0.02	1.02±0.03
10	10.3±0.33	0.83±0.02	1.18±0.03
13	10.7±0.39	0.86±0.03	1.22±0.05
16	11.9±0.59	0.92±0.03	1.30±0.04
19	15.0±1.06	1.12±0.07	1.57±0.09
22	16.8±0.54	1.22±0.03	1.72±0.05
25	18.3±0.53	1.32±0.03	1.86±0.04
30	21.2±0.64	1.49±0.05	2.10±0.07

Mean± standard deviation, Lt: standard length (mm), AB: length of upper jaw (mm), MH: width of mouth (mm).

Size of mouth is one of important factors affect the ability to catch the prey of fish. For peacock eel, the size of mouth increased with the growth of fish. When experiment began (3 DAH), the width of mouth was 0.44±0.05 (mm), corresponding to body length of 5.06±0.30 mm. At the end of experiment (30th day), width of mouth was 2.10±0.07mm while body length was 21.2±0.64mm (Table 3). Width of mouth determine size of prey that fish can eat. According to Shirota, 1970 fish can eat the prey with maximum size by 45% width of mouth.

Correlation of natural food in digestive tract of peacock eel and environment**Phytoplankton in the environment**

There was four species of phytoplankton was found in the environment: chlorophyta, euglenophyta, cyanophyta and diatom. Among the four species, density of chlorophyta was the highest during experiment, followed by euglenophyta. Cyanophyta and diatom was the lowest density. In eutrophic environment, chlorophyte and euglenophyta is often dominant (Vu Ngoc Ut and Duong Thi Hoang Oanh, 2013).

The density by percent of phytoplankton species in environment varied by age of fish. Density by percent of phytoplankton was presented in figure 1.

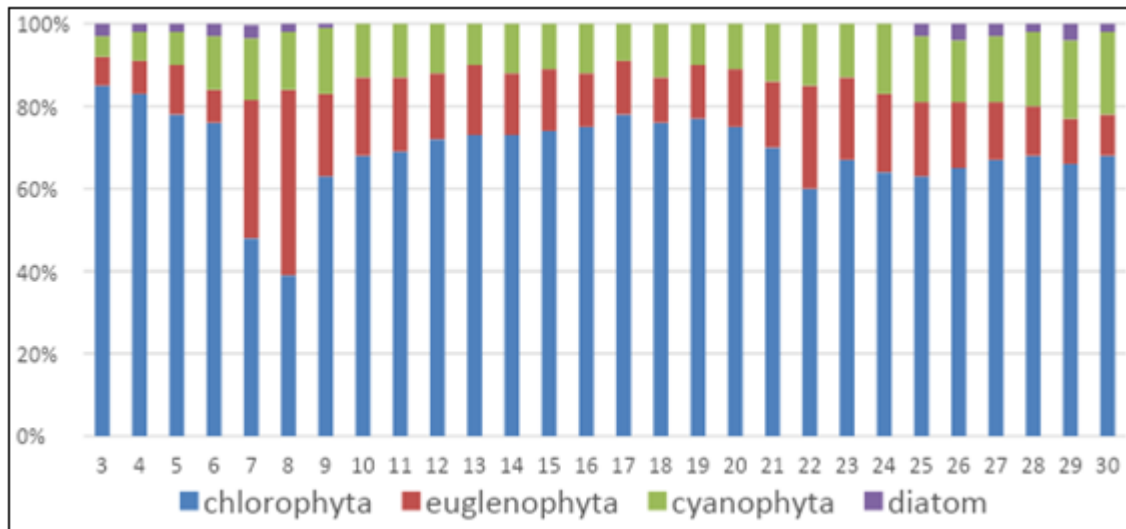


Figure 1: Density by percent of phytoplankton in the environment

Figure 1 shown the density by percent of phytoplankton in the environment. From the 3rd day to 6th day, chlorophyte was dominant in the water (76%-85%). From 7th day to 8th day, euglenophyta developed with high density in the environment (accounting for 39-48%), this make density of chlorophyte reduced to 34-48%. Chlorophyta and euglenophyta developed with high density in the water indicate the environment was enough nutrition (Vu Ngoc Ut and Duong Thi Hoang Oanh, 2013)

Zooplankton in the environment

There were five species of zooplankton in the water: Rotifera, copepod, cladocera, Nauplius and protozoa. Rotifera, copepod and cladocera were dominant during the experiment. Density of zooplankton in the environment was also change over time. The density by percent of zooplankton was shown in figure 2.

