International Journal of Fisheries and Aquaculture Sciences.
ISSN 2248-9975 Volume 4, Number 1 (2014), pp. 5-14

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Effect of Various Doses of Ovaprim on the Reproductive behaviour of Rainbow Trout *Salmo Gairdneri Gairdneri* Inhabiting Trout Hatchery Farm at Kokernag Anantnag Kashmir

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Abstract

In the present study, artificial reproduction in *Salmo gairdneri gairdneri* of 1.0 Kg to 1.3Kg weight was carried out using various doses of OVAPRIM viz., 0.30, 0.35, 0.40, 0.45 and 0.50 per kg of body weight. It was found that number of eggs released by a fish and the mean weight of the eggs ovulated increased with an increase in OVAPRIM concentration. But in any case hatching rate and survival rate increased at 0.35 OVAPRIM concentration and beyond which both hatching and survival rates declined down drastically.

1. Introduction

Kashmir valley spread over an area of 15,520.3 Km^2 is bounded by Himalayas on all sides except southern side where it is delineated by Pir Panjal Range. Located at an average elevation of 5300ft above mean sea level between 34.5^0N Latitude and 76.82^0E Longitude, Kashmir valley is embellished with a myriad of water bodies like streams, rivers, lakes, ponds, reservoirs etc which abounds in fishes especially trout.

Trout in Kashmir as sport fisheries was introduced 90 years ago by Mr. Frank J. Mitchell, a carpet dealer who is known as father of "Kashmir Trout Fishers". He managed to import twice the trout eyed ova from Scotland in 1904 which was reared at his house in Srinagar using the Jhelum water. It was a decade later when state government, took over cold water fisheries and introduced trout eggs in its freshwater bodies. Since then trouts are being propagated which are thriving well in many areas of the state. Eyed ova of rainbow trout were first transplanted from Kashmir to Kulu in Himachal Pradesh in 1919 and in Arunachal Pradesh in 1965. Subsequently rainbow

trout (*Salmo gairdneri gairdneri*) and other species were transplanted elsewhere in the country. Trout was introduced to high attitudes of Leh Valley in 1964 by State Fisheries Department where it is now well acclimatized.

However the natural population of trout and other indigenous species of fishes started dwelling very fast in Kashmir streams but due to heavy angling pressure and indiscriminate fishing, the restocking of streams with trout eggs thus became a major priority with State Fisheries Department. Realizing the necessity of restocking the Kashmir freshwaters with trouts, Department of Fisheries, Government of Jammu and Kashmir established first of its State of Art Trout Farm at Kokarnag with the assistance of European Economic Community (E.E.C.). The intake capacity of the hatchery for first phase was 90,000 eyed ova and consequently 90,000 eyed ova were imported from UK annually and 90% survival rate was achieved, which is double than envisaged. The survival target rate achieved in hatchery surpassed the targets achieved by some well established commercial firms in Europe. The production of 15-20 metric tonnes was achieved annually against 10 metric tonnes envisaged in the project report. But to further enhance the fishery production in the state, Department of Fisheries constructed various village raceways at different places in the valley like Dandipora, Larnor, Achhabal, Lairbal, Tricker, Pahalgam, Panzeth, Verinag etc. The department has also setup various trout farms on the various snow-fed streams of Kashmir such as Veshow, Lidder etc. To cater to the needs of fish seed in these fish farms and hatcheries, Department of Fisheries used induced breeding/artificial reproduction in trouts for enhanced fish production.

Since brown and rainbow trout only succeeded in acclimatizing to the water conditions of valley and of which enough investigations have been made on Brown trout, so present study was initiated to assess the impact of artificial reproduction on the spawning and spawn size of rainbow trout (*Salmo gairdneri gairdneri*) and the development thereon.

