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TEST OF DIFFERENT FEEDING REGIMES AND DIETS FOR REARING *CYPRINUS CARPIO* LARVAE IN CLOSED RAS

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The aim of the present paper was to rear carp larvae in the closed recirculating aquaculture system (RAS) for industrial application. Two issues were investigated: starter selection and transition period from live food (*A. salina*) to starter providing good survival and growth rate. A 26-day feeding trial proceeded in two stages: 12 days (phase I) and 14 days (phase II). The carp larvae (crossbreed of HSM x M72) arrived at University of Life Sciences from University of South Bohemia four days post-hatching. The larvae were divided into A, B and C group; 10 000 larvae in each group. Additionally, 3000 larvae were placed into a mud pond (control group).

The carp feeding started on the fourth day post-hatching. Group A was given starter Aller ArtEX right away. Group B was fed on *A. salina* for nine days, followed by a co-feeding period on Aller Futura MP EX. Group C was fed on *A. salina* for three days, followed by a four-day co-feeding period on Perla Larva Proactive 6.0, terminated in feeding on Perla Larva Proactive 6.0. The most efficient transition diet was in group B. On the 16th day post-hatching, the larvae total length was 13.82±0.65 mm and survival rate was 99.93 per cent.

The second phase of the test continued with the most livable group (B). It was divided into two groups (D, E), 4995 larvae in each group. Group D continued with the diet of group B for six days, terminated on Aller Futura MP EX on the 21st day post-hatching. Group E had a co-feeing diet on hatched *A. salina* and Veronesi VITA 0.2 for six days, terminated on Aller Futura MP EX on the 21st day post-hatching. The experiment showed that starter Aller Futura MP EX meets well the carp needs. On the 26th day post-hatching the carp total length was 25,05±0,65 mm and survival rate was 91,99 per cent. In four month, the carp in RAS weighted 195.5 grams, whereas the carp in the mud fishpond weighted 22.2 grams.

Key words: carp larvae rearing, live food, artificial feed, fish growth.

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INTRODUCTION

The carp (Cyprinidae) are the most cultured commercial freshwater fish in the world. This is due to the characteristics of the carp: easy to handle, fast growth, high tolerance to increased stocking density, diseases, environmental and water hydrochemical conditions (Kirpatchnikov 1999, Solomon et al. 2015, Adib et al. 2015).

In Estonia, several attempts to breed carp in fish farms have been made with varying degrees of success since 1970s. The main obstacles to produce commercial size fish in three years are large losses of fish per unit and high prime cost. According to Jelkić et al. (2012), Feldlite et al. (1999) and Jirásek et al. (2001), in the context of production conditions and mud fishponds, 50-90 per cent of losses occur in the larvae to fry growth period due to water temperature fluctuation, lack of oxygen, pollution, large zooplanktons, natural predators (e.g. birds, frogs, snakes etc.) or feeding conditions.

A closed recirculating aquaculture system (RAS) for rearing carp larvae and juvenile may be an efficient alternative to mud ponds. The closed RAS is independent of weather and seasonal conditions. As a result, some species of fish can be reared all year round. An extensive research by Myszkowski and Wolnicki (1998 – 2012) confirmed the fact that "in comparison to the traditional outdoor technologies, indoor methods can ensure either very low fish losses or considerable improvement in growth and development rates".

As is the case with any other fish species, success of rearing most cyprinid larvae in the RAS heavily depends on the availability of suitable diets that can be ingested, digested and, as a result, provide the required nutrients to support fish growth and health (Giri et al. 2002). The rearing of cyprinid larvae requires live food in sufficient quantities. Nauplii of A. salina are commonly used as a source of live food (Sorgeloos et al., 2001). Barr et al. (2007) showed that common carp larvae rearing in the RAS instead of ponds and feeding larvae on plankton as first feeding for the first 12 days increases the common carp larval survival rate from 30 per cent to 80-90 per cent.

However, the process of preparing live food requires a high level of expertise and organization, it is time-consuming and costly. Since the development of commercial fish culture over the years, the demand for *Artemia* cyst has gradually increased (Sorgeloos et al. 2001) and resulted in a high price. Ruyer et al. (1993) estimated that the expenses on live food make up to 79 per cent of total fry production costs in marine aquaculture (Jelkić et al. 2012).

