First feeding strategy for hatchery produced Beluga sturgeon, *Huso huso* larvae

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Abstract

Co-feeding of fish larvae with live food and formulated diet has been at the focus of fish nutritionists since last decade. In this study we tried to refine the feeding practices of great beluga sturgeon (*Huso huso*) larvae using different combinations of newly hatched *Artemia urmiana* nauplii and trout starter diet. Three replicate groups (250 fish/replicate) of first-feeding *Huso huso* larvae were fed on the basis of four main feeding regimens: (1) live food (live nauplii of brine shrimp *Artemia urmiana*); (2) indirect transition (5 days live food followed by gradual transition to formulated diet); (3) direct transition (using different combinations of live and formulated diet from start feeding); (4) formulated feed (FD). It was found that combining live food and manufactured diets (co-feeding) from first feeding stage (direct transition) significantly improves the weight gain in *H. huso* larvae followed by indirect transition, live food and FD. But survival was significantly higher in larvae fed on pure live food and direct transition regimens compared to indirect transition and FD. It was concluded that co-feeding of *H. huso* could be started immediately from commencement of exogenous feeding.

Keywords: *Huso huso*, *Artemia* nauplii, Formulated diet, Co-feeding, Growth, Survival

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Introduction

Sturgeon fish have been the focus of much attention in many countries including Iran over the last decade because they are particularly interesting species in terms of rearing value. These fish are mainly cultured for the production of caviar, as a result of the sharp decrease in production capacity of caviar from natural resources such as the Caspian Sea. Additionally they are also an important source of commercially valuable fish meat. However, the feeding patterns of these species on natural food have only been studied on a small scale. This is especially true for the larval and juvenile stages, which are the most critical stages in the commercial production of these species.

Different sturgeon species possess an anatomically complete digestive tract at the onset of exogenous feeding (Buddington and Christofferson, 1985; Gawlicka et al., 1995; Gisbert et al., 1998). Artificial larval diets have been used for intensive commercial culture of several Acipenserid species from the onset of exogenous feeding onwards (Charlon and Bergot, 1991; Giovannini et al., 1991; Huang, 1991; Gisbert and Williot, 1997). However, the end of the lecithotrophic stage and transition to exogenous feeding are still characterized by considerable larval mortality (Buddington and Christofferson, 1985; Giovannini et al., 1991; Gisbert and Williot, 1997; Bardi et al., 1998). This observation suggests that nutritional problems are associated with the digestion and assimilation of artificial diets, which are normally formulated for salmonids or marine fish species (Hung, 1991; Gisbert and Williot, 1997) or bad restoration (Sepahdari et al., 2010). Therefore it has been suggested that combined feeding of live and manufactured diets (referred to as co-feeding) from the start of exogenous feeding or from an early larval age, could be considered as an alternative strategy (Rosenlund et al., 1997).

Mohler et al. (2000) indicated high survival in Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* using *Artemia* nauplii and a commercial feed. They reported a complete transition to formulated feed with less than 25% mortality in a 20–26 days feeding trial. Dilauro et al. (1998) offered five different formulated diets in combination with live brine shrimp *Artemia* sp., to larvae of the lake sturgeon *Acipenser fulvescens*. They reported no dietary effect ($P \leq 0.05$) on mean survival, but significantly higher growth in fish fed only brine shrimp. Ware et al. (2006) investigated the effects of six feeding regimes on the survival and growth of shortnose sturgeon *Acipenser brevirostrum* fry over 30 days using formulated diets and co-feeding. They reported significantly higher survival and growth in groups co-fed with *Artemia* compared to live food and commercial feed as sole diets. Bardi et al. (1998) reported more than 95% survival in Gulf of Mexico sturgeon *Acipenser oxyrinchus desotoi* larvae fed brine shrimp, but nearly complete mortality (>99%) when a formulated feed was used during a three weeks feeding trial. According to their findings survival and growth rate of first-

feeding larvae increased if they were fed brine shrimp for one week and then switched to an experimental microdiet.

