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Effects of *Daphnia magna* and *Artemia* Nauplii on Growth Performance in Persian Sturgeon *Acipenser persicus* Larvae

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Abstracts: This study was carried out in a completely randomized design 3×3×4 factorial arrangement in three periods of rearing in spring 1997. Density of 300 larvae was assigned for each oval tank with 0.1m^2 surface area, 30cm deep, a volume of 20lit and 0.25 lit/min of water flow per minute at Shahid Rajaie fish breeding and culture center in Sari, north of Iran. The fries were divided into three groups according to their feeding regimes and fed with each of *Artemia or D. magna* speratley, and mixture of the two (50% *Artemia*+50% *Daphnia*) in ratioes of 80, 100 and 120% of their body weights in the first period and 30,50,70% of their body weights in the second and third period of rearing. Each period lasted 72 hours. The result indicated, significant differences ($p<0.005$) between food organisms, body weight gain and Food conservation Ratio (FCR) in the first and third period of rearing. The highest body weight gain was found in 120% food ratio in the first period. The best survival rate achieved with fries feeding at 70% body weight of *Artemia* nauplii and mixed food in the second period of rearing. The highest growth rate, survival rate and the best FCR obtained when *Artemia* fed as live food to fries. There were no significant differences between *Artemia* and mixed food in this study. It seems that mixed food (50% *Artemia* +50% *Daphnia*) given at 70% of larval body weight results in a better performance.

Key Words: *Daphnia magna ,Artemia ,Acipenser persicus*, Growth performance

Introduction

Breeding and cultivation of the sturgeon have been carried out in southern part of the Caspian Sea since 1971 and in the present time there are five special hatcheris located on the Iranian coast involving with this activities (Shabanpour, 1998).

Live foods are important diets on initial feeding of sturgeon larvae and are been used with different composition as supplementary diet to achieve different chemical composition in the diet (Kohneshary & Azari, 1974).

The living organisms that are generally used as diets are *Daphnia sp*., Cyclops, Rotifers, Oligochate, Chironomids, Gammarids and *Artemia*. Among the above mentioned live foods, the *Daphnia sp.,* Oligochate and *Artemia* are considered as the best and the majority of fish farms try to cultivate *D. magna* in concrete tanks or earthen ponds. Generally, the fish farmer uses the *Artemia* nauplii after hatching cysts in proper environmental conditions (Kohneshary & Azari, 1974).

When the yolk sac of fish larvae is completely absorbed, the larvae begins its exogenous feeding and its digestive system will be able to digest and absorb food materials. The timing of initial feeding begins with the excretion of a black material called "melanin plug" from the larvae gut (Gisbert & Williot, 1997). At the beginning of the exogenous feeding the sturgeon larvae usually weighs about 40 mg, and the swimming ability of larvae is improved greatly (Kohneshary & Azari, 1974).

Kohnehshahri and Azari (1974) showed that the food conversion rate of sturgeon larvae fed with *D. magna* and *Artemia salina* was 5-6 and 4 respectively. They also suggested the feeding ratio of 100-120% the body weight per day at the start and decreasing down to 50% at the end of the larvae culture in the Veniro tanks. Prior to external feeding, the larvae feed on yolk sac (endogenous feeding). After a few days, depending on the water temperature and during the endogenous feeding larvae intends to feed on live food organisms (exogenous feeding) (Kohnehshary & Azari, 1974). The Persian sturgeon larvae usually begin endoexogenous feeding 10-12 days after hatching at the water temperature of 17-22°C (Shabanpour, 1998).

The brine shrimp (*Artemia sp.*) is regarded as a valuable live food for feeding of varieties of fish larvae, shrimps, mysis and mysid. Due to its availability

(Watanabe 1987), ease of hatching (Sorgeloos *et al.*,1983) and its packing potential, dormant cysts of *Artemia* are used as a good source of food for larvae. Enrichment of *Artemia sp.* has been used by several researchers to culture aquatic animals (Lussier *et al.*,1988; Kuhn *et al.*, 1991; Domingues *et al.*, 1999).

Watanabe (1987) indicated that due to high digestibility and sufficient Fe-content, *Artemia* is a good food for feeding the fish larvae. Leger *et al.* (1987) recommend *Artemia* for its bright color, constant motion, low speed and simple method of culture, as an important food for fish larvae.

