International Multidisciplinary Research Journal 2011, 1(10):31-35
ISSN: 2231-6302
Available Online: http://irjs.info/

INTERNATIONAL MULTIDISCIPLINARY RESEARCH JOURNAL

Captive breeding of *Oncorhynchus myskiss*, a threatened and endemic species to the Western Ghats, India- A conservatory aspect

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Abstract
A comparative study on the reproductive performance of *Oncorhynchus myskiss*, two different doses of Ovaprim and Luteinizing-hormone-releasing hormone (LHRH) was conducted. Different doses of Ovaprim ranging from0.60 to 2.05ml/kg for male fishes and 2.05 to 2.50ml/kg for female fishes was injected. 0.0310 to 0.355 ml LHRH/kg and 0.0640 to 0.0730 ml LHRH/kg was administrated to male and female fishes respectively. The egg production, deformatities and survival rates were observed for all treatments. All the observed values were tabulated and statistically analyzed. The maximum hatchability rate percentage of Ovaprim was at the dose of 2.40ml/kg (84±0.640) where as LHRH showed maximum performance at 0.0710 ml/kg dose (85±0.750). The outcome of this study can be effectively used for the captive breeding and conservation of Rainbow trout, *O. myskiss* an endangered cold water fish of Western Ghats, India.

Keywords: Captive breeding, *Oncorhynchus mykiss*, Ovaprim, LHRH

INTRODUCTION

Aquaculture is an ancient art that has been sophisticated by scientific practices for the need of supplying essential protein foods and economic activity in majority of countries through commercial fishing business. The sustainable utilization of genetic resources, including fish is vital part in the standard of living in a populous country like India [1]. The wild population has steadily declined mainly due to the loss of habitat, introduction of alien species, diseases, pollution, siltation, poisoning, dynamite, and other destructive fishing [2]. Ecological studies focusing on patterns of biodiversity are a vital foundation for natural resource management and conservation [3].

Over the years, the cultural practices have undergone considerable intensification and with the possibility of obtaining high productivity levels there has been a state of flux between different farming practices. Studies on physicochemical parameters and reproductive fecundity of endangered and commercially important fishes are required for management and conservation of fish population in natural water bodies (4].

The Western Ghats a rich biodiversity situated in the region of Tamilnadu and is aptly classified as one of the 25 richest mega biodiversity hotspots of the world [5]. Approximately, 750 freshwater fishes have been recorded in India out of which 350 estimated as endemic to Western Ghats [6 & 7]. Rainbow trout, *Oncorhynchus myskiss* an endangered cold water fish of Western Ghats, are increasingly utilized as high-value "domesticated" aquaculture [8]. It can also be reared extensively, but its farming is mostly characterized by intensive feeding with high protein content for higher production with successful captive breeding protocol and larval rearing development [9,10&11]. Hormonal preparations applied in fish aquaculture allow improving artificial reproduction techniques during the spawning season and off season (12,13,14,15 &16). One of the difficulties in working with a new candidate fish for culture is obtaining reliable quantities of viable eggs, and it is often necessary to use exogenous hormone treatment to stimulate ovulation, to achieve this. Successful manipulation of reproductive processes is dependent on some level of understanding of reproduction in the target species [17].

The production of good quality seed depends on adequate supply of nutritional requirements as well as on the range of their tolerance to the different environmental factors [18] in development of feed formulations gives improved utilization of nutrients to fish larvae [19]. The mixture of different levels of defatted soybean meal, corn gluten meal and meat meal could replace up to 90% of the fishmeal to be used if the combination of these ingredients produces the profile of amino acids comparable to the fishmeal diet [20]. Our present study which compared the effect of LH-RH and Ovaprim hormones in induced breeding of *O. myskiss* , had the following specific objectives thus: to compare the level of egg productivity, egg hatchability, deformatities, survival and Starter formulated feed fed fishes were examined with regard to growth rate, survival and Standard Growth Rate (SGR).

