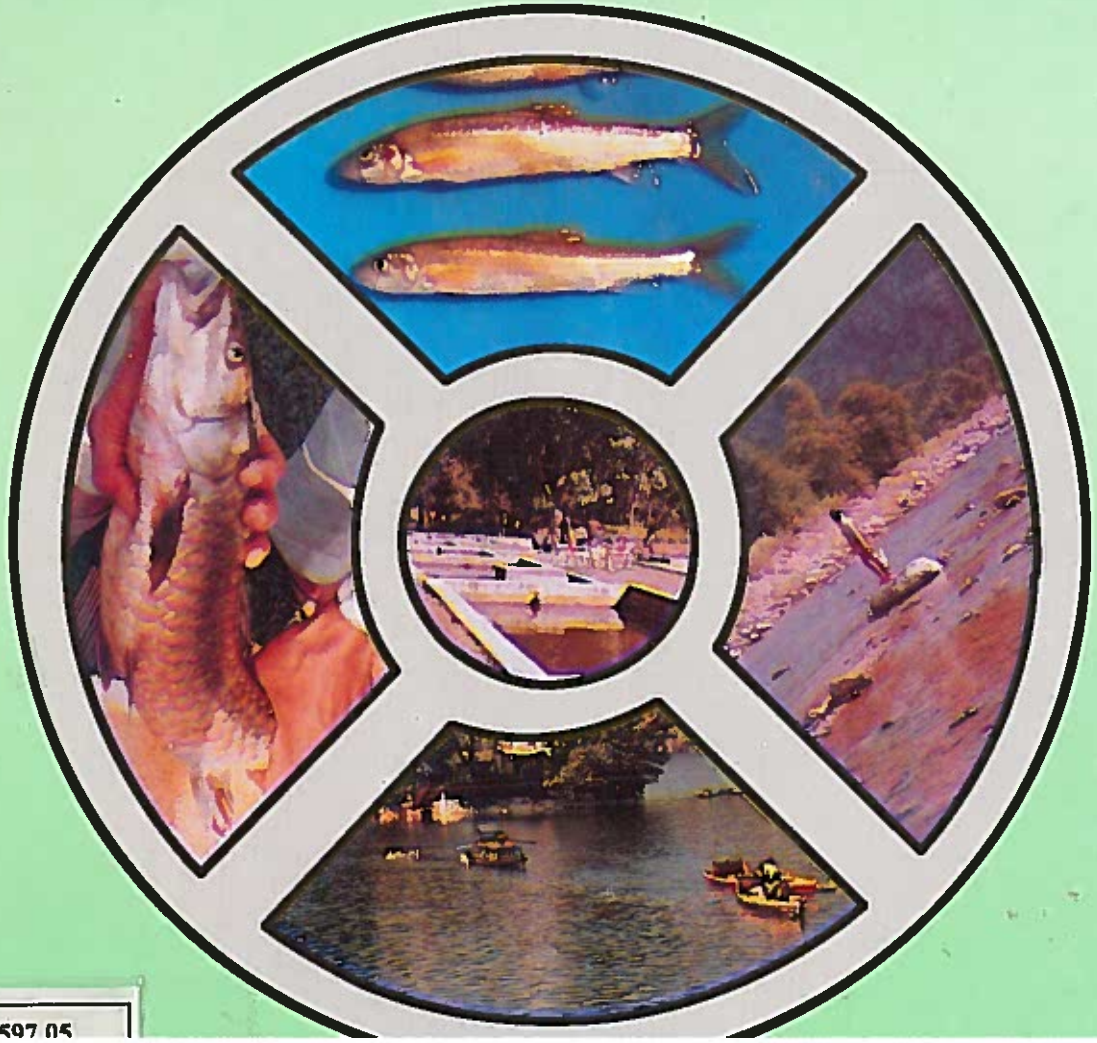
Biochemical Status of Coldwater Fishes



Biochemical Status of Coldwater Fishes

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FOREWORD

The tools of biochemistry, apart from providing important insight into various biological processes, are now being utilized for practical applications in medicine, agriculture, veterinary and industry. Understanding of critical biological processes of fish at molecular level has provided basic scientific inputs upon which modern aquaculture is based.

Nutrition biochemistry has provided the detailed knowledge of the nutrient requirements of fishes, bioavailability of nutrients, composition of feed ingredients and the requirements of essential amino acids, fatty acids and vitamins for computing a balanced diet for fishes inhabiting at different climatic zones having varying physiological needs.

Technological advances, particularly in relation to processing and preservation have played an important role in the rapid development and effective utilization of fishery resources especially in marine sector. All these developments were made possible due to use of various biochemical techniques/processes in this field.

The research needs in the fields of reproductive biochemistry, biochemical genetics, nutritional biochemistry as well as clinical biochemistry have an immediate relevance to aquaculture especially in the inland sector in which coldwater fisheries is one of the important sub-sector. The biochemical investigations on tropical fishes are being carried out in the country in several R & D organisations and significant amount of information has been generated which has helped us to increase the fish production.

In comparison, such studies in our country on coldwater fishes have been very limited. It is in the context that NRCCWF has initiated to take-up investigations on the fish species which are very important in our Himalayan regions. The institute has compiled the existing information in this important and frontier field of science and also presented the information generated on coldwater species. The document **"Biochemical Status of Coldwater Fishes"** sumarizes the research highlights of NRCCWF in this field. I am hopeful that the document will receive adequate response from researchers and students connected with inland aquaculture and particularly coldwater fishes in India.