D. magna can also be used as live food for sturgeon larvae because of it's high protein value and effective aid in digestion. The objectives of the present study were to determine the FCR, body weight gain and survival rate of Persian sturgeon larvae, fed by *Artemia* and *Daphnia magna* as food source in small-scale oval tank condition during early life stages.

Materials and Methods

The present experiment was carried out in a flow-through culture system at Shahid Rajaii Sturgeon Hatchery in southeast of the Caspian Sea, during March and April 1997. Persian sturgeon larvae were obtained from a batch of eggs of a single female in each period. After absorption of the yolk-sack contents, the larvae were fed with live *Daphnia magna* and *Artemia*. *Daphnia magna* of about1mm size were obtained from a stock produced in an earthen pond. The *Artemia* nauplii prepared from cysts collected from a natural water body stock of Fars province (south of Iran).Two grames of cysts of *Artemia* was added to one liter of water with the salinity of 25ppt and then transferred to 100lit zug jar. After 16-24 hours about 70% of the cysts in 29°C hatched to nauplii and fed to the larvae. Three hundreds of larvae were transferred to each oval tank with 0.1m^2 surface area, 30 cm deep, and water volume of 20 liters and 0.25 liters per minute. Water flow water supply was from the same source as the hatchery was supplied with a temperature ranged between 16-20°C.

The study was carried out in a completely randomized design as a 3×3×4 factorial arrangement with three replicate and repeated in three periods. The larvae were divided into three groups according to their feeding regimes and fed with *Artemia*, *D. magna* and a mixture of the two (50%*Artemia*+50% *Daphnia*).

The average initial weight of larvae in three periods of experiment was 64.4±4.1mg. The daily feeding rate during the first periods was 80, 100 and 120%, and during the second and third periods daily feeding rate was 30, 50, and 70% of body weight. A reserve group was also established to provide replacement for the likely mortality of larvae during the study and daily weighting of larvae instead of handling and imposing stress upon other tanks. As a result a total of 36 tanks were used in each period.

After weighing and released larvae were into the tanks. Daily feeding ratio was determined according to the daily weight measurement of fishes weight and feeding conducted was four times a day during the experiment. At the end of each feeding day 20-25 larvae were caught from the appropriate reserve tank, dried and weighted to the nearest 0.1 gramme, and then the average daily weight gain calculated. Daily mortality counted in each tank and dead fries were replaced with the new ones from the reserve tank to keep the density at 300 individual constantly per tanks. Each period lasted for 72 hours and the water temperature was measured four times a day during feeding time. The food conversion ratio (FCR) was calculated as fallows:

$$FCR = \frac{\text{The amount of food consumed (gramme)}}{\text{Total weight gained (gramme)}}$$

All data were subjected to one-way analysis of variance and Duncans Multiple Range test (Duncan, 1955) to determine significant differences, MSTATC-test software. The mean, standard deviation and standard error of the mean were calculated using the minitab software.

Results

The average initial weight of larvae and water temperature during the first period were 63.1 mg. and 16°C respectively. The final weight of larvae after feeding at three levels of 80, 100 and 120% of body weight, fed with *D. magna*, *Artemia* nauplii and mixture of these two, were measured 89.9, 86.1, 94.2 mg, 107.3, 108.3, 108.5 mg and 108.2,103.8, 110.4 mg, respectively.

The average initial weight of larvae and water temperature for the second period were 61.2 mg and 18.2° C, respectively. The final weight of larvae after feeding at three feeding levels of 30, 50 and 70% of bodyweight, fed with *D. magna, Artemia* nauplii and mixture of the two, were 73.6, 81.2, 85.0 mg, 105.7, 113.5, 108.3, mg, and 92.1, 96.7, 111.7 mg, respectively.

Due to shortage of sturgeon fries at the third period, fries from two brooders were used. The average initial weight of larvae in third period for those fed with *D. magna* was 76.0 mg and for those fed with *Artemia* and mixture, was 65.5mg, respectively. The average of water temperature during this period was 20°C.

