STUDIES ON HABITAT ECOLOGY, CAPTIVE BREEDING AND LARVAL REARING OF CYPRINID FISH *GARRA LANGLUNGENSIS* EZUNG, SHANGNINGAM & PANKAJ, 2021

by

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CERTIFICATE

This is to certify that, the thesis entitled **"Studies on Habitat Ecology, Captive Breeding and Larval Rearing of Cyprinid Fish *Garra langlungensis* Ezung, Shangningam & Pankaj, 2021"** is an authentic record of research work carried out by **Ms. Sophiya Ezung** under my guidance and supervision. The thesis has fulfilled the standard requirement of Ph.D regulation of Nagaland University and the work is worthy for consideration for the award of Ph.D degree. This is also to certify that the content of the thesis has not been the basis for the award of any degree, diploma, fellowship or similar title or any university or institution.

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I, **Ms. Sophiya Ezung**, bearing Ph.D registration No. 855/2020 *w.e.f.* 16/08/2016, hereby declare that the thesis entitled **"Studies on Habitat Ecology, Captive Breeding and Larval Rearing of Cyprinid Fish *Garra langlungensis* Ezung, Shangningam & Pankaj, 2021"** is a record of genuine work carried out by me under the supervision of Dr. Pranay Punj Pankaj, Assistant Professor, Department of Zoology, Nagaland University, Lumami – 798627 and that the content of thesis has not been the basis for the award of any degree, diploma, fellowship or similar title or any university or institution.

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Sophiya

Sophiya Ezung

DEDICATED

To

My Parents and Siblings

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ABBREVIATIONS

˚ C	Degree Centigrade
˚ E	Degree East
˚ N	Degree North
A	Anal fin
AFBL	Anal Fin Base Length
ANOVA	Analysis of variance
APHA	American Public Health Association
BD	Body Depth
BW	Body Weight
C	Caudal fin
cm	Centimeter
D	Dorsal fin
DCP	Depth of Caudal Penduncle
D.F	Degree of Freedom
D.O	Dissolved Oxygen
DSLR	Digital Single Lens Reflex
ED	Eye Diameter
EOS	Electro-Optical System
F.R	Fertilization Rate
gm	Gram
GnRH	Gonadotropin-releasing hormone
GSI	Gonadosomatic index
GtH	Gonadotropin hormone
HAF	Height of Anal Fin
H.D	High Dose
H.R	Hatchling Rate
hrs	Hour
HW	Head Width
IOS	Inter – Orbital Space
l	Litre
L.C	Least Concern
LCF	Length of Caudal Fin
L.D	Low Dose
LD	Length of Disc
LDFB	Length of Dorsal Fin Base
LDPE	Low Density Polyethylene
Ll	Lateral line scales
LP	Length of Peduncle
LPI	Length of Pulvinus
LPF	Length of Pectoral Fin
LSID	Life Science Identifier
Ltr	Lateral transverse scales
LVF	Length of Pelvic Fin
m	Meter
max	maximun
M.D	Medium Dose
M:F	Male:Female
mg	milligram
min	minimum
mins	minutes

HDF	Height of Dorsal Fin
HHO	Height of Head at Occiput
HL	Head Length
N.E	Not evaluated
NER	Northeastern Region
N.S	Not Significant
N.T	Near threatened
OL	Ovary Length
OW	Ovary Weight
P	Pectoral fin
PAL	Pre-Anal Length
PAsL	Pre-Anus Length
PDL	Pre-Dorsal Length
PPL	Pre-Pectoral Length
ppm	Parts per million
PVL	Pre-Pelvic Length
S	Significant
ml	milliliter
S.D	Standard Deviation
mm	millimeter
MW	Mouth Width
sec	Second
SL	Standard Length
SnL	Snout Length
S.R	Survival Rate
Sq.Km	Square Kilometer
TL	Total Length
V	Pelvic fin
VAFO	Pelvic and Anal Fin Origin Distance
VtAFO	Vent and Anal Fin Origin Distance
WD	Width of Disc
WPI	Width of Pulvinus
ZSI	Zoological Survey of India

CONTENTS

Abstract

The labeonine genus *Garra* Hamilton, 1822 belongs to the family Cyprinidae. Fishes of genus *Garra* are generally small-medium sized and categorized as ornamental fish as well as food fish for some of the species of *Garra*. The snout morphology and its associated tubercle pattern and distribution play a taxonomic significance in distinguishing *Garra* species and is considered a highly adaptive modification that enables fish to resist the fierce current of the water. The fishes of this genus inhabit rapid flowing streams and rivers comprising river beds mainly of boulders, stones, rocks, and gravel by clinging to the substratum. These species are commonly known as log suckers or stone suckers due to their habit of clinging to or licking the stones in the substratum. *Garra langlungensis* is commonly said to be in the local dialect as pathor maas, Engoro in Lotha naga tribe, Angad in Ao naga tribe, Aghungu in Sumi naga tribe. *Garra* fishes, in general, are very much relished for their taste in Nagaland and have high economic value in the local market. However, the majority of the catch in this region is made primarily from the wild habitat leading to declining fish populations. Therefore for sustaining the aquatic resources and natural restocking of the fish, a proper captive breeding program is the absolute necessity for repopulating the wild stock and also for its conservation aspects. The habitat ecology, taxonomy, length-weight relationship, and reproductive biology were studied to establish a captive breeding protocol of *Garra langlungensis*. Physicochemical parameters were studied to check the water quality of the natural habitat of the test species. All the water parameters showed seasonal variation during the study period. Air and water temperature was in the range of 24.75 –31.66 ˚C and 27.16 – 34.53 ˚C, water velocity

0.74 – 3.89 m/sec and water transparency in the range 25.5 – 0.75 cm. The pH was in the range of 7 – 8, Dissolved oxygen 11.6 – 5.2 mg/l, total alkalinity 70 – 35.9 mg/l and total hardness in the range 112.5 – 30 mg/l. The taxonomy study revealed that *Garra langlungensis* is diagnosed as member of the snout with proboscis species group having weakly-developed unilobed proboscis, a distinct transverse lobe with 8–12 small sized unicuspid acanthoid tubercles, 8–9 pre-dorsal scales, 30–32 lateral line scales and 13–15 circumpeduncular scales. The morphological study showed that the body of *Garra langlungensis* is elongated and laterally compressed. Fin formula was recorded as D ii 8½; P i 11-13; V i 7½; A ii 5½; C 10+9; LI 30-32; Ltr 3½|1|3. The length-weight relationship estimated for male, female and pooled populations deviated from the cube law b=3. The 'b' values in each population were less than '3', which showed negative allometric growth. The condition factor of the fish was in the range of 1.445-2.267, which indicated healthy condition or good well-being of the fish. *Garra langlungensis* exhibited sexual dimorphism only during the breeding season displaying secondary external morphology characters in body size, body shape, fin characters and anal opening. The sex ratio of males and females was 1:0.43 from natural collection interpretation. The fecundity ranged between 319 – 844, with an average mean of 565. The maximum ova produced was 844 by a female weighing 5.31 gm, with a total length of 77.9 mm. The minimum ova produced was 319 by a female weighing 2.19 gm with a total length of 55.2 mm. The GSI ranged between 0.3 to 8.1 in males and between 0.5 to 11.5 in females. The highest peak in GSI was observed during the month of April and the lowest in the month of October. The breeding season of the fish extends from February to May. *Garra langlungensis* showed spawning migration in its wild habitat

by flowing upstream against the water current towards the canal in the shallow water with boulders and stones as the substratum. *Garra langlungensis* spawn in fast-flowing shallow waters with boulders and stones in its wild habitat. For this, the breeding tank was highly aerated and set using river boulders and stones as a substratum for hiding purposes. The male and female brooders were grouped at a ratio of 2:1 for captive breeding. The brooders displayed courtship behaviour after 2.30 hrs of hormonal administration. Breeding was successful using Ovaprim as the hormonal agent for inducing breeding, and breeding occurred at 5.15 hrs after hormonal injection. The brooders did not show any parental care. A study on the effects of different doses of Ovaprim in captive breeding resulted in a dose 0.02 ml/gm as the optimum dose for breeding this fish. The eggs hatched out at 36.45 hrs after fertilization. The newly hatched hatchlings measured an average length of 4.1mm ± 0.1. The yolk sac is completely absorbed by 5 days. The hatchlings were fed with infusoria and boiled eggs after the absorption of the yolk sac. The gular disk started developing in the hatchlings by 30 days. The fish resembled the adult within 2 months after hatching, attaining an average length of 20.1 mm.

Keywords: *Garra*, Physico-chemical parameter, Morphological study, Length weight relationship, Sex ratio, Fecundity, Gonado-somatic index, Captive breeding, Breeding behaviour, Embryonic development, Larval development

Chapter 1

General Introduction

Contents

1.1 Introduction

Freshwater diversity

Most forms of life on Earth can be found in freshwater ecosystems, including rivers, lakes, and wetlands that make up less than one percent of the surface of the Earth. Despite this, they support a vast array of life, give a wealth of benefits to humans, and provide a home for around 10% of the world's described species, including a quarter of all vertebrates (Strayer & Dudgeon, 2010). Although freshwater accounts for approximately 3%, which is an extremely small proportion, it is home to more than 40% of all fish species, with relatively higher species richness than marine and terrestrial biodiversity. Nearly half of all species of vertebrates are fish, including about 17,948 marine and 18,397 freshwater species (Fricke *et al*., 2022).

Fishes represent one of the most valuable resources on a global scale, and their significance to human society cannot be ignored. However, human interference rapidly decreases this number. Humans are linked to the majority of threats to the freshwater environment primarily due to growth in population and economic development, leading to increasing demands for natural resources and space. Freshwater systems are one of the most endangered habitats in the world and have alarming rates of species extinction (Sala *et al*., 2000). Biodiversity is being lost more rapidly in freshwater ecosystems than in other ecosystems. The major threat classes to freshwater biodiversity are habitat degradation, over-exploitation, alien species invasion, river flow modification, and water pollution (Dudgeon *et al*., 2006).

Freshwater diversity of India

India has the largest land mass of the Indian subcontinent covering an area of 32, 87,590 sq. km. It is one of the most mega-diverse countries when it comes to freshwater biodiversity. In the world, India is considered one of the mega-biodiversity nations, and in terms of freshwater mega-biodiversity, it holds the ninth position (Mittermeier & Mittermeier, 1997). Four global biodiversity hotspots are situated in India, *viz.*, the Himalayas, the Western Ghats, the North-east and the Nicobar Islands, which harbors 3, 287 fish species which constitute about 9.41% of the known fish species of the world (Mittermeier & Mittermeier, 1997; Pande & Arora, 2014; Chandra *et al*., 2017). This diversity comprises the rich freshwater fish fauna in the world and ranks third among Asian countries after Indonesia and China (Kottelat & Whitten, 1996; Nguyen & De Silva, 2006).

Approximately 9.7% of the total fauna are associated with freshwater ecosystems, several of which are endemic and unique to India. For instance, the Ganga-Brahmaputra system exhibits the highest richness of large-bodied freshwater species (freshwater mega-fauna) worldwide and supports unique and threatened species. India is endowed with vast and varied resources, possessing a rich river ecological heritage and biodiversity. Of the 3287 fish species reported from India, 1027 are reported from freshwater fish. India has rich freshwater fish diversity, with as many as 1027 species, comprising primary, secondary, and alien freshwater fishes. The primary freshwater fishes consist of 858 species, which are divided into 167 genera, 40 families, and 12 orders. In addition, India is home to 137 species of secondary freshwater fish that frequently enter and flourish in freshwater reaches of rivers. Alien fishes that have become naturalized in Indian

freshwater bodies account for 32 species, of which 16 are considered to be potentially invasive. More than 60.3% of the primary freshwater fishes of India are endemic to the country (Chandra *et al*., 2017). India exhibits a great variety of ecological habitats in the form of rivers, streams, ponds, lakes, reservoirs, floodplain wetlands, and innumerable other small water bodies, which harbor rich fisheries resources due to their unique geographical location.

Freshwater diversity of North East India

The Northeast region of India (NER) lies between 21°57′ and 29°23′N and between 87°58′ and 97°09′ E with its diversified lotic and lentic water bodies. It comprises the eight sister states, *viz.*, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura, and Sikkim. This part of India is considered one of the world's hotspots of freshwater fish biodiversity (Kottelat & Whitten, 1996). The region is drained by four major significant drainages, the Brahmaputra, the Barak-Surma-Meghna, the Kaladan, and the Chindwin. Out of the 34 biodiversity hotspots listed by Conservation International (Roach, 2005), two biodiversity hotspots, i.e., the Himalayas and Indo-Burma, lie in northeast India.

The whole of Arunachal Pradesh and Assam, north of Brahmaputra and Sikkim, belong to the Himalayas. In contrast, Mizoram, Assam, south of the Brahmaputra, Meghalaya, Nagaland, and Manipur, belong to the Indo-Burma. This region harbors 512 fish species from 128 genera, 40 families, and 12 orders (Sarma *et al*., 2022). The ichthyofaunal diversity in this region got its richness by forming part of the Himalayas and Indo- Burma (Allen *et al*., 2010). The Himalayas have large mountains and deep gorges, which results in the high diversity of the region; on the other hand, the Indo-

Burma supports high diversity and endemism due to its weather patterns, peculiar topography, soil characteristics, and rainfall pattern. This locale harbors various fancy angles with enormous commercial importance (Mandal *et al*., 2007). Nevertheless, 16 % of the fish diversity in northeast India comes under the threatened category for which proper conservation measures are desirable. About 33% of fishes come under the data deficient and not evaluated categories, and their conservation status needs more attention (Sarma *et al*., 2022).

Ichthyofaunal diversity of Nagaland

Nagaland is located between 25˚ 6' N-27˚4'N latitude and 93˚ 20E - 95˚ 15' E longitude having a geographical area of 16,579 Sq. Km. It is the smallest hilly state situated at the extreme north-eastern end of India. The state shares its boundary with Assam on the West, Myanmar on the East, Arunachal Pradesh and parts of Assam on the North, and Manipur on the south. The altitude in this region varies from 194 m to 3048 m. The average annual rainfall varies from 2000 mm to 2700 mm. The distinctive piscine fauna of Nagaland is attributed to: the drainage pattern comprising three entirely different river systems *viz*., Barak drainage system, Brahmaputra river system, and Chindwin river system. The major rivers of Nagaland are Doyang, Dikhu, Dhansiri, Tzu, Tsurang, Nanyang, Desai, Tsumok, Menung, Dzu, Langlung, Zunki, Likimro, Lanye, Dzuza, and Manglu. All these rivers are dendritic in nature. Dhansiri, Doyang, and Dikhu flow westward into the Brahmaputra. The Tzu River, on the other hand, flows towards the east and joins the Chindwin River in Burma. Eleven major and ten minor rivers flow through the state, besides some other streams and purls (Ao *et al*., 2008). The diverse habitat is an abode for ichthyofauna due to variations in climate and altitude.

A couple of researchers have documented the ichthyofaunal diversity of Nagaland. Ao *et al*. (2008) compiled the fish species available throughout the state, revealing 149 fish species. Goswami *et al.* (2012) listed 187 fish species from the state. Recently the compilation of the fish species reported from the state was carried out by Ezung *et al*. (2020a), where they listed 197 species belonging to 10 orders, 26 families, and 87 genera. Various researchers have witnessed the recent addition of literature on taxonomic research of new species and the additional first record from this area (Ezung *et al.*, 2020b; Praveenraj *et al.*, 2021; Ezung *et al*., 2021; Praveenraj *et al.*, 2022 and Ezung *et al*., 2022). Despite recent contributions to the state fish fauna, accounts of species compositions in many water bodies remain undocumented and awaiting exploration. Many lotic systems have yet to be explored because most rivers are located in unapproachable mountainous, steep terrain with dense forest cover.

Status of *Garra* species

Garra (Hamilton, 1822), generally known as log suckers, belongs to the sub-family Labeoninae under the family Cyprinidae. Fishes of the genus *Garra* are small-medium sized, having an elongated sub-cylindrical body, flattened abdomen, head slightly depressed, mouth inferior, semicircular; lips thick and fleshy; upper lip fimbriated; lower jaw covered by thick labial fold; lower lip with a gular disc consisting of semi cartilaginous pad called pulvinus, the snout is more or less rounded or slightly conical, heavily tuberculated or absent, somewhat depressed and projecting beyond the mouth, either smooth or with a proboscis, transverse groove present or absent (Menon, 1964; Darshan *et al*., 2019; Kottelat, 2020). The generic recognition among these genera is mainly based on the morphology of the gular disc. Members of the genus share a

conservative body plan and show relatively slight variation in basic color patterns and meristic characters. However, adults of many species have been reported to have conspicuous projections and enlarged tubercles on the head (Menon, 1964; Nebeshwar & Vishwanath, 2013).

Hora (1952) ascertained that any structural changes and adaptation of organisms to their environments are part of the evolutionary process and that such adaptations are achieved through changes in functions. Several hill stream freshwater fishes go through evolutionary trends as adaptive strategies to adapt differently and differentially to the environment they inhabit (Nagar *et al.,* 2012). The genus has undergone remarkable changes in shifting vent position forward away from the base of the anal fin and the development of proboscis on the snout, which is considered a major evolutionary significance (Menon, 1964). The snout morphology and associated tubercle pattern and distribution also play a taxonomic significance in distinguishing *Garra* species. It is considered a highly adaptive modification that enables fish to resist the fierce current of the water. Nebeshwar & Vishwanath (2017) classified *Garra* into five species groups based on snout morphology: 1. Smooth snout species group: species in which the dorsal surface of the snout is usually more or less rounded or flattened. 2. Transverse lobe species group: species with an elongated transverse fold demarcated by a groove over the anterodorsal surface of the snout. 3. Rostral flap species group: species which possess a fleshy triangular lateral projection, broadly rounded or truncate, not elevated from the surface of the snout, and is situated on the lateral side of the snout a little distance anterodorsally to the base of the rostral barbel. 4. Rostral lobe species group: species that possess a fleshy triangular lateral projection, narrowly pointed, and located in a distally

deep widened sublachrymal groove. 5. Proboscis species group: species having a fleshy structure extended over the dorsal surface of the snout.

The genus *Garra* usually has a basic color pattern and is placed under non-classified ornamental fish. According to Menon (1964), the labeonine genus *Garra* is geographically restricted and is widely distributed from Sub–Saharan Africa to Borneo through the Arabian Peninsula, Southern Asia, and southern China (Zhang & Chen, 2002). *Garra* is a bottom-dweller and usually inhabit mountain torrents, rapidly flowing rivers and streams, typically solitary under rocks or among stones, boulders, and riverbeds consisting mainly of boulders, rocks, and gravels. These fishes primarily feed on algal felts, mats, and periphyton that they scrape off stones (Nagar *et al*., 2012).

