Bionomics, Cryopreservation of Gametes and Captive Breeding Behaviour of Threatened Hill Stream Cyprinid, *Garra surendranathanii* (Shaji, Arun & Easa, 1996)

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By

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Certificate

This is to certify that the thesis entitled **"Bionomics, Cryopreservation of Gametes and Captive Breeding Behaviour of Threatened Hill Stream Cyprinid, *Garra surendranathanii* (Shaji, Arun & Easa, 1996)"**is an authentic record of research work carried out by Mr. Sunesh Thampy under my guidance and supervision in the School of Industrial Fisheries, Cochin University of Science and Technology in partial fulfillment of the requirements for the degree and no part thereof has been presented before for the award of any degree, diploma or associateship in any University

Kochi-16
July, 2009

Dr. A. Ramachandran

Declaration

I do hereby declared that the thesis entitled **"Bionomics, Cryopreservation of Gametes and Captive Breeding Behaviour of Threatened Hill Stream Cyprinid, *Garra surendranathanii* (Shaji, Arun & Easa, 1996)"** is a genuine record of research work done by me under the supervision of Dr. A. Ramachandran, Professor, School of Industrial Fisheries, Cochin University of Science and Technology, Kochi 682 016 and no part of this work has previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title or any university or institution.

Kochi-16
July, 2009

Sunesh Thampy

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..... dedicated to my loving family

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Chapter 1

General Introduction

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1.1 Introduction

Fresh water makes up only 0.01% of the total water body of the World and approximately 0.8% of the Earth's surface. Yet this tiny fraction of global water supports at least 1, 00,000 species out of approximately 1.8 million – which is almost 6% of all the described species (Dudgeon *et al.*, 2006). Inland water and freshwater biodiversity constitute a valuable natural resource, in terms of economic, cultural, aesthetic, scientific and educational means. However, freshwater ecosystems are considered as the most endangered ecosystems all over the world. Declines in biodiversity are far greater in fresh waters than in the most affected terrestrial ecosystems (Sala *et al.*, 2000). If trends in human demands for water remain unaltered and species losses continue at current rates, the opportunity to conserve much of the remaining biodiversity in fresh water will vanish within few years. Conservation of fresh water biodiversity is complicated by the landscape, geomorphology, position of rivers and effluent discharge. Protection of freshwater biodiversity is perhaps the ultimate conservation challenge because it is influenced by the upstream drainage network, the surrounding

land, the riparian zone etc. Such prerequisites are hardly ever met. Immediate action is needed where opportunities exist to set aside intact lake and river ecosystems within large protected areas. For most of the global land surface, trade-offs between conservation of freshwater biodiversity and human use of ecosystem goods and services are necessary because their conservation and management are critical to the interests of all humans, nations and governments (Dudgeon *et al.*, 2006).

Inland fisheries is the major component of freshwater biodiversity which have the potential to provide good quality protein and their products can benefit the people without the need for complex and expensive harvesting, processing, marketing and transportation infrastructures. Potential fish yields vary from system to system and are a function of interacting abiotic and biotic factors. These fishery resources, upon which people depend on, are renewable when managed scientifically, on the other hand, when abused, they are delicate and can become extinct. Unfortunately, the fishing sector seems to follow the latter path. Due to increased pressure from growing population and rapid modernization, ichthyobiodiversity is now getting depleted at an unprecedented rate. In order to prevent decline of biodiversity due to human intervention or otherwise, it is necessary to understand how the diversity of life is maintained under natural conditions. The assessment of biodiversity in an ecosystem by and large depends on making detailed inventories of species but this is a formidable task.

A very small number of countries located in the tropical belt contain a high percentage of biodiversity and high degree of endemism and these are considered as "Megadiversity" countries. A dozen of countries are identified as megadiversity countries and India is one among them (Mc Neely *et al.*,

1990). India occupies just 2.5 percent of the global geographic area but it supports over 7 percent of plant and 64 percent of animal population on a global basis (Padmakumar, 2007). It has two major biodiversity "hot spot" areas, *viz.*, Himalayas and Western Ghats (Meyers, 1990). Areas rich in endemism are concentrated in regions of North East India, Western Ghats and North Western and Eastern Himalayas. The Western Ghats is a mountain chain running north–south parallel to the western coast of India. It runs rather continuously between 8 and 21^0 N latitudes, covering a distance of *c.* 1600 km, being interrupted just once by a 30-km wide Palghat gap at around 11^0 N (Fig. 1.1). The narrow coastal strip that separates the hill chain from the Arabian Sea in the west varies in width from 30 to 60 km; the narrowest being between 14 and 15^0 N. Hills are generally of elevations between 600 and 1000 m. However, there are higher hills of 1000–2000 m between 8 and 13^0 N and 18–19^0 N. Peaks over 2000 m are found only in the Nilgiris, Palanis and Anamalais. The Nilgiris and Palanis are spurs from the main hill chain, which extend the Western Ghats eastwards to *c.* 78^0 E (Dahanukar *et al.*, 2004). With respect to freshwater species, the streams and rivers originating from Western Ghats have been identified as one of the few sites in the world exhibiting high degree of endemism and exceptional biodiversity and rightly recognized as a 'hotspot' area of biodiversity for conservation (Kottelat and Whitten, 1996). Whereas, in case of endemic fish taxa, Western Ghats is known as the richest region in India encompassing around 192 endemic species of the total 287 species of fishes reported from that region (Shaji *et al.*, 2000). These specialties have attracted the attention of ichthyologists all around world towards Western Ghats.

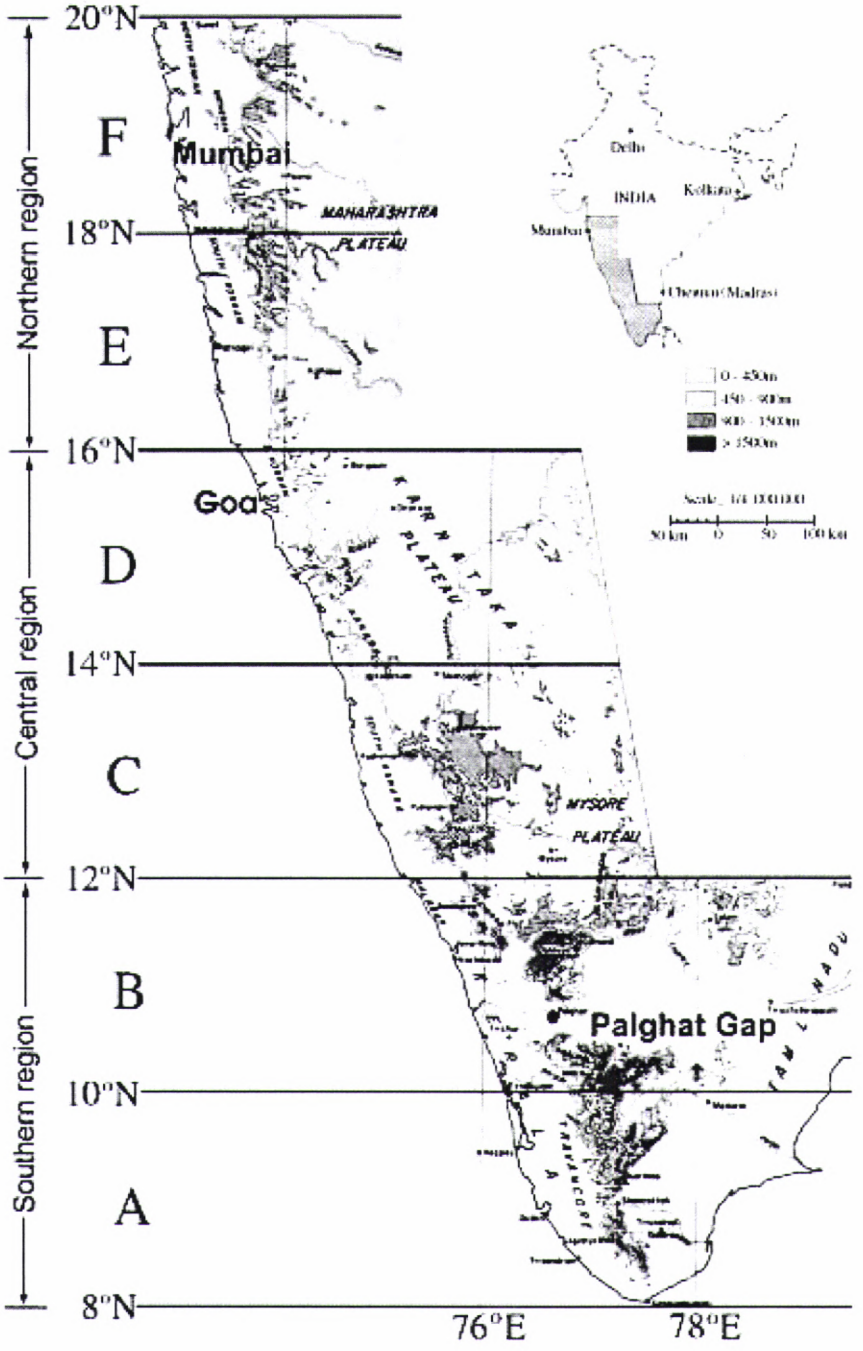

Fig. 1.1. Major physiographical units of the Western Ghats. Map adapted from Samant *et al*. (1996).

The most diverse and complex ecosystems, which are usually those with the richest faunas are likely to be those that are most sensitive to perturbation (May, 1975). The Western Ghats region, like other parts of the tropics, is undergoing rapid transformation. The damming of the rivers, hydroelectric projects, sand mining, deforestation, pollution are activities that dwindled the fish population. The destructive fishing methods such as mass poisoning, dynamiting, electric fishing etc. depleted the resource indiscriminately and introduction of exotic fishes in reservoirs which escape into rivers resulted in the replacement of ecological niches of native species from their habitat (Ramachandran, 2002). However, among the 734 species of threatened fishes listed in the IUCN Red Data Book from all over the world, only two species namely *Horaglanis krishnai* (Family: Claridae) and *Schistura sijuensis* (Family: Balitoridae) are included from India (IUCN, 1990). *Horaglanis krishnai*, a blind catfish, which is endemic to Kerala, is known only from the deep wells of its type-locality, Kottayam. None of the fish taxa from India is treated as being threatened in the Indian Red Data book prepared by the Zoological Survey of India, by and large, it pointed towards the lack of information on the conservation status of Indian freshwater fishes.

Later, National Bureau of Fish Genetic Resources (NBFGR) and Indian Council of Agricultural Research, Govt. of India in collaboration with the Zoo Outreach Organization (ZOO), Coimbatore, Tamil Nadu conducted Conservation Assessment and Management Plan (CAMP) workshop for freshwater fishes of India from 22nd to 26th September 1997 at Lucknow. The purpose of the workshop was to assess the conservation status of Indian freshwater fishes, according to the latest IUCN criteria, under the Biodiversity Conservation Prioritisation Project (BCPP). Out of 650 freshwater fish taxa reported from India, the CAMP workshop assessed the status of 327 fishes

including 92 species of the Western Ghats. Totally 227 taxa were included under threatened category which comprised of 47 critically endangered (CR), 98 endangered (EN) and 82 vulnerable (VU). The studies of Shaji *et al.* (2000); Gopi (2000); Ajithkumar *et al.* (2000); Kurup (2000); Ramachandran (2002); Dahanukar *et al.* (2004); Kurup *et al.* (2004); Radhakrishnan (2006); Kurup and Radhakrishnan (2006) and Mercy *et al.* (2007) clearly shows that many of these species, especially, many endemic fishes of Kerala are still under threat.

Endemism enhances the conservation value of a species (Molur and Walker, 1998). According to Musick (1999) species that are endemic or restricted in range to some relatively small, contiguous geographic entry (*i.e.*, island, archipelago, river system etc.) in which the habitat is or many be under threat of degradation or destruction should be classified at least as vulnerable. Maitland (1993) stated that in the case of fish, the actual numbers of individuals in the population are less important than the number of sites because fish are often so confined within their habitat that one incident (eg: a poisoning, land-slide) is likely to destroy every fish present. According to this author, it would be much safer to have 100 fish in each of 10 lakes is than 10,000 or more fish in one lake.

Conservation of these threatened stocks can be done either through *in-situ* or *ex-situ* methods. In the former the threatened fish species is conserved by protecting the ecosystem in which they occurs naturally or the habitat restoration is done. Such ecosystems are declared as national parks, biosphere reserves and sanctuaries. But, the constraints in *in-situ* conservation are the need for large investment of finance and trained manpower. Also, reorientation and modification of development project like river-valley projects will have to

be carried out (Padhi, 1987). In *ex-situ* conservation, the fish species is conserved outside its natural habitat. This includes (1) Live Gene Bank where the threatened species is reared in captivity and bred there-in and (2) Gamete/Embryo Bank where cryopreservation of milt, eggs and embryos is carried out (Pandey and Das, 2002).

1.2 Review of literature

"The fishes of Malabar" published by Day (1865) is perhaps the only book on the fishes of Kerala during the 19th century. Later in 20th century, a great deal of work had been done by many scientists and the faunal status of Kerala waters was strengthened during this period with the description of many new species. However, in the present study, literatures were scanned and special attention has been given to threatened freshwater fishes of Kerala, its biology, conservation and management programmes. Literature pertaining to biology, resource characteristics and conservation measures are given in the respective chapters of the thesis.

Reviewing the literature shows that the first account on the threatened fishes of Kerala river system is that of Kurup (1994), who listed 25 fish species as threatened from Kerala waters comprising of 6 endangered, 10 vulnerable and 9 rare and endemic species. Menon (1997) published a list of 18 fish species which were treated as rare and endangered fishes of Malabar, Kerala. CAMP report (Molur and Walker, 1998) was the major report which evaluated the conservation status of 327 species and in which 98 species belonged to Western Ghats and 92 to Kerala. Among the 92 species assessed from Kerala, 69 were categorized as threatened and in that 19 belonging to critically endangered, 29 endangered and 21 vulnerable species. CAMP report revealed that 35 species of the total 92 species evaluated from Kerala were endemic to

Kerala waters and among them, 32 were threatened. Of this 13 species belonged to the Critically Endangered category while an equal number of species were found in the Endangered category. The remaining 6 species were having vulnerable status. Recently there has been an upsurge in the publications on freshwater fish fauna of Kerala. However, majority of these works are either compilation of the past work by scanning the available literature or covers only highly restricted locations. A consolidated list of 106 species of economically important fishes endemic to Western Ghats with information on their distribution, maximum size attainable, etc. was prepared by Gopalakrishnan and Ponniah (2000). Shaji *et al.* (2000) catalogued 287 endemic, exotic transplanted and widely distributed fishes found in Western Ghats. During the period from 1993-1997, 165 freshwater fish species from Kerala together with their occurrence and relative abundance were reported by Gopi (2000) based on the faunistic survey programmes of Zoological Survey of India, Calicut, which also embodies the distribution and abundance of selected freshwater fishes seen very rarely in Kerala waters. Arunachalam *et al.* (2000) described the conservation status, habitat and ecology of 37 species of fishes, including 12 economically important cultivable species and 13 important ornamental fishes, from rivers of Waynad.

Ajithkumar *et al.* (2000) documented 83 fish species from Chalakudy River and listed various threats faced by them. Mini (2000) discussed the conservational aspects of fish fauna of Periyar River emphasizing the importance of banning over fishing, dynamiting, and eradication of introduced species and prohibition of fishing during closed season. Gopalakrishnan and Basheer (2000) cautioned about the threats from gradual establishment of Indian major carps in river systems of Kerala. Biju *et al.* (2000) surveyed 39 river systems of both northern and southern Kerala and studied both species

diversity as well as abundance. The authors reported the presence of seven critically endangered 28 endangered and 28 vulnerable species in that study.

Kurup (2000) proposed few management strategies such as strengthening database on population size and distribution, generating precise information on migration, breeding season, behaviour and spawning grounds, developing captive breeding techniques etc. to alleviate the declining freshwater fish diversity of Kerala. Kurup (2002) enlisted 170 freshwater fishes from Kerala and evaluated their biodiversity status as per IUCN red data list categories. Of the 170 species reported, 52 species were listed under threatened category and among them, 18 species belonged to the category of Critically Endangered fishes and 34 to Endangered while 31 were vulnerable in their status. The study listed out the various factors which aggravated the degree of threat and suggested relevant conservation and management measures required for the maintenance of the freshwater fish biodiversity of Kerala.

Ramachandran (2002) in his study observed considerable ambiguity exists among scientific community regarding conservation status of many fish species of Kerala and highlighted the importance of statistically significant exploratory studies for confirming the conservation status of such species. Further to this he stressed the importance of development of captive breeding techniques especially in the case of endangered species to meet demand in ornamental fish industry so that the fishing pressure on natural stock can be reduced considerably. Kurup and Radhakrishnan (2006) has surveyed 25 river systems of Kerala for a period of four years (2001-2004) and 144 fish species were collected and identified including 8 new species. The biodiversity status of the fishes was assessed based on the IUCN criteria and showed that 8 critically endangered, 36 endangered and 15 vulnerable. Of this 78 species were endemic

to Western Ghats while 21 species were found strictly endemic to Kerala. In this *Puntius denisonii, Nemecheilus keralensis, Osteobrama bakeri, Chela dadiburjori, Gonoproktopterus micropogon periyarensis, Silurus wynaadensis, Neolissochilus wynaadensis, Puntius ophicephalus, Garra surendranathanii* and *Garra Menoni* are showing high degree of endemism. Distribution of these species was found to be varying within a river system and also between the river systems. More over many of these fishes are characterized by highly restricted geographical distribution pattern. They also suggested the replenishment of endemic populations through development of captive breeding and massive seed production technologies.

1.3 Objectives of the Present Study

The literature review revealed that, many fishes which are endemic to Kerala are under severe threat. Immediate efforts should be taken to protect and conserve these threatened species, as per the order of priority. According to Molur and Walker (1998) endemic species deserves priority over the other fish species.

Garra surendranathanii is a hill stream cyprinid endemic to Kerala. According to IUCN based classification, *G. surendranathanii* is grouped under the threatened category. This endemic fish is having highly restricted and fragmented distribution and reported only from 5 river systems *viz.* Chalakudy, Periyar, Pamba, Achenkoil and Bharathapuzha. Categorization of this fish as a potential ornamental candidate can invariably add more pressure on the threat status of this particular species. Hence, this species is considered as one which requires foremost attention for conservation. Hitherto, no information is available on the bionomics, resource characteristics and any conservation attempts of *G. surendranathanii*. Studies on detailed life history traits and

development of captive breeding technique are indispensable for successful fishery management.

The present study was undertaken with the following objectives:

To study the Length-weight relationship and condition factor to ascertain the relationship between length and weight and general wellbeing of the fish

To study the age and growth to understand the age composition of the exploited stock, age at first maturation and life span of the species.

To study the reproductive biology of *G. surendranathanii* to gain insights in the process of gametogenesis, spawning, sex ratio, fecundity and other related aspects which are essential for developing captive breeding technology of this species.

To develop captive breeding technology and cryopreservation of gametes of *G. surendranathanii* for conservation

1.4 General organization of the thesis

The thesis is organized under eight chapters. In the **first chapter**, the General Introduction, works done on the threatened freshwater fishes of Kerala have been reviewed and the importance of the present study is emphasized. The objectives of the present study are highlighted and the general organization of the thesis is described. The **second chapter** deals with the salient features of *G. surendranathanii* together with its systematic position. The earlier reports of this species from Kerala are documented along with its distribution and IUCN status.

The **third chapter** brings out the relationship between total length and body weight in both the sexes. The results of age and growth studies of the populations

are also given in this chapter. The **fourth chapter** deals with reproductive biology of the species. The processes of spermatogenesis and oogenesis of the fish species are illustrated with the help of the histological studies of testis and ovary respectively at different stages of maturity. Maturity stages of males and females, monthly percentage occurrence of fish with gonads in different stages of maturity, pattern of progression of ova during different months, Gonado-Somatic Index, length at first maturity, sex ratio, fecundity and its relationship to various body parameters are discussed in this chapter.

Experiments on captive breeding and the effect of different hormones and their doses on the breeding performance of the fish are discussed in **chapter five**. The breeding behaviour of the fish is also addressed in this chapter. The developmental biology of the species is explained in **chapter six**.

Experiments on milt characteristics, suitability of different extenders, cryopreservation and post thaw motility, cryoprotectant toxicity and fertility trials are given in **chapter Seven**. Summary and recommendations are embodied in **Chapter eight**. The salient findings of the present study are consolidated under summary. Based on results of the present study, a few management measures relevant for the conservation of threatened and endemic fish germplasm of the rivers of Kerala are also proposed.

In general, each chapter is subdivided into brief introduction, materials and methods, results and discussion. Tables, graphs and photographs are inserted at appropriate places. The list of references consulted is appended at the end of the thesis.

Chapter 2

Systematics of *Garra surendranathanii*

Contents

2.1 Introduction

The diverse inland water bodies of Kerala, occupying an area of 3,55,037 hectares are represented by 44 rivers, 30 brackish water estuaries, 25 reservoirs, several fresh water lakes and innumerable ponds constituting 5% of India's total freshwater wealth (Ramachandran, 2001). From the 44 river systems, Kurup *et al.* (2004) described 175 species and grouped them under 106 ornamental and 67 food fishes. In this, the largest family is Cyprinidae.

The genus *Garra* (Hamilton) is represented by 24 species in Indian subcontinent (Jayaram, 1999) and among them, 19 species are distributed in India including the new species reported in the past two decades. This genus was represented by 7 species in Kerala until the description of four new species viz. *G. emarginata, G. mlapparaensis, G. travancoria* from Periyar River and *G. nilamburensis* from Chaliyar River recently. *G. cylonensis*, a Srilankan species under the genus *Garra* from Periyar River was also newly reported. Thus, the total species known from Kerala is 12 (Kurup and Radhakrishnan, 2006).

The species selected for the study, *Garra surendranathanii* is an endemic fish of Kerala. It is locally used as food fish and has been prioritized recently as a

candidate indigenous ornamental fish (Ponniah and Sarkar, 2000; Kurup and Radhakrishnan, 2006; Mercy *et al.*, 2007). This fish is categorized as threatened as per IUCN criteria. Several ichthyologists classified *G. surendranathanii* as endangered (EN) (CAMP, 1997; Shaji *et al.*, 2000; Gopi, 2000; Kurup, 2000; Ajithkumar *et al.*, 2000; Dahanukar *et al.*, 2004; Kurup *et al.*, 2004; Radhakrishnan and Kurup, 2006; Mercy *et al.*, 2007; Raghavan *et al.*, 2008) and a few described under vulnerable (VU) category (Radhakrishnan, 2006; Kurup and Radhakrishnan, 2006), which is depicted in Table. 2.1.

2.2 Description of the species

Garra suredranathani, an endemic hill stream cyprinid of Kerala, is coming under the stone sucker group (*Garra*), which are mostly found at the middle and upper stretches of the river systems of Western Ghats. This fish is commonly known as Periyar Garra Nilgiri Garra and locally known as 'Kallemutty' or 'Kallotty'

***Garra surendranathanii* (Shaji, Arun & Easa, 1996)**

Systematic Position

Phylum	**Chordata**	**Order**	**: Cypriniformes**
Sub-Phylum	**Vertebrata**	**Family**	**: Cyprinidae**
Super Class	**Gnathostomata**	**Sub Family**	**: Garrinae**
Class	**Actinopterygii**	**Genus**	**: *Garra***
Sub Class	**Neopterygii**	**Species**	**: *surendranathanii***
Division	**Teleostei**		

This species exhibits the following diagnostic characteristics.

D ii 8; P i 12; V i 7; A i 5; C.18; Ll. 36-37, Ltr 4.5/3

Body is elongated. Head broad with patches of black dots. Snout elongated without transverse groove but a weakly developed protuberance as in adult specimens and with spinate tubercles which is the distinguishing character of this species. A small sucking disc is present on the ventral side. Two pairs of barbels are present. Dorsal fin is close to snout than caudal. Caudal forked. Body uniformly scaled. Body is golden brown during juvenile stage and it transforms to brownish-black. Flanks are greenish brown. Scales have black edges which appear as interrupted bands or sometimes patches of spots. But these bands are prominent during the juvenile stage and not prominent/absent in adults. Fins are purple in colour at their bases with tips marked orange.

This fish is very attractive with its bands during the juvenile stage and even the adults are useful in aquariums because they graze on the algae attached to the bottom substratum or glasses of the aquarium tanks. The slow movements arouse curiosity and these reasons make it a candidate species to promote as ornamental fish.

Geographical Distribution: India: Western Ghats of Kerala (Jayaram, 1999). *G. surendranathanii's* distribution in Chalakudy, Periyar and Pamba rivers is reported by Shaji *et al.* (1996); Gopi (2000); Ajithkumar *et al.* (2000) and Kurup *et al.* (2004). Radhakrishnan (2006) reported this fish in Achenkoil and Bharathapuzha rivers as well.

Habitat: This species is found in Cascades, rapids and riffles with bedrock, cobbles and gravels as substratum.

2.3 Earlier reports

Shaji *et al.* (1996) described *Garra surendranathanii* from the Southern Western Ghats and the later reports are given below:

Table. 2.1 The previous reports / citation of *G. surendranathanii* and its distribution and threat status

S.No	Report	Distribution	IUCN Status
1	Shaji, C.P., Arun, L.K. and Easa, P.S., 1996. *Garra Surendranathanii* – A new cyprinid from the Southern Western Ghats, India. *J. Bombay Nat. Hist. Soc*, 93: 572-575	Chalakkudy, Periyar & Pamba	
2	CAMP (Conservation Assessment & Management Plan) workshop.1997		EN
3	Jayaram, 1999. The freshwater fishes of the Indian region. Narendra publishing house, New Delhi. xxvii + 509 pages		
4	Biju, C.R., Thomas Raju, K. and Ajithkumar, C.R., 1999. Fishes of Parambikulam Wildlife Sanctuary, Palakad district, Kerala. *J. Bombay Nat. Hist. Soc*, 96(1): 82-87		
5	Gopalakrishnan, A. and Ponniah, A.G., 2000. Cultivable, ornamental, sport and food fishes endemic to peninsular India with special reference to Western Ghats.		

