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Food utilization and fecundity of Guppy (*Lebistes reticulates*) fed with bioencapsulated *Artemia franciscana*

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Abstract
The feeding physiology of *Lebistes reticulates* fed on bioencapsulated *Artemia franciscana* were investigated under laboratory conditions. Multifactorial experimental designs were used to study food utilization and fecundity. Pre-adult individuals of *A. franciscana* were enriched with herbal products having aphrodisiac and tonic characters. In the present work herbal products of *Withania sominifera* and *Tinospora cordifolia*, were mixed to prepare Compound-I and *Myristica fragrans* and *Terminalia belerica*, were mixed to prepare Compound-II, 100% enrichment of *Artemia* with both compounds were fed to the *L. reticulates*, the gross and net conversion efficiency in fish *L. reticulates* fed with bioencapsulated *Artemia* was found to the more than control. The medicinal plant products having aphrodisiac and tonic characteristics play a significant role in enhancing growth and increasing fecundity of *L. reticulates*.

1. Introduction

The aquarium fishes are rapidly gaining importance due to their immense commercial value in the export trade, the world over. Guppy fish body is short, cylindrical, compressed, head and body with scales, pre maxillaries slightly protractile; mandible prominent, teeth on jaws, plate dentate. Young males are easily distinguished from the females by their beautiful orange red and black dots all over the body and fins. The most striking feature of sexual behavior in this species is the manner in which the males persistently pursue the females. Structurally, a viviparous fish (bearing living young) does not differ essentially from and oviparous (egg-laying) fish. Eggs are present in the female's body in both cases. But, with the viviparous fish, the eggs hatch just before being laid. Guppies, in spite of their tiny size, an useful citizens of what ever pond or stream they inhabit as one of their favourite foods is the "Mosquito wiggler". In the present experiment, bioencapsulated *Artemia franciscana* was altered to the fish as food.

Artemia is a non-selective. Obligate filter feeder is important as they are extensively used in aquaculture as a diet for fish and many invertebrates (Sumitra vijayaraghavan *et al.,* 1987). *Artemia* is a suitable live food for cultivable organisms than most artificial feeds. *Artemia* have discontinuous distribution both in temperate and tropical coastal marine and inland saline lakes. Selection of a suitable diet is normally determined by a number of criteria which is based on both the culturist and the view point of cultured animal. From the physical point of view, the purity, availability and acceptability, energetic requirements and nutrients requirements must be considered. *Artemia* is a suitable live food for cultivable organisms than most artificial feeds (Legar and Sorgeloos, 1994).

The particle size of the *Artemia* food should be under 50 μm diameter, because the *Artemia* is a filter feeder (Dobbeleir *et al.,* 1980). Feed accounts for 40-60% of the total coast in aquaculture and among the feed ingredients proteins is most expensive. (Tacon and Jackson, 1985) For the culturists, simplicity of food production technology for producing *Artemia* is much simpler than culturing algae, as their nauplii produced from a virtually inert, powder consisting of dormant, encysted embryos. The nauplii are produced by incubating the cysts for about 24 hrs in sea water under optimal condition of temperature, salinity, pH, Oxygen, cyst density and illumination (Sorgeloss *et al.,* 1986). A major drawback in feeding *Artemia* to freshwater organisms is that the *Artemia* die after 30 to 60 min in freshwater. Therefore, they are not continuously available to the predator, as they could be in marine systems, and must be fed intermittently every 2 to 3 hrs. They play an important role as energy and essential fatty acid (EFA) sources in fish.

Besides the functions, they are important carriers of non-fat materials such as fat-soluble Vitamins and Carotenoid pigments. The advantages of exploiting adult *Artemia* are, i) Protein content increases from 42 to 60% and fat content decreases from 20% to less than 10% of its dry weight, ii) Stimulates predation by its movement and iii) Reduces the temporal cost of feeding. The main aspect of versatility of *Artemia* involves its use as carrier. Medicines are applied directly to the shrimp larvae; these can be transferred through filter feeding organism.

