THE EFFICIENCY OF SIBERIAN STURGEON LARVAE FEEDING WITH ARTEMIA NAUPLII HATCHED FROM DECAPSULATED CYSTS

Liliana B. Athanasopoulos[1*], Desimira M. Stroe[1], D. Mirea[1], Fl.M. Dima[1], N. Patriche[1], Magdalena Tenciu[1], Veta Nistor[1], Elena Sîrbu[1]

[1]*Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture Galaţi, Romania*

Abstract

Aquaculture worldwide uses Artemia salina brine shrimp due to the cysts which have the advantage of being stored and incubated as needed and can quickly become, through the freshly hatched nauplii, the most important source of live food for fish larvae. This study looks at new methods for decapsulating Artemia cysts and the effect of using hatched nauplii from decapsulated or undecapsulated cysts in Siberian sturgeon larvae feeding. Decapsulation of cysts is an optional operation carried out in order to increase the hatching rate of nauplii and to reduce the mortality (up to 10%) of fish larvae which are fed on freshly hatched nauplii and whose separation from the shells or unhatched cysts cannot be total. Decapsulation avoids a possible obstruction of the fish larvae intestine with the Artemia cysts and the related mortality.

Key words: decapsulation, nauplii, Siberian sturgeon larvae, mortality

INTRODUCTION

The brine shrimp *Artemia salina* has been used in last decades in world larviculture [10] for fish larvae feeding, especially for the species that have a very small mouth opening or for marine fish with a high content of unsaturated fatty acids requirement, such as sea bream (*Sparus aurata*) and sea bass (*Dicentrarcus labrax*) [9].

In Romania, Artemia nauplii are used to feed fish larvae with high economic value since the early stages of exogenous feeding [1]. The high content of protein [7] and unsaturated fatty acids [5] varies depending on the species of Artemia and environmental conditions. This crustacean is a good biofiltrator and can be enriched, depending on the needs, with nutrients or active substances such as antibiotics, hormones, vitamins, probiotics, unsaturated fatty acids, etc. Obtaining live biomass by rapidly hatching cysts [8], makes the brine shrimp *Artemia salina* the main source of live food administered in the larviculture or aquaristics.

Depending on the variations of the environmental conditions *Artemia* can reproduce by ovoviviparity or by oviparity. In extreme conditions of living environment, the female adult lays cysts that can remain in diapause, an metabolic dormancy [11], dehydrated for long periods of time, until the optimal conditions for their hatching are restored. They can be dried, stored, incubated and used as needed, throughout the year, which recommends Artemia cysts as the best source of live food for aquaculture. The paper aims to study the efficiency of decapsulation techniques of Artemia cysts used for feeding sturgeon larvae.

Artemia cysts decapsulation consists in treating them with chemical substances for the chorion corrosion, until it is completely dissolved. The advantages of decapsulation are:

- by the action of corrosive chemicals the cysts suffer a decapsulation and a disinfection from possible pathogenic germs;
- as the process of separating hatched nauplii from cysts shells cannot be complete, cysts decapsulation avoids the cysts shells from entering in the rearing system and the danger of being ingested by the fish [3];

[*]Corresponding author:
lilianablondina@yahoo.com
The manuscript was received: 07.10.2021
Accepted for publication: 19.10.2021

-cysts decapsulation will help fish to avoid the ingestion of shells or undecapsulated cysts, which cannot be digested and can obstruct the intestine, which would eventually lead to increased mortality among fish larvae;

- *Artemia* nauplii hatched from decapsulated cysts have an increased energy content and an increased individual weight 30-35% higher than the nauplii hatched from undecapsulated cysts;

- because the lighting is not needed and the incubation period are shorter for the decapsulated cysts, the energy costs are considerably reduced;

- decapsulated eggs can be used directly for fish larvae feeding, eliminating the costs of incubation.

Because the separation of freshly hatched *Artemia* nauplii from shells or unhatched cysts cannot be achieved in proportion of hundred percent, these may cause indigestion or intestinal obstruction for fish larvae, the present experiment aims to establishes their effect on the feeding process of Siberian sturgeon larvae by monitoring mortality in different feeding variants [6].