There are five specially designed hatcheries and culture centers in Iran involved in propagation, weaning and resource restocking of the sturgeon fish. The larvae are mainly fed on *Artemia* nauplii and daphnia during early stages of growth. The fry are then released into fertilized earthen ponds containing different zooplankton population, mostly daphnia and chironomids , when their average weight reaches to about 100-150 mg. Introduction to the Caspian Sea or concrete culture ponds takes place at the average weight of 10 g (Personal correspondence with related authorities at Shahid Marjani and Shahid Behshty sturgeon hatcheries). None of the hatcheries use formulated diet during early stages of growth. This imposes huge expenditure for purchasing/production of live food and involvement of expert personnel. The aim of the present work was to contribute to reduce the massive mortality in the culture of these sturgeon species, associated with the dietary transition from live food to formulated feed. More specifically, we wanted to investigate under controlled laboratory conditions how different feeding regimes, using live food as a sole diet or co-fed with an inert diet through various weaning regimes, influence the survival and growth in *H. huso* larvae. We also aimed at replacing the long term live food regime with combination of live food and formulated diet in shortest period helping to reduce the expenses and excessive involvement of expert staff.

Materials and methods

Larval fish culture conditions

Polyethylene larval culture tanks (43 cm length, 30 cm wide, 35 cm height, 45 L total volume) contained 25 L UV treated fresh water obtained in a flow through system from a well with a flow rate of approximately 1 L/min. Dissolved oxygen was maintained above 7 mg/L using constant aeration and fish larvae were exposed to a natural photoperiod of approximately 12:12 L:D. Tanks were siphoned daily in the morning to remove trapped feces. Water temperature, O2, pH, TAN and nitrite were 20±1°C, 7.7±0.5, 7.4±0.1, 0.23±0.08 and 0.01±0.01 mg/L respectively, throughout the experiment. Temperature, pH and dissolved oxygen were monitored once or twice daily, but other parameters were measured once a week due to the constant quality of the well water and the low water retention time in the tanks (Noori et al., 2011). Yolk sac stage larvae were collected from Shahid Marjani sturgeon hatchery (Northeast of Iran). The larvae were transferred to the lab in oxygenated plastic bags. After acclimatization to the new environment the larvae (14 days post-hatch) were randomly distributed over the 45-L larviculture tanks (300 larvae/tank) and the feeding experiment started after absorption of the yolk sac at the time of mouth opening and commencement of external feeding. Each feeding treatment was run in 3 replicate tanks (Noori et al.,

2011). Two feeding strategies were adopted; in the first strategy the fish larvae were fed newly hatched *Artemia urmiana* nauplii (N) for 5 days followed by gradual replacement with a commercial formulated trout starter diet (FD). The basal diet containing 40% protein, 14% lipid, 4% fiber, vitamin and mineral premixes, was purchased from Chineh Co., Iran. In the second strategy the fish larvae were fed different combinations of newly hatched *Artemia* nauplii and FD from the first day of exogenous feeding. Feeding rations were based on wet body weight: initially 35% of body weight (first 5 days), followed by 25% (days 6-10), 15% (days 11-15) and 10% of body weight (days 16-20) (Agh et al., unpublished data). To determine the daily feeding rations, actively swimming newly hatched *Artemia* nauplii were transferred into a big beaker, aerated and 15 sub-samples (250 µl each) were collected, weighed, dried and weighed again in order to calculate their wet and dry weight. Daily rations were divided into six equal portions fed at intervals of four hours. The feeding rates were adjusted according to the daily mortalities in each tank. Each feeding strategy included several feeding treatments which varied in the rate of transition from a live food to a formulated diet. Additionally, two controls were included in the experimental set-up, one with live food, and one with formulated diet throughout the entire culture period. All feeding treatments were continued for 20 days until transition to FD had been completed for all of them.