In that respect, the versatility of *Artemia* as carrier for various dietary components, such as essential nutrients, pigments, hormones, prophylactics. Therapeutics or vaccines, is great importance in the larviculture. Ayurveda has incorporated a number of plant species in the treatment of many diseases of human beings and domestic animals. The present work investigates the effect of some ayurvedic medicinal plants products, like *Withania sominifera*, *Tinospora cordifolia*, *Myristica fragrans* and *Terminalia belerica* having the properties such as aphrodisiac and tonic, on the bioencapsulation characteristics of *Artemia franciscana* as well as effect of feeding the bioencapsulated *Artemia* on food utilization and fecundity in Guppy fish (*Lebistus reticulates*).

2. Materials and Methods

2.1. Fish collected and Maintained

The guppy fish were obtained from a commercial ornamental fish farm (Southern India Aquarists Pvt ltd) kadaichanaynthal near Madurai, Tamil nadu, reared in the laboratory, and their fry were collected and maintained in the culture tank. The fishes were acclimatized in well water supplied to the laboratory as dechlorinated water. During the period of acclimatization, the fishes were fed with groundnut cake. soybean, dried yeast, egg based artificial diet. The aquaria were cleaned well and the water was changed at 2 days interval. All these procedures were made before the start of experiment.

2.2. Morphometric and gravimetric analysis

The experimental fishes were selected according to their weight (i.e. The fish belongs to the same age group. The length of the fishes was measured. In guppy, the length was 1.5 cm in male and 2 cm in female. Fish weighing approximately 160 mg - 350 mg was preferred for this experiment.

2.3. Experimental design and fish feeding

Experimental fishes isolated from the stock were divided into three groups namely control, fishes receiving compound-I and fishes receiving compound-II. Each group contained six individuals. Out of six individuals three were males and three were females. They are fed with enriched and non enriched *Artemia*. For laboratory hatching of *Artemia* cysts, the dried cysts are allowed to float on the surface of filtered salt water of 35 ppt, where they hatch in 24 - 48 hrs, depending upon the ambient temperature. Vigorous aeration and suitable temperature regime (26 - 30^0C) may happen hasten the eggs hatching process. The hatched nauplii could be attracted using a light source and collected by a very fine meghed net and transferred to culture tank. *Artemia* is a continuous non-selective, particle filtering organisms. The Coupling of propulsion, respiration and filtration by the Thoracopods results in a practically continuous filter feeding.

2.4. Bioencapsulation study

To enrich the *Artemia* with the herbal products 400 mg of *Withania seminifera* and 400 mg of *Tinospora cardifolia* were taken and dissolved in 200 ml of water. It was poured into a 500 ml beaker and the 100 pre-adult *Artemia* (10^{th} stage) were introduced. A strange aeration was given. The other two herbal products of *Myristica fragans* and *Terminalia belerica* were also used the same. The gut of *Artemia* was observed under the microscope at regular intervals of time (5minutes). The gut saturation time as well as gut retention/gut evaculation were recorded. The enriched pre adults were made ready for feeding of Guppy.

Experimental design of feeding of *Lebistes reticulates* is illustrated in figure 2. About 18 individuals of *Lebistes reticulates* were isolated from the stock. The average weight of the fish was 150 - 350 mg. Eighteen individuals were divided into three groups, each contain 3 males and 3 females. Pre-adult (150 – 200 mg) encapsulated *Artemia* with compound - I and II were fed to the fish twice a day. The fish were fed with bioencapsulated *Artemia*. The unfed *Artemia*, within 30 minutes, were removed from the culture trough. Since the fish was fed with definite number of *Artemia*, it becomes necessary to find the relationship between number and dry weight of *Artemia*. After 21 days of feeding the total number of *Artemia* offered to a group was multiplied by the dry weight to get the amount of dry food consumed.