The genus *Garra* was established by Hamilton in 1822 as a sub genus of Cyprinus. Though the genus was established in 1822, few species were known until the nineteenth century. Menon (1964) monograph on *Garra* recognized 38 species. However, several new species have been identified in recent years, making the genus the most specious group of labeonines, with 168 valid species (Fricke *et al*., 2022). This genus is represented by 80 species in different water bodies of India, of which 56 species are distributed in different drainages of northeast India. The genus *Garra* in Nagaland was represented by 11 species: *Garra annandalei* Hora, 1921; *Garra gravelyi* Annandale, 1919; *Garra gotyla* Gray, 1830; *Garra kempi* Hora, 1921; *Garra lamta* Hamilton, 1822; *Garra lissorhynchus* McClelland, 1842; *Garra McClelland* Jerdon, 1849; *Garra notata* Blyth, 1860; *Garra naganensis* Hora, 1921; *Garra nasuta* McClelland, 1838; *Garrarupicola* McClelland, 1839, until the description of two new species, *viz*., *Garra chathensis* Ezung, Shangningam & Pankaj, 2020b and *Garra langlungensis* Ezung,

Shangningam & Pankaj, 2021 from Chathe river and Langlung river and an additional record of *Garra birostris* Nebeshwar & Vishwanath, 2013 from Dikhu and Doyang river in the recent report. Hence, the total number of species of *Garra* known from Nagaland is 14 (Ezung *et al*., 2022). The details of the *Garra* species from Nagaland and its conservation status (IUCN, 2022) are represented in Table 1.1.

Table 1.1: Details of *Garra* species in Nagaland

S. No	Scientific name	Economic importance	Distribution (within Nagaland)	Conservation Status
1	*Garra annandalei*	Food and Ornamental	Dikhu, Seidzu and Zungki	L.C
2	*Garra birostris*	Food and Ornamental	Dikhu, Doyang	N.E
3	*Garra chathensis*	Food and Ornamental	Chathe	N.E
4	*Garra gotyla*	Food and Ornamental	Meguiki, Milak, Tepuiki, Tesuru, Dhansiri and Doyang	L.C
5	*Garra gravely*	Food and Ornamental	Doyang	N.T
6	*Garra kempi*	Food and Ornamental	Tsuru and Tizu	L.C
7	*Garra lamta*	Food and Ornamental	Doyang	L.C
8	*Garra langlungensis*	Food and Ornamental	Langlung	N.E
9	*Garra lissorhynchus*	Food and Ornamental	Chathe, Doyang, Milak, Tizit, Tsurang and Tizu	L.C

10	*Garra mcclellandi*	Food and Ornamental	Arachu, Doyang, Dzuna, Lanyi, Likhimro, Seidzu, Tepuiki, Tesuru and Zunki	L.C
11	*Garra naganensis*	Food and Ornamental	Tizu, Meguiki and Doyang	L.C
12	*Garra nasuta*	Food and Ornamental	Intanki, Likhimro, Milak, Seidzu and Tizit	L.C
13	*Garra notate*	Food and Ornamental	Zungki	L.C
14	*Garra rupecula*	Food and Ornamental	Doyang	N.T

Abbreviations: L.C = Least Concern, N.T = Near Threatened, N.E = Not Evaluated

Importance of captive breeding

Captive breeding is the process of spawing fish in captivity by triggering gonadal maturation with an external agent under controlled conditions. It is one of the major steps undertaken in restocking and conservation of many fish species. It is also one of the most extensively utilised management tools for the endemic and endangered species of fish population to re-establish and conserve with concurrent improvisation and improvement of fisheries (Flemming, 1994). Captive breeding is essential in reintroducing endangered, threatened populations or species, or supplementing declining populations by releasing captive-bred individuals or translocating wild individuals (Attard *et al*., 2016). A similar reintroduction program called 'Supportive breeding' is also an approach to captive breeding that aims to restore wild populations of endangered species, using wild fish parents to breed in captivity and returning offspring into the wild at an early stage development (Blanchet *et al*., 2008). Minckley & Deacon (1991) reported that captive breeding is one of the proven techniques of saving endangered species from extinction to

increase their population size with the help of sound breeding techniques under controlled conditions.

However, several limitations exist to employing seed production and stocking methods for commercially farmed aquatic species. Firstly, many species are restrained from maturing and spawn in captivity. It is often difficult to simulate in captivity the environmental conditions, cues, and triggers necessary for successful reproduction, particularly the unique river habitat situations essential and conducive for spawning.

Despite several limitations, captive breeding of aquatic organisms and ranching seeds is considered the most helpful aid to conservation management. In the context that many of the endemic fish species in these places are of value either as food or ornamental species, there is a dire need to standardize the technology for mass production of seeds to replenish and restore them in natural habitats through a judicious ranching program. Mass multiplication of such rare and endangered seeds by captive breeding will also facilitate their sustainable utilization on commercial lines. Unless actions are taken to protect biodiversity, we will lose the opportunity of reaping its full potential benefit to humankind forever. As the maintenance of diversity will depend on conservation choices, the ultimate conservation strategy should allow the operation of natural forces by which both wild and domestic species evolve and maintain gene pools and genetic traits that may prove valuable in the future.

1.2 Review of literature

Physicochemical characteristics of aquatic habitats are of utmost significance for aquatic organisms, including fishes, as it profoundly influences survivability and biological activity. Water quality maintenance is also crucial for survival and growth in

aquaculture. According to Swann (1992), fish culture success or failure was reliant on the nature of water, *viz.*, physicochemical characteristics such as dissolved oxygen, temperature, pH, hardness, and mineral content. A considerable quantity of research has been carried out on the physicochemical in freshwaters of India. Bhandari & Nayal (2008) studied the physicochemical parameters of Kosi river water. Joshi *et al.* (2009) assessed the water quality of Ganga river for drinking purposes. Khan *et al.* (2012) analyzed the physicochemical parameters of Jhelum river in Kashmir. Das (2013) studied the ecology of the Pagladia river of Assam. Kumar *et al.* (2016) carried out the water quality and pollution status of Rawasan river. Gupta *et al.* (2017) studied the effect of physicochemical and biological parameters of Narmada river. Assessment on the water quality index of Vishwamitri river, Gujarat was carried out by Magadum *et al.* (2017). Khan & Mir (2018) carried out a limnological profile study of Kishanganga river in Kashmir. Vijayan *et al.* (2018) conducted a physicochemical analysis of water samples from Cauvery river, Tamil Nadu. Khan & Wen (2021) evaluated the physicochemical and heavy metals characteristics of the Ganga basin, India.

In recent years, several researchers have worked on the limnology of Nagaland. Gurumayum *et al*. (2014) studied the seasonal variation in the physicochemical parameters of water and soil in Doyang, Dikhu, Tserang, Sidzu, Tezu and Zungki river. Baidya & Biswas (2015) also studied the seasonal variation in the physicochemical parameters of Chathe river. Temjen & Singh (2018) assessed the water quality status of Milak river. Longchar *et al.* (2018) and Sarmah *et al.* (2020) studied the seasonal variation in the water quality of Dikhu river of Nagaland. Longkumer *et al*. (2020) assessed the water quality status of Doyang river. Semy & Singh (2021) also study the

water quality assessment of Tsurang river. The literature review reveals no record of any work carried out on the Langlung river.

The morphological study is crucial in fish biology because fish morphology is the primary source of taxonomy study information, categorized as morphometric and meristic characters (Brraich & Akhter, 2015). Morphometric and meristic analysis of fish species is an essential tool for accurate identification of the species by measuring length, weight, counting fins, counting spines, and other parameters (Cavalcanti *et al*., 1999). Various researchers have reported morphological studies on *Garra* species. Kanwal & Panthani (2011) studied the mophometrics of *Garra lamta*. Qayoom *et al*. (2015) and Brraich & Akhter (2015) reported on the morphometric characters and meristic counts study on *Garra gotyla*. Cıcek *et al*. (2016a) studied the morphological difference in *Garra variabilis*; Sabaridasan *et al*. (2017) also studied the morphometric divergence of *Garra mullya*. Keivany *et al*. (2015), Cıcek *et al*. (2016b) and Zamani-Faradonbe *et al.* (2020) worked on the morphometric and meristic divergence on *Garra rufa*.

The mathematical computation of length-weight is an important index for fish biologists to estimate growth in length to growth in weight or *vice-versa*, gonadal development and general condition of the fish. The length-weight relationship is based on the cube law, which represents the growth in fish. Growth is an adaptive property that is ensured by the species' and environment's compatibility (Nikolsky, 1963). Studies on the length-weight relationship indicate the variations in expected weight from the length groups, which indicate fatness and their suitability to the environment (Le Cren, 1951). Length-weight relationship and condition factor of fish species indicate the demographic and biological differences affected by ecological factors of their habitats. Length-weight

relationship and condition factor are very important quantitative parameters determining present and future population success by their strong impact on fish survival, growth, and reproduction (Hossain *et al*. 2006).

The length-weight relationship and condition factor of *Neolissochilus hexagonolepis* and *Garra lissorhynchus* from Jatinga river in Assam was reported by Kar *et al*. (2005). Baby *et al*. (2011) observed the length-weight relationship and condition factor of *Garra gotyla stenorhynchus* from Chaliyar and Bhavani rivers and found that the population from Chaliyar river followed an isometric like growth while the population from Bhavani rivers followed negative allometric growth. Mir *et al*. (2012) observed negative allometric growth throughout the year except March, July and October where the growth was isometric in *Schizopyge curvifrons* from Jhelum river. Kashyap *et al*. (2014) observed isometric growth in the *Channa punctatus* from River Gomti and negative allometric growth from ponds of Kolkata and Malihabad. Gerami *et al*. (2013) studied length-weight relationship in male, female and combined sex in *Garra rufa* form Cholvar river in which all three population was found to have positive allometric growth. Kumar *et al*. (2017) reported positive allometric growth in *Clarias batrachus* from Gaurmati fish farm of Kawardha. Basumatary *et al*. (2017) observed negative allometric growth in *Garra birostris, Garra annandalei , Raiamas bola,* and *Johnius coitor* from the Brahmaputra River basin in Assam. Zamani-Faradonbe *et al.* (2018) also observed positive allometric growth in *Garra amirhosseini* and *Garra gymnothorax* and negative allometric growth in *Garra mondica* and *Garra rosica* in Iranian basins. Renjithkumar *et al*. (2018) reported two cyprinid fishes, *Hypselobarbus thomassi* and *Hypselobarbus kurali*an isometric like growth from Kallada river of Southern Western Ghats. Keivany &

Siami (2020) observed negative allometric growth for both sexes of *Capoeta coadi* from Beheshtabad river.

Fish culture and a successful fishery management program require knowledge of reproductive biology. The studies on the reproductive biology of fish provide improved knowledge about the annual restoration of their stock. Information on sexual dimorphism, sex ratio, fecundity and gonadal-somatic index of a species make subsequent studies on the fish's spawning season, which is important for its management. Rautela *et al*. (2006) reported on the maturation biology of *Garra lamta* in Khoh river. Gong *et al*. (2022) studied the reproductive characteristics of *Garra tibetana* in Tsangpo river. Patimar *et al*. (2010) and Abedi *et al*. (2011) reported on the life history aspects of *Garra rufa*. Bindu & Padmakumar (2014) also reported on the reproductive biology of *Etroplus suratensis* in the Vembanad wetland system of Kerala. Studies on reproductive biology of *Garra tana* and *Garra regressus* was reported by Geremew *et al*. (2015). Kanwal (2017) studied the reproductive biology of *Garra lamta* of Suyal river. Bahuguna *et al*. (2021) also reported on the reproductive potential of *Puntius ticto* of Aasan river.

Sex ratio is the study of male and female percentage in a population. Study of sex ratio is useful for determination of female spawning biomass. Joadder (2013) reported the male to female sex ratio of 1:1.15 in *Labeo bata.* Jega *et al*. (2017) observed the female to male ratio of 1.30:0.97 with female biased population in *Hemibagrus menoda* of Kangsha river. Hossain *et al*. (2019a) reported male dominating population with the male to female ratio of 1:0.90 in *Clupisoma garua* of Ganga river. Aung & Sein (2019) observed the male to female sex ratio of 1: 0.76 *Channa punctata*.

Fecundity is the number of mature eggs in the ovary of female fish prior to spawning (Bagenal & Braum, 1978). Fecundity which includes the quantity and size of eggs, provides information about reproductive potential of fish species which is important for productive aquaculture. Sivashanthini *et al*. (2008) reported a fecundity study of *Gerres abbreviatus* of Jafina lagoon. Rahman & Miah (2009) performed a fecundity study in *Mastacembelus pancalus.* Marimuthu *et al*. (2009) investigated the fecundity of *Anabus testudineus*. Kant *et al*. (2016) reported the study of fecundity on *Puntius sophore*. Rashid & Dobriyal (2020) investigated the fecundity analysis of *Mastacembelus armatus*.

Gonado-somatic index (GSI) indicates the relative development of the gonads (gonad weight) standardized against the total weight. The index has been frequently used to judge the relative development of the gonads with the assumption that the gonads are well developed in sexually active individuals and regress during the period of sexual inactivity (Vlaming *et al*., 1982). Borthakur (2018) reported the highest gonado-somatic index value during the month of July in *Xenontedon cancila*. Jewel *et al.* (2019) observed the breeding season of *Cirrhinus reba* from June to September, with the peak in August. Boonkusol *et al*. (2020) also reported the highest gonado-somatic index value during the month of July in *Channa striata*.

Hormonal manipulation has proven to be the usual method for captive breeding among fishes, especially for species that do not naturally breed in confinement. Culture and breeding of different fish species have been focused in recent years. Many workers have done captive and open-induced breeding practices through hypophysation on different species. A considerable number of researchers have worked on these aspects.

Nandeesha *et al*. (1990) reported induced breeding on major Indian carp. Sarkar *et al*. (2005); Bhattacharyya & Homechaudhuri (2009) and Singh *et al*. (2012) also reported on the captive breeding of *Anabas testudineus*. Banik *et al.* (2011) studied the captive breeding of *Ompok bimaculatus.* Mercy *et al*. (2015) reported on the captive breeding of *Sahyadria denisonii.* Ali *et al.* (2016) also reported on the induced breeding of *Heteropneustes fossilis.* Rahman & Awal (2016) studied the development of captive breeding techniques of *Channa striatus*. Hasan *et al.* (2016) reported on the captive breeding of *Mastacembelus pancalus.* Vijayakumar *et al*. (2020) studied the captive breeding of *Neolissochilus tamiraparaniensis*. Mercy *et al*. (2021) reported captive breeding on *Dravidia fasciata*.

A perusal of the literature reveals that limited work has been carried out on breeding *Garra* species. Somvanshi (1980) reported on the spawning biology of *Garra mullya.* Sundarabarathy *et al.* (2005) observed breeding and larval rearing of *Garra cyclonensis*. Thampy (2009) also reported breeding and larval rearing of *Garra surendranathanii.* Vazirzadeh *et al.* (2015) studied on the spawning induction in *Garra rufa*.

Fish exhibit considerable differences in their breeding behaviour. Most of them are seasonal breeders and spawn during the rainy season. Literature reviews revealed that several scientists have reported information on spawning behaviour (Stacey, 1976; Kyle & Peter, 1982; Esteve, 2005; Vincent & Thomas, 2008; Thampy, 2009; Colin, 2010; Domeier, 2012; Walsh *et al*., 2013; Angami, 2012; Amenla, 2014; Dey *et al*., 2014; La Mesa *et al*., 2021).

An understanding of the early development of a fish species is also considered to be an important step for fish culturists. Many researchers have worked on the early development of different fish (Mercy *et al*., 2003; Arezo *et al*., 2005; Sahoo *et al*., 2007; Swain *et al*., 2008; Rahman *et al*., 2009; Rahaman *et al*., 2011; Nica *et al*., 2012; Rakhi *et al*., 2015; Mahapatra & Krishna, 2016; Islam *et al*., 2017; Islam & Rani, 2021; Yasmine *et al*., 2022)

The fishes of the genus *Garra* are generally small to medium-sized, basic coloured with ornamental and food value. In Nagaland, this species is highly relished for its taste. However, most of the catch in this region is mainly from the wild habitat, which, in process, is declining the fish resources in the wild and, in the long term, may threaten the fish species. If we look into the conservation status of the *Garra* species from Nagaland, out of the 14 species: 09 species fall under the least concern category; 03 species fall under the not evaluated category due to their recent description, and two species fall under the near threatened category. The conservation status suggests that if the random collection of this species continues, it may threaten the species in the long term.

Due to ecological degradation and overutilization, some species are unavailable frequently, although earlier found as dominant species. In recent years the fish species and their natural habitat are facing prevailing threats due to destructive fishing methods such as using poisons and dynamite for fish harvesting; destruction of wild habitats such as boulder digging, sand digging; and various anthropogenic activities hazards such as the construction of dams, irrigation for human needs and waste deposit. In the last 3-4 decades, in natural waters, there has been a rapid decline in the population of fish species,

and there is a need to sustain their population through conservation and aquaculture techniques or, better, through principles of conservation aquaculture. An excellent sustainable management practice can check the further extinction of these fishes from this region.

Therefore, after reviewing all concerned literature, it came to be known that there is very scarce information on the fish species available in this region, and there is a huge lack of detailed study on the biology, captive maturation and sustainable breeding of fish species specifically from Nagaland.

1.3 Objectives of the present study

Garra langlungensis is a hill stream fish of Nagaland with a prospective ornamental value which is highly valued for its delicacy and commercial value. However, no information is available on any work on this fish. For domestication, breeding, and culture under the captivity of the species, detailed eco-biological information is utmost importance. Keeping view on this, the present work was carried out to study the life history trait and development of captive breeding technique with the following objectives:

1. To study the habitat ecology of the *Garra langlungensis*.
1. To examine the length-weight relationship and condition factor to ascertain the relationship between length and weight and the general well-being of the fish.
2. To study the reproductive biology of *Garra langlungensis* to gain insights into the sexual dimorphism, sex ratio, fecundity and gonadal-somatic index.
3. To develop in-house breeding technology and propagation of *Garra langlungensis* for conservation.

Chapter 2

Ecology of Langlung River, Nagaland

Contents

2.1 Introduction

Water is essential for the growth and survival of all living organisms. Water quality is related to all other hydrological properties such as physical, chemical, and biological characteristics, which is crucial for fish production. It is widely used for drinking, irrigation and other purposes. Interactions between physical and chemical properties of water contribute to the composition, distribution, and abundance of aquatic organisms. It also provides insight into the relationship between organisms and their environment and is used to determine the water quality and productivity of the water body (Haruna *et al*., 2006).

