Int Aquat Res (2009) 1: 67-72
ISSN 2008-4935
Available online at www.intelaquares.com

International
Aquatic Research

Effect of diets containing artemia enriched with unsaturated fatty acids and vitamin C on angel fish *Pterophyllum scalare* propagation

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Received: 10 August 2009; Accepted: 5 November 2009

Abstract

Artemia urmiana was added to diets of angel fish *Pterophyllum scalare* and various factors including fecundity, fertilization rate, hatching rate and larvae survival rate and interval between spawning and total time for the eight spawning cycles were determined. Broodstock angel fish (18 pairs) were kept separately in a 50 L aquarium. Diets containing artemia and fatty acids were most effective with the average rate of fecundity of 378 eggs, 98.9% fertilization, 97.5% hatching for fish fed the live *Artemia urmiana* + fatty acid diet. *Artemia urmiana* + fatty acid emulsion + 0.5 g vitamin C resulted in the highest larvae survival (93.3%) which was also significantly higher than the control group ($P < 0.05$). The shortest interval for the eight spawning cycles (43 days, 6 day intervals) was for fish fed the *Artemia urmiana* + fatty acid + 1 g vitamin C diet. Feeding live food reduced the time for the eight spawning cycles evaluated and increasing fecundity, fertilization rate, hatching rate and larvae survival rate. Adding *Artemia urmiana* decreased this time between spawns and would be a useful ingredient to include in diets for broodstock in continuous spawning operations and is likely to be economical for broodstock operations because of increased efficiency even though the feed cost would be higher.

Keywords: *Pterophyllum scalare*, Enriched *Artemia urmiana*, Propagation, Unsaturated fatty acid, Vitamin C

Introduction

Ornamental fishes are popular pets around the world and their cultivation can be quite lucrative. Fresh water ornamental fishes are found in different areas in the world and the aquarium industry has developed methods for cultivation and propagation of many common species. Angel fish *Pterophyllum scalare* is native of Central America and can live and adapt to a variety of climate or environmental conditions. This fish is commonly sexually mature at less than one year of age. Its life spawn depends upon how well proper culture conditions are maintained, but it seems that this fish would survive for 2-3 years under ideal conditions. It can spawn 10-15 times on average, each time producing hundreds of eggs. However, brood stock tend to become weaker after continuous spawning, and proportionately the fecundity rate, fertilization rate, hatching rate and larvae survival rate are reduced. Brood stocks are one of the most valuable assets for an ornamental hatchery, so any reduction in their fertility rate or larvae survival can influence the hatchery output. A common strategy has been to obtain the maximum larvae production over the shortest period of time.

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Diet plays a critical role for broodstock fecundity. Brood stock fed live food often perform better because the live food may have greater nutritional value, be more palatable and easier to digest and can positively influence gonadal development and propagation. Successful fish production depends upon access to suitable food for feeding that is healthy and supports growth for brood stock and for the newborn stages for the larvae (Lim 2002a). Fish breeding requires suitable food with high quality that it is accepted and digested easily by the fish (Lim 2002b). Using live food has many advantages in fostering shrimp larvae, fish and salt water fishes and ornamental fishes. Among numerous sources and variety in live food, *Artemia urmiana* is particularly important. *Artemia urmiana* can be used as carrier of some nutritive materials such as long chain unsaturated fatty acids and vitamins especially vitamin C (Coutteau et al. 1997). Researchers indicate that fatty acids play a critical role in the reproductive physiology of teleost fishes (Kim et al. 1996) such as maintaining immune function, osmoregulatory systems and endocrine system function. Moradkhani (2008) and Javaheri (2006) conducted similar tests using *Cichlasoma severum* and *Salmo trutta Caspius* fed-enriched *Artemia urmiana* that has been fed fatty acids and vitamin C.

Vitamin C plays an important role in maintaining immune response and is required for numerous biological functions in fish and other vertebrates, for example, maintaining skeletal integrity, growth and survival and physiologic coefficient such as resistance against stresses, poisoning and immune activities improve in different species of aquatic larvae with usage of vitamin C complements (Dhert and Sorgeloos 1995).

In this research the affect of feeding angel fish broodstock artemia (*Artemia urmiana*) which had been specifically fed an emulsion of unsaturated fatty acids and vitamin C was tested to determine if feeding these enriched artemia would improve the efficiency of propagation of angel fish.