2.5. Food Utilization

The scheme of mass budget followed in this work proposed by Petrusewicz and Macfadyen (1970). Metabolism was obtained as the difference between A and P. Feeding rate(cr), Assimilation rate(Ar), Conversion (or) production rate (Pr) and metabolic rate(Mr) were calculated by dividing the respective amount by the products of mid body weight(g) of the animal and the duration(Day). The rates are expressed in terms of mg/ g live fish/ day. Mid body weight (MBW) of the test individuals is the mid point in the growth of the animal for the test period. It is calculated by the following formula:

MBW = Initial live weight of test fish (g) + final live weight of test fish/ 2

Feeding rate (cr) = Food Consumption (mg/ individual)/ MBW (g) × Duration (day)

Absorption rate (ar) = Food assimilated (mg/ individual)/ MBW (g) × Duration (day)

Conversion rate (pr) = Food Converted (mg/ individual)/ MBW (g) × Duration (day)

Metabolic rate (Mr) = Amount of food metabolished (mg/ individual)/ MBW (g) × Duration (day)

Absorption efficiency (Ae %)

This is expressed as a percentage of food that is absorbed in relation to the food is consumed.

Ae = Food absorbed/ Food Consumed × 100

Conversion Efficiency

There are two types of conversion efficiency there are gross conversion efficiency (k_1) and net Conversion efficiency (k_2)

i) Gross Conversion efficiency (k_1) (%) = Food converted/ Food consumed × 100

ii) Net Conversion efficiency (k_2) (%) = Food Converted/ Food assimilated × 100

2.6. Fecundity study

In the present experiment, after feeding the fish for 21 days to estimate growth, fishes were allowed to breed to study fecundity males and female fish were kept in the same trough for 2 days to initiate mating. After 2 days, they were separated and fed continuously until the female lays young ones. Results obtained in the present study were subjected to statistical analysis.

3. Result

Fish were fed with enriched and non enriched *Artemia*. To enrich *Artemia* four herbal products with Aphrodisiac and tonic characters were chosen. They are,

1. *Myristica fragrans* (Aphrodisiac).
2. *Withania sominifera* (Aphrodisiac).
3. *Terminalia belerica* (Tonic).
4. *Tinospara cardifolia* (Tonic).

3.1. Aphrodisiac compound

1. *Myristica fragrans*

Artemia (7 mm) was enriched with *Myristica fragrans* at room temperature. The time taken for enrichment decreased with increase in concentration of herbal products for instance at 100 mg/100 ml *Artemia* required 48 minutes which decreased to 35 minutes at 400 mg/100 ml concentration.

2. *Withania Somnifera*

And the *Withania somnifera* was given at different concentrations. The time taken for 100% enrichment was 38±1.26 minutes at the lowest concentration of herbal products (100 mg/100 ml) which decreased to 30±0.73 minutes at 400 mg/100 ml of concentration (Figure-1).

3.2. Tonic Compound

3. *Terminalia belerica*

When *Terminalia belerica* was given at 100 mg/100 ml of concentration, the time taken for 100% enrichment was 20±1.73 minutes which decreased to 12 minutes at 400 mg/100 ml of concentration.

4. *Tinospora cordifolia*

For *Tinospora cordifolia*, the time taken for complete enrichment was 32±0.96 minutes at 100 mg/100 ml concentration which decreased to 15 minutes at 400 mg/100 ml concentration (Figure-2).

3.3. Bio encapsulation

Artemia franciscana preadult measuring about 7 mm±1.16 was used for feeding the *L. reticulates*. It was enriched with compound-I, containing equal amount of herbal products *Withania somnifera*, *Tinospora cordifolia* and compound-II containing equal amount of *Terminalia belerica, Myristica fragrans. Artemia* took 25±1.32 and 30±1 minutes to reach the saturation level for compound-I & compound-II, respectively. The time taken for 100% enrichment is given in Figure-3.

3.4. Mass Budget

L. reticulates were reared at 27±1°C for 34, 36 and 39 days. The fishes where divided into 3 groups, each group having 3 males and 3 females. The first group, the control, received non-enriched *Artemia* two times a day. The second group received *Artemia* enriched with compound-I two times a day. The third group received *Artemia* enriched with compound-II, two times a day. Date on mass budget for the *L. reticulates* fed with non enriched *Artemia* and *Artemia* enriched with compound-I and compound-II are given in tables-1, 2 & 3.

Table: 1 Mass budget of *Lebistes reticulates* (Guppy) fed with non enriched *Artemia* at 27±1°C for 39 days. $\overline{X}$ ± SD of three observations.