In the case of cyprinid larvae, various attempts to substitute live food with cheap artificial starters have not succeeded yet. Until the beginning of 80s, there was a strong opinion that carp larvae cannot be fed artificially by starter-feed if live zooplankton was not given first (Bryant et al. 1980). The first attempts to use Ewos commercial carp starters C10 and C20 or Ekvizo starter formulated by Ostroumova et al. (1980) were unsuccessful (Myszkowski et al. 2012). Recent research conducted on common carp with the complete replacement of live food with artificial feed ascertained the most commonly observed effects of commercial starters on cyprinid fish: low growth rate and/or low survival rate, poor food conversion, high variability of individual size, lowered biological quality and body deformities (Sierra et al. 1995, Quiros et al. 1997, 1998, 2002, Wolnicki et al. 1999, 2000, 2002). According to Wolnicki et al. (2002), at least some of these effects result from the high fat content in commercial starters.

In 90s, the research into carp larvae starter problem has taken new directions. A few attempts have been made to find the best transition period from live food to artificial. The experiments focus on reduction of the dependence of common carp larvae on live food by switching partially or totally to artificial feed at various times (Jelkić et al. 2012).

The aim of the present study is twofold:

- to determine the survival rate and the length increment of carp larvae in the

early acceptance of the artificial feed while decreasing the amount of live zooplankton;
- to compare growth and survival rate of common carp larvae fed on different starters under controlled conditions.

MATERIAL AND METHODS

Closed RAS

The RAS was build at the University of Life Sciences and had the following components:
- three intermediate bulk containers (3x700 l)
- a settling tank for solids removal (600 l)
- container for filters (740 l)
- RK-Plast floating biofilter pellets (total surface of 75 m^2) activated by NH_4CL
- trickling filter (total surface of 0,27 m^3 and usable surface of 54 m^2)
- oxygen injection (1000 mg/h)
- air diffuser
- water pump (400 W)

The total volume of the closed RAS was 4150 l. The exchange of water in fish tanks was adjusted to the tap. The water flow in the RAS was maintained at 138 l/min. Automatic heaters maintained water stable temperature.

Water quality parameters and monitoring

Various water quality parameters such as temperature (˚C), dissolved oxygen (mg/l), pH, and redox potential were recorded on the spot every day throughout the experiment. Ammonia nitrogen (mg/l), nitrate and nitrite concentration (mg/l) in the closed RAS were measured weekly in the chemical analysis lab of the University of Tartu (Appendix 1).

Larvae transportation and preparation for the experiment

Carp spawning (crossbreed of HSM x M72) and egg incubation took place at University of South Bohemia České Budějovice in Czech. On the second day after hatching, ca 33 000 carp larvae were sent to Estonia by plane in plastic containers filled with water and oxygen. The temperature in the container was 19 degrees Celsius. The larvae were brought from the airport to the University of Life Sciences by car. Transportation took 48 hours in total. The mortality of the larvae was insignificant. Mainly a knot of the container caused it. On the arrival to the closed RAS, the carp larvae were four days after hatching (one day after the acceptance of exogenic feeding).

The larvae were randomly distributed between three tanks of the RAS (10 000 larvae per 700 l tank) with stock density of 14.3 larvae per litre. Before placing the larvae into the tanks, the tank water temperature was decreased by 1 degree within two hours to match the water temperature of the transportation container (19 degrees Celsius). In the next nine days, the water temperature in the RAS was gradually increased to 27 degrees Celsius.

A control group of 3 000 larvae was intended to be reared in the 0.1 ha mud fishpond of a private fish farmer Riina Kalda. On the 30th May 2014, the water temperature in the pond was 14.6 degrees Celsius. Therefore, the water temperature in the transportation container was gradually decreased to counterbalance the difference in the temperature.

Diets and feeding

The experiment was designed in two stages and tested four commercial starters:
- Aqua Aller Futura MP EX
- Aqua Aller ArtEX
- Perva Larva Proactive 6.0
- Veronesi VITA 0.2

The details and the components of the starts are shown in Appendix 2.

