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STUDIES ON THE NUTRITIONAL EVALUATION AND BIOENRICHMENT OF *ARTEMIA PARTHENOGENETICA* NAUPLII FOR THEREARING OF ORNAMENTAL FISH *XIPHOPHORUS MACULATUS*

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Abstract: The present study aims at optimizing the enrichment protocols of *Artemia* nauplii with different oils containing highly unsaturated fatty acids. The bioenriched *Artemia* nauplii is used as an enriching feed of the ornamental fish *Xiphophorus maculatus* for their healthy growth. The bioenrichment of the nauplii has been done throughthe use of different types of plant oils such as, olive and corn oil, besides fish oils such as, those of sardine and sharks. *Artemia* nauplii enrichedwith olive oil fedfishes have shown higher growth and survival rate compared with unenriched *Artemia* nauplii fed fishes (control) and other oil enriched *Artemia* nauplii fed fishes. Olive oil contains fatty acids which include omega-3 fatty acids, linoleic acids, Eicosa Pentanoic Acid (EPA) and Decosa Hexaenoic Acid (DHA). The linoleic acids are very important because theypromote larval growth in fishes. Fatty acid profile of differently oil-enriched *Artemia* fed fishes was done by gas chromatography. This experiment contribution examines the bioenrichment study of *Artemia*in order to establish that it is the best live food in aquaculture field.

Key words: *Artemia parthenogenetica,* Bioenrichment, *Xiphophorus maculatus*, gas chromatography

INTRODUCTION

Aquaculture is an ancient art that has been advanced by scientific practices. Ornamental fish keeping is becoming popular as an easy and stress relieving hobby (Ghosh *et al*., 2008). Nutrition plays an important role in maintaining good health and normal behaviour of fishes, enhancing external appearance and improving reproductive performance of any fish species, including ornamental fish. Feeding of ornamental fish is based on the extrapolations of nutrient requirements and practices derived from food fish under intensive culture conditions aimed at reaching maximum growth in a shorter time (Keshavanath and Patil, 2006). Naupliar stages of the crustacean *Artemia* are widely used in aquaculture for the larval rearing of many fish species. Generally, the use of *Artemia* nauplii requires the improvement of their nutritional value by means of enrichment procedures. The process by which beneficial substances are included inside the body of live preys is known as bioencapsulation. This boosting process consists of an incubation of live preys in a medium with enrichment product. Non-selective feeding behaviour on live preys makes the dispersed particles of the enrichment product incorporate in the composition and digestive tract of the prey. The present study mainly point out that the nauplii of *Artemia* are bioenriched with two different type of plant oils such as olive oil and corn oil and two different types of fish oil such as sardine oil and shark liver oil. Olive oil contains fatty acid which includes omega 3 fatty acids and Alpha Linoleic Acid (ALA), EPA and DHA. It also contains polycyclic aromatic hydrocarbons and chlorophylls. It promotes the formation of O_2radical and speeds up oxidation and acts as antioxidant. Corn oil is a light coloured oil and contains high level of linoleic and oleic acids. The fish oils such as sardine and shark liver oil contain a high level of oleic acid, linoleic acid, EPA and DHA and a low amount of myristic acid,

palmitic acid, stearic acid, arachidonic acid, behanic acid and lignoceric acid (Kinsella, 1989). The amount of EPA and DHA is very important for *Artemia* larval growth and development. They are also related to food value as well as the price of *Artemia*. DHA plays a critical role as an essential fatty acid in the maintenance of physiological process in fishes (Sargent *et al.,* 2002). The DHA/EPA ratio in live feed may be an important factor in the pigmentation of several flat fishes and ornamental fishes (Reitan *et al*., 1994). By bioenrichment or bioencapsulation, specific amount of particulate or emulsified products rich in highly unsaturated fatty acids in the *Artemia* metanauplii and the nutritional quality of the *Artemia* can be further tailored to suit the predator requirements. The objective of this study was to determine the effect of length, weight, survival rate, growth estimates and fatty acid composition of *Xiphophorus maculatus* fed with different commercial oil or enriched and unenriched *Artemia parthenogenetica*nauplii. The commercial oil such as olive oil, corn oil, sardine oil and shark liver oil and the unenriched *Atremia* nauplii was taken as control.