Parameters	Male	Female
Growth (mg)	65±0.47	108±0.96
Food Consumption (C) (mg Dry wt/individual)	198.2±0.95	325±1.00
Faeces (F) (mg Dry wt/individual)	49.55±0.58	81.25±1.73
Assimilation (A) (mg Dry wt/individual)	148.65±1.16	243.75±0.58
Metabolism (R) (mg Dry wt/individual)	83.65±1.53	135.75±1.58
Feeding rate (Fr) (mg Dry wt/g live wt/day)	23.15±0.66	29.76±0.73
Assimilation rate (Ar) (mg Dry wt/g live wt/day)	17.36±1.06	22.32±0.96
Conversion rate (Cr) (mg Dry wt/g live wt/day)	7.59±0.58	9.89±1.00
Metabolic rate (Mr) (mg Dry wt/g live wt/day)	9.77±1.53	12.43±1.15
Assimilation efficiency (Ae) (%)	75±1.73	75±1.00
Gross conversion efficiency (K1) (%)	33	33
Net conversion efficiency(K2) (%)	44	44

Table: 2 Mass budget of *Lebistes reticulates* (Guppy) fed with enriched (Compound- I) *Artemia* at 27±1°C for 39 days. $\overline{X}$ ± SD of three observations.

Parameters	Male	Female
Growth (mg)	82±1.73	131±1.00
Food Consumption (C) (mg Dry wt/individual)	238±1.00	368.9±1.73
Faeces (F) (mg Dry wt/individual)	58.74±1.53	84.84±1.16
Assimilation (A) (mg Dry wt/individual)	179.26±0.58	283.16±0.58
Metabolism (R) (mg Dry wt/individual)	97.26±1.15	152.16±1.15
Feeding rate (Fr) (mg Dry wt/g live wt/day)	29.66±1.58	35.98±1.73
Assimilation rate (Ar) (mg Dry wt/g live wt/day)	22.34±1.16	27.62±0.58
Conversion rate (Cr) (mg Dry wt/g live wt/day)	10.22±1.00	12.77±1.13
Metabolic rate (Mr) (mg Dry wt/g live wt/day)	12.122±1.73	14.84±1.15
Assimilation efficiency (Ae) (%)	75.32±0.58	76.75±1.16
Gross conversion efficiency (K1) (%)	34	36
Net conversion efficiency(K2) (%)	46	46

Table: 3 Mass budget of *Lebistes reticulates* (Guppy) fed with enriched (Compound- II) *Artemia* at 27±1°C for 39 days. $\overline{X}$ ± SD of three observations

Parameters	Male	Female
Growth (mg)	97±1.00	150±1.00
Food Consumption (C) (mg Dry wt/individual)	252±1.73	386.4±1.15
Faeces (F) (mg Dry wt/individual)	50.54±1.53	77.28±1.15
Assimilation (A) (mg Dry wt/individual)	201.46±1.58	309.12±1.00
Metabolism (R) (mg Dry wt/individual)	104.46±1.16	159.12±1.58
Feeding rate (Fr) (mg Dry wt/g live wt/day)	29.47±1.53	34.29±1.53
Assimilation rate (Ar) (mg Dry wt/g live wt/day)	23.56±0.58	27.43±0.58
Conversion rate (Cr) (mg Dry wt/g live wt/day)	11.34±1.15	13.31±0.58
Metabolic rate (Mr) (mg Dry wt/g live wt/day)	12.21±1.00	14.12±1.15
Assimilation efficiency (Ae) (%)	79.94±1.53	80±1.73
Gross conversion efficiency (K1) (%)	38	39
Net conversion efficiency(K2) (%)	48	49

Table: 4 Growth of *Lebistes reticulates* (Guppy) fed with non enriched *Artemia,* enriched *Artemia* compound-I and II at 27 ± 1°C for 36 days. $\overline{X}$ ± SD of three observations.