PREFACE

The National Research Centre on Coldwater Fisheries apart from concentrating its efforts in developing technologies for farming of important indigenous fish species inhabiting Himalayan uplands is also generating scientific database on various biochemical factors which influence the growth behaviour and environmental responses of these important fishes. The information generated will be of great help in understanding reproductive, nutritional, and clinical biochemistry requirements in coldwater fishes. These will be effective tools for initiating biochemistry requirements in coldwater fishes. These will be effective tools for initiating biotechnological approach in developing a complete production system in Temperate Aquaculture.

The present document apart from reflecting the work done in this field of science with respect to fisheries highlights the work done by NRCCWF in this important area. The information presented is based on the sustained efforts of our biochemistry and fisheries scientists involved in such projects from time to time. All the workers of the institute who contributed in preparation of this document deserve appreciation. I am hopeful that the document will be a baseline information for different researchers to initiate work on biochemistry of coldwater fisheries in the country about which very little is known.

(K.K. Vass)
Director NRC on
Coldwater Fisheries

Date : August 7, 1999

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Introduction

Biochemistry is the study of the molecular basis of life which has its roots in two major scientific disciplines, Biology and Chemistry. Past several years have witnessed exceptionally rapid pace of discovery in this area. The tools of biochemistry apart from providing important insights into various biological processes are now being utilized for practical applications in medicine, agriculture, veterinary and industry. In recent past a considerable amount of new information has been generated concerning fisheries. Understanding of the critical biological processes of fish at molecular level has provided basic scientific inputs upon which modern aquaculture is based. Credit of advances made in the concept of nutrition and feeding practices of fishes, management of health and diseases, controlled maturation of sex organs for artificial breeding and fish stock improvement by identifying molecular markers goes to the science of biochemistry.

Nutritional Biochemistry

Detailed knowledge of the nutrient requirements of fishes, bio-availability of nutrients, composition of feed ingredients and vitality of essential amino acids, fatty acids and vitamins for computing a balanced diet for fishes inhabiting different climatic zones depending upon their physiological needs has been acquired. Recently, thyroid and growth hormones (somatotropins) have been attracting interest as potential feed additives for accelerating the growth rates of fishes. However, in the absence of any economic source of somatotropin, production of recombinant somatotropins in microbes and their subsequent testing in fish has been increased with the advent of genetic engineering. But now, alternative to the direct administration of growth regulatory peptides and proteins to fish focus is shifting towards production of transgenic fish by microinjection of DNA having desirable characteristics directly into the fertilized eggs.

Clinical Biochemistry

efforts made to better understand the piscine immuno-chemistry especially as it relates to protection against infectious agents. Highly specific monoclonal antibodies are now used in the diagnosis of infections, specific identification of parasites, detection and identification of parasite antigen(s) in host tissue/faeces, differentiation of morphologically similar parasites and study of parasite antigens in systematics (Kapila and Kapila, 1997).

Reproductive Biochemistry

Information on endocrine control of fish reproduction has made hormone treatment a routine practice in fish farming because of its relative ease and efficiency. This has enabled farmers to spawn cultivable fishes in a synchronized manner which do not spawn in captivity or in a season when desired. On the other hand, successful reversal of sex as well as artificial control of physiological sex in some of the fish species have also become possible by treatment of steroid hormones which has great significance because there are differences in growth rate, behavior pattern, breeding time, body colour, shape, or size between male and female in each cultured species. Fish breeders or culturists may want to breed males and females separately or to achieve a monosex culture depending on biological or economic traits.

Biochemical Genetics

Similarly, biochemical techniques proved to be useful in fish stock assessment through the study of genetic variability within and among populations of fishes. Gel electrophoresis coupled with histochemical staining are rapid, relatively inexpensive methods for separating and visualizing proteins, to reveal patterns of variation of ontogenetic, phylogenetic, or zoogeographic interest. By the early 1980s, direct examination of the gene was also become possible by determining differences in the sequence of nucleotides, components that comprise DNA molecule.

Fish Technology

Technological advances, particularly in relation to preservation, processing and quality control have played an important role in the rapid development and effective utilization of fishery resources. Knowledge of autolytic tissue degradation by enzymes and understanding of biochemical changes occurring in fish tis

In India, researches on coldwater fisheries started in 1963 under the aegis of Central Inland Fisheries Research Institute (ICAR) which were subsequently extended by National research Centre on Coldwater Fisheries (ICAR) after its coming in existence in 1988. As a result, considerable information has accumulated in about thirty five years, on resource management in selected areas, biological productivity of mountain streams, lakes, etc., assessment of seed resources of commercially important coldwater fish, biology of trouts, schizothoracids and mahseer and estimation of subsistence fish catches from natural waters, hatchery and nursery management and fish production in raceways. But unfortunately, biochemical studies carried out on cold water fishes in our country are still in infant stage and whatever the information is available is scattered though the foregoing discussions clearly depicts that extensive work on several aspects of fish biochemistry has been conducted in other parts of the world. Hence an attempt has been made in present communication to overview the current areas of work and research needs in the fields of reproductive biochemistry, biochemical genetics, nutritional biochemistry and clinical biochemistry which have immediate relevance to aquaculture in uplands of the country.

1. Nutritional Requirements of Fish

Studies on food conversion efficiency of fishes have created an immense interest among fish biologists and environmental biologists, because of their applications in resource management. Intensive fish culture is based on efficient feeding. The dietary formulation of a nutritionally balanced diet to meet the requirements is one of the major aspects in the development of aquaculture.