MATERIALS AND METHODS

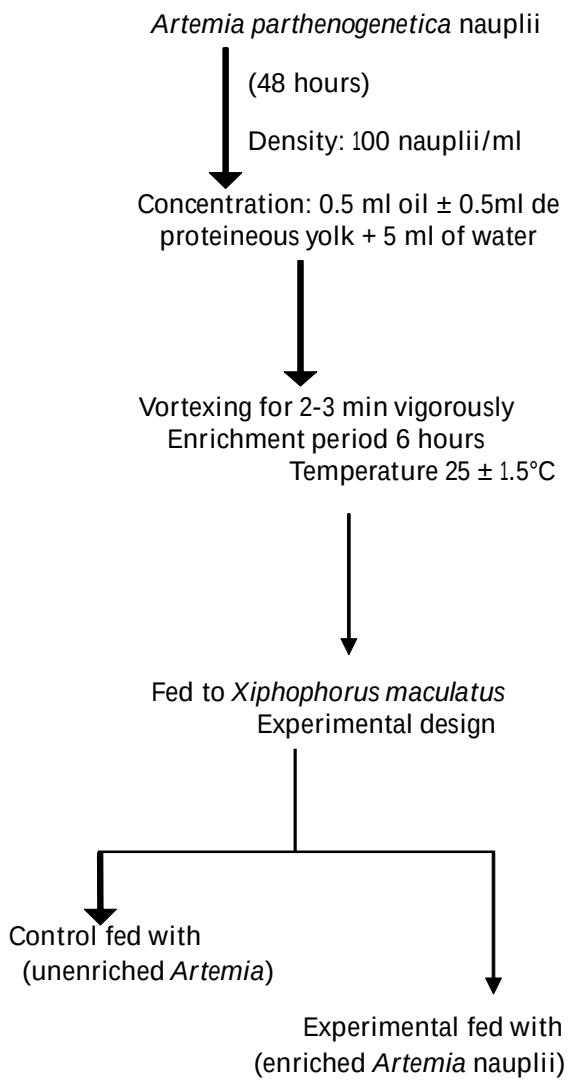
Artemia parthenogenetica was used to study the nutritional evaluation of ornamental fish *Xiphophorus maculatus.* The nutritional study was carried out by using the bioenrichment of *Artemia parthenogenetica* nauplii fed with *Xiphophorus maculatus.*

Feed experiments with enriched nauplii

The fryof *Xiphophorus maculates* were maintained in aquarium tanks and fed with *Artemia parthenogenetica* nauplii (without fatty acid enrichment served as control) and the seedlings fed with fatty acid enriched nauplii served as experimental. In each tank, 50 numbers seedlings were maintained. The period of experiment was 60 days. Before starting the experiment, the initial weight and length were measured. At the end of the experiment (after 60 days) the survival rate, length and weight, growth estimates and fatty acid analysis in each experimental tank was determined.

Methodology For Enrichment Studies

Flow Chart



Statistical analysis: The mean body length and body weight of different oil enrichments and control fishes before and after experiments were analyzed by using ANOVA. Tukey's Multiple Comparison Test was done to assess the significant difference at 1% level..