The physicochemical parameters for water quality assessment provide a proper indication of the water body status, productivity, and sustainability (Djukic *et al*., 1994). The role of physicochemical parameters like temperature, velocity, transparency, dissolved oxygen, pH, alkalinity and hardness provides information for maintaining a healthy aquatic environment for survival and optimal fish growth. Generally, the well-being of fish is correlated to the water quality in an aquatic environment. Therefore, it is critical to study and assess the physicochemical properties of any water body for a better understanding and to maintain the biodiversity of an aquatic environment.

Seasonal fluctuation in physicochemical properties of water for different rivers in Nagaland have been reported by Baidya & Biswas (2015), Longchar *et al*., (2018), Baidya *et al*., (2018), Temjen & Singh (2018), Longkumer *et al*., (2020) and Semy & Singh (2021).

Looking at the importance of studying physicochemical parameters and understanding a fish species natural habitat could assist in overall management and

planning for any captive breeding program. The genus *Garra* is known to inhabit fast-flowing streams and rivers. Hitherto, there has been no report on the study of physical and chemical parameters of the Langlung river. Hence the present study aims to analyze the physicochemical parameters of the Langlung river to understand the wild habitat of *Garra langlungensis*.

2.2 Materials and methods

2.2.1 Study area and sample collection

For physicochemical study of Langlung river, sample collection was done seasonally, i.e. winter (Dec, Jan, Feb), spring (Mar, April, May), summer (June, July, Aug) and autumn (Sept, Oct, Nov) for one year. Sampling was carried out in five sites at the end of every month from March 2017- February 2018 between 9-12 AM. Sampling sites, *i*.e., Site 1 (25˚42′57.45″ N, 93˚39′50.85″ E), Site 2 (25˚43′9.84″ N, 93˚39′46.68″ E), Site 3 (25˚43′20.36″ N, 93˚39′53.28″ E), Site 4 (25˚43′31.43″ N, 93˚39′45.10″ E) and Site 5 (25˚43′50.90″ N, 93˚39′40.10″ E) were selected along the stretch of Zutovi village having a distance of about 400 – 800 m between each sampling sites. The sampling sites and Langlung river in different seasons are shown in Figure 2.1(A-E).

2.2.2. Temperature

Both surface water and air temperature were measured using a mercury thermometer graduated from 0 to 100 ˚C.

2.2.3 Water velocity

Water velocity was measured using a float, a stop clock and a measuring tape. Time taken by the drifted float to cover a particular distance represents the velocity of

running water. The velocity of water was determined after Saha (2010) following the empirical formula:

$$V = \frac{d}{1.2t}$$

Where,

V = velocity (m/sec)

d = distance between the two poles (meter)

1.2 = a constant

t = time required (sec)

2.2.4 Water transparency

Transparency was determined using a Secchi disc. The transparency of the water was computed as follows-

$$Secchi\ disc\ light\ penetration = \frac{A+B}{2}\ (cm)$$

Where,

A= depth at which Secchi disc disappears

B= depth at which Secchi disc reappears

2.2.5 pH

The pH of water samples was measured using a portable digital pH meter.

2.2.6 Dissolved oxygen

It was estimated using a compact water analysis kit Aquamerck 1.11151.0001.

2.2.7 Total alkalinity

It was estimated by titration method as suggested by APHA (2005).

2.2.8 Total hardness

It was estimated using a compact water analysis kit Aquamerck 1.11151.0001.

2.2.9 Statistical analysis

All the statistical analysis was calculated using Microsoft Office Excel 2007.

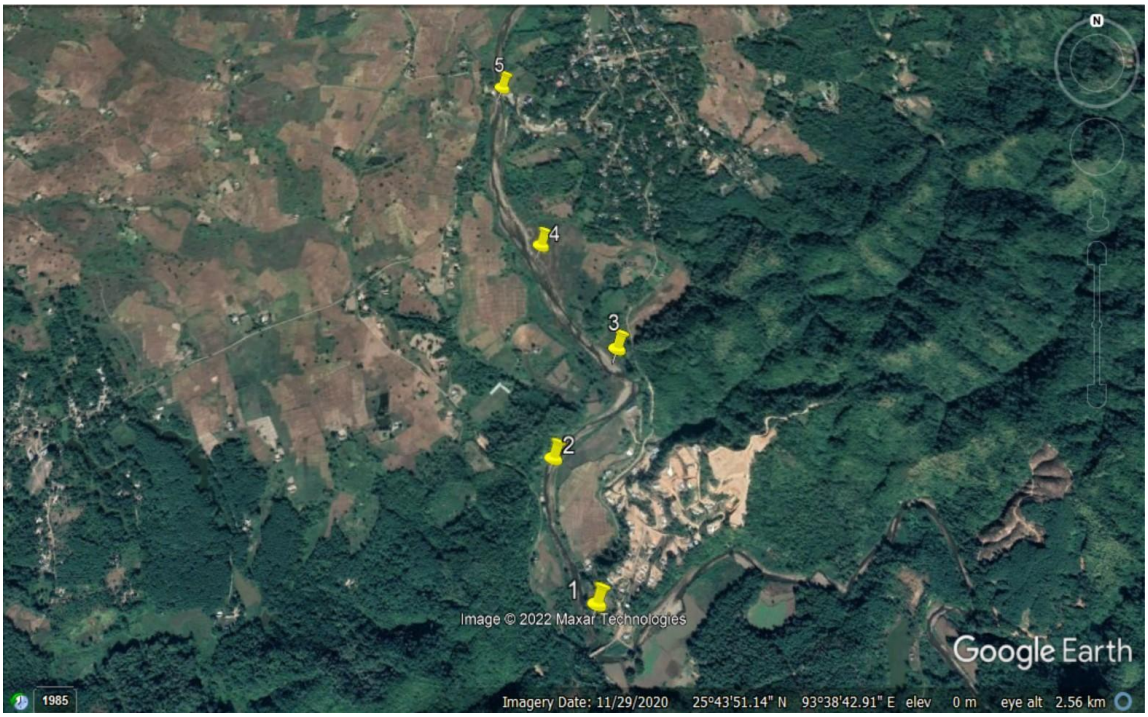


Figure 2.1A: Google Earth image of Langlung river, Dimapur, India, Yellow map pin showing the sampling sites

Figure 2.1B: Langlung river during winter season

Figure 2.1C: Langlung river during spring season

Figure 2.1D: Langlung river during summer season

Figure 2.1E: Langlung river during autumn season

2.3 Results

2.3.1 Physical parameters

2.3.1.1 Air temperature

The air temperature ranged from 27.16 – 34.53 ˚C, with an annual mean of 31.26 ± 2.11 ˚C. Summer air temperatures were the maximum in selected Langlung river area, with its peak value during June in site 5, followed by spring and autumn. Winter temperatures were the minimum, with their lowest during January in site 4. The mean values of seasonal variations in water temperature have been represented in Table 2.1.

At site 1, the air temperature ranged between 27.50 – 34.53 ˚C, with an average mean of 31.44 ± 2.18 ˚C. The range of air temperature variation at site 1 was 7.03 ˚C

At site 2, the air temperature ranged between 27.66 – 34.43 ˚C, with an average mean of 31.29 ± 2.15 ˚C. The range of air temperature variation at site 2 was 6.77 ˚C

At site 3, the air temperature ranged between 27.75 – 34.06 ˚C, with an average mean of 31.22 ± 2.06 ˚C. The range of air temperature variation at site 3 was 6.31 ˚C

At site 4, the air temperature ranged between 27.16 – 34.30 ˚C, with an average mean of 31.13 ± 2.25 ˚C. The range of air temperature variation at site 4 was 7.14 ˚C

At site 5, the air temperature ranged between 27.33 – 34.60 ˚C, with an average mean of 31.21 ± 2.27 ˚C. The range of air temperature variation at site 5 was 7.27 ˚C. The air temperature was highest in June and the lowest in January for all the study sites. The monthly and seasonal variation of air temperature in Langlung river is depicted in Figure 2.2 and 2.3.

Table 2.1: Seasonal variations of air temperature (˚C) in Langlung river during the period from March 2017 to February 2018 (Mean ± S.D)

	Study Sites	Seasons				Annual Air temperature (˚C)
		Winter	Spring	Summer	Autumn	
Air temperature (˚C)	Site 1	28.58 ± 1.01	31.88 ± 1.60	33.74 ± 0.86	31.54 ± 1.12	31.26 ± 2.11
	Site 2	28.49 ± 0.76	31.85 ± 1.50	33.51 ± 0.97	31.29 ± 1.46	
	Site 3	28.44 ± 0.60	31.66 ± 1.57	33.40 ± 0.71	31.38 ± 1.02	
	Site 4	28.21 ± 1.05	31.75 ± 1.73	33.46 ± 0.95	31.08 ± 1.12	
	Site 5	28.16 ± 0.84	31.73 ± 1.38	33.69 ± 0.93	31.25 ± 1.20	

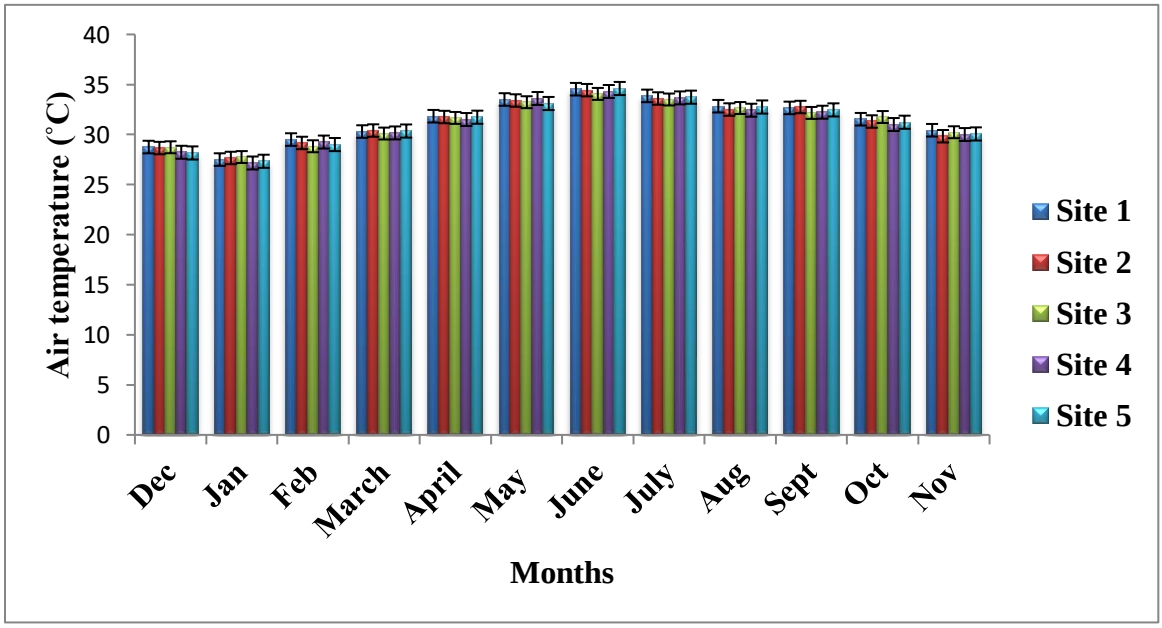


Figure 2.2: Monthly variations of air temperature (˚C) in Langlung river during the period from March 2017 to February 2018

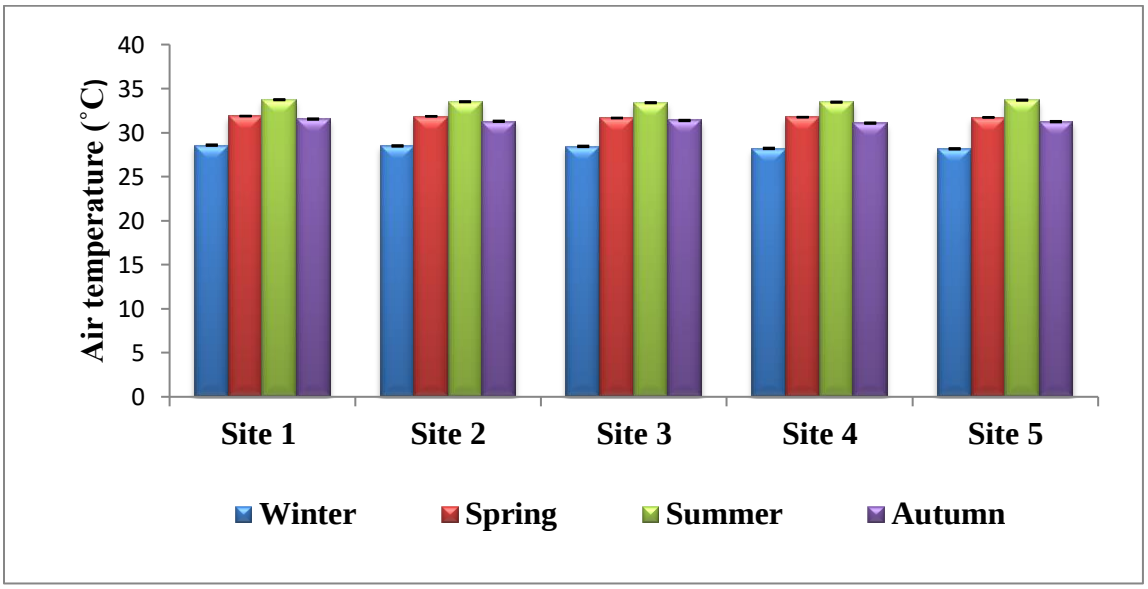


Figure 2.3: Seasonal variations of air temperature (˚C) in Langlung river during the period from March 2017 to February 2018

2.3.1.2 Water temperature

The water temperature ranged from 24.75 – 31.66 ˚C, with an annual mean of 28.41 ± 2.04 ˚C. Water temperatures in Langlung river were the maximum in summer with its peak value during June in site 5, followed by spring and autumn, while winter temperatures were the minimum with their lowest during January in site 2. The mean values of seasonal variations in water temperature have been represented in Table 2.2.

At site 1, the water temperature ranged between 25.00 – 31.40 ˚C, with an average mean of 28.54 ± 2.08 ˚C. The range of air temperature variation at site 1 was 6.4 ˚C

At site 2, the water temperature ranged between 25.16 – 31.53 ˚C, with an average mean of 28.34 ± 2.08 ˚C. The range of air temperature variation at site 2 was 6.37 ˚C

At site 3, the water temperature ranged between 25.35 – 31.30 ˚C, with an average mean of 28.50 ± 2.08 ˚C. The range of air temperature variation at site 3 was 5.95 ˚C

At site 4, the water temperature ranged between 24.75 – 31.25 ˚C, with an average mean of 28.24 ± 2.08 ˚C. The range of air temperature variation at site 4 was 6.5 ˚C

At site 5, the water temperature ranged between 24.86 – 31.66 ˚C, with an average mean of 28.42 ± 2.23 ˚C. The range of air temperature variation at site 5 was 6.8 ˚C. The highest water temperature in all the study sites was recorded in June and the lowest in January. The monthly and seasonal variations of water temperature in Langlung river is depicted in Figure 2.4 and 2.5.

Table 2.2: Seasonal variations of water temperature (˚C) in Langlung river during the period from March 2017 to February 2018 (Mean ± S.D)

	Study Sites	Seasons				Annual Water temperature (˚C)
		Winter	Spring	Summer	Autumn	
Water temperature (˚C)	Site 1	25.78 ± 0.75	28.98 ± 1.39	30.83 ± 0.52	28.60 ± 1.20	28.41 ± 2.04
	Site 2	25.68 ± 0.50	28.66 ± 1.52	30.74 ± 0.70	28.27 ± 1.22	
	Site 3	25.75 ± 0.59	28.83 ± 1.42	30.79 ± 0.41	28.56 ± 1.21	
	Site 4	25.55 ± 0.75	28.54 ± 1.45	30.58 ± 0.58	28.26 ± 1.12	
	Site 5	25.46 ± 0.75	28.74 ± 1.43	30.88 ± 0.59	28.47 ± 1.06	

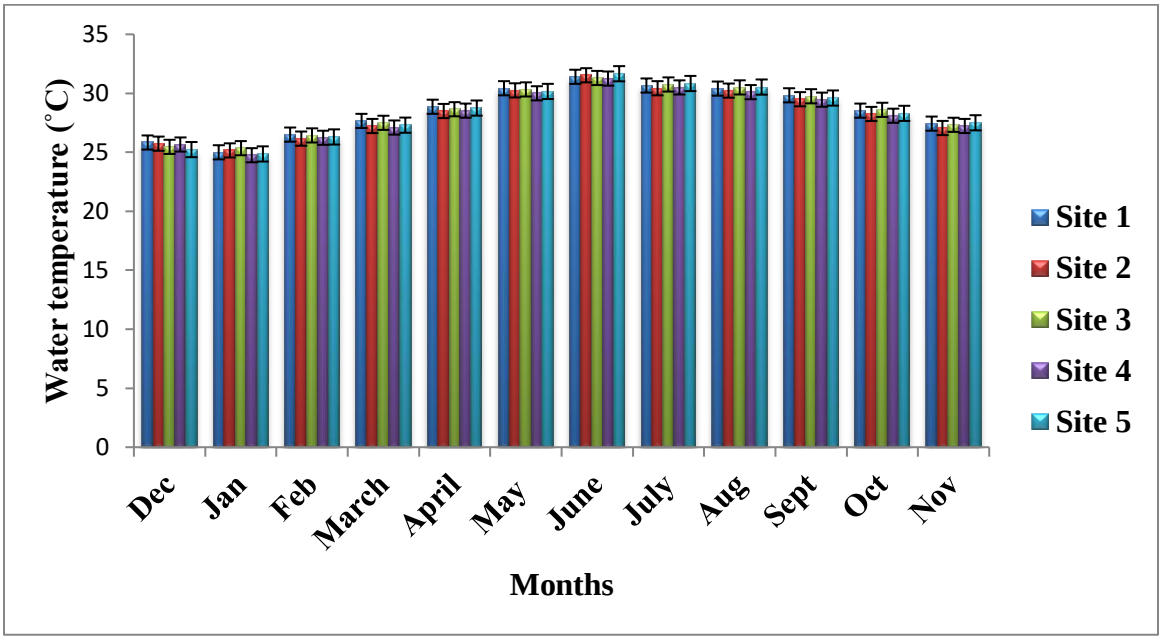


Figure 2.4: Monthly variations of water temperature (˚C) in Langlung river during the period from March 2017 to February 2018

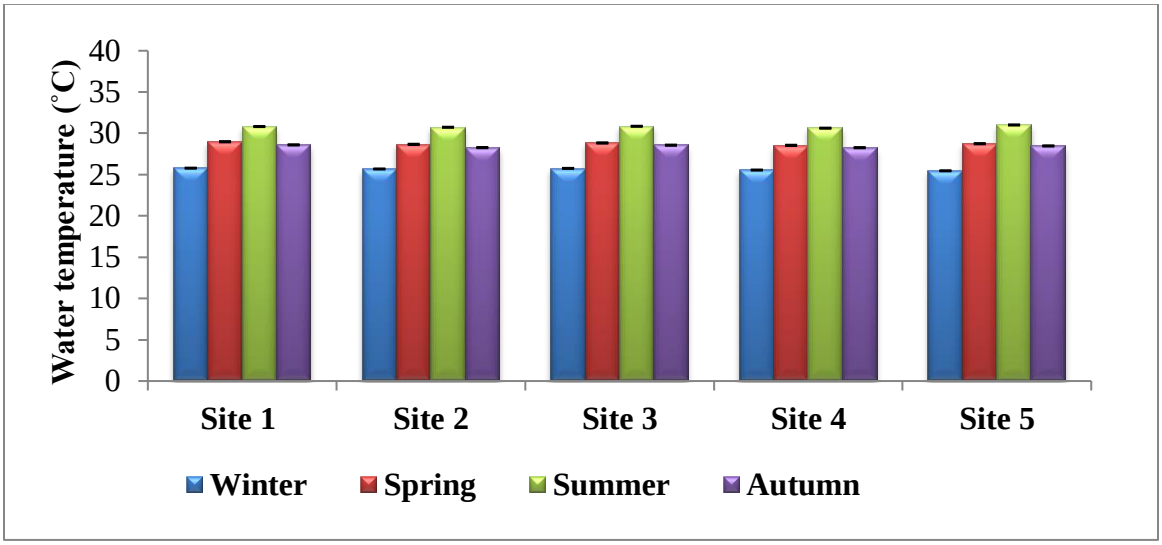


Figure 2.5: Seasonal variations of water temperature (˚C) in Langlung river during the period from March 2017 to February 2018

2.3.1.3 Water velocity

The water velocity ranged from 0.74 – 3.89 m/sec, with an annual mean of 1.84 ± 0.76 m/sec. A wide variation was observed in the water velocity of the Langlung river, reaching its peak value during July at site 4 and its lowest in March at site 1. The maximum water velocity was recorded during summer, followed by autumn and spring, while the minimum was recorded during winter. The mean values of seasonal variations in water velocity have been represented in Table 2.3.