2. MaterialS and MethodS

Kashmir valley is noted for being one of the leading trout fish seed producing state in India from where the trout fish seed is transported to distant places. Of all the trout farms established by Department of Fisheries in the state, Trout Fish Farm at Kokernag is considered as one of the most renowned and successful one not only in the state but also in the country. Since the trout farm at Kokernag is well equipped with all the modern facilities, hence present study on the artificial reproduction of rainbow trout (Salmo gairdneri gairdneri Richardson) in natural water was conducted at this popular trout hatchery farm.

The trout fish farm at Kokernag is located at an elevation of 7000ft above mean sea level and at a distance of 24 Kms from the district headquarters at Anantnag. By 1990, the total area of the farm was 5.0 hectares but presently the total area of the farm complex is more than 10.5 hectares which accommodates 20 raceways, that are meant to rear and propagate the young ones upto marketable size of about 250 gms. This trout

farm breeds and propagates Rainbow trout (*Salmo gairdneri gairdneri*) and Brown trout (*Salmo trutta fario*) in farm conditions provided all required favourable environmental conditions are simulated. For the sake of present study, rainbow trout *Salmo gairdneri gairdneri* has been selected.

For artificial reproduction and enhanced production of seed, synthetic hormone "OVAPRIM" was used. For breeding purposes, healthy breeders (female of 2 years age and male of 3-4 years age) were selected from the captive brood stock at Kokernag Trout hatchery farm before the onset of actual breeding season (December -January). Brooders selected for artificial breeding were acclimatized to the environment and diet controls. Male and female brooders were selected on the basis of gonadal maturation stage with average weight of the fish being 1 to 1.5 kg. Moreover mature males were also collected and kept separately. A total of 5 females and 5 male trout fish were collected for inducing breeding in them.

For induced breeding with the help of "OVAPRIM" hormone, 5 concentrations were prepared and for each concentration 5 female rainbow trouts were exposed to different spawning tanks. The female fishes were injected with a single dose intra muscularly into the dorsal muscle above the lateral line and below the anterior part of dorsal fin. The selected brooder females were injected with 0.30, 0.35, 0.40, 0.45 and 0.50ml OVAPRIM/kg of body weight. Each treatment was repeated thrice. The injected fishes were introduced into the artificial spawning tank in the ratio of 1:1.

3. Procurement of Eggs and Milt

For obtaining gametes, both male and female fishes were anaesthetized and then by holding the caudal peduncle with one hand and supporting the head with other hand, anaesthetized fish are cleaned in freshwater to wash off the anesthetic.

For the sake of present study, only 0.5g of ovulated eggs were taken from each female per treatment by wet stripping method. The "Wet Stripping" of anesthetized female (1-5) was carried first in plastic basin followed by stripping of milt from (1-3) males. Both ova and milt in basin were mixed by hand or feather mixing was done. After 30 seconds, when fertilization of eggs take place, water is added to the basin and then fertilized eggs are rinsed in clean water which removes the excess milt and foreign material and the eggs are then transferred to new basin. Fresh water is added is added and container is kept for an hour till eggs get swollen (absorption of water increases the size of egg by about 40%), but in the entire process temperature is maintained at 6-12^{0}C. The breeding shows 1700-1900 hundred eggs per kg. body weight.

4. Estimation of Hatchability Percentage

This entire process of breeding takes place in natural conditions but in captive conditions fish might not reproduce at the advantageous time owing to which alteration in spawning cycle is thought to be desirable. So due to changes in breeding cycle, the fish could be made to reproduce at other times of the year due to which survival of fertilizing and incubating eggs could be enhanced. Twenty Six hours after fertilization the unviable dead eggs were siphoned out to prevent infection and subsequent mortality of the fertilized eggs.

The unhatched eggs were counted physically and the percentage hatchability was expressed as:

$$\frac{\text{Total No. of Eggs Incubated} - \text{No. of Unhatched Eggs}}{\text{Total No. of Eggs Incubated}} \times 100$$

5. Results and Discussions

Artificial propagation of trouts is undertaken to meet the seed requirement. The trout culturing involves spawning, incubation of eggs, rearing of young fry in nursery ponds, raising fingerlings in rearing ponds and producing yearling in race ways. Bovine liver with slaughter house waste is given as supplementary feed at Trout Hatchery Farm at Kokernag.