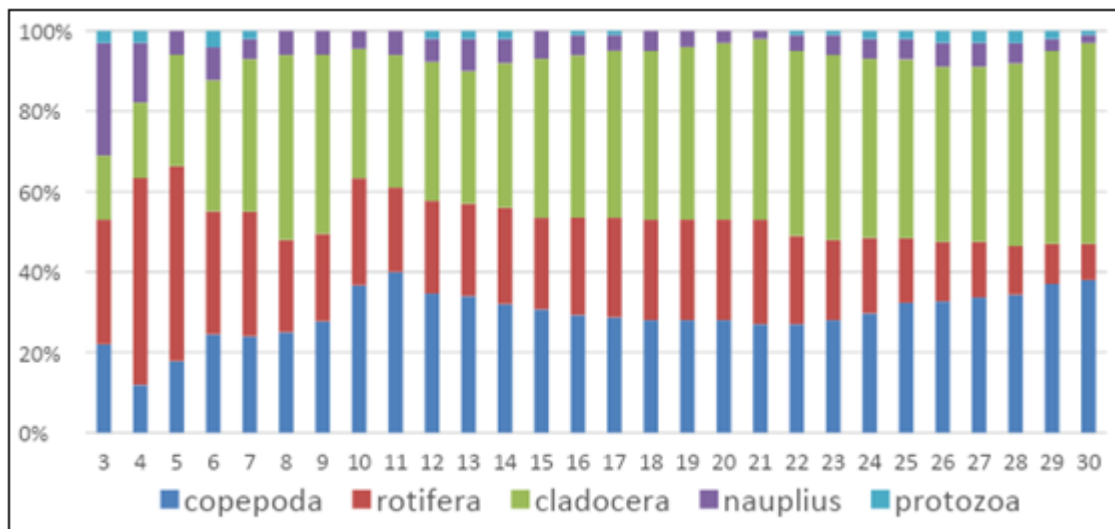


Figure 2: Density by percent of zooplankton in the environment

Figure 2 shown the density variation of five species of zooplankton. At the beginning of the experiment (3rd day), density of nauplius and rotifer were the highest (33% and 31%). On the 4th day, density of rotifer was the highest (52%). From the 5th day onwards, density of rotifer and Nauplius reduced gradually while density of copepoda and cladocera increased over time. Density of copepod reached the highest proportion on the 11th day (40%) and density of cladocera was the highest on the 30th day (50%). The density of zooplankton which has big size (cladocera and copepoda) tend to increase time by time, it was easier for fish to select the food that fits their mouth size.

Analyzing food in digestive tract of peacock eel

The amount of each type of food in digestive tract of peacock eel was varied by day old.

The proportion of each type of food in digestive tract of fish was presented in figure 3. Figure 3 indicated that the food in digestive tract of peacock eel on 3th day were rotifera and nauplius with 68% and 32% respectively. These zooplankton appeared in digestive tract of peacock eel with the trend of increasing rotifer and decreasing Nauplius. On the 6th day, the proportion of nauplius reduced to 13% while the proportion of rotifera increased to 52%. On the 6th day, peacock eel tend to catch the prey which has bigger size (rotifer) instead of Nauplius. Besides that, density of rotifera

in the environment increased sharply from the 4th day and maintain with high proportion in the water (40%) on the 6th day (figure 2). In this study, peacock eel at the 6th day tended to catch the prey which fits their mouth with high density in the environment.

From the 5th day, cladocera (moina) appeared in digestive tract of peacock eel (17%), the proportion of rotifera and nauplius reduced to 58% and 25% respectively. From the 8th day onwards, nauplius was not found in digestive tract of fish. Cladocera increased gradually and reached the highest proportion on the 9th day with 71%, the proportion of

rotifera reduced to 21%. Copepod appeared in digestive tract of peacock eel on the 9th day (8%). The proportion of copepod increased continuously and reached the highest proportion on the 16th day with 39%. From the 23th day, zooplankton appeared in digestive tract of peacock eel with the trend of increasing cladocera and decreasing copepoda.

On the 10th day, the other kind of food (tubifex worm) appeared in the digestive with the small proportion (4%) then fish began to choose worm as main food from the 30th day with the highest proportion (36.17%).