1.1 Protein

It is considered as the most important and expensive component of fish diets. Proteins are mainly needed for tissue growth and tissue replacement. Although they can be used for energy supply too and in fact often serve as such, calories provided in this way will always be expensive. The characteristics of the protein sources used in fish diets should be considered, since the response of the fish could vary depending on protein quality and gross protein level of the diet. A protein requirement as such is less important. The requirements for different amino acids both essential and non-essential are of primary importance. It is shown up till now that 10 amino acids are essential for salmonids, viz. **lysine, arginine, methionine, threonine, leucine, isoleucine, tryptophane, histidine, phenylalanine** and **valine (EIFAC, 1971).**

The protein requirement of culturable fishes like the carps and tilapias, the mullets and milkfish, which are herbivores/omnivores, is in the range of 25-30%, as against 30-40% protein required by the carnivorus fishes like the salmon and trout. This carnivorous nature of trout is further confirmed by Joshi and Joshi, (1984) who observed that mortality of the brood stock of brown trout was higher when they were fed on vegetable products as compared to animal meals. On the other hand, series of experiments conducted with purified test diets (casein based) and formulated diets of 21.0-50.0% protein to ascertain the best growth performance of the juveniles of golden mahseer *(Tor putitora)* revealed the diets containing

whole mahseer fry which indicated high protein content to the tune of 50.27% as compared to 20.27% in adult fishes.

Most tropical fish like tilapias, carps and milkfish display a faster specific growth rate then temperate fish such as trout. The relatively faster growth displayed by tropical fish demand nearly two times higher dietary protein requirements than their temperate counterparts. This is further confirmed by Pandian and Vivekanandan (1985), who observed that the feeding rate of tropical fish is 180% higher than that of temperate species, and that the elevation in feeding rate is nearly 2.5 times higher than that (70%) observed for maintenance metabolism. Chinook Salmon *(Oncorhynchus tshawytscha)* need food containing 40% protein in water temperature of about 8°C for optimum growth, whereas in temperatures of about 14°C the same fish will need food containing 55% protein. At lower temperatures, food containing more than 40% protein produce stress due to excess of ammonia released from gills (DeLong *et al.*, 1958).

1.2 Lipids

Fats and lipids are major sources of metabolic energy in fish. They are also the sources of essential fatty acids for cell structure and membrane function and are carriers of oil-soluble vitamins. The fatty acid profile like amino acid profile can be used as an index of quality of dietary lipids. Fish oils contain a greater variety of fatty acids in comparison with other oils and fats. Fish oils contain polyunsaturated fatty acids of considerable longer chain (20 or 22 carbon chain length), most of which belong to the n-3 family of fatty acids (Stansby 1982). The long chain fatty acids generally make up one-fourth to one-third of all fatty acids in fish oils. The composition of fatty acids in fish is affected by number of environmental factors foremost of which are salinity, temperature and diet. If the trends in fatty acid composition can be taken as a clue to the essential fatty acid (EFA) requirements of fish, the n-3 requirement would be greater for coldwater fish. Warmwater fish may do better with a mixture of n-6 and n-3. Coldwater fish are likely to be more demanding in their requirement for essential fatty acids than warmwater fish because constrains imposed in maintaining membrane fluidity are greater at low temperature (Hazel 1979). Studies have demonstrated that the EFA requirements of fish differ considerably from species to species. The EFA requirements of

were observed to be suitable for better growth and survival of mahseer fry (Raina *et al.*, 1993).

1.3 Carbohydrates

Carbohydrates are the most abundant and relatively least expensive source of energy. These may consist of easily digested sugars to complex cellulose which is difficult to digest. Based on results of research on carnivorous species, doubts have been expressed on the value of carbohydrates in fish feeds, but practical experience in fish culture shows that digestible carbohydrates can be an energy source if kept in proper balance with other nutrients. The ability to assimilate starches depends on enzymatic activity (production of amylase). In herbivores, amylase occurs through the entire digestive tract whereas cellulase activity associated with cellulolytic microflora. Metabolizable energy values of carbohydrates may range up to 3.8 kcal/g for easily digestible sugars, while for undigestible cellulose it may be near zero. Values for raw starch range from 1.2-2.0 kcal/g. When processed in high moist temperatures, for making pelleted feed, starch gelatinizes and its digestibility therefore improves. When digested, the products of hydrolysis are assimilated into the blood stream, where their known function is to provide energy. Therefore they have a protein sparing action and excess is partly stored in the liver as glycogen and partially converted into visceral and muscular fat. Successful fish feeds contain a certain amount of carbohydrates, as for example 20% for coldwater fish feeds and 30% for warm water fish feeds (Pillay, 1990). Similarly, diets containing 15-20% carbohydrates resulted in better growth and survival of Himalayan golden mahseer fry (Raina *et al.*, 1993). Besides providing energy, they have the physical function of texturizing manufactured feeds and acting as a binder in the formulation of pellets.