Breeding: Artificial breeding of trout was conducted in January. Trout attains maturity by the end of 2nd year and start ovulating inside the body cavity. Female brooders of 2years old and male brooders of 3 years old were selected for undertaking breeding. Brood stock were kept under constant observation till the breeding season commenced. There are two breeding season at Kokernag, one during November - December and the second time during February - March.

The breeding operation started as soon as breeders were ready. About two to three weeks before the breeding operations, male and female brooders were segregated and released in separate ponds for fine rearing. Only fully matured breeds are selected for

breeding during winter season in the extreme colder months. The farm rearing brood attained maturity rather little earlier since they take nutritious food.

In the selection of female breeds, the bulging belly and the dark red column on lateral side of the body were considered fully mature female. In case of selecting male breeders for breeding, milt comes out on pressing the belly near the vent. Males also develop hook on the lower jar.

The 5 gravid female rainbow trout selected for the present study were injected with the synthetic hormone 'Ovaprim'. It is worthwhile to mention here that induced breeding in *Oncorynchus mykiss* is not practised at Kokernag Trout Hatchery farm. After injecting synthetic hormone Ovaprim, the brooders were introduced into artificial raceways/spawning tanks wherefrom they were subjected to stripping.

Since anesthetizing makes breeder easy to handle and since work can be carried out in confidence, breeder were normally anesthetized during experiments. This also ensured safeness of the breeders. Brooders were dipped into anesthesia 1-5 minutes. Benzocaine (1:3000) or Tricaine Methane Sulphonate (1:10,000), which is commonly known as MS-222, was generally used as anesthetics. A stock solution was first prepared by adding 20 gms of MS-222 per liter of water and then 5ml of stock solution was added to every liter of water in the tub.

Stripping of anesthetized female (1-5) was carried first in Plastic basin followed by stripping of milt from one to three males. Both ova and milt mixed together by hand or father. Mixing was also done by shaking the basin gently. After mixing the eggs were shifted into another container. Repeated washing of the eggs with clear water was done till the excess milt and foreign materials washed out. After washing some quantity of fresh water was added to the container and kept them for an hour till the eggs were water hardened fully swollen eggs per c.c. by volumetric counted 8-11 nos., and by gravimetric method it accounted 7-13 per gm. With the water absorption the size of the eggs increased about 40%. The temperature of the water of the basin containing eggs should not be too low otherwise eggs may spoil. The suitable temperature of water has been found to be about 6-12^{0}C. The entire stripping operation carried out was by wet method. 1004-1225 ova per kg body weight of rainbow trout appeared in full stripped breeding. Each ovum collected from the brooders had a diameter that varied from 0.3-0.4mm.

After stripping the fish used for spawning were kept in well oxygenated water till the fish recovered where after they were released back in raceways. The stripped fish were kept separately.

The impact of different doses of "OVAPRIM" on the fecundity, hatching and survival rate of *Salmo gairdneri gairdneri* is presented in the table 1 below.

The minimum and maximum weight of the fish used for OVAPRIM injection varied from 1kg 5gm to 1kg 295gm. The concentration of ovaprim injected increased with an increase in the weight of the fish. It has been found that with an increase in the weight of the fish, number of eggs released and mean weight of each ovulated egg increased. Similarly Ventakachalam *et al.* (2011) also reported that from lighter females light weighed eggs were obtained. Like wise hatching rate and survival rate of

the fish eggs decreased with an increase in the Ovaprim concentration with maximum hatching and survivality rate being at 0.35 Ovaprim concentration. Similar positive response has also been reported by Mudnakudu *et al*. (1990) and Olubiyi *et al*. (2005) in Mrigal carp induced with OVAPRIM at 0.3ml/kg indicating high potency of this ovulating agent. Moreover Brzuska (2003) observed deterioration of egg quality with an increase in the doses of gonadotropin hormone, which is agreement with the present finding.