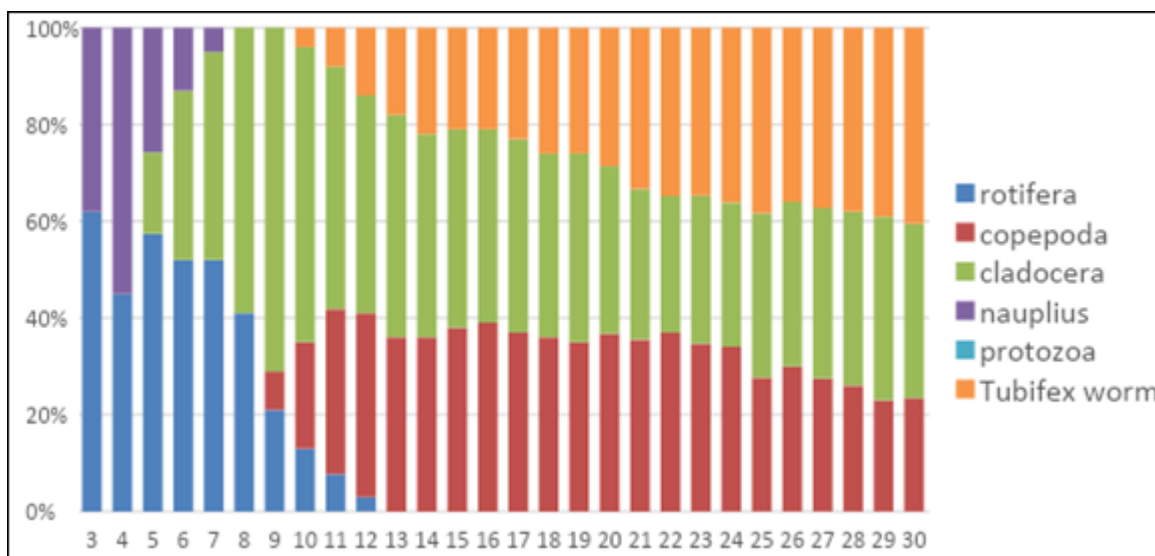


Figure 3: Food types in digestive tract of peacock eel

The food selection index of peacock eel

The digestive system of peacock eel on the 3rd day old can be found the presence of Rotifera and nauplius with the selection index were 0.378 and 0.101 (table 3). This proved that peacock eel on the 3th day old can use food in

the outside. Natural food such as rotifera, cladocera and copepoda have high nutrition value which plays an important role nursing, especially in larval stage (Vu Ngoc Ut and Duong Thi Hoang Oanh, 2013)

Table 3: Food selection index of peacock eel from the 3th day to 30th day

Day Food	3	4	5	6	7	8	9	10	11
Protozoa (tintinopsis)	-1	-1	-1	-1	-1	-1	-1	-1	-1
Rotifera (brachionous)	0.101	-0.072	0.084	0.268	0.253	0.281	-0.023	-0.297	-0.463
Cladocera (moina)	-1	-1	-0.244	0.045	0.062	0.124	0.224	0.356	0.205
Copepoda (mesocyclop)	-1	-1	-1	-1	-1	-1	-0.556	-0.2	-0.081
Nauplius	0.378	0.571	0.625	0.238	0	-1	-1	-1	-1

Day Food	12	13	14	15	16	17	18	19	20
Protozoa (tintinopsis)	-1	-1	-1	-1	-1	-1	-1	-1	-1
Rotifera (brachionous)	-0.778	-1	-1	-1	-1	-1	-1	-1	-1
Cladocera (moina)	0.111	0.165	0.077	0.012	0	-0.024	-0.05	-0.049	-0.118
Copepoda (Mesocyclop)	0.027	0.029	0.059	0.101	0.147	0.121	0.125	0.111	0.135
Nauplius	-1	-1	-1	-1	-1	-1	-1	-1	-1

Day Food	21	22	23	24	25	26	27	28	29	30
Protozoa (tintinopsis)	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Rotifera (brachionous)	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Cladocera (moina)	-0.193	-0.239	-0.201	-0.203	-0.128	-0.128	-0.11	-0.11	-0.116	-0.163
Copepoda (mesocyclop)	0.126	0.156	0.107	0.063	-0.073	-0.048	-0.107	-0.133	-0.233	-0.236
Nauplius	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