	Sex	Growth							Spawning
		Initial wt of fish (mg)	Day						
			5	10	15	21	26	31	36/34	39
Non enriched *Artemia*	Male	184 ± 1.00	192 ± 0.58	200 ± 0.96	209 ± 0.58	220 ± 0.46	225 ± 1.53	233 ± 0.56	244 ± 0.46	249 ± 0.21
	Female	226 ± 1.00	236 ± 1.16	246 ± 0.58	257 ± 0.46	272 ± 1.06	286 ± 1.16	305 ± 0.96	325 ± 0.42	334 ± 0.18
Enriched *Artemia* (Compound I)	Male	193 ± 1.00	203 ± 0.16	215 ± 0.46	230 ± 0.58	248 ± 0.46	255 ± 1.16	267 ± 0.76	275 ± 0.18	
	Female	236 ± 1.00	250 ± 0.58	268 ± 0.36	286 ± 1.00	307 ± 0.96	327 ± 0.58	351 ± 0.46	367 ± 0.21	
Enriched *Artemia* (Compound II)	Male	189 ± 1.00	201 ± 1.00	214 ± 1.73	230 ± 1.56	249 ± 1.36	257 ± 1.00	270 ± 0.58	286 ± 1.15	-
	Female	238 ± 1.00	253 ± 1.73	271 ± 0.58	291 ± 1.16	314 ± 0.58	334 ± 1.00	359 ± 0.96	388 ± 1.73	

Figure: 1 Time taken for 100% enrichment in *Artemia franciscana* fed with herbal products (Aphrodisiac)

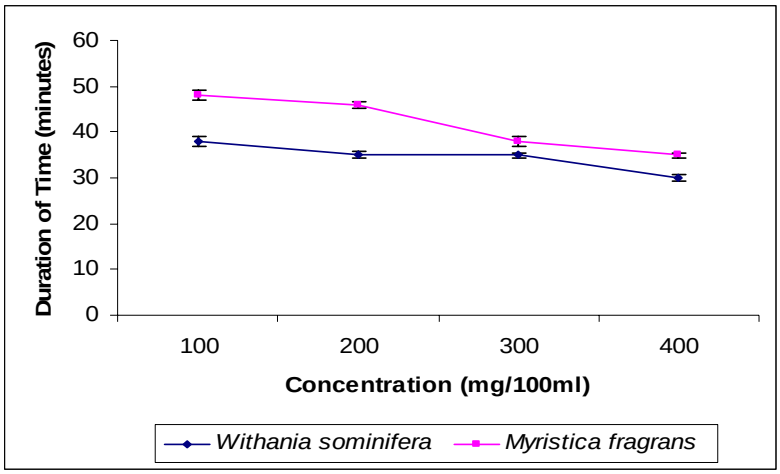


Figure: 2 Time taken for 100% enrichment in *Artemia franciscana* fed with herbal products (Tonic)

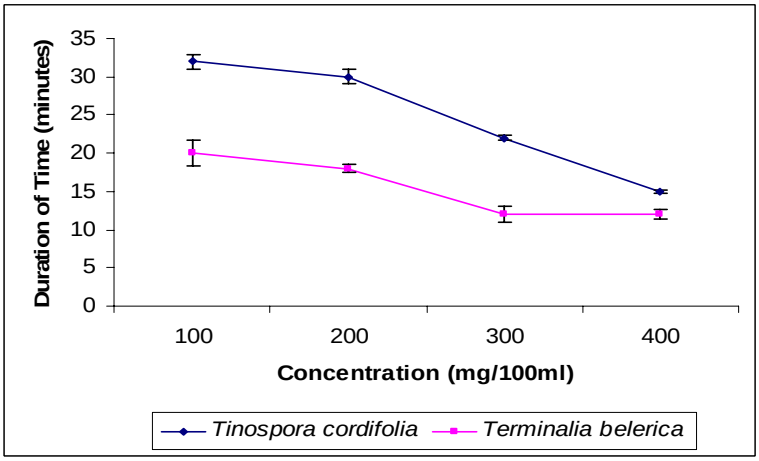
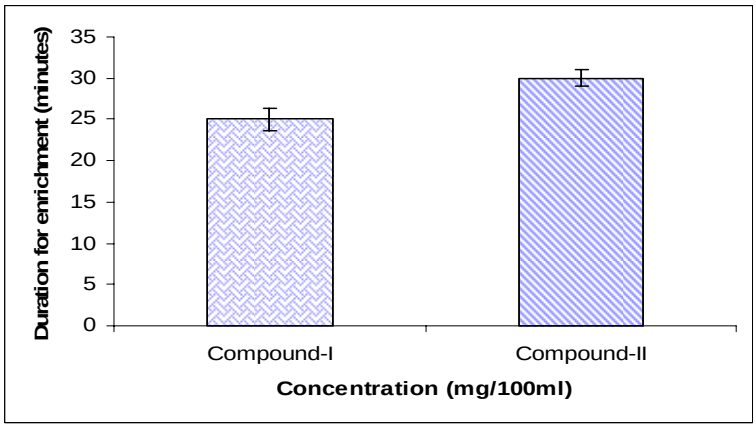


