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Potential to use the native freshwater rotifer, *Brachionus calyciflorus* in feeding *Acipenser persicus* larvae

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ABSTRACT

Acipenser persicus is the most important sturgeon species in the south Caspian Sea region, showing high mortality during larval culture. The aim of this study was to use the freshwater rotifer, Brachionus calyciflorus to feed A. persicus larvae to improve survival rates and enhance resistance in these larvae at the onset of exogenous feeding. Three experimental groups were used in this study; Control group similar to the feeding protocol of the hatchery was initially fed decapsulated cysts of Artemia and then fed daphnia, group 1 was fed a mixed diet of decapsulated Artemia cysts, daphnia and freshwater rotifers, group 2 was fed only on freshwater rotifers, and group 3 was fed freshwater rotifers enriched with vitamin C (ascorbic acid 6-palmitate). A total of 45 larvae were stocked in each experimental tank (100 l capacity) filled with 30 l of water. Three replicates were used for each experimental group. Larvae were fed four times a day at the rate of 30% of body weight per day for 8 days. Mean dissolved oxygen, pH and water temperature throughout the experimental period were 9.58±0.2 mg/l, 8.5±0.1 and 22.5±0.5 ºC, respectivel y. DNA sequence of genome DNA extracted from the native freshwater rotifer under study was determined and registered. Results showed significant differences in specific growth rate (SGR), percentage weight gain (WG), feed conversion ratio (FCR), and condition factor (cf) of experiment group 1 and group 3. Survival rates of the experiment groups 2and 3 were significantly different from those of the experiment control group and 1. twenty first feeding day of the study showed ration oral size to body length was evaluated by (0 /1 ± 0/01) mm. This study demonstrated that Brachionus calyciflorus is a suitable live food for larval feeding and can be cultured, enriched and used along with other freshwater rotifers.

Key words: *Brachionus calyciflorus*, *Acipenser persicus*, fatty acid, vitamin C, survival rate, larval culture.

INTRODUCTION

Increasing survival rates and improving overall performance of artificial breeding and rearing programs can be considered effective in achieving sustainable use of sturgeons [1]. *Acipenser persicus*, a native species of Iran, shows the highest hatching rates. However, high mortality in larvae during early larval feeding and at the time of their release into the sea, is of major concern. In recent years sturgeon stocks and catch figures have dropped severely as a result of decreased available juvenile rearing habitat[2]. Persian sturgeon stocks, mainly belonging to the Iranian shores, play a significant role in the annual catch figures[3]. Sturgeon fingerlings confined in freshwater tend to lose their tolerance to salinity. Hence when releasing into the sea is the principle objective of artificial breeding programs, fingerlings should be fed with food items found in their natural environments to gradually increase their ability to hunt. Persian sturgeon larvae were fed freshwater rotifers enriched with vitamin C at the onset of exogenous feeding to meet their dietary requirements during larviculture. Success during early larviculture guarantees faster growth rates, better health and higher survival rates in fish in the later stages of growth and after their release [4]. Application of vitamins during larviculture makes fish resistant to diseases [5]. Rotifers are the most commonly used marine zooplankton as live feed for fish larvae cultures and an ideal feed source for large quantity fish cultivation [6].The freshwater rotifer, *Brachionus calciflorus*, in the ideal size range of180-220 microns [7] and with a high potential for reproduction appears to be an ideal live food for several freshwater species [8]. *Brachionus calciflorus* is one of the strong against environmental stress and to be able to ingest and survive on a diet of toxic *Microcystis aeruginosa* [9] Previous investigations have shown that even when using formulated diets, alternating between formulated diets and freshwater rotifers influences growth parameters and survival rates in fish [10]. Rotifers show a high potential for enrichment with lipids and vitamins [11][12][13] In the present study, pure semi mass cultures of freshwater rotifers, native to Iranian waters were obtained and enriched with ascorbic acid palmitate to improve survival and growth rates during early development and then used in the feeding trials.