4. Discussion

Fry often choose zooplankton as main feed for the early stage (Pham Minh Thanh and Nguyen Van Kiem, 2009). From the 3th to 6th day old, it was clearly shown that fish larvae choose nauplius to feed with the selection index ranging from 0.378 to 0.625. From the 6th to 8th day old, peacock eel began to choose rotifera with the selection index from 0.268 to 0.281 (table 3). The food selection of fry is critical to their survival in the nursery process. In common, fry choose the food that fits their mouth size (Trieu, 2016). Nauplius has small size (size of nauplius from 0.06-0.1mm) fits the mouth size of fish from the 3th day to the 6th day (0.44-0.73mm). According to Shirota (1970), the majority of fish can eat the prey with the maximum size by 45% mouth size. From the 3th day old to the 5th day, fish had the mouth size from 0.44-0.60 mm, the size of Nauplius is 0.069 mm equal 11.5%- 15.68% size of mouth. From the 6th to the 8th day, peacock eel tended to use rotifera. Size of rotifera from 0.1-1mm and different from species (Vu Ngoc Ut and Duong Thi Hoang Oanh, 2013). In addition, zooplankton with different size is often used by small fish (Pham Thanh Liem and ctv., 2002). From the 9th day, peacock eel chose cladocera as main feed with selection index was 0.224. From the 9th day until the end of the experiment, copepod and cladocera appeared in the digestive tract of peacock eel with high proportion (figure 3). When fry become bigger, catching ability of them is higher (Gill and Hart, 1994).

Plankton density has a great influence on the ability of the fish to catch prey, high density of zooplankton in the environment, higher chance for fish to catch prey (Pham Minh Thanh and Nguyen Van Kiem, 2009). When the experiment began, density of Nauplius and rotifera were very high (density of Nauplius was 28% and density of rotifera was 31%) and the selection index of peacock eel for nauplius and rotifera was high (0.378 and 0.101 respectively).

The moving organs of small fish is not complete, so they can only catch the prey with slow and simple moving style (Senoo et al, 1994). Peacock eel from the 3th day old had the passive swimming mechanism, so they can only catch the prey with slow moving style. According to Vu Ngoc Ut and Duong Thi Hoang Oanh, (2013) nauplius has zigzag movement pattern from the bottom up and vice versa. This type of movement is suitable for small fish (from 2th to 5th day) to catch prey. It was clearly shown in selection index of fish for nauplius from 3 to 5 days of age (0.378-0.625) (table 3).

From the 13th day until the end of the experiment, the proportion of zooplankton in digestive tract of peacock eel were cladocera (30-42%) and copepoda (23-38%). At this stage, mouth size of fish reached 1.22-2.10 mm, so they can catch the prey with big size as moina (0.4-1.6mm) and mesocyclops (>1mm).

From the 9th day to 14th day, peacock eel chose moina with selection index from 0.077-0.224 then they chose copepoda from the 15th day to 23th day with selection index from 0.101-0.156.

On the 10th day, some pieces of tubifex worms were found in digestive tract of peacock eel (4%) then fish used worms as main feed beside cladocera and copepoda. From the 24th onwards, the proportion of tubifex worm in digestive tract of fish was the highest (figure 3).

In conclusion, peacock eel from 3 days to 5 days old chose Nauplius. From the 6th day to the 8th day, fish chose rotifera. Peacock eel chose cladocera from the 9th day to the 14th day. From the 15th day to the 24th day, they chose copepoda to feed. From the 24th day onwards, fish can feed on blood worms. Peacock eel did not choose phytoplankton to feed at this stage.

5. Conclusion

Hatching period of peacock eel was 32-42 hours at 29-30^o C, mouth size of peacock eel larvae was 0.44 ±0. 05 mm (3 DAH)

Ratio of intestinal length and body length of fry from 0.44-0.78 mm. Peacock eel larvae had carnivorous feeding habit (Li/Lt<1). Size of mouth from 3th day to 30th day was from 0.44- 2.10 mm. From 20th day onwards, external morphology of the peacock eel was nearly the same as adult fish.

Peacock eel began to eat outside from 3 DAH. Nauplius and rotifera were the original food of fish. Peacock eel from 3th day to 5th day chose nauplius to feed on. From the 6th day to the 8th day, fish chose rotifera. Peacock eel chose cladocera from the 9th day to the 14th day. From the 15th day to the 23th day, they chose copepoda to feed. From the 24th day onwards, fish can feed on blood worms. Peacock eel did not choose phytoplankton to feed at this stage. Factors such as prey size, density and movement pattern of prey affected the food choices of peacock eel.

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