	Endemic fish diversity of Western Ghats,NBFGR-NATP publication No.1,Lucknow.p. 13 – 32		
6	Shaji, C.P., Easa, P.S. and Gopalakrishnan, A., 2000. Freshwater fish diversity of Western Ghats. *In:* Endemic fish diversity of Western Ghats, NBFGR-NATP publication No.1,Lucknow.p.33-55		EN
7	Gopi, K.C., 2000. Freshwater fishes of Kerala State. *In:* Endemic fish diversity of Western Ghats, NBFGR-NATP publication No.1, Lucknow.p.56-76	Chalakkudy, Periyar & Pamba	EN
8	Ajithkumar, C.R., Sunny George and Nayar, C.K.G., 2000. Fish genetic resources of Chalakudy River. *In:* Endemic fish diversity of Western Ghats, NBFGR-NATP publication No.1, Lucknow.p.157-159	Periyar	
9	Kurup, B.M., 2000. Management plans to arrest the decline of freshwater fish diversity of Kerela. *In:* Endemic fish diversity of Western Ghats, NBFGR-NATP publication No.1, Lucknow.p.164-166	Central & Northern rivers of Kerala	EN
10	Ajithkumar, C.R., Biju, C.R. and Thomas, K.R., 2000. Ecology of hill streams of Western Ghats with special reference to	Chalakkudy, Periyar & Pamba	EN

	fish community, Final report 1996-1999, Project report submitted to Bombay Nat. Hist. Soc. Mumbai. pp.203		
11	Easa, P.S. and Shaji, C.P., 2003. KFRI hand book No.17, Biodiversity documentation for Kerala, Part 8: Freshwater Fishes 127 pages		
12	Dahanukar, N., Raut, R. and Bhat, A., 2004. Distribution, endemism and threat status of freshwater fishes in the Western Ghats of India. *J. Biogeogr.* 31: 123-136		EN
13	Kurup, B.M., Radhakrishnan, K.V. and Manojkumar, T.G., 2004. Biodiversity status of fishes inhabiting rivers of Kerala (S.India) with special reference to endemism, threats and conservation measures. *In*: Proc. Second International Symposia on larger rivers, Cambodia. FAO Feb 2004	Periyar, Chalakudy	EN
14	Radhakrishnan, K.V., 2006. Systematics, Germplasm evaluation and pattern of distribution and abundance of freshwater fishes of Kerala (India). PhD thesis submitted to Cochin University of Science and Technology.	Periyar, Pamba, Chalakudy, Achenkoil, Bharathapuzha	VU
15	Kurup, B.M. and Radhakrishnan, K.V., 2006. Status of freshwater germplasm		VU

	resourses of Kerala, India. *In:* Sustain Fish. Kurup, B.M. & Ravindran, K. (Eds.), School of Industrial Fisheries, CUSAT, Cochin, India		
16	Radhakrishnan, K.V. and Kurup, B.M., 2006. Distribution and stock size of freshwater ornamental fishes of Kerala (S. India) with special reference to sustainability issues. *In:* Sustain Fish Kurup, B.M. & Ravindran, K. (Eds.), School of Industrial Fisheries, CUSAT, Cochin, India	Chalakudy, Bharathapuzha	EN
17	Mercy, A.T.V., Gopalakrishnan, A., Kapoor, D. and Lakra, W.S., 2007. *In*: Ornamental Fishes of the Western Ghats of India. Published by: National Bureau of Fish Genetic Resources, Lucknow, India. pp. 235.		EN
18	Raghavan, R., Prasad, G., Anvar Ali, P.H. & Pereira, B., 2008. Fish fauna of Chalakudy River, part of Western Ghats biodiversity hotspot, Kerala, India: patterns of distribution, threats and conservation needs. *Biodivers Conserv.* 17: 3119–3131		EN

EN-Endangered, VU-Vulnerable

Ever since the description of *G. surendranathanii* in 1996 by Shaji *et al.*, virtually nothing has been added to our knowledge on this species except the new report sighting the fish from two more rivers (Radhakrishanan, 2006). But, even then, the status of this species which is having immense ornamental potential is remained as threatened and there was an urgent need to initiate the conservation of this species. The present study was undertaken to address the issues pertain to life history traits and possible conservation measures of this valuable species.

Chapter 3

Population Characteristics and Stock Assessment

Contents

3.1 Introduction

In the studies of biological profile of a species, age and growth has an important role. Knowledge of these parameters is essential to understand the dynamic features of the population and forms the basic key to determine the quantity of fish that could be produced in a population against time. Once the addition (weight) in a fish stock in relation to time is determined, the optimum size of age can be fixed for rational exploitation of a fishery. Further the loss in a given fish stock due to natural and fishing mortality is to be estimated for arriving at maximum sustainable yield and biomass estimation. Thus, the knowledge on the age, structure and growth rate is essential pre-requisite for successful fishery management.

Age and growth studies are important for population dynamics research, fishery forecasts, fish culture in natural habitats, acclimatization, racial studies and rational commercial exploitation. Most of the methods employed for assessing the state of exploited fish stocks rely on the availability of age

composition data (Ricker, 1975). Information on growth rate, natural and fishing mortality, age at maturity and spawning, age composition of the exploited population, etc. can be evolved from age data of fish populations. Such information provide essential tools for scientific interpretation of the fluctuations in fish populations over space and time and also in formulating scientific and economic management policies for the fisheries in question (Seshappa, 1999).

The growth process is species specific; however, it can differ in the same fish inhabiting different geographical locations and is easily influenced by several biotic and abiotic factors. Growth is an adaptive property, ensured by the unity of the species and its environment (Nikolsky, 1963). A comparison of rate of growth from different localities may help in identifying suitable environmental conditions for the sustenance of a stock. The purpose of growth studies in any fish species is to determine the amount of fish that can be produced with respect to time (Qasim, 1973).

The age and growth rate of fishes are determined by both direct and indirect methods. The direct methods include rearing fishes in captivity under controlled conditions and observing their growth and also by using mark recapture method (tagging programmes). Dissection of annual rings lay down on scales, otoliths and other hard parts of the body and length frequency analysis are the indirect methods mostly relied upon. As the direct methods have limited scope due to practical difficulties, biologists prefer the indirect methods for age and growth studies. The annular rings on scales and other hard parts of the body are effectively used in temperate regions where, during winter seasons, slow growth leaves clear rings of closely placed circuli. On the other

hand, in tropics, the age determination based on direct counting of check marks is difficult because the growth rings do not necessarily represent year marks.

While studying the age and growth of a species, studies on length-weight relationship are very important in assessing whether the fish maintains its dimensional equality during its growth phase. According to Haniffa *et al.* (2006), for successful development, management, production and ultimate conservation, it is essential to understand the relationship between length and weight of a species in a natural environment. Knowledge of length – weight relationship is of paramount importance in fishery biology as it serves several practical purposes. The general length-weight relation equation provides a mathematical relationship between the two variables, length and weight, so that the unknown variable can be easily calculated from the known variable. This expression had been extensively used in the study of fish population dynamics for estimating the unknown weights from known lengths in yield assessments (Pauly, 1993), in setting up yield equation for estimating population strength (Beverton and Holt, 1957; Ricker, 1958), in estimating the number of fish landed and in comparing the populations over space and time (Sekharan, 1968; Chanchal *et al.*, 1978). The mathematical relationship between length and weight of fishes is a practical index suitable for understanding their survival, growth, maturity, reproduction, and general well being (Le Cren, 1951) and therefore, is useful for the comparison of body forms of different groups of fishes. The length –weight relationship also has a biological basis as it depicts the pattern of growth of fishes. According to the general cube law governing length-weight relationship, the weight of the fish would vary as the cube of length. However, all fish species do not strictly obey the cube law and deviations from the law are measured by condition factor (Ponderal index or K factor). Le Cren (1951) proposed relative condition factor (Kn) in preference to

K as the former considers all the variations like those associated with food and feeding , sexual maturity, etc., while the latter does so only if the exponent value is equal to 3. Thus 'K' factor measures the variations from an ideal fish, which holds the cube law while Kn measures the individual deviations from the expected weight derived from the length- weight relationship.

The length frequency analysis method of Petersen (1895, 1903) is well known, in which, peaks of length distribution are assumed to represent the different age groups. Length-frequency method is widely used by fishery biologists in fishes inhabiting tropical waters. A computer based method for the analysis of length frequency data, ELEFAN (Electronic Length Frequency Analysis) (Gayanilo *et al.*, 1988), has been effectively used to separate the composite length frequency into peaks and troughs and the best growth curve passing through maximum number of peaks is selected using a goodness of fit ratio of ESP (Explained sum of peaks)/ASP(Accumulated sum of peaks)(Rn) (Pauly and David, 1981; Gayalino *et al.*, 1988). The peaks are believed to represent individual cohorts. The module is incorporated into the FiSAT (FAO-ICLARM Fish stock assessment tools) Software (Gayanilo and Pauly, 1997).

The age and growth of freshwater fishes of India were studied by several scientists (Jhingran, 1959; Qasim and Bhatt, 1964; Bhatt, 1969; Kamal, 1969; Khan and Siddiqui, 1973; Murty, 1976; Chatterji *et al.* 1979; Pathani, 1981; Reddy, 1981; Mathew and Zacharia, 1982; Tandon and Johal, 1983; Shree Prakash and Gupta, 1986; Desai and Shrivastava, 1990; Devi *et al.*, 1990; Johal and Tandon, 1992). Qasim (1973) made a critical evaluation on the various methods used for age and growth studies in India and described the difficulties encountered in determining the age in tropical fishes. Some of the recent works on age and growth include those of Kurup (1997) in *Labeo dussumieri*, (Singh *et al.*, 1998) in *L.*

rohita, (Kamal *et al.*, 2002) in *L. calbasu*, (Narayani and Tamot, 2002) in *Tor tor* and (Nautiyal, 2002) and Nautiyal *et al.* (2008) in *Tor Putitora*.

The length- weight relationship of cyprinids from India has also been subjected to detailed studies, notably by Jhingran (1952); Bhatnagar (1963); Natrajan and Jhingran (1963); Sinha (1972); Pathak (1975); Chatterji (1980); Chatterji *et al.* (1980); Vinci and Sugunan (1981); Sivakami (1982); Choudhary *et al.* (1982); Malhotra (1982, 1985); Mohan and Sankaran (1988); Kurup (1990); Reddy and Rao (1992); Biswas (1993); Pandey and Sharma (1998); Sarkar *et al.* (1999); Sunil(2000); Mercy *et al.* (2002); Kumar *et al.* (2006) and Prasad and Anwar Ali (2007).

Garra surendranathanii is an endemic threatened fish of Kerala and no attempt was made to study the age and growth or length-weight relationship of this species. Hence a pioneer study is attempted in this direction.

3.2 Materials and methods

3.2.1 Length-weight relationship

268 specimens of *G. surendranathanii* comprising of 164 males and 56 females and 48 indeterminate collected from Periyar river system were used for the present study. After blotting the specimens to remove excess water, total length to the nearest millimeter and weight to the nearest 0.01 gram were recorded. Total length was measured from the tip of the snout to tip of the longest ray in the caudal fin (Jayaram, 1999). Total length of male and female varied between 75 to 142 mm and 90 to 209 mm respectively whereas weights of males and females ranged from 3.33 to 26.73 g and, 6.36 to 87.45g respectively. The data so generated was used for fitting length-weight relationship following Le Cren, 1951.

$$w = a\, l^b$$

The logarithamatic transformation of which gives the linear equation:

$$\log w = a + b \log l$$

where w = weight in gram, l = length in mm, a= a constant being the initial growth index and b= growth coefficient. Constant 'a' represents the point at which the regression line intercepts the y-axis and 'b' the slope of the regression line.

3.2.2 Age, growth and population dynamics

A total of 268 specimens of *G. surendranathanii* comprising of 164 males and 56 females and 48 indeterminate collected from Periyar river system were used for the present study. All specimens were measured to the nearest mm in total length (TL). Length frequency data in respect of males and females were grouped into 10 mm class interval. Growth was estimated separately for males, females and the pooled population. The Von Bertalanffy growth formula (VBGF) (Bertalanffy, 1938) was used to describe the growth. The equation in growth in length is given by:

$$L_t = L_\infty [1 - \exp^{-K(t-t_0)}]$$

Where L_t = length at age t.

L_∞ = asymptotic length or the maximum attainable length if the organism is allowed to grow.

K = growth coefficient

t_0 = age at which length equals 0, i.e. the theoretical age at zero length

The growth parameters for both the sexes were estimated separately using the ELEFAN 1 programme of FiSAT software (Gayanilo and Pauly, 1997). Age length key was prepared from ELEFAN I. The estimate of t_0 was

made using von Bertalanffy plot (1934). Based on the growth parameters arrived at instantaneous rate of total mortality (Z), natural mortality coefficient, probabilities of capture, relative yield per recruit (Y/R) were worked out using FiSAT software (Gayanilo and Pauly, 1997). The recruitment pattern of pooled population of G. surendranathanii was obtained from FiSAT programme. The exploitation rate (Beverton and Holt, 1957) and exploitation ratio (Sparre and Venema, 1992) were also worked out. Growth performances of both male and female populations were compared by Munro's PHI prime index, ϕ (Munro and Pauly, 1983) which was computed from the equation:

$$\phi = \log_{10} K + 2 \log_{10} L\alpha$$

where K and $L\alpha$ are Von Bertalanffy's growth parameters.

3.3 Results

3.3.1 Length-weight relationship of *G. surendranathanii*

Length – weight relationship of males, females and pooled population of *G. surendranathanii* can be expressed as follows:

Males $\quad \log W = -5.06486 + 3.004 \log l, \; r = 0.99$

Females $\quad \log W = -5.23378 + 3.087 \log l, \; r = 0.98$

Pooled $\quad \log W = -5.1825 + 3.059 \log l, \; r = 0.99$

The logarithmic relationship between length and weight of males, females and pooled population of *G. surendranathanii* together with correlation coefficient is depicted in Figs: 3.1, 3.2 and 3.3 respectively. The correlation coefficient 'r' between log length and log weight is given below.

	n	a	b	r
Male	164	**-5.06486**	**3.004**	**0.99**
Female	56	**-5.23378**	**3. 087**	**0.98**
Pooled	268	**-5.1825**	**3.059**	**0.99**

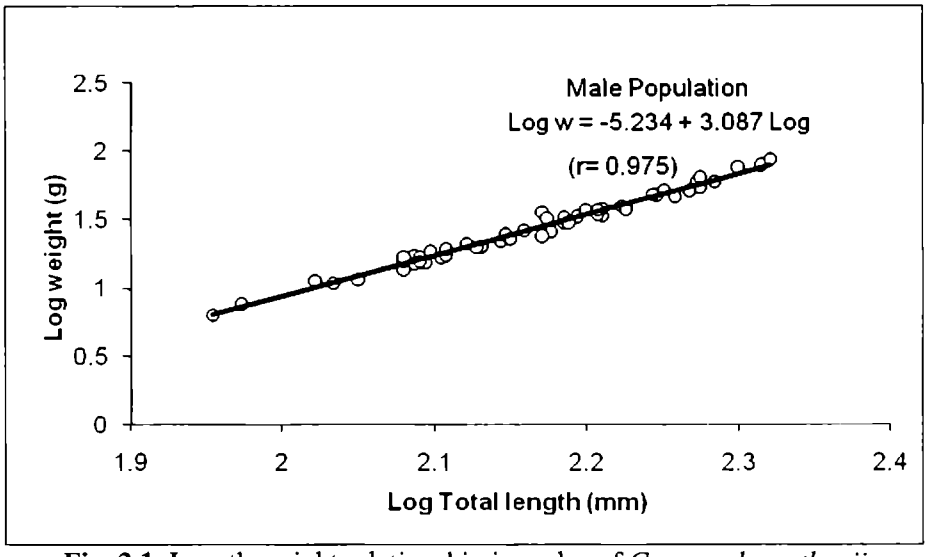

Fig. 3.1 Length weight relationship in males of *G. surendranathanii*

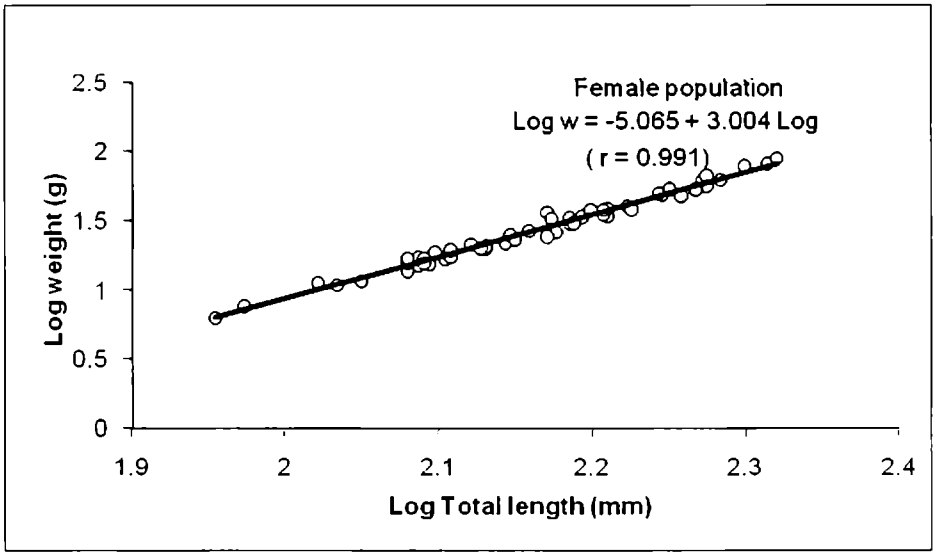

Fig.3.2 Length weight relationship in females of *G. surendranathanii*

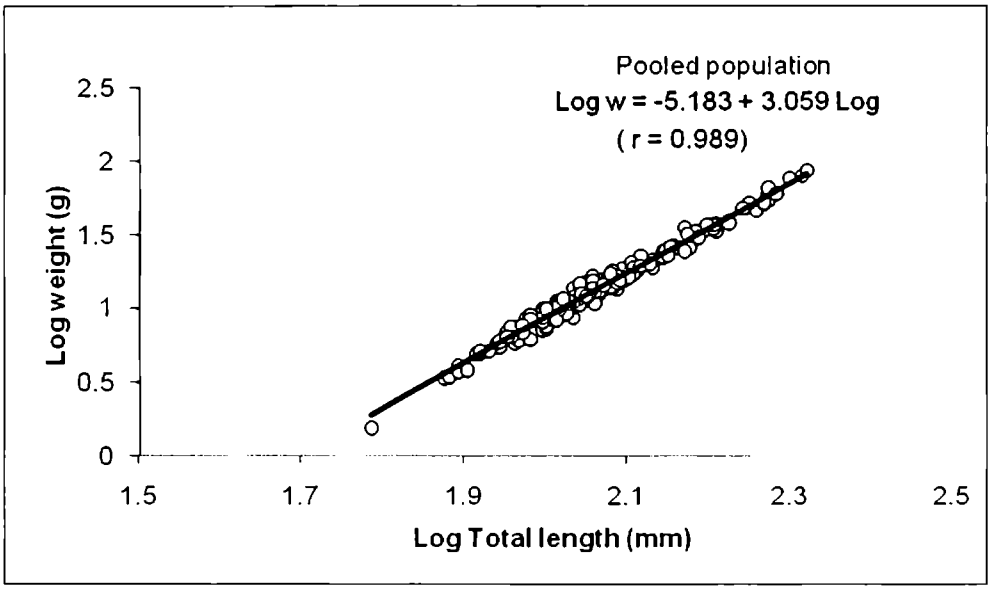

Fig.3.3 Length weight relationship in pooled population of *G. surendranathanii*

3.3.2 Age and growth of *G. surendranathanii* in River Periyar

The exploited population of *G. surendranathanii* in River Periyar during the period 2004 January to December was constituted by individuals ranging from 75 to 209 mm. The highest length class recorded among males was 141-150 mm while the same in female population was 201-210 mm. The fishery was predominated by individuals in the size range 111-120 mm among males while fishes of size ranges 121-130 mm formed the dominating size groups among females.

3.3.2.1 Age and growth of male population of *G. surendranathanii*

The growth parameters estimated in the male population of *Garra surendranathanii* using ELEFAN I programme are given in Table.3.1. The FISAT output of restructured length frequency data of male population of *Garra surendranathanii* in river with superimposed growth curve fitted with highest levels of Rn is given in Fig.3.4. The VBGF in terms of male, arrived at

based on the growth parameters worked out using ELEFAN (Gayanilo *et al.*, 1996) and Bertlanffy plot (1934) can be expressed as follows.

$$L_t = 154\ [\ 1 - \exp^{-0.59(\ t\ +0.2883)}]$$

The lengths attained by male *G. surendranathanii* following VBGF equation at the end of I, II, III, IV and V years were estimated to be 82mm, 114mm. 132mm, 142mm and 147mm respectively. The growth performance index (ϕ) in respect of males was worked out as 4.15

Table. 3.1 The growth parameters and growth performance index worked out in male, female and pooled population of *G. surendranathanii* in River Periyar using ELEFAN I programme

	L ∞	**K**	**Rn**	**ϕ**
Males	154	0.59	428	4.15
Females	220	0.60	442	4.46
Pooled	222	0.61	322	4.48

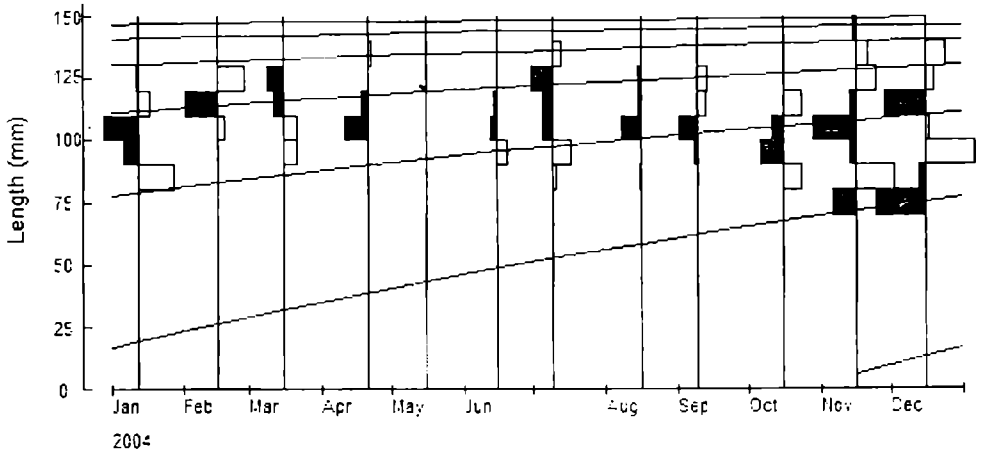

Fig.3.4 Growth curve of male *G. surendranathanii* as estimated using ELEFAN 1 programme

3.3.2.2 Age and growth of female population of *G. surendranathanii*

The growth parameters estimated in the female population of *Garra surendranathanii* using ELEFAN I programme given in Table.3.1. The FISAT output of restructured length frequency data of female population of *G. surendranathanii* in River Periyar with superimposed growth curve fitted with highest levels of Rn is given in Fig.3.5. The VBGF in terms of female arrived at based on the growth parameters can be expressed as follows.

$$L_t = 220\ [\ 1 - \exp^{-0.60(\ t\ +0.3659)}]$$

The lengths attained by female following VBGF equation at the end of I, II, III, IV and V years were estimated to be 123mm, 167mm, 191mm, 204mmand 211mm, respectively. The growth performance index (ϕ) in respect of females was worked out as 4.46.

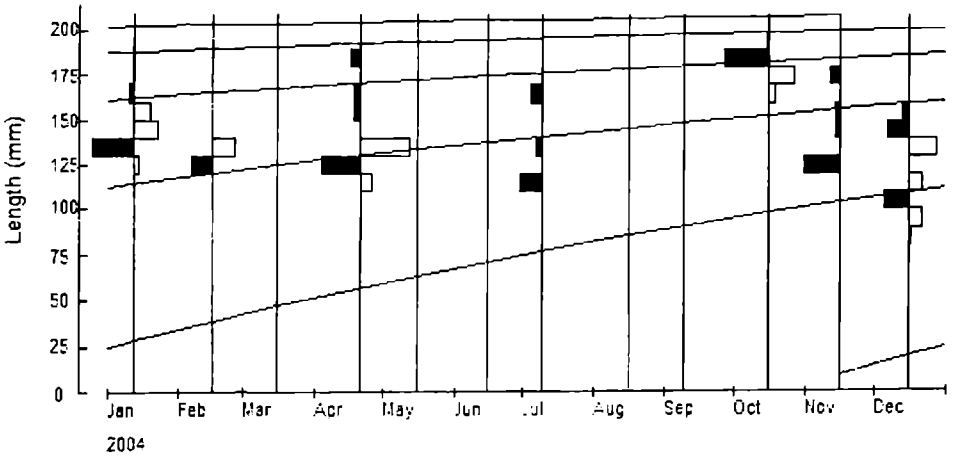

Fig.3.5. Growth curve of female *G. surendranathanii* as estimated using ELEFAN 1 programme

3.3.2.3 Age and growth of pooled population of *G. surendranathanii*

The growth parameters estimated in pooled population of *G. surendranathanii* using ELEFAN I programme are given in Table.3.1. The FISAT output of restructured length frequency data of pooled population of *G. surendranathanii* in river Periyar with superimposed growth curve fitted with highest levels of Rn is given in Fig.3.6. The VBGF in terms of pooled population, arrived at based on the growth can be expressed as follows.

$$L_t = 222[\ 1 - \exp^{-0.61(t+0.3681)}]$$

The lengths attained by pooled *Garra surendranathanii* following VBGF equation at the end of I, II, III, IV and V years were estimated to be 126mm, 170mm, 194mm, 207mm and 214mm respectively. The growth performance index (ϕ) in respect of pooled population was worked out as 4.48.

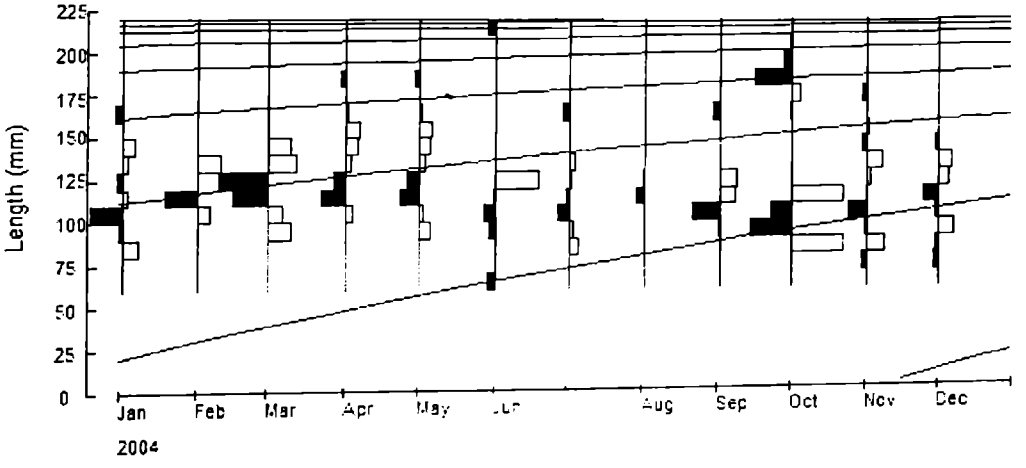

Fig.3.6. Growth curve of pooled *G. surendranathanii* as estimated using ELEFAN 1 programme

3.3.3 Mortality estimates and exploitation of *G. surendranathanii*

The FISAT output of mortality estimates of pooled population of *G. surendranathanii* in river Periyar by catch curve method is depicted in Fig.3.7. The total mortality (Z) was estimated to be 2.85. The estimates of natural mortality (M) were recorded as 0.63. The values of fishing mortality coefficient (F) and Exploitation rate (E) were worked out as 2.22 and 0.78 respectively (Fig.4.). The optimum length (l- opt) was worked out to be 165mm.

The estimates of probabilities of capture and lc values were worked out using the length converted catch curve method. The values obtained by probabilities of capture were L 25 = 91.78mm, L50= 99.51mm and L 75= 107.23mm (Fig.3.8). These values were used as inputs for relative Y/R of Beverton and Holt (Y'/R). The Lc/Lα and M/K values used for Y'/R analysis were 0.448and 1.0328 respectively. The relative yield per recruit and biomass per recruit in *G. surendranathanii* is depicted in Fig.3.9. The relative yield per recruit reached a maximum at an exploitation rate of 0.621 and with an increase in the exploitation rate, Y'/R decreased. It may be noted that the present exploitation rate E (0.78) exceeds the optimum exploitation rate Emax= 0.621. The values of E0.1 and E0.5 were estimated as 0.511 and 0.351 respectively. The results of length based virtual population analysis showed that F increases to a maximum of 2.41 at 120-130mm size (Fig.3.10). The catch increases substantially from 80-90mm size groups and attains maximum at 100-120mm size groups.