At site 1, the water velocity ranged between 0.74 – 3.31 m/sec, with an average mean of 1.72 ± 0.74 m/sec. The range of water velocity variation at site 1 was 2.57 m/sec.

At site 2, the water velocity ranged between 0.9 – 3.01 m/sec, with an average mean of 1.77 ± 0.71 m/sec. The range of water velocity variation at site 2 was 2.11 m/sec.

At site 3, the water velocity ranged between 0.81 – 3.47 m/sec, with an average mean of 1.95 ± 0.81 m/sec. The range of water velocity variation at site 3 was 2.66 m/sec.

At site 4, the water velocity ranged between 0.98 – 3.89 m/sec, with an average mean of 2.12± 0.93 m/sec. The range of water velocity variation at site 4 was 2.91 m/sec.

At site 5, the water velocity ranged between 0.89 – 2.9 m/sec, with an average mean of 1.63 ± 0.60 m/sec. The range of water velocity variation at site 5 was 2.01 m/sec. The water velocity was highest in the month of July and the lowest in the month of March in all the selected study sites. The monthly and seasonal variation of water velocity in Langlung river is depicted in Figure 2.6 and 2.7.

Table 2.3: Seasonal variations of water velocity (m/sec) in Langlung river during the period from March 2017 to February 2018 (Mean ± S.D)

	Study Sites	Seasons				Annual Water velocity (m/sec)
		Winter	Spring	Summer	Autumn	
Water velocity (m/sec)	Site 1	1.25 ± 0.21	1.34 ± 0.68	2.78 ± 0.47	1.52 ± 0.21	1.84 ± 0.76
	Site 2	1.23 ± 0.10	1.57 ± 0.83	2.73 ± 0.25	1.55 ± 0.28	
	Site 3	1.33 ± 0.25	1.54 ± 0.88	3.01 ± 0.41	1.93 ± 0.36	
	Site 4	1.40 ± 0.23	1.65 ± 0.96	3.37 ± 0.47	2.06 ± 0.37	
	Site 5	1.24 ± 0.30	1.35 ± 0.57	2.44 ± 0.42	1.47 ± 0.13	

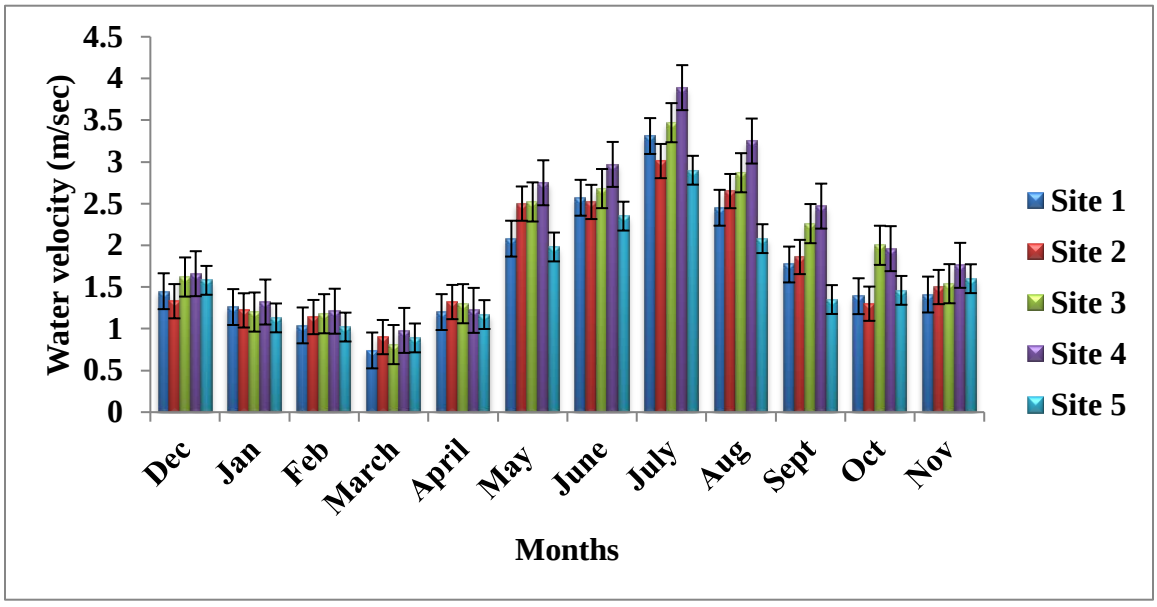


Figure 2.6: Monthly variations of water velocity (m/sec) in Langlung river during the period from March 2017 to February 2018

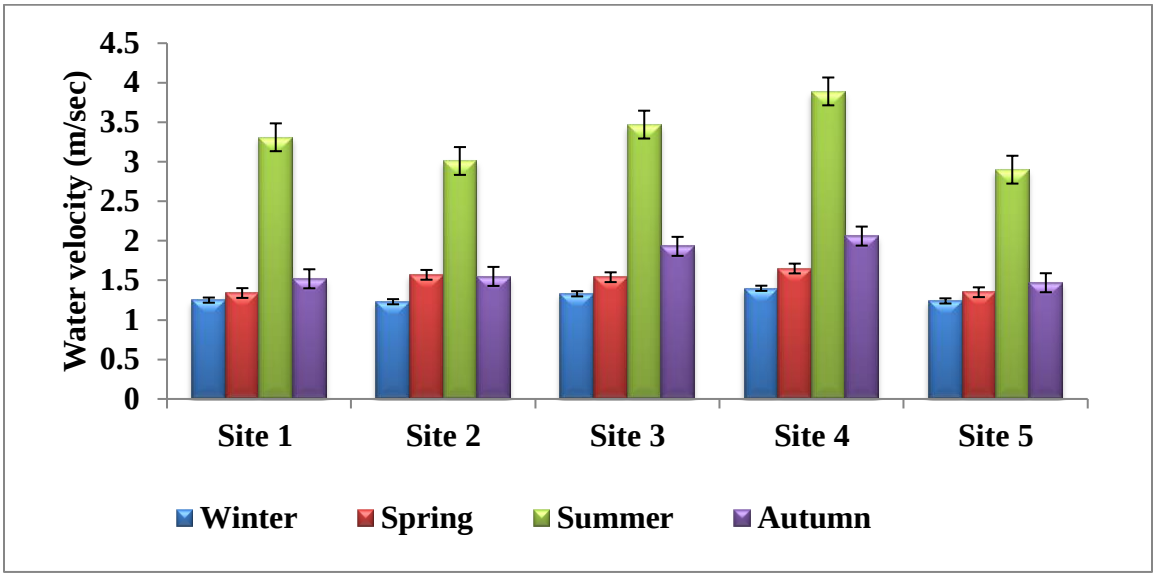


Figure 2.7: Seasonal variations of water velocity (m/sec) in Langlung river during the period from March 2017 to February 2018

2.3.1.4 Water transparency

The water transparency ranged from 0.75 – 25.5 cm, with an annual mean of 10.01 ± 7.51 cm. During autumn season, the maximum water transparency was recorded, peaking in October at site 1, followed by winter and spring. The minimum water transparency was recorded during summer, with its lowest value in July at site 4. The mean values of seasonal variations in water transparency have been represented in Table 2.4.

At site 1, the water transparency ranged between 1.5 – 25.5 cm, with an average mean of 11.85 ± 8.83 cm. The range of water transparency variation at site 1 was 24 cm.

At site 2, the water transparency ranged between 1.25 – 21.2 cm, with an average mean of 9.83 ± 7.08 cm. The range of water transparency variation at site 2 was 19.95 cm.

At site 3, the water transparency ranged between 1.5 – 23.8 cm, with an average mean of 10.23 ± 7.70 cm. The range of water transparency variation at site 3 was 22.3 cm.

At site 4, the water transparency ranged between 0.75 – 25 cm, with an average mean of 8.83 ± 7.44 cm. The range of water transparency variation at site 4 was 24.25 cm.

At site 5, the water transparency ranged between 1 – 20.8 cm, with a mean of 9.32 ± 7.33 cm. The range of water transparency variation at site 5 was 19.8 cm. The water transparency was recorded as highest in October in site 1, 3, 4 and November in site 2, 5 and the lowest in July in all the study sites. The monthly and seasonal variation of water transparency in Langlung river is depicted in Figure 2.8 and 2.9.

Table 2.4: Seasonal variations of water transparency (cm) in Langlung river during the period from March 2017 to February 2018 (Mean ± S.D)

	Study Sites	Seasons				Annual Water transparency (cm)
		Winter	Spring	Summer	Autumn	
Water transparency (cm)	Site 1	18.43 ± 2.27	7.27 ± 4.29	2.30 ± 0.83	19.42 ± 9.27	10.01 ± 7.51
	Site 2	14.83 ± 1.26	5.67 ± 2.38	2.38 ± 1.08	16.42 ± 7.44	
	Site 3	13.93 ± 3.37	6.25 ± 3.38	2.53 ± 1.05	18.18 ± 8.63	
	Site 4	12.03 ± 2.04	4.67 ± 2.18	1.83 ± 1.01	16.80 ± 9.17	
	Site 5	14.80 ± 3.50	4.92 ± 2.50	1.75 ± 0.75	15.80 ± 7.32	

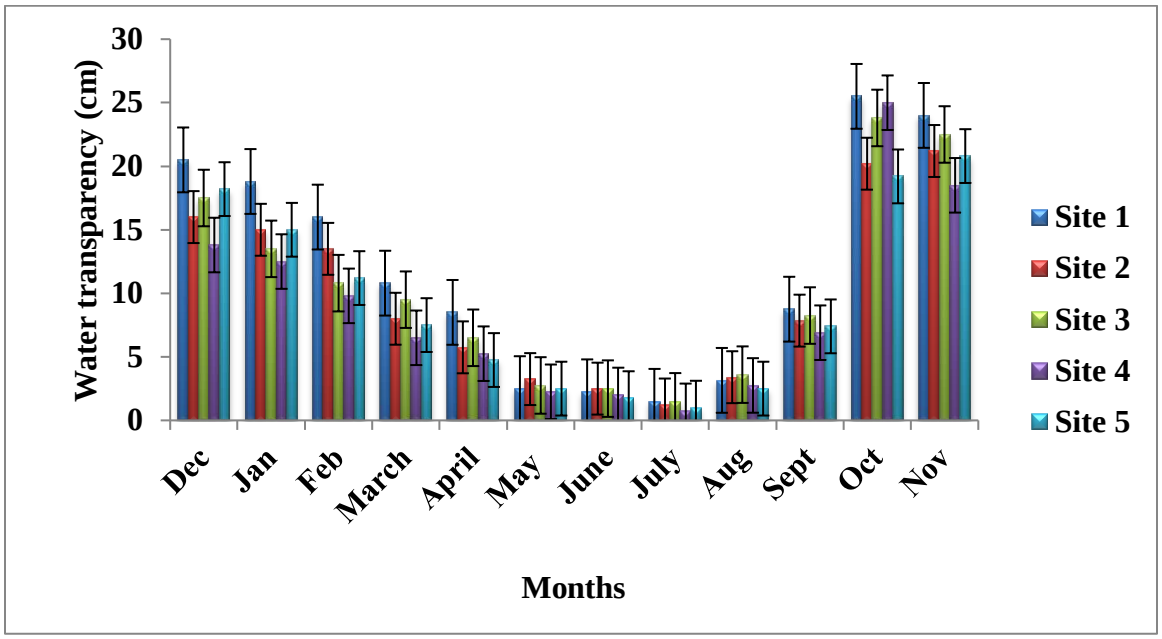


Figure 2.8: Monthly variations of water transparency (cm) in Langlung river during the period from March 2017 to February 2018

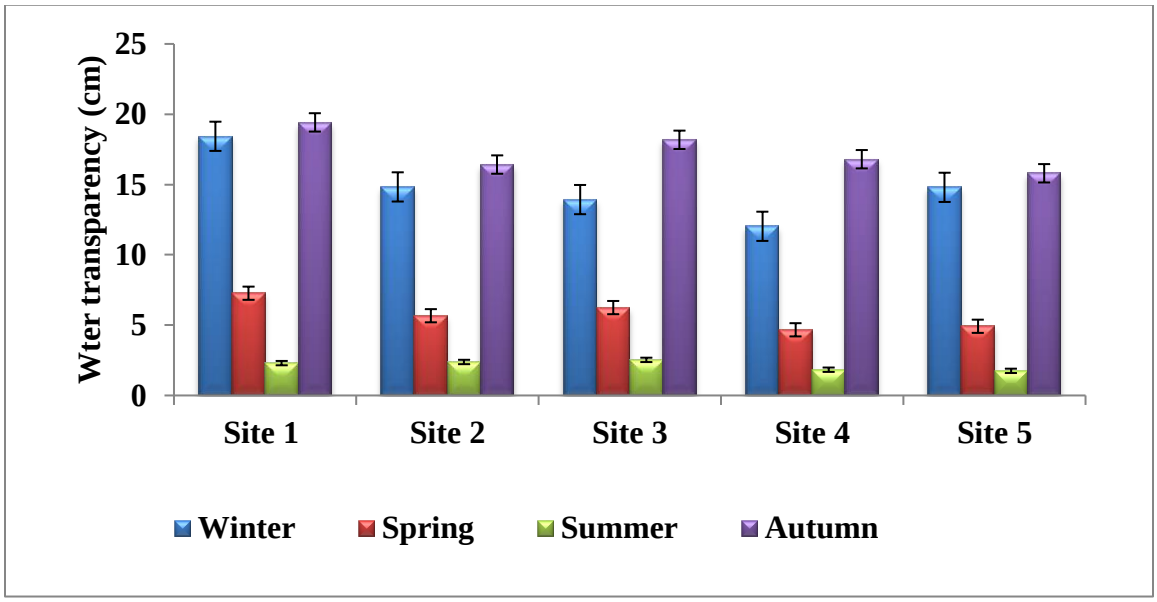


Figure 2.9: Seasonal variations of water transparency (cm) in Langlung river during the period from March 2017 to February 2018

2.3.2 Chemical parameters

2.3.2.1 pH

The pH of Langlung river ranged slightly during the year, ranging from 7 – 8, with an annual mean of 7.57 ± 0.26. The pH of the Langlung river reached its peak value during December, January and February at site 3 and site 4 and its lowest in June and July at sites 2 and 4. The maximum pH was recorded during winter, followed by autumn and spring, while the minimum was recorded during summer. The mean values of seasonal variations in pH have been represented in Table 2.5.

At site 1, the pH ranged between 7.1 – 7.8, with an average mean of 7.53 ± 0.21. The pH was recorded as highest in November and December and lowest in July. The range of pH variation at site 1 was 0.7.

At site 2, the pH ranged between 7 – 7.8, with an average mean of 7.53 ± 0.27. The pH was recorded as highest in November and lowest in July. The range of pH variation at site 2 was 0.8.

At site 3, the pH ranged between 7.1 – 8, with an average mean of 7.63 ± 0.31. The pH was recorded as highest in December and lowest in July. The range of pH variation at site 3 was 0.9.

At site 4, the pH ranged between 7 – 8, with an average mean of 7.60 ± 0.28. The pH was recorded as highest in November and January and lowest in June. The range of pH variation at site 4 was 1.

At site 5, the pH ranged between 7.1 – 7.9, with a mean of 7.56 ± 0.24. The pH was recorded as highest in December, January and February and lowest in June and July.

The range of pH variation at site 5 was 0.8. The monthly and seasonal variation of pH in Langlung river is depicted in Figure 2.10 and 2.11.