Feeding experiments conducted on some hill stream fishes *(Schizothorax richardsonii, S. progastus, Tor putitora* and *Barilius bendelisis)* fed on natural food, suji (Protein, 8.9-11.9%; Fat, 1.2-2.1%; and carbohydrate, 68.4-72.9%) and commercial fish meal (Protein, 16.0-17.0%; Fat, 2.0-2.2% and Carbohydrate, 20-21%) revealed highest feeding rate in *S. richardsonii* followed by *Barilius bendelisis* and *S. progastus.* The feeding rate is not the factor responsible for a better growth but the stress, choice food, quality and quantity of food and temperature. Besides, food conversion ratio was recorded to be highest in *S.*

1.4 Vitamins and minerals

Vitamins are chemically diverse group of organic substances that are either not synthesized by the organisms or are synthesized at rates insufficient to meet the organisms' needs. They constitute only a minute fraction of the diet and are more catalytic in their function and are critical for the maintenance of normal metabolic and physiological functions. The following vitamins can be classified as essential: vitamins **A, D, E, K,** and **C** and from the B-complex : **B1, B2, Niacin, Pantothenic acid, B6, B12, Choline, Folic acid, Biotin** and **Inositol** (EIFAC, 1971). Water soluble vitamins (C and B group) are generally lost during food processing, storage and leaching in water. Vitamins become unavailable due to interaction and chelation with other dietary ingredients. Recent studies have established the role of vitamin B complex, C and E in cellular metabolism, improving immunity to diseases and reproductive performances. The effect of dietary ascorbic acid in broodstock feed on egg hatchability is now known. It is essential for vitellogenesis and embryonic development in fish. A feed made for rainbow trout, brood stock supplemented with ascorbic acid (1g/Kg) produced eggs with significantly improved hatching rate and successful larval development compared to ascorbic acid deficient diet which was otherwise identical in its gross composition (Nandi *et al.*, 1997). In order to assess the impact of vitamin fortified diets on fish growth and survival in coldwaters, a basal diet fortified with vitamin-A(8000 IU/Kg), vitamin-E(100 IU/Kg) and vitamin-C(400mg/Kg) in different combinations, were trial tested at Chirapani fish farm of the institute (NRCCWF, Annual Report, 1997-98). After nine months of experiment the dietary groups having combinations of vitamin E+C and vitamin A+E+C exhibited significantly higher ($P<0.05$) growth of fry by 10.2% and 17.4%, respectively as compared to control in *Schizothorax richardsonii.* Similarly higher weight gain of 21.2% and 32.9% was recorded in golden mahseer fingerlings fed with diets supplemented with combinations of vitamin A+C and vitamin A+E. On the other hand feed containing vitamin E+C and vitamin A+E+C recorded 35.30% and 20.6% increase in growth in terms of weight gain in case of common carp.

Minerals in diet are highly essential for various physiological functions such as the formation of bones, respiration, digestion and osmoregulation. Calcium, Phosphorus, Magnesium, Iron, Sodium, Potassium, Chlorine, Manganese, Zinc,

1.5 Growth promoting additives

Dietary hormonal manipulation is one of the means by which growth rate of the animal can be accelerated. Steroid hormones such as androgens, estrogens and progestogens and non steroidal hormones such as thyroxin are used as growth promoters in animal feeds. In the case of Coho salmon *(Oncorhynchus kiisutch)* higher growth was recorded when 17-L-Methyl-testosterone was given at a dose of 10mg/Kg. Similarly, rainbow trout fed with diet having methyltestosterone at the rate of 2mg/Kg exhibited higher growth (Weatherley and Gill, 1987). However, use of steroids in aquaculture practices is a state of controversy whether they can be safely used without any health hazards. Shirgur (1996) reported "Bhimasu" formerly termed as `Biovet' a product marketed by Wockhardt as a effective growth promoter of mahseer fry *(Tor khudree)* when incorporated in feed.

2. Haematological and Serological Investigations

The application of haematological and serological techniques have proved valuable for fishery biologists in assessing the health of fish and monitoring stress responses either due to fluctuations in environmental conditions or due to sublethal concentrations of pollutants. Haematology concerns mainly investigations on cells present in the blood viz. Red blood cell (RBC) count, Haemoglobin concentration (Hb), Packed Cell Volume (PCV), White blood cell (WBC) count, Mean corpuscular haemoglobin (MCH), Mean Corpuscular haemoglobin concentration (MCHC) and Mean corpuscular volume (MCV). Whereas, serology deals with the constituents in the fluid part of blood, such as proteins, enzymes, minerals, carbohydrates, pigments, hormones, immune bodies, etc. The values of haematological parameters determined in various upland fishes are summarised in table 1.

2.1 Toxicological and pathological studies

Measurement of the activities of plasma/serum non specific enzymes had diagnostic potential in fish toxicology and pathology because enzymes activities can often be related to cell damage in specific organs. For example, the liver is rich in glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvate transaminase (GPT), and changes in plasma levels of these enzymes may be indicative of liver dysfunction. In rainbow trout treated with carbon tetrachloride (CCl_4), a known hepatotoxicant, plasma GOT and GPT levels increased significantly. Likewise, levels of lactate dehydrogenase (LDH), GOT and GPT increased many folds when rainbow trout was infected with *Aeromonas* (Racicot *et al.*, 1975). A variety of environmental contaminants in water are toxic to fish. The primary effect of many of these contaminants is impairment of gill ion-regulation and therefore disruption of plasma electrolyte balance function. Chronic exposure of fish to sublethal

atological Values in Some Coldwater Fish Species.