Materials and methods

Broodstocks were the eighteen pairs of fish with 9-10 months of age and 8-11 cm in length. They were divided into 6 treatment groups and each treatment replicated 3 times. Selected pairs were ready to spawn. The ideal spawning conditions involved separating brood stock from the colony (100 fish in two aquariums with 500 liter of water) and placing each breeding pair into a separate aquarium with 50 liter of aerated, dechlorinated municipal water (28.0 ± 0.1^{o}C, pH= 7.5 ± 0.5, and hardness > 170 ppm). To remove chlorine, water for the aquaria was drawn from 1000 liter tanks which had been intensely aerated for at least 24 hours. To stimulate spawning, a ceramic plate was placed at an angle of 45° against an inside wall of the aquarium to stimulating spawning and provide a locus for egg deposition. Selected brood stock fishes were acclimated on the treatment feed for at least during 7 days before the first experimental spawning.

After spawning, eggs were incubated in a 30 liter aquarium by placing the ceramic plate containing the eggs into it. An antifungal drug (1 ppm Methylene blue, Merck, Frankfurter, Germany) used to the aquarium containing the incubating eggs.

This research was conducted over 90 days period until the end of the 8th spawning in last pairs of fishes with similar conditions maintained for each tank. Fish diets for the various treatments included a control diet which was a common concentrated food for ornamental fishes (crude protein 40%, crude fiber 5%, crude fat 6%, moisture 12%, ash 10%) (Energy Company, Thailand).

Artemia urmiana were recovered from Lake Urmia in Iran located in Azerbaijan province and in geographical location of 37 -38/30 north width and 45-46 east length. This lake has 102 large and small islands.

Live *Artemia urmiana* was transferred to the ornamental fish farm and enriched in 1.5 L dishes with 33 g/L salt, 28 °C and under mild aeration. *Artemia urmiana* does not do well with intense aeration and this leads to higher losses. The density of the *Artemia urmiana* was 200 animals per liter when fed to the fish. To produce the artemia high in unsaturated fatty acid the following feeding regime was followed: *Artemia urmiana* was suspended in a small tank containing an emulsion INVE Co (ICES 30/4/C, INVE Co., Belgium) which consist of 30% unsaturated fatty acid methyl esters at 30% dry weight of the emulsion). This emulsion was 4 to 1 of dicosahexenoic (DHA) and eicosapentaenoic (EPA). Preparation of standard emulsion is done on the basis of Leger's formulation 1989 (Lim 2003). Five ml of the fatty acid emulsion was blended with 50ml dechlorinated fresh water and blended in an electric blender for 3 minutes at room temperature. For *Artemia urmiana* enriching with fatty acids and vitamin C, ascorbyl palmitate was added at a ratio of either 10% or 20% of the fatty acid emulsion as proposed by (Merchie et al. 1995, 1997) and Agh and Sorgeloos (2005). The ascorbyl palmitate at 0.05 g or 1 g per 5 ml of emulsion was blended with 50 ml of water for a total of 55 ml. Five ml of this blend was then added to the 1.5 L tank for enrichment. These prepared emulsions blends were stored in tightly sealed containers which excluded light under a nitrogen head space to limit oxidation and the suspension kept in the refrigerator for up to 10 days until it was used for enriching the *Artemia urmiana*. For each spawning cycle a new batch of enriched artermia was prepared.

For artemia enrichment, 2ml of one of the emulsions described above was added to per 1liter of water (Leger et al. 1987; Coutteau and Sorgeloos 1997). After 12 hr, enrichment solution effective saturation of the artemia was achieved. A treatment of 4 ml per liter water was added with the *Artemia urmiana* and incubated for 24 hr. After 24 hr, the enriched *Artemia urmiana* were recovered. At this point, we wrapped the artemia in a clean and wet cloth and held them in a refrigerator (10°C) until use. Holding the artemia in this manner kept them in a dormant states and from utilizing the fatty acids and vitamin C. The enriched artemia were fed within 24 hours to the broodstock angel fish.

Brood stocks were feed 4 meals a day at 7:00, 12:00, 17:00, 23:00 hours. The control group was fed with a commercial concentrated food using the same feeding schedule. We determined in preliminary experiments that angel fish was fed to satiation at an intake of 35-45 mature *Artemia urmiana* per day. As a result, we fed 40-50 mature *Artemia urmiana* to each angel fish daily or 10-12 *Artemia urmiana* at each feeding.

