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Ex-Situ Study on Density Dependent Survival after Handling Yolk Sac Larvae of Snow Trout, *Schizothorax plagiostomus* Heckel

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Abstract
Snow trout, *Schizothorax plagiostomus* (Heckel) is most important commercial food fish of Garhwal Himalaya inhabited among the snow fed tributaries of river Ganga. In present study the induced breeding were carried out by stripping method and it was noticed that the survival of larvae were high, near (95-100%), at high density and the lowest survival rate was observed at the lowest density (less than 5%). The mechanism responsible for the observed density dependent survival was not under stood. It is possible that the concentration of substance secreted by larvae as well as change in larval behaviors is responsible factors for survival in higher concentration.

***Key words*:** Survival, *Schizothorax plagiostomus*, density, Garhwal Himalaya

Introduction

Snow trout *Schizothorax plagiostomus* Heckel is the most important food fish of Garhwal Himalaya inhibits in snow fed rivers *viz* Alaknanda, Mandakani, Pinder, Nandakani, Bhagirathi, etc (Bodola, 1979). Geographically *Schizothorax Spp*. spread in different rivers, lakes and tributaries throughout Himalaya extending to confines of China, eastern Afghanistan, Pakistan, Turkistan, Nepal, Ladakh, Tibet, Bhutan and North east India (Day, 1958). In India, *Schizothorax spp.* is the most important food fish of Himalaya region including Kashmir, Himanchal Pradesh, Uttarakhand, Uttar Pradesh foot hilis (Terai area), Assam etc. (Day, 1958) and also plays an important role in commercial fish production (Bahuguna, 2002). *Schizothorax spp*. is recorded 2.3 kg weight and 50 cm in length with 85% total catchments (Sharma, 1994).

During seed production of marine fishes higher mortality occurs during larval stages and this mortality was in yolk sac stage or prior to first feeding (Kjorsvik *et al.*, 1990) and expected to be due to egg quality or some other environmental factors. Early mortality is a serious problem in seed production of economically important species such as the coral trout *Plectropomus leopardus* (Masuma *et al*., 1993), Blue fin tuna *T. thynnus* (Kaji *et al.*, 1996) and yellow fin tuna *Thunnus albicus* (Kaji *et al*., 1999).

In past years, some valuable work about the density dependent survival after hatching was reported by Tagawa *et al*.

(1997) in yolk sac larvae of Japani flounder (*Paralichthys olivaceus*) and in *Thunnus thynnus, Thunnus albacares* and in *Plectropomus leopardus by* Kaji (2000) using small plastic plates as fish container. In India no work has been made to know the density dependent survival among the larva of fresh water fishes. So an effort has been made to know the survival of snow trout larvae during handling stress and density suitable for their survival because density dependent survival of larvae plays an important role in seed production of economically rich fish.

Materials and methods

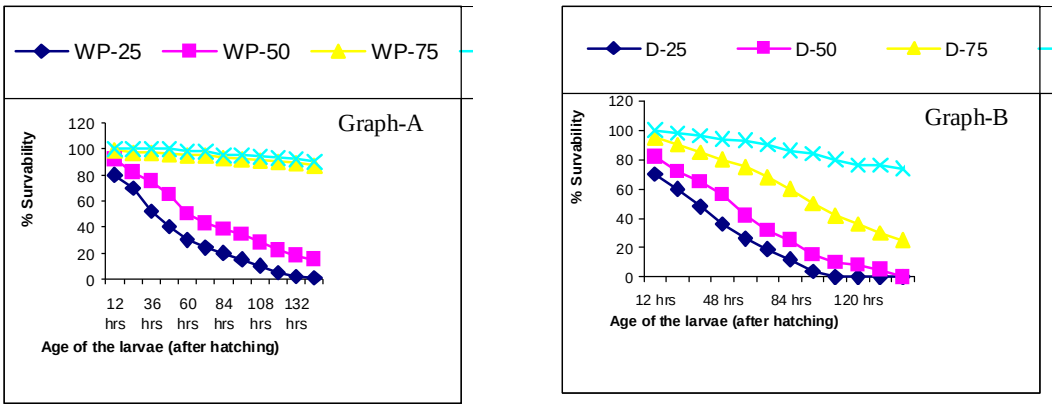
For experimental work mature *Schizothorax plagiostomus* brooders were collected from the snow fed river Alaknanda located between Latitude 30°10' to 30°15'N, Longitude 78°45' to 78°50'E and at elevation of 500 to 600 meter by using gill net. To obtaining the fertile egg, artificial breeding were carried during the month of September-October, 2006 and 2007 at the bank of river Alaknanda (water temp. 16 to18°C) by stripping method as followed by Bahuguna (2002, 2006). Fertilized eggs were kept in the hatching tray with proper aeration under controlled temperature and light. Hatched larvae were taken after 120 hrs of the fertilization and experimental grouped in 9 cm plastic disc and 6 wells tissue culture plates and were placed in laboratory hatchery, Department of Zoology and Biotechnology, HNB Garhwal University, Srinagar, Garhwal, Uttarakhand, India.