Experimental analysis

Control I	-	Unenriched *Artemia* nauplii
Experimental I	-	Olive oil enriched *Artemia* nauplii
Experimental II	-	Corn oil enriched *Artemia* nauplii
Experimental III	-	Sardine oil enriched *Artemia* nauplii
Experimental VI	-	Shark liver oil enriched *Artemia* nauplii

Growth estimates

Increase in biomass=final weight–initial weight

Increase in biomass (%) = ((final weight – initial weight) / Initial weight) x 100

SGR =((log final weight - log initial weight) / No. of days of experiment) x 100

RESULTS AND DISCUSSION

Growth was found to be higher in the fishes fed with olive oil enriched *Artemia* nauplii. The growth and survival rate of *Xiphophorus maculatus* fed with olive oil enriched *Artemia* nauplii, corn oil enriched *Artemia* nauplii, sardine oil enriched *Artemia* nauplii, shark liver oil enriched Artemia nauplii and unenriched *Artemia* nauplii (control) after the duration of experiments are presented in Table 1. The survival rate of olive oil enriched *Artemia* nauplii was high (96%) whereas the survival rate of shark liver oil enriched *Artemia* nauplii showed 94%, that of sardine oil enriched *Artemia* nauplii showed 93%, that corn oil enriched *Artemia* nauplii showed 92% and control showed 90%. From the result, it was evident that olive oil enriched *Artemia* nauplii is the best food for larval rearing of *Xiphophorus maculatus*. Fatty acid content of *Xiphophorus maculatus* fed with unenriched and enriched *Artemia parthenogenetica* nauplii has been depicted in Table 2 and Figures 1, 2, 3, 4 and 5. Linoleic acid level was high compared with that of other fatty acid and its value was high in olive oil enriched *Artemia* nauplii fed fishes. The statistical analysis showed that there was significant difference ($p<0.05$) in the length and weight of fishes during different oil enrichments and control. The length and weight of the fishes were high in olive oil enriched *Artemia parthenogenetica* fed fishes(Tables 3&4;Tables 6&7). Similarly Post Hoc Test was used to analyze the differences in length and weight of fishes in different oil enriched *Artemia* nauplii fed fishes compared with un-enriched *Artemia* nauplii fed fishes. In this analysis it was concluded that growth ratebetween the olive oil and control showed the highest the differences. It means that olive oil enriched *Artemia* nauplii fed fishes had a high growth rate than others (Tables 5&8). The level of significance was 5%.

The growth estimates such as increase in biomass, percent increase in biomass and specific growth rate were found to be higher in olive oil enrichment than in others. The increase in biomass in fishes fed with olive oil enriched *Artemia* nauplii, corn oil enriched *Artemia* nauplii, sardine oil enriched *Artemia* nauplii, shark liver oil enriched *Artemia* nauplii and un-enriched *Artemia* nauplii was found to be 1.91 ± 0.007, 1.758 ± 0.001, 1.85 ± 0.0427, 1.318 ± 0.01 respectively. The increase in biomass (%) in fishes fed with olive oil enriched *Artemia* nauplii, corn oil enriched *Artemia* nauplii, sardine oil enriched *Artemia* nauplii, shark liver oil enriched *Artemia* nauplii and un-enriched *Artemia* nauplii was found to be 194 ± 0.987, 195 ± 0.087, 188.7 ± 0.0301, 186.2 ± 0.480, 134 ± 0.521 respectively. Specific growth rate (SGR) in fishes fed with olive oil enriched *Artemia* nauplii, corn oil enriched *Artemia* nauplii, sardine oil enriched *Artemia* nauplii, shark liver oil enriched *Artemia* nauplii and unenriched *Artemia* nauplii was found to be 3.18 ± 0.010, 293 ± 0.17, 3.08 ± 0.019, 3.04 ± 0.005, 2.196 ± 0.002 respectively (Table 9).

Live feeds are a convenient and often essential food source for the larvae of some culture species, especially those without a fully developed digestive system. Freshly hatched *Artemia* nauplii are a high-value-feed for fish larvae and fry.

Table 1. Growth and survival rate of *Xiphophorus maculatus* fed with different oil emulsions.