MATERIALS AND METHODS

Fish supply and culture system

Acipenser persicus larvae with yolk sac were transferred to the laboratory at the Ghazian coast Research Station on 10 June 2008 in plastic bags with one-third water and two-thirds oxygen. Larvae were then stocked in fiber glass tanks after adapting to new conditions for three days. About 45 larvae were stocked in each 100 l tank filled two-thirds with water. Water entered the tanks from the top at a rate of 0.5 l/min and drained out at the bottom from the outlet installed with a filter. Throughout the 8 days culture period water temperature was 22±0.5 ºC, pH was 9.5±0.2 and dissolved oxygen was recorded as 8.5±0.12 mg/L. Biometry was performed every other day to determine the biomass. Three experiment groups were studied; Group 1fed a mixed diet of decapsulated Artemia cysts, daphnia and rotifer, Group 2 fed rotifers as the starter diet and Group 3 fed a starter diet of rotifers enriched with vitamin C. A control group was also studied which used the feeding protocol followed in the hatchery (first two days decapsulated Artemia cysts followed by daphnia along with micro planktons found in pond water. Three replicates were used for each experiment group.

Semi mass culture of freshwater rotifers and enrichment with ascorbic acid palmitate

Rotifers under study from the Anzali Lagoon were isolated and purified under a stereomicroscope and transferred to 20 ml test tubes containing EPA culture medium (96 mg $CaHCO_3$+ 60 mg $MnSO_4$+4 mg KCl in 1L water). Rotifers were fed algae at a rate of $1x10^6$ cells/ml). With an increase in biomass they were gradually transferred to 100 L tanks until they reached a stocking density of 640±40 ind/ml. To harvest the required weight of rotifers to feed sturgeon larvae, 100 rotifers were dried and weighed and the mean weight of each rotifer under the culture conditions was calculated. Rotifers used to feed larvae in Group 3 were enriched with ascorbic acid palmitate (Manufactured by Basel, Switzerland) for 24 h at a rate of 1000 mg/g dry weight of rotifers.

Algae culture

Mass culture of *Chlorella vulgaris* was carried out in plastic bags (7 L capacity) using culture medium Z-8±N [14] under continuous aeration, at a temperature of 25±1ºC and 3500±350 lux light.

Daphnia culture

Daphnia required for feeding larvae in the control group were cultured in outdoor concrete tanks using cow dung extract, while pure daphnia required for other groups were cultured in well water *in vitro* in 500 L tanks mixed with algae and protozoa.

Artemia culture

Decapsulated cysts of *Artemia parthenogenetica* (2 g/L) were placed into hatching containers (zougs of 100 L capacity) in salt water at a salinity of 25 ppt and water temperature of 29 ºC. After 36 h more than 70% of the cysts hatched into nauplii producing an average of 80000 nauplii/g cysts *Artemia* that were used to feed sturgeon larvae.

DNA extraction from freshwater rotifer

To register the race of freshwater rotifer native to Iran DNA was extracted from the freshwater rotifer *Brachionus calciflorus* following the phenol-chloroform method. The quantity of DNA was assessed using 1% Agarose gel electrophoresis and nanodrop instrument. PCR reaction was performed on a small quantity of diluted target DNA (100 ng) using 18 Sr DNA. The sequence obtained was as follows:

Forward; 5' GGTCAACAAATCATAAAGATATTGG 3'
Rev: 5' TAACTTCAGGGTGACCAAAAAATCA 3'

Thermal PCR cycles

PCR amplification was carried out in a DNA thermal cycler under at 65 ºC for 15 minutes and temperature was lowered to 56 ºC for annealing.

Statistical analyses

The data were statistically processed using software such as SPSS ver 13 and Excel. Data were analyzed for normal distribution and equal variance using the Shapiro Wilk test. One Way Analysis of Variance was used for normal distribution and in case of non-normal distribution Kruskal-Wallis One Way Analysis of Variance was used.