The final weight of fries after feeding at three feeding levels of 30, 50 and 70% of body weight, fed with *D. magna, Artemia* nauplii and mixture were 81.2, 85.2, 90.5 mg, 96, 113.1, 114.6mg and 84.6, 96.2, 97.4 mg, respectively.

Because of excessive food availability to fries in the first period, there were no significant differences between the total weight gains of the larvae at assigned feeding levels. Consequently, the feeding levels decrease to 30, 50, 70, percent of body weight in the second and third period of experiment.

Significant differences ($p<0.005$) exist between food organisms, total body weight gain and FCR values in all periods of study. No significant differences between food organisms and survival number were found at the first and third period of the study (Table 1).

I-Effect of food organisms

A-Total weight gain

The highest biomass of larvae was obtained at the first period of study, when the excessive food was provided in each tank, and the lowest biomass was obtained at third period of rearing. Highest and lowest weight gains of 14.40 and 2.71gr were achieved when larvae fed with *Artemia* nauplii in the second and with *D. mana* in the third period of rearing, respectively (Table 2). .

On average the highest (13.520.85) gr, and lowest (5.450.50) gr, weight gain of larvae in three period of experiment occurred when using *Artemia* nauplii and *D. magna* (Fig. 1).

Table 1: Analysis of variance for FCR, survival rate and total body weight gain of Persian sturgeon fed by Artemia and *D. magna*

Period	Mean square(MS) of Specifications			DF	SOV
	FCR	Survival rate(%)	Total body weight gain (gr)		
I	0.96^{ns}	155.81^{ns}	2.59^{ns}	2	Repeat
	15.51^{***}	200.84^{ns}	86.76^{***}	2	Food organisms
	7.30^{***}	81.93^{ns}	5.06^{ns}	2	Feeding levels
	0.73^{ns}	24.15^{ns}	1.23^{ns}	4	F.organ×F.levels
	0.46	59.52	1.77	16	Error
	12.84	2.95	11.46		CV%
II	0.42	49.37^{ns}	1.19^{ns}	2	Repeat
	20.35^{***}	501.93^{***}	180.71^{***}	2	Food organisms
	4.00^{***}	128.26^{***}	24.85^{***}	2	Feeding levels
	0.30^{ns}	50.65^{*}	7.48^{*}	4	F.organ×F.levels
	0.24	13.62	0.98	16	Error
	19.49	**1.29**	**9.41**		CV%
III	***10.08***^{ns}	***4.93***^{ns}	***3.86***^{ns}	2	Repeat
	318.48^{***}	8.04^{ns}	224.40^{***}	2	Food organisms
	5.69^{ns}	5.15^{ns}	41.45^{***}	2	Feeding levels
	8.92^{ns}	12.37^{ns}	3.10^{ns}	4	F.organ×F.levels
	6.39	10.01	1.1	16	Error
	37.95	**1.08**	**13.35**		CV%

ns= nonsignificant *= $p<0.05$ ***=$P<0.005$

SOV= Sources of Variation DF= Degree of freedom CV= Cofficients of variation

Table2: Average specifications in the experiment by Duncan-test

Period	Food organisms and ration amaunt	Total body weight (Biomass gr)	Survival (%)	FCR
I	*Daphnia magna*	8.02±0.7^{b}	86.7±0.73^{a}	6.68±0.62^{a}
	Artemia nauplii	13.45±0.09^{a}	85.56±0.13^{a}	4.51±0.54^{b}
	Mixed food	13.31±0.56^{a}	88.74±1.02^{a}	4.52±0.54^{b}
	80%	11.53±0.46^{a}	86.06±0.46^{a}	4.23±0.68^{b}
	100%	10.89±0.65^{a}	86.86±1.02^{a}	5.65±1.05^{a}
	120%	12.36±0.61^{a}	88.11±1.36^{a}	5.83±0.43^{a}
II	*Daphnia magna*	5.62±1.01^{c}	92.83±1.51^{b}	5.11±0.3^{a}
	Artemia nauplii	14.40±0.69^{a}	97.6±0.3^{a}	2.29±0.54^{b}
	Mixed food	11.67±1.78^{b}	96.11±0.57^{a}	2.77±0.34^{b}
	30%	8.78±0.27^{b}	94.50±2.28^{b}	2.73±0.97^{b}
	50%	10.78±0.19^{a}	95.20±1.12^{b}	3.37±0.83^{b}
	70%	12.14±0.23^{a}	96.83±0.87^{a}	4.07±0.81^{a}
III	*Daphnia magna*	2.71±0.80^{c}	97.53±0.54^{a}	13.49±1.56^{a}
	Artemia nauplii	12.71±1.78^{a}	97.43±0.23^{a}	2.47±0.32^{b}
	Mixed food	8.16±1.22^{b}	96.66±0.08^{a}	4.06±0.26^{b}
	30%	4.85±0.46^{b}	97.03±0.26^{a}	7.56±4.55^{a}
	50%	8.13±0.21^{a}	97.36±0.29^{a}	6.02±3.09^{a}
	70%	8.93±0.52^{a}	97.53±0.48^{a}	6.43±2.66^{a}