Commercial	Feeding period (days)	Average body length (cm)		Average body weight(g)		Survival (%)
enrichment		Initial	Final	Initial	Final	
Olive oil	60 days	1.2	3.42	0.98	2.854	96
Corn oil	60 days	1.2	3.08	0.98	2.738	92
Sardine oil	60 days	1.2	3.18	0.98	2.83	93
Shark liver oil	60 days	1.2	3.08	0.98	2.804	94
Control	60 days	1.2	2.84	0.98	2.298	90

Because of the size of the naupliar stage, *Artemia* also represents the only practical food source for the early stages of many a fish and crustacean larvae (Bardach, 1972). The nauplii must be fed to the larvae as soon as possible after hatching or be stored at low temperatures to decrease their rate of metabolism. For many fish species live food still gives better results in terms of growth and survival than artificial diets (Dabrowski, 1984).

Artemia adults provide additional benefits as they have been used for improving the nutritional requirement of a wide variety of organisms. It can increase the biomass concentration and specific growth rate of fishes. The olive oil enriched *Artemia* nauplii contain a high level increase in biomass and specific growth rate. It can be concluded from the results that vegetable oil had higher nutritional value than marine fish oil because of their higher levels of n-3 HUFA, which contained high linoleic acid and showed better nutritional value. Olive oil rich linoleic acid had a higher nutritional value than others rich in linolenic acid. It indicates that linoleic acid appears to be essential in the diet of fish larvae were required maximum growth, survival, feed efficiency. Neither linoleic acid nor linolenic acid, or their combination improved growth significantly compared with fishes fed on a basal diet which contained only palmitic or stearic acids. All HUFA (Highly Unsaturated Fatty Acids) showed higher nutritional values than PUFA (Poly Unsaturated Fatty Acids) and produced significantly higher weight gain and total lipid content in fish muscle (Gomez-Requeni*et al.,* 2003; Han *et al.,* 2005).

The enrichment process is time dependent. As the fatty acids are taken up by the *Artemia*, their fattyacid profile changes according to the duration of enrichment period. After enrichment of 12 hours the total fattyacids increased significantly and the *Artemia* had significantly higher amounts of essential fatty acids in the n-3 and n-6 families and total fatty acids were achieved. The results indicate that convenience and the length of enrichment process should also be considered when preparing *Artemia* nauplii as a food for the larvae of ornamental fish. Freshwater ornamental fish producers in Hawaii who are utilizing enrichment procedures have improved consistently higher survival levels in larval discs, improved growth and survival of gold fish larvae (*Carassius auratus*), increased fecundity of angel fishes (*Poecillia reticulata*) and guppies (*Pterophyllum altum*) (Chapman *et al.,* 1997).

Fatty acids are ubiquitous in all living organisms and their importance in energy metabolism, in the control of physical properties of cell membranes and in regulating cell metabolism has been well established. In order to sustain normal life, humans and many other animals, including fishes, have an absolute requirement for certain poly unsaturated fatty acids, considered essential for the species concerned. Enrichment of *Artemia* with these lipids containing high level of PUFA which improve the growth and survival rate in

Table 2. Fatty acids profile of *Xiphophorus maculatus* fed with un-enriched and enriched *Artemia parthenogenetica* nauplii (Expressed in % concentration)

Sl. N	Fatty acids	Unenriched *Artemiaparthenogenetica* nauplii	Enriched *Artemiaparthenogenetica nauplii*			
			Olive oil	Corn Oil	Sardine oil	Shark liver oil
1	Lauric acid	0.6935	0.3572	0.4848	0.4515	0.2432
2	Myristic acid	0.2385	0.037	0.1125	0.1727	11.9675
3	Palmitic acid	16.0503	11.1203	14.4836	13.1007	1.1918
4	Palmitolic acid	4.1796	0.6945	2.3542	2.7536	5.6027
5	stearic acid	6.2785	5.8043	5.8746	6.0959	31.1015
6	oliec acid	34.7061	28.23	32.6541	32.9715	34.928
7	linoleic acid	29.7851	47.1979	36.1831	38.2166	44.9418
8	linolenic acid	0.2753	0.1346	0.397	1.4982	-
9	Arachidic acid	2.5099	1.3871	2.6299	-	1.9009
10	Behanic acid	0.5509	0.4967	0.7056	0.8219	0.1372
11	EPA	1.2637	2.0282	1.3886	1.5811	1.4319
12	Lignoceric acid	0.4659	1.1191	0.3068	0.4814	0.0611
13	DHA	1.232	1.3246	2.23	1.48	1.3205
	Total fatty acids	98.22%	99.93%	99.80%	99.62%	99.99%

