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Original Article

Optimizing the Co-feeding strategy of Persian sturgeon (*Acipenser persicus*) larvae using *Artemia* nauplii and formulated diet

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Abstract: High mortality and labor costs are associated with first-feeding sturgeon culture, particularly during the period of dietary transition from live to formulated feed. Therefore we investigated the effects of various feeding treatments on the survival and growth of the Persian sturgeon (*Acipenser persicus*) larvae during a 20-day culture period. Three replicate groups (250 fish/replicate) of first-feeding larvae were fed according to four main feeding regimes: (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 the start feeding onwards); (4) formulated feed (FD) from the start feeding. Results indicated that growth and survival were higher in the indirect transition feeding regime than in other regimes. Based on our study, Co-feeding of *A. persicus* should start five days after prior feeding with live food.

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Introduction

Sturgeon 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. The Persian sturgeon (*Acipenser persicus*) as a sturgeon fish is a migratory species which are especially adaptable to changes in their environment and in food supply; thus, they can occur and attain satisfactory growth in various climatic zones. These fish species have been the focus of much attention in Iran over the last decade because they are particularly interesting species in terms of rearing value.

At the onset of exogenous feeding, different sturgeon species possess an anatomically complete digestive tract with a marked specialization at different segments (Buddington and Christofferson, 1985; Gawlicka et al., 1995; Gisbert et al., 1998; Asgari et al., 2013). 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; Hung, 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

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Table 1. Weight of sturgeon larvae at different stages from hatching to introduction into earthen pond and the normal feeding strategy in Iranian sturgeon fish hatcheries.

	Weight of larvae at different stages until transfer into earthen ponds			Feeding strategy		
	at hatching (mg)	at start feeding (mg)	at transfer to pond (mg)	*Artemia* nauplii (days)	*Artemia* nauplii + *Daphnia* (days)	*Daphnia* (days)
Huso huso	20-22	60-65	100-120	1	1	8-9
Acipenser persicus	16-17	40-42	80-100	2	2	5-6
Acipenser gueldenstaetii	16-17	40-42	80-100	2	2	5-6
Acipenser nudiventris	10-14	32-38	150	6	4	8-10
Acipenser stellatus	7-9	28-30	150	6	4	8-10

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). 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 to reduce larval mortality (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 day 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 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 week 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.

In Iranian sturgeon fish hatcheries, the sturgeon larvae are mainly fed on *Artemia* nauplii and *daphnia* during early stages of growth (Table 1). The fry are then released into fertilized earthen ponds containing different zooplankton population, mostly daphnia and chironomidae, when their average weight reaches to about 150 mg. Introduction to the Caspian Sea or concrete culture ponds takes place at an average weight of 10 g. 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. Moreover, huge mortality has been reported on sudden transition from live food feeding in earthen ponds into formulated diet in concrete ponds.

The aim of the present work was to contribute to reduce the massive mortality in the culture of *A. persicus*, 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

Table 2. The chemical composition of *Artemia* nauplii and formulated diet. (The values indicate the averages values of replicates with standard deviations).

Feed	Crude protein (% DW)	Crude lipid (% DW)	Carbohydrate (% DW)	Ash (% DW)	Energy (cal g^{-1})
Formulated diet	50 ± 2	12 ± 1.5	12.5 ± 1	13.5 ± 1	4000 ± 31
Nauplii	61.6 ± 0.8	11.6 ± 2.1	20 ± 2.9	6.8 ± 2	5013.1 ± 88.3

food as a sole diet or Co-fed with an inert diet through various weaning regimes, influence the survival and growth in *A. persicus* larvae. We also aimed at replacing the long term live food regimen 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^{-1}. Dissolved oxygen was maintained above 7 mg l^{-1} using constant aeration. 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, DO, 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^{-1} 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.