Figure: 3 Time taken for 100% enrichment in *Artemia franciscana* fed Compound-I and Compound-II



3.5. Growth

Male *L. reticulates* receiving non enriched *Artemia* exhibited a growth of 249±0.21mg in 39 days. However female *L. reticulates* exhibited a growth of 334±0.18 mg in 39 days. However, Male *L. reticulates* receiving *Artemia* enriched with compound-I grew from 193±1.00 mg to 275±0.18 mg in 34 days. Female exhibited growth of 367±0.21mg in 34 days. Fish receiving *Artemia* enriched with compound-II exhibited a higher growth than other fish. Fish receiving *Artemia* enriched with compound –II showed a growth of 286±1.15 mg in male and female 388±1.73 mg in 36 days (Table-4).

3.6. Food consumption

Dry weight of food consumed by *L. reticulates* different in different groups. Male and females receiving non-enriched *Artemia* consumed 198.2±0.95 and 325±1 respectively in 39 days. Among the fish receiving enriched *Artemia* (male & females), with compound-I consumed 238±1 and 368.9±1.73 mg Dry wt of food, respectively where as fish receiving *Artemia* enriched with compound-II consumed 252±1.73 and 386.4±1.15 mg dry wt of food (Table-1, 2&3).

3.7. Assimilation

Dry wt of food absorbed by fishes of different groups differed. Fish (male & female) receiving *Artemia* enriched with compound-I showed an assimilation of 176.26±0.58 and 283.16±0.58 mg dry wt. of food, respectively. And the non-enriched *Artemia* could absorb only 148.65+0.58 and 243.75±1.73mg dry wt of food. Fish receiving *Artemia* enriched with the compound-II showed better assimilation than others (Table-1, 2&3).

3.8. Feeding rate

Feeding rate of the fish belonging to different groups did not differ much. Feeding rate was higher in fish receiving *Artemia* enriched with compound-I and compound-II than the control fish.

3.9. Assimilation rate

Assimilation rate was higher in group receiving *Artemia* enriched with compound-II and it was 23.56±0.58 and 24.43±0.58 mg dry wt/gm live wt/day, in male & female, respectively. Those receiving *Artemia* enriched with compound-I showed an assimilation rate of 22.34±1.16 and 27.62±0.58 mg dry wt/gm live wt/day, in male and female, respectively. Fish belonging to control group exhibited a very low assimilation rate and it was 17.36±1.0 6 and 22.32±0.96 mg dry wt/gm live wt/day in male & female, respectively (Table-1, 2&3).

3.10. Conversion rate

Conversion rate was higher in group receiving *Artemia* enriched with compound-II and it was 11.34±1.05 and 13.31±0.58 mg dry/wt/gm live wt/day in male & female, respectively. Fish receiving *Artemia* enriched with compound-I showed a conversion rate of 10.22±1 and 12.77±10.13 mg/dry wt/gm live wt/day in male & female, respectively. Conversion rate of the fish receiving non enriched *Artemia* was 7.59±0.58 and 9.89±1 mg dry wt/gm live wt/day in male & female, respectively (Table-1,2&3).