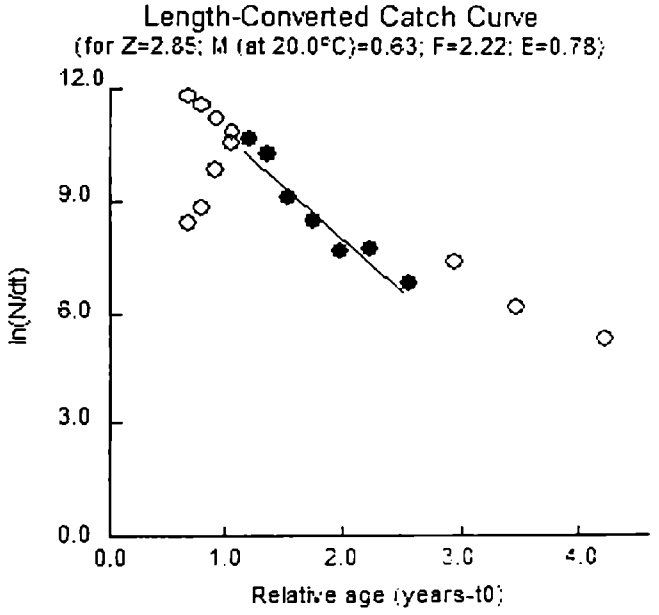

Fig.3.7. Mortality estimates of pooled population of *G. surendranathanii*

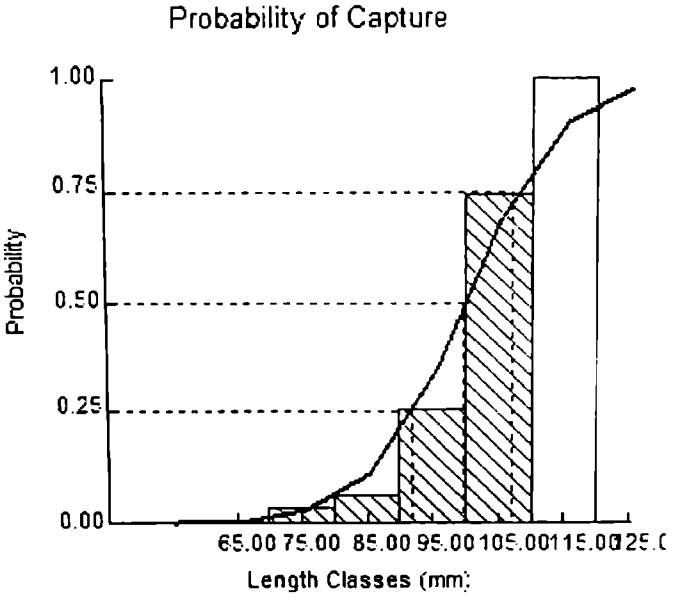

Fig: 3.8. Probability of capture of pooled population of *G. surendranathanii*

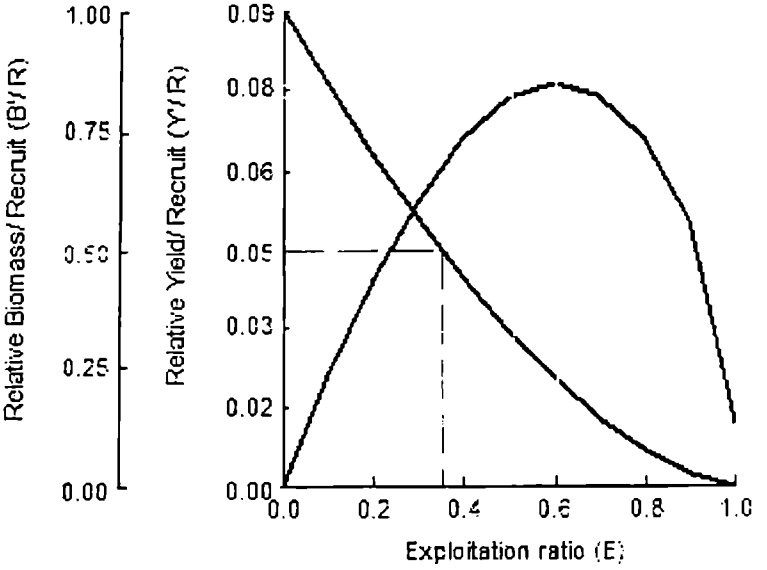

Fig.3.9. The relative yield per recruit and biomass per recruit in *G. surendranathanii*

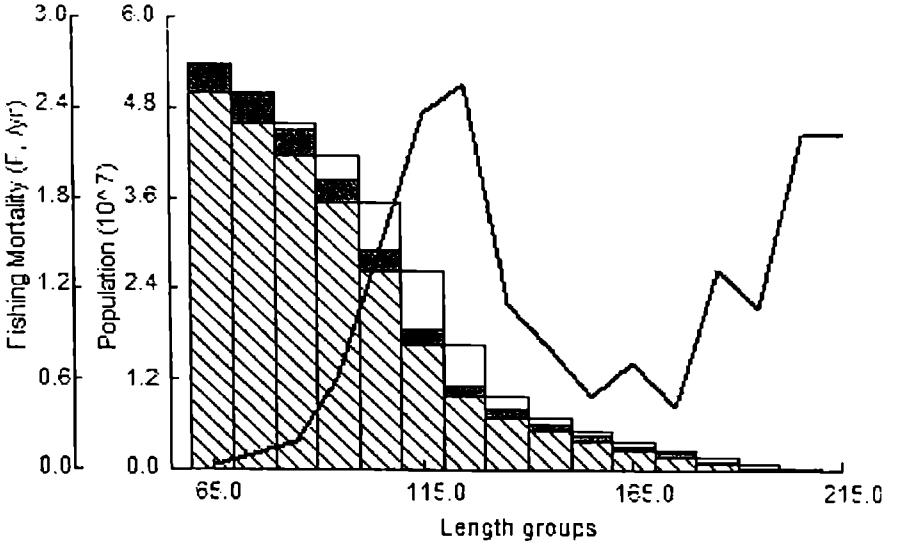

Fig3.10. Length based virtual population analysis of pooled population of *G. surendranathanii*

3.3.4 Recruitment

Recruitment percentage of the pooled population is given in Fig.3.11. It shows a peak in the months of March (15.91%), April (20.99%), May (13.47) and June (11.42).

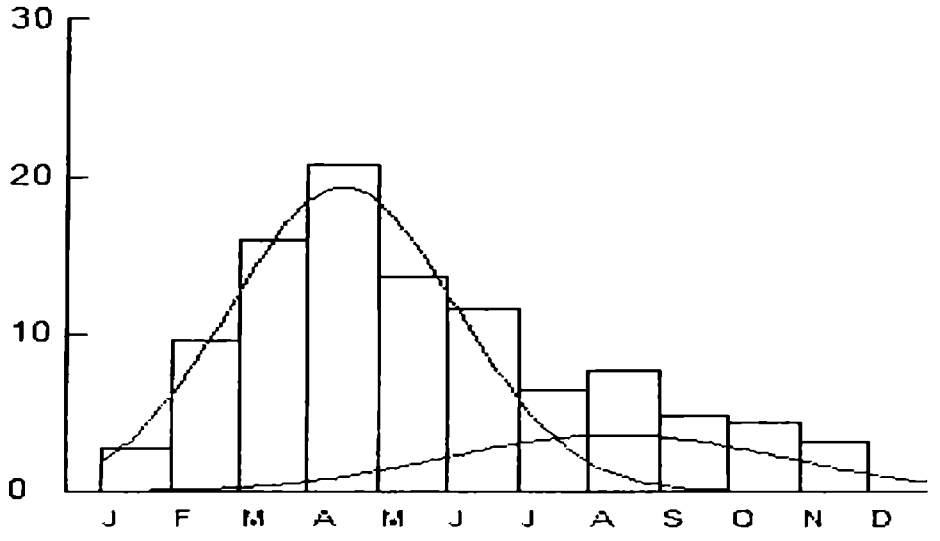

Fig. 3. 11. Recruitment percentage of the pooled population of *G. surendranathanii*

3.4 Discussion

Length-weight relationship was expressed by the cube formula $W=aL^3$ by the earlier workers (Brody, 1945; Lagler, 1952; Brown, 1957). Allen (1938) supported this law and declared that for an ideal fish, which exhibits isomeric growth, the value of regression coefficient should not be different from 3. The cube law confers a constancy of form and specific gravity to an ideal fish. However, adverting the inadequacy of the cubic law in explaining the length-weight relationships in fishes, many researchers adopted the general formula in the form $W=aL^b$ LeCren (1951)suggested that the deviations from the cube law might be contributed to the condition of the fish, reproductive activities, taxonomic differences or environmental factors. Ricker (1958) explained that

due to changes in body proportions during the various life stages of fishes, their body form and specific gravity can vary and hence cube law does not hold true for them. According to Rounsefell and Everhart (1953), generally the value of 'b' is 3 in fishes but the cube law need not always hold good.

In the present study, the highest 'b' value was arrived at in females of *G. surendranathanii* followed by pooled population. The exponential value of 3.059 implies that the females gain weight at a faster rate in relation to its length. But, the exponential values of 3.059 and 3.004 of pooled and males and pooled indicate that the growth rate does not vary much from females. It may be concluded that during the entire period of life, all the population grows isometrically, more or less obeying cube law.

Reports on the length-weight relationship of cyprinid fishes showed that many of them strictly follow cube law while there are many in which the weights of fishes either tend to increase or decrease in proportion to the cube of length. Isometric growth pattern has been reported in *Cirrhinus mrigala* and *Labeo rohita* (Jhingran, 1952), *Labeo calbasu* (Pathak, 1975), *Puntius sarana* (Salim and Shamsi, 1981), *Puntius dorsalis* (Sivakami, 1982), *Catla catla* (Choudhury *et al.*, 1982; Kartha and Rao, 1990) and *Schizothorax plagiostomus* (Bhagat and Sunder, 1983). All these earlier reports are in compliance with the present findings on the length-weight relationship in males, females and pooled populations of *G. surendranathanii* in which the 'b'value was very close to the isometric value of 3.

As there have been no reports on the age and growth and population dynamics of the species, the growth parameters worked out in the present study could not be compared with similar species. In the present study, $L\alpha$ computed by ELEFAN I, showed the highest in pooled population (222), followed by

females(220) and males(154). The 'K' value and growth performance index (Φ) were 0.59 and 4.15 in males,0.60 and 4.46 in females and 0.61 and 4.48 in pooled category. This shows the better growth rate in females compared to males.

The largest size of male *G. surendranathanii* recorded during the present study was 142 mm and that of female as 209 mm. The length of males at the end of first, second, third, fourth and fifth years of life were estimated to be 82, 114, 132, 142 and 147 mm respectively. Females attained a length of 123 at the end of I year, 167 at the end of II year, 191 at the end of third year and 204 at the end of IV year and 211 by V th year. Based on the results of the present study, it can reasonably be inferred that the longevity of *G. surendranathanii* is around five years. Since majority of the males fall in the length class 111-120mm and females in 121-130mm, it can be postulated that the exploited stock of males and females invariably belonged to one year age group.

The highest and only peak in the recruitment percentage was observed during March to June. This is indicating that the breeding season of *G. surendranathanii* may be before March with a single spawning season. The growth curves obtained using ELEFAN I also strongly corroborate the possible existence of a single brood in a year.

The estimates of total instantaneous mortality rate (Z) and the natural mortality coefficient (M) worked out to be 2.85 and 0.63 respectively. According to Gulland (1971), fishes which grow slowly have low natural mortality, Similar results are also reported in fishes belonging to the same family; *Tor khudree* (Kurup *et al.*, 2007); *Puntius denisonii* (Kurup *et al.*, 2008). To verify the estimates of M or K, the M/K ratio is taken as a parameter which usually ranges from 1 to 2.5 in fishes (Beverton and Holt, 1959). M/K

ratio in the present study was found to be 1.03 which lies well within the limits. Similar values were reported in *Labeo dussumieri* by Kurup (1998); *L. calbasu* by Alam *et al.* (2000) and *L. rohita* by Nurulamin *et al.* (2001). The exploitation ratio (E) is worked out as 0.78 which is well beyond the optimum exploitation rate, Emax = 0.621. Also, the present level of exploitation for the species is higher than the exploitation rate ($E_{0.5}$) which will maintains 50% of the unexploited stock biomass. Similar instances have already reported in *Tilapia zilli* (Mehanna, 2004). For management purpose, the exploitation rate must be reduced from 0.621 to 0.351 to maintain a sufficient biomass. This can be achieved by reducing the number of fishing days or the number of fishing trips or declaring the closed season as a conservation measure.

G. surendranathanii is an endemic species to Kerala having the threatened status. Non availability of enough specimens belonging to all size groups at regular intervals had been one of the major limiting factors in pursuing the studies on length frequency or age and growth using more refined methods. Since, there is total lack of knowledge on these aspects of *G. surendranathanii*, the results of this pioneer work on these parameters would definitely advance our knowledge on the biology of fish species and immensely help in formulating relevant conservation and management programmes for the protection and preservation of the germplasm of endemic fishes of Kerala.

Chapter 4

Reproductive Biology

Contents

4.1 Introduction

The sustainable utilization of genetic resources, including fish plays a vital role in improving the standard of living of human society. Concern over declining harvests and an obvious reduction in biodiversity of fish species has lead to a more holistic approach to fisheries management and research. Unfortunately, many ichthyofauna are in decline and some have become endangered due to a combination of natural and anthropogenic stresses. If any species is to be managed, conserved and exploited scientifically, a thorough knowledge on the various intricacies of reproduction is of paramount importance. The main purpose of such studies is to understand and predict the biological changes undergone by the population as a whole during the year (Qasim, 1973). Information on related aspects such as ecological conditions which lead to the synchronization of maturity and breeding activity in males and females, size at first maturity, breeding migration, sex ratios, sexual dimorphism, fecundity etc, are having immense application for the conservation and management of fish stocks and also for developing captive breeding

techniques and undertaking aquaculture programmes. These studies are also essential in assessing strength of broods, spawning time and space requirement and sex composition of the exploited stock (Kurian and Inasu, 2003).

Information on the size at first maturation is essential for avoiding over exploitation of immature juveniles and ensuring the spawning of the individual fishes at least once in life (Euphrasia and Kurup, 2008). A precise knowledge on the maturity stage, breeding period, fecundity in relation to size/age is of great practical utility in fish culture programmes for proper planning of successful hatching and nursery operations. Knowledge about fecundity of a fish is essential for evaluating the commercial potentialities of its stock, life history, practical culture and actual management of the fishery (Lagler, 1956; Doha and Hye, 1970) and also in stock size estimation and stock discrimination (Holden and Raitt, 1974). Fecundity studies have been considered useful in tracing the different stocks or populations of the same species of fish in different areas (Gupta, 1968). Species-wise information is ineludible before venturing into seed production in aquaculture or conservation of natural fauna because fishes exhibit extreme variations in all aspects of breeding. The knowledge on the maturing time, breeding migration, breeding grounds and aggregation assume importance in various fishery regulation and conservation programmes.

In recent decades, much attention has been given by research workers on the gonadal cycle, reproductive physiology and induced breeding of many species of fresh water fishes from Indian waters (Chonder, 1977; Ritakumari and Nair, 1979, Joshi and Khanna, 1980; Thakre and Bapat, 1981; Geevarghese and John, 1983; Badola and Singh, 1984; Shrestha, 1986; Sunder, 1986; Reddy and Rao, 1992; Kaul, 1994; Kurup, 1994; Kurup and Kuriakose, 1994; Nath,

1994). Review of literature showed that hitherto no information is available on the reproductive biology of *G. surendranathanii* and literature available on the related species are of *Garra mullya* (Somvanshi, 1980, 1985; Joseph and Wesley, 2000), *Garra lamta* (Ojha, 2002) and *Garra cylonensis* (Sundarabarathy *et al.*, 2005).

As a rare species, it is important to understand all aspects of biology of *Garra surendranathanii* for developing proper conservation measures. However, the reproductive biology of this species was unknown and in view of this; the present study was conducted.

4.2 Materials and Methods

Monthly samplings of the fishes were done from Periyar River at uniform intervals during the period from January 2004 to December 2004. The study was based on 220 specimens of *G. surendranathanii*, 164 males and 56 females ranging in total length from 75 mm to 142 mm and 90 to 209 mm respectively and weight between 3.33 to 26.73 g and, 6.36 to 87.45g in males and females respectively. The specimens were preserved in 8% formalin after making some perforation in the vent region and brought to the laboratory for further investigation. After removing the excess water by blotting, lengths (to the nearest mm) and weight (to the nearest 0.01g) were recorded for each fish. Fishes were then dissected out to identify the sex and the condition of the gonad. Gonads were taken out and their length and weight were recorded to the nearest millimeter and milligram respectively. After assessing the stage of maturation, the ovary was preserved in 4% formalin for ova diameter and fecundity studies. For histological studies, gonads were taken out from freshly killed specimens and washed; adhering fat was removed and immediately fixed in Bouin's fixative. Conventional histological techniques were followed for

processing testis and immature and spent ovaries (Weesner, 1960). Since routine wax-embedding method leads to crumbling and collapse of yolk-laden oocytes, yolky oocytes were processed following the double embedding method of Khoo, 1979 as modified by Gopalakrishnan, 1991. The spawning season was delineated on the basis of: (1) quantification of maturity stages, (2) the monthly percentage occurrence of fish with gonads in different stages of maturity, (3) pattern of progression of ova during different months and (4) variation in gonadosomatic index. Based on the scheme proposed by Qayyum & Qasim, 1964 a,b,c, and Qasim, 1973, the testis and ovary were grouped under five maturity stages. Quantification of maturity stages was done following morphological characteristics of the gonad such as appearance, colour, degree of distension, relative space occupied in the body cavity and ova diameter measurement. To trace the development of ova, ova diameter was measured from ovaries belonging to all the five stages of maturity, following the method of Clark, 1934. A total of 36 ovaries in different stages of maturation were examined. Altogether 300 ova with 100 each from the anterior, middle and posterior region of each ovary were taken for ova diameter study. Measurements of ova diameter were taken by an ocular micrometer.

Gonadosomatic Index (GSI) was calculated month-wise, applying the formula of June, 1953 and Yuen,1955.

$$GSI \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

The percentage occurrence of males and females in maturing stages in different length groups of the fishes examined was plotted to calculate the length at first maturity. The length when 50% of the fish attained maturity was taken as the minimum length at first maturity (Kagwade, 1968; Geevarghese &

John, 1983). Sex-ratio data was analyzed month wise and size-wise. Chi-square formula (Snedecor & Cochran, 1967) was employed to test whether the observed ratio between males and females deviated from the expected 1:1 ratio for the two sexes using the formula:

$$X^2 = \sum \left[\frac{(O - E)^2}{E} \right]$$

Where O = Observed number of males and females in each month/length group.

E = Expected number of males and females in each month/length group.

Fecundity was estimated on the basis of 17 ripe females of *G. surendranathanii* in the length range of 120 mm to 209 mm. Sub samples from the anterior, middle and posterior regions of the ovary were weighed and the number of ova in each sub-sample was counted manually. Fecundity was estimated by the gravimetric method, applying the formula:

F = nG/g

where F = Fecundity

n = number of eggs in the sub-sample

G = Total weight of the ovary

g = weight of the subsample

Fecundity indices such as the number of ova produced per gram weight of the body or relative fecundity (Bagenal, 1963), the number of ova produced per gram ovarian weight and the gonadosomtic index or the ovarian weight in relation to the fish weight excluding the ovary weight

(Somavanshi, 1985) were worked out. Regression analysis was employed to find out the correlation between fecundity and various body parameters such as total body length, total body weight, ovary length and ovary weight and also between ovary weight and parameters such as total body length and total body weight.

4.3 Results

As in most teleosts, the gonads in the males and females of *G. surendranathanii* are paired, elongated structures lying ventral to kidneys. The ovary is attaches to the dorsal wall of the body cavity my mesovarium and the testis by means of mesorchium.

4.3.1 Gametogenesis

Gametogenesis involves the differentiation of primordial germ cells into mature gametes passing through a series of cellular stages. Spermatogenesis is the development of spermatozoa from sperm mother sells while oogenesis is the process of transformation of oogonia into ripe egg, both processes involving complicated changes occurring in cytoplasm as well as nucleus.

4.3.1.1 Spermatogenesis

The different stages of spermatogenesis in *G. surendranathanii* are as follows:

1. **Primary Spermatogonia:** The primary spermatogonia are the largest spherical cell types among the spermatogenic cells in the testes of *G. surendranathanii* and especially being in higher concentration in the peripheral lobules as well as in the vicinity of lobular wall. Under light microscope they appear to have a large nucleus with deeply stained eccentric nucleolus and nuclear membrane. The cytoplasm of these cells

exhibits less affinity towards basic dyes such as haematoxylin-eosin etc.(Fig.4.1).

2. **Secondary Spermatogonia:** Similar to primary spermatogonia except in size these are smaller round cells with less cytoplasm.(Fig.4.1).

3. **Primary Spermatocytes:** They are much smaller than secondary spermatogonia with reduced cytoplasm. Cytoplasm stains faintly and nucleus purple with haematoxylin-eosin. Nucleus is still distinct and large compared to the size of the cell. Nucleolus is not visible in all cells (Fig.4.2).

4. **Secondary Spermatocytes**: They are formed by the meiotic division of primary spermatocytes. The cytoplasmic connections between the dividing primary spermatocytes persist in most cells. Cytoplasm is less. Nucleolus is no longer visible (Fig.4.2).

5. **Spermatids:** The spermatids are much smaller compact dot like structures, which are formed by the second meiotic division of secondary spermatocytes. They appear deeply stained with haematoxylin-eosin. Nucleolus is absent(Fig.4.3).

6. **Spermatozoa:** The transformation of spermatids to spermatozoa is spermiogenesis. Spermatozoa are small cells with distinct tail and darkly stained nucleus. The lumen of the seminiferous tubules in ripe males is richly packed with mature sperms (Fig.4.3).

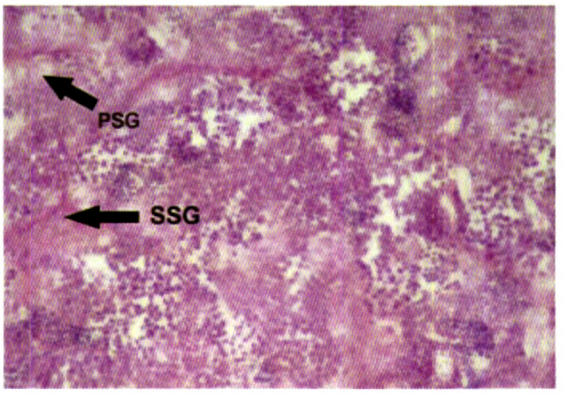

Fig.4.1 T.S. of testis showing primary and secondary spermatogonia

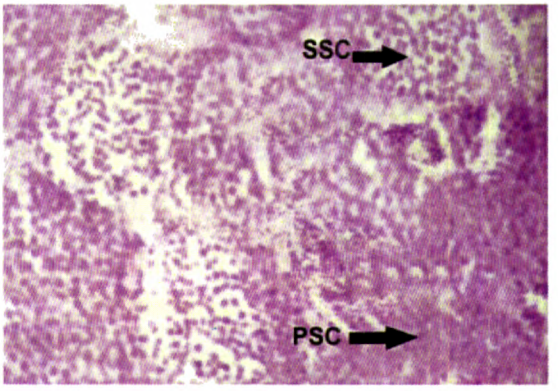

Fig: 4.2 T.S. of testis showing primary and secondary spermatocytes

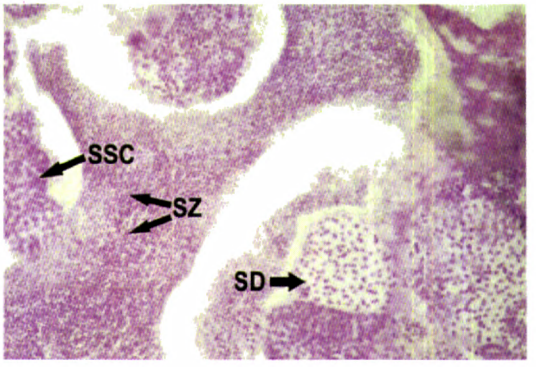

Fig. 4.3. T.S. of testis showing secondary spermatocytes, spermatids and spermatozoa

PSG – Primary spermatogonia
SSG – Secondary spermatogonia
PSC – Primary spermatocytes
SSC – Secondary spermatocytes
SZ – Spermatozoa
SD – Spermatid

4.3.1.2 Oogenesis

Each ovary is covered by a thin peritoneum beneath which lies the thick tunica albuginea containing blood vessels, connective tissue and smooth muscles. The innermost layer is a layer of germinal epithelium which projects into the ovocoel forming ovigerous lamellae. The oogonia appear on these lamellae. Each oogonium in *G. Surendranathanii* passes through the following stages to form mature ovum:

The following phases of the reproductive cycle of *G. surendranathanii* could be identified through histological study of gonads:

I. Immature Stage

a) Chromatin nucleolus stage:

At this stage, oocytes were small and almost spherical. The cytoplasm is highly basophilic and nucleus is relatively large. Nucleus contains 1 - 4 nucleoli. The diameter of ova was varying from 0.05 to 0.09 mm. The nucleus is distinct and the nucleoli remained scattered in the ooplasm (Fig.4.4).

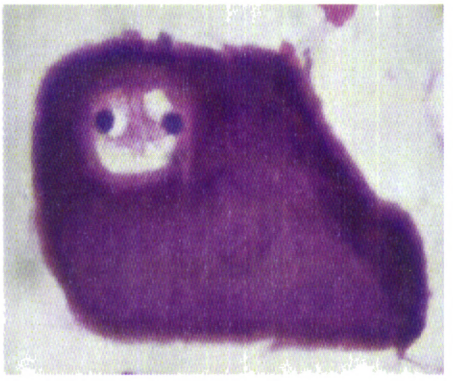

Fig: 4:4. Chromatin nucleolus stage

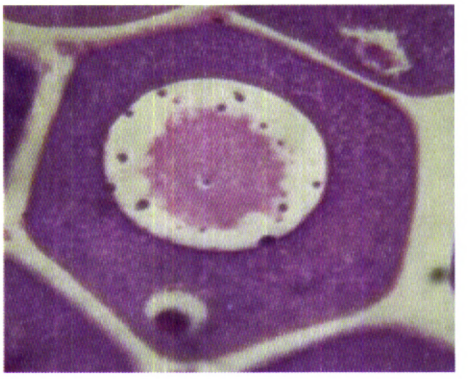

Fig: 4.5. Early perinucleolus stage

b) Early perinucleolus stage

In this stage, the size of oocytes and number of nucleoli increase. The nucleus is relatively large and the quantity of cytoplasm increases and becomes highly basophilic. The diameter of ova varied from 0.10 to 0.15 mm. In some cells, yolk nucleus was very distinct. (Fig: 4.5).

c) Late perinucleolus stage

In this stage, the size of the ova increased further and the cytoplasm becomes highly basophilic. The diameter of ova was varying from 0.18 to 0.23 mm. The number of nucleoli show considerable increase and were arranged like a ring just near the nuclear membrane. Most of the cell possesses yolk nucleus, which was very clear (Fig: 4.6).