Experimental feeding regimes:

1. *Artemia* nauplii (N) throughout the experiment
2. N for first 5 days + 10% daily replacement of N with FD from day 6 (total conversion to FD occurring on day 15)
3. N for first 5 days + 30% replacement of N with FD on day 6 and 10% daily additional replacement with FD from day 7 (total conversion to FD occurring on day 13)
4. N for first 5 days + 50% replacement of N with FD on day 6 and 10% daily additional replacement with FD from day 7 (total conversion to FD occurring on day 11)
5. N (90% feed weight) and FD (10% feed weight) on day 1 + 10% daily replacement of N with FD from day 2 (total conversion to FD occurring on day 10)
6. N (70% feed weight) and formulated feed (30% feed weight) on day 1 + 10% daily replacement of N with FD from day 2 (total conversion to FD occurring on day 8)
7. N (50% feed weight) and formulated feed (50% feed weight) on day 1 + 10% daily replacement of N with FD from day 2 (total conversion to FD occurring on day 6)
8. FD throughout the experiment.

Survival

The dead larvae were counted daily and removed from each culture tank. Based on these figures the mean survival in each treatment was calculated.

Growth

At the beginning of the experiment 10 randomly collected fish larvae from each

species were weighed, and the initial feed requirement for each feeding regime was based on this wet weight value. Every alternate day 6 individuals were collected from each culture tank to measure the total length of the larvae with the help of a stereomicroscope equipped with drawing tube and micrometer. Drawings were later digitized using a digitizer (Graphica, Japan) connected to a computer. The same specimens were also used for determination of wet and dry weight (dried at 60°C for 24 h). Based on the increase of wet weight of the fish larvae in each tank, the amount of feed needed for the next two days was calculated. Specific Growth Rate (SGR) (Huang et al., 2008) and Food Conversion Rate (FCR) (Turchini et al., 2003) were calculated at the end of the experiment.

Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA, SPSS version 13). Significance of differences between means was calculated by the Tukey test. All tests used a significance level of $P \leq 0.05$. The normal distribution was tested with the Shapiro-Wilk test and the homoscedasticity was checked with Levene's test and the survival percentages were transformed using Arcsine method (Sokal and Rohlf, 1969).

Results

Results obtained from the experiments are presented in Figure 1. Growth of fish was significantly higher in all co-fed groups compared to the fish fed only *Artemia* nauplii or FD ($P < 0.05$). Fish larvae receiving 70% *Artemia* nauplii and 30% commercial feed on the first day followed by 10% daily replacement with FD (treatment 7) showed the highest growth. Lowest SGR and highest FCR were obtained in fish larvae fed FD and *Artemia* nauplii, respectively ($P < 0.05$). Although SGR was highest in treatment 7, no statistical differences were observed among different co-feeding groups. We found that feeding *H. huso* larvae with a combination of live food and FD from the beginning of exogenous feeding (direct transition) resulted in higher weight gain compared to the indirect mode of transition to FD. However, the difference was not statistically significant. Very little growth and high mortality (mostly due to cannibalism) were observed in fish fed uniquely on FD compared to all other feeding treatments. Significantly higher survival was observed in the fish fed solely on live food (70.6%) compared to all other treatments except treatments 6 and 7 (65.7% and 59.7% respectively).

Summarizing, early co-feeding resulted in higher survival and weight gain compared to feeding on *Artemia* nauplii for 5 days and gradual shifting to FD. FCR was lower in all transition treatments compared to fish fed on live food and FD. Use of *Artemia* nauplii as live food for the larval stages of commercially important fresh water and marine fish species has been documented worldwide. The production of this zooplankton is usually very high and the nutritional quality of *Artemia* harvested from different geographical sources are variable

(Sorgeloos, 1980; Watanabe et al., 1983). On the other hand it has been proved that using live food alone cannot support acceptable growth rates in many fish species, especially in marine fish, due to the restricted food intake and its low nutrient content and restricted food intake (Olsen et al., 1992).

