Arch. Pol. Fish. (2013) 21: 53-61
DOI 10.2478/aopf-2013-0006

RESEARCH ARTICLE

Results of the larviculture of Atlantic sturgeon (*Acipenser oxyrinchus*) fed different types of diets

Iwona Piotrowska, Bożena Szczepkowska, Michał Kozłowski, Krzysztof Wunderlich, Mirosław Szczepkowski

Received – 29 August 2012/Accepted – 17 November 2012. Published online: 31 March 2013; ©Inland Fisheries Institute in Olsztyn, Poland
Citation: Piotrowska I., Szczepkowska B., Kozłowski M., Wunderlich K., Szczepkowski M. 2013 – Results of the larviculture of Atlantic sturgeon (*Acipenser oxyrinchus*) fed different types of diets – Arch. Pol. Fish. 21: 53-61.

Abstract. The aim of this experiment was to assess the impact live and formulated diets had on the growth and survival of larval Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill. In the first experiment, the results of feeding the fish decapsulated (group D) and undecapsulated (group ND) *Artemia* sp. cysts were compared. Increases in the body weights and lengths of the fish fed decapsulated and undecapsulated *Artemia* sp. were similar at values that were not statistically significant. Survival in the larval group fed undecapsulated *Artemia* was higher in comparison to the group fed decapsulated *Artemia* (95.0 and 82.8%, respectively). In the second experiment, three commercial starter feeds were used: Aller ArtEX (Aller Aqua, Poland; group A), Nutra HP (Skretting, France; group H), and Perla Prolarvae (Spa Hendrix, Italy; group P). Following 35 days of culture, no differences were noted in fish growth with lengths in the different groups ranging from 28 to 30 mm and weights from 119 to 134 mg. Fish survival in group A was 55% and was significantly statistically higher than that in groups H and P at 31.7 and 35.7%, respectively. The results of the experiment suggest that the best feed for the early stages of Atlantic sturgeon culture is undecapsulated *Artemia* during the beginning of exogenous feeding followed by an *Artemia*-based feed in the initial period of feeding with formulated diets.

Keywords: sturgeon, *Acipenser oxyrinchus, Artemia*, decapsulation, feeding, recirculating aquaculture system

I. Piotrowska, B. Szczepkowska, M. Kozłowski, K. Wunderlich, M. Szczepkowski [✉]
Department of Sturgeon Fish Breeding
Inland Fisheries Institute in Olsztyn,
Pieczarki 50, 11-610 Pozezdrze, Poland
tel. +48 428 3666, e-mail: szczepkowski@infish.com.pl

Introduction

The Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill, occurs in North America where it ascends rivers such as the St. Lawrence and St. John to spawn (Leim and Scott 1966). Genetic testing (Ludwig et al. 2002) confirmed that this species also occurred in Europe, and the restoration of this species to the Atlantic Sea basin was begun several years ago (Gessner et al. 2005, Kolman et al. 2008, 2011). The Atlantic sturgeon possesses a range of characters that differ from other sturgeon species which means its culture is significantly more difficult. While several studies have contributed to the development of many aspects of the biotechnology required for culturing Atlantic sturgeon (Kynard and Horgan 2002, Mohler 2003, Kolman 2006, Szczepkowski et al. 2011), difficulties are still encountered at the youngest stages of larva and fry. Studies to date indicate the Atlantic sturgeon will not accept formulated feed when it begins exogenous feeding (Mohler et al. 2000, Szczepkowski et al. 2011), and positive culture results have only been achieved with *Artemia*, but even this strategy poses problems and fish mortality associated with its use is high (Szczepkowski et al. 2010). Effective culture methods for the youngest sturgeon stages are crucial to conservation efforts because they permit producing stocking material and creating broodstocks. The

aim of the current experiment was to assess the impact live and formulated feeds have on the growth and survival of Atlantic sturgeon larvae.

Materials and methods

Experiment I

Atlantic sturgeon larvae were obtained through artificial reproduction of wild spawners from the St. John River in Canada. The fertilized eggs were transported in aerated bags to the Inland Fisheries Institute's Department of Sturgeon Fish Breeding in Pieczarki, where they were placed in McDonald hatching jars. Immediately after hatching, the larvae were transferred to tanks with volumes of 40 l each that were part of a recirculating aquaculture system. The stocking density in each tank was 200 larvae with mean body weights of 0.007 ± 0.001 g. Two types of live feed were supplied: decapsulated (group D) and undecapsulated (group ND) *Artemia* cysts. There were four replicates of each group, and the same type of *Artemia* was used in each one (OF, INVE, Holland). The cysts were decapsulated with Bielnar (Narwam, Poland) containing a hypochlorite solution (Szczerbowski and Łuczyński 1994). The decapsulated *Artemia* cysts were incubated in mini-jars, but the undecapsulated cysts were incubated in Weiss jars because of the differences in the hatching period of 24 and 30 h, respectively (Szczerbowski and Łuczyński 1994, Sorgeloos et al. 2001).