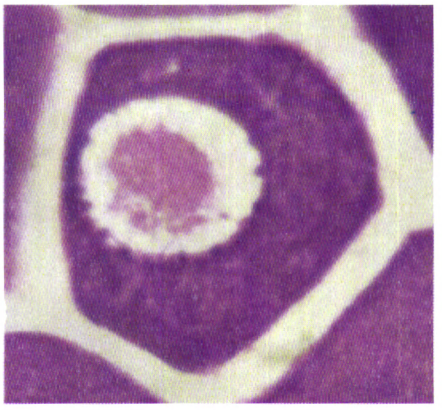

Fig.4.6. Late perinucleolus stage

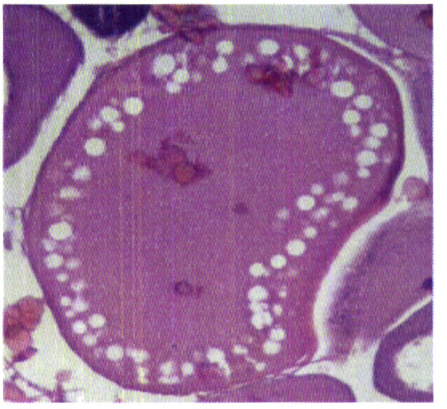

Fig.4.7. Late cortical alveolar stage

II. Maturing stage

a) Early cortical alveolar stage

This stage was characterized by the development of cortical alveoli (lipid droplet) which contain yolk elements on the periphery of the oocytes.

The size of the ova varied from 0.28 to 0.46 mm and the number of nucleoli increased. The nucleus was well defined with nuclear membrane.

b) Late cortical alveolar stage

Slight changes in the shape of nuclear membrane were seen. The diameter of ova increased and varied from 0.52 to 0.92 mm. Nucleolus increased in number and its size decreased and was seen scattered inside the nucleus. Cortical alveoli increased in its size and arranged themselves into two to three layers in the peripheral ooplasm. Cytoplasm is seen as granular and less basophilic when compared to early stages (Fig. 4.7).

III. Mature stage

a) Early yolk deposition stage

The characteristics of this stage is the appearance of acidophilic yolk globules that were seen in between the nucleus and the alveolar layers. Cortical alveoli became larger in size and the nucleus became more irregular in shape. The size and diameter of ova increased further and was in between 0.1 to 1.12 mm. The number of nucleoli increased and they were smaller than in early stages.

b) Late yolk deposition stage

Further accumulation of yolk continued and the increase in number of both yolk globules and lipid droplet were clear. The whole oocyte was consequently filled with yolk globules and cortical alveoli. Thickness of zona radiata increased greatly. The size of oocytes increased considerably due to yolk deposition. The diameter of ova increased and measured from 1.15 to 1.36 mm. Size of nuclei was reduced due to the increase in yolk deposition.

IV. Ripe egg stage

The nucleus was no longer visible. The increasing yolk granules pushed the cortical alveoli and ooplasm to a thin zone on the periphery of the oocyte. The egg was fully ripened and ready to spawn. The diameter of ova was varying from 1.42 to 2.16 mm (Fig: 4.8).

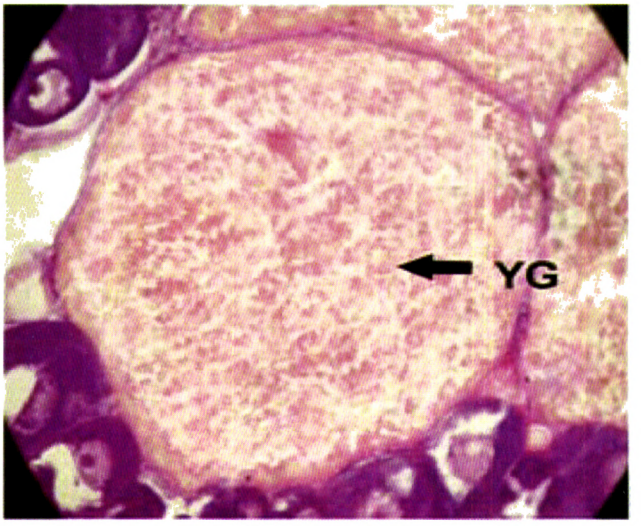

Fig. 4.8. Ripe egg

YG – Yolk globules

V. Spent stage

At this stage, the ovary after spawning was composed of many post-ovulatory follicles, immature oocytes, and mature eggs. Some atretic oocytes were also seen.

4.3.2 Stages of maturation

The following stages of maturation were identified in the males and females of *G. surendranathanii.*

Degree of Maturation	Description
Immature virgins	**Ovaries:** Slender, elongated jelly-like, flesh coloured, occupy a little more than ¼ of the body cavity. Ova invisible to the naked eye. **Testes:** Extremely thin, thread-like, translucent, occupy nearly ½ of the body cavity.
Maturing virgins/ Recovered spents	**Ovaries:** Somewhat flattened pale yellow, occupy ½ of the body cavity. **Testes**: Opaque, firm, white, occupy more than ½ of the body cavity.
Ripening	**Ovaries**: Slightly cylindrical, yellow. Opaque, occupy ¾ of the body cavity, the inner side slightly depressed to accommodate the gut. Usually asymmetry observed between the two lobes of ovary. **Testes**: Creamy white, lobulated with irregular outer margin occupy ¾ of the body cavity.
Ripe	**Ovaries**: Considerably enlarged, occupy nearly the entire length of the body cavity, golden yellow in colour, distended outer membrane, loosely arranged and clearly visible mature and ripe ova. The ovary is highly vasculated with rich blood supply (Fig: 4.9) **Testes**: Very soft, cream coloured, occupy the entire body cavity (Fig: 4.10)
Spent	**Ovaries**: Shrunken, flaccid, blood shot, transluscent, occupy a little more than ½ of the body cavity. Few residual eggs, which are in different stages of maturity were observed. **Testes**: Shrunken, flabby, partly opaque and partly semitransparent occupy less than ½ of the body cavity.

Fig.4.9. Ripe ovary

Fig. 4.10. Ripe testis

4.3.3 Monthly percentage occurrence of fish with gonads in different stages of maturity

The monthly percentage occurrence of males and females in different stages of maturity during 2004 January to December is shown in Fig.4.11. In males the immature individuals (Stage I) appeared from April onwards and reached the maximum in May and June and were contributed 100% After June the stage I individuals showed a sharp decline and after September their presence in the catch was not observed. Recovering spent (Stage II) fishes started to appear in the catch from July onwards and reached a peak during September with a contribution of 76.47%. From October onwards the recovering spent individuals showed a sharp decline. Fishes with gonads in stage III or ripening individuals appeared in the catch from July onwards and reached the peak during October and contributed to 35.71%. Ripe (stage IV) individuals were available in the catch from October onwards and reached the peak during November, contributed to 100 %. Spent (stage V) fishes were present from December onwards and reached the peak during March and showed their presence in the catch up to April.

In females the immature (stage I) individuals appeared in the catch from April to August and reached the peak during June and August with a

contribution of 100 %. Maturing virgins or fishes with gonads in Stage II appeared in the catch only in July and November. Ripening (Stage III) fishes appeared in the catch in September and the maximum also observed in the same month Fishes with gonads in stage III condition showed their presence in the catch in October too. Ripe (Stage IV) fishes appeared in the catch from October to December and reached its peak during November with a contribution of 75. Spent (stage V) fishes appeared in the catch from January to March.

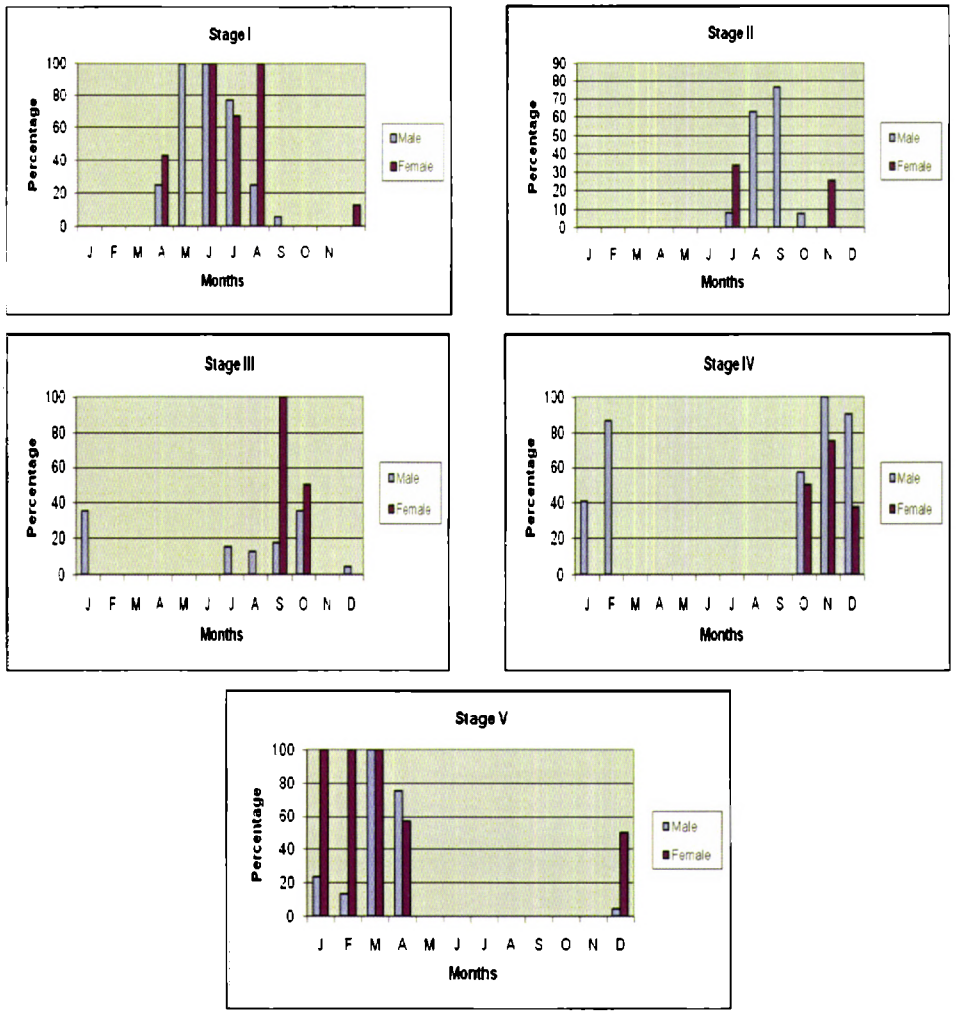

Fig: 4.11. Monthly percentage occurrence of gonads in different stages of maturity in *G. surendranathanii* during Jan-Dec 2004.

4.3.4 Pattern of progression of ova during different months

The pattern of progression of ova during June to December is studied during the study. All the ova less than 0.23 mm diameter were immature. The next group of ova between 0.28 – 0.92mm was identified as maturing ones. The ova in the range between 1.00-1.36 mm were belonged to the ripening eggs. Ova measuring 1.42 mm and above were in fully ripe condition. The development of ova during different months showed the preponderance of immature and maturing ova during June and August. Oocytes up to 1.21 mm were appeared in September with a major mode at 0.8-0.9 mm. Thereafter; the progression of ova was very rapid with the result that ripening oocytes were very prominent with the mode shifting to 1.1-1.3mm in October. During November and December major portion of the ova diameter ranged between 1.4 2.1mm size class and another batch of immature ova of 0.12- 0.23mm also observed.

4.3.5 Gonadosomatic index

The mean monthly variation of gonadosomatic index (GSI) values of males and females during January to December 2004 are depicted in Fig.4:12. The testicular weight started increasing from July and attained the peaks in October and November. Thereafter the GSI showed a drastically declining trend. The trend was more or less the same in case of females also and the peak observed was in November and then a declining trend.

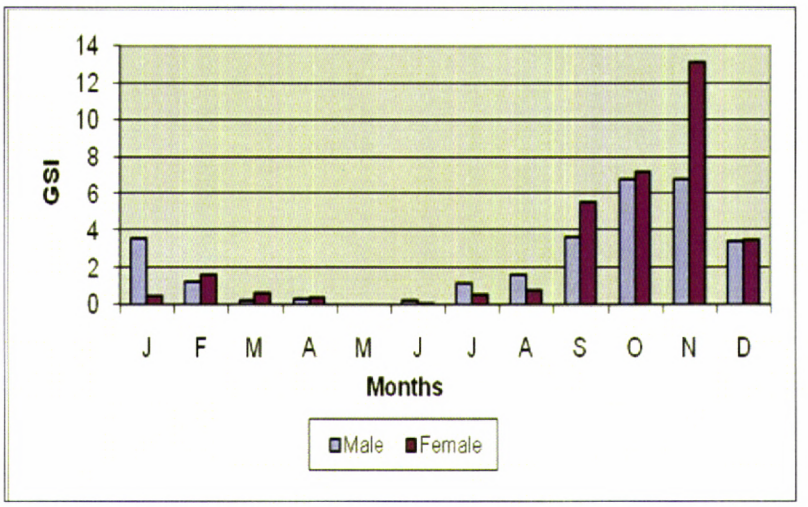

Fig.4:12. Monthly variation of gonadosomatic index in *G. surendranathanii* during Jan-Dec 2004

4.3.6 Length at first maturity

Occurrence of males and females at different stages of maturity in various size groups are shown in Table. 4.1 and 4.2 respectively. Fig.4.13. represents the relation between maturity and length of the male and female *G. surendranathanii*. It appeared that in males specimens up to 70 mm total length and in females, specimens up to 100 mm were belonged to immature and maturing fishes. The percentage of ripening fishes increased rapidly after that stage. The smallest ripe male belonged to the 71-80 mm TL size group while the smallest ripe female belonged to 101-110mm TL group. The length at which 50% of the specimens attained maturity, taken as the mean length at which maturity is attained (Kagwade, 1968), were 85 mm for males and 115 mm for females. Thus males were found to mature at a lower size than their female counterpart.

Table. 4.1. Maturity stages (in %) in different length groups of male *G. surendranathanii*

Length Group(mm)	Stage of Maturity (Male)				
	I	II	III	IV	V
71-80	80.00	20.00			
81-90	44.44	33.33	11.11	11.11	
91-100	9.52	14.29	14.29	52.38	9.52
101-110	20.00	17.78	15.56	24.44	22.22
111-120	13.04	8.70	2.17	32.61	43.48
121-130	9.68	16.13	9.68	19.35	45.16
131-140			20.00	40.00	40.00
141-150	50.00			50.00	
151-160					
161-170					
171-180					
181-190					
191-200					
201-210					

Table.4.2. Maturity stages (in %) in different length groups of female *G. surendranathanii*

Length Group(mm)	Stage of Maturity (Female)				
	I	II	III	IV	V
71-80					
81-90					
91-100					
101-110	100.00				
111-120	75.00			25.00	
121-130	20.00	10.00	10.00	10.00	50.00
131-140	14.29			14.29	71.42
141-150				33.33	66.67
151-160	20.00			40.00	40.00
161-170	16.67	16.67	33.33	16.67	16.67
171-180			33.33	66.67	
181-190			50.00	33.33	16.67
191-200			66.67	33.33	
201-210				100.00	

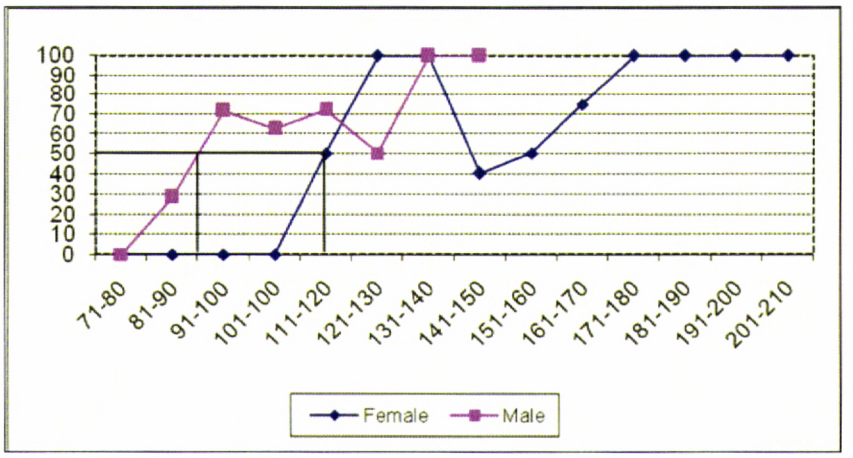

Fig. 4.13. Percentage occurrence of mature males and females in *G. surendranathanii*

4.3.7 Sex ratio

Altogether 268 specimens were examined in the laboratory to determine the sex-ratio. Due to the absence of sexual dimorphism in *G. surendranathanii*, the fishes were sexed by internal examination. Out of the 220 specimens examined, 164 were males, 56 females and the remaining 48 indeterminate. The month wise distribution of the two sexes (Table 4.3) revealed that the sexes were disproportionate in the population. Males outnumbered the females in all months except October where the representation was equal. Chi-square test confirmed the significant dominance of males in the population (Table 4.3). Though there was considerable variation in the distribution of the sexes in some of the months, the overall sex ratio showed significant dominance of males ($P<0.01$). The mean ratio of males to females was 1:0.34 and the respective chi-square value of 53.02 lend to support to the above observation that the sex ratio significantly skewed from the expected 1:1 ratio ($P<0.01$).

Table 4.4 shows the variation in sex ratio among the various size groups. Males were predominating up to 130 mm TL and thereafter the percentage occurrences of males were reduced and females showed higher contribution in

the fishery. Beyond the 150 mm TL, only females were observed in the fishery. The chi-square value of 53.02 for the overall sex ratio showed that the variation was highly significant ($p<0.01$).

Table: 4.3. Sex ratio of *G. surendranathanii* during January –December 2004.

Months	Total	Male	Female	M-F Ratio	Chi-square	Probability
January	23	17	6	0.35	5.26	P<0.05
February	19	15	4	0.27	6.37	P<0.05
March	23	20	3	0.15	12.57	P<0.01
April	19	12	7	0.58	1.32	P>0.05
May	1	1	0			
June	6	5	1	0.20	2.67	P>0.05
July	16	13	3	0.23	6.25	P<0.05
August	10	8	2	0.25	3.60	P>0.05
September	18	17	1	0.06	14.22	P<0.01
October	28	14	14	1.00	0.00	P>0.05
November	25	21	4	0.19	11.56	P<0.01
December	32	21	11	0.52	3.13	P>0.05
Total	220	164	56	0.34	53.02	P<0.01

Table:4.4. Sex ratio in *G. surendranathanii* in each 10mm length group

Length Group(mm)	Total	Male	Female	M-F Ratio	Chi-square	Probability
71-80	5	5	0			
81-90	10	9	1	0.11	6.40	P<0.05
91-100	22	21	1	0.05	18.18	P<0.01
101-110	47	45	2	0.04	39.34	P<0.01
111-120	50	46	4	0.09	35.28	P<0.01
121-130	41	31	10	0.32	10.76	P<0.01
131-140	12	5	7	1.40	0.33	P>0.05
141-150	8	2	6	3.00	2.00	P>0.05
151-160	5	0	5			
161-170	6	0	6			
171-180	3	0	3			
181-190	6	0	6			
191-200	3	0	3			
201-210	2	0	2			
Total	220	164	56	0.34	53.02	P<0.01

4.3.8 Fecundity

The average values of fecundity indices of *G. surendranathanii* are given in Table.4.5. Relationship of fecundity with total body length, body weight, ovary length and ovary weight were worked out by regression analysis and the results are depicted in Fig: 4.14 – 4.17. Fig: 4.18 and 4.19 represent the regression of ovary weight on total body length and body weight.

Table: 4.5. Average Value of Fecundity indices in the spawners of *Garra surendranathanii*

Length Group (mm)	Average fish length (mm)	Average fish weight (g)	Average Ovarian weight (g)	No.of fishes examined	No. of ova per g fish weight	Per g ovarian weight	Gonadosomatic Index	Absolute fecundity
111-120	120	17.02	1.68	1	25	257	9.87	431
121-130	0	0.00	0.00	0	0	0	0.00	0
131-140	0	0.00	0.00	0	0	0	0.00	0
141-150	149	34.43	6.21	2	51	280	17.96	1740
151-160	156	35.36	6.07	2	21	124	8.74	756
161-170	164	39.15	3.09	2	29	369	7.82	1139
171-180	177	52.36	8.04	4	47	307	15.41	2470
181-190	187	57.18	4.41	3	26	342	7.15	1506
191-200	199	77.32	13.21	1	70	409	17.09	5402
201-210	208	83.92	9.50	2	31	274	11.49	2605

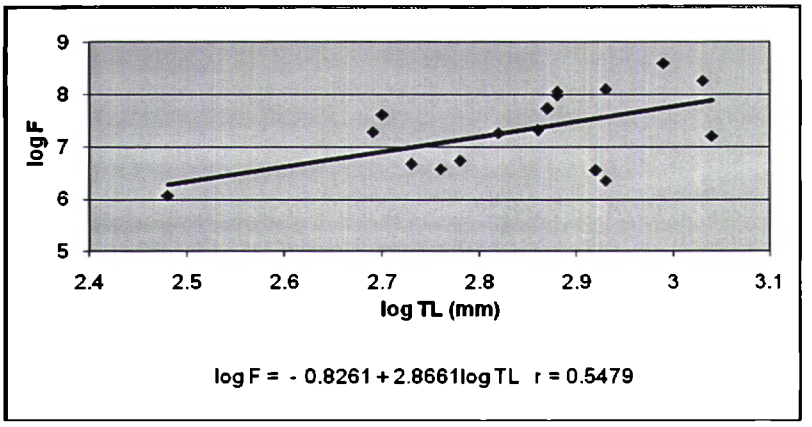

Fig. 4.14. Relationship between fecundity and total weight

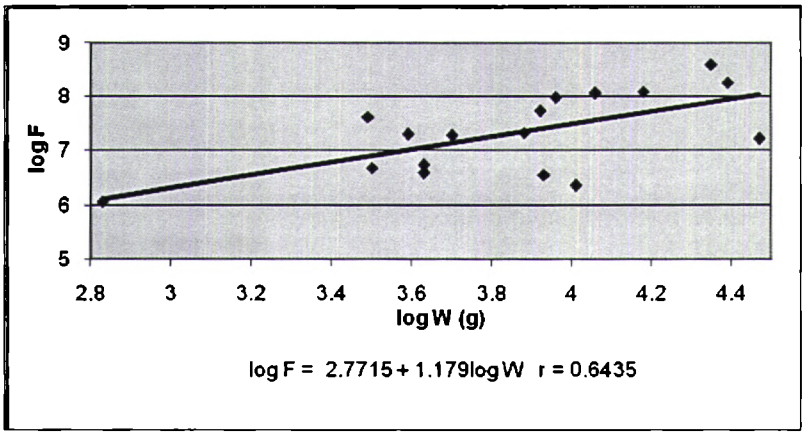

Fig. 4.15. Relationship between fecundity and body weight

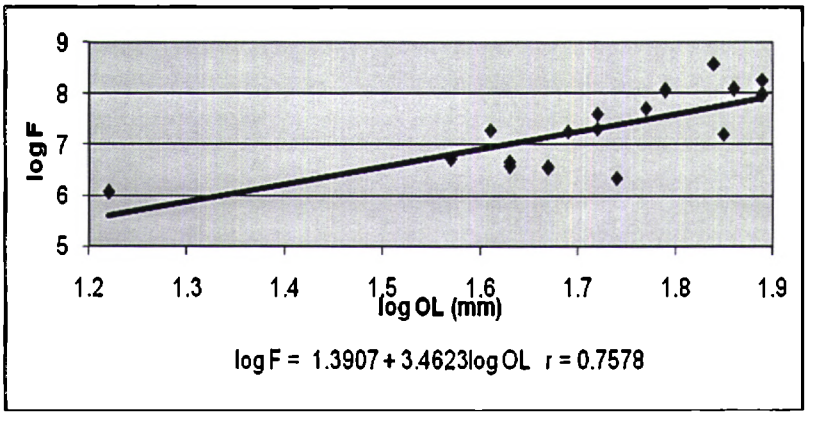

Fig. 4. 16. Relationship between fecundity and ovary length

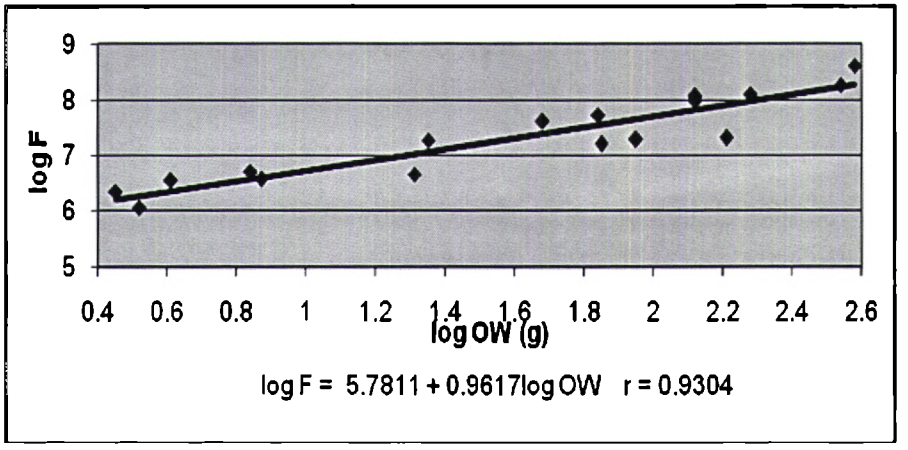

Fig. 4.17. Relationship between fecundity and ovary weight

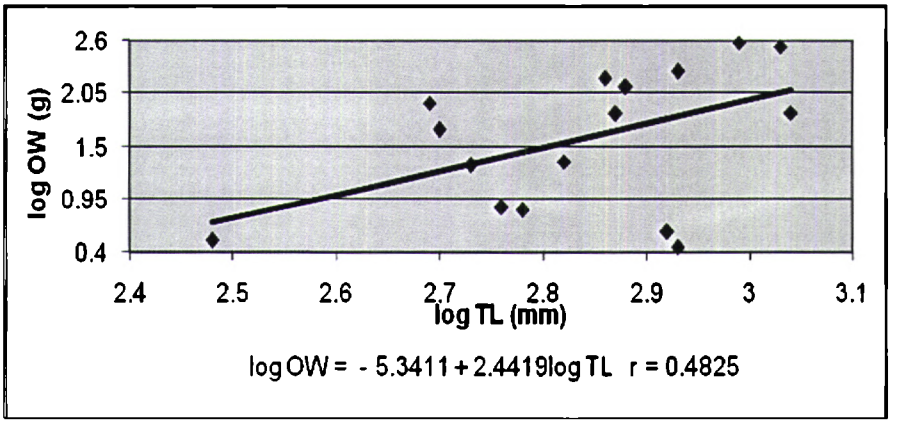

Fig. 4.18. Relationship between total length of fish and ovary weight

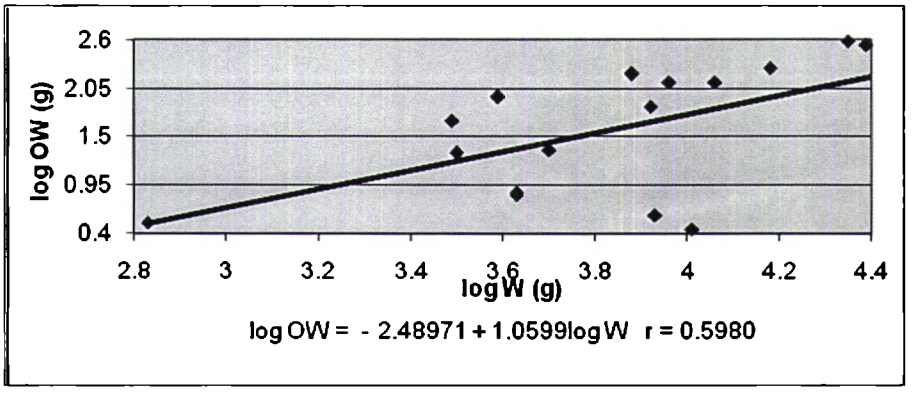

Fig. 4.19. Relationship between body weight of the fish and ovary weight

4.3.8.1 Fecundity indices

The absolute fecundity varied from 431-5402 eggs in specimens ranging from 120 –199 mm in total length and the average was worked out to be 1924 ova. The relative fecundity was estimated to be vary between 10 (188 mm TL) and 70 (199mm TL) with an average of 36, while the number of ova per gram ovarian weight varied between 21 and 70, with the average 17. Gonodosomatic values varied in different length groups. The gonosomatic values varied between 7.15 (181-190mm size group) and 17.96 (141-150mm size group).