Yolk sac stage larvae were collected from Shahid Marjani sturgeon hatchery (Gorgan city, NE Iran). The larvae were transferred to the lab in oxygenated plastic bags. After acclimatization to the new environment they 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, 14 and 10 days post-hatch for beluga and Persian sturgeon larvae, respectively. Each feeding treatment was run in three replicate tanks.

Two feeding strategies were adopted for both fish species. In the first strategy, the fish larvae were fed with newly hatched *Artemia urmiana* nauplii (N) for 5 days followed by gradual replacement with a commercial formulated starter diet (FD) provided by Joosen-Luyckx, Turnhout, Belgium. 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, 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 fed live food, and the other fed formulated diet throughout the culture period. All feeding treatments were continued for 20 days until transition to FD were completed for all treatments.

Experimental feeding regimes used in this study were as follows:

1. *Artemia* nauplii (N) throughout the experiment.
2. FD throughout the experiment.
3. N for first 5 days + 10% daily replacement of N with FD from day 6 (total conversion to FD occurring on day 15).
4. N for first 5 days + 20% replacement of N with FD on day 6 and 10% daily additional replacement

Table 3. Final length, wet weight (WW) and dry weight (DW) (mean ± standard deviation) of *A. persicus* larvae fed on different combinations of live food and commercial feed (initial total length, wet weight and dry weight were 20.7 mm, 39.1 mg and 4.5 mg respectively).

Treatments	Final length (mm)	Final WW (mg)	Final DW (mg)
1	45.7 ± 2.0^{fg}	557.5 ± 36.3^{e}	66.9 ± 4.3^{e}
2	27.3 ± 2.0^{a}	87.7 ± 15.8^{a}	11.8 ± 3.7^{a}
3	48.9 ± 1.5^{g}	657.5 ± 36.3^{f}	78.9 ± 4.4^{f}
4	46.1 ± 0.6^{fg}	592.5 ± 24.8^{ef}	71.7 ± 3.0^{ef}
5	$1.1^{ef} \pm 43.7$	550.7 ± 17.2^{e}	66.9 ± 2.0^{e}
6	42.4 ± 1.1^{def}	446.4 ± 25.1^{d}	53.6 ± 3.0^{d}
7	40.3 ± 1.6^{de}	417.0 ± 30.9^{cd}	50.5 ± 3.7^{d}
8	40.4 ± 2.2^{de}	435.0 ± 3.0^{d}	53.1 ± 4.3^{d}
9	37.9 ± 1.8^{cd}	380.0 ± 1.0^{bcd}	45.6 ± 1.8^{cd}
10	34.2 ± 1.7^{bc}	345.1 ± 20.0^{bc}	41.0 ± 2.4^{bc}
11	32.3 ± 1.2^{b}	329.6 ± 13.5^{b}	38.9 ± 1.6^{bc}
12	29.6 ± 1.4^{ab}	307 ± 14.0^{b}	35.3 ± 1.6^{b}

Different superscripts in each column indicate significant difference among treatments ($P < 0.05$).
Treatments: (1) N; (2) FD; (3, 4, 5, 6, 7) indirect transition; (8, 9, 10, 11, 12) direct transition.

with FD from day 7 (total conversion to FD occurring on day 14).
5. 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).
6. N for first 5 days + 40% 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 12).
7. 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).
8. 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)
9. N (80% feed weight) and formulated feed (20% feed weight) on day 1 + 10% daily replacement of N with FD from day 2 (total conversion to FD occurring on day 9).
10. 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).
11. N (60% feed weight) and formulated feed (40% feed weight) on day 1 + 10% daily replacement of N with FD from day 2 (total conversion to FD occurring on day 8).

