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Increasing in Growth of *Rutilus frisii kutum* Larvae with Using Slurry (Fermented Organic Manure) in Yosefpoor Propagation and Rearing Center (Iran)

[1]M. Fallahi Kapoorchali, [2]S.M. Reza Fatemi, [2]G. Vosoghy, [3]M. Matinfar and [4]M. Sharifian
[1]Department of Marine Aquaculture Research,
National Inland Water Aquaculture Institute, P.O. Box 66, Bandar Anzali, Iran
[2]Department of Marine Biology, Faculty of Marine Science and Technology,
Science and Research Branch, Islamic Azad University, P.O. Box 14515/775, Tehran, Iran
[3]Iranian Fisheries Research Organization, P.O. Box 14155-6116, Iran
[4]Ph.D Student, Science and Research Branch, Islamic Azad University (IAU),
P.O. Box 14515/775, Tehran, Iran

Abstract: The main objective of this study is to investigate the effect of slurry on larval growth in comparison with common methods; thus, cow manure was fermented under anaerobic conditions for 40 days. Then the following two treatments were examined: concentrated food as common method and slurry as the new method. To study, the two treatments and each with three replications, 6 ponds were selected, each measuring 1.7 ha with depth of 1.7 m. The larvae population density was 1.7 m ha^{-1}. The results showed that the mean weight and length, in slurry treatments were 77.16±25.3664 mg and 22.03±2.31 mm and in control treatment were 63.13±17.8552 mg and 19.6±2.296 mm, respectively. Condition factor was found to be 0.9 for slurry and 0.8 for control treatment. During culture period some other factors such as DWG (Daily Weight Gain), DLG (Daily Length Gain), CF (Condition Factor) and SGR (Specific Growth factor Rate) were measured. In comparison with control group, all parameters had higher values in slurry treatment. The statistical analysis indicated that there is a significant difference ($p<0.001$) for parameters of length and weight for both treatments in the first week of culture and also there was a significant difference ($p<0.05$) for the items in the 2nd week too. The obtained results showed that the slurry, due to high concentration of nutrients, had more effect on growth and increased the abundance of zooplanktons, which are the main food of larvae in early stages of life cycle.

Key words: Slurry, *Rutilis frisii kutum*, larvae, Caspian Sea

INTRODUCTION

Kutum is known as one of the commercial species in Iranian waters of the Caspian Sea and has the main role in the economy of the region. Unfortunately, during previous years, its resources have been declined because of various reasons such as overexploitation of brood stocks by beach-seine especially during spawning season, changes of rivers, decrease of water flow of rivers, pollution increase, gravel and sand removals in rivers which are all caused the decrease of natural breeding of fishes.

The world aquaculture has increased from 7.72 m mt in 1985 to 26.38 mmt in 1996 mmt in 1996.This increase in fish production has corresponded with an increase in the consumption of fish meat as an alternative or substitute for red meat (Sehgal and Segal, 2002). In Iran, two hundred million

Corresponding Author: M. Fallahi Kapoorchali, Department of Marine Aquaculture Research,
National Inland Water Aquaculture Institute, P.O. Box 66, Bandar Anzali, Iran

fingerlings of Kutum, with one gram weight produced annually by breeding centers, are released into the rivers for rehabilitation (Abdolhay, 1997). Meanwhile, high percentages of larvae are killed before reaching the fingerling stage. Some of larvae take a long time to reach this fingerling stage and some of them are also underdeveloped. Necessity of success in culture and breeding of fishes is to have enough and thorough knowledge of life cycle stages, the larvae life cycle stages (feeding from yolk sac and breathing via skin), with adaptation with the next growth stage (external feeding and breathing with gills), are known as vital stages. Initiation of feeding is function of the rate and efficiency at which the yolk sac material is consumed (Bisbal and Bengtson, 1995), in that transferring from internal feeding to out-resource food causes mortality. The most critical period at onest of feeding in pikeperch *Stizostedion lucioperca* is short (5 days at 20°C). The larvae are sensitive to prey density during the first week of exogenous feeding (Ljunggren, 2002). Behavior of larval Siberian sturgeon and the effect of the timing of first feeding (5, 7, 9, 11, 13, 15 and 17. Feeding Siberian sturgeon larvae during the phase of transition between yolk-sac nutrition to exogenous feeding (5-7 days post hatch), did not provide any advantage.