Table 2.5: Seasonal variations of pH in Langlung river during the period from March 2017 to February 2018 (Mean ± S.D)

	Study Sites	Seasons				Annual pH
		Winter	Spring	Summer	Autumn	
pH	Site 1	7.77 ± 0.06	7.53 ± 0.15	7.27 ± 0.15	7.57 ± 0.06	7.57 ± 0.26
	Site 2	7.77 ± 0.06	7.47 ± 0.15	7.20 ± 0.26	7.70 ± 0.10	
	Site 3	7.97 ± 0.06	7.60 ± 0.20	7.23 ± 0.15	7.70 ± 0.20	
	Site 4	7.87 ± 0.15	7.60 ± 0.10	7.27 ± 0.31	7.67 ± 0.15	
	Site 5	7.83 ± 0.06	7.50 ± 0.10	7.27 ± 0.21	7.63 ± 0.06	

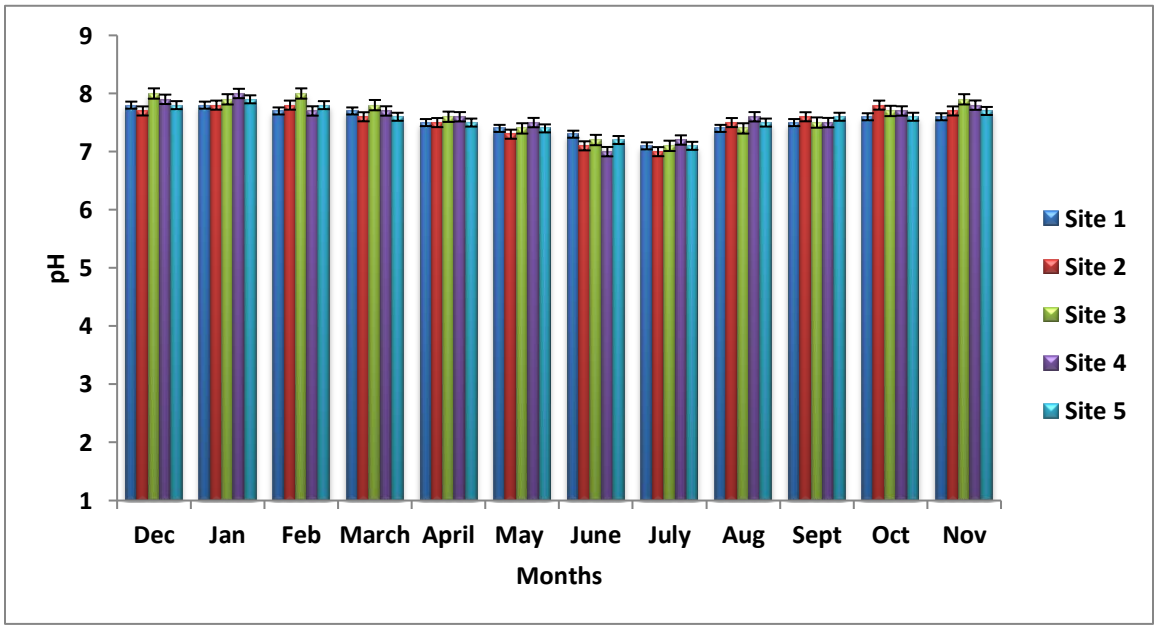


Figure 2.10: Monthly variations in pH of Langlung river during the period from March 2017 to February 2018

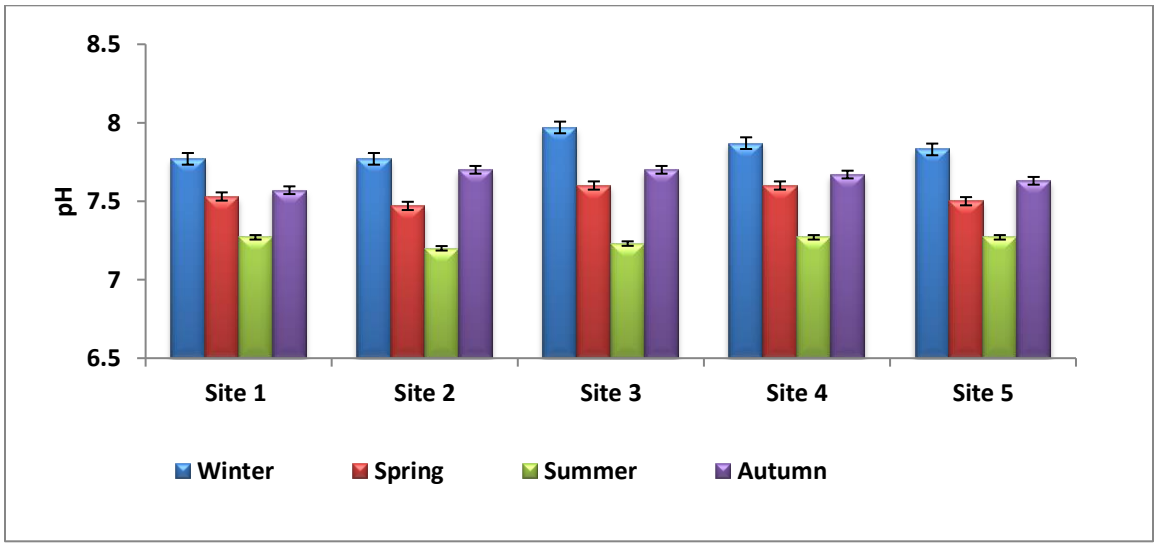


Figure 2.11: Seasonal variations in pH of Langlung river during the period from March 2017 to February 2018

2.3.2.2 Dissolved oxygen

The dissolved oxygen ranged from 5.2 – 11.6 mg/l, with an annual mean of 7.60 ± 1.73 mg/l. The maximum dissolved oxygen was recorded during the winter, peaking in January at site 4, followed by autumn and spring. The minimum dissolved oxygen recorded during the summer declined to its lowest value in July at site 5. The mean values of seasonal variations in dissolved oxygen have been represented in Table 2.6.

At site 1, the dissolved oxygen ranged between 5.5 – 10.6 mg/l, with an average mean of 7.52 ± 1.73 mg/l. The range of dissolved oxygen variation at site 1 was 5.1 mg/l.

At site 2, the dissolved oxygen ranged between 5.4 – 11.2 mg/l, with an average mean of 7.47 ± 1.81 mg/l. The range of dissolved oxygen variation at site 2 was 5.8 mg/l.

At site 3, the dissolved oxygen ranged between 5.6 – 10.9 mg/l, with an average mean of 7.77 ± 1.77 mg/l. The range of dissolved oxygen variation at site 3 was 5.3 mg/l.

At site 4, the dissolved oxygen ranged between 5.9 – 11.6 mg/l, with an average mean of 7.98 ± 1.83 mg/l. The range of dissolved oxygen variation at site 4 was 5.7 mg/l.

At site 5, the dissolved oxygen ranged between 5.2 – 10.5 mg/l, with an average mean of 7.25 ± 1.72 mg/l. The range of dissolved oxygen variation at site 5 was 5.3 mg/l. The dissolved oxygen was recorded highest in January and lowest in July in all the study sites. The monthly and seasonal variation of dissolved oxygen in Langlung river is depicted in Figure 2.12 and 2.13.

Table 2.6: Seasonal variations of dissolved oxygen (mg/l) in Langlung river during the period from March 2017 to February 2018 (Mean ± S.D)

	Study Sites	Seasons				Annual Dissolved oxygen (mg/l)
		Winter	Spring	Summer	Autumn	
Dissolved oxygen (mg/l)	Site 1	9.93 ± 0.59	7.13 ± 1.11	5.80 ± 0.44	7.20 ± 1.11	7.60 ± 1.73
	Site 2	9.93 ± 1.14	6.90 ± 1.15	5.77 ± 0.40	7.27 ± 1.10	
	Site 3	10.10 ± 0.70	7.27 ± 1.40	5.97 ± 0.40	7.73 ± 1.06	
	Site 4	10.53 ± 0.93	7.50 ± 1.05	6.17 ± 0.38	7.73 ± 1.07	
	Site 5	9.53 ± 0.85	6.63 ± 1.17	5.53 ± 0.42	7.30 ± 1.14	

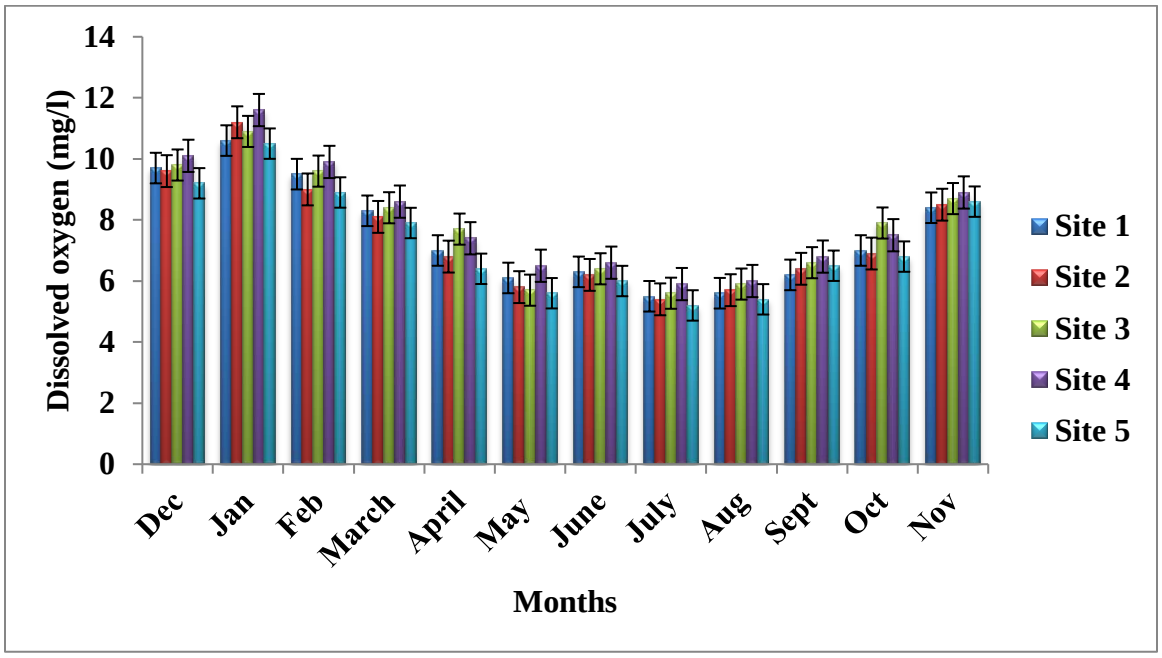


Figure 2.12: Monthly variations in dissolved oxygen (mg/l) of Langlung river during the period from March 2017 to February 2018

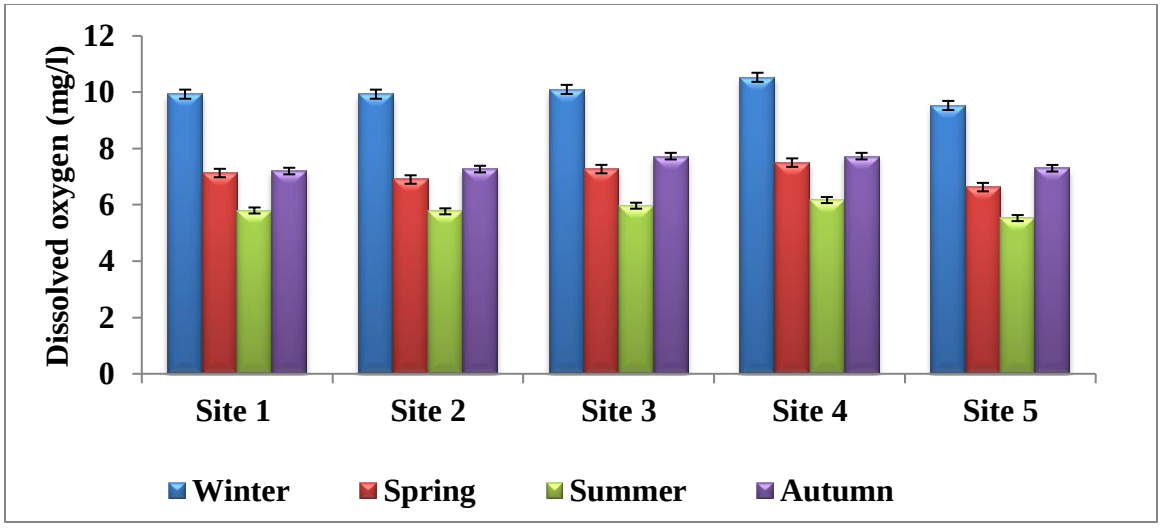


Figure 2.13: Seasonal variations in dissolved oxygen (mg/l) of Langlung river during the period from March 2017 to February 2018

2.3.2.3 Total alkalinity

The total alkalinity ranged from 34.1 – 70 mg/l, with an annual mean of 50.14 ± 11.65 mg/l. The maximum total alkalinity was recorded in winter, peaking in January at site 4 while the minimum was recorded in summer with the lowest in August at site 1. The mean values of seasonal variations in total alkalinity have been represented in Table 2.7.

At site 1, the total alkalinity ranged between 34.1 – 61.90 mg/l, with an average mean of 46.90 ± 11.05 mg/l. The range of total alkalinity variation at site 1 was 27.4 mg/l.

At site 2, the total alkalinity ranged between 34.6 – 63.4 mg/l, with an average mean of 48.61 ± 11.49 mg/l. The range of total alkalinity variation at site 2 was 28.8 mg/l.

At site 3, the total alkalinity ranged between 37.6 – 67.5 mg/l, with an average mean of 52.14 ± 12.11 mg/l. The range of total alkalinity variation at site 3 was 29.9 mg/l.

At site 4, the total alkalinity ranged between 40 – 70 mg/l, with an average mean of 53.76 ± 12.55 mg/l. The range of total alkalinity variation at site 4 was 30 mg/l.

At site 5, the total alkalinity ranged between 35 – 65 mg/l, with an average mean of 49.31 ± 11.67 mg/l. The range of total alkalinity variation at site 5 was 30 mg/l. The total alkalinity was recorded as the highest in January and the lowest in August in all the study sites. The monthly and seasonal variation of total alkalinity in Langlung river is depicted in Figure 2.14 and 2.15.

Table 2.7: Seasonal variations of total alkalinity (mg/l) in Langlung river during the period from March 2017 to February 2018 (Mean ± S.D)

	Study Sites	Seasons				Annual Total alkalinity (mg/l)
		Winter	Spring	Summer	Autumn	
Total alkalinity (mg/l)	Site 1	60.70 ± 0.75	47.33 ± 8.00	35.40 ± 1.54	44.17 ± 10.43	50.14 ± 11.65
	Site 2	62.73 ± 0.59	49.13 ± 8.86	36.43 ± 1.68	46.13 ± 10.62	
	Site 3	66.53 ± 1.34	52.50 ± 9.54	39.13 ± 1.33	50.40 ± 11.95	
	Site 4	69.00 ± 1.00	53.50 ± 11.26	40.87 ± 1.10	51.67 ± 11.55	
	Site 5	63.33 ± 2.89	49.87 ± 8.89	36.87 ± 1.76	47.17 ± 11.14	

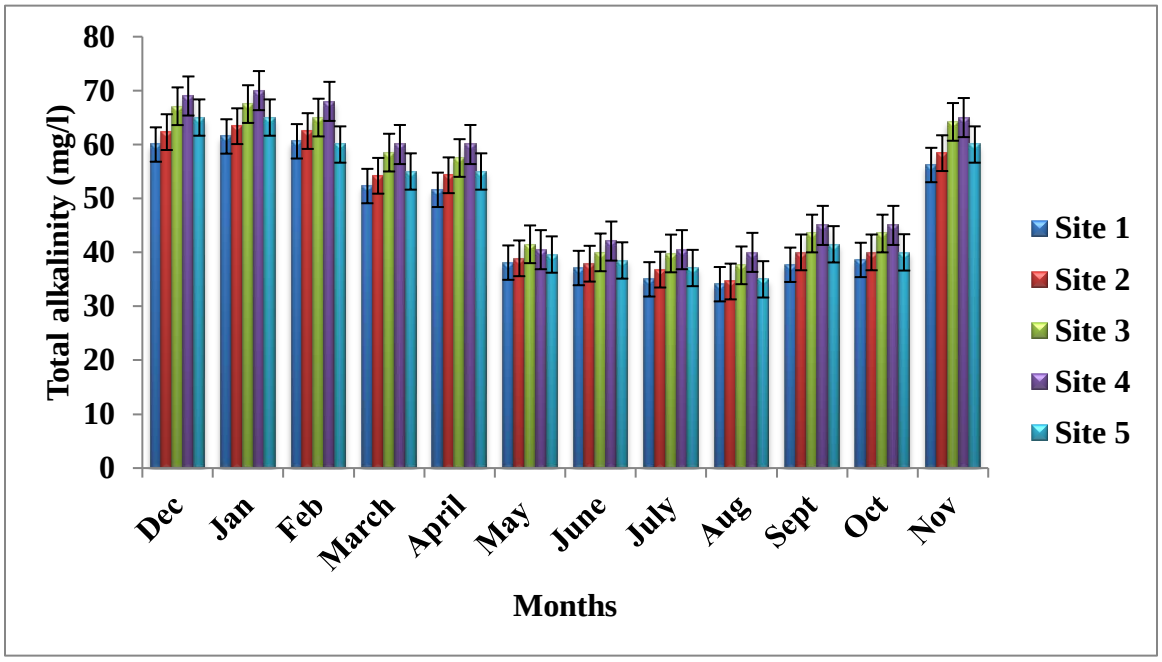


Figure 2.14: Monthly variations in total alkalinity (mg/l) of Langlung river during the period from March 2017 to February 2018

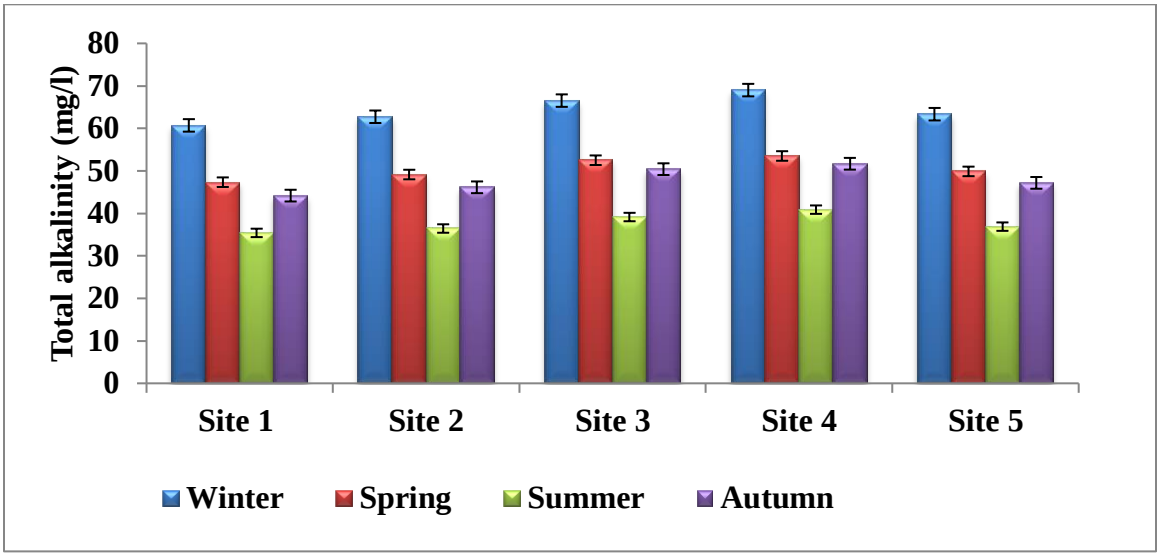


Figure 2.15: Seasonal variations in total alkalinity (mg/l) of Langlung river during the period from March 2017 to February

2.3.2.4 Total hardness

The total hardness ranged from 30 – 112.5 mg/l, with an annual mean of 65.44 ± 24.20 mg/l. The maximum total hardness was recorded during winter, reaching its peak value during February at site 1, followed by spring and autumn. The minimum was recorded during summer, with its lowest value in July at site 2. The mean values of seasonal variations in total hardness have been represented in Table 2.8.