The initial daily ration of *Artemia* nauplii (recalculated from cyst dry weight) and undecapsulated cysts was 14% of fish biomass (Szczepkowski et al. 2010). *Artemia* nauplii were supplied on day 10 post hatch. The fish were fed once daily by placing the nauplii on the tank bottom using a graduated pipette with a volume of 10 ml. The tanks were cleaned of excrement and waste every morning, and the number of dead fish were counted. During larviculture, water exchange in all of the tanks was maintained at 1.6 l min^{-1}. The mean water temperature during the experiment was 18.8 ± 0.5°C, water oxygen content at the tank outflows did not decrease below 6.95 mg l^{-1}, and the water pH was 7.07. The concentration of total ammonia nitrogen (TAN) at the tank outflows did not exceed 0.31 mg l^{-1}, while that of nitrites (NO_2-N) did not exceed 0.037 mg l^{-1}. The TAN content was determined with direct nesslerization, and that of NO_2-N with the sulfanilic method (Hermanowicz et al. 1999) with a Carl Zeiss 11 spectrophotometer (Carl Zeiss, Germany).

Experiment II

The experimental material was cultured larval Atlantic sturgeon aged 25 days post hatch with mean body weights of 0.033 ± 0.002 g and body lengths of 1.9 ± 0.1 cm. The fish were stocked into tanks with volumes of 40 l each that were part of a recirculating aquaculture system. The stock density in each tank was 160 individuals. The water flow in each tank was 1.5 l min^{-1}. Three commercial feeds were used: Aller ArtEX (Aller Aqua, Poland; group A), Nutra HP (Skretting, France; group H), and Perla Prolarvae (Spa Hendrix, Italy; group P) (Table 1). There were three replicates of each experimental group. All of the groups were fed live nauplii *Artemia* supplied ad libitum once per day and feed twice per day. The feed was soaked and then placed on the tank bottom with a graduated pipette with a volume of 10 ml. Initially, the daily ration of *Artemia* nauplii (recalculated from cyst dry weight) was 14% of the fish biomass, and it was gradually decreased to 5% of the fish biomass by the end of the experiment (Szczepkowski et al. 2010). The daily feed ratio was 10% initially, and from the sixth day it was increased to 13% of the fish biomass (Mohler 2000). For the first 22 days of rearing in groups A and P, the feed supplied was of a smaller particle size (Aller ArtEX 1 and Perla Prolarvae 6.0). For the subsequent five days of the experiment, this feed was mixed with a feed of larger particle size (Aller ArtEX 2 and Perla Prolarvae 5.0) at a proportion of 50:50. Feed with the largest particle size was supplied exclusively from day 28 of culture. However, group H received only Nutra HP 0.3

Table 1
Characteristics of the experimental feeds used in the experiments (according to manufacturer data)

	Feed				
Parameter	Aller ArtEX 1	Aller ArtEX 2	Nutra HP 0.3	Perla Prolarva 6.0	Perla Prolarva 5.0
Protein (%)	50	50	57	62	62
Fat (%)	15	15	17	11	11
Carbohydrates (%)	8	8	10	10	10
Particle size (mm)	0.05-0.15	0.15-0.40	0.20-0.60	0.10-0.30	0.20-0.40

feed for the duration of the experiment. Unconsumed food was cleaned from the tanks daily, and any dead fish were counted and removed. Additionally, the digestive tract contents of all the dead fish were analyzed to determine whether these individuals had consumed unhatched *Artemia* cysts. These observations were performed with a Nikon SMZ-10A binocular microscope (Japan). The experiment was 35 days long. The water temperature was maintained at 18.2 ± 0.5°C, and water pH was 6.6. The concentration of total ammonia nitrogen (TAN) at the tank outflows did not exceed 0.44 mg l^{-1}, and nitrite (NO_2-N) content did not exceed 0.048 mg l^{-1}. The content of dissolved oxygen in the water at the outflows did not decrease below 6.8 mg l^{-1}.