4.3.8.2 Relationship between fecundity and body parameters

The relationship between total length (x) and number of ova (y) was calculated and the result is depicted in Fig: 4.14. The regression equation after logarithamatic transformation of the variables can be expressed as follows:

Log F= -0.8261+2.8661 log TL; r = 0.5479

The logarithamatic relationship between fecundity and fish weight (Fig: 4.15) was found to be

Log F=2.7715+1.1791 log W; r = 0.6435

Fecundity was related to the measurements of ovary, the ovary length (OL) (Fig:4.16) and ovary weight (OW)(Fig: 4.17) which can be expressed as follows:

Log F =1.3907+3.4623 log OL; r = 0.7578

Log F = 5.7811+0.9617 log OW; r =0.9304

The regression equation of ovarian weight (OW) on body length (W) (Fig: 4.18) and body weight (TL) (Fig: 4.19) are given below.

Log OW = -5.3411+2.4419 log TL; r = 0.4825

Log OW = -2.4897+1.0599 log W; r = 0.5980

4.4 Discussion

The male and female reproductive organs of *Garra surendranathanii* are built on the general telestean pattern as observed in other teleosts. The paired testes in teleost fishes are either fused along the entire length or completely separate or fused posterior. In *G. surendranathanii*, the testes are united at the posterior region to form a spermatic duct as reported in *Barbus tor* (Rai, 1965), *Channa gachua* (Sanwal and Khanna, 1972), *Schizothorax richardsonii* (Bisht and Joshi, 1974) and *Schizothorax plagiostomus* (Agarwal, 1996).

It is well known that the differentiation of primordial germ cells into gametes (Spermatogenesis and Ooogenesis) is an orderly process and follows a distinct pattern. The various stages involved in the development of spermatozoa, *viz.* the primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa, as observed in *G. surendranathanii*, have been identified and described in other freshwater teleosts such as *Mystus seenghala* (Sathyanesan, 1959), *Clarius macrocephalus* (Mollah, 1988), *Puntius dukai* (Joshi and Joshi, 1989), *Schizothorax plagiostomus* (Agarwal, 1996) and in the related species *Garra lamta* (Ojha, 2002).

Teleostean ovaries of many Indian fish species have been studied in details by many authors and few are by Sathyanesan (1959); Belsare (1962); Sobhana and Nair (1974); Agarwal (1996); Shafi (2000); Ram *et al.* (2001) and

Euphrasia and Kurup (2008). The results of the present study on Oogenesis in *G. surendranathanii* lend support to the findings of the above authors.

Breeding season of fishes was ascertained by applying indirect methods such as quantification of maturity stages, monthly occurrence of gonads in different stages of maturity, monthly progression of ova towards maturity and seasonal variations in the gonadosomatic index. Results of the one data have shown that as far as occurrence of gonads in different stages of maturity is concerned, males mature slightly earlier than females. Majority of the fishes underwent ripening rapidly and by the end of December majority of the males and females were in the maturing virgin stage. From April onwards the maturation in males was a slow process and from the end of September onwards ripe males appeared in the population. While maximum number of ripe males appeared in the population during November. In the case of females, ripe fishes were observed in the population from September to December with a peak during November. The male fishes might have a longer spawning cycle, as manifested by the total absence of mature fishes from March. Where as in females, only spent stages were observed after December. Based on the results of the present study, it can well be concluded that *G. surendranathanii* inhabiting Periyar River has a prolonged spawning period extending from October to February with a distinct peak during October –November.

It is well known that ova diameter measurements can give reliable evidence about the time of spawning and spawning periodicity of fishes. Clark (1934) made the first attempt to study the maturity of California sardine (*Sardina caerulea*) based on the size frequency of ova in the ripe ovary. This method has been successfully applied for delineating the spawning period of many Indian fishes by several authors (Prabhu, 1956; Qasim and Qayyum,

1963; Sathyanesan, 1962; Bhatnagar, 1967; Desai and Karamchandani, 1967; Qasim, 1973; Murthy, 1975; James and Baragi, 1980; Jayaprakash and Nair, 1981; Thakre and Bapat, 1981; Geeevarghese and John, 1983; Kurup, 1994).

In *G. surendranathanii*, all the ova measuring 1.42 mm and above were fully ripe while the group having diameter between 1-1.36 mm were the ripening ones. Those falling below 1mm were adjudged as maturing and immature categories. From the appearance of largest oocytes of 1.4-2.1 mm in fully ripe conditions in November and December, it can be reasonably concluded that this species starts spawning during November and this is in close agreement with the spawning season delineated for *G. surendranathanii* in the present study. During November and December immature ova were seen with the mature suggests that this species is a single spawner. One batch of mature ova for the present spawning season while the immature for may be reserve oocytes for the following spawning season. Similar observation was seen the related species *Garra cylonensis* (Sundarabarathy *et al.*, 2005).

Marza (1938) described three categories of rhythm in the maturation of oocytes.(1)Total synchronism- all oocytes in the ovary develop synchronously as in *Onchorhyncus masou*(Yamamoto *et al.*, 1959) (2) Group or partial synchronism-two groups of oocytes are distinguished indicating spawning once a year within a short and definite period as in *Clarius batrachus* (Lehri,1968).(3) Asynchronism –oocytes in different stages of development are present indicating a long spawning season with several spawning within the season as in *Schizothorax richardsonii* (Bisht and Joshi,1975). In *G. surendranathanii*, a batch of oocytes is passing through a single stage at a time and hence the fish exhibited total synchronism in oocyte maturation as in the case of *Garra mullya* (Somvanshi, 1985).

The maturation of germ cells in fish gonads is associated with an increase in the weight of gonad and this increase is expressed by the gonadosomatic index (GSI). However, the process of maturation is not exactly identical in males and females. In ovary, as the oocytes grow, they accumulate metabolites leading to an increase in their weight (Nagahama, 1983). GSI is indicative of fish spawning in temperate and tropical regions (Bouain and Sian, 1983; Biswas *et al.*, 1984; Phukon and Biswas, 2002). GSI values of both males and females followed more or less the same trend. Low GSI values in January - July is concomitant with a period of occurrence of spent fishes and early development of gonads. The slightly high values observed from August to September reflected a diversity of gonad stages including a large number of maturing (II stage) and ripening (III stage) gonads. The peak GSI values encountered during October and December in both females and males. During spawning season, the GSI show a plummeting due to the release of the gonadal products. Hence breeding season ensues the months with maximal GSI. Reduced GSI in females is a consequence of release of ova from the ovary while in males, it may result from the combined effect of elimination of residual body followed by initiation of spermiation (Stoumboudi *et al.*, 1993). These values confirms that, Garra surendranathanii breeds only once a year and the peak breeding season is October – December.

Based on the occurrence of large number of ripe fishes and ripening individuals with advanced stages of oocytes in the ovary, the appearance of spent individuals, the presence of ripe ova and the high GSI values, it can reasonably be inferred that males of this species are reproductively active for 4-5 months (September-January) and females for 3 months (October – December).

Prabhu (1956) classified fishes into 4 distinct groups on the basis of the spawning pattern.

Type A: Spawning taking place only once in a year during a definite short period. 2 batches of ova, mature and immature, are found in mature ovaries.

Type B: Spawning taking place only once in a year but with a longer duration. The range in size of the mature ova will be nearly half of the total ranges in the size of the whole intra-ovarian eggs.

Type C: Spawning twice a year. Ovaries contain distinct ripe as well as maturing ova.

Type D: Spawning throughout the year but intermittently. Ovaries contain different batches of eggs which are not sharply differentiated from one another.

On the basis of ova diameter frequencies, Qasim and Qayyum (1961), classified fishes into 3 categories.

Category I: Fishes with a well-marked single batch of maturing eggs in their ovaries. Breeding occurs only once a year.

Category II: Fishes with more than one group of maturing oocytes. The breeding season is long.

Category III: Fishes with oocytes of all sizes ranging from the smallest to the largest without well-marked batches. They have non-seasonal breeding.

It would thus appear that *G. surendranathanii* fits into Type 'A' of Prabhu (1956) and category I of Qasim and Qayyum(1961). *G.*

surendranathanii was found to breed only once in a year in the Periyar River with ovaries containing single group of maturing oocytes. The breeding season was observed to be moderately long.

Usually fishes attain maturity at a particular length of the individuals. The onset of maturity differs considerably inter-specifically as well as intraspecifically (Nikolsky, 1963). Information on the size of maturation is essential for avoiding over exploitation of immature juveniles and ensuring the spawning of the individual fishes at least once in life. The minimum size of maturity has been estimated earlier by several workers (Qayyum and Qasim, 1964a; Parameswaran *et al.*, 1972; Selvaraj *et al.*, 1972; Sobhana and Nair, 1974; Somavanshi, 1980: Nautiyal, 1984; Sunder, 1986; Kurup, 1994; Agarwal, 1996, Euphrasia and Kurup, 2008). In *G. surendranathanii*, the males and females were found to be mature at 85 and 115 mm respectively. Thus, males attain sexual maturity at a smaller length than the females. Similar observations had been reported in many freshwater fishes such as *Cyprinus carpio* (Parameswaran *et al.*, 1972), *Labeo boggut* (Selvaraj *et al.*, 1972), *Barbus sarana* (Murthy, 1975), *Tor tor* (Chaturvedi, 1976), *Labeo gonius* (Siddiqui *et al.*, 1976a), *Labeo bata* (Siddiqui *et al.*, 1976b), *Noemacheilus triangularis* (Ritakumari and Nair, 1979), *Schizothorax longipinnis* (Sunder,1986) and *Labeo dussumieri* (Kurup, 1994). The first appearance of ripe and spent individuals in 81-90 mm size group in males and 111-120 mm size group in females of *G. surendranathanii* suggest that this roughly corresponds to the minimum size group at which the females and males attain ripeness and start spawning. It is a generalized fact that among fishes, males usually grow to a smaller size than females (Sivakami, 1982). In *G. surendranathanii* also, females are larger in size. The maximum size of the males and females encountered during the present investigation is 146 mm and 209mm

respectively. During the present study, the year wise growth, estimated using Von Bertalanffy growth formula also revealed the differential growth among males and females (Chapter 3). The difference in the size at first maturity and the maximum size attained in the two sexes may be due to differential growth rate or due to the fact that females live longer and hence attain a larger size (Murthy, 1975).

A proper knowledge of sex ratio is important in the management of fishery. It indicates features such as the movement of sexes in relation to season, strength of spawning stock, catch composition, etc. Considerable variation was observed in the ratio of males and females of *G. surendranathanii* in some of the months. However there, was a preponderance of males during almost all the months. This is a serious problem which should be taken into consideration. The natural spawning and reproduction of the species could be strongly hindered by the variation in sex ratio. The size group wise variation of the species showed that the significant variations are also observed for sex ratio at different stages of its life history. Females were reported more with the advancement in length group. Siddiqui *et al.*, (1976b) while studying the life history of natural populations of *Labeo bata* (Ham.) observed the dominance of males in higher groups and stated that this might be due to heavy mortality of females in smaller size groups either due to natural death or fishing pressure as they are caught more easily or more exposed to predation. But, in the present study's observation we can say that the females due to their larger size may be exploited more for food compared to males and it is easy to catch them during their breeding migration to shallow waters (Chapter 5).

The ideal sex-ratio in natural population is close to 1:1(Nikolsky, 1980). A definite ratio of males and females during the spawning season is a

prerequisite for most effective fertilization of eggs deposited by spawning females. The deviation in sex ratio from the ideal one during the spawning season encountered during the period of study with a distinct predominance of males may be a contributing factor to the endangerment of *G.surendranathanii.* Nautiyal (1994) and Singh (1997) reported that spawning migration of fishes can lead to alterations in sex ratio drastically. The changing sex ratios may be associated with the shoaling habits of fishes, which might be a contributing factor for the dominance of either of the sex in the catch composition of different days. Differential mortality may be another cause of skewness in sex ratio (Bhatnagar, 1972).

The higher occurrence of males in lower and females in higher size groups as observed in *G. surendranathanii* are corroborating with the findings in a number of fish species (Bailey, 1963; Bhatnagar, 1972; Chaturvedi, 1976; Siddiqui *et al.*, 1976a; Somavanshi, 1980, Vinci and Sugunan, 1981; Kurup, 1994). According to Makeeva and Nikolsky (1965), variation in sex ratio at different sizes and age groups exists even in species with an overall 1:1 ratio. Nikolsky (1980) assigned the dominance of males in smaller size groups to the tendency of males to mature earlier and live less longer. According to Qasim (1966), the disparity in growth rate between sexes led to the preponderance of one sex and the preponderant sex attains a bigger size. This is at variance with the present observation in *G. surendranathanii* in which the males were dominant in the sample population, although the minimum size at maturity and the maximum size of the individual were found to be higher in females.

Lowe-McConnell (1975) defined the fecundity as the number of eggs produced by an individual fish in its lifetime. Bagenal (1978) considered it as the number of ripening eggs found in female prior to spawning and termed it as

individual or absolute fecundity. Fecundity is generally regarded as the number of ova in an organism, which has the potential to give rise to the offsprings. Thus, the reproductive potential is a function of the fecundity of fishes. Fecundity varies both within and between fish populations and numerous factors such as nutritional state (Scott, 1962; McFadden *et al.*, 1965; Stauffer, 1976), time of sampling and maturity stage (Healey, 1971), racial characteristics (Bagenal, 1966) and environmental conditions such as rainfall and salinity (Joshi and Khanna, 1980). Fecundity in teleosts range from a few hundred to several lakhs.

The fecundity estimates of important freshwater cyprinids have been reported by several authors. Fishes such as *Labeo calbasu* (Rao and Rao, 1972; Vinci and Sugunan, 1981), *L.rohita* (Varghese, 1973), *Cirrhinus mrigala* (Chakrabarty and Singh, 1967), *L.dero* (Bhatnagar, 1967), *Cyprinus carpio* (Parameswaran *et al.*,1972), *L.fimbriatus* (Bhatnagar, 1972), *L. gonius* (Joshi and Khanna, 1980) and *L.dussumieri* (Kurup, 1994) are highly fecund fishes with several lakhs of eggs. *Puntius vittatus*(Ibrahim, 1957)with 26 to 302 ova, *Barilius bendelisis* var. *chedra* (Desai and Karamchandani, 1967)with 305-1168 ova, *Glyptothorax kashmirensis*(Kaul, 1994) with 692-1392 ova and *Noemacheilus triangularis* (Ritakumari and Nair, 1979) with 800-2126 ova are some freshwater fish species with less number of ova in their mature ovaries. The fecundity of other cyprinids are 2368-8590 ova in *Puntius ticto* (Ibrahim, 1957), 3340-6160 in *Crossocheilus latius diplocheilus* (Kaul, 1994), 3416-53139 in *P.stigma* (Ibrahim, 1957)I4245-58330 ova in *P.dorsalis* (Sivakami, 1982) and 58327-139934 ova in *P.sarana* (Sinha, 1975). In the related species *Garra mullya* 1700-6259 ova were observed (Somvanshi, 1985). In *G. surendranathanii* the fecundity ranged from 431-5402. Comparatively bigger sizes of the eggs may be identified as one of the reasons for the low fecundity

of *G. surendranathanii*. Bulkley (1976) discussed the influence of egg size on fecundity in steel head trout, *Salmo gairdneri* and stated that it is possible that a fish producing fewer eggs could produce larger eggs within limits than if it were producing numerous eggs. Fecundity is higher in those fishes in which eggs are smaller in size than those in which the eggs are larger (Kaul, 1994).

The reproductive potential of fishes of different size groups had been expressed as the number of ova produced per gram body weight called relative fecundity. (Bagenal, 1963; De Silva, 1973) or comparative fecundity (Das, 1964). Relative fecundity provides a better comparison of fecundities and eliminates the alteration in absolute fecundity with fish age and size (Sheila and Nair, 1983). The present study revealed that the average relative fecundity of *G. surendranathanii* was 36. This value is very low when compared to a relative fecundity of 252 in *L.calbasu* (Pathak and Jhingran,1977), 256 in *L.rohita* (Varghese, 1973), 285 in *L.bata* (Alikunchi, 1956), 275 in *Barilus bendelisis* (Dobriyal and Singh, 1987), 271 in *L.gonius* (Joshi and Khanna, 1980), 228 in *P.vittatus* (Ibrahim, 1957),227 in *P.sarana sunasutus* (Sobhana and Nair, 1974),201 in *L.calbasu* (Vinci and Sugunan, 1981) and 180 eggs in *L.dussumieri* (Kurup, 1994). It can therefore be concluded that the very low relative fecundity of *G. surendranathanii* when compared to other species is a major reason for its threatened status in the natural waters.

Fecundity is often correlated with length, weight and age of fish and also with the length, weight and volume of ovary. The relationship between total length and fecundity differ in different species of fishes. Clark (1934) opined that the fecundity of a fish increased in proportion to the square of its length. Simpson (1951) established that the fecundity of plaice was related to the cube of its length and was thus directly proportional to fish weight. Many authors

have supported Simpson's view of fecundity being related to fish length by a factor closer to the cube (Bagenal, 1957; Sarojini, 1957; Pillai, 1958; Pantalu, 1963; Varghese, 1973, 1976; Kurup, 1994). After surveying 62 fish species, Wooton (1979) concluded that the exponent value varied from 1 to 5 with most of the values lying between 3.25 and 3.75 and invariably higher values were reported in marine species than in freshwater forms. Jhingran (1961) and Qasim and Qayyum (1963) have reported the exponential value to range around 3. In the present study, the exponential value of *G. surendranathanii* was observed to be 2.8661 which did not deviate significantly from the value of '3' and this finding is in total agreement with the above reports.

Fecundity was found to have a linear relationship to body weight. The 'b' values of 1.1791 did not significantly deviate from unity in other words, the number of ova increased in proportion to body weight. Linear relationship between fecundity and body weight has been reported in *L.fimbriatus* (Bhatnagar, 1972), *P.sarana* (Sinha, 1975), *L.rohita* (Khan and Jhingran, 1975), *L.bata* (Siddiqui *et al.*, 1976b), *L.dero* (Raina and Bali, 1982) and *L. Dussumieri* (Kurup, 1994). The observations of some early workers (Bagenal, 1957; Sarojini, 1957; Gupta, 1968; Varghese, 1973) also lend support to the linear relationship between fecundity and body weight.

The coefficient of correlation of the various statistical relationships derived between fecundity, body length, body weight, ovary length and ovary weight revealed significant relation between fecundity and the body parameters. The highest degree of correlation was seen between fecundity and ovary weight. This is in agreement with the observations of Chathurvedi (1976) in *Tor tor*, Joshi and Khanna (1980) in *L.gonius*, Qadri *et al.*(1983) in *Schizothorax richardsonii*, Sunder (1986) in *S.longipinnis* and Kurup (1994) in *L.dussumieri*.

It is well known that the weight of ovaries of a fish is mainly influenced by the ova contained in them. The 'r' value between ovary weight and body length and ovary weight and body weight exhibited a fair correlation between the variables.

From the multivariate analysis, ovary weight was identified as the most appropriate predictor of ovarian egg count. But, it is undesirable to sacrifice the fish to determine the gonad weight. Bagenal (1957) has stated that fish length, being easier to measure in the field, is more suitable to make prediction of fecundity when large samples are to be dealt within limited time. Fecundity in *Garra surendranathanii* was found to be almost close to the cube of length and directly proportional to the fish weight and these results would be invaluable in enumerating the fecundity without sacrificing the specimens.

Chapter 5

Captive Breeding and Breeding Behaviour

Contents

5.1 Introduction

Many factors, including habitat degradation, over harvesting, exotic species introductions, and other anthropogenic influences, have severely reduced the range or population size of many species, where as some species are considered as threatened due to their restricted area of distribution. Species recovery efforts generally focus on *in situ* actions such as habitat protection. However, supplemental breeding is an intensive population management strategy wherein adults are captured from nature and spawned in controlled settings, and the resulting offspring are later released into the wild (Fiumera *et al.*, 2004) and is a good conservation method for endangered freshwater fish species (Philippart, 1995; Poncin and Philippart, 2002). Captive breeding also can provide critical life history information, as well as helping supplement of existing or restoring extirpated populations and allows discovery of important behavioral or life history characteristics that may constrain reproduction of rare species in altered natural habitats. (Rakes *et al.*, 1999).

A major breakthrough in fish breeding research was the finding that dopamine acts as an inhibitory factor for synthesis of gonadotropins (Peter *et al.*, 1998). Several hormones and chemical agents intervene at different levels of the hypothalamus-pituitary-ovarian axis in maturation and breeding. Research on these aspects had lead to the development of artificial breeding techniques through hypophysation mainly with pituitary extract and was accepted worldwide. But, using pituitary glands for the same has some disadvantages like difficulty in standardizing its gonadotropic potency. Hence, alternative sources like Human Chorionic Gonadotropin (HCG) (Inyang and Hittiarachehi, 1994; Kurup, 1998; Haniffa *et al.*, 2000), Ovaprim (Kurup, 1998; Haniffa *et al.*, 1996, 2000), Ovatide (Das, 2000; Mijkherjee *et al.*, 2002) etc. have been employed for the same. Since, gonadal maturation ultimately depends on the endocrine system, hormonal manipulation appears as the most direct approach to broodstock development.

The knowledge about the reproduction modes of the species is important in developing conservation strategies (Johnston, 1999). A breeding behaviour, which may be lasted only for a second can reveal an animal's adaptations for spawning and can be a major breakthrough in developing its captive breeding. Thus, studies on spawning behavior have a far wider application than has commonly been supposed.

Even though the family Cyprinidae is the largest family of freshwater fishes (Nelson, 1994), many cyprinid species endemic to Western Ghats are classified as endangered. *Garra surendranathanii* is an endemic fish of Kerala part of Western Ghats. This species is listed as threatened in all the recent studies (Table 2:1). It has a restricted distribution in five rivers and is showing high habitat preference (Kurup, 2002). Since, breeding under captivity is

considered as a major bottleneck in most of the conservation programmes, the present study was aimed to breed this threatened fish using different hormones and to analyze the effects of different doses on its breeding performance. The reproductive behaviour of *G. surendranathanii* in captivity was also studied.

5.2 Materials and Methods

5.2.1 Spawning Migration

Observations were made at collection sites on the chances of spawning migration in *G. surendranathanii* as in the case of many hill stream fishes.

5.2.2 Collection, transportation and acclimatization

Live specimens of *G. surendranathanii* were collected using cast net from River Periyar, from the upstreams of Pooyamkutty tributary ($09^0$57'51N and $076^0$16'58E), Western Ghats, Kerala during late evening and night hours (Fig. 5.1). The fishes were packed in oxygenated bags filled with river water and transported to the School of Industrial Fisheries, Cochin University of Science and Technology, Kochi. Transportation of captured fishes was carried out in LDPE bags. Each bag was then placed in master cartons and brought to the laboratory under normal atmospheric temperature. The fishes are dipped in 10ppm potassium permanganate solution and were released to large FRP storage tanks containing dechlorinated tap water with aeration for acclimatisation. Injured fishes were separated. The tanks were covered with nets to prevent fishes jumping out of the water. Feeding was restricted to once daily during acclimatisation period. After 1 week, fishes were transferred in to glass tanks (3x1.25x1.5 feet) provided with biological filtration systems and continuous aeration (Fig.5.2). Since, these fishes prefer to rest/ hide in-between the stones, sufficient number of boulders / rock pieces were provided in all the tanks.

Fig. 5.1 Brood stock collection of *G. surendranathanii*

Fig. 5.2 Breeding tanks

5.2.3 Brood Stock maintenance

In the successful captive breeding and larval rearing experiments of the related species *Garra ceylonensis*, feeds such as dried swine liver, artemia and also formulated feeds were given (Sundarabarathy *et al.*, 2005). Brood Fishes of *Garra surendranathanii* were fed with mosquito larvae as major and commercial pellet feed. frozen tubifex worms and fresh prawn meat to check its adaptability to these food items. Periodical assessment of water quality parameters such as temperature (28 ± 2^{0}C), DO (5.9±0.2mg/l) and pH (6.8 – 7.2) were recorded.

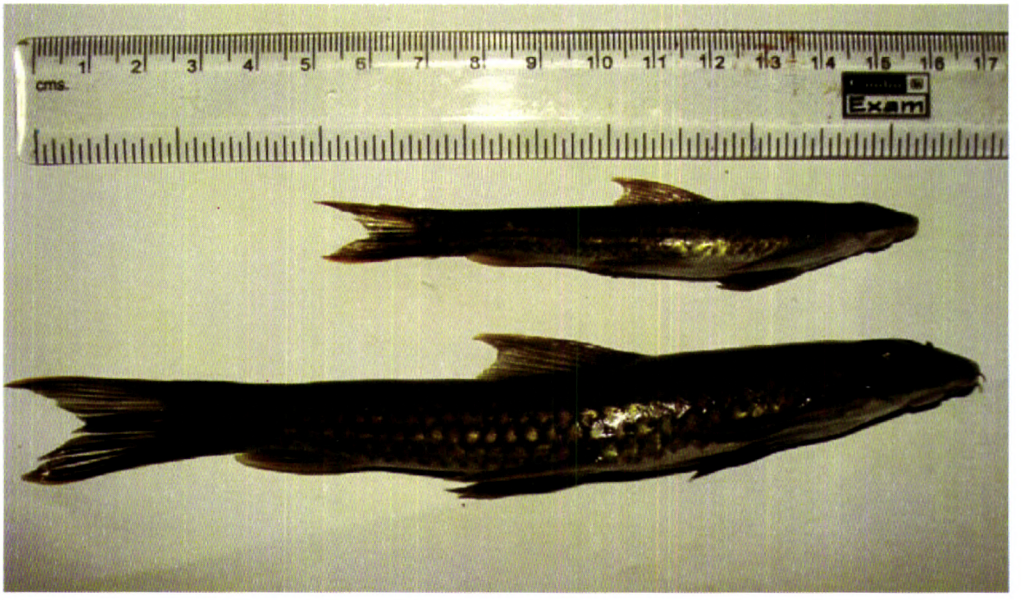

Fig. 5.3. Male (small) and female (Large) *G. surendranathanii*

5.2.4 Induced breeding

Mature healthy males and females were selected based on external morphological characters. (Table: 5.1). In addition, the eggs oozed by gently pressing the ventral side of the fish were observed for confirming the maturity of female and selected based on the position of the nucleus germinal vesicle

(Wallace and Selman, 1978). The position of the nucleus was observed under Hertel & Reuss microscope (Germine).