Study size of the oral

30 Larvae start feeding from 20 days of heaven sinks workshop was fixed within 20% formalin. Biocom software size measure oral Nikon camera(E-600) attached to the loop and the physical size of the oral Sturgeon Research Institute reviewed.(pic 1 & 2)

RESULTS

Highest final body weight, percentage weight gain and SGR and lowest FCR were recorded in experimental group 3 (Table 1 figure 1-6) followed by experimental group 1 ($P<0.05$), whereas lowest values for growth parameters were recorded in experimental control group. Highest value for condition factor (0.79±0.07) belonged to experimental group 3 while the lowest belonged to experimental group 1(Table 1 &figure7) ($P<0.05$).Survival rates recorded in experimental groups 2 and 3 were significantly higher ($P<0.05$) than those recorded in experimental group 1 and in the control group.

The study measured the size of the mouth showed that the size of the mouth to the body of the twenty first feeding day performance was evaluated by 0 /1 ± 0/01 mm was the length of the increase the size of oral feeding and weight gain during the process is shown in the table and figure below (figur 8 & 9) .

DNA sequence of a single sample of PCR product of the 18s rDNA belonging to *Brachionus calyciflorus* from the Anzali Lagoon was determined the length of which was estimated as 780bp. This was registered in the NCBI gene bank (bankit1256895 GQ502286) as a pure species of the Anzali Lagoon. To ensure To ensure the amplification of the 18s rDNA, the PC product was electrophoresed on a 1.5% agarose gel 9 pic,3).

DISCUSSION

The freshwater rotifer *B. calyciflorus* is widely used in aquaculture as a live food to raise larvae of many species during the first two weeks of exogenous feeding. No other live food has been developed for larval feeding that can replace rotifers [15].

The two types of protease enzymes in rotifers assist fish larvae to digest them [16]. Weight, rearing environment and feeding protocol are among the key factors affecting the migration and fishery returns of fish fingerlings produced by artificial breeding programs for the purpose of restocking and rehabilitation of stocks [17].

Gilber found that the geographical distribution of *B. calyciflorus* associated with their genetic differentiation is mainly due to the cosmopolitan behavior, body size and their high potential in aquaculture applications[18]. The genomic sequence extracted from the Iranian species and registered was 780 bp in length.

Growth trials in *A. persicus* larvae showed highest growth performance in experimental group 3 and lowest in experimental group 1. Suitable growth recorded in the control group may be because during early larval feeding in vniro tanks at the sturgeon hatchery, along with daphnia larvae were fed freshwater rotifers (*Brachionus calyciflorus, Keratella sp., Syncheata sp.,*

Asplanchna sp.) and copepod (nauplii *Cyclops, Cyclops sp*.) in high densities. Similar results were obtained in the qualitative and quantitative studies of sturgeon rearing ponds [19].In another similar investigation Jafarian conducted feeding trials for 10 days with *A. persicus* larvae at the onset of active feeding and reported a weight gain of 180 mg when larvae were fed probiotic enriched *Artemia* nauplii as compared to 56 mg in larvae fed vitamin C enriched *Artemia* nauplii[20][21]. Feeding fish larvae with probiotic enriched rotifer not only increased growth parameters but also improved survival rate [22].These results agree with the findings of the present study.

Arimoro [23]documented that batch cultures of *B. calyciflorus* significantly affect survival rates in fish larvae. He further observed that any variation in their density influences feeding rates, excretion and growth performance in fish larvae. Condition factor is an important parameter associated with survival in fish. Condition factor in larvae fed enriched rotifers was higher up to day 8 as compared to that in other experimental groups. Similar results were reported by Haddadi for larvae of *A. persicus* fed with the marine rotifer *B. plicatilis* during the first seven days of early feeding[24].

According to recent investigations, improved growth and survival rates in larvae are related to the increased free amino acid (FAA) content of rotifers after enrichment. In one study the AA pools (free and protein-bound) of the rotifer *Brachionus rotundiformis* and of *Artemia parthenogenetica metanauplii* used to feed fish larvae were analyzed after enrichment. Results indicated a higher relative FAA content in rotifers than in *Artemia metanauplii* [25]. Moreover amino acids are known to elevate feeding behavior in several species [26]. The presence of amino acids during the transition from endogenous to exogenous feeding and also during early exogenous feeding greatly influenced survival rate of larvae [27]FAA also serves as a source of energy during yolk sac absorption and at the onset of exogenous feeding affecting larval growth [28].