Availability of feed significantly affected survival, body size and specific growth rate of fish larvae ($p<0.05$), but no abnormal behaviour was observed as a consequence of food deprivation. Fry with the highest survival rates (76-83%), growth in weight and length (288-308 mg and 35.9-37.2 mm, respectively) and specific growth rates (13.4-13.1% day^{-1}) were those fed at days 5, 7 and 9 after hatching. Condition factor decreased dramatically from 1.39 to 0.44-0.49 during the endogenous feeding phase. However, from the beginning of the exogenous feeding to 22 days after hatching, condition factor slightly increased and achieved adult shape values (0.60-0.63). These results confirm the importance of the initial feeding time and suggest that food should be offered to sturgeon larvae at 9 day after hatching, coinciding with the disappearing of schooling behaviour, the scattering of larvae across the bottom of the tank and the apparent full resorption of yolk-sac reserves (Gisbert and Williot, 1997).

Kutum larvae in early stages of feeding need foods containing protein and lipid with small-size and slow movements and on the other hand containing high chain unsaturated fatty acid. One of the best life foods are Rotifera and Zooplanktons which the decrease in the cost of production and increase in the growth and survival. Sustainable aquaculture depends upon -friendly and economically and socially viable culture serves the dual purpose of cleaning the environment (by avoiding the problem of waste disposal) and providing economic benefits. The recycling of animal wastes in fish pond for natural fish production is important to sustainable aquaculture and reduces expenditure on costly feeds and fertilizers which form more than 50% of the total input cost (Dhawan and Kaur, 2002). Slurry is one of the products which can increase the production of zooplankton in ponds. Biogas slurry (BGS) applied at 52.1 L ha^{-1} day^{-1} (6.4% dry matter) resulted in a significant increase in total zooplankton over the control (Harjeet *et al*., 1991a).

Digestion of the livestock waste under closed anaerobic conditions, followed by oxidation in open shallow basins with natural algae providing the free oxygen through photosynthesis, before letting the treated waste effluent flow into the fish pond, can convert almost 100% of the organics into inorganics, which will not consume any oxygen to deprive the fish of this important life-sustaining item. So, theoretically, it is possible to increase the quantity of waste ten-fold into the pond without any risk of pollution. By treating the livestock wastes an aerobically in digesters, with additional production of biogas energy and aerobically in shallow basins, their amount can be increased ten-fold in the system, increasing the fertilizer and feed in the pond accordingly, but without using any of the dissolved oxygen (Chan, 2004).

In fact, making use of living foods at early stages causes the decrease of mortality and increase of fast growth and survival and recruitment and decrease in production costs. This investigation was carried out to enhance the mean weight of released Kutum into rivers and the survival ratio as well.

MATERIALS AND METHODS

Two treatments with three replicates each were carried out in six ponds allocated according to a Completely Randomized Design (CRD). Each pond has an area of 1.7 ha and 1.7 m deep. The first treatment (Control) was the common culture method of Kutum. In the beginning of the experiment, fishes were fed with concentrated food daily and cow manure was added directly to the ponds. In the second treatment, fermented cow manure (slurry) was added to the fish ponds. This matter generally is used as fertilizer (Quynh Trame *et al*., 2004).

Construction and Management of Slurry Pools

Six concrete pools with dimension 2.19×14.2×1.4 m^3 were constructed. The mixture ratio was 60:50 up to 50:50 and after mixing cow fertilizer. A little yeast was added as starter. Then all pools were kept in anaerobic condition for 40 days.

Preparation and Management of Cultured Pools

The bottom and sides of ponds were treated with lime $CaCO_3$ (50 kg ha^{-1}). This process caused bacteria decomposition, destroying pathogens and eradicates predators. Wild fish and possible predators were eradicated with *Deris roots* (*Deris eliptica*) or quick lime (Dang *et al*., 2006). The ponds were preserved in a constant condition to be dried for 10 days and afterwards filling water process was done and also for primarily preparation of pond, cow fertilizer was used with a density of three tones per hectare. After a week, when density of Rotifera reached 3 cm^3 after filtering 100 L of ponds water, *Kutum* larvae were introduced to ponds in a level of 1.7 m ha^{-1}.