As live food, nauplii of *Artemia salina* were used. The nauplii were hatched in a cone shaped hatcher under controlled conditions according to the manufacturer's instruction.

Experiment stages

First feeding (day 0) was four days after hatching

when larvae exhibited an inflated swim bladder. The experiment consisted of two stages (Appendix 3). The first stage of the experiment was aimed at the transition period from the live food to the artificial feed. The second stage of the experiment was aimed at the comparison of starters.

Stage I

The first period lasted from 30th May 2014 till 10 June 2014, in total 12 days. Larvae feeding began four days after hatching (Fig. 1).

Group A was fed over the entire period of rearing (12 days) on dry food Aller ArtEX (crude fat 15 per cent, crude protein 50 per cent). Group B1 was given *A. salina* nauplii for nine days, followed by four days of co-feeding on Aller Futura MP EX (crude fat 17 per cent, crude protein 60 per cent). Group C was given *A. salina* for three days, followed by five days of co-feeding on Perla Larva Proactive 6.0 (crude fat 11 per cent, crude protein 62 per cent), switching to the starter only for the rest four days. The larvae in the control group were fed with Zivim feed produced in Latvia. The feed contained natural

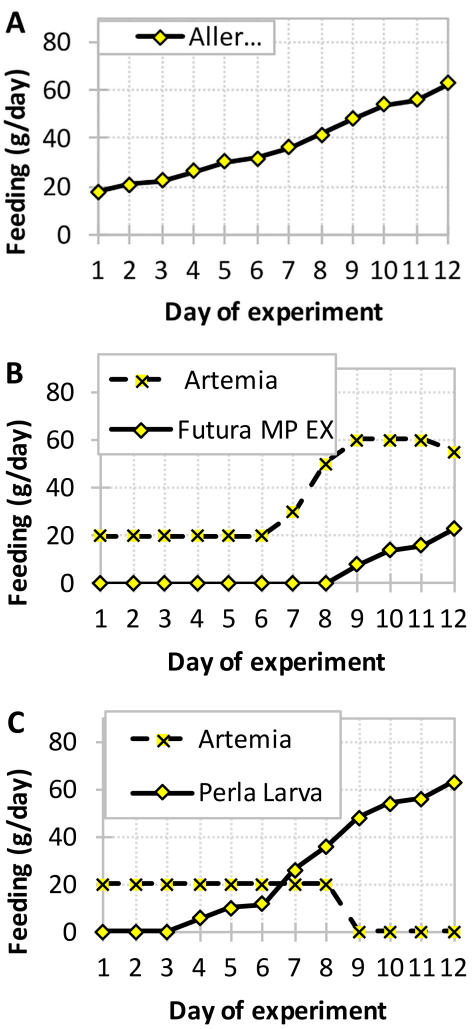

Fig.1. Feeding regimes for each group.

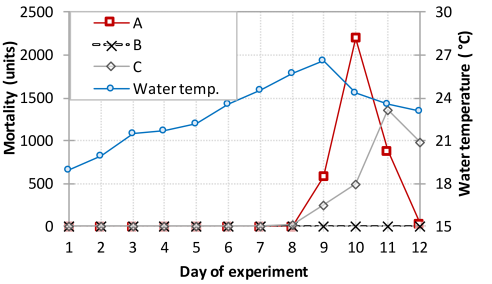

Fig. 2. Water temperature in RAS and larvae mortality.

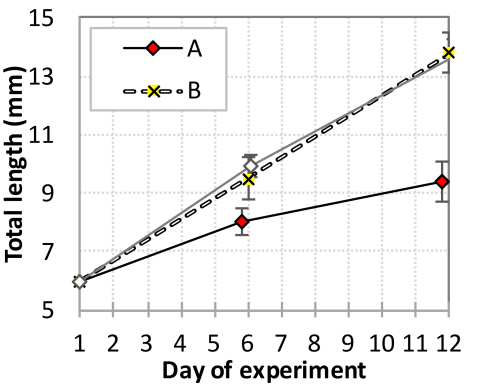

Fig. 3. Average length.