	Body weight (g)	RBC ($x10^6/mm^3$)	Hb (g%)	PCV (%)	WBC ($x10^3/mm^3$)	MCH (pg)	MCHC (%)	MCV (um^3)	Source
	99.36	3.21	10.42	50.27	10.78	32.46	20.72	156.60	Sharma and joshi, 1986
	470.5	2.42	9.87	52.94	10.15	41.00	18.62	234.64	NRCCWF,
	42.02	2.09	9.53	40.88	8.77	47.70	23.62	201.47	-do-
s	116.12	2.68	14.43	43.2	7.28	53.96	33.54	161.74	Bhatt and Singh, 1985
	90.43	3.15	10.21	48.63	10.58	31.41	20.99	154.38	Sharma, 1986
isis	45.62	3.11	9.81	47.18	10.35	31.54	20.79	151.70	-do-
	36.94	3.09	9.34	46.43	9.87	30.22	20.11	150.25	-do-
	4.65	3.01	8.00	44.47	11.84	26.57	17.98	147.74	-do-
	5.73	3.04	8.15	45.14	12.46	26.80	18.05	148.48	-do-
	Adult specimen	1.42	6.99	-	22.00	-	-	-	Rodriguez, 1995
	Adult specimen	0.98	9.65	46.50	-	98.46	20.55	497.00	Sandnes *et al*., 1988

2.2 Non-pathological factors

Factors like temperature, pH, salinity, reproductive state, season sex etc. have profound effect on haematological and serological parameters. Creatine phosphokinase activity in trout plasma increased when acclimation temperature was increased from 3.5°C to 10°C but then declined at15°C. Alkaline phosphatase activity decreased when acclimation temperature was increased from 6°C to 19°C (Sauer and Haider, 1977). Table 2 depicts the activities of some serum enzymes in coldwater fish species.

The exposure of *Schizothorax richardsonii* to different temperatures and pH at Institute's laboratory, brought forth changes in haematological parameters and enzyme activities (NRCCWF, Annual Report, 1997-98). A progressive decrease in RBC count, Hb concentration and PCV at low temperature (5°C and 10°C) as compared to the control (20°C) was observed. Besides, at higher temperature of 30°C, RBC, PCV and Hb exhibited drop in values after 6hrs. On other hand remarkable increase was observed in the activities of LDH and GOT at 5°C. Further,it has been observed that GPT and ALP changed insignificantly at different temperatures while the activities of ACP were recorded highest at 20°C and 30°C (Figure 1). In case of exposure of snow trout to different pH, a remarkable increase in the values of various haematological parameters (RBC, Hb. and PCV) and serum enzymes (LDH, GOT, GPT, ACP and ALP) at pH values of 5.0 as compared to the control (pH 7) were noticed.

Haematological and enzymatic studies on both the sexes of matured *Tor putitora* (Ham.) indicated that males have significantly higher values for RBC, Hb and PCV as compared to their female counterparts having remarkably increased values of WBC during breeding season. Effect of capture stress on serum enzyme activities in golden mahseer resulted in considerable increase in the values of LDH, GOT, GPT, ACP and ALP in fishes netted out by gill nets, besides, all other enzymes except, ALP and ACP also exhibited significantly higher levels of activities when fishing was done by angling as compared to cast nets and drag nets (NRCCWF, Annual Report, 1996-97). The blood cells present in Himalayan mahseer are depicted in Figure 2.

Table 2. The activity of nonspecific serum enzymes in different coldwater fish species.

Species	Enzyme activity (Units min^{-1} l^{-1}) LDH	GOT	GPT	ACP	ALP	Reference
Rainbow trout *Oncorhynchus mykiss*						
Fed	1111	244	7.2		138	Sauer and Haider (1979)
Starved	730	157	4.9		68	-do-
Fresh water	610	171	8.0		136	-do-
10% Salinity	491	192	7.8		191	-do-
20% Salinity	579	267	17.9		111	-do-
3.5°C	150	125	7.0		72	Sauer and Haider (1977)
10°C	825	275	11.5		140	-do-
15°C	910	310	15.2		122	-do-
Atlantic salmon *Salmo salar*		278	6.0		853	Sandnes *et al.*, (1988)
Pink salmon *Oncorhynchus gorbuscha*						
Nonspawning	518	309				-do-
Prespawning	2427	500				-do-
Spawning	2463	797				-do-
Golden Mahseer *Tor putitora* (Ham).						
Matured Male	1862	265	85	310	29	NRCCWF
Matured Female	1569	277	80	288	20	-do-
Snow tourt *Schizothorax richardsonii*						
Prespawning (June-July)	944	156	54	556	12	-do-
Spawning (Aug-Oct)	808	143	52	472	10	-do-
Postspawning	731	126	62	421	14	