At site 1, the total hardness ranged between 33.4 – 112.5 mg/l, with an average mean of 66.00 ± 25.53 mg/l. The range of total hardness variation at site 1 was 79.1 mg/l.

At site 2, the total hardness ranged between 30 – 110 mg/l, with an average mean of 65.63 ± 25.36 mg/l. The range of total hardness variation at site 2 was 80 mg/l.

At site 3, the total hardness ranged between 31.7 – 111.7 mg/l, with an average mean of 65.90 ± 25.69 mg/l. The range of total hardness variation at site 3 was 80 mg/l.

At site 4, the total hardness ranged between 35 – 108.4 mg/l, with an average mean of 65.00 ± 24.43 mg/l. The range of total hardness variation at site 4 was 73.4 mg/l.

At site 5, the total hardness ranged between 32.5 – 105 mg/l, with an average mean of 64.66 ± 24.25 mg/l. The range of total hardness variation at site 5 was 72.5 mg/l. The total hardness was recorded as highest in February and the lowest in July in all the study sites. The monthly and seasonal fluctuation of total hardness in Langlung river is depicted in Figure 2.16 and 2.17.

Table 2.8: Seasonal variations of total hardness (mg/l) in Langlung river during the period from March 2017 to February 2018 (Mean ± S.D)

	Study sites	Seasons				Annual Total hardness (mg/l)
		Winter	Spring	Summer	Autumn	
Total hardness (mg/l)	Site 1	97.23 ± 15.01	63.90 ± 25.56	39.50 ± 5.35	63.37± 12.05	65.44±24.20
	Site 2	94.47 ± 15.03	65.83 ± 28.27	38.33 ± 7.64	63.90 ± 9.13	
	Site 3	96.10 ± 13.62	66.10 ± 27.13	37.23 ± 5.08	64.78 ± 8.78	
	Site 4	95.83 ± 11.07	65.00 ± 24.11	39.17 ± 5.20	60.27 ± 9.27	
	Site 5	95.00 ± 10.00	62.23 ± 24.28	39.73 ± 7.10	61.58± 12.58	

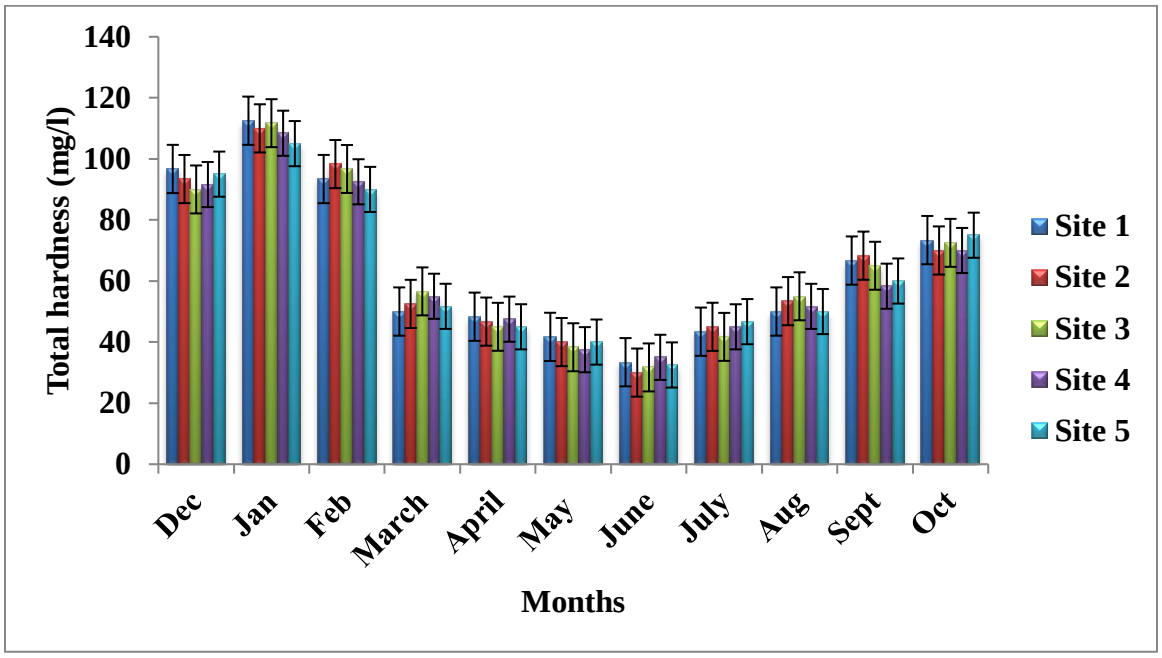


Figure 2.16: Monthly variations in total hardness (mg/l) of Langlung river during the period from March 2017 to February 2018

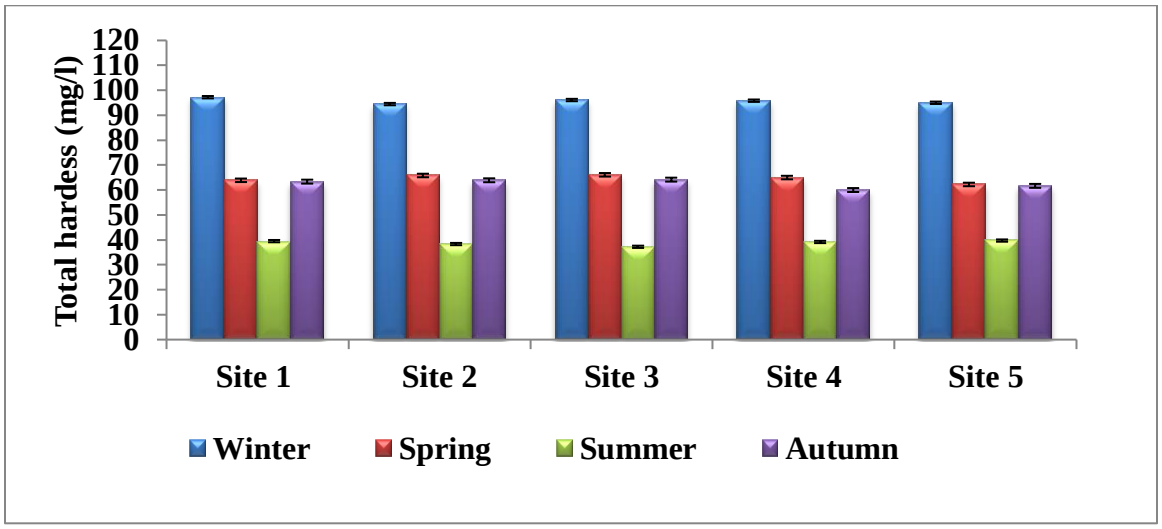


Figure 2.17: Seasonal variations in total hardness (mg/l) of Langlung river during the period from March 2017 to February 2018

2.4 Discussion

Knowledge of the physicochemical parameters of any water body is regarded as critical because it is capable of influencing and maintaining a healthy aquatic environment and providing information on water quality (Junaid *et al*., 2018; Rameshkumar *et al*., 2019). Good quality water is distinguished by proper temperature, transparency, adequate oxygen, limited levels of metabolites and other environmental factors that influence fish culture (Bhatnagar & Devi, 2013). Water quality determines the survival and growth of fishes in any water body. Consequently, the analysis of physicochemical parameters of any water body indicates the condition of water for aquacultures, such as fish production, agricultural purposes, and other uses (Rehman *et al*., 2015).

Temperature is a crucial physical factor in an aquatic environment that influences physical and chemical properties of water. It is also regarded as one of the most important factors as it affects aquatic vegetation and organisms and has a major influence on their biological activities and growth (Dwivedi & Pandey, 2002; Sajitha & Vijayamma, 2016). In the present study, the maximum air temperature (33.74˚C ± 0.86) was recorded in site 1 during summer and the minimum (28.16 ˚C ± 0.84) in site 5 during winter. Similarly, water temperature also showed the maximum (30.85 ˚C ± 0.41) in site 3during summer and the minimum (25.46 ˚C ± 0.75) in site 5 during winter. Seasonal variation in both air and water temperature was observed during the study, showing an upward trend of increasing temperature from February to June, followed by a downward trend from July onward.

Similar trends in seasonal variation of air and water temperature were reported by Kumar *et al*. (2016) in Rawasan stream, air temperature ranging of (18.48 – 34.96 ˚C) and water temperature ranging of (15.2 – 27.6 ˚C). Imnatoshi (2013) observed seasonal variation of air and water temperature in Doyang river and the variations ranged from 10 – 29 ˚C in air temperature and 9 – 26 ˚C in water temperature. The fluctuation in water temperature showing seasonal variation has been reported by Verma & Khan (2015) at the range of (14.1 – 29.5˚C) in the Fateh Sagar lake of Bagar. Vijayan *et al*. (2018) has recorded water temperature range of (24 – 32 ˚C) of the Cauvery river of Thanjavur district, Tamil Nadu. Borkar (2015) stated that water temperature of 26 – 32 ˚C is the ideal temperature for the proper growth of fish. The present finding showed that the water temperature in Langlung river ranged between 24.75 – 31.66 ˚C and is approaching the ideal range of water temperature, indicating that Langlung river is favorable for fish growth.

Water velocity is the rate at which water moves or flows in a fast-flowing river. Water velocity varied widely in the current study, ranging from 0.74 – 3.89 m/sec with a ranged variation of 3.15 m/sec. The maximum water transparency recorded to be 3.37 m/sec ± 0.47 in site 4 during summer and the minimum of 1.23 m/sec ± 0.10 in site 2 during winter. Water velocity in Langlung river was lowest in winter having slightly higher variation in spring and drastically peaking during summer and gradually decreasing during the autumn season. The declining water velocity could be attributed to low discharge during the dry season. While high water velocity begins during the rainy season in the summer, this indicates that rain is directly related to water velocity. Although the Langlung river is a shallow river, it was observed during the study period

that the river did not dry up despite its shallow depth during the dry season. A similar observation of water velocity during the winter and summer seasons was reported by Joshi *et al*. (2009) with the range of (0.39 – 2.18 m/sec) in Ganga river of Haridwar district. The increasing trend of water velocity during summer and declining during winter season was also reported by Das (2013) in the Pagladia river of Assam at the range of (0.31 – 1.30 m/sec). Imnatoshi (2013) reported the similar variation in water velocity with the range of (0.303 – 0.966 m/sec) in the Doyang river of Nagaland. Kumar *et al*. (2016) also recorded the range of (0.27 – 0.65 m/sec) of the Rawasan stream in Garhwal Himalaya.

Water transparency measures the clarity of the water; the higher the penetration of light in the water, the higher the transparency. In the present studies, the maximum water transparency (19.42 cm ± 9.27) was recorded to be in site 1 during autumn and the minimum of (1.75 cm ± 0.75) in site 5 during summer. During the study period, it was indicated that water was clear till the bottom during winter and spring but had low transparency during summer, which could be attributed to the shallow depth of the river, which causes heavy suspension of mud, clay, and other substances during the rainy season. Observation of low transparency during the rainy season was reported by Manjare *et al.* (2010) in a Tamdalge tank with a range of (6.0 – 92.0 cm). Das (2013) also reported similar variation in water transparency in the Pagladia river of Assam ranging from (3 – 41 cm). Chakravarty & Gupta (2021) reported the water transparency in Jatinga river of Assam (2.63 – 60.67 cm). Laishram & Dey (2014) reported higher water transparency range between 29-162 cm in Loktak lake of Manipur.

The pH measures hydrogen ion concentration of water and it is a critical component in every aquatic ecosystem since it influences biological processes and regulates fish development and survival. The maximum pH (7.97 ± 0.06) was recorded in site 3 during winter and the minimum (7.20 ± 0.26) in site 2 during summer. The present findings showed that the water of Langlung river is neutral to alkaline during the study period March 2017 to February 2018. A pH range of 6.5 – 9.0 is considered most suitable for fish production (Adebisi, 1981), and the standard pH range of 6.5 – 8.5 is considered good quality of water as prescribed by the World Health Organisation (Nayar, 2020). Similar observations of pH values were reported by Joshi *et al.* (2009) with a pH value of 7.06 – 8.35 from Ganga river. Malik *et al*. (2012) reported a pH range of 7.09 – 8.03 from Asan reservoir of Dehradun. Sarmah *et al*., (2020) also reported a pH range of 7.0 – 8.2 from Dikhow river of Nagaland. The pH of the Langlung river in the present finding ranged between 7 – 8, indicating a good quality water.

Dissolved oxygen (D.O) is one of the most crucial water parameters as it influences the growth, survival, and distribution of any aquatic organism. It also acts as a water quality indicator and provides detailed information on the water body health (Solis, 1988; Bramley & Roth, 2002; Faithful & Finlayson, 2005). The maximum dissolved oxygen (10.53 mg/l ± 0.93) was recorded in site 4 during winter and the minimum (5.53 mg/l ± 0.42) in site 5 during summer. The dissolved oxygen level in the Langlung river varies seasonally and fluctuates alongside temperature, with the maximum level during the dry winter season and the lowest during the summer season. Dissolved oxygen level of 5–14.5 mg/l is ideal for any natural water, but a D.O range of 4 to 6 mg/l provides healthier aquatic life in any water body (Gupta *et al*., 2017). Seasonal variations in

dissolved oxygen have also been reported by several researchers. Joshi *et al*. (2009) reported maximum dissolved oxygen content 11.71 mg/l in winter season and minimum 7.08 mg/l in rainy season. Laishram & Dey (2014) also reported a similar trend of seasonal variation of D.O in Loktak lake of Manipur (4.05-14.18 mg/l). Verma & Khan (2015) from Fateh Sagar lake at Bagar reported D.O range of (4.32 – 6.02 mg/l). Khan & Mir (2018) reported seasonal variation in D.O ranging between (6.2- 12.9 mg/l) in Kishanganga river of Kashmir. Chakravarty & Gupta (2021) also reported a range of (5.8 – 10.1 mg/l) in Jatinga river of Assam. According to the present finding, the Langlung river is well-oxygenated, making it an ideal River for fish growth and survival.

The alkalinity of the water is the ability to interact with the hydrogen ions or resist changes in hydrogen ions (Bhatnagar & Devi, 2013; Umar *et al*., 2017). The maximum alkalinity (69.00 mg/l ± 1.00) was recorded to be in site 4 during the winter, and the minimum of (47.33 mg/l ± 8.00) in site 1 during the summer. During the study period in Langlung river, total alkalinity tends to increase from autumn, peaking in winter and gradually decreasing from spring and lowest in the summer season. Total alkalinity showed seasonal variation during the study period. The declining trend in alkalinity corresponded to the rainfall during the summer season. A similar observation of seasonal variation in alkalinity was reported by Joshi *et al*. (2009) ranging from 34.35 mg/l in winter season to 90.5 mg/l in summer season. Sajitha & Vijayamma (2016) reported a similar trend of total alkalinity, ranging from 10 to 60 mg/L. Laishram & Dey (2014) reported the range of total alkalinity value between 35-90 mg/l from Loktak lake of Manipur. Sarmah *et al*. (2020) also reported seasonal variation in total alkalinity ranging between 46.2 – 63.33 mg/l.

The hardness of water is caused mainly by the presence of salts of calcium and magnesium. It is a measure of its capacity to form precipitates with soap and scales with certain anions present in the water. According to Durfor & Becker (1964), water below 60 indicates soft water, between 61 – 120 mg as moderately hard water, between 121 – 180 mg as hard water and more than 181 mg as very hard water. Total hardness in the present study recorded the maximum (97.23 mg/l ± 15.01) in site 1 during winter and a minimum (37.23 mg/l ± 5.08l) in site 3 during summer. Total hardness showed seasonal variations, recording highest during the winter months and gradually decreasing during the rainy seasons in the summer months. Several workers have reported seasonal variations in total hardness. Baidya & Biswas (2015) reported soft to moderately hard water (25.33 – 100 mg/l) during physicochemical assessment in Chathe river. Verma & Khan (2015) also reported the total hardness ranging from 33.16 – 102.54 mg/l. Sarmah *et al*. (2020) also reported seasonal variation in total hardness from Dikhu river ranging from 42.9 – 86.8 mg/l. The recommended hardness value for fish culture ranges between 30-180 mg/l (Santhosh & Singh, 2007). The hardness of the water ranged between 30 – 112.5 mg/l in Langlung river, showing soft and moderately hard water and an ideal river for fish growth and survival during the study period from March 2017 to February 2018.

Chapter 3

Description of New Species *Garra langlungensis* (Cyprinidae: Garrinae) from Nagaland, India

Contents

3.1 Introduction

The fundamental principle for any kind of biological study is the identification of a species. A most reliable and clear picture of taxonomic information is obtained from the detailed examination of the species (Vecchione *et al*., 2000). The labeonine genus *Garra* (Hamilton, 1822) is widely distributed from Sub–Saharan Africa to Borneo through the Arabian Peninsula, Southern Asia, and Southern China (Zhang & Chen, 2002). A distinctive feature of the genus *Garra* is the presence of a gular disc and having diverse snout morphology, such as the development of the proboscis, transverse lobe, and distribution pattern of the tubercles on the snout (Nebeshwar & Vishwanath, 2013; Kottelat, 2020). The genus *Garra* found in southern and Southeastern Asia is divided into five groups by Nebeshwar & Vishwanath (2017) based on snout morphology such as smooth snout species group, transverse lobe species group, proboscis species group, rostral flap species group, and the rostral lobe species group. The snout morphology and its associated tubercle patterns and distribution in *Garra* play a taxonomic significance in distinguishing between the species within the genus.

Nagaland records a good number of *Garra* species and it is represented by 11 species *viz., Garra annandalei*, *Garra gravelyi*, *Garra gotyla*, *Garra kempi*, *Garra lamta*, *Garra lissorhynchus*, *Garra McClelland*, *Garra notata*, *Garra naganensis*, *Garra nasuta* and *Garra rupicola* as reported by Ezung *et al*. (2020a). Recent reports on the ichthyofauna exploration contributed to the description of two new species *viz.*, *Garra chathensis* Ezung, Shangningam & Pankaj, 2020b and *Garra langlungensis* Ezung, Shangningam & Pankaj, 2021 from Chathe river and Langlung river respectively, together with an additional record of *Garra birostris* Nebeshwar & Vishwanath,

2013 from Dikhu and Doyang river (Ezung *et al*., 2022). Hence, Nagaland is a home to 14 species of the genus *Garra.*

The present chapter deals with the detailed description of *Garra langlungensis.* This species was collected from Langlung river near Zutovi village, during preliminary survey carried out in the rivers of Dimapur district. Seven samples of *Garra* with a weakly-developed proboscis and a transverse lobe on the snout were collected during the survey and on further study, became reported as new to science. The detailed description of *Garra langlungensis* along with the systematic account of the species and key to *Garra* species of Nagaland is given in this chapter.