Significant differences were detected in survival rates of larvae in the different experimental groups studied (Table 1). These results are in agreement with previous studies conducted on sunshine bass [29] and on the burbot (*Lota lota*) larvae [30] using the freshwater rotifer *B. calyciflorus*. In these studies the freshwater rotifer was seen to greatly influence growth parameters during the first two weeks of active feeding.

In another study, the percentage survival of African catfish larvae, Clarias *anguillaris* reared on monospecies culture of freshwater rotifers was relatively high over a 24 day rearing period in comparison with a mixed zooplankton diet [31].

Percentage survival of larvae in experiment group 3 was relatively higher than that in experiment group 1 ($P>0.05$). Enriched rotifers fed to *A. persicus* larvae enhanced their growth and survival during larval culture. The need to use rotifers at an earlier stage and in the first weeks of larviculture has been documented in beluga sturgeon [32]. Similarly the influence of probiotic enriched daphnia and *Artemia* on growth and survival in *A. persicus* have also been reported by [33][34]. Other researchers have supported the fact that the use of growth supplements is very effective in the successful culture of *A. persicus* and beluga larvae in the first ten days of active feeding [20][21].

Investigation has shown that 40 days of sturgeon in the actual structures of the teeth can be seen. [35]This study showed that initional 7 daysfeeding the mouth size is suitable for feeding freshwater zooplankton.Increasing the size of the mouth to the body length according fig (8&9) was of about (0 /1 ± 0/01)mm. During this period, larvae and weigh up to 100 mg wrer of food also to feed 180 to 500 microns. Rotifers wrer seen in the stomach of fish .

Study of Bradaran tahori showed These fish can weigh up to 400 to 800 mgr of daphnia food and then eating the benthos and other words of zooplankton that feed the 400 to 800 mgr [36]of be Since microscopic examination of the digestive system of these fish larvae show that the full development of the digestive system takes about fifteen to twenty days since this study was performed during 20 days of larval feeding,[37][38]Success in hunting these fish, since the olfactory organ does not a role when it comes to food under the snout or whiskers or have been at a distance 0/7 to 1 cm nthos and Completely are fed from the benthos.[35].

According to this study in the early days of rotifer can be easily fed and dont prohibitive that smaller size.The interpretation section for feeding larvae and daphnia actually in shahid beheshty center (control treatment) a significant amount of the mixture of freshwater rotifer and feeding larvae are harvested.Aguado study in 2007 on the relationship did between size of fish larvae and live prey size showed The group of fish including *Ameca splendens* With the increasing success of hunting prey fish size is smaller than the larger prey [39].

The main purpose of artificial reproduction for *Acipenser persicus* is the lacking of their stocks in the Caspian sea .The fingerling fish after primary cultivation ,will be release in basine river.Immigration to sea will be successfully overed just for a group of fish that have a good ability for osmoregulation,adaptation by their new environment and replacement of artificial food by natural ones.So the most important gist in caltivation of this species is the utilization of live foods based on the natural rivers fauna and release the fish by standard weight.

Table 1 Growth performance and feed utilization of juvenile *A. persicus* in different experimental groups live food for 8 days (initial weight=43.0±0.5 mg)

Growth Parameters	Control Group	Experimental Group 1	Experimental Group 2	Experimental Group 3
FBW m(g)	75.14±0.93^{b}	62.36±0.85^{a}	77.6±1.41^{c}	99.32 ±0.68^{d}
WG (%)	72.61±0.62^{b}	45.18±0.66^{a}	77.77±2.3^{c}	124.4±0.62^{d}
SGR (%/day)	6.98±0.05^{b}	4.65±0.06^{a}	7.38±0.18^{c}	10.47±0.4^{d}
FCR	2.75±0.03^{c}	4.48±0.07^{d}	2.47±0.08^{b}	1.51±0.01^{a}
CF(K)	0.68±0.03^{b}	0.62±0.05^{a}	0.72±0.04^{bc}	0.79±0.07^{c}
Survival (%)	84.46 ±5.87^{a}	84.4±4.45^{a}	95.5±2.25^{b}	97.8±2.2^{b}

Values within each row not sharing a common superscript letter are significantly different(P<0/05). Each data represent the(mean ± SD)of triplicate tanks (45 fi sh per tank)

FBW=Final body weight.