MATERIAL AND METHOD

The experiment was conducted between 26.02-18.03.2021 within the Recirculating System of I.C.D.E.A.P.A Galati and includes two stages:

Stage 1 includes preliminary tests regarding the efficiency of *Artemia* cyst decapsulation carried out on 26.02-27.02.2021 by two methods, stereomicroscope measurements and determination of the hatching percent.

Estimating the degree of decapsulation was accomplished using an Olympus SZ 61 stereomicroscope equipped with an SC 50 video camera. The hatched nauplii were counted after 24 hours, the incubation was performed in triplicate, using a density of 30 cysts per 10 ml of 5 ppt. saline, for establishing the efficiency of cysts decapsulation through the nauplii hatching percentage The cysts used were from Ocean Nutrition, with hatching characteristics of 230000 cysts per gram, were previously hydrated for 90 minutes in fresh water in cylindroconical containers with bottom aeration, at water temperature of 25°C.

The experiments for cyst decapsulation were performed in three variants:

V1-decapsulation for 6 minutes with a solution of 250 mg sodium hydroxide in 0.5 ml saline solution 5 ppt and 5 ml sodium hypochlorite 5% refrigerated, for 0,5 wet mass hydrated cysts (which have been hydrated in fresh water with aeration at 25°C for 90 minutes), then rinsed for 10 minutes with tap water and neutralized with 0,1 N hydrochloric acid [4];

V2-decapsulation for 6 minutes with a mixture of 250 mg sodium hydroxide in 0.5 ml saline solution 5 ppt. and 5 ml peracetic acid 15%, for 0.5 wet mass of hydrated cysts, rinsed and neutralized just like in the previous variant (original method);

V3-decapsulation for 4 minutes with 10 ml peracetic acid 15% rinsed for 10 minutes with tap water (original method). Peracetic acid is a mixture of acetic acid and hydrogen peroxide that decomposes reversibly into peracetic acid and water [2]. The advantages of its use are due to the strong oxidation reaction of this acid which is not toxic to the environment or to operators.

Freshly hatched nauplii from each experimental variant were fixed with a few drops of Lugol's solution, counted on the Kolkwitz chamber and the results were presented in Table 2.

Stage 2 consists in monitoring the results of feeding Siberian sturgeon larvae with *Artemia* nauplii obtained from cysts through the decapsulation methods, used in stage 1, and the undecapsulated cysts used for control:

The *Acipenser baerii* larvae six days old, with an average mass of 19.3±1.6 mg and an average length of 11.5±0.6 mm, from the C1, C2, C3, C4, C5 square fiberglass tanks, with water volume of 0,67 m^3, populated with 2.000 larvae per rearing tank, at a water temperature of 19±0.8°C, were fed freshly hatched nauplii from cysts:

-undecapsulated, control variant H1, distributed in the rearing tanks C1 and C2;

-decapsulated (through method V1), variant H2, distributed in rearing tanks C3 and C4;

-decapsulated (through method V2), variant H3, distributed in rearing tanks C5 and C6;

The incubation of the cysts was achieved in cylindroconical containers with a volume of 2 liters provided with aeration from bottom as to keep all the cysts in suspension, in a constant water temperature of 25°C, in saline solution 35 ppt with an addition of 2 g sodium bicarbonate for maintaining an alkaline pH, and for the undecapsulated cysts was used a supplementary an illumination of 2000 lux.

The quantity of cysts used per incubation unit was 1.5 g, and the total quantity calculated for the two stock tanks from each experimental variant was 12 g of Artemia cysts calculated with the formula:

$$X=[Vt(ml)\ xNo.n/larv./ml]x[\%HrxNo.cy/g]^{-1}$$

whereas:
Vt=total volume of all rearing tanks (ml);
No.n/larv/ml=number of nauplii required to feed one larvae/ml;
%Hr=percentage hatch rate;
No.cy=number of cysts per gram.

The Siberian sturgeon larvae were fed with freshly hatched nauplii during 04.03-11.03.2021 with 8 meals per 24 hours. Between 11.03-18.03.2021 a small amount of fodder was introduced in order to gradually accommodating with artificial food for the larvae.

RESULTS AND DISCUSSIONS

The results of the experiment for the cyst decapsulation depending on each method used were evaluated through the estimation of the decapsulation degree (the average value of the decapsulated cysts diameter - photo 1, 2, 3 and table 1) and the hatching percent (photo 4 and table 2).