In each column the average with the same symbol showed no significant differences.

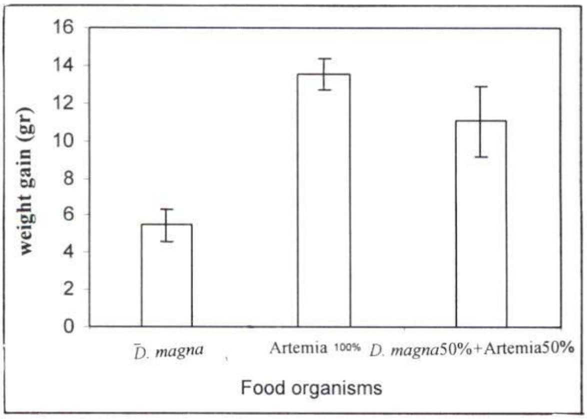

Fig 1: Food organisms and average weight gain with different live food in three periods of study

B- Survival rate

There were found no significant differences between using different live food and survival rates at the first and third period of study. Larvae fed with *Artemia* nauplii and mixed food at the second period of rearing showed no significant differences. The highest (97.6±0.3) and lowest (85.56±0.13) survival rates obtained at the second and the first periods of study, both fed with *Artemai* nauplii, respectively (Table 2).On average the highest (93.84±2.60) and lowest (92.35 ±3.13) survival rates of larvae in three periods of experiment occurred when using mixed food and *D. mana,* respectively (Fig. 2).

C-FCR

There were significant differences ($p<0.005$), between FCR and the kind of food organisms during all periods of study and the best and worst FCR (2.29±0.54 and 13.49±1.56) achieved by feeding the larvae with *Artemia* nauplii and *D. magna* in the second and third periods of rearing, respectively (Fig. 3).

On average the best and worst FCR of larvae in three periods has been found with *Artemia* nauplii (3.1±0.7) and *D. magna* (8.5±2.5), respectively.

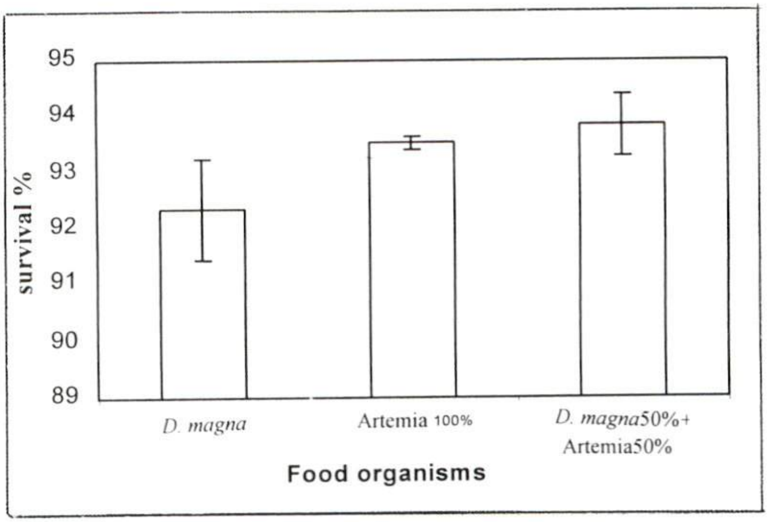

Figure 2: Food organisms and average survival rate (%) of larvae of Persian sturgeon with different live foods in three periods of study