MATERIALS AND METHODS

The Endangered Rainbow trout *O.myskiss* is found in India only at higher altitude of Himalayas and Western Ghats at a temperature range between 3 to 5°C. Brooders were collected from upper Bhavani reservoir located Avalanche (Longitude 11°15'21"N 76°33'1"E, Latitude 11°19'12"N 76°36'55"E). In the present study of *Oncorhynchus myskiss* induced breeding was done using Synthetic hormone Ovaprim (Virbag Pvt. Ltd, Mumbai) and LH-RH (Sigma, Bangalore). The rainbow trout is a hardy fish that is easy to spawn, fast growing, tolerating a wide range of environments and handling stresses. Fry can be easily accepts an artificial diet (usually feeding on zooplankton). This fish is capable of occupying many different habitats, ranging from an anadromous life

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history.

Healthy breeders were selected from the captive brood stock before the onset of the breeding season (September to January - Monsoon). Brooders were acclimatized to the environment and diet controls. Male and Female brooders are selected based on their gonadal maturation stage, average weight of the fish (400gm). The maturity assessments of the fishes are done by ovarian biopsy. Ova of matured female fishes will be around 2-4mm in diameter. Both male and female brooders will ooze out gametes if their abdomen is pressed gently.

Induced breeding of *Oncorhynchus myskiss* was done using synthetic hormone Ovaprim [21]. For each concentration 10 male and 10 female fishes (20 groups) were exposed to separate spawning tanks. Both male (2ml/kg) and female (2.5ml/kg) fishes were injected with a single dose intra muscularly. The dosage level has been standardized as LHRH [22] at 60-70 µg/kg body weight for females and 30-35 µg/kg body weight for males. Both the hormones were injected at 10 different concentrations as shown in the Table. I. The injected fishes were introduced into the artificial raceway spawning tank at 1:1 ratio. The spawning rate, hatching rate, deformities, and survival rate were analyzed using standard procedure [23]. Boiled hens egg was given as feed for the alvins and reared in rearing pond of 3x5x3 feet.

The prepared nursery for starter formulated pellet of 30% dried Beef liver, 30% Fish meal and 30% Fresh water mussel powder each feed mixed with 20% Soya bean , 20%Ground nut cake,10% Rice brawn, 10% Topiacco powder,5% Minerals and 5% Vitamins ingredients were prepared adopting the method of [24 & 25]. The freshly prepared pellet (0.5 to 1 mm) was stored in air tight container until required. The experimental period was restricted to 50 days due to earlier growth state. Statistical analysis of one way ANOVA - version 1.95 were used to perform hormonal dosages (LHRH and ovaprim), spawning rate, hatching rate, deformities and survival rate [26].