WG=Weight gain (WG) = ((Final weight-initial weight))/initial weight)) × 100.

SGR= Specific growth rate (% BW day)1) = 100 · [(Ln Final weight – Ln Initial weigh)/day].

FCR=Feed conversion ratio = Feed intake/weight gain.

CF= Condition factor (K) = 100 · [Final body weight mg/final standard length, mm].

Survival=(final number fish-initial number fish)×100

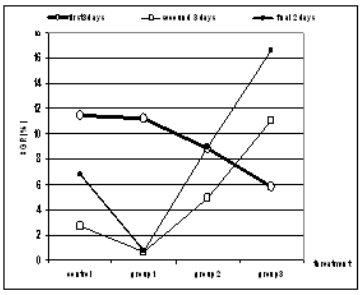


Figure1: comparative SGR (%) in different experimental groups

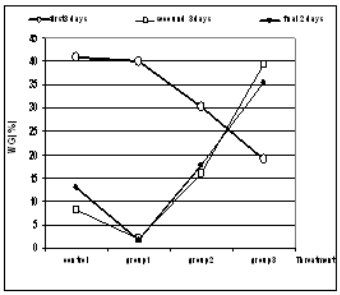


Figure2: comparative WG (%) in Different experimental groups

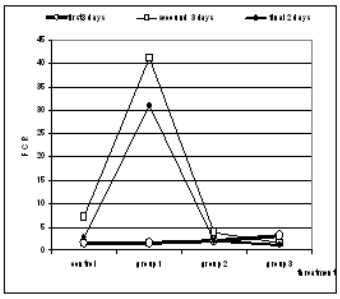


Figure3: comparative FCR (%) in different experimental groups

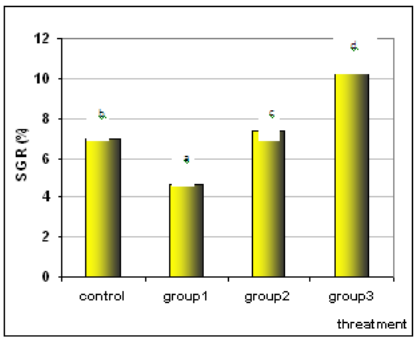


Figure 4: comparative SGR (%) in different experimental groups in final research

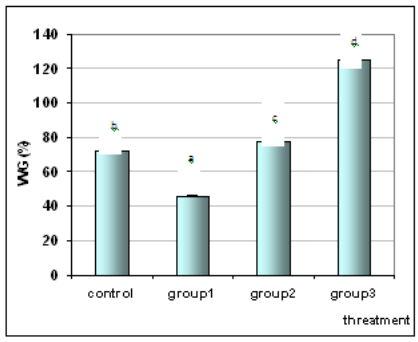


Figure 5: comparative WG (%) in different experimental groups in final research

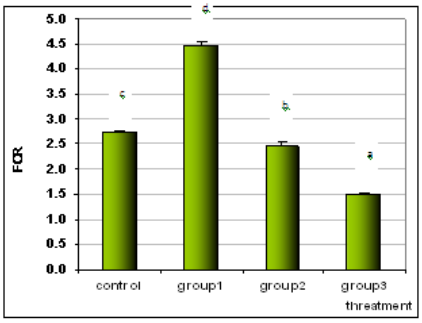


Figure 6: comparative FCR (%) in different experimental groups in final research

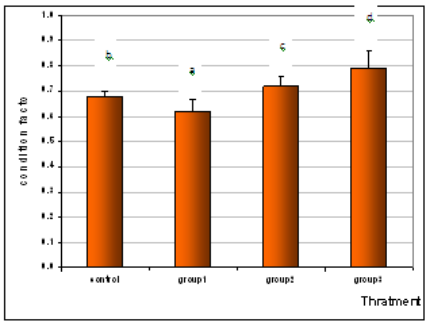
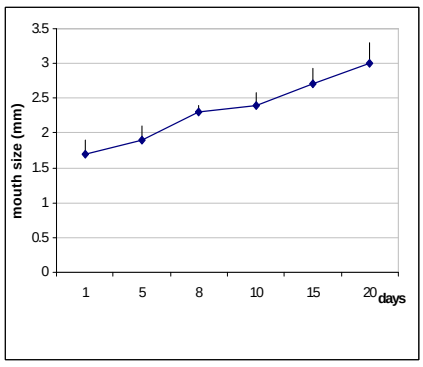