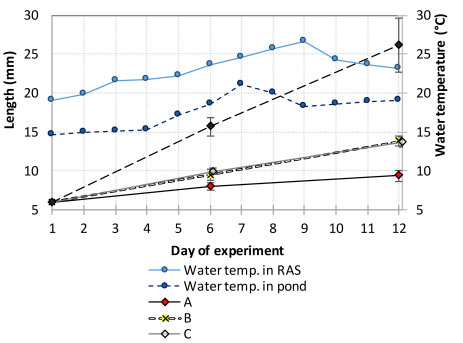

Fig. 4. Water temperature in RAS and fishpond, larvae growth.

Table 1. The growth and survival rate

	Group A	Group B	Group C	Control group
Total length on 6th day of experiment (mm)	7.99±0,45	9.49±0.72	9.92±0.39	15.73±1.18
Total length on 12th day of experiment (mm)	9.38±0,7	13.82±0.65	13.69±0.56	26.18±3.42
Survival rate (%)	<63.01	99.93	68.81	-

Table 2. The growth and survival rate

	Group D	Group E
Starter	Aller Futura MP EX	Veronesi VITA 0.2
Total length (mm) on the 18th day of the second stage	18,7±0,72	17,95±0,45
Total length (mm) on the 22nd day of the second stage	25,05±0,65	22,39±0,7
Survival rate on the 26th day of the second stage (%)	91,99	36,67

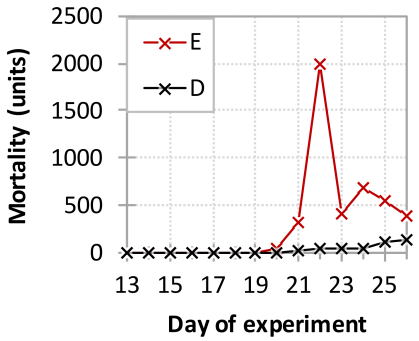

Fig. 5. Larvae mortality.

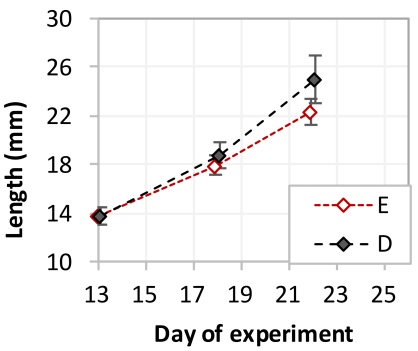

Fig. 6. Carp length

components, 24.16 per cent crude protein and 3.99 per cent crude fat.

All larvae in the RAS were fed in excess (*ad libitum*). The artificial feed was served by an automatic feeder every 32 minutes 24 times a day between 8 am and 9 pm. Nauplii of *A. salina* was given twice a day for the first six day, followed by three times a day regime for the next six days. The larvae of group A learned how to catch the floating particles on the surface of the water by the third day of the experiment. On the contrary, the larvae in group B and C instinctively caught moving food as early as the first feeding of larvae on *A. salina* nauplii. Good lightning of tanks was crucial because carp larvae use their sense of sight in the first days of life. On the sixth day of the experiment, group C caught artificial food intensively. The growth of the larvae and the survival rate are shown in Table 1 and Fig. 2-4.

Jelkić et al. (2012) suggested rearing larvae at the temperature between 26 and 30 degrees Celsius. However, at the temperature of 27 degrees Celsius high mortality was observed and the water temperature in RAS was decreased to 24 degrees Celsius.

Stage II

The second stage of the experiment lasted from 11 June to 24 June 2014, in total 14 days. Since group B had the least mortality (99.93 per cent), it was used in the second stage of the experiment. Group B was divided into groups (D and E), ca 4 996 larvae in each group. Both groups were co-fed with *A. salina* for six days. Group D was fed on Aller Futura MP EX. Group E was fed on Veronesi VITA 0.2. Both groups were fed in excess. On the sixth day of the second stage of the experiment (18th day of feeding), the co-feeding ceased. The mortality in group E increased dramatically (40 per cent). Next day it was decided to switch back to Aller Futura MP EX. The mortality slightly decreased (Table 2, Fig. 5, Fig. 6).