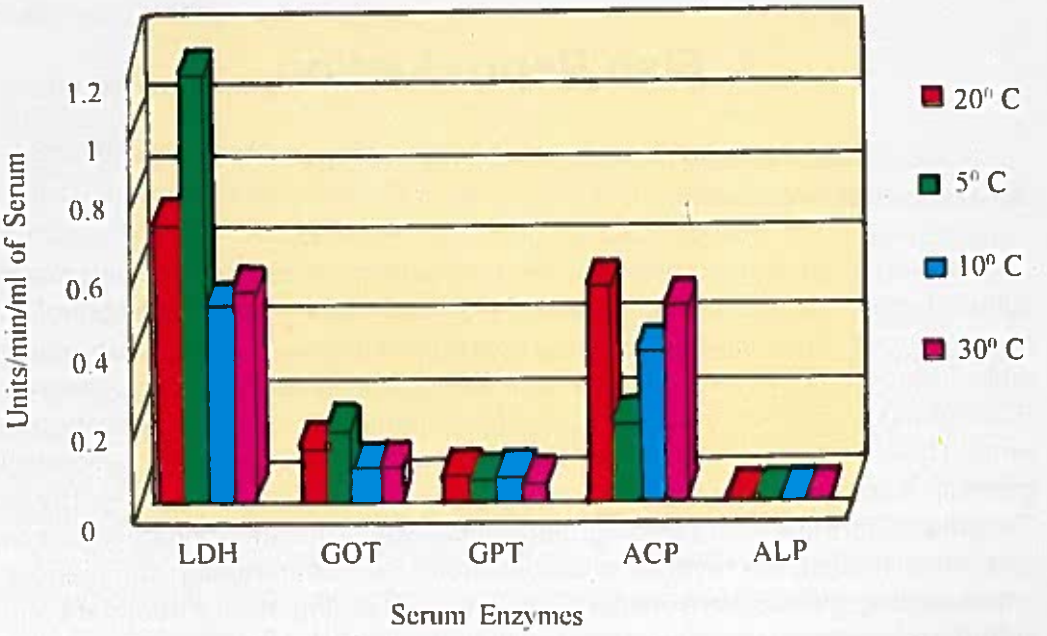
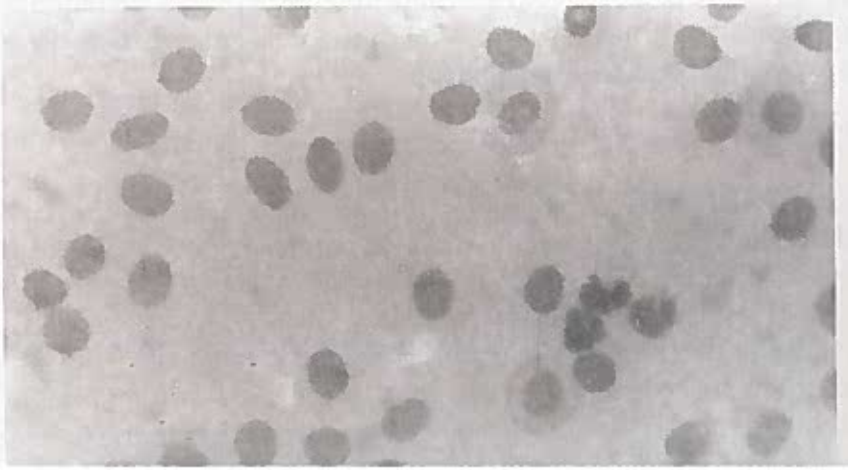


Figure 1. Impact of temperature variations on serum enzymes activities of *Schizothorax richardsonii*



3. Fish Reproduction

3.1 Hormonal sex control

The control of sexual development to produce monosex or sterile stocks of cultured species is economically desirable. Sexuality of the fish can be controlled by sex steroids. Functional sex reversal from female to male is carried out by using male hormones (Testosterone) while feminization is induced by female hormones (Estrogens). In rainbow trout, the optimum concentration of methyltestosterone ranged between 0.1-3.0 mg/kg diet is required which is quite low than needed by goldfish, tilapia and zebra fish for inducing masculinization, (Yamazaki, 1983). Treatment with more than 10mg/kg. diet induces sterile or hermaphroditic gonads and rarely induces sex reversal in rainbow trout. Sex differentation commences after hatching, either before or after the initiation of feeding. Hence treatment with steroids is usually carried out by feeding during early stages of life for a specified period depending upon fish species. However in salmon, male sex differentiation is initiated before feeding commences, so other procedures are used, such as immersion of larvae or alevins. An alternate method of producing monosex stock is to induce sterility, and this can be done by the administration of high doses of sex steroids or by chromosomal manipulation.

3.2 Chromosomal engineering

Chromosomal manipulation for inducing polyploidy, gynogenesis and androgenesis has been studied with a view to controlling sex, as well as for rapid breeding. Manipulation become feasible during the nuclear cycles of cell division, and since fertilization in fish is external, artificial means can be employed to either gamete before fertilization, or to the fertilised eggs. The triploids and tetraploids could be produced by subjecting the eggs to pressure or temperature shock shortly after fertilization. Generally the coldwater species respond to heat shock

candidate species of coldwater in which polyploidy gynogenesis and androgenesis have been successfully induced by many workers (Kaul and Srivastava, 1989).

3.3 Induced breeding

Studies on reproductive biochemistry and physiology from the aquaculture point of view proved very useful for developing techniques to control and induce breeding. Sexual maturation is regulated by environment. Photoperiod and temperature are one of the most important environment factors which can be manipulated to control maturation and spawning of fishes. For example, the maturation period and breeding time of farmed salmonids has been shortened by changing photoperiod (MacQuarrie *et al.*, 1981). Likewise, an understanding of the sequential mechanism of acceleration or delaying of the various steps leading to final ovulation by using exogenous hormones at different stages for inducing maturation and ovulation is prerequisite for successful long term viable aquaculture. Human chorionic gonadotropin (HCG), fish pituitary gland extract (PGE), purified fish gonadotropin, salmon gonadotropin releasing hormone (SGnRH), mammalian LHRH and its analogue (LHRH-a), dopamine antagonists such as pimozide (PIM) and domperidone, and steroids are generally used for such manipulation. Many successful attempts of induced breeding of coldwater fishes have been made in the developed countries.

One such attempt of induced breeding of mahseer, *Tor putitora* was made at Mahseer seed production unit, Bhimtal (Joshi, 1988). The females were administered the preliminary dose of fish pituitary extract @ 6mg/kg body weight, whereas the males were not given any injection before releasing into the hapa along with the injected females. However, males were given a light dose of fish pituitary extract @ 2-4 mg/kg. body weight at the time of administration of the second booster dose (8mg/kg body weight) to the females. Fertilization was carried out after 24 hrs. of the first injection and fertilization rate ranged from 34 to 92% was achieved.