3.2 Materials and methods

3.2.1 Collection

For taxonomic study, fish samples were collected from different sites along the course of Langlung River and care was taken to keep the external morphology intact during collection. Fishes were caught using cast nets, gill nets of different mesh sizes and scoop nets. Photographs of live specimens were taken in the field before preservation.

3.2.2 Preservation, identification and measurements

All the samples collected in the field study were fixed in 10% formaldehyde. Detailed taxonomic studies were carried out at Fish Biology and Fisheries Laboratory, Department of Zoology, Nagaland University, Lumami as well as at Freshwater Fish Section, Zoological Survey of India, Kolkata. The fish specimens were taxonomically identified and confirmed after Menon (1964), Jayaram (1981), Datta Munshi & Srivastava (1988), Talwar & Jhingran (1991), Jayaram (1999), Vishwanath *et al*. (2007), Ao *et al*. (2008) and Vishwanath *et al*. (2014). Comparative materials, whenever

necessary, were compared with type specimens deposited at the Freshwater Fish Section, Zoological Survey of India, Kolkata.

All measurements were made using digital calliper, point to point on the left side of the specimen closest to 0.1 mm. Counts, measurements and terminology follow Nebeshwar & Vishwanath (2013). Gular disc terminology follows (Kottelat, 2020). Dorsal and anal fin rays follow Kottelat (2001). Fin rays and the number of scales were counted using the Huvitz stereo zoom microscope. Head length and measurements of body are expressed in percentage of standard length (%SL); subunits of head in percentage of head length (%HL); pelvic-anal distance in the percentage of vent anal distance; caudal peduncle depth in the percent of caudal peduncle length.

3.3 Results

3.3.1 Description of the species

Systematic position

Kingdom	-	Animalia
Phylum	-	Chordata
Subphylum	-	Vertebrata
Infraphylum	-	Gnathostomata
Parvphylum	-	Osteichthyes
Gigaclass	-	Actinopterygii
Class	-	Actinopteri
Subclass	-	Teleostei
Order	-	Cypriniformes
Family	-	Cyprinidae
Subfamily	-	Labeoninae
Genus	-	*Garra*
Species	-	*langlungensis*

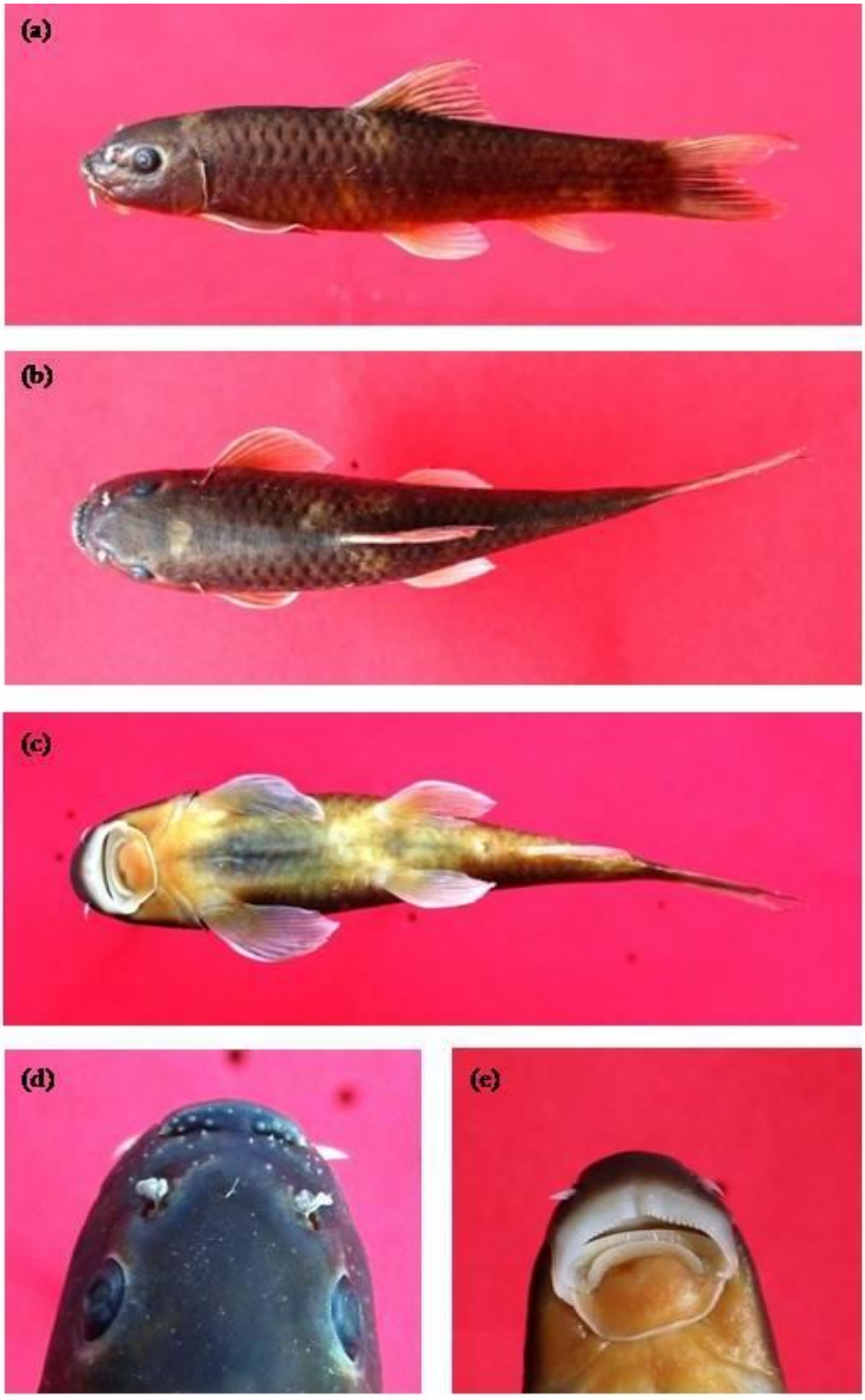


Figure 3.1: *Garra langlungensis*: (a) lateral view (b) dorsal view (c) ventral view (d) snout morphology (e) oromandibular structure

Holotype: ZSI FF 7152, 54.9 mm SL, India, Nagaland, Langlung river near Zutovi village, Dimapur district, Brahmaputra Basin; 25˚43′N, 93˚39′E

Paratypes: 6 exs, 54.8 – 70.2 mm SL, same data as above. Other materials: (non type) 213 unregistered exs, 82.4 – 45.8 mm, collected from the river stretch of 2149.27 m, from Langlung river around Zutovi village (Dimapur district, Nagaland, India). Ezung *et al*., February 23, 2017- January 26, 2019.

Diagnosis: *Garra langlungensis* is a member of the snout with proboscis species group, can be distinguished from other members of this group in having the following combination of characters: weakly-developed unilobed proboscis, a distinct transverse lobe with 8–12 small sized unicuspid acanthoid tubercles, 8–9 pre-dorsal scales, 30–32 lateral line scales and 13–15 circumpeduncular scales. Vent closed to the anal-fin origin than pelvic-fin origin. Dorsal, ventral, lateral view, snout morphology and oromandibular structure of *Garra langlungensis* is shown in Figure 3.1. Description of *Garra langlungensis* is shown in Figure 3.2.

Figure 3.2: Description of *Garra langlungensis* Ezung, Shangningam & Pankaj, 2021: LSID (LSID urn:lsid:zoobank.org:act:4C8A5C5E-0093-4BDA-B269-A72C833C0849)

Description: Body elongate, laterally compressed, more towards the caudal peduncle. Dorsal head profile rising gently over the snout, slightly convex, more or less continuous with dorsal body profile to dorsal-fin origin, then gently sloping towards caudal peduncle. Ventral profile from head to chest straight and profile from chest to anal-fin origin more or less convex. Head moderately large, depressed with slightly convex inter-orbital area; height less than length; width greater than height. Eyes dorso-laterally located, closer to posterior margin of opercle than to snout tip.

Snout rounded, with a distinct transverse lobe covered with 8–12 small-sized unicuspid acanthoid tubercles, demarcated posteriorly by a narrow moderately deep transverse groove. Proboscis is weakly developed, unilobed, with small tubercles on its margin.

Barbels two pairs; rostral barbel anteroventrally located, shorter than eye diameter; maxillary barbel at the corner of the mouth, shorter than rostral barbel. Rostral cap well–developed, its distal margin highly fimbriate, papillate ventral surface moderately wide; separated from upper jaw by deep groove and laterally continuous with the lower lip. Upper jaw entirely covered by the rostral cap. Disc elliptical, shorter than wide and narrower than head width through roots of maxillary barbel; labellum of lower lip distinct; torus well developed with papillae, not covered by the rostral cap; toral groove between the posterior torus and pulvinus deep; papillae on inner half of the whole length of labrum larger and coarsely arranged; anterior marginal surface of pulvinus with coarsely arranged fleshy papillae; posterior most margin of labrum extending vertical to eye.

Dorsal fin with two simple and 8½ branched rays; distal margin concave; origin nearer to snout tip than to caudal-fin base, inserted anterior to vertical through pelvic-fin origin. Pectoral fin with 1 simple and 11 or 12 branched rays, reaching beyond midway to pelvic fin origin; margin subacuminate. Pelvic fin with 1 simple and 7½ branched rays; second branched ray longest, reaching beyond midway to anal-fin origin, surpassing anus; origin closer to anal-fin origin than to pectoral-fin origin. Anal fin with 2 simple and 5½ branched rays; first branched ray longest, not reaching base of caudal fin; distal posterior margin slightly concave, origin closer to caudal-fin base than to pelvic-fin origin. Vent closer to the anal-fin origin than to pelvic-fin origin. Caudal fin forked with 10+9 principal caudal rays; upper lobe slightly longer; tip of lobes pointed.

Lateral line complete, scales along lateral line 28, 29 or 30 + 2 on caudal-fin base. Transverse scale rows above lateral line scale 3½; between lateral line and pelvic-fin

origin 3; between the lateral line to anal fin origin 3½. Circumpeduncular scales 13, 14 or 15. Pre-dorsal scales 8 or 9; scales regularly arranged. Chest and belly with well-developed scales. One long axillary scale at the base of the pelvic fin, its tip reaching the posterior end of pelvic-fin origin. Dorsal-fin base scales 7 of which last three to four connected to the base of the dorsal fin. Anal-fin base scales 4 of which last three to four connected to the base of the anal fin. Scales between the vent and anal-fin origin 2 or 3. The morphometric data of *Garra langlungensis* is depicted in Table 3.1.

Coloration: In fresh specimens, head and body greenish-brown dorsally and laterally. Mouth, chest and abdomen white. Dorsal, pectoral, pelvic, anal and caudal fins orange yellowish, fin rays moderately spotted. In preservative, head, dorsal and lateral side dark grey. Mouth, chest and abdomen yellowish white. A black spot at the upper angle of the gill opening. Dorsal, pectoral, and pelvic fins with thin melanophores. Anal and caudal fins greyish-yellow. Six narrow black stripes on the lateral side more prominent towards the caudal peduncle. Median rays and tips of upper and lower lobe of caudal fin dark brown.

Etymology: This species is named after its type locality, Langlung river, Nagaland.

Distribution: *Garra langlungensis* is known only from the type locality, Langlung River near Zutovi village, Dimapur district, Nagaland, India. The type locality of *Garra langlungensis* is shown in Figure 3.3.

Common local names: Pathor maas (Nagamese dialect), Engoro (Lotha naga tribe), Angad (Ao naga tribe), Aghungu (Sumi naga tribe).

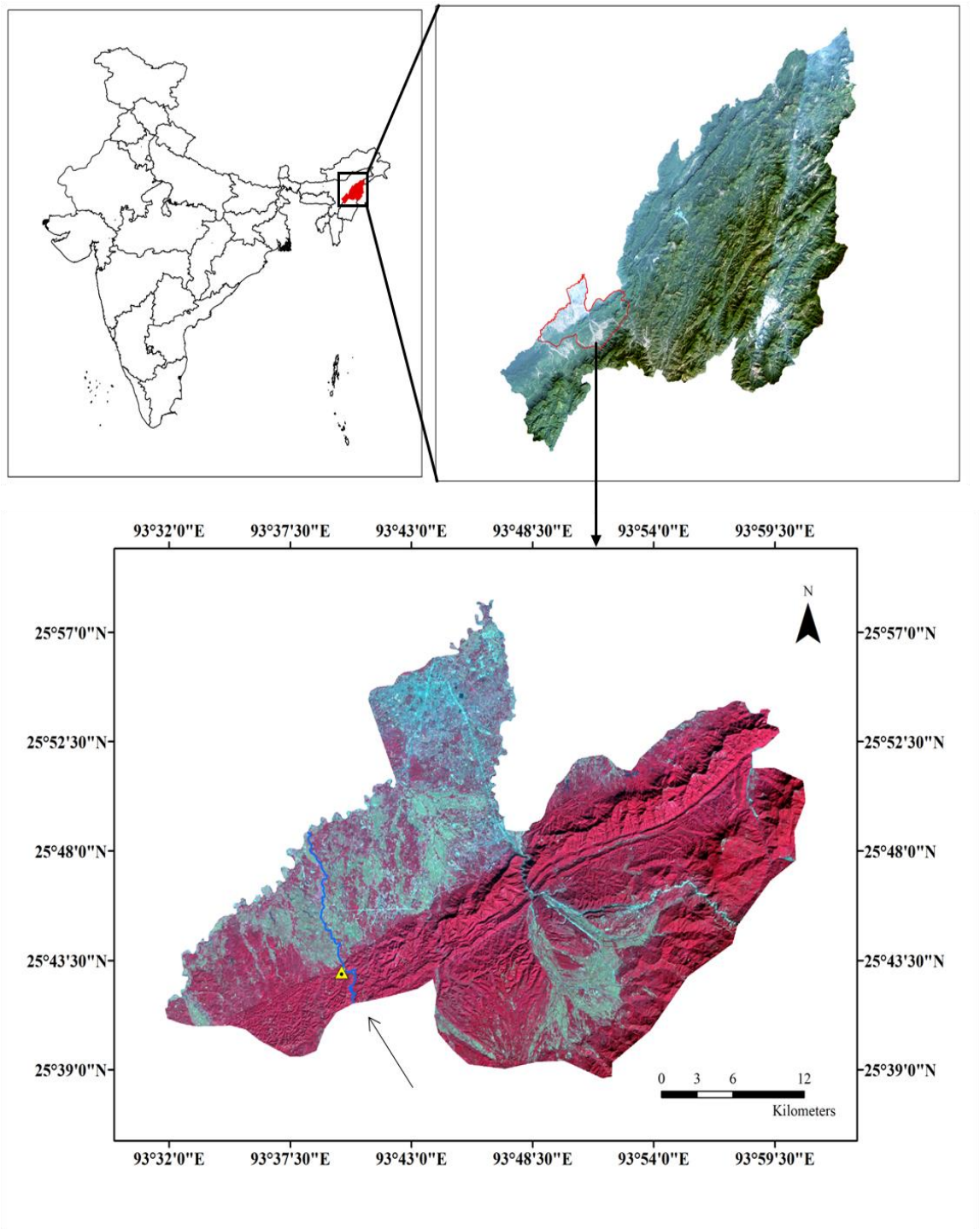


Figure 3.3: Type locality of *Garra langlungensis*

Table 3.1: Morphometric data of *Garra langlungensis*. Range includes the value of holotype. n, number of specimens; SD, standard deviation

	Garra langlungensis- (n=7 including holotype)			
	holotype	range	mean	S.D
Standard length (in mm)	54.9	54.8 – 70.2		
In percentage of standard length (% SL)				
Head length	26.2	24.9 – 27.9	26.4	1
Body depth at dorsal-fin origin	23.5	20.9 – 25.9	23.5	1.6
Pre-dorsal length	48.7	47.1 – 49.8	48.7	0.9
Pre-anus length	67.9	66.6 – 69.6	67.7	1
Pre-anal length	74.1	74.1 – 77.4	75.5	1.1
Pre-pectoral length	22.4	21.4 – 22.6	22.1	0.5
Pre-pelvic length	50.9	50.9 – 53.9	52	1
Dorsal-fin base length	16.4	16.3 – 19.0	17.3	0.9
Dorsal-fin length	25.1	23.2 – 25.4	24.1	0.9
Pectoral fin length	23.2	18.4 – 23.9	22.3	1.8
Pelvic fin length	19.7	18.5 – 20.3	19.6	0.6
Anal-fin base length	7.1	6.4 – 7.6	7	0.4
Anal-fin length	19.5	16.9 – 20.1	19.1	1.1
Distance from vent to anal fin	5.5	4.8 – 7.8	6.4	1.2
Caudal-peduncle length	16.3	16.3 – 19.8	18	1.2
Caudal-peduncle depth	15.2	14.2 – 15.7	14.8	0.6
disc length	8.5	8.5 – 9.8	9.4	0.5
disc width	13	12.5 – 13.7	13	0.4
Pulvinus length	5.7	5.7 – 6.6	6.1	0.3
Pulvinus width	9.5	8.6 – 9.5	9.1	0.3
In percentage of pelvic-anal distance (% pelvic-anal distance)				
Distance from vent to anal fin	23.1	19.7-31	25.7	4.2
In percentage of head length (% HL)				
Head depth at occiput	75.1	68.5 – 77.9	72.4	3.3

Snout length	55.4	50.8 – 56.1	53.7	1.9
Interorbital distance	45.5	43.8 – 49.8	46.7	2.2
Eye diameter	26.4	20.1 – 26.4	23.9	2.3
Disc length	32.5	32.5 – 38.9	35.8	2.3
Disc width	49.7	46.1 – 54.7	49.6	2.7
Pulvinus length	21.7	21.1 – 25.7	23	1.6
Pulvinus width	36.1	33.2 – 36.2	34.5	1.2
In percentage of Caudal peduncle length (%caudal peduncle length)				
Caudal peduncle depth	93.4	77.0 – 93.4	82.2	5.5
Meristic count				
Dorsal fin rays	ii8½	ii8½		
Pectoral fin rays	i11½	i11-12½		
Pelvic fin rays	i7½	i7½		
Anal fin rays	ii5½	ii5½		
Caudal fin rays	10+9	10+9		
Pre-dorsal scales	9	8-9		
Lateral line scales	30	30-32		
Transverse scales	3½|1|3	3½|1|3		
Circumpeduncular scale rows	15	13-15		

Differential diagnosis: *Garra langlungensis* belongs to the proboscis species group and is compared with 32 valid *Garra* congeners belonging to this group, *viz*., *Garra dengba* Deng *et al*. (2018), *Garra kalpangi* Nebeshwar *et al.* (2012), *Garra gravelyi* Annandale (1919), *Garra bimaculacauda* Thoni *et al.* (2016), *Garra clavirostris* Roni *et al*. (2017), *Garra kangrae* Prashad (1919), *Garra montisalsi* Hora (1921), *Garra parastenorhynchus* Thoni *et al.* (2016), *Garra simbalbaraensis* Rath *et al.* (2019), *Garra stenorhynchus* Jerdon (1849), *Garra substrictorostris* Roni & Vishwanath (2018), *Garra arunachalensis* Nebeshwar & Vishwanath (2013), *Garra biloborostris* Roni & Vishwanath (2017),

Garra birostris Nebeshwar & Vishwanath (2013), *Garra bispinosa* Zhang (2005), *Garra chathensis* Ezung *et al*. (2020b), *Garra chindwinensis* Premananda *et al*. (2017), *Garra cornigera* Shangningam & Vishwanath (2015), *Garra gotyla* Gray (1830), *Garra litanansis* Vishwanath (1993), *Garra motuoensis* Gong *et al*. (2018), *Garra quadratirostris* Nebeshwar & Vishwanath (2013), *Garra qiaojiensis* Wu (1977), *Garra rotundinasus* Zhang (2006), *Garra yajiangensis* Gong *et al.* (2018), *Garra bicornuta* Rao (1920), *Garra koladynensis* Nebeshwar & Vishwanath (2017), *Garra nasuta* M'Clelland (1838), *Garra paratrilobata* Roni *et al*. (2019), *Garra surgifrons* Sun *et al*. (2018), *Garra tamangi* Gurumayum & Kosygin (2016), and *Garra trilobata* Shangningam & Vishwanath (2015).