Control of Zooplanktons

Since in early life stages larvae feed from small and slow movement zooplanktons such as *Rotifer*, it was tried to eliminate other zooplanktons (excluding Rotifers). For this purpose, before introducing Kutum larvae to the ponds, treechlorophen poison (0.5 ppm) was added to water. This poison helps to destroy harmful zooplanktons, apus and lepthesteria.

Concentration of N (Nitrogen) and P (Phosphorous) of cow fertilizer were measured before and after microbial fermentation process. Also measurements of some parameters e.g., CO_2, O_2, temperature, transparency, conductivity and pH as well as nutrients (NO_3, NO_2, NH_4 and PO_4) were carried out.

Feeding of Larvae in Treatments

As a common method, Kutum larvae in control pond were fed with concentrated foods with a ratio of 15% (p to 6 days) and after that, 500 kg row cow fertilizer was added until the end of the second week. In experiment treatment, larvae were fed with slurry. The amount of slurry was adjusted in accordance with density of zooplanktons. Regarding to measure the growth rate, fishes (at least 50 Kutum fish) were sampled. Fish sampling was done weekly in order to determine the growth rate of the fish by recording the length and weight of the sample. The fish were aught with a seine net and were taken randomly as the sample and finally weighted by using a weigh scale (±1.0). The length was measured from the tip to the mouth to the caudal fin with a ruler. The growth rate was calculated with the following formula:

$$D_{wg}\ (\text{g day}^{-1}) = (W_t - W_0)$$

Where:
W_0 = Primary weight
W_t = Final weight
t = Experiment duration

$$D_{lg}\ (\text{m day}^{-1}) = (L_t\text{-}L_0)$$

Where:
L_0 = Primary weight
L_t = Secondary length

$$S_{gr} = 100\ (\text{Ln}\ (W_t) - \text{Ln}\ (W_0))/t$$

Where:
W_t = Final weight
W_0 = Primary weight
t = Duration of experiment

$$CF = W/L^3 \times 10^5$$ (Biswas *et al*., 2008)

Where:
CF = Condition factor
W = Weight of larva
L = Length of larva

RESULTS

During the first two weeks of larvae life cycles, growths of Kutum's larvae were studied under condition of two different treatments. The results showed that during the first week, the most total length of larvae was 15 mm in pond No.6 of slurry treatment and the least mean total length of larvae with 10.8 mm was observed in pond No.1 of control treatment (Table 1).

During the first week of the culture, the range of changes in total length of larvae was 0.20-0.25 mm for control group and 0.25-0.40 mm for slurry treatment. On the other hand, the mean weight of larvae for slurry treatment was estimated 19.64±7.266 mg which has increased 26.57% in comparison with common method.

During the second week, the range of total length of larvae for control group (Common Method) and slurry treatment were estimated 0.40-0.75 and 0.35-0.50 mm, respectively.

The comparison between two treatments indicated that change in mean Total Length (TL) of Kutum larvae during two weeks was about 7.6% more in slurry treatments. At the end of the second week, the range of changes of TL for slurry showed lower quantity in comparison with common method, on the other hand in slurry samples, not only have the mean TL of larvae been increased, there was also a regular higher length-growth. During the second week of the culture, mean weight of larvae for slurry treatments was calculated 77.16±25.3664 mg, which showed 22% more growth in comparison with control group (Table 2).

Table 1: A comparison between mean total length and weight of Kutum' larvae during the first week of culture

		Total length (mm)			Weight (mg)		
Treatment	No. of pond	Mean	Max	Min	Mean	Max	Min
First treatment	1	10.8	12.0	10.0	10.2	12	9.2
(Common method)	2	13.5	14.5	12.0	19.7	28	13.0
	3	12.8	13.0	11.5	13.37	15	12.0
Total mean	-	12.3±1.278	-	-	14.42±4.536	-	-
Second treatment	4	13.1	14.0	12.5	13.29	16	12.2
(Slurry)	5	13.8	14.5	12.0	22.85	36	12.0
	6	14.1	15.0	11.0	22.8	34	12.0
Total mean	-	13.66±1.031	-	-	19.64±7.266	-	-