Table. 5.1 Sexual dimorphism in *G. surendranathanii* during breeding season

Sl. No	Male	Female
1	Size –small	Large
2	Body short and slender	Body long and fleshy
3	Abdomen normal	Conspicuously bulging abdomen
4	Genital papilla elongated and pointed	Genital papilla raised, prominent and oval in shape

Migration of the nucleus to the periphery indicated the ripeness of the egg and the readiness of the fish for spawning. The fish showing the highest percentage of mature oocytes having germinal vesicle (GV) either in the centre or in the initial stage of migration were selected for the hormonal treatment (Billard *et al.*, 1984)

Free oozing males and ripe female were taken in the ratio of 2:1, respectively, for breeding. Three types of synthetic hormones viz. Ovaprim, HCG and Ovatide were tested independently in 3 doses for induced breeding. Fishes were taken in a wet cloth to avoid maximum stress and the hormones were injected intramuscularly to the base of dorsal fin region (Fig. 5.4) of both males and females in a single dose. Immediately after administering the hormone, the brooders were released back to the glass tanks, provided with stones for hiding purposes. Three sets of experiments were conducted for three different doses (Table.5.2) in separate tanks. A control was also maintained where distilled water in same doses was injected. Immediately after injections,

each set (two males and one female) was released to glass tanks provided with boulders rock pieces as substratum and high aeration was given using air stone as well as jet pump.

Table.5.2 Different doses of hormones used for induced breeding of *G. surendranathanii*

1. Ovaprim		
	Low dose (LD)	0.1 ml
	Medium dose (MD)	0.2 ml
	High dose (HD)	0.3 ml
2. HCG		
	Low dose (LD)	1000 IU
	Medium dose (MD)	2000 IU
	High dose (HD)	3000 IU
3. Ovatide		
	Low dose (LD)	0.1 ml
	Medium dose (MD)	0.2 ml
	High dose (HD)	0.3 ml

Fig. 5.4. Intramuscular injection

5.2.5 Breeding Behaviour

The breeding behaviour was documented using Canon A 610 Digital camera cum movie recorder at intervals of one hour. The behaviour patterns were studied by frame-by-frame analysis of video footages.

5.2.6 Breeding response and collection of eggs

The brooders were monitored for their response to hormone administration. After spawning, eggs were collected from each tank and the percentage of fertilization, hatching and survival were estimated following Lagler (1956) and Sarkar *et al.* (2005) and examining at least 100 eggs from each tank. A few hundred eggs were allowed to hatch and grow along with parents in the breeding compartment to observe the parental care.

5.2.7 Statistical Analysis

The effect of different doses of Ovaprim, HCG and Ovatide on the latency period, hatching period and fertilization rate in *G. surendranathanii* was studied and the percentage fertilization rates were arcsine-square root transformed prior to statistical analysis. Data were analysed by one-way ANOVA and two-way ANOVA. When, one way ANOVA showed differences among means, multiple comparisons were made using Tukey's multiple range test (Zar, 1984)

5.3 Results

5.3.1 Spawning migration

During breeding season, i.e., October to December, the fishes had shown breeding migration to nearby canals of the main river. The habitat observed in these canals was the presence of boulders, rocks and the water was shallow (Fig.5.5). The fishes tend to move upwards against the water current. All the fishes observed were mature and the result was confirmed by the presence of

larvae and juveniles in the upstream of these channels in the months of November to February. Another interesting observation made was, only 10% of the population was females and the rest was males.

Fig. 5.5 Spawning ground of *G. surendranathanii*

5.3.2 Food and Feeding

Garra surendranathanii became well accepted mosquito larvae and frozen tubifex worms and adapted to pellet feeds and fresh shrimp meat in 1 week time. No mortality observed during the stocking period.

5.3.3 Breeding Behaviour

Initially, all the 3 fishes were rested on the bottom of the tank in different locations without any spawning activity. After three hours of hormone administration, one male fish started to move actively and within 15 minutes the second male also became very active. But the female was not attentive to it. By fourth hour the female also started to move when the male fishes started touching its vent region with snout and within 30

minutes, the swimming movements of the male pair became synchronized with the movement of female on both sides. All the three fishes moved to the surface and then to the bottom of the water. They had shown a special tendency to mainly in the area where aeration was given i.e., preferred to swim through the air bubbles. In between the face to face and side to side movements, the males started showing the nipping behaviour by touching the urogenital region of female with its snout and gradually moving to the flanks and ending in the head region.

After 5 hours, males began to quiver the female and the female responded by either continuing to move with no apparent alteration of swimming speed or vertical position, or by stopping and sinking to the bottom. At this point, all the fishes drift slightly forward; no movement of their tails was recorded. Once on the bottom, the female resumed traveling followed by males. At the end of the 10th hour, the frequency of nipping or butting activity by the males increased. Spawning occurred when a male pressed the female against substratum i.e. rock pieces at the corner of the tank with the individuals keeping their bodies laterally compressed against each other and simultaneously the other male nibbled the female's vent region. This was lasted for 2-3 seconds and female released the eggs with a trembling movement and a batch of eggs was released. This activity continued till the spawning was completed which took about 2-3 hrs. The eggs were scattered and non sticky. The different stages of development seen in the eggs collected from the same brood fish indicated that the eggs were released in batches during the prolonged spawning act. It was observed that the female is spawning simultaneously with two males and inter-male aggression was never observed (Fig. 5.6).

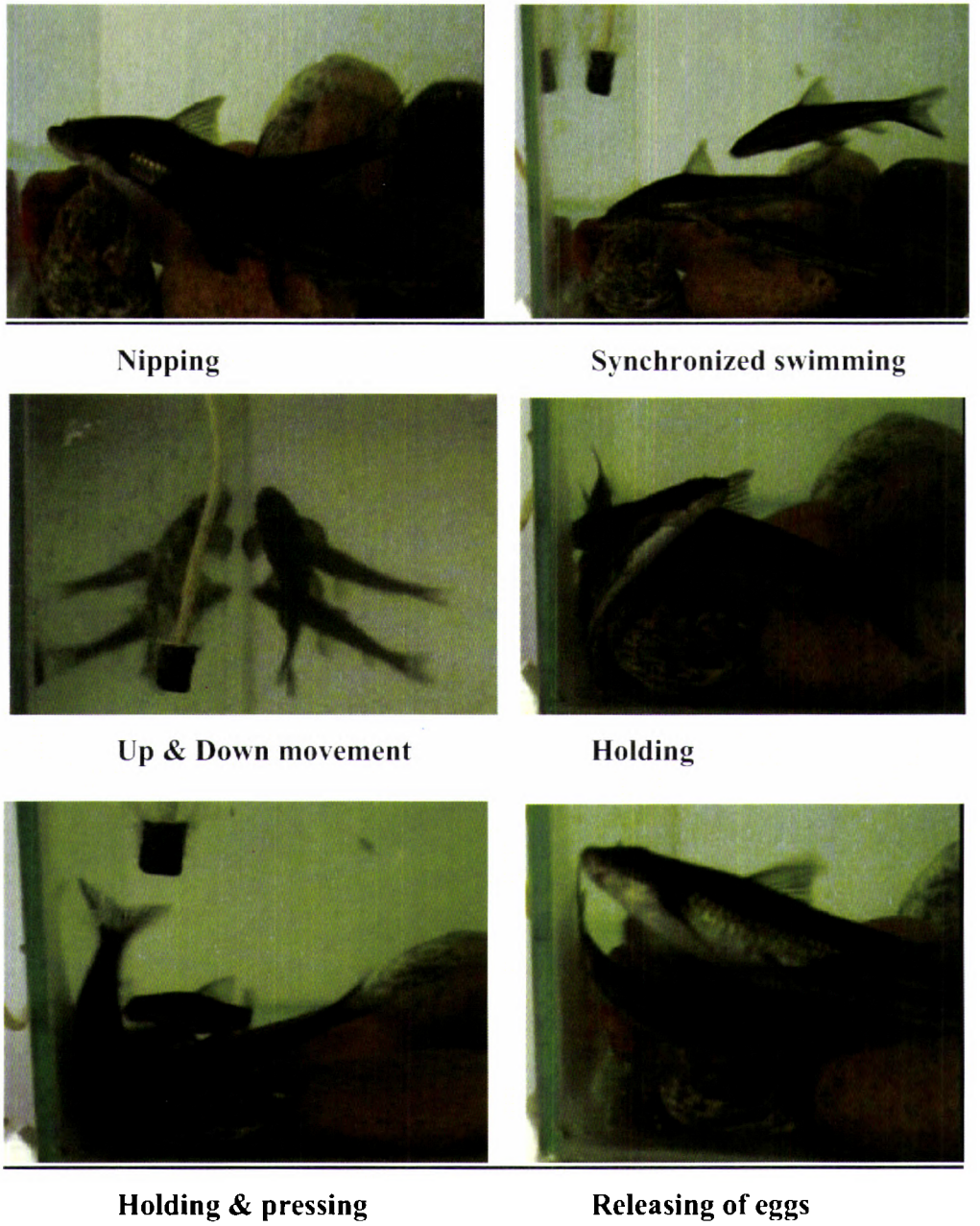

Fig. 5.6. Breeding behaviors of *G. surendranathanii* (Large sized- Female, Small-Males)

5.3.4 Parental care

No parental care was noticed in breeders of *G. surendranathanii*. After spawning, neither female nor males showed any sign of aggressiveness and remained in different locations of the breeding tank.

5.3.5 Effect of hormones on induced breeding

Effects of different doses (low, medium and high) of Ovaprim, HCG and Ovatide on the induced spawning of *G. surendranathanii* were investigated. No spawning was observed in control *i.e* fishes injected with distilled water. The results of the induced spawning experiments are given in Table 5.3

Low dose of Ovaprim (0.1ml/kg bw) resulted in high latency period (14.2 ± 0.3 hrs) whereas it was low (10.3 ± 0.3 hrs) in high dose (0.5 ml/kg bw). An inverse trend was noticed between the dose and latency period. Between low and high doses, latency period was significantly different ($P < 0.05$) and a significant relationship was noticed between all pairs of Doses (Table: 5.4). The hatching period ranged from 35.2 ± 0.3 to 36.9 ± 0.4 hrs among the different doses of hormone. The low dose Ovaprim produced high hatching period (36.9 ± 0.4) whereas in the high dose Ovaprim injected individuals, the same was low (35.2 ± 0.3). Difference in the hatching period due to increase in dose of hormone was statistically significant, but the differences between medium and high doses were not significant (Table: 5.5). The fertilization rate (90.1 ± 1.1 %), hatching rate (88.8 ± 0.4%) and survival rate of hatchlings (87.5 ± 0.6) were high in medium dose injected individuals. The fertilization rate was significantly different ($P< 0.05$) among low, medium and high doses and the difference between all the pairs of dose were again significant.

Table 5.3. Effects of different hormones on induced breeding in *G. surendranathanii*

Hormone	Weight of Spawners (g)		Dosage of Hormone (/kg)	Latency Period (h)	hatching Period (h)	Fertilization (%)	Hatching (%)	Survival (%)
	Male	Female						
Ovaprim	12.33 14.67	37.33	0.1ml	14.2 ± 0.3	36.9 ± 0.4	80.3 ± 2.7	80.0 ± 1.0	80.5 ± 1.0
	11.00 12.00	35.33	0.3ml	11.60 ± 0.4	35.80 ± 0.5	90.1 ± 1.1	88.8 ± 0.4	87.5 ± 0.6
	11.67 11.67	38.33	0.5ml	10.3 ± 0.3	35.2 ± 0.3	86.1 ± 0.8	83.8 ± 0.7	79.4 ± 0.5
HCG	12.67 14.67	37.67	1000 IU	13.2 ± 0.3	37.3 ± 0.4	75.6 ± 1.2	70.6 ± 0.7	68.0 ± 0.5
	10.67 11.67	35.67	2000 IU	11.1 ± 0.2	36.1 ± 0.1	85.9 ± 1.0	83.8 ± 0.9	81.9 ± 0.5
	12.00 11.00	36.33	3000 IU	9.9 ± 0.4	35.2 ± 0.3	76.8 ± 0.7	67.7 ± 0.9	64.3 ± 1.1
Ovatide	12.00 12.33	36.00	0.1ml	18.6 ± 0.4	38.0 ± 0.2	64.8 ± 0.2	60.5 ± 1.2	56.0 ± 2.8
	11.33 12.33	34.33	0.3ml	18.1 ± 0.1	37.4 ± 0.2	81.8 ± 0.9	76.8 ± 2.1	73.6 ± 0.2
	13.00 12.00	36.00	0.5ml	17.6 ± 0.4	37.1 ± 0.1	73.7 ± 1.1	67.4 ± 1.6	62.8 ± 1.6

Table. 5.4 One way ANOVA showing the effect of different Doses of Ovaprim on the Latency Period of *G. surendranathanii*. The means were compared by Tukey's Multiple Range Test.

Source of Variation	Sum of Squares	df	Mean Square	F-value	P-ılue
Dose	24.009	2	12.004	127.106	0.000
Error	0.567	6	0.094		
Total	24.576	8			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
LD Vs MD	2.600	0.251	0.000	Reject $\mu_{LD} = \mu_{MD}$
LD Vs HD	3.933	0.251	0.000	Reject $\mu_{LD} = \mu_{HD}$
MD Vs HD	1.333	0251	0.004	Reject $\mu_{MD} = \mu_{HD}$

Table. 5.5. One way ANOVA showing the effect of different Doses of Ovaprim on the Hatching Period of *G. surendranathanii*. The means were compared by Tukey's Multiple Range Test.

Source of Variation	Sum of Squares	df	Mean Square	F-value	P-value
Dose	4.096	2	2.048	13.355	0.006
Error	0.920	6	0.153		
Total	5.016	8			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
LD Vs MD	1.033	0.320	0.041	Reject $\mu_{LD} = \mu_{MD}$
LD Vs HD	1.633	0.320	0.005	Reject $\mu_{LD} = \mu_{HD}$
MD Vs HD	0.600	0.320	0.225	Accept $\mu_{MD} = \mu_{HD}$

Table. 5.6. One way ANOVA showing the effect of different Doses of Ovaprim on the Fertilization of *G. surendranathanii*. The means were compared by Tukey's Multiple Range Test.

Source of Variation	Sum of Squares	df	Mean Square	F-value	P-value
Dose	96.390	2	48.195	26.283	0.001
Error	11.002	6	1.834		
Total	107.392	8			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
LD Vs MD	-8.002	1.106	0.001	Reject $\mu_{LD} = \mu_{MD}$
LD Vs HD	-4.406	1.106	0.017	Reject $\mu_{LD} = \mu_{HD}$
MD Vs HD	3.597	1.106	0.040	Reject $\mu_{MD} = \mu_{HD}$

In different doses of HCG, latency period for low, medium and high doses were 13.2 ± 0.3, 11.1 ± 0.2 and 9.9 ± 0.4 hrs respectively. Hatching period varied from 35.2 ± 0.3 to 37.3 ± 0.4 for three different doses. Fertilization, hatching and survival rate at hatching were high (85.9 ± 1.0, 83.8 ± 0.9 and 81.9 ± 0.5 % respectively) in medium dose injected individuals compared to low and high doses. There was significant difference between ($P < 0.05$) low, medium and high doses of latency period and a significant difference in all the pairs too (Table.5.7). Again, there was significant difference ($P < 0.05$) between low, medium and high doses of hatching period and a significant difference in all the pairs (Table: 5.8). Statistical analysis indicated that significance difference ($P < 0.05$) was found between the low dose and medium dose fertilization rate of HCG injected individuals compared to the high dose and low dose where no significant difference was noticed (Table:5.9).

Table. 5. 7. One way ANOVA showing the effect of different Doses of HCG on the Latency Period of *G. surendranathanii*. The means were compared by Tukey's Multiple Range Test.

Source of Variation	Sum of Squares	df	Mean Square	F-value	P-value
Dose	17.102	2	8.551	114.866	0.000
Error	0.447	6	0.074		
Total	17.549	8			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
LD Vs MD	2.133	0.223	0.000	Reject $\mu_{LD} = \mu_{MD}$
LD Vs HD	3.333	0.223	0.000	Reject $\mu_{LD} = \mu_{HD}$
MD Vs HD	1.200	0.223	0.004	Reject $\mu_{MD} = \mu_{HD}$

Table. 5.8. One way ANOVA showing the effect of different Doses of HCG on the Hatching Period of *G. surendranathanii*. The means were compared by Tukey's Multiple Range Test.

Source of Variation	Sum of Squares	df	Mean Square	F-value	P-value
Dose	6.709	2	3.354	40.253	0.000
Error	0.500	6	0.083		
Total	7.209	8			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
LD Vs MD	1.267	0.236	0.004	Reject $\mu_{LD} = \mu_{MD}$
LD Vs HD	2.100	0.236	0.000	Reject $\mu_{LD} = \mu_{HD}$
MD Vs HD	0.833	0.236	0.028	Reject $\mu_{MD} = \mu_{HD}$

Table. 5.9. One way ANOVA showing the effect of different Doses of HCG on the Fertilization of *G. surendranathanii*. The means were compared by Tukey's Multiple Range Test.

Source of Variation	Sum of Squares	df	Mean Square	F-value	P-value
Dose	102.740	2	51.370	101.317	0.000
Error	3.042	6	0.507		
Total	105.782	8			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
LD Vs MD	-7.454	0.581	.000	Reject $\mu_{LD} = \mu_{MD}$
LD Vs HD	-0.827	0.581	.388	Accept $\mu_{LD} = \mu_{HD}$
MD Vs HD	6.718	0.581	.000	Reject $\mu_{MD} = \mu_{HD}$

Effects of different doses of Ovatide on ovulation and hatching were studied. In the present study, the latency and hatching periods were more in low dose (18.6 ± 0.4 and 38.0 ± 0.2 hrs respectively) injected fishes and less in high dose(17.6 ± 0.4 and 37.1 ± 0.1 hrs respectively) injections. The difference in latency period was statistically significant (Table: 5.10). Moreover, the hatching period was significantly different ($P<0.05$) among the different doses. The differences between medium and high doses were significant in hatching period (Table: 5.11). The fertilization rate, hatching rate and survival rates were achieved high (81.8 ± 0.9, 76.8 ± 2.1 and 73.6 ± 0.2 % respectively) in medium dose injected fishes but the same was poor in (64.8 ± 0.2, 60.5 ± 1.2 and 56.0 ± 2.8% respectively) in low dose injected fishes. Fertilization rate was significantly different ($P <0.05$) between all the pairs of dosages (Table: 5.12)

Table. 5.10. One way ANOVA showing the effect of different Doses of Ovatide on the Latency Period of *G. surendranathanii*. The means were compared by Tukey's Multiple Range Test.

Source of Variation	Sum of Squares	df	Mean Square	F-value	P-value
Dose	1.602	2	0.801	8.101	0.020
Error	0.593	6	0.099		
Total	2.195	8			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
LD Vs MD	0.533	0.257	0.175	Accept $\mu_{LD} = \mu_{MD}$
LD Vs HD	1.033	0.257	0.016	Reject $\mu_{LD} = \mu_{HD}$
MD Vs HD	0.500	0.257	0.206	Accept $\mu_{MD} = \mu_{HD}$

Table. 5.11. One way ANOVA showing the effect of different Doses of Ovatide on the Hatching Period of *G. surendranathanii*. The means were compared by Tukey's Multiple Range Test.

Source of Variation	Sum of Squares	df	Mean Square	F-value	P-value
Dose	1.362	2	0.681	26.652	0.001
Error	0.153	6	0.026		
Total	1.515	8			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
LD Vs MD	0.633	0.131	0.007	Reject $\mu_{LD} = \mu_{MD}$
LD Vs HD	0.933	0.131	0.001	Reject $\mu_{LD} = \mu_{HD}$
MD Vs HD	0.300	0.131	0.132	Accept $\mu_{MD} = \mu_{HD}$

Table. 5.12. One way ANOVA showing the effect of different Doses of Ovatide on the Fertilization of *G. surendranathanii*. The means were compared by Tukey's Multiple Range Test.

Source of Variation	Sum of Squares	df	Mean Square	F-value	P-value
Dose	187.737	2	93.868	299.171	0.000
Error	1.883	6	0.314		
Total	189.619	8			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
LD Vs MD	-11.187	0.457	0.000	Reject $\mu_{LD} = \mu_{MD}$
LD Vs HD	-5.561	0.457	0.000	Reject $\mu_{LD} = \mu_{HD}$
MD Vs HD	5.626	0.457	0.000	Reject $\mu_{MD} = \mu_{HD}$

The hatching period was more or less similar (Table.5.3) in all the treatments. A common trend was noticed among the treatments *i.e.* the medium dose of all hormones produced better results of fertilisation, hatching and survival rate. Among the different hormones, Ovaprim was highly effective. Statistical analysis indicated that, there was a significant difference ($P<0.05$) in the latency period in Ovatide between ovaprim / HCG where as the difference was not found between Ovaprim and HCG. Two way ANOVA (Table. 5.13) also confirmed the same trend. Similar results were found in the hatching period also (Table. 5.14). Analysis by two way ANOVA showed that the difference in fertilization rate was statistically significant ($P< 0.05$) between all the 3 hormones (Table.5.15). Means were compared by Tukey's Multiple Range Test.

Table. 5.13. Two-way ANOVA showing the effect of different Doses of Ovaprim, HCG and Ovatide on the latency Period of *G. surendranathanii*. The Tukey's Multiple Range Test.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Hormone	165.014	1	165.014	397.890	0.000
Dose	36.026	4	9.006	21.717	0.000
Error	8.294	20	0.415		
Total	209.334	25			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
Ovaprim Vs HCG	0.611	0.304	0.135	Accept $\mu_{OP} = \mu_{HCG}$
Ovaprim Vs Ovatide	-6.056	0.304	0.000	Reject $\mu_{OT} = \mu_{HCG}$
HCG Vs Ovatide	-6.667	0.304	0.000	Reject $\mu_{HCG} = \mu_{OT}$

Table. 5.14. Two-way ANOVA showing the effect of different Doses of Ovaprim, HCG and Ovatide on the Hatching Period of *G. surendranathanii*. The means were compared by Tukey's Multiple Range Test.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Hormone	10.125	1	10.125	104.202	0.000
Dose	11.797	4	2.949	30.352	0.000
Error	1.943	20	0.097		
Total	23.865	25			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
Ovaprim Vs HCG	-0.233	0.147	0.274	Accept $\mu_{OP} = \mu_{HCG}$
Ovaprim Vs Ovatide	-1.500	0.147	0.000	Reject $\mu_{OT} = \mu_{HCG}$
HCG Vs Ovatide	-1.267	0.147	0.000	Reject $\mu_{HCG} = \mu_{OT}$

Table. 5.15. Two-way ANOVA showing the effect of different Doses of Ovaprim, HCG and Ovatide on the Fertilization of *G. surendranathanii*. The means were compared by Tukey's Multiple Range Test.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Hormone	338.550	1	338.550	285.404	0.000
Dose	379.070	4	94.767	79.891	0.000
Error	23.724	20	1.186		
Total	741.344	25			

Comparison	Mean Difference	Std. Error	P-value	Conclusion
Ovaprim Vs HCG	4.674	0.513	0.000	Reject $\mu_{OP} = \mu_{HCG}$
Ovaprim Vs Ovatide	8.674	0.513	0.000	Reject $\mu_{OT} = \mu_{HCG}$
HCG Vs Ovatide	4.000	0.513	0.000	Reject $\mu_{HCG} = \mu_{OT}$

5.4 Discussion

5.4.1 Spawning migration

The spawning migration of the hill stream fishes to upstream was reported by many. Thomas (1897) noted mahseer travelling long distances for the sake of food and spawning and the migration of mature fish in relation to the breeding season was later mentioned by Cordington (1946). Desai (1973) states that the Tor mahseer showing breeding migration to upper reaches when the monsoon showers cause flooding in River Narmada. From the observation made during the present study related to the breeding behaviour of this fish in captivity, *G. surendranathanii* prefers a habitat with boulders / rocks for its breeding. Presence of clear water with high oxygen in the small canals during rainy season may be another habitat requirement essential for its breeding. In

captivity also, the fishes showed tendency to spend more time in the areas were more aeration was given.

5.4.2 Breeding behaviour

This study describes for the first time the reproductive behaviour and the spawning tactics of *G.surendranathanii* and provides data on a potential technique for breeding this fish species in captivity. The increase of the general activity of fishes in the onset of the breeding episode observed was also described as an indicator for several cyprinid species (e.g. Svardson (1952), *Rutilus rutilus*; Breder & Rosen (1966) *Cyprinus carpio*). Cyprinid fishes in general, exhibit some common patterns in their courtship and reproductive behaviour (Turner, 1993; Mercy *et al.,* 2003). But, one of the salient features breeding behaviour of *G. surendranathanii* is the absence of agonistic behaviour in male-male interactions, also related to absence of territoriality which was not very common in cyprinids. But, the same type of co-operation was observed in the endangered Iberian cyprinid *Chondrostoma lusitanicum* (Carvalho *et al.*, 2002). Simultaneous mating of a female with more males was also seen in *L. ghigii* which is considered as polygamous i.e. each male can mate successively with several females and each female can mate simultaneously and successively with several males (Turner, 1986). This character is agreeing with the observations made during the present study that, during breeding season *G. surendranathanii* migrates to small streams and in the migrating population, only 10% of the fishes were females and the rest 90% are males.

The reproduction mode observed is broadcasting (e.g. release and abandonment of eggs and sperm over an unprepared substrate; Johnston, 1999), common to many other cyprinids like C. *lusitanicum* (Carvalho *et al.,* 2002),

north American species of minnows and for fishes in general (Johnston & Page, 1992). The spawning sequences described above seem to be specially suited for the type of environment to which this species migrates for breeding like shallow streams with plenty of rocks (Fig .....) and these rocks may be playing a crucial role as substratum for breeding. Spending more time in the aeration zone also showing its preferred breeding habitat in wild with high oxygen.

5.4.3 Parental Care

Parental care of the eggs and hatchlings by the male and female breeders was not observed in *G. surendranathanii* like in the case of most of the tropical cyprinids.

5.4.4 Effect of hormones on induced breeding

G. surendranathanii is an important species mainly due to its threatened status as well as endemism. Another important factor is its projection as a potential species for ornamental fish industry. These fishes didn't spawn spontaneously when held in captivity. To facilitate the breeding of this species, oocyte maturation and ovulation need to be induced. Maturation of gonads is stimulated by factors such as environmental parameters, feed quality and administration of hormones. Among these factors, injection of hormones is assumes as a major role in the maturation and ovulation in teleosts (Bhattacharya, 1999). Various hormones were used to for inducing maturation and spawning in a variety of cyprinids especially carps.