Table 1: Artificial Spawning Of *Salmo Gairdneri Gairdneri* Using Various Doses of Ovaprim

Dosage level (ml/kg body wt.)	Mean Wt. of female before Stripping (gm)	Number of Eggs released	Mean wt. of ovulated egg (gm)	Hatching Rate (%)	Survival Rate (%)
0.30	1000.5	1004	0.151	95%	90%
0.35	1075.3	1050	0.154	97%	92%
0.40	1100.6	1090	0.156	93%	83%
0.45	1250.9	1160	0.160	91%	78%
0.50	1295.0	1225	0.165	87%	70%

Developmental stage of "*Salmo gairdneri gairdneri*" post fertilization: The period of incubation of rainbow trout varied from three to eleven weeks in water temperature from 4^0C -15^0C.

(i) **One hour after fertilization:** During incubation period, the flow of water was maintained @ 10-12 liters per minute with the depth of 10 cms. The fertilized egg is somewhat spherical in shape the color is white green and the average diameter of about 0.2-0.3 mm.

(ii) **Five hour after fertilization:** The fertilized egg is somewhat spherical in shape the color is dark green and the average diameter of about 0.3-0.4 mm.

(iii) **Green ova stage:** The fertilized egg is spherical in shape the color is green and the average diameter of about 0.4 mm. Green eggs, immediately after fertilization and till the eyes appeared in the egg were kept in each tray and three to four such trays were kept in a single trough for hatching.

(iv) **Incomplete Eyed stage:** The egg is round or spherical in shape with average diameter of 11mm. The one eye appears as a black spot and another one is slightly visible. The green eggs on further development passed to eyed eggs, the next stage of development of the embryo, when one eye was visible and other eye remained in deep.

(v) **v) Eyed stage:** The fertilized egg developing fish oval or spherical in shape the color is green and the average diameter of about 0.9 mm. The two eyes appear as a black spot hence called complete eyed stage. At this stage sorting out of bad eggs was done by shocking and siphoning through a rubber pipe. Removal

of bad eggs was done within 48 hours of fertilization at eyed-ova stage. The water temperature and the dissolved oxygen level of water used in the hatchery during the first experiment was maintained between 6.2^0C and 12^0C and 6.2 ppm to 9.4 ppm respectively.

(vi) **Alevin sac stage:** The shape of developing fish changes because it shows the distinguished head and two eyes on each side the emergence of caudal fin is distinguished clear and thin transparent and yolk is attached at cephalic region and the average diameter of developing fish is about 19 mm. The water flow was maintained at 13-14 liters per minute with the depth of 25 cm. during this stage. Alevin or sac fry remained on the bottom of the container for some days.

The hatching may last for about two weeks from the date of hatching and the shells of the hatched eggs were removed regularly to avoid pollution. Water flow regulation was also made at this stage for obtaining a better size of swim-up fry.

(vii) **(xiv) Alevin sac stage after thirty two days:** The body is clearly distinguished into interior, middle and posterior portion region in the in the interior head is demarked from the middle part of the body with two prominent eyes. The posterior end is still tapering Yolk sac is also attached but slightly reduced. The length of the developing fish reached upto 15 mm.

(viii) **(xv) Alevin sac stage after thirty three days:** The body is clearly distinguished into interior, middle and posterior portion region in the in the interior head is demarked from the middle part of the body with two prominent eyes. The posterior end is still tapering. Yolk sac is also attached but slightly reduced. The length of the developing fish reached upto 17 mm.

(ix) **(xvi) Alevin sac stage after thirty five days:** The body of a developing fish shows three distinct regions the interior is one head region with distinct eyes and posterior tail region in the middle the yolk sac is attached to the main body and hence called complete Alvin sac stage the body length in the 18 mm. the dorsal fin shows there appearance.