Statistical measurements and calculations

Fifteen individuals from each group were collected at the conclusion of the experiment, body weight was determined to the nearest 0.001 g, and body length was measured to the nearest 0.1 cm. The total fish biomass in each tank was also determined. These data were used to calculate the specific growth rate (SGR, % d^{-1}), the condition coefficient (K), the body weight coefficient of variation (CV, %), the feed conversion ratio (FCR), and the protein efficiency ratio (PER).

Statistical calculations were performed using Statistica 5.0PL (StatSoft Inc.). The significance of differences was confirmed with one-way ANOVA. Tests of significance among the experiment variants were performed using mean values from replicates in given variants. Tukey's multiple comparison test was used to evaluate the significance of differences, which were considered statistically significant at $P \leq 0.05$.

Results

Experiment 1

Fish growth in the experimental groups was similar. At the end of the experiment, the fish in group D had attained mean body weights of 0.034 ± 0.002 g, while those in group ND were 0.032 ± 0.002 g ($P>0.05$; Table 2). The specific growth rates were 10.4 and 10.1% d^{-1}, in groups D and ND, respectively. Variation in fish size was similar at the end of the experiment, and the values of the body weight coefficients of variation (CV) were 25.3 and 25.4% ($P>0.05$; Table 2). No differences were noted between the fish condition coefficients (K), and in both groups it was 0.47. Fish survival was significantly statistically higher in group ND at 95%, while in group D it was only 82.8%. The first losses were noted in both groups as early as the second day after the tanks had been stocked. The most losses were noted in group ND on the seventh day of larviculture at 1.4% of the stock day^{-1} (Fig. 1). Losses in group D were significantly higher, and on the ninth day of larviculture mortality reached 9.6% of the fish stock. Smaller individuals that had not consumed feed and with visible anomalies in the tail section of the body predominated among dead fish. At the conclusion of the experiment, the share of individuals with tail anomalies in both groups was 2.8%.

Table 2
Selected production indicators of larval Atlantic sturgeon (*A. oxyrinchus*) fed decapsulated (group D) and undecapsulated (group ND) *Artemia sp.* (mean values ± SD; N = 3)

Parameter	group D	group ND
Initial body weight (g)	0.007 ± 0.001[a]	0.007 ± 0.001[a]
Final body weight (g)	0.034 ± 0.002[a]	0.032 ± 0.002[a]
Specific growth rate SGR (% d^{-1})	10.4 ± 0.4[a]	10.1 ± 0.4[a]
Body weight coefficient of variation CV (%)	25.3 ± 3.4[a]	25.4 ± 4.2[a]
Initial body length (cm)	1.1 ± 0.1[a]	1.1 ± 0.1[a]
Final body length (cm)	1.9 ± 0.1[a]	1.9 ± 0.1[a]
Condition coefficient K	0.47 ± 0.01[a]	0.47 ± 0.00[a]
Survival (%)	82.8 ± 4.5[a]	95.0 ± 1.1[b]

Groups with the same letter index in the same row do not differ significantly statistically (P > 0.05)

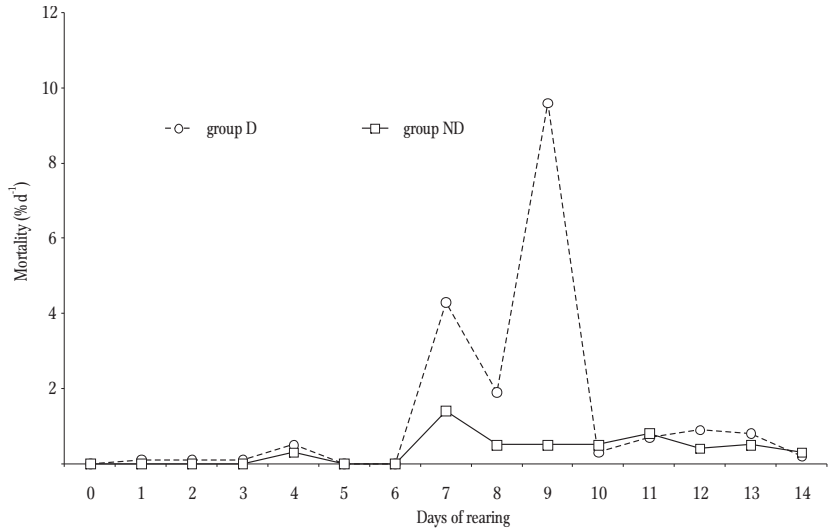

Figure 1. Mortality during the larviculture of Atlantic sturgeon (*A. oxyrinchus*) fed decapsulated (group D) and undecapsulated (group ND) *Artemia* sp.