For determination of fecundity after the end of spawning, we removed the ceramic plate from brood stock aquarium and replaced it with a new ceramic plate for next spawning cycle. We counted the eggs that were attached to the ceramic plate carefully and quickly and then transferred them immediately to an incubation aquarium and added methylene blue (1 ppm) to water in the aquarium. This level of methylene blue caused that color of water becomes dark blue and light transmission to the eggs was reduced. We also placed the aquarium containing the eggs into an area with less light so that there was no direct light shining on the egg in the aquarium.

Table1. Description of diet test treatments

Treatment No.	Type of feeding	Type of enrichment
1	Concentrated food* (common concentrate food for ornamental fishes (40% protein) that is made of Energy Company of Thailand)	-----
2	Concentrate food + mature *Artemia urmiana*	-----
3	Ripe *Artemia urmiana*	-----
4	Artemia + fatty acids	Unsaturated fatty acids (5 ml in a liter water containing 200 *Artemia urmiana*)
5	Artemia +fatty acids + vitamin C (0.5)	Unsaturated fatty acids (5 ml in a liter water containing 200 *Artemia urmiana*) + 0.5g ascorbic acid
6	Artemia + fatty acids + vitamin C (1.0)	Unsaturated fatty acids (5ml in a liter water containing 200 *Artemia urmiana*) +1g ascorbic acid

Notes:
* Chemical composition of the commercial concentrated fish food (Energy Co., Thailand) for ornamental fishes: crude protein 40%, crude fiber 5%, crude fat 6%, moisture 12%, ash 10%, (Ingredient materials: fish flour, corn gluten flour, fish oil, calcium phosphate, vitamin and mineral mix).

After 6 hours after transferring the eggs to the incubation aquarium, the number of unfertilized (white eggs) was counted. The rate percentage of fertilization eggs was calculated. In 48 hr, the rate of hatching was determined by counting the total number of hatchlings and the number of unhatched eggs. Since eggs detach from the ceramic plate after hatching and drop to aquarium floor, any eggs remaining on the plate after 27 hours were counted as unhatched eggs. After 4 to 5 days, the number of actively swimming larvae was counted. This provided data for computing larvae survival rate.

To determine uptake of long chain fatty acids in the enriched *Artemia urmiana*, fatty acid profiles were measured (n=3) by the gas chromatography system (GC). Data were analyzed by analysis of one-way ANOVA, and Duncan’s test in $P < 0.05$ for comparison of averages using SPSS 16 software.

Results

The average of EPA and DHA content is presented in Table 2. The concentration of EPA and DHA in the artemia increased after enrichment with the emulsion from 3.48-3.82g and 6.89-6.77 g percent of the total fatty acids and vitamin C for EPA and DHA respectively.

For preparing samples for chemical testing, *Artemia urmiana* is tested after enriching immediately. The level of EPA was 1/25(%) and DHA was 1/59(%) in concentrate food. The comparison in fecundity, fertilization percent, hatching percent and larvae survival and total time for spawning cycles is shown in Table 3 for the eight spawning cycles.

Table 2. EPA and DHA fatty acid concentration in different diet treatments (percentage of total fatty acids)

	Unenriched Artemia	Artemia + unsaturated fatty acid	Artemia + unsaturated fatty acid + 0.5g vitamin C	Artemia + unsaturated fatty acid + 1g vitamin C
EPA (%)	1.92	2.65	3.48	3.82
DHA (%)	0	2.94	6.89	6.77

In the control treatment for the second repetition for the 5th spawning, no fertilized eggs were recovered. As result, all values were set to zero for this treatment. This is the reason for the wide variation in experimental data for the control treatment.

As shown in table 3, treatment 4 (live artemia enriched with fatty acids) had the highest average fecundity (378 eggs), 98.9% fertilization average, 97.5% hatching average had the most output in comparison with other treatments. Adding vitamin C did not increase egg fertility or larval survival but did decrease the days before the next spawning cycle and the number of days the fish spawned.