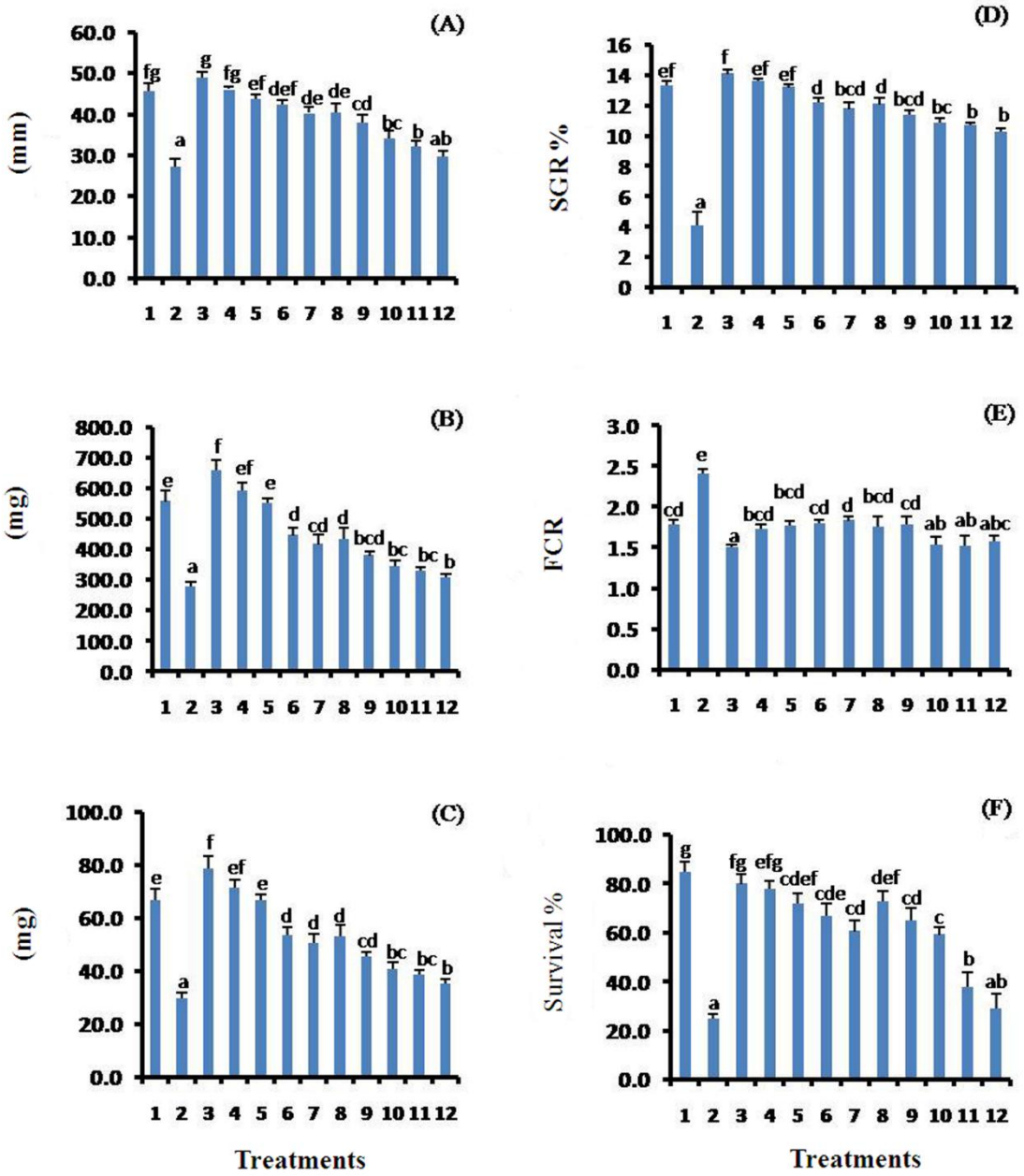

Figure 1. Final length (A), final wet weight (B), final dry weight (C), SGR (D), FCR (E) and % survival (F) of *A. persicus* larvae fed on different combinations of live food and commercial feed (initial total length, wet weight and dry weight were 20.7 mm, 39.1 mg and 4.5 mg, respectively). Different superscripts in each column indicate significant difference among treatments (P<0.05). Treatments: (1) N; (2) FD; (3, 4, 5, 6, 7) indirect transition; (8, 9, 10, 11, 12) direct transition.