Experiment 2

The final body weights of the larval Atlantic sturgeon fed different feeds were similar at 0.134 ± 0.010 g (group A), 0.119 ± 0.006 g (group H) and 0.134 ± 0.014 g (group P) (Table 3). The specific growth rates (SGR) were from 3.56% d^{-1} in group H to 3.87% d^{-1} in group A, and were not statistically significant (Table 3). The final body lengths ranged from 2.8 ± 0.1 cm in group H to 3.0 ± 0.1 cm in groups A and P. No differences were noted in the condition coefficient (K) (P > 0.05; Table 3). At the end of the experiment, however, differences were noted among the groups in the body weight coefficient of variation. The highest value of the body weight coefficient of variation (CV) was noted in group H at 53.5%, while the lowest was in group P at 40.2%, and these differences were statistically significant (P<0.05; Table 3). Differences in fish survival were also confirmed among the groups fed different feeds. In group A it was 55.3% and was statistically higher (P<0.05) that it was in groups H (31.7%) and P (35.7%).

Two periods of increased fish mortality were observed during the experiment in all of the experimental groups. The first occurred just after the beginning of feeding, but only in the group fed ArtEX did mortality not exceed 2% of the fish stock per day (Fig. 2). In the other two groups, the highest mortality was observed on day 10 of the experiment at 6.3% of the

Table 3

Selected production indicators of larval Atlantic sturgeon (*A. oxyrinchus*) fed three types of commercial starters: Aller ArtEX (group A), Nutra HP (group H), and Perla Prolarvae (group P) (mean values ± SD; N = 3)

	Group		
Parameter	A	H	P
Initial body weight (g)	0.033 ± 0.002	0.033 ± 0.002	0.033 ± 0.002
Final body weight (g)	0.134 ± 0.010[a]	0.119 ± 0.006[a]	0.134 ± 0.014[a]
Specific growth rate SGR (% d^{-1})	3.87 ± 0.22[a]	3.56 ± 0.13[a]	3.86 ± 0.31[a]
Body weight coefficient of variation CV (%)	43.9 ± 3.5[ab]	53.5 ± 1.3[a]	40.2 ± 2.7[b]
Initial body length (cm)	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Final body length (cm)	3.0 ± 0.1[a]	2.8 ± 0.1[a]	3.0 ± 0.1[a]
Condition coefficient K	0.49 ± 0.01[a]	0.49 ± 0.00[a]	0.49 ± 0.00[a]
Protein efficiency ratio (PER)	0.286 ± 0.046[a]	0.030 ± 0.033[b]	0.078 ± 0.013[b]
Survival (%)	55.3 ± 2.2[a]	31.7 ± 3.3[b]	35.7 ± 2.6[b]

Groups with the same letter index in the same row do not differ significantly statistically (P > 0.05)