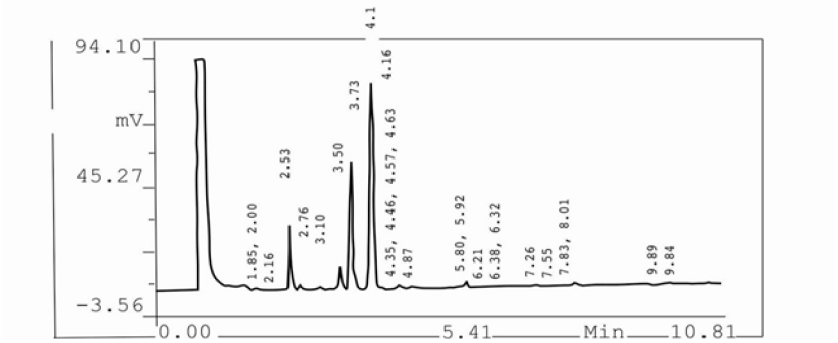


Fig. 1. Fatty acid composition of *Xiphophorus maculatus* fed with olive oil enriched *Artemia parthenogenitica* (value expressed in percentage)

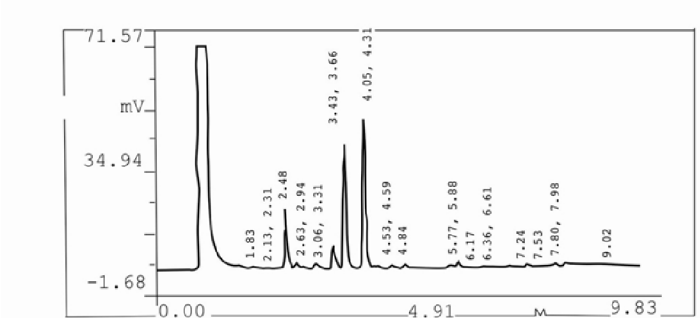


Fig. 2. Fatty acid composition of *Xiphophorus maculatus* fed with Sardine oil enriched *Artemia parthenogenitica* (value expressed in percentage)

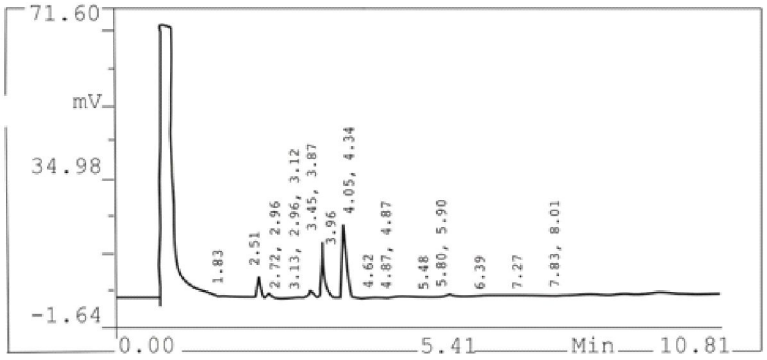


Fig. 3. Fatty acid composition of *Xiphophorus maculatus* fed with Shark oil enriched *Artemia parthenogenitica* (value expressed in percentage)