Table 2: A comparison between mean total length (TL) and weight of Kutum's larvae during the second week of culture

		Total length (mm)			Weight (mg)		
Treatment	No. of pond	Mean	Max	Min	Mean	Max	Min
First treatment	1	18.4	21.5	16	59.5	95	33
(Common method)	2	18.7	23.0	19	62.0	89	51
	3	21.7	20.5	13	67.9	92	51
Total mean	-	19.6±2.296	-	-	63.13±17.855	-	-
Second treatment	4	22.7	21.0	16	64.0	129	37
(Slurry)	5	21.7	25.0	21	86.7	120	63
	6	21.7	23.5	20	80.8	120	57
Total mean	-	22.03 ±2.31	-	-	77.16±25.3664	-	-

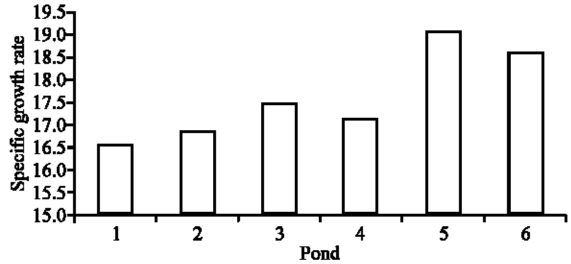

Fig. 1: Specific growth rate of Kutum larvae measured in different ponds

At the end of two weeks culture the maximum and minimum TL of larvae were estimated as 25.0 mm for the second treatment and as 18.4 mm for the first treatment (Table 2). For mean weight parameter, the Kutum larvae in pond No.5 were 86.7 mg belonging to slurry treatment and had the maximum weight (Table 2). From specific growth rate standpoint, comparison between different ponds and treatments found that pond No. 5 with 19.08 and pond No.6 with 18.61 indicated maximum growth rate and mean growth rate for slurry and control treatment was measured 18.28 and 16.95, respectively (Fig. 1).

The Daily Weight Growth (DWG) comparing in the second week of culture, mean (DWG) for the second treatment was estimated as 4.81 mg per day and this was 24% more than control treatment. Comparison among different ponds of Kutum larvae, it was found that the maximum DWG with 5.44 mg per day was in pond No. 5 for slurry treatment ring in the second week of culture, mean DWG for the second treatment was estimated as 4.81 mg per day, namely 24% more than control treatment. Comparison among different ponds of Kutum larvae, it was found that the maximum DWG with 5.44 mg per day was in pond No.5 for slurry treatment.

The daily length growth coefficient (DLG) showed the same trend as DWG. The mean DLG comparison between slurry and control treatments indicated that this value for slurry treatment was estimated 0.74 mm day^{-1} and with 15.6% higher growth comparing to control No. 1. The results of comparison among different ponds from daily length growth standpoint also indicated that the maximum DLG with 0.84 mm day^{-1} was for pond No.5 of slurry treatment and the minimum value with 0.11 mm day^{-1} for pond No. 1 of control treatment (Fig. 3).

According to the results obtained from Fig. 4, the growth trend of slurry treatment showed ascending trend from 13.66±1.031 mm of TL in the first week of culture up to 22.03 ±2.31 mm in the second week (Fig. 4).

From weight growth parameter standpoint, this value for slurry treatment showed an ascending trend from 19.64±7.266 mg in the first week of culture to 77.16±25.366 mg in the second week, namely 57.52 mg increased (Fig. 5).