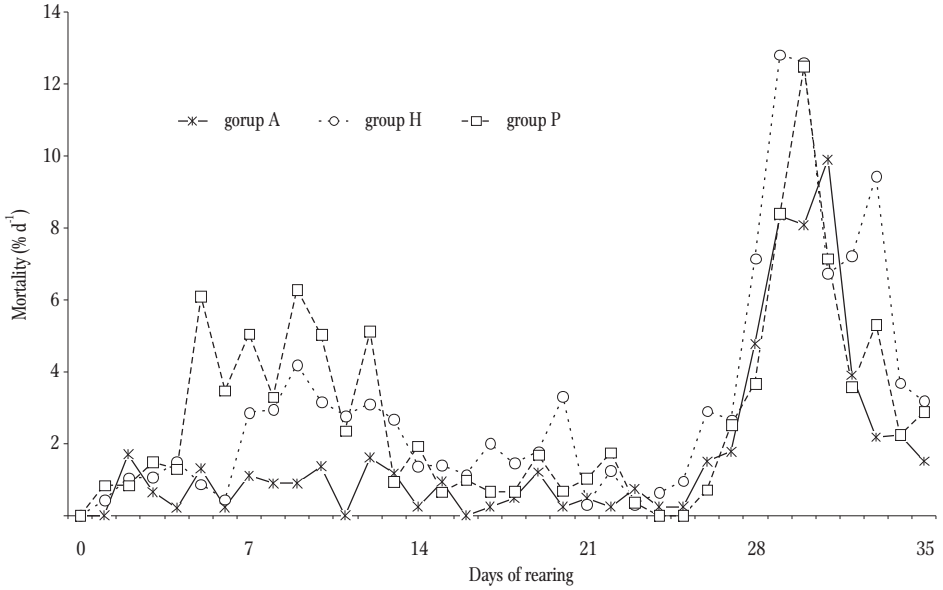

Figure 2. Mortality during the larviculture of Atlantic sturgeon (*A. oxyrinchus*) fed different diets: Aller ArtEX (group A), Nutra HP (group H), and Perla Prolarvae (group P), N = 3.

stock in group P and 4.2% in group H. The second period of increased mortality began on day 26 of rearing, when it exceeded 12% d^{-1} in groups H and P (Fig. 2).

The feed conversion ratio was low with FCR values ranging from 7.33 in group A to 21.9 in group P. At the conclusion of the experiment in group H the value of this coefficient was negative. The highest protein efficiency ratio (PER) was noted in the fish fed Aller ArtEX feed at 0.286, which was significantly statistically higher than the values of the other groups ($P<0.05$; Table 3).

Discussion

Immediately after beginning exogenous feeding larval Atlantic sturgeon do not accept formulated feed and require live food. To date, *Artemia* sp. have been demonstrated to be the best food for this period (Mohler 2003), but even with this food mortality can be as high as 50% of the stock (Szczepkowski et al. 2010).

Studies to date have failed to identify unambiguously the cause of such high mortality, but possible causes include the presence of cyst shells and

undecapsulated *Artemia* that can obstruct the digestive tract (Szczepkowski et al. 2010). *Artemia* cysts that have been decapsulated during a fast-acting chemical reaction that simultaneously disinfects them and removes the indigestible shells are used in the culture of many fish species (Van Stappen 1996). These organisms retain all of their nutritional properties, and can be used as food in this state (Vanhaecke et al. 1990) or they can also be rinsed, after which they are more desirable to juvenile fish (Kossakowski 1992). Some fish species, for example, tench, *Tinca tinca* (L.); Garcia et al. 2011 and hybrids of Siberian sturgeon, *Acipenser baerii* Brandt and Russian sturgeon, *Acipenser gueldenstaedtii* Brandt and Ratzeburg; Szczepkowska et al. 2009), exhibited greater preferences for live nauplii than for decapsulated *Artemia* that had not been rinsed. Larval Atlantic sturgeon did not exhibit any interest in consuming dried, decapsulated *Artemia* cysts, which probably stemmed from this food's lack of movement (Szczepkowska, unpublished data). The observations from the current study confirmed that larval Atlantic sturgeon generally exhibited greater interest in actively swimming nauplii than they did in passive feed, which is why the decapsulated cysts used in the present study were incubated.

The cyst preparation method (decapsulation or a lack thereof) did not impact fish growth since the fish in both groups had attained similar body weights and lengths by the end of the experiment. This indicates that the decapsulation process itself does not lower the nutritional values of the nauplii. This is confirmed in the study by Garcia-Ortega et al. (1998) in which no differences were noted in the nutritional components or enzymes between *Artemia* nauplii or decapsulated cysts. However, higher larval survival rates were noted in the group fed undecapsulated *Artemia*, and this could have resulted from different levels of food acceptance between the two groups. Van Stappen (1996) observed that undecapsulated *Artemia* nauplii were more intensely colored and swam more actively than did decapsulated nauplii, which might have affected the feeding preferences of the fish. Since sturgeon do not rely on their vision when searching for food, the color of it is not really significant; however, it seems that these differences could stem from the varied behavior of the nauplii and their greater or lesser availability to the larvae. The results of the experiment indicate that undecapsultaed *Artemia* were more readily available and accepted.

The highest mortality in both groups was observed between days 14 and 16 post hatch, which corresponded to that recorded in earlier studies (Kolman et al. 2006, Piotrowska et al. 2010). Mortality during this period is characteristic of other sturgeon species and stems from problems encountered during the transition to exogenous food once resources of endogenous food are depleted (Bardi et al. 1998). Mortality was noted in both groups studied mainly among weaker individuals that did not feed and often exhibited wounds in the caudal section of the body. Fish observed to have inflicted wounds were the victims of the cannibalistic behavior of faster-growing individuals (Baras et al. 2000).