3.11. Assimilation efficiency

Fish fed with *Artemia* enriched with compound-II exhibited a high assimilation efficiency of 80±1.73% compared to fish receiving *Artemia* enriched with compound-I, which showed an assimilation efficiency of 76.75±1.16%. However, not much difference could be observed between the assimilation efficiency of fish receiving non-enriched *Artemia* and fish receiving *Artemia* enriched with compound-I and it was showed 75±1% (Table-1, 2 & 3).

3.12. Gross conversion efficiency [(k^1) (%)]

Fish receiving *Artemia* enriched with compound-I and compound-II showed a high Gross conversion efficiency of 39% and 36%. Fish receiving Non-enriched *Artemia* showed a conversion efficiency of 33% (Table-1, 2 & 3).

3.13. Net conversion efficiency: (k^2) (%)

Fish receiving *Artemia* enriched with compound-I and compound-II showed a high net conversation efficiency of 46% and 49% respectively. However, fish fed with non enriched *Artemia* showed a slightly low net conservation efficiency of 44% (Table-1, 2 & 3).

3.14. Fecundity

After studying the growth of fish for 21 days males and females were paired for mating. Number of young ones produced and their weight were found. *L. reticulates* fed with non enriched *Artemia* produced 13 offspring in the first spawning. Fish receiving *Artemia* enriched with compound-I produced considerable number of off springs in the first spawning and it was 18. Fish fed with *Artemia* enriched with compound-II showed considerable increase in the number off springs produced than those fed with *Artemia* enriched with compound-I and control. They produced 22 off springs in first spawning.

4. Discussion

The main objective of developing rearing strategies for ornamental fish is the establishment of a feeding regime that will result in optimal growth, survival and health of the fish. Increase in availability of food having higher calorific value promotes consumption, assimilation, conversion, metabolism and their rates and efficiencies are more than that seen in fish exposed to non availability of food of fish exposed to lesser caloric valued foods. The enzymatic breakdown of the food determines the type of nutrients available for absorption. The versatility of *Artemia* involves in its use as carries for various dietary components such as essential nutrients, pigments, hormones, prophylactics, therapeutics or vaccines. *Artemia* has become an indispensable food source for a large

number of aquatic organisms like fish and marine/ freshwater crustaceans. Kinne (1977) cites that more than 85% of the organisms under cultivation depend on *Artemia* as a food source. Bioencapsulation to enhance the nutritional quality of *Artemia* was conducted only when the *Artemia* failed to meet the fish requirement. Ayurvedic products have generally been found more effective in supporting larval growth and survival of *Penaeus monodon* (Devi, 1995). Fish fed with Juvenile *Artemia* bioencapsulated with 10% or 20% of animal proteins for 20 days did not show any significant difference in stress resistance from those fed with control *Artemia* Juveniles, fed fish (Lim *et al.,* 2000). Plant products which promote stress resistance and growth have been selected to enrich *Artemia* pre-adult and improve their quality (Citarasu, 1994; Chitra, 1995; Babu, 1999). In the present work *L. reticulates* divided into three groups were fed with non-enriched *Artemia, Artemia* enriched with compound-I and *Artemia* enriched with compound - II, to study the food utilization and fecundity. Fish receiving *Artemia* enriched with compound - II exhibited 43% increase in body weight in 21 days. However fish fed with *Artemia* enriched with compound-I exhibited 23% increase in body weight. The fish receiving non enriched *Artemia,* exhibited lesser growth than fish receiving compound-I and compound-II. Here, the maximum growth was observed in fish receiving *Artemia* enriched with compound-II. It is infered from the above observation that the compound-II promotes the growth of fish. Fish fed with *Artemia* enriched with compound-I produced 18 young ones and fish receiving *Artemia* enriched with compound-II produced 22 young ones. In the present investigation, *Artemia* was used as a delivery agent to introduce herbal medicines in Guppy (*L. reticulates*). To deliver the desired herbal medicines, bioencapsulation technique has been practiced in marine fish and shrimp hatcheries (Sorgeloos *et al.,* 1987). Results obtained in the present work reveal higher growth and fecundity in fish fed with *Artemia* enriched with herbal products and it is a testimonial to the role of herbal products on fish growth and fecundity.

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