Higher latency period was observed in all the 3 hormones at the dose of high dose indicates difference in the mode of action of the hormone. Similar observation was reported by Habibi *et al.* (1989) in *Carassius auratus* and Haniffa *et al.* (2000) in *Channa striatus*. In all the three hormones, the latency

period was less in the highest dose was injected. But, the hatching period was almost same in case of all the hormones as well as for doses.

Ovaprim has been adjusted as the most superior inducing agent for carps and also characterised with high spawning success, percentage of fertilization and hatching rate (Nandeesha *et al.,* 1990). *Labeo dussumieri* when induced bred with Ovaprim, carp pituitary extract (CPE) combination of CPE and HCG and LHRH and pimozide, the best result was observed in Ovaprim induced fishes (Kurup, 2001). The same result was also found in *Channa striatus* when induced bred with Ovaprim, LHRHap, pituitary extract and HCG (Haniffa *et al.*, 2000). Due to its high success rate, Ovaprim is the most used commercial spawning aid by the producers of ornamental cyprinids in United States (Hill *et al.*, 2009). The experiments showed that, *G. surendranathanii* can be bred successfully with all the 3 hormones used but the results vary with hormones and doses. The best hormone identified during the study was Ovaprim at a dosage of 0.3 ml/kg body weight and can be recommended good results in this fish.

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Chapter 6

Developmental Biology

Contents

6.1 Introduction

Embryonic and larval developmental studies provide sufficient information regarding the successful rearing of larvae (Mathew *et al.*, 1996). Refining the techniques of larval rearing is very important for practical and commercial applications (Liao, 1993). A good number of reports are available on the early life stages of fishes and their characteristics (Kendall *et al.*, 1984; Kimmel *et al.*, 1995; Arockiaraj *et al.*, 2003; Schmidt and Starck., 2004). Great variation in size exists among species in young fishes before they become free living forms [free from yolk sac (after 3-5 days)] (Diwan and Dhakad, 1995). Apart from the academic interest, experimental based information on these early stages is required for progress in the advanced fields of fish culture.

Despite the success in artificial propagation of *G. surendranathanii*, by induced spawning (chapter 5), it is necessary to understand the developmental biology of the species which is a prerequisite for successful rearing of the species and commercialization of the induced breeding techniques. It is an undisputed fact that larval rearing remains the most critical and crucial phase to

decide the success of freshwater fish for seed production. Development of suitable protocols for the mass rearing of larval fish represents one of the important barriers for the successful propagation of most of the freshwater species (Thakur *et al.*, 1974; Thakur, 1980). The main problems arise in larval fish rearing is the relatively smaller size of the mouth and limited yolk reserves of the larvae (Shirota, 1970).

The rearing of early life stages by fish culturists is important because the requirement of young fish change rapidly with every hours or days. Working definitions of developmental stages for aquaculture are, therefore, practically very useful (Haylor, 1992). Breeding of *G. surendranathanii* is a pioneering effort and, hence the details of developmental biology of this fish are scarce and hence taken up. The literature on the related species was that of captive breeding of *Garra cylonensis* (Sundarabarathy *et al.*, 2005) and even in that, the developmental biology is unaddressed. The present chapter is an attempt to explain the developmental stages of *G. surendranathanii*

6.2 Materials and Methods

After spawning, the developing eggs were carefully collected from the breeding compartments using a dropper and transferred to aerated tubs of 10 liter capacity each at a density of 200 eggs per tub (temperature (28 ± 1^0C), DO (5.9±0.2mg/l) and pH (6.8 – 7.2)). The developing eggs were sampled every 30-min interval till hatching and every 3 hours for the next 3 days and thereafter only once in a day at 9 a.m. The observation was continued for two months until the fingerling attained shape and characters of adult. Descriptions of the developing stages were made on the basis of examining live specimens under a trinocular sterio microscope (Nikon SMZ 800,) and microphotographs of the developmental stages of eggs and larvae were taken.

6.3 Results

6.3.1 Description of fertilized eggs:

Fertilized eggs of *G. surendranathanii* are almost rounded, translucent, demersal, non- adhesive containing a proportionately large amount of yolk. The individual egg appears to be pearly and the diameter of the fully swollen fertilized eggs averaged 2.46mm.

6.3.2 Development of the Embryo

First cleavage was observed 30 minutes after fertilization. Two celled embryo formed after 40 minutes transformed in to a four celled embryo in 55 minutes which is seen shrunk within the egg creating more yolk-free space. Within 2 hours after fertilization, the embryo became 32 celled and transform to multi-celled (more than 64-celled) in 3.35 hours. At this stage the dividing cells looks like a cap placed on the animal pole. At 5^{th} hour the fast dividing cells at the periphery of animal pole begin to epibolize over the yolk, it seems like a sac about to cover a sphere. During this time it was also noticed that the yolk part of the embryo began to shrunk, so that the whole embryo became elongated. Also beginning of organogenesis was commenced during this period and after 10 hours the embryo became more elongated and the presumptive head region can be well distinguished at this period. At 12 hours somites are well distinguishable and the embryo attains a characteristic elongate and curved shape. Soon after 12 hours the embryo was found to be much more elongated with a curved pear shaped yolk region. Tail gets differentiated and the

development of eyes can be well noticeable during this time. After 13 hours the tail started developing sideways and the whole embryo became more elongated. At seventeenth hour, the shape of yolk sac was changed to almost spherical shape with a narrow bud like extension on one side and the embryo exhibited wriggling movements within the egg. At twenty first hours the embryo was found to be more active, and the whole egg was changed in to an elliptical shape with one side more rounded. Shape of the yolk was peculiar during this period ; it has a bulbous anterior region with a narrow and elongated posterior part which lies in the posterior part of the embryo. The embryo continues to wriggle within the egg and during twenty sixth hours the egg attained a transparent cylindrical shape having both ends irregularly rounded. During this time the posterior region of yolk started to get elongated. Eyes became clearly distinguishable during 32 hours and the post yolk region of the embryo was further elongated and pointed at the end Egg membrane was found to getting its shape continuously changed as the embryo keeping on struggling inside. Eggs were found hatched during 36 hours post fertilization The major developmental features (Fig 6.1) and the respective time period of *Garra surendranathanii* is given in and Table 6:1.

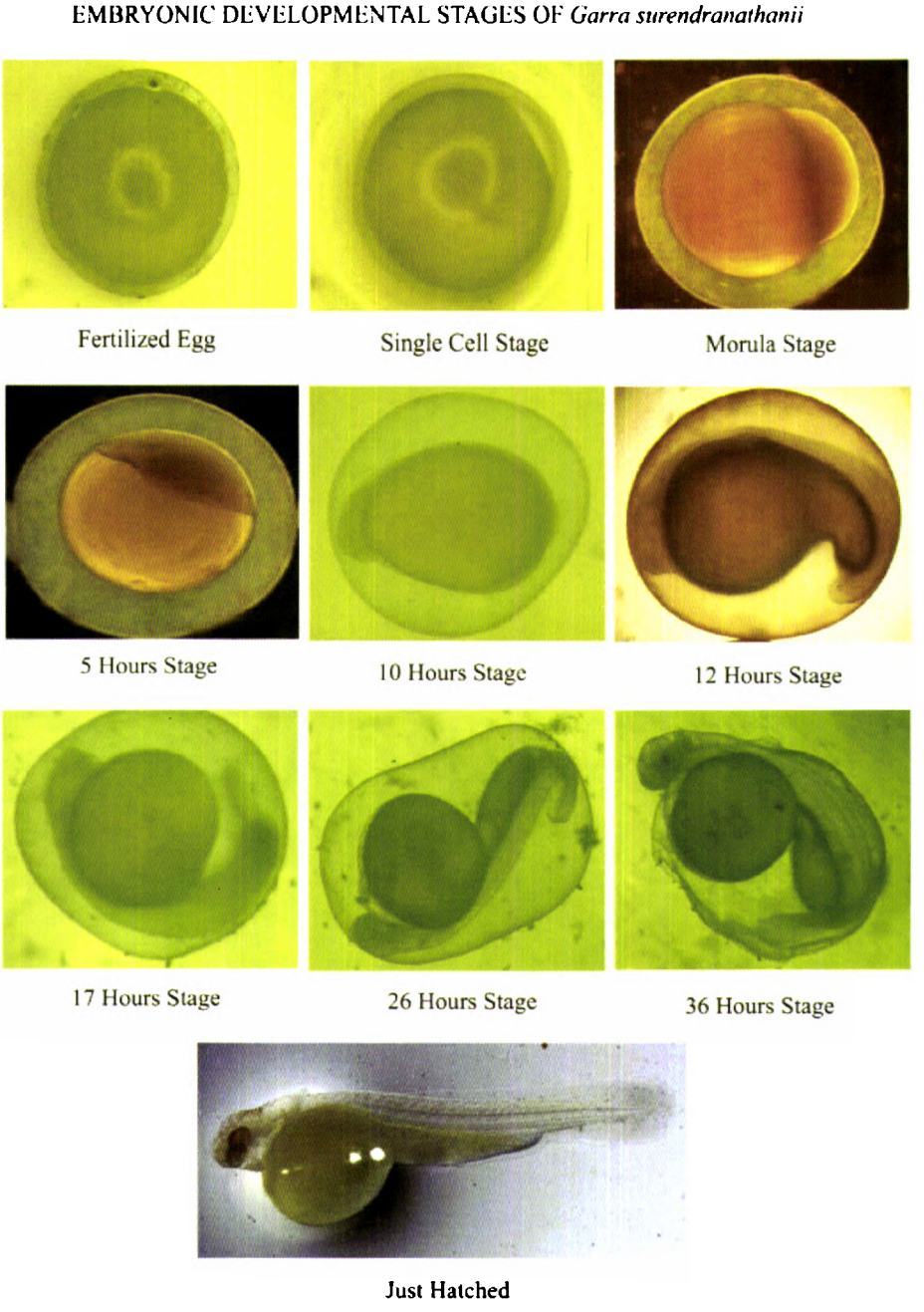


Fig. 6.1. Embryonic developmental stages of *G. surendranathanii*

Table. 6.1. Development of fertilized egg of *G. surendranathanii*

Developmental Features	**Hours after fertilization**
First cleavage	0.30
Second cleavage	0.40
4 celled stage	0.55
6 celled stage	1.05
8 celled stage	1.20
16 celled stage	1.45
32 celled stage	2.00
Morula stage	3.35
Yolk plug stage	5.05
Elongation of the yolk mass	7.30
Appearance of embryonic rudiment	10.25
6 somite stage	11.10
8 somite stage	11.50
12 somite stage	12.35
Elongation of tail	13.30
Appearance of heart rudiment	14.10
Appearance of gill rudiment	15.55
Appearance of pectoral fin buds	16.05
Tail separation& twitching movement of the embryo	17.00
Period of incubation	36 hrs.

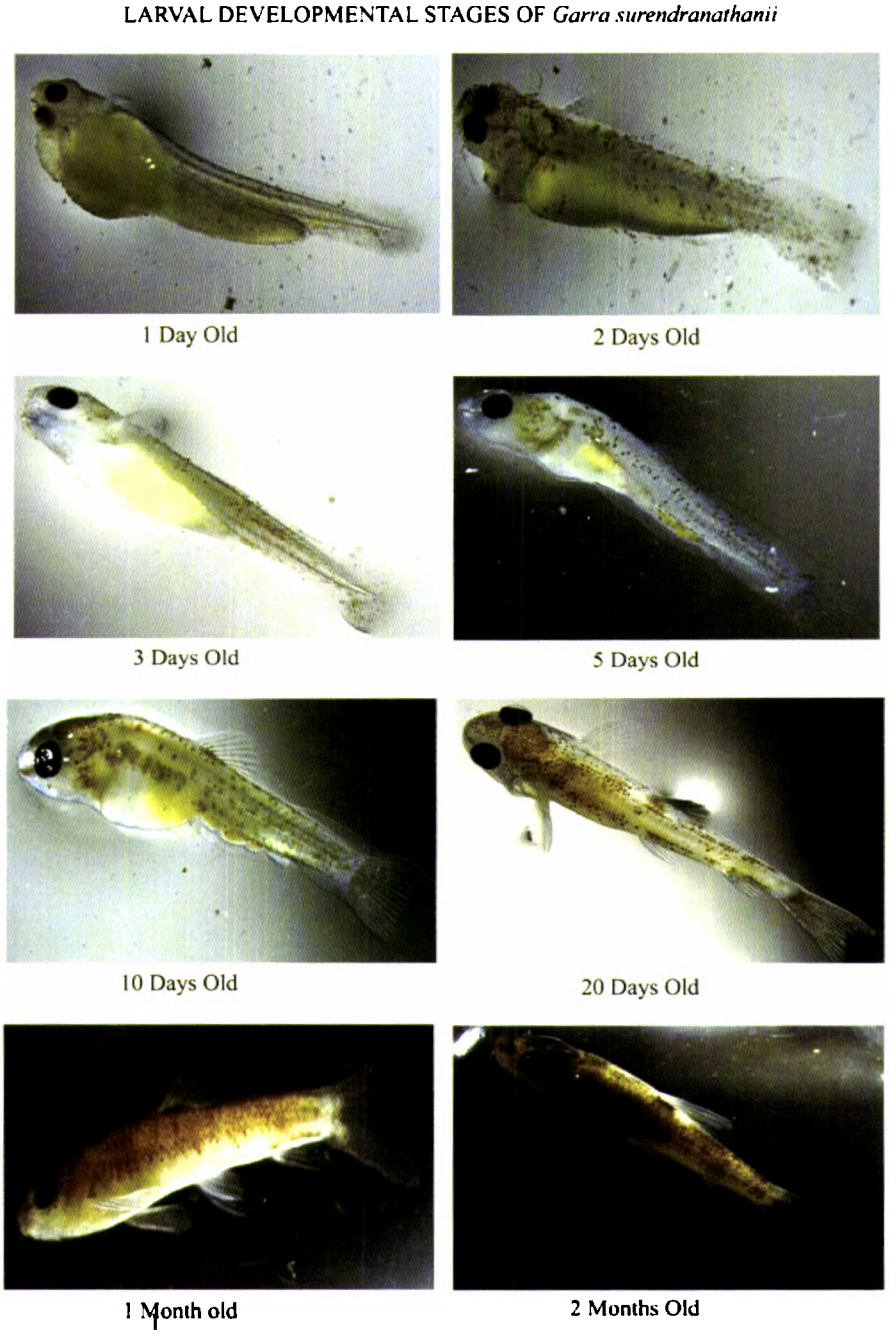


Fig. 6.2. Larval development stages of *G. surendranathanii*

6.3.3 Larval Development

1. **Hatchlings:**

   Average total length is 5.86 mm. Average total yolk length 3.85 mm and yolk width 1.66 mm (bulbus portion).The just hatched larvae is slender, transparent and greenish. The anterior portion of the yolk sac is prominent by bulbous whereas the posterior part is narrow, elongated tubular and ends bluntly in a point. A rudimentary dorsal fin fold is present. Heart is situated anterior to the yolk sac. Optic vesicles are clear. Notochord is distinct with 23 pre-anal and 14 post-anal myotomes

2. **Six hours after hatching:**

   Average total length is 5.94mm. Average total yolk length 3.84 mm and yolk width 1.57 mm (bulbus portion). Chromatophores started developing above the eyes especially on the head region. An irregular darting movement was observed and the larvae frequently touched the bottom.

3. **Twelve hours after hatching:**

   Average total length is 6.08 mm. Average total yolk length 3.72 mm and yolk width 1.48 mm (bulbus portion). Chromatophores on eyes became darker in shade. Rudimentary pectorals appear. Mouth cleft clear. Length of yolk sac reduced.

4. **Twenty four hours (1 day old) after hatching**

   Average total length 6.53 mm. Average total yolk length 3.06 mm and yolk width 1.35 mm (bulbus portion). Head width averaging 1.2mm. Alimentary canal straight. Striations on caudal fin developing. Pectoral fin bud appears. Notochord slightly bend upwards at posterior end. More chromotophores developed all along the dorsal side of body.

5. **Forty eight hours (2 days old) after hatching**

   Total length averaging 6.73 mm. Yolk length 3.1 mm and yolk width 1.05mm. Yolk stared diminishing. Mouth well developed with distinct lips and upper jaw and lower jaw is developed. Anterior profile of yolk sac convex. The eyes become darker. Gill and operculam developed. Alimentary canal is visible.

6. **Seventy two hours (3 days old) after hatching**

   Total length 7.3 mm. Hatchlings greenish yellow. Dorsal profile of the embryo golden yellow. The yolk sac becomes elliptical and slender. Dorsal and ventral fin folds commencing almost from the same level. Gill rakers clear. Dark chromatophores between eyes and extending up to the auditory vesicle. 3-4 rows of black asterisk shaped chromatophores scattered from the auditory vesicle to the tip of the notochord.

6.3.4 Post Larval Development

1. **Five days old :** Total length- 8.1 mm. Rudimentary rays in the form of streaks started appear on both caudal fin and dorsal fin. Yolk is is almost absorbed. Demarcation of Dorsal fin is clear, but still confluent with the caudal fin. Caudal fin rays are clearly visible. Head and dorsal region became greenish yellow in colour.

2. **Ten days old :** Total length 12.2 mm. Dorsal fin with distinct 9 fin rays. Alimentary canal became coiled. Rudimentary pelvics present. Distributions of chromatophores are more over less uneven and dark brown chromatophores are more at the head region. More Chromatophores are seen at the base of the dorsal fin

3. **20 days old.** Total length- 18mm. Lips are thick. Chromatophores at the head region are prominent. Dark chomatophores at the base of caudal fin. Anal fin is well developed with 6-7 rays. One pair of tiny mandibular barbell appears. Head with dark brown tinge. Distinct caudal peduncle.
4. **1 month old.** Total length- 24 mm Chormatophores are more prominent on dorsal side while very few on ventral side. Sucker started developing. Barbels more clear.
5. **2 months old.** Total length – 42mm. At this stage fish resembled adult. The sucker development almost complete. The scale had black edges and appeared like bands. The caudal fin is deeply forked and all other fins are well developed.

6.4 Discussion

Changes in the pattern of whole structure of an organ or specific organ in relation to environment are decisive for evaluating the developmental patterns of a species (Balon, 1999). The ontogenic events during the ovular phase (cleavage stage) did not differ markedly from most of the cyprinid fishes (Kurup, 2001). The shift in structure emphasizes the thresholds between embryonic-larval-post larval development from the onset of cleavage or epiboly or at the time of organogenesis respectively (Kovac, 2000).

In *G. surendranathanii* the yolk content was high compared to other cyprinid fishes. The hatching time taken for *G. surendranathanii* was 36 hrs where as in few other cyprinid fishes like *Labeo dussumery* – 22 hrs (Kurup,

2001), *Danio malabaricus* - 24 hrs (Mercy *et al.*, 2002), *Danio aequipinnatus*- 30-36 hrs and in *Puntious ticto* – 28-36 hrs (Swain *et al.*, 2008). But, the related species *Garra cylonensis* showed the same hatching time as in *G. surendranathanii* (36 hrs) (Sundarabarathy *et al.*, 2005).

In most of the cyprinids, the yolk absorption is completed by third day (Kurup, 2001), where as in *G. surendranathanii* the same was on 5th day. Even though, the yolk absorption was completed by 5th day, the larvae started feeding on artemia and moina from day 3 onwards. The length of the larval phase and the capabilities of larvae at hatching varies depending upon the species. Obviously, species that have well developed first feeding larvae with short larval phase are better cultured candidate species. This clearly indicates the suitability of this species for breeding under captivity. From 30th day onwards the larvae started consuming pelleted feed.

The sucker appeared by 30-32 days and the development completed between 55-60 days. During the development of the sucker, post larvae tended to move in the water column. However, it was observed that during the final phase of development i.e. 30-35 days post hatch, they tended to climb on the tank walls and observed to feed in all areas of the tank. But, on completion of the development of the sucker, post larvae tended to spend more time at the bottom of the tank and they remained on the bottom like adults.

Chapter 7

Cryopreservation of Gametes

Contents

7.1 Introduction

Cryopreservation is a branch of cryobiology which relates to the long term preservation and storage of biological material at very low temperatures, usually at −196°C employing liquid nitrogen. At this temperature, cellular viability can be retained in a genetically stable form since the rates of biological processes will be too slow to affect cell survival (Lakra, 1993). The basic technique of cryopreservation involves collection of fish gametes in which specific diluent (extender) with cryoprotectant (such as dimethyl sulfoxide, glycerol, ethylene glycol and methanol) is added. After a period of specific of equilibration, it is frozen rapidly and stored in liquid nitrogen. After thawing, the milt can be activated for use in fertilizing the eggs. In teleosts, spermatozoa are held within the testis in an immotile state by the chemical composition of seminal plasma. Under natural conditions, motility is initiated when the semen or milt is diluted with water on release during spawning. During cryopreservation, the sperms are to be retained in inactive condition and it is the extender that keeps the sperm in live but in immotile state so that ideal dilution of cryoprotectant can be made to mix with the milt. Cryopreservation is helpful

in overcoming the problem of males maturing before females, allows selective breeding and stock improvement and enables the conservation of genomes (Harvey, 1983). It has been estimated that sperm from 200 fin fish and shell fish species has been successfully cryopreserved (Scott and Baynes, 1980; McAndrew *et al.*, 1993 and Billard *et al.*, 1995). However, appropriate to each species, optimizations of technology are needed. The successful cryopreservation of fish spermatozoa might be used to increase the number of offspring from genetically superior males, aid in the transport of semen and provide a year-round supply of male gametes. Furthermore, cryopreservation can increase the economic utilization of males and is a prerequisite for the establishment of gene banks (Munkittrick and Moccia, 1984).

Since the first work of Blaxter (1953) fish sperm cryopreservation has been attempted on many freshwater as well as marine species (Erdahl and Graham, 1980; 1987). Techniques of sperm management have been established in some freshwater fish species such as cyprinids (Billard *et al.*, 1995), siluroids (Legendre *et al.*, 1996) and in salmonids (Scott and Baynes, 1980; Billard, 1992). Among these techniques, sperm storage and cryopreservation are of special interest. For a successful cryopreservation technique, inadequate understanding of the mechanisms at the cellular level, associated injuries and the absence of appropriate cellular models are much required (Wolfe and Bryant, 2001). During freezing, the cells encounter changes in the membrane phase states, ice formation and increased concentration of all other solutes and they must respond to these changes within the finite time allowed by cryopreservation protocol (Mazur and Cole, 1989; Schneider and Mazur, 1984; Formicki, 1997). With all these considerations, an appropriate protocol for maximizing cell survival is required.

The spermatozoa of several economically important species have been cryopreserved in the recent past (Lakra, 1993; Lakra *et al.*, 2007). It includes rainbow trout (Wheeler and Thorgaard, 1991; Baynes and Scott, 1987; Schmidt-Baulain and Holtz, 1989), Atlantic salmon (Anderson and Macneil, 1984), Tilapias (Harvey, 1983; Harvey and Kelley, 1988; Chao *et al.*, 1987; Rana and McAndrew, 1989) and Cyprinids including Common, Chinese and Indian carps (Koldras and Bienarz, 1987; Kurokura *et al.*, 1984; Lakra, 1993). In India, Bhowmic and Bagchi (1971) reported for the first time successful preservation of *Labeo rohita* sperm in Holt-freter's and frog Ringer's solutions. The cryopreservation protocols have now been standardized for spermatozoa of Indian carps and catfishes (Lakra, 1993; Lal, *et al.*, 1996 and Lakra and Krishna, 1997).

Garra surendranathanii with its endemic as well as threatened status, requires special attention for conservation. To conserve and rehabilitate this fish species, no conservation strategies have been devised other than the captive breeding technology developed during the present study. Hence, cryopreservation of gametes, which is considered as another important option for conservation of threatened species, is attempted.

7.2 Material and methods

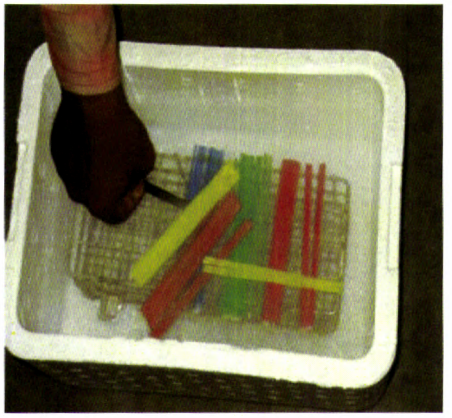
7.2.1 Collection of Sperm

Live specimens of *Garra surendranathanii* were collected using cast nets from lower reaches of Athirampally waterfalls, from river Chalakkudy during night hours with the help of local fishermen during breeding season. Initially, the fishes were kept in a hapa in the site itself and later transported in oxygenated polythene bags to the lab and kept in rearing tanks. Fishes were checked for milt availability by pressing the belly and those fishes oozed milt

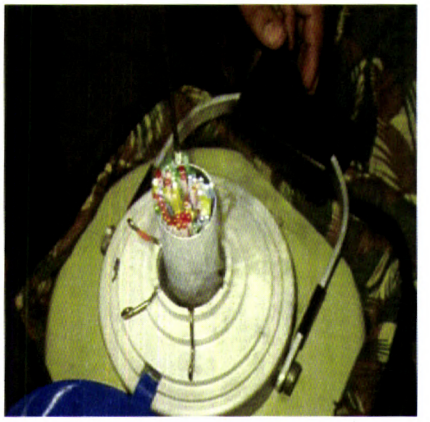
were taken for milt cryopreservation experiments. Fishes weighing 15-30 g were administered Ovaprim (Glaxo, India Ltd) injection at the rate of 0.3 ml per kg body weight and after 12 hrs milt was collected by gently pressing the belly to a clean plastic box (Fig. 7 1). Individual collections were made from all fishes. Care was taken not to mix any water or body fluid from the fish to the milt.

Collection of Milt

Pre-cooling of filled straws



Keeping in Liq.nitrogen vapour phase



Cryopreserved milt straws

Fig. 7.1. Different stages in milt cryopreservation of *G.surendranathanii*

7.2.2 Milt characteristics

The collected milt was evaluated for motility time, pH, spermatocrit percentage and sperm density. Motility of the milt was checked using tap water as activator and scores were recorded in the scale of 0-5. Spermatocrit was measured using a microcapillary tube, drawn the milt in the tube, sealed the ends and centrifuged at 3000 rpm for 3 minutes. Sperm count was done using a haemocytometer after diluting the milt with an extender.

7.2.3 Extenders

Motility of the milt was checked with a number of extenders and based on its performance, five extenders (Extender A, Extender B, Extender C, Extender D and Extender E, modified from Scott and Baynes (1980) were used for cryopreservation (Table 7.1).

Table. 7.1. Extender compositions used for Cryopreservation of milt of *G.surendranathanii* (Modified from Scott and Baynes (1980))

Chemicals (mg/100ml)	**Extender A**	**Extender B**	**Extender C**	**Extender D**	**Extender E**
NaCl	750	750	750	750	750
KCl	40	40	40	40	40
$CaCl_2$			20	70	20
$NaHCO_3$	200	200	50		20
$MgSO_47H_2O$		20	20	20	
NaH_2PO_4		50	40	40	
Glucose	100	100	100	100	100
Glycine		500			500
Mannitol			250	250	

7.2.4 Cryoprotectant

Initially DMSO (10%) was used as cryoprotectant, but as the post thaw motility was very less in subsequent experiments ethylene glycol (10%) was used as cryoprotectant (Melo and Godinho, 2004) The ratio of milt, extender and cryoprotectant was kept as 1:3.5:0.5. Cryoprotectant was added just before filling the straw to reduce the toxicity to milt.