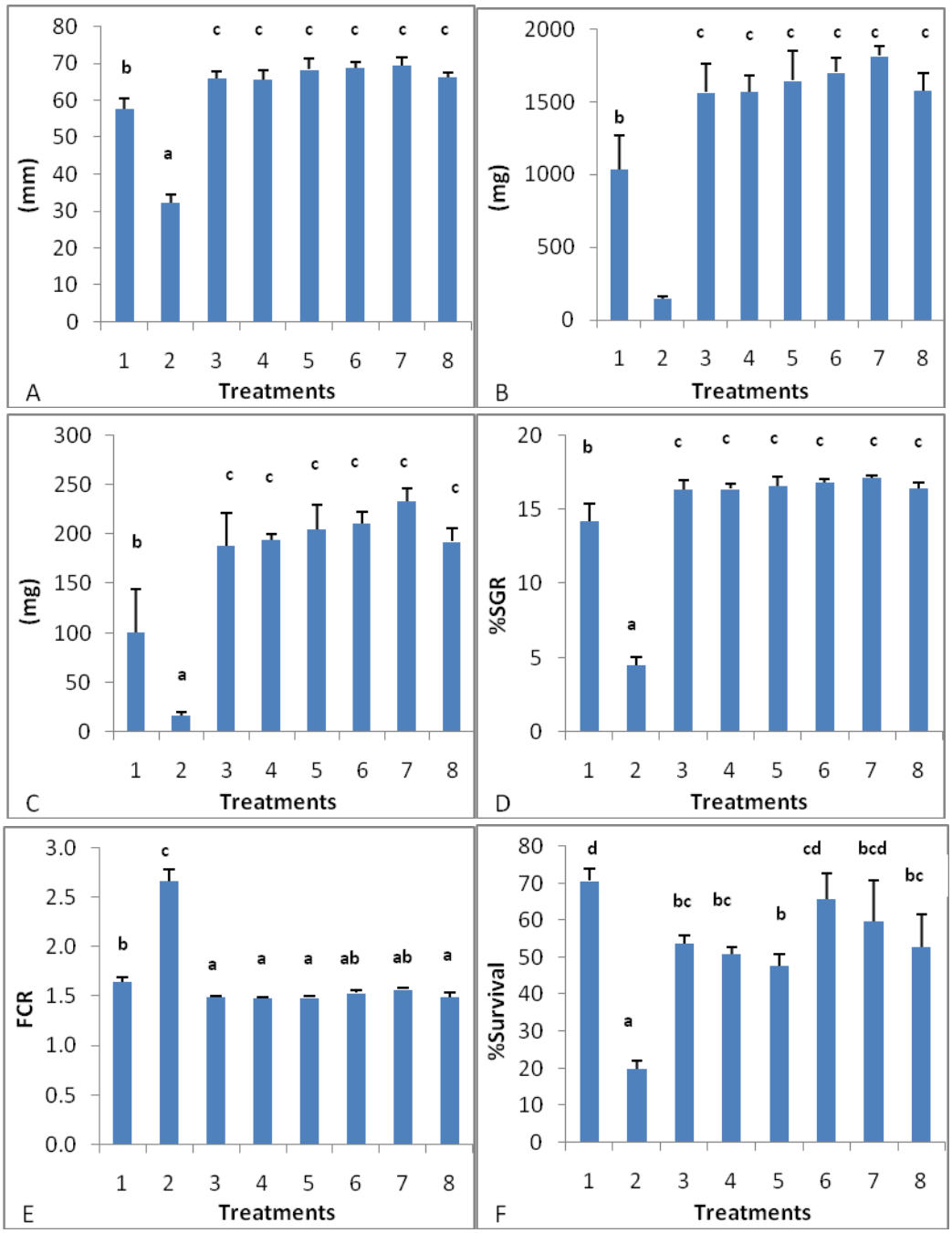

Figure 1: Final length (A), final wet weight (B), final dry weight (C), SGR (D), FCR (E) and % survival (F) of *H. huso* larvae fed on different combinations of live food and commercial feed (initial total length, wet weight and dry weight were 25 mm, 59 mg and 10.3 mg respectively). Error bars correspond with standard deviation.Different superscripts on top of each bar indicate significant difference among treatments (P < 0.05).Treatments: (1) N; (2) FD; (3, 4, 5) indirect transition; (6, 7, 8) direct transition