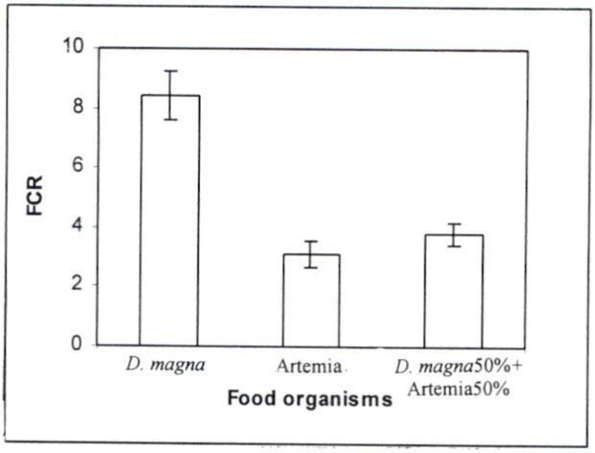

Figure 3: Food organisms and average food conversion ratio (FCR) of larvae of Persian sturgeon with different live foods in three periods of study

II-Effect of ration size

A- Body weight gain

No significant differences were found between body weight gain and ration size except for ration size of 30% of body weight at the second and third periods. The highest and lowest body weight gains12.36±0.61 and 4.85±0.46gr achieved by 120% and 30% of body weight feeding levels at the first and third periods of study (table 2). The highest weight increase was obtained at the highest ration levels in all periods of experiment (Fig. 4).

B-Survival

No significant differences were found between survival and ration size except for ration size of 30 and 50% at the second period of rearing (Table 2). The highest and lowest survival rates were achieved with 70% (97.26±0.3) and 80% (86.06±0.46) of ration size (Fig .5). The highest mortality, however, occurred in the first period of the study (Table 2).

C- FCR

No significant differences were found between FCR and ration size of 100 and 120% of body weight of the first period, 30 and 50% of body weight of the second period, and all ration sizes at third period of study (Fig. 6). The best and the worst FCR were obtained with (2.73±0.97) 30% and (7.56±4.55) 30% of ration size at second and third periods of study (Table 2).

On average in three period of experiment the best (4.23±0.68) and the worst (5.83±0.43) FCR were obtained with 80% and 120% of ration size respectively (Fig. 6)

Significant differences were not found between integration of food organisms × feeding levels on total weigh gain, survival rate and FCR at the first and third periods of the study. However, significant differences ($p<0.05$) were found between integration food organisms × feeding levels on total weight gain and survival number at the second period of study (Table 1). As shown, the larvae grew best when fed with *Artemia* nauplii (Fig.1) on 120% of ration size (Fig.4).

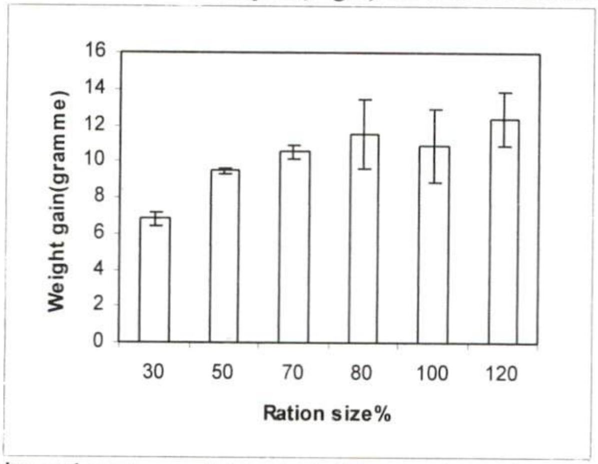

Figure 4; Ration size and average weight gain (gr) larvae of Persian sturgeon with different live foods in three periods of study