Figure 7: comparative cF in different experimental groups in final research



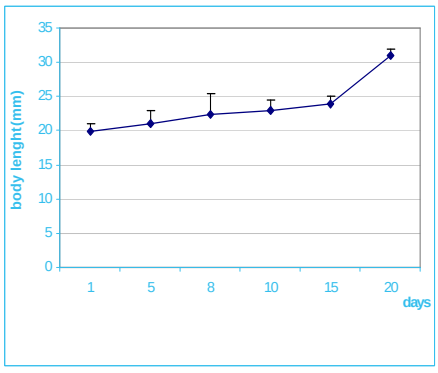
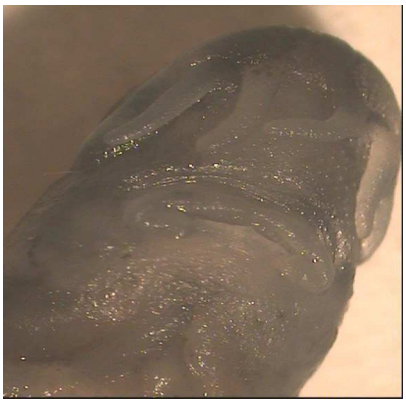
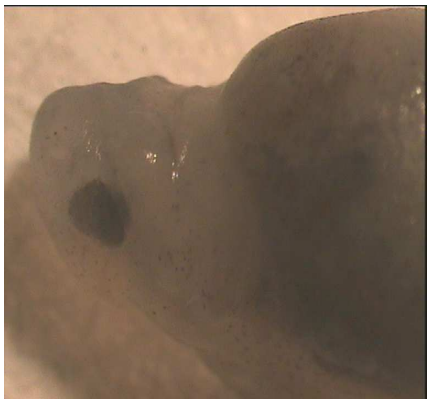


Figure 8: Increased the oral during examination

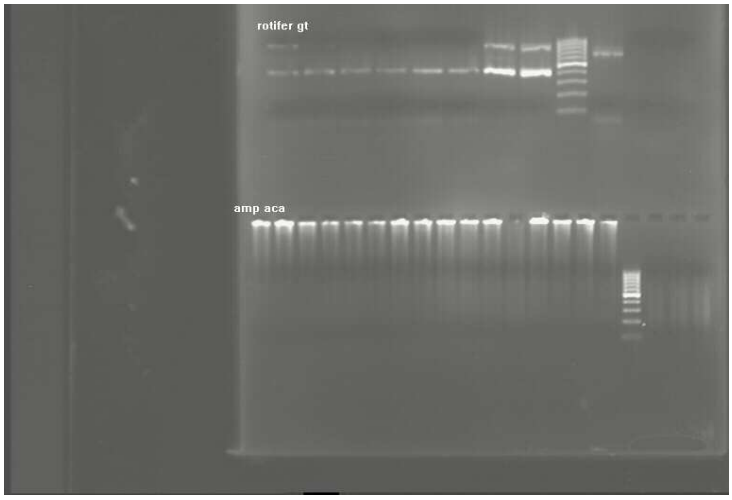


Pic 1 : Acipenser persicus larval ,6 days after starting food

Figure9 :Incraesed the oral size during exaexamination



Pic 2 : Acipenser persicus larval ,in sleep time



Pic.3, Amplified gene of *B. calyciflorus* in agarose gel

In view of the results obtained from the use of rotifers in the present study to ensure an overall satisfactory performance of larviculture in vniro tanks it is suggested that the fish culturist fill and fertilize a series of ponds at estimated times to provide an abundance of rotifers and subsequently copepod naupli and cladocerans to have a supply of live food at different stages of fry development.

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