DISCUSSION

In a very large number of fish feeding tests (Szlaminska et al. 1985), it has been concluded that feeding on both zooplankton and dried feed in excess results in the larvae highest growth rate. Unfortunately, in the production conditions, it is impossible to feed the fish with both feeds in excess. The main problem is connected to the live food. Firstly, the preparation of hatched *A. salina* is labour-intensive. Secondly, the feeding on live food cannot be automated. Therefore, fish farms are interested to make the transition period as short as possible.

Matty et al. (1981) reported that 9.5 mg carp larvae could be fed on a starter for trout with survival rate of 80 per cent. Bambroo (2012) found more efficient schema: the carp larvae can be successfully given only artificial feed at the weight of 15.2 mg with zero per cent mortality. The present experiment confirmed Bambroo's conclusion.

In group C, the transition period was the shortest. On the sixth day, it was observed that the group had the highest growth rate (9.92 ±0.39 mm) than group B (9.49 ±0.72 mm). However, on the 12th day, group C lost it growth rate advantage. On the contrary, group B had the most stable growth rate. There might be several causes why group B did not grow fast. Firstly, it took the larvae time to learn to catch the feed from the water surface. Secondly, even if the larvae catch the particles, it cannot digest the feed. Group A did not have the transition period. It was fed on artificial feed in excess. However, we assume with great probability that the group had been starving from the first day of the experiment. The observation can be explained by the fact that the carp do not have the gaster.

The comparison of two different starters during the second phase of the experiment showed that only Aller Futura MP EX had good result. The causes of the high mortality rate in the second group fed on Veronesi VITA are not known. We assume the cause of a lower growth rate of the second group can be explained by different protein sources (although the chemical contents of both starters are alike). The fish farmer V. Saluste confirmed the hypothesis.

The present experiment proved that successful carp rearing in the RAS depends mainly on the continuous feeding, with both life and artificial feed. However, it is not easy to ensure all the time the optimal feeding norm of artificial feed by automated feeders. The majority of the machines can be programmed to increase the daily norm of the feed by 10 per cent. As the present experiment showed the carp larvae are be able to eat 15 - 20 per cent of own weight. According to Bryant et al. (1980), the optimal norm of feed lies between 15 and 17.5 per cent. The growth rate increase in consequence of water high temperature in the RAS was not observed. On the contrast, the feeding and the dietary activity increased at the temperature of 23-24 degrees Celsius. Therefore, the conclusion of Jelkić et al. (2012) to rear carp larvae in the RAS at 26-30 ˚C was not confirmed. The optimum water temperature can be considered 24 ± 0,5 degrees Celsius. If the rate of the dissolved oxygen in the RAS cannot be technically maintained, it is suggested to slightly decrease the water temperature.

The density of 14,3 larvae per litre during the first phase of the experiment may be considered optimal. Generally, the population density of 25 larvae per litre is considered to be low (Sharma et al. 1999). Nevertheless, the increase in the growth rate of the larvae at the first stage was so significant that two consecutive experiment phases would not have been able to withstand without density decrease in the tank. On the contrast, 50 and 100 larvae per litre will cause a significant increase in mortality and a decrease in weight gain. In order to grow 0.5-gram fish, the original density should be between 5 and 7 larvae per litre.

Post experiment

After the second stage of the experiment, the larvae were transported to the closed RAS of a fish farm. The larvae were kept in the same conditions as at the University of Life Sciences for the next five months.

By the end of the summer, the fish rearing in the closed RAS weighted seven times more than the fish reared in the mud pond (Fig. 7).

Importantly, the growth rate of the larvae in the mud pond stopped in the middle of October whereas it continued to increase with the same rate in the closed RAS.

CONCLUSION

The present paper and the conducted pilot experiment had two goals. The first goal was to study the primary feeding habits of the carp larvae using different diets as well as to evaluate the efficiency of the changeover in diets from live food to artificial aqua feed. Secondly, the same pilot experiments facilitated to study larvae rearing in the closed RAS and compare the results of rearing in a traditional pond farming. The research methodology consisted of the experiment on teaching the larvae to feed on live food first and then to change over to the artificial feed. The control group was reared in the mud pond.