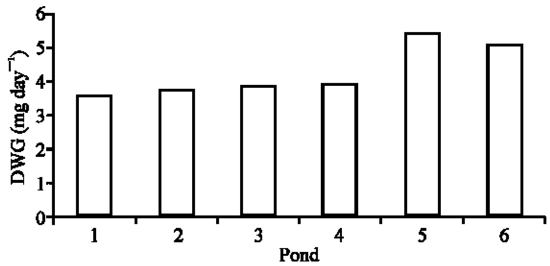

Fig. 2: DWG comparison among different culture ponds of Kutum larvae

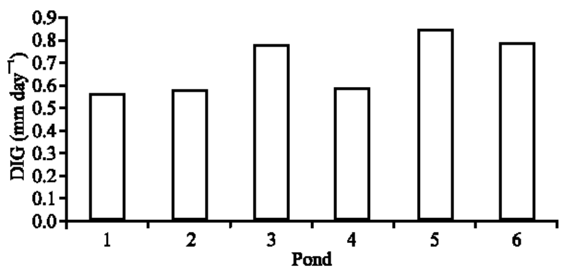

Fig. 3: DLG comparison among different culture pond of Kutum larvae

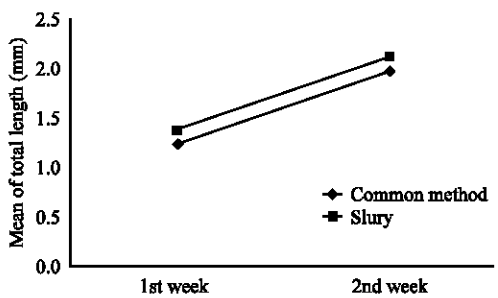

Fig. 4: Mean length growth comparison of Kutum larvae between control and slurry treatments during 2 weeks culture

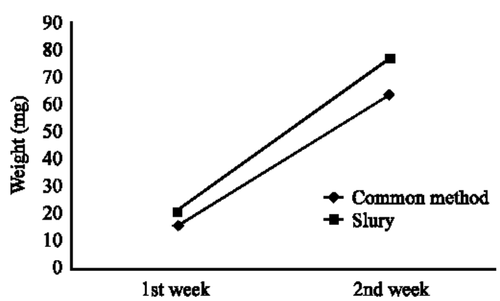

Fig. 5: Mean weight growth comparison of Kutum larvae between slurry and Control treatments during 2 weeks culture

Table 3: Chemical analysis of cow fertilizer (raw and fermented) of one or more of the nutrient elements in soil and water which may be caused by other

	Chemical compounds (dry material %)						
Type	Protein	Ash	Fat	Ca	P	K	Others
Slurry	15.13	54.73	1.71	2.24	0.8	2.11	23.28
Raw	10.4	50.43	1.43	1.77	0.48	0.58	34.91

Statistical analysis for the two treatments indicated significant difference ($p<0.001$) between both length and weight parameters in the first week of culture, meanwhile in the second week the significant difference was observed at statistical level of $p<0.05$. The length measurement during the first week of the culture were compared and it was found that TL of larvae for slurry treatment with 13.66 mm showed 11.38% increase more than control group (Table 1). On the other hand the condition factor measurement results showed these parameters were calculated 0.9 for slurry treatment and 0.8 for control treatment during two weeks of culture and it can be concluded that there is a highly significant increase in slurry.

The chemical analysis of row cow fertilizer and fermented (slurry) is shown in Table 3, which can be seen that nutrients are abundant in slurry.

DISCUSSION

Growth rate comparison between two treatments of slurry and control showed that in all the biological parameters such as total length and body weight of larvae, condition factor, specific growth rate, DWG and DLG, slurry had higher values in comparison with control treatment ($p<0.001$ for the first week and $p<0.05$ for the second week). The BSG seems to have promoted a heterotrophic food chains responsible for higher fish growth (Harjeet *et al*., 1991a, b). Effect of biogas slurry, a by-product of biogas plant on the survival and growth of common carp *Cyprinus carpio* var. *communis* over 270 days of culture were determined. Growth rates of fish (In terms of weight) were 3.45 times higher in biogas slurry treated tanks than in controls (Kamaldeep *et al*., 1987). Biogas slurry (BGS) applied at 52.1 L/ha/day (6.4% dry matter) resulted in faster maturation of common carp *Communis* (Kamaldeep *et al*., 1993).

There is no doubt that larvae stage (especially after absorbing yolk sac), is a sensitive stage and to decrease or ban the mortality, providing suitable foods for larvae is essential The main characteristic of this phase is that the source of nutrient and energy necessary to continue the larval development changes from the yolk reserves to the ingested food. To achieve this transition with success it is necessary that all structures and organs related with food uptake, digestion and assimilation are ready in due time and that the appropriate food is available (Yúfera and Darias, 2007). Most such mortality occurs during the pelagic larval stage ,particularly after yolk absorption, when young fish have to begin feeding their own.