Discussion

This has prompted a great deal of interest in the development of an artificial larval microdiet (MD) as an economic alternative to live food (Kolkovski et al., 1997). However, a lower performance is commonly reported when inert diets are fed to larvae from the onset of exogenous feeding. This may be due to the composition, palatability, or physical characteristics of the dry feed (Person Le Ruyet et al., 1993), to the larva's inability to properly digest the feed (Holt, 1993; Kolkovski et al., 1993; Walford and Lam, 1993; Zambonino Infante and Cahu, 1994), or to the low attractiveness of the non-mobile particle for the fish larvae. However, the performance of MD's for a variety of fish species was considerably enhanced when co-fed with live zooplankton (Kanazawa et al., 1982; Szlaminska and Przybyl, 1986; Ehrlich et al., 1989; Fermin and Bolivar, 1991; Marte and Duray, 1991; Tandler and Kolkovski, 1991; Walford et al., 1991; Person Le Ruyet et al., 1993; Lavens et al., 1995). The successful use of *Artemia* nauplii alone or co-fed with a commercial diet at the start feeding or during early development of different sturgeon species has been reported by a number of researchers (Dilauro et al., 1998; Bardi et al., 1998; Mohler et al., 2000; Volkman et al., 2004).

Results obtained in the present study indicated that a carefully programmed diet of live food co-fed with a commercial diet could be successfully used in first feeding of beluga sturgeon (*Huso huso*). Co-feeding *H. huso* with live food and FD from start feeding onwards resulted in the best growth performance. This supports the findings of Ware et al. (2006) that shortnose sturgeon *Acipenser brevirostrum* exhibited higher survival and growth in different types of co-feeding regimes compared to a live food diet. *H. huso* larvae demonstrated significantly higher growth when live food was combined with FD at start feeding compared to FD and nauplii alone. Although highest survival was observed in larvae fed only *Artemia* nauplii, there was no significant difference with treatments 6 and 7 (direct transition groups beginning with 10 and 30% FD from day 1). Unlike the findings of Dilauro et al. (1998) and Bardi et al. (1998), our findings proved that *H. huso* larvae readily accept co-feeding of live food and FD from first feeding onwards, and are easily weaned to FD as monodiet within seven days from the onset of exogenous feeding.

It has been proved that co-feeding enhances larval performance beyond the level achieved by feeding either type of feed alone (Kanazawa et al., 1989; Holt, 1993; Leu et al., 1991; Abi-Ayad and Kestemont, 1994), and that it permits weaning in a shorter time (Person Le Ruyet et al., 1993). This is the case as well for *H. huso*. An increased supply of more suitable nutrients may be the main reason for better performance of fish larvae accepting FD along with live food. However, the fish larvae suffered significantly higher mortality caused by

starvation when they were offered only formulated diet from first feeding. The empty digestive tract suggested low attractiveness of the formulated diet for the larvae. Feeding on FD alone resulted in high cannibalism in *H. huso* larvae, indicating that the commercial trout feed alone did not meet all nutritional requirements at first feeding. Apparently there is a specific period during development, related to feeding behavior and physiological capacity, when sturgeon fish larvae accept manufactured diets. Successful co-feeding thus depends on the ability of the fish larvae to eat dry feed when live food is also present.

Summarizing, *H. huso* larvae could be co-fed with a combination of *Artemia* nauplii and commercial feed from the onset of exogenous feeding, with a rapid total conversion to FD within seven days. Co-feeding resulted in a considerable reduction of costs needed for consumables (including *Artemia* cysts), infrastructure, labor and space needed for feed preparation and for the feeding process.

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