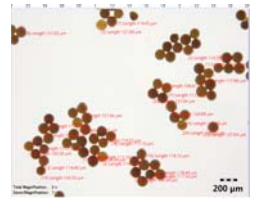


Photo no. 1- Decapsulation of *Artemia* cysts in V1

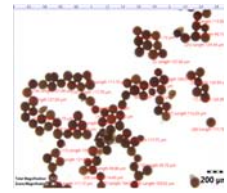


Photo no. 2- Decapsulation of *Artemia* cysts in V2

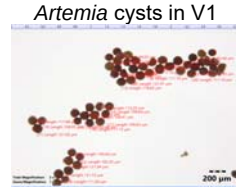


Photo no. 3- Decapsulation of *Artemia* cysts in V3

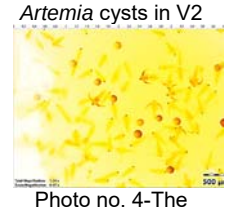


Photo no. 4-The counting of nauplii and unhatched *Artemia* cysts V1

From the centralized data in Table 1 it can be observed, the best decapsulation was performed in the V3 variant that shows a low hatching percentage compared to the one obtained in V2 variant (according to Tab. 2).

This could be explained by the aggression of the substance used for decapsulation on the embryo resulting in its death or by visual measurement errors of the stereomicroscopic method.

Table 1 Average diameter of decapsulated cysts

Experimental variant	Average diameter of decapsulated cysts (µm)
V1	116.38
V2	115.66
V3	114.45

Table 2 The percentage of hatched *Artemia* nauplii

V1 (Incubation interval 26.02-27.02.2021, hours 10.44)	Nauplii in different stages of development	25	40%
	Unhatched cysts	10	60%
V2 (Incubation interval 26.02-27.02.2021, hours 11.43)	Nauplii in different stages of development	20	67%
	Unhatched cysts	10	33%
V3 (Incubation interval 26.02-27.02.2021, hours 13.05)	Nauplii în toate stadiile Nauplii in different stages of development	17	57%
	Unhatched cysts	13	43%

Following tests with the three variants for decapsulation, variant V3 was excluded from the experiment. For variants V1 and V2 was found that cysts have different degrees of decapsulation.

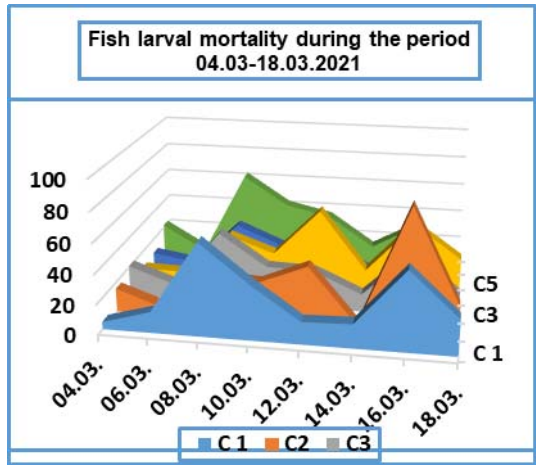
During the period 04.03-18.03.2021, the experiment of feeding the Siberian sturgeon larvae with *Artemia* nauplii was initiated in duplicate, with the three feeding variants H1, H2, H3, mortality was monitored and presented in table 3 and graph 1.

The mortality values paired with each feeding variant on two rearing tanks, are presented in table and graph 2.

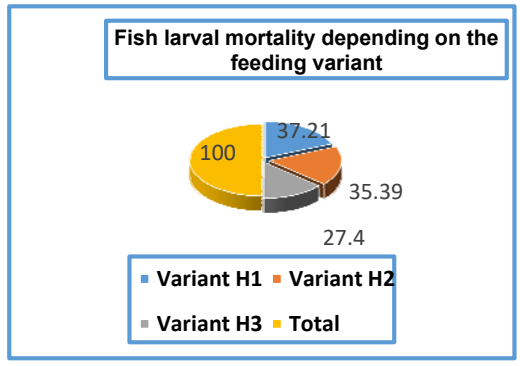
Table 3 Siberian sturgeon larvae fed in different variants

Variant H1	37.21%
Variant H2	35.39%
Variant H3	27.40%
TOTAL	100.00%

Total mortality is recorded and may include cannibalism and other causes, but the culture conditions and the food rations are uniform for all the experimental premises within the recirculating system.