(x) **(xvii) Swim up fry stage after forty days:** The body of a developing fish becomes elongated and strengthened and body length upto 19mm. the body becomes opaque and pigmented and more darker. The fish shows appearance of caudal fin. The head regions more prominent or distinct and bigger than the body becomes more darker and appeared dorsal anal fin become more visible and also shows slide increase of the body. But yolk sac is still attached to the truck region of the body. During this stage, the flow of water was maintained at 20 liters per minute with 30 cms depth in fry rearing circular pools.

(xi) **(xviii) Swim up fry stage after forty two days:** The body of a developing fish becomes further elongated body length reaches upto 22mm. the dorsal anal and caudal fin are visible the body color becomes darker but yolk sac is still attached to the trunk region and is greatly reduced in size.

(xii) **(xix) Swim up fry stage after forty fifth days:** The body of a developing fish becomes further elongated body length reaches upto 23mm. the body

color becomes darker the caudal and dorsal fin are still in developing stage the yolk sac still attached the body at trunk region but almost absorbed. The swim-up fry, which comes up to the surface for search of food was collected and stocked in circular pools. Proper care was taken for feeding of swim-up fry which were then shifted to large circular tanks for rearing upto fingerling stage. The circular tanks, usually made of concrete or fabricated fibre glass material, metal or any other hand substance that proves disinfectant, handy and easily be erected or dismantled, were used. The diameter of a circular tank used was varying from 1-10 meter with 1.0- 1.5 meter deep depending on the size of fish to be reared. The circular pools having slope towards the centre and equipped with a central exhaust filter pipe was provided with water supply through other pipes arranged from the top. Zinc coated or G.I. pipes, which appear to be harmful to alevins, since they are susceptible to zinc concentration of 0.04 ppm, are avoided. Small circular pools of these types were used for rearing fry and fingerlings, where as large size circular tanks used to serve grow out ponds. The stocking density of fish in the circular ponds was maintained at 10-15 kg/m^3 of water.

(xiii) **(xx) Finger ling stage after fifty days:** Fingerlings produced is circular tanks were collected and stocked in the raceways, grow-out ponds while rearing them upto marketable size. The grading was done before stocking fingerlings in raceways or ponds to avoid cannibalism and unequal growth.

Raceways may be completely solid concrete construction or may have concrete sides with natural bottom. A natural raceway is an oblong trench with earth walls and bottom. A series of two raceways or double series of four raceways are large installations, each having an area of 30m x 25m with 70cm of water depth. Screen sluices are water connections in between upper and lower raceway. The screens are cleared time to time for maintaining proper water flow through it. High flow of water was maintained at high stocking density of fish. The stocking density varied from 15-35 kg/m^3 of water.

The body of a developing fish becomes further elongated and strengthened due to the formation of muscles; the body length reaches upto 24mm; the body color becomes darker the caudal, dorsal and anal fins clearly appeared. There is complete absorbance of yolk sac.

(xiv) **(xxi) Finger ling stage after fifty five days:** The fish is fully developed. The body size reaches upto 25 mm. The mouth gap is also seen here. Dorsal, anal and caudal fins fully appear in this stage. The fish develops streaks on the body.

(xv) **(xxii) Finger ling stage after sixty days:** The fish is fully developed elongated and strengthened due to formation of body organs. The body length of the fish reaches up to 27 mm. The dorsal and anal fins together with the caudal fin develop in this stage.

(xvi) **(xxiii) Finger ling stage after sixty five days:** The developing fish is usually elongated but the body shape of the fish changes due to increasing size and

weight. The fish moves about freely in the tanks while feeding on the supplemented feed. The body size reaches upto 28 mm.

(xvii) **(xxvi) Finger ling stage after seventy days:** The developing fish increase in weight. The shape and size also increases. The body length of the developing fish measures upto 29mm. The head region further increases in size. The fish caudal, anal, dorsal, pectoral and pelvic fins also appear. **(xxvii) Finger ling stage after seventy five days:** The developing fish further increase in weight. The shape and size also increases and the fish attains upto 30 mm body length. The developing fish appears like an adult fish.

The produced young rainbow trout of this farm are then restocked in stocking pond or natural waters of the state.

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