Recently, induced spawning and hatching of golden mahseer *(Tor putitora)*

3.4 Biochemical changes during reproductive cycle

In addition to induced breeding, studies on biochemical changes during reproductive cycle have also been carried in some hills steam fishes. The increasing protein content in the gonads of *Shizothorax richardsonii* and *Glyptothorax pectinopterus* during the gonadal maturation is indicative of the fact that rapid protein synthesis is necessary during the development and maturation of the oocytes and sperms. Likewise, increase in the cholestrol levels in the testes of both the fishes during maturation were observed which subsequently exhibited sharp decline during spent stage contrasting to the ovaries of these species having lowest cholesterol levels at immature stage and the highest at the spent stage. On the basis of these observations, it is considered that high cholesterol level in the gonads acts as a reservoir to meet the cholesterol demand of the maturing gonads and the decreased level might be due to increase in the rate of steroidogenesis. (Singh and Nauriyal, 1990).

The change in carbohydrate reserve in fish seems mostly to reflect the requirements of developing gonads specially females. Glycogen and glucose have both been reported to accumulate during maturation in the ovary of *Oncorhynchus nerka* (Chang and Idler, 1960). Similarly, in *Garra mullya,* ovarian glycogen was highest and that in muscle was lowest during spawning season probably due to the depletion of glycogen from muscles and its transfer to ovary for maturation (Khan, 1994). Declination in the levels of protein, fat and glycogen in the muscle tissues of *Tor putitora* (Ham.) during spawning season suggesting their utilization in spawning cycle, was also observed in institute's laboratory (NRCCWF, Annual Report, 1996-97).

3.5 Cryopreservation of fish gametes

In many species, the maturation of gonads in the two sexes in not synchronous. Males often show testicular recrudescence earlier during the season because of this, ripe males occur during the beginning of the season, when the females are not yet mature and ready for spawning. Under such circumstances, it will be most advantageous to have a suitable means of preserving the gametes for artificial fertilization, when needed. Cryopreservation of gametes also offers immense benefits to selective breeding, cross breeding and protecting endangered species by gene banking programmes.

potentiality of gamete preservation extensive researches were carried out worldwide with special emphasis on the temperate zone salmonid species (Stoss, J and Refstie, T., 1983) but it is only recently, studies with some success have been initiated by National Bureau of Fish Genetic Resources, Lucknow in collaboration with the NRCCWF, Bhimtal by cryopreserving milt of some upland species. Fertility trials were carried out with milt of *Tor putitora* cryopreserved with two cryprotectants (Glycerol and DMSO) under three equilibration times of 60, 120 and 180 minutes resulted in production of viable hatchings (0.53 to 2.17%) after one year of storage. (NBFGR, Annual Report, 1992-93).

4. Genetic Markers/Molecular Probes

Fishery biology has much to gain from the advances in technology that now permit the study of products and components of genes themselves. Several of the most important questions in fisheries, e.g., those of stock assessment and biochemical identification, can be addressed readily through the study of genetic variability within and among populations of fishes. Up till now the population structure of fish species has been studied predominantly using the quantitative morphological traits such as the number of vertebrae, the fin rays, the gill rakers, etc. which do not seem to be adequate in differentiating the populations. On the other hand, even small variations in the genome can be detected relatively easily by electrophoretic methods (Kapila and Kapila, 1996). The use of electrophoretic techniques has led to the discovery of a large number of polymorphic biochemical systems. Several modern methods used to resolve molecular characteristics, including restriction enzyme analysis and direct sequencing techniques of mitochondrial and nuclear DNA also use an electrophoretic step to separate components.

4.1 Protein polymorphism

Genetic profile of endangered *Tor putitora,* collected from Beas river of Himachal Pradesh was studied by NBFGR, Lucknow (NBFGR, Annual Report, 1995-96) Analysis of eye lens proteins by isoelectronic focussing revealed 40 distinct bands. Out of 53 specimens studied 17 individuals revealed variable band pattern. Comparative enzymatic profile of endangered *Torputitora* and *Schizothorax richardsonii* was also carried out using 7 enzyme systems (G6PD, GPI, PGM, IDHP, AAT, SOD and G2PDH). Similarly, some preliminary investigations of