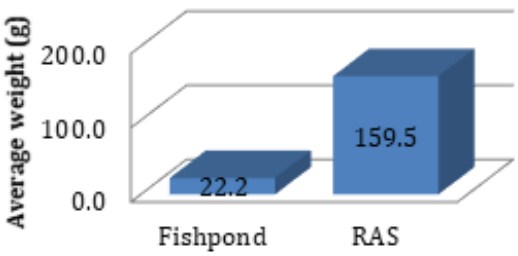

Fig.7. Carp weight in four month (in the fishpond and RAS).

The conducted experiment on feeding the carp larvae in the closed RAS was successful when the feeding schedule was as following: the first 18 days the carp larvae were fed on hatched *A. salina* larvae; in addition, from the ninth day the carp larvae were given the artificial feed (Aller Futura MP EX 0,2 mm). Furthermore, out of four starters, Aller Futura MP EX showed the most reasonable results. The described feeding schedule and starter had the highest survival rate than the other diets (99.93 per cent). The presented experiment also showed that the carp larvae are possible to rear in production quantities in the closed RAS with great success if the population density is about 14 fish per litre.

The results of present research confirmed the conclusions of previous research papers: there is no determined age or weight of larvae, which guarantees successful changeover to the artificial food. The success depends rather on the food, feeding strategy and methodology. Furthermore, the current experiment did not confirm the statement that high temperature of the water is a top priority.

Considering carp farmers and their interest, the following conclusion was reached: it is feasible to schedule spawning to an earlier period and shorten the production cycle up to a year by bringing carp larvae to Estonia a few month earlier and rearing them in warm water (24 degrees Celsius).

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APPENDIX 1. WATER PARAMETERS

Table 4. Water chemical parameters in RAS

	NH_4-N (mg/l)	NO_2-N (mg/l)	NO_3-N (mg/l)
26.05.2014	0.014	0.002	36
02.06.2014	0.12	0.33	36.2
09.06.2014	0.27	0.072	30.5
18.06.2014	0.45	0.165	26.5

Table 3. Water parameters in RAS and fishpond

Date	Day of experiment	Air	Water	O_2 (tank A)	O_2 (tank B)	O_2 (tank C)	O_2 (settling tank)		Water change per day	pH	Conductivity	Redox	Water in pond
		C^0	C^0				Mg/l	%	l		µs/ms	mV	C°
28.05.14		18	19.7	7.3	7.2	7.4	7.4	77		8.27	0.955	160	
29.05.14		18	19.2	7.3	7.7	7.4	7.3	79		8.3	0.97	192	14.1
30.05.14	1	17	19	7.4	7.7	7.7	7.4	79	2	8.3	0.1851	185	14.6
31.05.14	2	17	19.9	7.2	7	7	7.2	78	40	8.27	4.49	208	14.9
01.06.14	3	17.4	21.5	5.7	6.5	6.5	6.3	72	32	8.09	6.28	153	15.1