After hatching the yolk sac larvae were put in 9 cm plastic disc (area 64 cm^2, depth 0.5 cm^2 and volume 32 ml) and 6 well tissue culture plates (area 9.4 cm^2, depth 0.5 cm^2 and volume 4.7 ml), filled with natural Alaknanda water having temperature 18°C. The fixed quantity of water (32 ml in 9 cm plastic disc and 4.7 ml in 6 well tissue culture plates) were maintain in all plates and established the density of hatched larvae in plates and well as per given in Table 1. After transfer all the containers were kept in the dark place at controlled temperature (20°C) without aeration. Dead larvae were removing and counted after every 12 hrs interval. Water was not renewed and food was not supplied during the experiments. Examinations of the larvae were conducted just after hatching to 7^{th} day. Data analysis was done by using computer added statistical software. Data processing was done in tabular as well as in graphical mode.

Results

Fertilized yolk sac larvae of *Schizothorax plagiostomus* were transferred to small plastic disces at various densities, and were reared without diet. Dead larvae were counted and removed after every 12 hours. In plastic disc survivality reccorded upto 60% in lower density and upto 98 % in higher density on 1^{st} day (after 24 hrs), second day mortality increased, and survivality decreased recorded upto 36% in lower density and 94% in higher density. In 3^{rd} day 20% larvae were survive in lower density in compare to 90 % in higher density. In lower density rate of mortality were increased day by day and all larvae were death upto 5^{th} days but in higher density survival of larvae were recorded mutch better in compare to low density upto 74% (Graph A).

In tissue culture plates 70% survivality recorded in lower density in compare to 100% survivality in higher density on 1^{st} day. On second and third day mortality was increased and survival of larvae was decreased recorded upto 24% in lower



Graph A-B. Relationship between the age of the larvae and survivability among the larvae of *S. plagiostomus*

Table 1. Experiment design conducted in the research Lab.

Group	Container	Area (cm^2)	Depth (cm^2)	Volume (ml)	Number of larvae	Density (Ind/ml)
D-25	9 cm disc	64	0.5	32	25	0.78
D-50					50	1.56
D-75					75	2.34
D-100					100	3.125
WP-25	6 Well Plate	9.4	0.5	4.7	25	5.32
WP-50					50	10.64
WP-75					75	15.96
WP-100					100	21.28

D-25= 25 larvae in 9 cm disc, WP-25= 25 larvae in 6 well tissue culture plates.

Table 2. Experimental study of density dependent survival after handling in yolk sac larvae of snow trout *Schizothorax plagiostomus* (Heckel).

Exp.	% Survibility (after hatching)											
	1st day		2nd day		3rd day		4th day		5th day		6th day	
	12 hrs	24 hrs	36 hrs	48 hrs	60 hrs	72 hrs	84 hrs	96 hrs	108 hrs	120 hrs	132 hrs	144 hrs
D-25	70	60	48	36	26	19	12	04	0	-	-	-
D-50	82	72	65	56	42	32	25	15	10	08	05	00
D-75	95	90	85	80	75	68	60	50	42	36	30	25
D-100	100	98	96	94	93	90	86	84	80	76	76	74
WP-25	80	70	52	40	30	24	20	15	10	05	02	01
WP-50	92	82	75	65	50	43	38	34	28	22	18	15
WP-75	99	97	97	96	95	95	93	92	91	90	89	87
WP-100	100	100	100	100	98	98	95	95	94	93	92	90

density in compared to 98 % in higher density. After third day mortality increased day by day and survivality recorded upto 4% in lower density in compare to 90 % in higher density on seventh day (Graph B).

Results indicate high mortality on 1st day after the transferring in the experimental purpose, which would be related to handling stress. The survival of larvae at the first observation was high near in 100% at high densities (95-100%) and lowest survival rate was observed at lowest densities of snow trout. In more shape (tissue culture plate) the rate of survival was better in compare to plastic disc because concentration of larva in tissue culture plates is higher in compare to the density of plastic disc and survival rate is also higher (97-100%). Hence results indicate the better survival is in higher density in compare to lower density.