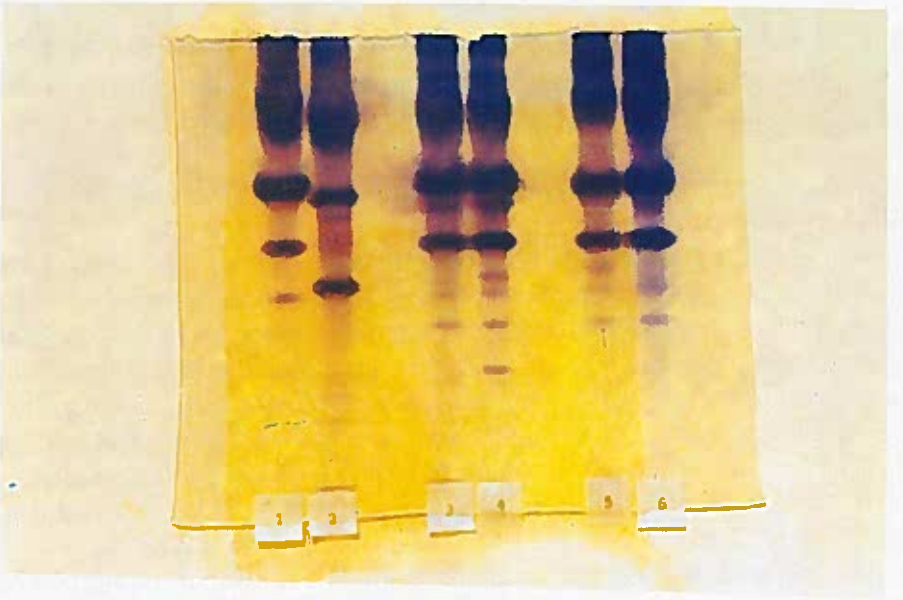
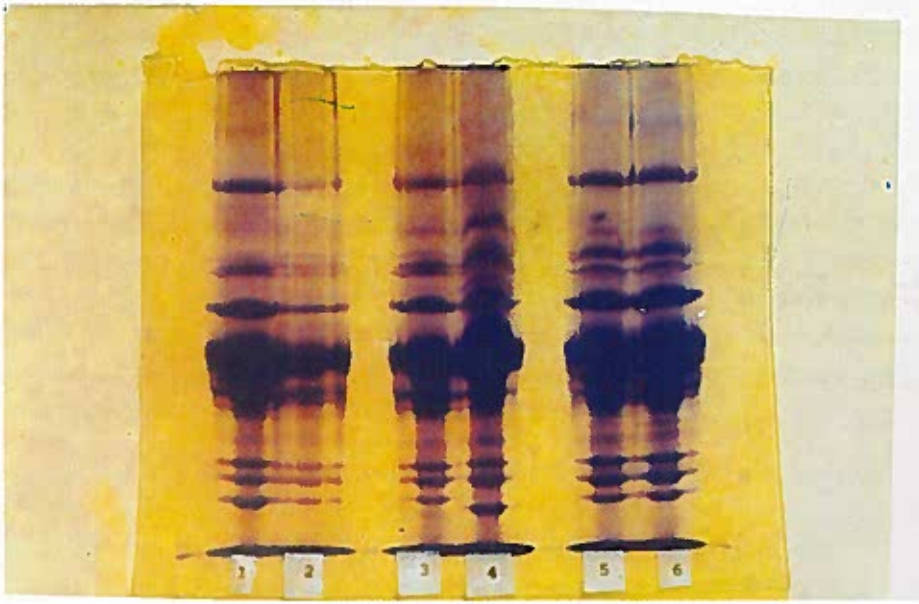


Figure 3. Electrophoretic profile (PAGE) of soluble muscle proteins in important hill stream fishes



(Figure 3 & 4). The variations in band pattern of an animal species mostly occurred as a result of their genetic constituents and the influence of environmental and physiological factors (NRCCWF, Annual Report, 1997-98).

4.2 DNA polymorphism

The nucleotide sequences of DNA typically offer more than 3 billion potentially informative markers. Molecular techniques, once tedious, have advanced so that rapidly analysing the numerous samples typically required to resolve fisheries problems is now possible. To date, most molecular analyses in fisheries science have focussed on analysis of protein polymorphism, but newer approaches involving mitochondrial DNA (mt DNA) and nuclear DNA (n DNA) show great promise of providing more information regarding genetic diversity. Large number of DNA based genetic markers have been developed for trout and salmon (Ferguson and Danzmann, 1998) in other parts of world but researcher in India have just begun to exploit this wealth of genetic information using biochemical techniques.

5. Proximate Composition of Fish

Fish growth may be defined as an accretion of body constituents (protein, lipid, water, carbohydrate, inorganic substances) by the process known collectively as anabolism. The examination of anabolic growth process can be approached by proximate analysis of body composition using biochemical procedures and subsequently relating the changes in composition to corresponding changes in the bulk of the body and its constituents tissues. Detailed studies on the partitioning of protein and lipid in the fish body depending upon sex, age, size, migration, starvation, feeding frequency, season and different stages in the reproductive maturation cycle, could lead to more accurate optimization of catch in commercial fisheries by concentrating on those specimens in which protein is at its highest level. Such analysis could assist in better management of capture fisheries. In addition, aquaculturists and genetics could benefit from an improved knowledge of protein and lipid dynamics in various tissues of the fish body during growth by aiming to maximise the growth of the more useful tissues. A knowledge of the proximate composition of fish is also of paramount importance while evaluating its nutritive value, particularly when the fish meat has to be processed and incorporated in various specialty products. The processor, the nutritionist, the cook and the consumer all have a direct interest in the composition of fish. The processor wants to know the nature of the raw material before he can apply correctly the techniques of chilling, freezing, smoking or canning; the nutritionist needs to know what contribution of fish can make up the diet and the health, while the cook must know whether the fish is lean or fatty in order to prepare it for table. The consumer is interested whether the fish tastes good and also whether it is nutritious with all necessary protein vitamins and minerals in desirable proportions.

Generally, fat and water constitute about 80% of the weight of fresh or `wet' fish muscle. Depending upon the fat content, the fish may be classified as fatty (fat content > 8%), semi fatty (fat content between 1% to 8%) and lean (fat content <1%). Apart from fat and water, the rest is protein, varying from 6 to 28%

The powerful and sophisticated experimental approaches of modern biochemistry have made a profound impact on nutrition, clinical, reproductive and genetic fields of aquaculture in the past three decades. We have examined the prospects and implications of this innovative technology for aquaculture in coldwater areas of the country and provided examples in specific aspects of current research in this field. Judicious application of new approaches in this area will not only provide answers to the various queries but will also help to preserve and potentially improve the rich aquatic hill resource in harmonious and beneficial ways.

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