Date	Day of experiment	Air	Water	O_2 (tank A)	O_2 (tank B)	O_2 (tank C)	O_2 (settling tank)		Water change per day	pH	Conductivity	Redox	Water in pond
		C^0	C^0				Mg/l	%	l		µs/ms	mV	C°
02.06.14	4	17.3	21.7	6.7	6.8	6.8	6.7	75	234	8.17	6.27	164	15.2
03.06.14	5	17.8	22.2	6.3	6.2	6.3	6.1	69	5	8.16	6.02	207	17.1
04.06.14	6	25	23.6	6.3	6.3	6.2	6.2	73	3	8.11	6.1	218	18.5
05.06.14	7	22.5	24.5	5.8	5.8	5.8	6	71	0	7.96	6.14	222	21.1
06.06.14	8	23.3	25.7	5.8	5.7	5.3	5.7	68	63	8.05	6.19	222	20
07.06.14	9	24	26.6	5.8	5.4	5.5	5.5	67	71	8.03	6.19	167	18.3
08.06.14	10	21.4	24.3	5.7	5.4	5.5	5.2	61	64	7.94	6.2	149	18.5
09.06.14	11	20.5	23.6	5.9	5.6	5.7	5.6	67	152	7.91	6.17	155	18.9
10.06.14	12	20	23.1	6.2	5.9	6	6	70	598	8.01	6.12	159	19.1
11.06.14	13	22	23.6	6.3	6	6	5.9	69	54	7.93	5.82	159	19.8
12.06.14	14	23	23.7	5.7	5.7	6	5.9	65	113	8.04	5.73	153	18.9
13.06.14	15	22.8	23.6	6.2	5.8	6.3	6.2	72	214	8.11	5.55	156	18.1
14.06.14	16	23	23.7	6.2	5.4		6	70	171	8	5.37	161	18.1
15.06.14	17	23	24.2	5.8	5.1		5.8	68	110	7.98	5.27	168	18
16.06.14	18	23	24.3	6	5.1		5.9	69	133	8.02	5.25	166	17.2
17.06.14	19	23	24.2	5.9	5.1		5.8	68	106	7.96	5.04	171	16
18.06.14	20	22	23.9	5.4	4.3		5.1	59	113	7.78	4.94	148	14.7
19.06.14	21	21.5	23.6	4.8	4.5	6	4.6	54	303	7.73	4.7	133	14.7
20.06.14	22	21	22.9	4.7	4.5		4.6	54	319	7.41	4.18	120	15.1
21.06.14	23	21.5	23.5	5.6	4.8	5.1	5	58	144	7.69	4.24	114	15.3
22.06.14	24	22	23.5	5.4	4.6	4.8	4.8	56	322	7.57	3.94	100	14.9
23.06.14	25	21.5	23.6	5.5	4.1	4.4	4.5	52	141	7.55	3.94	128	14.9
24.06.14	26	21.5	23.8				4.4	53			3.82	136	15.4
Average		**21.1**	**23.1**	**6.0**	**5.7**	**6.1**	**5.8**	**66.9**	**3507**	**8.0**	**4.9**	**163.4**	**16.9**

APPENDIX 2. STARTER PROPERTIES

Table 5. Starter properties

	Hatched *Artemia salina*	Aqua Aller Futura MP EX	Aqua Aller ArtEX	Perla Larva Proactive 6.0	Veronesi VITA 0.2
Size (µm)	ca 400	200	50-150	100-300	250
Fish flour				77,6 %	
Corn flour				6,4 %	
Crustacean flour				5 %	
Wheat gluten				5 %	
Dry yeast				2.5 %	
Soybean lecithin				2.5 %	
Fish oil				1%	

	Hatched *Artemia salina*	Aqua Aller Futura MP EX	Aqua Aller ArtEX	Perla Larva Proactive 6.0	Veronesi VITA 0.2
Artemia flour			mostly		
Crude protein	ca 45 %	60 %	50 %	62 %	62 %
Crude fat	ca 23 %	17 %	15 %	11 %	12 %
Carbohydrate		5.3 %			7.6 %
Cellulose		0.5 %	2 %		0.8 %
Ash		10.5 %	8 %	10 %	8.6 %
P		1.8 %	1%	1.1 %	1.35 %
Na		0.5 %			
Ca		1.8 %			
Vitamin A		10 000 IU/ kg	25 000 IU/kg	15 000	
Vitamin D3		1000 IU/kg	3000 IU/kg	2 000	1000 mg/kg
Vitamin E		300 mg/kg	250 ppm	330	850 mg/kg

APPENDIX 3. FEEDING REGIMES

Table 6. Experiment stages, feeding regimes and growth properties on the 12th and 24th day of the experiment (ED – experiment day (ED1 = four days post-hatching); TL – total length (mm); SR – survival rate (%))

			Group A	**Group B1**		**Group C**
STAGE 1	Hatched *A. salina* (Inve Aquaculture)			ED1-FD9		ED1-ED3
	Artificial feed	Aller Futura MP EX		ED10-ED12		
		Perla Larva Proactive 6.0				ED4-ED12
		Aller ArtEX	FD1-FD12			
			TL= 9.38±0,7 SR<63.01	*TL= 13.82±0.65 SR=99.93*		*TL= 13.69±0.56 SR=68.81*
STAGE 2	Artifial feed	Hatched *A. salina*		ED13-ED18	ED13-ED18	
	Artificial feed	Aller Futura MP EX		ED13-ED26	ED20-ED26	
		Veronesi VITA 0.2			ED13-ED19	
				TL= 25.05±0.65 SR=91.99	*TL=22.39±0.7 SR=36.67*	