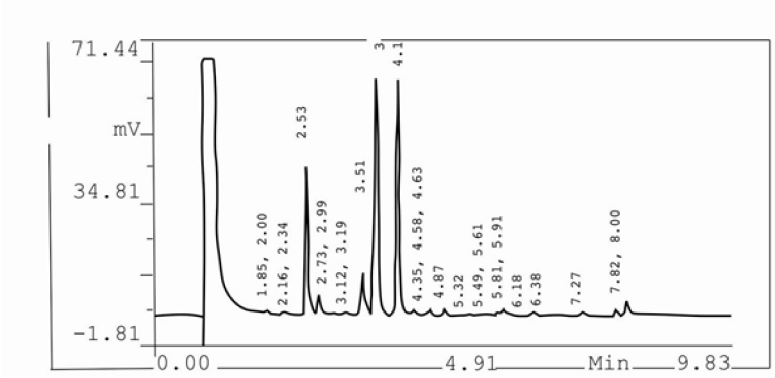


Fig. 4. Fatty acid composition of *Xiphophorus maculatus* fed with un-enriched *Artemia parthenogenitica* (value expressed in percentage)

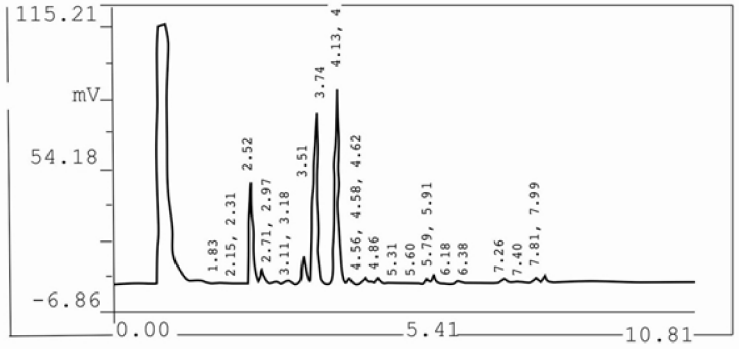


Fig. 5. Fattyacid composition of *Xiphophorus maculatus* fed with Corn oil enriched *Artemia parthenogenitica* (value expressed in percentage)

Table 3. ANOVA comparing length of the fishes between treatment days **(**Overall test of model for Y=Length, Test of effects for Y=Length)

Sources	Type111 SS	Df	Mean Sq.	F
Model	104647.5	19	55074.08	1013.584*
Error	15214.07	280	54.336	-
Total	1061622	299	-	-
Treatment	13214.13	4	25803.5	474.889*
Days	933199.5	3	311066.5	5724.872*
Treatment* Days	9993.87	12	832.823	15.327*

*significant (p<0.05)

Table 4. Mean (+_SE) value of length of fishes in un-enriched and enriched *Artemia parthenogenetica* fed fishes

	Treatments				
Days	Control	Olive oil	Corn oil	Sardine oil	Sharkliver oil
	Mean+_SE	Mean+_SE	Mean+_SE	Mean+_SE	Mean+_SE
15	96.11 +_1.11	140.00+_1.206	120.0+_1.959	129.4+_1.11	118.33+_2.025
30	100.00 +_1.627	163.889+_2.100	127.77+_2.656	127.77+_2.656	123.3+_3.832
45	156.667+_2.329	226.667+_2.329	173.889+_2.801	202.2+_1.514	170.0+_1.361
60	236.667+_.709	285.00+_0.690	256.889+_0.524	265.00+_0.586	256.66+_0.552

Table 5. Post Hoc test of comparing length in different oil enriched *Artemia parthenogenetica* fed fishes

Test	Group1	Group2	Mean Diff	Q
Tukeys test (Analysis of Multiple comparison in statistics)	Control	Shark oil	-19.722	20.725
		Sardine oil	-33.75	35.465
		Corn oil	-22.278	23.41
	Shark liver oil	Olive oil	-56.528	59.401
		Sardine oil	-14.028	14.741
		Corn oil	-2.556	2.685
		Olive oil	-36.806	38.676
	Sardine oil	Corn oil	11.472	12.055
		Olive oil	-22.778	23.936
	Corn oil	Olive oil	-34.25	35.991

Table 6. ANOVA comparing weight of the fishes between treatment days(Overall test of model for Y=Weight, Test of effects for Y=Weight)