7.2.5 Cryopreservation of Sperm

0.5 ml straws (CBS high density sperm strew) were used filling the milt extender solution. Since the quantity of the solution was less, straws were filled by mouth sucking. Straws were filled with the extended milt, sealed with PVA powder and kept on ice chamber (0°C) for 10 minutes, then liquid nitrogen vapour phase (~ -90°C) for 10 minutes and then plunged into liquid nitrogen. The straws were then stacked in canister and kept in cryocans containing liquid nitrogen.

7.2.6 Thawing

After 24 hours of cryopreservation, straws were taken, and thawed at 37°C in a hot bath for 20 seconds. One straw from each extender composition was thawed to estimate the post-thaw motility of sperm. Straws were then cut open and checked for the motility of the milt using tap water as activator.

7.2.7 Fertility trail

Eight female fishes (35-45 g) were given ovaprim injection @ 0.2 ml/kg body weight and after 12 of injection fishes were stripped for collecting ripe eggs. The eggs were collected in a clean dry plastic box and care has taken for not mixing with other fluids and water. Approximately 200 eggs were taken in a plastic tray and milt from a two straws used for fertilization. Fresh milt was taken as control. Milt was poured over the eggs, mixed then added tap water, as an activator. It was thoroughly mixed and washed with water and kept for

development. After eight hours dead eggs were removed and fertilization percentage was calculated. Hatching percentage was calculated after 48 hours.

7.2.8 Evaluation of cryoprotectants

Three reagents, dimethyl sulfoxide (DMSO), glycerol and Ethylene glycol were evaluated for their efficacy on sperm cryopreservation at two doses each, 5% and 10%. Sperm-extender solution was cryopreserved with three selected cryoprotectants and fertility trials were conducted. The result was statistically analyzed to evaluate the best cryoprotectant and concentration for long term cryopreservation of milt of *Garra surendranathanii.*

7.2.9 Statistical Analysis

All percent fertilization and hatching values were arcsine-square root transformed prior to statistical analysis. A one-factor analysis of variance (ANOVA) (MS-Excel) was used to compare different extender composition used for cryopreservation. Different cryoprotectant and their concentration were also analyzed using ANOVA. For all tests, Duncan's multiple range test was used to determine if significant differences existed among treatment means. Differences were considered significant at $P \leq 0.05$.

7.3 Results

7.3.1 Milt characteristics

The ripe fishes were smaller (12±1 mm Total length); hence the availability of the milt was less compared to medium size or large cyprinids (ranging from few drops to 1.5 ml per fish). The pH of the milt was checked and found in the range of 7.4 7.8. The motility, spermatocrit and sperm density of the milt was given the table: 7.2. The mean motility period of fresh *Garra surendranathanii* sperm was 76 ± 8 seconds (n = 10). Longest motility period observed was 90 seconds and after that all sperm were found immotile.

The spermatocrit values ranged from 33.3 to 44.4%. The average value of sperm count was 23.0 ± 2.1 x 10^9/ml (n = 10).

Table: 7.2. Fresh Milt characteristics of *Garra surendranathanii*

Sl. No	Motility time (in sec.s)	Sperm count (x10^9)	Spermatocrit (%)
1	90	27.4	44.4
2	85	21.3	39.4
3	80	20.6	38.3
4	70	22.1	39.1
5	70	23.8	39.7
6	75	22.1	38.2
7	70	22.2	39.0
8	80	24.0	40.0
9	65	22.7	36.9
10	70	19.8	33.3
Average ± SD	**76±8.0**	**23±2.1**	**39±2.8**

7.3.2 Cryopreservation and post-thaw motility

Cryopreservation of milt was carried out with five extenders. Post-thaw motility was checked after 24 hours. The highest post-thaw motility was observed with extender D (45 seconds, 60%), followed by extender C and E (40 seconds, 60%).

7.3.3 Cryoprotectant toxicity

With a view to select the ideal cryoprotectant, three chemicals (DMSO, Glycerol and Ethylene glycol) in varying concentrations were tried. Fertilisation and hatching rate of *Garra surendranathanii* using cryopreserved milt with different cryoprotectants is given in Table: 7.3. The percentage hatching are shown in Figure: 7.2. One way analysis of variance showed that all treatments significantly differed

from controls (Table: 7.4). Out of the six treatments ethylene glycol (10%) with extender D showed highest hatching percentage (92.3% as that of control) which was significantly different from other treatments ($p < 0.01$).

Table: 7.3. Percent fertilization and hatching of *G. surendranathanii* using cryopreserved milt with different cryoprotectants.

	Percent fertilisation Mean ±SD	**Percent hatching Mean ±SD**	**Percent as that of control**
DMSO 5%	16.5±0.4	21.1±1.1 [a]	46.3
DMSO 10%	19.8±1.7	29.9±2.0 [b]	65.6
Eth glycol 5%	21.1±1.6	30.3±4.7 [c, b]	66.4
Eth glycol 10%	23.8±1.4	42.1±2.2 [d]	92.3
Glycerol 5%	20.7±2.0	25.4±6.2 [a]	55.7
Glycerol 10%	20.2±1.8	24.8±4.2 [a]	54.4
Control	40.0±1.6	45.6±2.8 [e]	100.0

Different alphabets denotes values are significantly different between extenders ($P<0.01$)

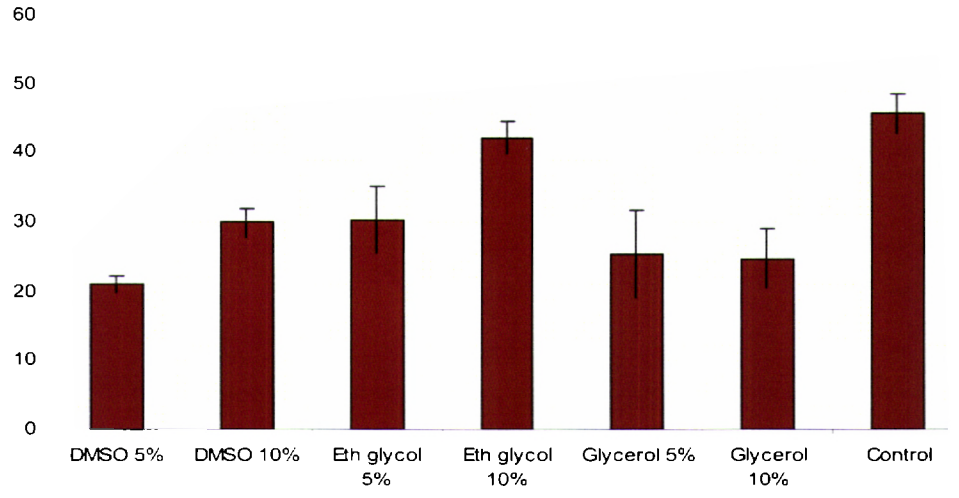


Fig: 7.2. Percent hatching (out of fertilized eggs) using cryopreserved milt with different cryoprotectants

Table. 7.4. One way ANOVA for Percentage hatching with different cryoprotectants

Groups	Count	Sum	Average	Variance
DMSO 5%	3	82.02024	27.3401	0.596882
DMSO 10%	3	99.48359	33.1612	1.569661
Eth glycol 5%	3	99.99551	33.3318	8.775801
Eth glycol 10%	3	121.2958	40.4319	1.707225
Glycerol 5%	3	90.55073	30.1836	16.1627
Glycerol 10%	3	89.51461	29.8382	7.616965
Control	3	127.3623	42.4541	2.657403

Source of Variation	SS	df	MS	F	P-value	F crit
Between Cryoprotectants	570.0161	6	95.00268	17.01397	1.07E-05	2.847727
Within Cryoprotectants	78.17327	14	5.583805			
Total	648.1894	20				

7.3.4 Fertility trial

Two fertility trials were carried out using cryopreserved milt and fresh milt as control. Fertilisation and hatching rates of *Garra surendranathanii* using cryopreserved milt are given in table: 7.5. The percentage hatching in two different trails are shown in Figure: 7.3. Fertility trials with frozen sperm showed significant differences from the controls (Table: 7.6). Hatching percentage using Extender D with 10% ethylene glycol (29.1±1.4) was close to that of control which differed significantly from other treatments showing that the combination of extender D with 10% ethylene glycol is the ideal combination for milt cryopreservation of *Garra surendranathanii.*

Table. 7.5. Percent fertilization and hatching of *G. surendranathanii* using cryopreserved milt with different extenders

Extenders	Percent fertilisation Mean ±SD	Percent hatching Mean ±SD	Percent as that of control
Extender A	11 7±0.6	21.2±3.8[a]	55.4
Extender B	19.2±1.0	24.2±0.6[b]	63.2
Extender C	22.9±1.2	25.3±1.7[a,b,c]	66.1
Extender D	24.0±1.8	29.1±1.4[c]	76.0
Extender E	31.4±2.6	26.5±2.6[a,b]	69.2
Control	36.8±1.6	38.3±1.5[c]	100.0

Different alphabets denotes values are significantly different between extenders (P<0.01)

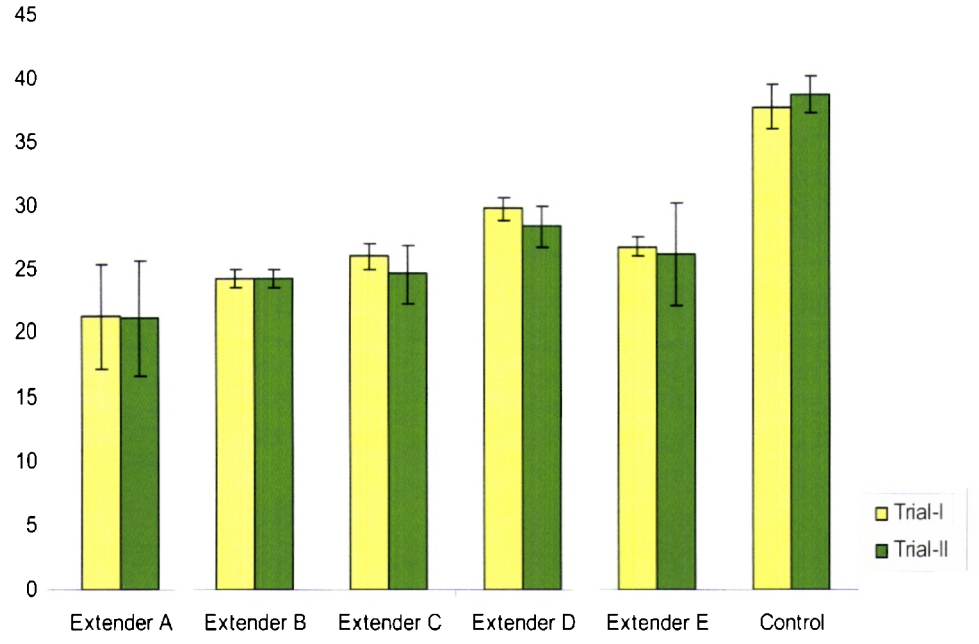


Fig: 7.3. Percentage hatching (out of fertilized eggs) in two trials

Table: 7.6. ANOVA for Percentage hatching with different extenders

Groups	Count	Sum	Average	Variance
Extender A	6	164.1219	27.35365	7.103975
Extender B	6	176.6635	29.44391	0.186448
Extender C	6	181.1219	30.18698	1.314724
Extender D	6	195.9704	32.66174	0.756967
Extender E	6	185.8294	30.97156	2.892677
Control O	6	229.327	38.22116	0.744638

Source of Variation	SS	df	MS	F	P-value	F crit
Between Extenders	419.6542	5	83.93084	38.73901	3.29E-12	2.533554
Within Extenders	64.99715	30	2.166572			
Total	484.6514	35				

7.4 Discussion

The amount of milt produced by fish during the reproductive season varies according to the species, the individuals, and the method of collection. In the present study the milt volume per fish was less even after ovaprim injection. Short-term treatment with hypothalamic or pituitary hormones induces a testicular hydration response that causes milt volume to increase (Billard *et al.*, 1995). Before milt collection, *Garra surendranathanii* males were treated with ovaprim injection at the rate of 0.3 ml/kg body weight. Bedore (1999) showed a significant increase in hand-stripped milt volume following crude carp pituitary extract (CCPE) treatment in *Brycon orbignyanus*. In smaller fishes, particularly

in ornamental fishes very low volume of milt only is produced (Harvey *et al.*, 1982). *Garra surendranathanii* is an ornamental fish which weighs maximum upto 50g in size. Hence, the volume of the milt is also less. However, in bigger cyprinids, milt yield was recorded as high as 8.9 ml/kg body weight from *Cirhinus mrigala* (Verma *et al.*, 2009). The mean motility period of fresh *Garra surendranathanii* sperm was 76 ± 8 seconds (n = 10). Longest motility period observed was 90 seconds and after that all sperm were found immotile. The motility of carp spermatozoa ranged from 80 to 110 sec in different species (Routray *et al,* 2006; Verma *et al.,* 2009). The maximum duration of spermatozoa motility in cyprinids is up to 120 seconds (Suzuki, 1959).

Fish sperm concentration, which is an important parameter in hatchery reproduction management, is highly variable and this also depends on species, individuals, fish size, and season (Glogowski *et al.*, 2002). The sperm density and spermatocrit has direct correlation in fishes (Routray *et al.,* 2006). The spermatocrit of *Garra surendranathanii* ranged from 33.3 to 44.4%. The average value of sperm count was 23.0 ± 2.1 x 10^9/ml (n = 10). In other cyprinids (Indian major carps and Chinese carps), the spermatocrit values ranged from 69 to 81% (Verma *et al.,* 2009). They also observed the sperm count between 2.6 x 10^{10}/ml to 3.5 x 10^{10}/ml in six species of carps and reported wide variation in the spermatocrit and sperm count in all the six carp species. They attributed this is due to the spermatozoa size and species-specific nature of carps. Routray *et al.,* (2006) observed that in *Labeo rohita* spermatocrit value of more than 70% is good for utilization in cryopreservation and fertilization process. In the present study, the spermatocrit values are lesser than other cyprinids and this might hampered the fertility rate and hatching rate of this species.

The large number of potential cryoprotectants and the different concentrations in which they can be used make the development of a suitable extender solution a complex task. In addition, depending on the concentration used, some cryoprotectants may be toxic (Leung and Jamieson, 1991). Thus, the effects of cryoprotectants on sperm viability should be evaluated when they are intended to be used for a species not previously tested. As this work was the first to deal with *Garra surendranathanii* sperm cryopreservation, three commonly used cryoprotectants (DMSO, Glycerol and Ethylene glycol) in fish sperm cryopreservation were tested. Out of the three cryoprotectants used at two doses (total 6 combinations) ethylene glycol (10%) with extender D showed highest hatching percentage (92.3% as that of control) which was significantly different from other treatments. Patil and Lakra (2005) found a combination of DMSO and glycerol (9:11 ratio) as a good cryoprotectant for *Tor khudree* and *Tor tor*. DMSO (dimethyl-sulfoxide) at various concentrations is generally used as a cryoprotectant for animal cells, for example 10% DMSO resulted in a high percentage of post-thaw survival of milkfish spermatozoa (Chao, 1991). However, 10% Dimethyl-acetamide (DMA) was more effective than 10% DMSO for cryopreservation of rainbow trout (*Ochorhynchus mykiss*) spermatozoa (Richardson, *et al.*, 2000) and African catfish (*Clarias gariepinus*) spermatozoa (Horvath and Urbanyi, 2001). Similarly, glycerol was found to be a more effective cryoprotectant than DMSO and ethylene glycol (EG) for European catfish (*Silurus glanis*) spermatozoa (Linhart *et al.*, 1993). In contrast, Marian and Krasznai (1987) found DMSO better than EG in European catfish, and propylene glycol (PG) in yellowtail flounder (*Pleuronectes ferrugineus*) (Richardson *et al.*, 1995). But, in the sperm cryopreservation of common carp, *Cyprinus carpio* (Cognie *et al.*, 1989) and *Brycon orthotaenia,* good results

were seen with ethylene glycol (Melo and Godinho, 2006). During the present study, out of the three cryoprotectants, ethylene glycol (10%) with extender D showed highest hatching percentage (92.3% as that of control) which was significantly different from other treatments.

Long term cryopreservation of milt will be successful only after conducting fertility trial with cryopreserved milt. From this fertility trial we can conclude the best extender and cryoprotectant combination for cryopreservation. In the present study, five extender combinations with cryoprotectant (10% ethylene glycol) were used for milt cryopreservation of *Garra surendranathanii*. The fertilization rate was ranged from 11.7±0.6% in Extender A, to 31.4±2.6% in extender E and hatching rate ranged from 21.2±3.8% in extender A to 29.1±1.4% in extender D Though there is higher fertilisation rate in extender E the higher hatching rate showed in extender D. Akcay *et al.* (2004) observed a higher fertilisation rate in one extender and higher hatching rate in another extender. They attributed this to damaged sperms which may be fertilizing the eggs but not facilitating the subsequent development. Hatching rate was subjected to one way ANOVA and it was found that all extender combination significantly differed from control. However, hatching rate (29.1±1.4) using Extender D with 10% ethylene glycol as cryoprotectant was close to that of control which differed significantly from other treatments. This extender combination slightly differed from other extender combinations in having glucose and mannitol and less of $NaHCO_3$. Simple sugar-based extenders have been tested on common carp sperm (Babiak *et al.*, 1997) but met with little success. Fructose and glucose were reported as components of different extenders for the freezing of sperm of other cyprinid species (Kumar, 1988; Zhang and Liu, 1991). In the present study also glucose

and mannitol based extender gave better result. Sugar extenders were successfully used for the cryopreservation of sperm of several other fish species such as African catfish (*Clarias gariepinus*) (Urbányi *et al.*, 1999) and various sturgeon species (Tsvetkova *et al.*, 1996; Glogowski *et al.*, 2002). The success of sugars as extenders can be explained by their role as external cryoprotectants and membrane stabilizers (Maisse, 1996). In the present study it is found that ethylene glycol was a far more efficient cryoprotectant for *Garra surendranathanii* sperm than DMSO. Methanol has also successfully used for the cryopreservation of cyprinid species such as the zebra fish (*Danio rerio*) (Harvey *et al.*, 1982). Babiak *et al.* (1997) who achieved his best results with dimethyl-acetamide (DMA) in common carp. The present work indicated that *Garra surendranathanii* spermatozoa can be successfully cryopreserved using extender D with 10% ethylene glycol as cryoprotectant.

Chapter 8

Summary and Conclusion

India is considered as a 'Mega Diversity' country due to its high percentage of biodiversity and high degree of endemism. It has two major biodiversity "hot spot" areas, *viz.*, Himalayas and Western Ghats. However, the Western Ghats region, like other parts of the tropics, is undergoing rapid transformation due to damming of the rivers, hydroelectric projects, sand mining, deforestation, pollution, etc. which resulted in dwindling the fish population. Added to this the destructive fishing methods such as mass poisoning, dynamiting and electric fishing have depleted the resources beyond sustainable level. Introduction of exotic fishes in reservoirs which escape into rivers resulted in the replacement of ecological niches of native species from their habitat. These all resulted in the decline of many of the endemic fauna in their own natural habitats. Unfortunately, lack of information on these species and their habitats has been a major handicap in taking timely steps in conservation. Keeping this in mind the present study was undertaken to investigate the life history traits, resource characteristics, captive breeding, developmental biology and cryopreservation of gametes of *Garra surendranathanii,* a highly endemic species found in limited locations in the hill streams of Kerala.

According to IUCN based classification, *G. surendranathanii* is grouped under the threatened category. This endemic fish is having highly restricted and fragmented distribution. In Kerala this species is reported from only 5 river systems *viz.* Chalakudy, Periyar, Pamba, Achenkoil and Bharathapuzha.

Categorization of this fish as a potential ornamental candidate can invariably add more pressure on the threat status of this particular species. Hence, this species is considered as one which requires foremost attention for conservation and the possibilities of captive breeding for commercial purposes. Hitherto, no information is available on the bionomics, resource characteristics and any conservation attempts of *G. surendranathanii*. Studies on detailed life history traits and development of captive breeding technique are indispensable for successful fishery management.

The present study was undertaken with the following objectives:

To study the Length-weight relationship and condition factor to ascertain the relationship between length and weight and general wellbeing of the fish

To study the age and growth to understand the age composition of the exploited stock, age at first maturity and life span of the species.

To study the reproductive biology to gain insights in the process of gametogenesis, spawning, sex ratio, fecundity and other related aspects which are essential for developing captive breeding technology for this species.

To develop captive breeding technology and cryopreservation of gametes for conservation

The salient findings of the study are summarized as below:

From length weight relationships it was found that during the entire period of life, all the population of *Garra surendranathanii* grows isometrically, more or less obeying cube law. The females were found to attain weight at a faster rate in relation to its length.

The largest size of male *G. surendranathanii* recorded during the present study was 142 mm and female 209 mm. The lengths of males at the end of first, second, third, fourth and fifth years of life were estimated to be 82, 114, 132, 142 and 147 mm respectively. Females attained a length of 123 at the end of I year, 167 at the end of II year, 191 at the end of third year and 204 at the end of IV year and 211 by V th year. Based on the results of the present study, it can reasonably be inferred that the longevity of *G. surendranathanii* is around five years. Since majority of the males fall in the length class 111- 120mm and females in 121-130mm, it can be postulated that the exploited stock of males and females invariably belonged to one year age group.

The male and female reproductive organs of *G. surendranathanii* are built on the general teleostean pattern as observed in other teleosts.

During the present of study, it was found that the peak breeding of *G. surendranathanii* is from October – December and only one breeding season is observed.

Length at first maturity was found to be 85 mm for males and 115 mm for females which invariably falls within one year.

The sex ratio significantly skewed from the expected 1:1 ratio and the mean ratio of males to females was 1: 0.34 and this can be pointed out as one of the reasons for its threatened status in wild.

The absolute fecundity varied from 431-5402 eggs in specimens ranging from 120 –199 mm in total length and the average was worked out to be 1924 ova. The relative fecundity was estimated to vary between 10 (188 mm TL) and 70 (199mm TL) with an average of 36, while the number

of ova per gram ovarian weight varied between 21 and 70, with the average 17. These showed the low fecundity as in the case of most of the threatened fishes. Ovary weight was identified as the most appropriate predictor of ovarian egg count.

The captive breeding of *G. surendranathanii* could be successfully done with 3 hormones viz. Ovaprim, HCG and Ovatide. Ovaprim @ 3 ml/ kg body was found to be the best hormone and dose for induced spawning of the fish. After fertilisation the eggs hatch with in 36 hrs.

The breeding behaviour studies revealed the habitat specificity of this species and emphasize the adverse effects of habitat destruction like deforestation, sand mining, and other man made modification of the ecosystem, etc.

The *G. surendranathanii* spermatozoa could be successfully cryopreserved using extender D with 10% ethylene glycol as cryoprotectant and this combination can be utilized for further use.

Recommendations

Based on the present investigation the following measures are suggested for the conservation of fish species in the rivers of Kerala.

1. The critically endangered and endemic fresh water fishes shall be brought under the purview of the list of similar fishes prepared by the Ministry of Environment And Forest, Government of India.
2. In view of the paucity of information on endangered fish on aspects related to population structure, distribution range, habitat, life history traits and the factors responsible for their threatened position, it is

recommended that research in these lines shall be initiated and strengthened.

3. The natural breeding grounds and nurseries of the threatened fishes shall be identified and declared as aquatic sanctuaries.

4. In view of the indiscriminate exploitation of brood-stocks of fresh water fishes, especially during the south west monsoon, imposition of a seasonal closure of fishing during this period is found necessary to maintain the stock recruitment relationship of freshwater fishes in general and threatened fishes in particular.

5. Regulate the human interventions, in the habitats of critically endangered species such as sand mining, conduct of unethical fishing practices, discharge of polluted water, diesel spillage from boats, etc.

6. Any fish species whose distribution is well restricted to a single location is always prone to extinction in near future due to natural or anthropogenic reasons. In such cases, translocation of such species would be a rewarding conservation activity. Identification of ideal habitats and translocation of critically endangered species which are restricted to a single location to new locations would also be worthwhile.

7. Development and standardization of captive breeding technology of the endemic threatened fish species are inevitable for their rehabilitation as a tool for the sustenance of stock.

8. More studies should be conducted for developing protocols for the cryopreservation of gametes of threatened fishes.

9. Government of Kerala should set up a fish hatchery exclusively for the breeding and propagation of critically endangered and endemic fresh water fishes of Kerala.

10. It is felt that there is inadequacy of appropriate legislation to curb the unethical and unscientific fishing methods and practices which are very rampant in the rivers and rivulets of Kerala. By totally conceiving this, immediate enactment of Kerala Inland Fisheries Regulation Act (KIFRA) is found indispensable for the conservation of threatened fishes of Kerala.

11. Sanctuaries, Reserves and National parks need to be set up for fishes as done for the protection and preservation of other wild animals. Display boards depicting the details of the sanctuary and legal measures taken against offenders should be exhibited at eye-catching places.

12. Introduction of exotic species should be allowed only after studying its biology, habitat and potential threats to native fish species and environment.

13. Traditional knowledge of ecology, behaviour and abundance of a species may prove invaluable in many cases. Documentation of knowledge and perception of the local people on biodiversity and conservation can be done using a questionnaire. Educated youth can be deployed for the purpose after giving proper training instructions and guidelines.

14. The 'Biodiversity Conservation Order' passed by the Government of Kerala in 2000 should be given wide publicity through mass media.

15. Successful fish conservation on long term basis is mainly dependant on habitat protection which in turn can be achieved only through public awareness. Educate the fishermen community, local people, governmental and non-governmental agencies, students and the general public regarding the importance of conservation of fish fauna through

group discussions, seminars, training camps and publicity through mass media. Awareness campaign need to be initiated by bringing out posters, stickers, stamps, showing clippings in electronic media etc. implementation of local specific conservation programmes giving due representation to inland fishermen at local body level is found very necessary for the protection of fast depleting icthyofauna of the state.

16. Students may be encouraged to observe 'Icthyofauna week' on the line of 'Wild Life Week' Postage stamps may be issued in this connection.

17. Aquarium keeping using indigenous fishes shall be promoted as a hobby. Collection of fishes from wild for domestication and export as ornamental fishes shall be regulated and fishes bred under captivity shall only be used for trade purposes.

18. A state-level apex body including representatives from governmental and non-governmental organizations and research centers shall be constituted for the conservation of fish biodiversity which can control, co-ordinate and evaluate the performance of various committees formed for regular monitoring of water bodies and implementation of mass awareness programme.

19. The State Fisheries Department and the research community of the state should start working together in a meaning manner fro the formulation and implementation of research projects and other appropriate measures for the conservation of fishes.

20. Installation of future hydro-electric projects must be realized only after assuming that fish movements are not hampered and their breeding grounds and nurseries are least disturbed. Fish passes, fish-ladders etc should be provided for fish movements.

21. Conservation management programmes can be implemented by generating financial assistance from various international and national funding agencies.

22. In the case of *Garra surendranathanii* it is to be concluded that it is a fish to be nurtured as it has several adverse features which adversely affect its survival in a disturbed environment like low fecundity, prolonged hatching time of egg, sluggish movement, selective breeding and feeding grounds and significantly skewed male to female ratio (1: 0.34).

23. The results on cryopreservation of gametes of *Garra surendranathanii* can be used as a basis for developing a commercial unit for the cyropreservation of gametes of all the threatened species.

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