Pond fertilization has assumed an important role to supplement nutrient deficiency and augment biological productivity through autotrophic and heterotrophic pathways. This is especially important in the extensive and semi-intensive culture systems by promoting the functioning of natural ecosystems in a benign environment (Das *et al*., 2003).

The results showed that slurry could increase number of methanobacters which are the food for zooplanktons and consequently zooplanktons are larvaes' food and increase the speed of growth rate (Kangmin and Hua, 2000). Applying slurry (as fertilizer) stimulates the production of organisms that serve as the first foods for fish and increases fry survival and growth. Fish fry feed primarily on zooplanktons. Organic fertilizers are broken down by bacteria, which in turn are food for many types of zooplanktons. In addition, the bacteria release nutrients that phytoplankton used (Ludwing *et al*., 1998).

A lot of work has been done on utilization if fish culture ponds, of animal manure, particularly farmyard manures, poultry droppings, cow manure and biogas slurry which are suitable substitutes for costly feeds and fertilizers (Schroeder, 1980; Dawan and Toor, 1989). Slurry was a quit acting fertilizer, containing several soluble nutrients (Table 3). The digestible crude protein and total digestible nutrients contents of slurry were found to be 7.6 and 38%, respectively, indicating that about 50% of protein of the biogas slurry was digested. The animals maintained weight on this feed. The biogas slurry was free from pathogenic organisms (Sexena *et al*., 1989). Agricultural wastes such as animal manure can produce methane anaerobic fermentation. Organic fertilizer with a higher percent crude protein contains greater amounts of Nitrogen. In the beginning of growth, the larvae have slow movements and foods are in contact with their mouth accidentally to be swallowed. Therefore, slurry can be useful in this regard. These researches also stated that slurry increases the amount of TN (Total Nitrogen) and TP (Total Phosphorous) up to 59.8 and 42.8%, respectively. The biological productivity of any aquatic body is generally judged by quantities estimation of planktons, which form the natural food of fish (Ahmed and Sing, 1989).

According to the chemical analysis, the slurry samples contain higher values of protein, fat, ash, P and K and it shows that fermentation process caused to increase amount of nutrients. The investigations carried out by Kangmin and Hua (2000), indicated that there was higher survival rate for carps cultured with slurry, than using chicken fertilizer. Long retention times are required for manure digestion, not only due to the presence of complex organic compounds, but also due to the presence of complex organic compounds ,but also due to the high concentration of ammonia nitrogen, which affects anaerobic decomposition (Alvarez *et al*., 2005). In Iran is to use retention time 40 days. The retention time of 40 days proved to be much better than shorter retention times in terms of total gas produced, methane percentage and degree of decomposition of organic matter (El Amin and Dirar, 1988). In a way that 1 kg of chicken fertilizer consumes 5000 mg Oxygen during 15 h, meanwhile 750 kg of enriched slurry consume 77 mg during 15 h. During this investigation it was found that, after anaerobic fermentation, the pathogen agents were destroyed; it means that occurrence of disease among fries fed by enriched slurry has been significantly decreased. According to measurements done by agricultural universities of SHENYANG, the protein content increased after anaerobic fermentation from 16.62 up to 46.90%. According to this study, amounts of essential amino acid's compound with vitamin B was also increased about 7.2 times more after fermentation process.

To use the study provides developed hygienic condition in culture ponds can destroy eggs of parasites and pathogenic factors produced after bacterial fermentation process. For instance 98% of bacterial Coil-forms and 99% of hook worms were killed. On the other hand, amount of BOD was uncrated up to 80% due to anaerobic fermentation (Kangmin and Hua, 2000).

On the other hand, amount of BOD was increased up to 80% due to anaerobic fermentation (Kangmin and Hua, 2000). These authors stated that 60% of chemical materials can be replaced by slurry, which also obtains different economical and ecological benefits and increases the growth of fingerlings.

Thus, it seems that BGS resulted in higher fish growth over the control by increasing the heterotrophic components of culture ecosystem.

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