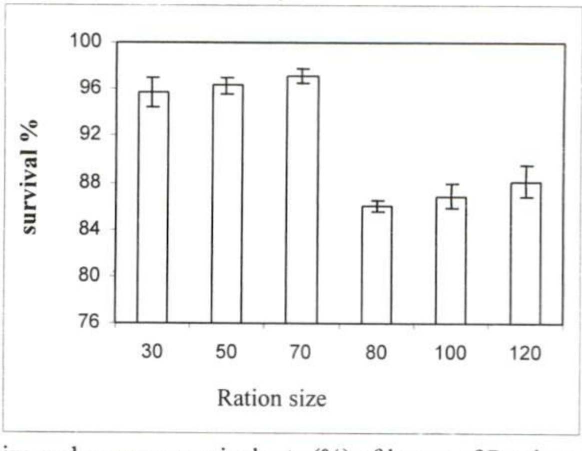

Figure 5: Ration size and average survival rate (%) of larvae of Persian sturgeon with different live foods in three periods of study

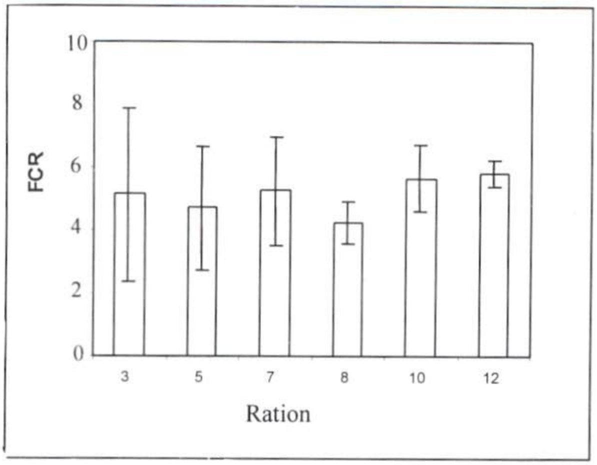

Figure 6: Ration size and average FCR of larvae of Persian sturgeon with different live foods in three periods of study

Discussion

It seems that the levels of feeding (80,100 and 120% of body weight) chosen at the first period were very high. An observation on the stomach of the larvae during the first rearing period and also remaining excessive food in the bottom of tanks indicated that large quantities of food wasted and washed away or caused pollution after decomposition, hence causing reduction in survival rate at the first period in comparison with the second and third periods. The mortality rate and FCR at the first period of experiment were very high and we chose to decrease feeding level at the second and third periods of experiment. According to Kohnehshari and Azari (1974), the food conversion ratio of Acipencerids larvae fed with *Artemia salina* and *D. magna* were 4 and 5-6 respectively. In another study, the FCR for Acipenserids larvae fed with *D. magna* and *Artemia* were 6 and 5 respectively (Azari, 1990). The same study reported that, the larvae fed with *Artemia* obtained higher body weight gain and higher survival rate than the larvae fed with *D. magna* or mixed diet (50% *Daphnia* + 50% *Artemia*). In an experiment in Rajaii Sturgeon hatchery, the calculated FCR for Russian sturgeon fed with *Artemia* and *D. magna sp*. were 6.15 and 11.1 respectively (Nazari, 1995). Kolkovski *et al*., (1993) at their experiment on Sea bass (*Dicentrachus labrax*) found that the live food, especially *Artemia* had an effective aid on digestive process as a result of amylase and tripsin content. In addition, they mentioned that addition of live food into artificial food of larvae increased the assimilation rate of 30%.

The FCR in Russian sturgeon (weighted 3.3-3.4 gr) fed with *Artemia* and *D. magna* were 6.15 and 11.1 respectively (Nazari, 1996). In the present study the minimum weight gain and maximum FCR observed in the third period of rearing when fishes were fed at 30% of body weight which means that there was sufficient food available, but the larvae were not be able to exploit it properly. Ability of larvae to consume and ingest different prey items depends on the water quality especially on temperature and dissolved oxygen (Huet, 1986). The size of mouth is also very important and determines the ability of the larvae to consume different food organisms (Huet, 1986). These mean if the size of prey is greater than the larvae mouth, they will starve and if the size of prey is very small the energy consumed for capturing a sufficient food will be very high. In this study, the size of *Artemia* and *D. magna* was less than 1mm, but the water temperature and oxygen level might have been varied. The water temperature at third period of rearing averaged 20°C, with constant water flow, which might have been too high for physiological activity, and so the larvae might have been. Our finding presents a useful guide for fish culturists to assess the quality and quantity of live foods, some new information was achieved which might be helpful for a better knowledge and understanding of Persian sturgeon in natural environments and provide insight into their ecology.

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