Discussion

In seed production of *Schizothorax plagiostomus*, high mortality frequently occurs during larval period. Mortality at yolk sac stage, prior to first feeding is expected to be due to eggs quality (Kjorsvic *et al*., 1990) and all environmental factors. Early mortality is a serious problem in the development of seed production of this economically important Garhwal hill stream snow trout (Bahuguna, 2002). Recently, presence of density dependent survival after handling was reported in yolk sac larvae of some fishes (Tagawa *et al.*, 1997) using small plastic plate as fish containers (Brown and Nunez, 1994). In the present study, survival after handling stress was examined in relation to larval density in the above mentioned *Schizothorax* species with more interest for the establishment of seed production procedures than previously used techniques.

I believe the initial mortality of larvae after the transfer is related to handling stress. The narrow tip of the pasture pipette could damage the larvae during the transfer from the stock tank to the plastic containers. Since larvae transferred to containers of higher densities showed better survival it appears that survival after acute stress or damage is improved by rearing fish at extremely high density. In some Japanese fish larvae have such type of problem which has been reported by Tagawa and Kimura (1991) also support our finding in snow trout fish larvae.

The second timing of severe mortalities in *Schizothorax* larvae would be related to the starvation, because the timing of the mortality later than the completion of yolk absorption. The information will be further discussion on the reason of larval mortality, which may be related to food quality development in relation to natural site and laboratory site. In natural site they may have enough quality food than laboratory site (Bahuguna, 2002; 2006).

Concentration of substances secreted from larvae as well as changes in larval behavior, in high densities are possible factors to be considered in future which help to know the causes of less mortality among higher density larvae. That investigation may improve our understanding of the larval response to stress and thus our seed production abilities. Present finding is based on a small experimental system with stress by pipeting and larval density, which is unrealistically high. The result cannot be applied directly to the technique of the seed production; they do suggest that further experiments of this type may help us to understand the mechanism of the larval death and or recovery after stress.

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References

Badola, S.P. 1979. *Ecological studies on the Ichthyofauna of freshwater resources of Garhwal region*. University of Garhwal, Srinagar (Garhwal), India. (D.Phil. Thesis)

Bahuguna, S.N. 2002. *Role of hormone, histochemical and bimolecular comparison of certain tissues with respect to migration of Himalayan mahseer Tor putitora in the Ganga river system in Uttarakhand*. Final Technical Report Submitted to *ICAR, Krishi Anusandhan Bhawan*, New Delhi.

Bahuguna, S.N. 2006. *Morpho-functional developmental study of Garhwal Himalayan snow trout Schizothorax plagiostomus (Heckel) Larvae from post hatching to yolk absorption stages*. Final technical report submitted to ICAR, Krishi Bhawan, New Delhi.

Brown, C.L. and J.M. Nunez 1994. Hormone action and application in embryogenesis. In *Perspective in comparetive endocrynology* (eds. K.G. Davey, R.E. Peter and S.S. Tobe). National Research Council Canada, Ottawa. pp. 333-339.

Day, F. 1958. *The fishes of India: Being a natural history of the fishes known to inhabit the seas and fresh waters of India, Burma and Ceylon, Vol. **1*** (text). William Dawson and Sons Ltd., London. 564p.

Kaji, T. 2000. *Studies on the early development of bluefin and yellowfin Tuna*. Kyoto University, Japan. (Ph.D. Thesis).

Kaji, T., M. Tanaka, Y. Takahashi, M. Oka and N. Ishibashi 1996. Preliminary observation on development of Pacific bluefin tuna *Thunnus thynnus* (Scombridae) larvae reared in the laboratory, with special reference to the digestive system. *Mar. Freshwater Res.* ***47*****:** 261-269.

Kaji, T., M. Tananka, M. Oka, S. Ohsumi, K. Teruya and J. Hirokawa 1999. Growth and morphological development of laboratory reared yellowish tuna *Thunnus albacares* larvae and early juvenile, with special emphasis on the digestive system. *Fish Sci.* ***65*****:** 700-707.

Kjorsvik, E., A. Mangor-Jenson and I. Holmefjord 1990. Egg quality in fishes. *Adv. Mar. Boil.* ***26*****:** 71-113.

Masuma, S., N. Tezuka and K. Teruya 1993. Embryonic and morphological development of larval ans juvenile coral trout, *Plectropomus lepardus. Japan. J. Ichthyol.* ***40*****:** 333-342.

Sharma, R.C. 1994. Qualitative and quantitative seasonal variation in feeding of snow trout, *Schizothorax plagiostomus* (Heckel) of Garhwal Himalaya. *Biol. Bulet. Ind*. ***6*****:** 20-25.

Tagawa, M. and R. Kimura 1991. Development and role of endocrine function. In *Early development in fishes* (Ed. M. Tanaka). Kohseisya kohseikaku, Tokyo. pp. 47-59.

Tagawa, M., T. Seikai and M. Tanaka 1997. Effect of thyroid hormone supplementation to eggs on the larval survival of Japanese flounder, Abst. 13. International Congress of Comparative Endocrinology. pp 48.