Table 1. Results of captive breeding using different doses of Ovaprim and LHRH

No.	Hormones (ml/kg)				Number of Egg Released (n)		Hatching Rate (%)		Deformities (%)		Survival (%)	
	Ovaprim		LHRH									
	M(10n)	F(10n)	M(10n)	F(10n)	Ovaprim	LHRH	Ovaprim	LHRH	Ova Prim	LHRH	Ova Prim	LHRH
1	1.60	2.05	0.0310	0.0640	554±1.00^{d}	451±1.15^{j}	67±0.90^{h}	77±0.85^{f}	12±0.72^{b}	15±1.72^{e}	56±0.07^{h}	71±0.70^{i}
2	1.65	2.10	0.0315	0.0650	532±2.08^{f}	454±0.81^{i}	70±0.32^{f}	73±0.73^{g}	10±0.56^{d}	17±0.80^{c}	66±2.40^{e}	78±0.77^{g}
3	1.70	2.15	0.0320	0.0660	466±2.51^{g}	547±0.49^{g}	66±1.22^{i}	71±1.60^{h}	6±0.32^{f}	14±1.27^{f}	62±0.77^{g}	74±1.83^{h}
4	1.75	2.20	0.0325	0.0670	544±2.64^{e}	670±1.15^{f}	69±0.75^{g}	66±2.30^{i}	2±0.05^{h}	6±0.75^{i}	54±1.20^{j}	80±0.21^{e}
5	1.80	2.25	0.0330	0.0680	383±1.52^{j}	543±0.66^{h}	70±0.60^{e}	84±1.93^{b}	15±1.00^{a}	19±0.55^{b}	65±0.84^{f}	82±0.42^{d}
6	1.85	2.30	0.0335	0.0690	389±1.73^{j}	676±0.65^{d}	76±0.40^{c}	78±0.55^{d}	7±0.251^{e}	11±0.66^{g}	70±0.55^{c}	79±2.05^{f}
7	1.90	2.35	0.0340	0.0700	432±2.51^{h}	764±0.81^{b}	79±0.47^{b}	66±1.21^{j}	11±0.585^{c}	24±0.35^{a}	73±0.84^{b}	83±1.48^{c}
8	1.95	2.40	0.0345	0.0710	564±2.00^{c}	674±0.47^{e}	84±0.64^{a}	85±0.75^{a}	0.0^{j}	6±0.75^{j}	86±0.77^{a}	87±0.98^{a}
9	2.0	2.45	0.0350	0.0720	614±1.52^{a}	781±1.25^{a}	75±0.50^{d}	77±1.67^{e}	0.0^{i}	8±0.28^{h}	68±1.20^{d}	86±0.98^{b}
10	2.05	2.50	0.0355	0.0730	593±1.15^{b}	755±1.05^{c}	56±0.529^{j}	80±1.45^{c}	3±0.29^{g}	16±1.20^{d}	47±0.49^{j}	70±0.21^{j}

Data (Mean ± SD), (n=2)
Means with different superscript within the same group are significantly different (P< 0.05)
Means with same superscript within the same group are not significantly different (P>0.05)

Table No. 2. Average weight of *Oncorchynchus mykiss* Brooder using Ovaprim and LHRH

No of Trials	No of Brooders		OVAPRIM Average Weight(g)		LH-RH Average Weight(g)	
	Male	Female	Male	Female	Male	Female
1	10	10	374.0±2.645	574.0±3.535	434.2±2.588	603.2±1.643
2	10	10	400.0±2.000	587.2±1.788	362.5±1.732	555.0±3.391
3	10	10	368.8±1.214	562.0±1.000	374.8±2.387	574.8±1.303
4	10	10	397.2±1.483	612.6±2.509	391.0±1.581	571.8±0.836
5	10	10	374.2±2.049	608.8±1.923	374.8±1.951	582.4±1.140
6	10	10	397.4±1.516	586.6±1.816	411.2±0.447	579.6±1.516
7	10	10	381.6±1.140	602.2±1.923	383.4±2.880	564.6±1.816
8	10	10	359.8±0.836	611.0±1.581	365.6±3.049	613.2±2.489
9	10	10	412.4±2.302	595.2±1.643	385.0±2.345	566.8±2.167
10	10	10	391.4±2.966	607.8±1.303	396.4±0.894	554.8±1.303

RESULTS AND DISCUSSION

In the present study we observed the successful captive breeding of *Oncorhynchus myskiss* with the administration of Ovaprim and LHRH. The different dose of the hormone significantly altered the percentage of fertilization, number of egg laid, hatching rate and deformatities (Table 1). The synchronization of ovulation after the stimulation is a very important aspect. Brzuska 2003 reported that the deterioration of the egg quality with increasing doses of gonadotropin [27, 28, 29 &30].

The percentage of fertilization of hatching eggs obtained in the present trial ranged 84±0.640 (2.40ml/kg Ovaprim) and 85±0.750. LHRH used at 0.0710 ml/kg of body weight. Similar findings were reported in rainbow trout *O.mykiss* [31]. Ovaprim [32] and LHRH have been successfully induce ovulation in a number of teleosts [33] [34].