Table 3. Average reproductive parameters for the eight spawning cycles

	Treatments					
	1*	2	3	4	5	6
Fecundity	258.5±150.5[a]	318±132.5[b]	370.7±133[c]	377.9±185[c]	318.6±120 [b]	371.6± 148.5[c]
Fertilized egg %	91.6± 50[a]	95.4± 3.4[b]	98.4± 2[c]	98.88± 5[c]	98.8± 3[c]	97.4± 5.17[c]
Hatched egg %	85.4± 44.1[a]	92.7± 7[b]	96.4± 3.9[c]	97.5± 8.7[c]	96.8± 5.17[c]	94.8± 6.8[c]
Survival rate %	74.9± 46 [a]	87.4± 10.4[b]	92.3±17.7[c]	93.2±12.3[c]	93.3± 7.6[c]	93± 5.7[c]
Time between continuous spawning (days)	8.1± 1.5[a]	7.4± 1[b]	6.85± 1[c]	6.1± 1[d]	6.2± 1[d]	5.9± 1[d]
The eight spawning times (days)	58.3± 1.5[a]	53± 1[b]	49± 2.5[c]	44.6± 1.5[d]	44.6± 1.5[d]	43± 1[d]

Notes:
*1= Concentrated food (control treatment), 2= Concentrate food + live ripe Artemia, 3=live ripe Artemia, 4=Enriched live ripe Artemia + unsaturated fatty acid, 5= Enriched live ripe Artemia + unsaturated fatty acid + 0.5g Vitamin C, 6= Enriched live ripe Artemia + unsaturated fatty acid + 1g Vitamin C.

Discussion

The effect of enriching live artemia with long chain unsaturated fatty acid and Vitamin C is has been evaluated in different species of fishes and shrimp in salt and fresh water, but only in a few ornamental fishes. Similar research with *Acipenser* sp., Indian white shrimp post larvae *(Tenaeus indicus)*, rainbow trout larvae *(Oncorhynchus mykiss)*, giant freshwater prawn (*Macrobranchium rosenbergii*) and milk fish larvae *(Chanos chanos)* reported and that enriched artemia with unsaturated fatty acid and Vitamin C increased larvae survival rate (Dhert et al. 2004; Gapasin 1998; Girri et al. 2002; Lim 2001; Lim 2002a). Here we saw an increase in survival between the control and the treatments using fatty acid enrichment, but adding vitamin C in addition to the unsaturated fatty acids did not appear to increase survival.

Moradkhani (2008) studied Cichlasoma *severum* and noticed effects of using live food include enriched foods on increased fecundity. Also, Tamaru et al. (2003) noted that the use of live food for angel fish brood stock and *Carassius auratus* increases fertilization percent. Lim et al. (2003), Tamaru et al. (2003), Dhert (2004) and Moradkhani (2008) researched feeding for *Poecilia reticulata, Xiphophorus helleri, Xiphophorus maculates, Poecilia sphenops, Hyphessobrycon herbertaxelrodi, Cichlasoma severum, Carassius auratus* and *Symphysodon aequifasciata* and found that enrichment of *Artemia urmiana* with unsaturated fatty acid and ascorbic acid and its use in feeding of brood stocks improved larvae survival rate.

Adding *Artemia urmiana* with unsaturated fatty acids and Vitamin C to broodstocks diets improved larval survival rate (Moradkhani 2008; Lim 2001a; Lim 2001b; Merchie 1997; Sorgeloos 1980; Tamaru et al. 2003). We also found that fecundity increased with live and enriched diets compared to controls ($P < 0.05$).

Significant differences were observed in hatching percent between all treatments and the control group. Using alive artemia enhanced propagation ($P < 0.05$) supporting the findings of earlier researchers. This is likely due to the higher concentration of unsaturated fatty acids in diet treatments. About larvae survival rate, acquired results showed that all treatments have significant difference with testimonial group in fecundity rate ($P < 0.05$). Since Vitamin C decreases stress, as result; decreasing effect of environmental stresses on larvae cause to increase its survival in treatment which had been enriched with ascorbic acid. Increasing unsaturated fatty acid and vitamin C content in the parents' food could transmit beneficial effects to the gametes through the fertilized eggs enhancing survivability and increasing the strength of the resulting larvae.

Moradkhani (2008) in similar research on *Cichlasoma severum* reported using live artemia in broodstock diets and observed a shorter interval between spawning cycles. Also, he reported that enriching artemia with unsaturated fatty acids and ascorbic acid resulted in a significantly shorter time interval between spawning compared to using artemia alone. Time between spawning cycles was more consistent for breeding pairs that had been fed higher levels of vitamin C ($P < 0.05$); this may possibly have reduced impacts from environmental stress.

Using live food such as artemia instead of a commercial concentrate increases fecundity and decreases the spawning cycle and spawning intervals. Enriching the artemia by feeding them to increase the EPA and DHA content and incorporate additional vitamin C increases egg hatch and larval survival. Improving efficiency of hatchery operations would make them more economically viable and also increase the number of offspring from breeding pairs of angel fish.

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