Source	Type111 SS	Df	Mean Sq.	F
Model	961040.702	19	50581.09	3165.377*
Error	4474.255	280	15.97	-
Total	965514.957	299	-	-
Treatment	54548.246	4	13637.06	853.411*
Days	891814.553	3	2972.518	18603.324*
Treatment* Days	14677.903	12	1223.159	76.546*

Table 7. Mean (+_SE) value of weight of fishes in un-enriched and enriched *Artemia parthenogenetica* fed fishes

Days	Treatments				
	Control	Olive oil	Corn oil	Sardine oil	Sharkliver oil
	Mean+_SE	Mean+_SE	Mean+_SE	Mean+_SE	Mean+_SE
15	114.524+_2.258	129.932+_0.23	123.33+_0.153	127.619+_0.263	124.184+_0.263
30	146.395+_2.019	180.102+_0.459	157.286+_0.894	175.034+_0.644	166.361+_0.455
45	183.435+_1.427	232.177+_0.208	215.570+_1.377	222.143+_0.806	210.578+_1.006
60	233.673+_124.184	295.156+_1.143	280.272+_0.855	288.776+_0.918	256.667+_0.552

Table 8. Post Hoc test of comparing weight in different oil enriched *Artemia parthenogenetica* fed fishes

Test	Group1	Group2	Mean Diff	q
Tukeys test (Analysis of Multiple comparison in statistics)	Control	Shark oil	-19.94	38.639
		Sardine oil	-33.886	65.662
		Corn oil	-24.594	47.656
	Shark liver oil	Olive oil	-38.835	75.252
		Sardine oil	-13.946	27.023
		Corn oil	-4.653	9.016
		Olive oil	-18.895	36.613
	Sardine oil	Corn oil	9.293	18.006
		Olive oil	-4.949	9.59
	Corn oil	Olive oil	-14.241	27.596

Table 9. Growth estimates of *Xiphophorus maculatus* fed with commercial oil emulsions and control

	Olive oil enrichment	Corn oil enrichment	Sardine oil enrichment	Shark liver oil enrichment	Control
Increase in biomass	1.91 ± .007	1.758 ± 0.001	1.85 ± 0.010	1.824 ± 0.0427	1.318 ± 0.01
Increase in biomass %	194 ± 0.987	175 ± 0.087	188.7 ± 0.03010	186.12 ± 0.48	134 ± 0.521
Specific growth rate	3.18 ± 0.010	2.93 ± 0.17	3.08 ± 0.019	3.04 ± 0.005	2.196 ± 0.002

the early stage of larval development (Van Stappen, 2007). In the present study, the growth and survival rate of fish larvae of *Xiphophorus mauculatus* enrichment with different commercial oils were improved (Chang and Southgate, 2001).

CONCLUSIONS

The ornamental fish *Xiphophorus maculatus* was fed with different commercial oils-enriched *A rtemia parthenogenetica* nauplii. Olive oil enriched *Artemia parthenogenetica* nauplii and fishes fed to them brought out increased length, weight, survival rate, and growth estimates compared with other oil enrichments and control. Similarly fatty acid analysis of olive oil enriched *Artemia* nauplii- fed fishes showed a high rate of linoleic acid, EPA and DHA. The linoleic acid is very important because it promotes larval growth in fishes. EPA and DHA are important polyunsaturated fatty acids, which when given as feed to *Artemia* nauplii, arehowever incapable of synthesizing highly polyunsaturated fatty acids. So the need is to feed them on enriched oils which contain a high level of PUFA. DHA has high biological value during larval development and is selectively incorporated into neural tissue for contributing pigmentation. The

above mentioned oils, such as olive oil, corn oil, sardine oil and shark liver oil, particularly rich inDHA, EPA, oleic acid and arachdonic acid are required for the growth and survival of larvae as well as for contributing to egg and sperm quality when used in brood stock for inducement of breeding.

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