Certain drugs such as LHRH and Human chorionic gonadotropin (HCG) have been induced in spawning fishes with variation in the percentage of breeding [35]. It also confirms suitability of Ovaprim in fish reproduction stimulation [36][37]. In India, most of the breeders have preferred Ovaprim, as a survey showed that only 10 to 15 % of fish breeders use extract due to the complexity of the technique [38]. Ovaprim (at a dose of 0.5 m/kg body weight) induce breed of 70% spotted murrel (*Channa punctatus*) and fertilization [39]. In *C. striatus* fertilization rate was 95 - 98% with Ovaprim as the hormonal material[40].

According to Table 1, the results of number of eggs released, deformities and survival rate had significant difference ($P<0.05$) whereas the hatching rate was not much significant ($P>0.05$) between the female broods injected with Ovaprim and with LHRH dosages. These results are in agreement with the study conducted by [41] in terms of fertilization and hatching rates, between the fish treated with LHRH 100 or 200 mg and the control fish which ovulated spontaneously[42]. [43] recorded a considerable number of dead larvae in the last phase of incubation of eggs obtained from *Silurus glanis* females treated with LHRH. The results of previous studies shows that in general the female fishes with high quantity of eggs are obtained in synthetic ovulation stimulators compared to the treatment with stimulators of natural origin. The eggs stripped injected with 1-1.5 ml Ovaprim/kg and stripped during 14-17 hr post-injection is regarded as the good quality due to high fertilization and hatching [44].

In the present experiment the average body weight of lighter and heavier females used for the reproduction differed about 2 kg and was not at all statistically significant between the means of traits determining the weight and quality of eggs (Table-2). However, the obtained results distinctly showed that from lighter females low weighed eggs was obtained. Feeds that differed in lipid content did not affect weight gain in rainbow trout, which is in agreement with the previous findings for this species[45,46&47]. In this study it was identified that 30% mussel feed fed larvae obtained maximum growth compared to Trash fish meal and beef liver fed larvae(Graph-1&2). Being carnivorous species, rainbow trout larvae need a diet rich in protein, which should be a concern in formulating feed, alternating to starter feed to explore nursing and rearing trout larvae [48]. The frozen raw buffalo liver feed has been proven to be one of the best alternatives, showing healthier and better growth compared to other feeds [49].

High hatching rate and survival rate was successfully obtained in *Oncorhynchus myskiss* when stimulated with appropriate dose of Ovaprim and LHRH. This threatened and endemic species of rainbow trout in Western Ghats can be successfully breed in captive conditions and its population can be reclaimed with immediate effects.

Figure 1.Breeding and Larviculture of *Oncorhynchus myskiss*

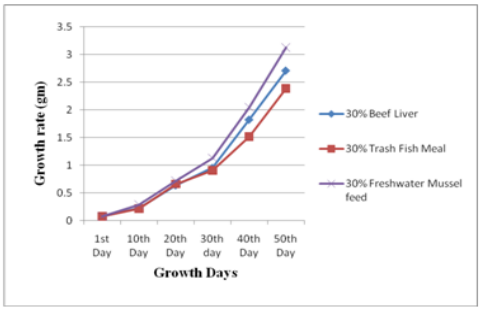

Figure: 2. Growth rate of *O. myskiss* fed with different formulated diets

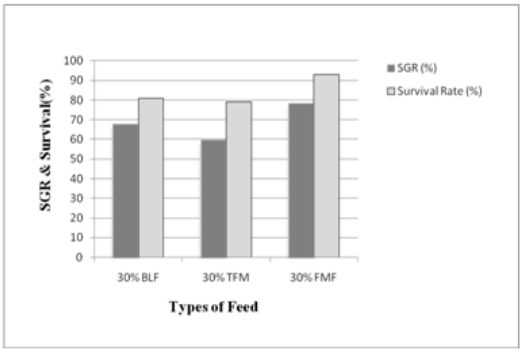

Figure: 3. Standard Growth rate and Survival rate of O. myskiss fed with different formulated diets

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