Graph no. 1 Mortality obtained in the experiment of feeding Siberian sturgeon larvae with *Artemia* nauplii



Graph no. 2 Percentage mortality of sturgeon larvae recorded in the three experimental feeding variants

It is observable from graph 1 and 2 that the higher mortality values are registered in feeding variant H1 (with *Artemia* nauplii from undecapsulated nauplii), and the lowest mortality were registered in variant H3 (nauplii hatched following the decapsulation cysts with sodium hydroxide and peracetic acid by the method V2).

CONCLUSIONS

1-The results show that the administration of freshly hatched nauplii together with the shells and undencapsulated cysts (from which they cannot be completely separated) can cause losses of up to 10% of the sturgeon larvae fed in variant H1;

2-The use of decapsulated cysts through method V2 is efficient, shows a 67% hatching percentage, is less polluting, and the mortality of the Siberian strugeon larvae fed with the H3 variant are lower – 27.4%;

3-The difference between the mortality recorded in the feeding variant H1 (37.21%) and H3 (27.4%) is 9.81%, which indicates that the use of decapsulated Artemia cysts can reduce the mortality of fish larvae by up to 10%;

4- Further research is recommended to find more precise methods for quantifying the effect of using decapsulated eggs in feeding fish larvae.

REFERENCES

[1] Bura M., The feeding of sturgeon larvae in Recirculating Aquatic System, The farm, 2010, shorturl.at/ntwzS;
[2] Dibo Liu et al., Peracetic acid is a suitable disinfectant for recirculating fish-microalgae integrated multi-trophic aquaculture systems, 2016, Aquaculture Reports, shorturl.at/bklKS.
[3] Garcia-Ulloa M. Gomez and Hernandez-Garciabada F., Effect of the feeding ration on growth performance of *Oreochromis mossambicus* (Peters) larvae using decapsulated *Artemia* cysts as dietary supplement, 2004, Hidrobiologica, ISSN 0188-8897, shorturl.at/bpEIM;
[4] Granvil D. Treece, Artemia Production for Marine Larval Fish Culture, 2000, Southern Regional Aquaculture Center, shorturl.at/pxN49;
[5] Herawati V.E., Hutabarat J., Radjasa O.K., Nutritional Content of Artemia sp. Fed with Chaetoceros calcitrans and Skeletonema costatum, 2014, Journal of Biosciences 21 (4), shorturl.at/noGH2;
[6] Kayiș Ș. et al., Bacterial pathogens and health problems of Acipenser gueldenstaedtii and Acipenser baerii sturgeons reared in the eastern Black Sea of Turkey, 2017, National Library OF Medicine from National Center for Biotechnology Information, shorturl.at/nAGKN;
[7] Maldonado-Montiel D.N.J.T, Rodriguez-Canche L.G., Biomass production and nutritional value of Artemia sp. (Anostraca Artemiidae) in Campeche, Mexico, 2005, Revista de Biologia Tropical vol. 53, no. 3-4 San Jose, shorturl.at/disDZ;
[8] Mocanu E., Patriche N., Tenciu M., Savin V., Popa M.D., Stimulating Disease Resistance for Common Carp (Cyprinus Carpio) Reared in Recirculating System, by Utilising Feeding Diets Supplemented with Fatty Acids, 2020, Scientific Papers-Animal Science Series: Lucrări Ştiinţifice - Seria Zootehnie, vol. 74, p. 116-123;
[9] Nolting M., Ueberschau B., Rosenthal H., Trypsin activity and physiological aspects in larval rearing of European sea bass (Dicentrarchus labrax) using live prey and compunds diets, 2001, Journal of Applied Ichtiology 15 (3) shorturl.at/huxP8;
[10] Sorgeloos P., Dhert P., Candreva P., Use of brine shrimp, Artemia spp., in marine fish larviculture, Aquaculture, Volume 200, Issues 1-2, Pages 147-159, shorturl.at/fvySW;
[11] Van Stappen G., Introduction, biology and ecology of Artemia, shorturl.at/kmESX;