12. 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).

The approximate chemical composition of the formulated food and *Artemia* nauplii used in this study is shown in Table 2.

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 ten randomly collected fish larvae from each species were weighed. The initial feed requirement for each feeding regime was based on this wet weight value. Every alternate day six individuals were collected from each culture tank to measure the total length of the larvae with the help of a stereomicroscope

equipped with a drawing tube and micrometer (Zhiss, Germany). 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) and Food Conversion Rate (FCR) were calculated at the end of the experiment.

Statistical analysis: Data were examined using analysis of variance (ANOVA) followed by the Tukey test.

Results

Results obtained from the experiments are presented in Table 3 and Figure 1. Significantly higher survival was observed in larvae fed live food (85 ± 4%) compared to other feeding treatments except the indirect co-feeding regimes 3 and 4 with 80 ± 4 and 78 ± 3% survival respectively (P<0.05). Significantly lower survival was observed in larvae when 40-50% live food was replaced with FD at start feeding. Highest growth was observed in fish of co-fed groups (indirect transition treatments 3 and 4) followed by larvae fed on *Artemia* nauplii, which was significantly higher compared to FD and direct transition groups (P<0.05). Direct transition treatments resulted in higher mortality of *A. persicus* larvae compared to indirect transition regimes. Growth and survival gradually and often significantly decreased in treatments receiving higher percentages of FD in both weaning strategies. Significantly lower growth and survival was observed in larvae fed on the inert diet solely (P<0.05).

Discussion

In most marine fish species, compound diets fed alone have a poor ability to sustain larval growth and development (Cañavate and Fernández-Díaz, 1999; Robin and Vincent, 2003; Curnow et al., 2006a). The low performance usually observed when feeding an inert diet to marine fish larvae from mouth opening onwards may be due to sub-optimal diet composition and the larva's poor ability to modulate its digestive enzymes (Cahu and Zambonino Infante, 2001). Therefore, the Co-feeding strategy has been proposed for farmed species, such as Dourado (Vega-Orellana et al., 2006), Asian sea bass (Curnow et al., 2006b), Pikeperch (Hamza et al., 2007), and Cod (Rosenlund and Halldórsson, 2007). *Artemia* nauplii are used world-wide as live food for the larval stages of commercially important fresh water and marine fish species. The costs of infrastructure, labor and energy to culture this zooplankton organism are considerable and the supply and nutritional quality of brine shrimp are variable (Sorgeloos, 1980; Watanabe et al., 1983). Furthermore, it seems that acceptable growth rates in a number of fish species cannot be maintained using exclusively live food due to its low nutrient content and restricted food intake (Olsen et al., 1992). This has prompted a great deal of interest in the development of an artificial larval microdiet (MD) as an economic alternative to live food. 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 larva. 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 and early larval feeding of Persian sturgeon (*Acipenser persicus*). The results obtained with different co-feeding regimes showed that maximum survival is obtained with live food but it was not significantly different with treatments 3 and 4 (indirect transition starting with 10 and 20% replacements of live food with FD from day 6). Maximum growth was obtained in the same indirect transition treatments, significantly higher than all other treatments except those fed solely on live food. This means that in *A. persicus*, the best results are obtained with a gradual transition process where the larvae are fed on *Artemia* nauplii for five days, and then slowly weaned to FD. This finding is in congruence with the data of Dilauro et al. (1998) for *Acipenser fulvescens* and of Bardi et al. (1998) for *Acipenser oxyrinchus desotoi*.

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 study proved that this feeding strategy is applicable for *A. persicus* too, but different transition regimes need to be adopted. 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 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. 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.

We may conclude that *A. persicus* prefers an early feeding with live food followed by gradual transition to FD from day 6 onwards. 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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