No unhatched cysts or shells were noted in the digestive tracts of the larvae fed decapsulated *Artemia*. Thus, it appears that when high-quality *Artemia* is incubated properly decapsulation is not required. This process could only be necessary when cysts have a relatively low hatch rate (Celada et al. 2011), or when they are of uncertain origin and could be a source of bacteria of the genus *Vibrio* sp., which can cause high mortality in cultured fish (Sorgeloos et al. 2001).

If fish culture is to be successful, then the transition from live to formulated food must go smoothly. Changing the type of food reduces the labor required to prepare and deliver *Artemia*, thus reducing culture costs. However, in contrast to most sturgeon species, larval Atlantic sturgeon do not readily adapt to consuming feed (Mohler 2000), and mortality rates as high as 25% of the stock can occur during this period (Mohler 2003). Larval adaptation to new feed is compounded by their unwillingness to accept new taste and scent stimulants (Kolman 2008), which both play decisive roles as sturgeon search for food (Kasumyan 1999). Therefore, appropriate feed must be chosen not only based on nutritional value, but also with regard to taste and scent (Kolman et al. 2006). Additionally, new feed should be introduced gradually to allow the larvae to become accustomed to it over a longer period of time (Szczepkowski et al. 2007). Currently, most

sturgeon species are fed salmonid starter feeds (Jodun 2004, King 2004, Ware et al. 2006, Kolman 2008), but these did not produce the desired results in Atlantic sturgeon. Similar observations were reported for larval white sturgeon, *Acipenser transmontanus* Richardson, among which were noted individuals with slow growth rates and various body deformations (Hung et al. 1987).

The proximate composition and particle size of the feed used in the current experiment complied with recommendations for Atlantic sturgeon. Mohler (2003) and Jodun (2004) reported that feed used in the larval culture of this species should comprise 48-59% protein, 16% fat, and 7-12% ash with a particle size of 0.2-0.4 mm (Kolman 2006), but the components used to produce the feed differed significantly. The best results were obtained with Art-Ex, the main component of which (*Artemia* cysts) is most similar to the natural food of larval sturgeon. Survival among the fish fed this feed was more than 20% higher in comparison with that in the groups fed the other feeds tested. The worst results were noted with HP feed, the main components of which are fish meal and crustacean meal.

Although the larvae consumed the feed, they did not fully assimilate the nutritional components in it (Mohler 2003), as is reflected in the high values of the feed conversion ration (FCR) as well as those of the specific growth rates (SGR; 3.56-3.87). In older fish the relative SGR exceeded 10% daily, and not until 60 days of culture did it decrease below 6% d^{-1} (Kolman et al. 2008). Thus, it can be concluded that none of the feeds used in the current experiment fully meets the nutritional requirements of Atlantic sturgeon. This is confirmed by the fact that mortality increased in all of the groups tested once the food source was switched to formulated feeds, as well as when feed particle size was changed. Simultaneously, differences in fish size were noted in all of the groups tested when the fish were fed formulated feeds. This is a common phenomenon during the culture of Atlantic sturgeon larvae and fry (Kolman et al. 2008), and it is linked with variable feed adaptation rates among individuals. Larvae that are beginning to consume formulated feeds grow very rapidly, and the occurrence of such fish can have a negative impact since they monopolize feed consumption and limit the possibility of other fish feeding. This can lead to starvation and then death in some fish. This phenomenon was most apparent in the group of fish fed Nutra HP starter, and in which the fewest fish consumed the feed.

The results of the experiment indicate that *Artemia* is the optimum food when exogenous feeding commences. Since the decapsulation of *Artemia* cysts did not improve the results of the experiment, it can be concluded that mortality in early stage culture of larval Atlantic sturgeon was not the result of indigestible shells in the food. Of the feeds tested in the experiment, the best results were obtained with that in which the main component is *Artemia* and is the most similar to the previous food of the larval sturgeon. Feeding behavior in this group changed relatively little, and these larvae most easily adapted to the new feed. Thus, this can be recommended for short-term larval culture, for example when producing stocking material. The results of the current experiment with formulated feeds also confirm observations that Atlantic sturgeon are highly sensitive to changes during culture, including changes in food sources.

Author contributions. I.P. and M.S. conceived of and implemented the experiment; I.P., B.S., M.K., K.W., and M.S. prepared and performed the experiment; I.P., B.S., M.K., K.W., and M.S. collected data; M.S. performed statistical analyses; I.P., B.S., and M.S. wrote the article.

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