Garra langlungensis most closely resembles *Garra dengba, Garra kalpangi, Garra gravelyi* and *Garra bimaculacauda* in having weakly developed proboscis on the snout. It differs from *Garra dengba, Garra kalpangi, Garra gravelyi* and *Garra bimaculacauda* in having fewer pre-dorsal scales and in having 13-15 circumpenduncular scale rows.

Garra langlungensis is distinguished from *Garra dengba* in having fewer pre-dorsal scales (8–9 *vs.* 14–16), fewer lateral-line scales (30–32 *vs.* 42–44), more branched anal fin rays (5½ *vs*. 4), branched dorsal-fin rays (8½ *vs*. 6), more circumpeduncular scales (13–15 *vs*. 12) and shorter disc width (46–54 *vs.* 57–73 % HL). It differs from *Garra kalpangi* in the absence (*vs*. presence) of black spot at the base of branched dorsal-fin rays, fewer pre-dorsal scales (8–9 *vs*. 10–11), fewer transverse row below lateral line (3 *vs*. 3½–4), fewer circumpeduncular scales (13–15 *vs*.16), longer pulvinus length (5.7–6.6 *vs*. 4.8–5.5 % SL) and greater pulvinus width (8.6–9.5 *vs*. 7.3–8.1 % SL). It differs

from *Garra gravelyi* in the absence (*vs*. presence) of black spots along dorsal-fin base, more branched dorsal-fin rays (8½ *vs*. 7), fewer branched pectoral-fin rays (11–12 *vs*. 14–15), fewer pre-dorsal scales (8–9 *vs*. 10–11) and more circumpeduncular scales (13–15 *vs*. 12). It is distinguished from *Garra bimaculacauda* in the absence (*vs*. presence) of two distinct black spot in the caudal fin, lesser branched pectoral-fin rays (11–12 *vs*. 14), fewer pre-dorsal scales (8–9 *vs*. 11–12), transverse scale rows from dorsal-fin origin to lateral line (3½ *vs*. 6), more circumpeduncular scales (13–15 *vs*. 12), shorter disc length (32–38 *vs*. 40–44 %HL).

It differs from *Garra clavirostris* in having weakly developed proboscis (*vs*. clubbed proboscis), lesser branched pectoral fin rays (11–12 *vs*. 14–15), transverse scale rows from dorsal origin to lateral line (3½ *vs*. 5½) and smaller disc length (32–38 *vs*. 50−65 % HL); from *Garra kangrae*, in having weakly-developed proboscis (*vs*. prominent quadrate proboscis), fewer branched pectoral fin rays (11–12 *vs*. 15) and fewer lateral line scales (30–32 *vs*. 34). It differs from *Garra montisalsi*, in having weakly developed proboscis (*vs*. prominent unilobed proboscis projecting upward above the transverse lobe), longer disc length (32–38 *vs*. 28 %HL), pulvinus length (21–25 *vs*. 18 %HL) and pulvinus width (33–36 *vs*. 22 %HL); from *Garra parastenorhynchus*, in having weakly-developed proboscis (*vs*. club-shaped overhanging proboscis), fewer pre-dorsal scales (8–9 *vs*. 10–11), circumpeduncular scales (13–15 *vs*. 16), more head length (24.9–27.9 *vs*. 28.5–30.7 %SL), lesser pre-anus length (66.6–69.6 *vs*. 70.1-74.2 %SL) and more interorbital width (43–49 *vs*. 34–39 %HL); from *Garra simbalbaraensis* in having weakly-developed proboscis (*vs*. prominent unilobed rounded proboscis), fewer circumpeduncular (13–15 *vs*. 16) and more pulvinus width (33–36 *vs*. 26–29 %HL). It

differs from *Garra stenorhynchus* in having weakly-developed proboscis (*vs.* prominent quadrate proboscis) and fewer lateral line scales (30–32 *vs.* 34). It differs from *Garra substrictorostris* in having weakly-developed proboscis (*vs.* narrow antrorse unilobed proboscis), fewer branched pectoral-fin rays (11– 12 *vs*. 15), fewer pre-dorsal scales (8–9 *vs.* 10), transverse scale rows from dorsal origin to lateral line (3½ *vs*. 5½), circumpeduncular (13–15 *vs.* 16), shorter pre-anus length (66.6–69.6 *vs.* 70.3–77.7 %SL), disc length (32–38 *vs.* 44– 55 %HL) and disc width (46– 54 *vs.* 53–66 %HL).

Garra langlungensis is also differentiated from *Garra arunachalensis, Garra biloborostris, Garra birostris, Garra chathensis, Garra bispinosa, Garra chindwinensis, Garra cornigera, Garra gotyla, Garra litanansis, Garra motuoensis, Garra quadratirostris, Garra qiaojiensis, Garra rotundinasus Garra yajiangensis* in having weakly-developed unilobed proboscis (*vs.* prominent bilobed or slightly bilobed) proboscis on the snout. It can be differentiated from *Garra bicornuta, Garra koladynensis, Garra nasuta, Garra paratrilobata, Garra surgifrons, Garra tamangi,* and *Garra trilobata* in having weakly-developed unilobed (*vs.* prominent trilobed) proboscis on the snout.

Materials compared

Garra biloborostris: ZSI FF 7928, 2 paratypes, 69.1– 75.6 mm; India, Assam, Chirang District, Kanamakra river, Brahmaputra basin, Sewali and Paraty.

Garra chathensis: ZSI FF 8037, holotype, 65.6 mm SL, India, Nagaland, Chathe river.

Garra chindwinensis: ZSI FF 5906, holotype, 120mm SL, India, Manipur, Senapati District, Laniye river nearLaii, Premananda.

Garra clavirostris: ZSI FF 6062, 2 paratypes, 71.2−83.0 mm SL; India, Assam, Dima Hasao District, Dilaima river at Boro Chenam village below the confluence of Dilaima and Dihandi Brahmaputra drainage.

Garra cornigera: ZSI FF 5995, 2 paratypes, 72.19−46.82 mm SL; India, Manipur, Ukhrul District, Sanalok river, Chindwin basin.

Garra elongata: ZSI FF 4157/1, paratype, 79.30mm SL; Hill stream near Tollai, Ukhrul District, Manipur, India, L. Kosygin.

Garra gravelyi: ZSI F 9694/1, type, 60.9mm SL; Myanmar, S. Shan States, he-ho stream, Annandale (1919).

Garra jenkinsonianum: ZSI F 5736/1 type, 57.9mm SL; Paresnath hills, Dr. J.T. Jenkin's and N. Annandale.

Garra kempi: ZSI F 7716/1, type, 86.6mm SL; India, Arunachal Pradesh, Siyom river below Damda the Abor hills, Abor expedition 1912, Dr. S.W. Kemp.

Garra montisalsi: ZSI F 9953/1, type, 100.8mm. SL; India, Punjab, Nilwan ravine near the Shapur salt ranges.

Garra mullya: ZSI F 8138/1, type, 56.32mm SL; Chanda, C.P, Museum collector.

Garra prasadi: ZSI F 9971/1, type, 77.3mm SL; A small stream flowline near Malwa tal, U.P, Dr. B. Prashad and S.I. Hora.

Garra simbalbaraensis: ZSI FF 8003, 60.8mm SL; India: Himachal Pradesh, Sirmaur District, Simbalbara river, Yamuna river basin.

Garra stenorhynchus: ZSI F 9957, 64.5mm SL; India, Mysore, hillstream, Coorg,

Garra tamangi: ZSI FF 5423, paratypes, 102.4mm SL; India, Arunachal Pradesh, Dikrong river at Hoj, Brahmaputra drainage.

Garra trilobata: ZSI FF 5994, 2 paratypes, 95.78−119.14 mm SL; India, Manipur, Ukhrul District, Sanalok river.

Key to the *Garra* of Nagaland

1.	Snout with proboscis and transverse lobe or only transverse lobe ..	…2
	Snout without proboscis or transverse lobe ….. ….. ….. …	…9
2.	Snout with both proboscis and transverse lobe ….. ….. …..	…4
	Snout with only transverse lobe ….. …… ….. ….. ….. …..	…3
3.	13 pre-dorsal scales ….. ….. …… ……. …… …… ……	*Garra kempi*
	8-10 pre-dorsal scales ….. ….. ….. …… ……. ……. …..	*Garra mcclellandi*
4.	Proboscis weakly developed ….. …… ….. …… …… …	…5
	Proboscis bilobed or trilobed or quadrat …… …… …… …	…6
5.	7 branched dorsal fin rays …… …… ……. ……. …….	*Garra gravelyi*
	8½ branched dorsal fin rays …… …… ……. ….... ……. …	*Garra langlungensis*
6.	Proboscis bilobed ….. ….. …… ……. ……. ….. …… …..	…7
	Proboscis trilobed or quadrat …… …… ……. ……. …….	…8
7.	Each lobe with tri or tetra acanthiod tubercules …… ……	*Garra birostris*
	Each lobe with bi acanthiod tubercules …… …… ….. …….	*Garra chathensis*
8.	Tri lobed proboscis presence of a pit between the nares …..	*Garra nasuta*

	Quadrate proboscis with or without a shallow depression in ... the middles to present a bilobed appearance	*Garra gotyla*
9.	Caudal fin with a "W" shaped band …… ……. ……. ……	…10
	No "W" shaped band on caudal ……. …… ….. …… ……	…11
10.	Presence of a pair of rostral flap …… …… ……. ……. …	*Garra lissorhynchus*
	Absence of pair of rostral flap ….. …… ……. ……. ……	*Garra rupecula*
11.	Lateral line 38-40 …… …… ….. …… …….. ……. …….	*Garra naganensis*
	Lateral line 34 and below ……. ……. ……. …… ….. …..	…12
12.	Lateral line 34 …… …… ……. …..... ……. …… …….	…13
	Lateral line 31-34 …… …… …… ……. ……. ……	*Garra lamta*
13.	Transverse scale row 3.5|1|3 or 3.5 …… ……. ……. …….	*Garra annandalei*
	Transverse scale row 5 or 5.5|1|4.5 …….. ……. …… ….	*Garra notata*

3.4 Discussion

The present study comprehensively explains the systematic position and description of *Garra langlungensis*, an indigenous new species reported from Langlung river near Zutovi village, Dimapur, Nagaland. The new species differentiated from its congeners in having weakly-developed unilobed proboscis, a distinct transverse lobe with 8–12 small-sized unicuspid acanthoid tubercles, 8–9 pre-dorsal scales, 30–32 lateral line scales, and 13–15 circumpeduncular scales. Vent closed to the anal-fin origin than the pelvic-fin origin. The genus *Garra* exhibits tremendous variation in its snout morphology and these variations play a vital role in differentiating and diagnoses of species of *Garra*. The new species *Garra langlungensis* was placed under the proboscis group in having weakly developed proboscis and transverse lobe in the snout as per description of snout

morphology by Nebeshwar & Vishwanath (2017). The comparative study was carried out between 32 valid species belonging to the proboscis species group, described from Brahmaputra and its neighboring river basins. The differential diagnosis between *Garra langlungensis* and its congeners belonging to the proboscis species group resulted into the finding that *Garra langlungensis* closely resembled *Garra dengba, Garra kalpangi, Garra gravelyi* and *Garra bimaculacauda* from the 32 congeners in having weakly developed proboscis. However, *Garra langlungensis* was found to differentiate from its closely related congeners in having fewer pre-dorsal scales and 13-15 circumpenduncular scale rows.

The description of *Garra langlungensis* thus enriches the ichthyofaunal diversity of *Garra* from Nagaland. Based on the snout morphology given by Nebeshwar & Vishwanath (2017), the species of *Garra* from Nagaland are represented by four groups, i.e., smooth snout species group, transverse lobe species group, proboscis species group and rostral flap species group and the keys to identification of *Garra* species from Nagaland are given herein this chapter. The distribution of the new species *Garra langlungensis* is hitherto endemic only to Langlung River; hence, the species-limited distribution highlights the significance and necessity to protect the species and its habitat from any threats, as well as its conservation.

Chapter 4

General Biology

Contents

4.1 Introduction

The study and knowledge on growth rate is an essential pre-requisite for fishery management. The morphometric analysis of fish plays a vital role in the study of fish biology. It acts as an efficient tool for the differentiation of taxonomic units. It is also used to measure discreteness between the same species (Naeem & Salam, 2005; Ambily, 2016; Hussain *et al*., 2012). Studies on morphometry also help to determine the degree of association of various characters and to establish an equation of one measurement into the other. Morphometric and meristic traits are very helpful for identifying and classifying any fish species in a laboratory or the fields (Bagenal & Tesch, 1978; Jayaram, 1999; Nawer *et al*., 2017).

Fish morphology has been an important data source for taxonomy and evolutionary studies and is the most basic and direct method of identifying fish (Ambily, 2016). These characteristics are generally classified into two types: morphometric and meristic. Morphometric characters include the measurable characters of a fish, and meristic counts, on the other hand, are the countable characters. Furthermore, they are utilized to measure intraspecific changes between species. Morphometric parameters of a fish species have a major role in ensuring whether there is any disparity between the same species of different geographic regions (Naeem *et al*., 2012). Variations in morphometric characters explain the evolutionary adaptations of the species; for instance, the mouth gap size of a species determines the feeding habit of the species (Wainwright & Richard, 1995).

The studies on the relationship between length and weight and the condition factor are critical in fish biology and stock assessment of fishery resources; it is also used

for comparing the condition, fatness, or well-being of a fish population (Tesch, 1968; Beyer, 1987; King, 2007; Bobori *et al*., 2010). Knowledge of the length-weight relationship also allows comparison of life history and morphology between different fish species or fish populations from different habitats or regions (Goncovales *et al*., 1997; Santos *et al.*, 2002). The mathematical relationship between the length and weight of different sexes and sizes from a specific area is a suitable index for research in biology, physiology, ecology, health management, and population dynamics for understanding the survival growth, maturity, reproduction, and general well being of the fishes (Le Cren, 1951).

The length-weight relationship represents the pattern of growth in fishes. Fish can attain either isometric growth, negative allometric growth, or positive allometric growth. Isometric growth (b=3) is associated with no change of body shape as an organism grows. Negative allometric growth (b<3) implies the fish becomes more slender as its increases in weight, while positive allometric growth (b>3) implies the fish becomes relatively stouter or deeper-bodied as it increases in length (Riedel *et al*., 2007).

The information on condition factor (k) in fisheries research allows understanding of the condition, fatness or well-being of the fish. Relative condition factor (Kn) measures the individual deviations from the expected weight derived from the length-weight relationship (Le Cren, 1951). Studies on condition factors indicate the suitability of a specific water body for the growth of fish and environmental differences in environmental conditions such as seasonal changes, nutritional quality, and type of aquatic system, e.g., rivers or lakes (Yilmaz *et al*., 2012; Alam *et al*., 2014; Mouludi-Saleh & Eagderi, 2019).

The selected fish for the study, *Garra langlungensis*, is an endemic fish of Nagaland. It locally has economic and food value; even so, there is no record of growth-related study on this species. Hence the current study was carried out in order to study morphology, length-weight relationship and condition factor of this fish.

4.2 Materials and methods

4.2.1 Collection and preservation

Fish specimens were collected from Langlung river on a monthly basis from March 2017 to February 2018 period. A total of 213 specimens of *Garra langlungensis* ranging from 45.8 to 82.4 mm in total length and 1.2 to 6.43 gm in weight were collected for the study. All the samples collected in the field were fixed in 10% formaldehyde.

4.2.2 Meristic and Morphometric measurements

All measurements were made using a digital calliper to the nearest 0.1 mm. Fin rays and the number of scales were counted using the Huvitz stereo zoom microscope. Thirty morphometric and seven meristic characters were studied following the standard procedure and terminology described by Nebeshwar & Vishwanath (2013). Dorsal and anal fin rays follow Kottelat (2001). Gular disc terminology follows (Kottelat, 2020). Head length and measurements of body are expressed in percentage of standard length (% SL); subunits of head in percentage of head length (% HL); caudal peduncle depth in the percent of caudal peduncle length (% caudal peduncle length).

4.2.3 Length – weight relationship

A total of 213 specimens of *Garra langlungensis* collected from Langlung river comprising 149 males and 64 females were used for the present study. After removing excess water from the specimens by blotting, total length and weight to the nearest 0.01

mm/gm were recorded using digital caliper and electronic weighing balance. The recorded data was used to generate length-weight relationships following Le Cren, (1951).

$$\mathbf{W = a\ L^b}$$

The logarithmic transformation of which gives the linear equation:

$$\mathbf{Log\ w = a + b\ log\ L}$$

Where,

w = weight in gram, 1 = 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.

The coefficient of condition is estimated using Le Cren's relative condition factor. The relative condition factor is the ratio between the observed weight and the expected weight based on the length-weight regression (Le Cren, 1951); it was computed using the formula.

$$Kn = \frac{W}{aL^b}$$

4.2.4 Statistical analysis

All the statistical analysis was done using Microsoft Office Excel 2007.

4.3 Results

4.3.1 Morphological studies

A total of 60 random samples of *Garra langlungensis* were selected for the present study. Morphological study was carried out for both male and female separately

taking 30 samples each for both populations. The total length of the studied fish ranged between 53.4 – 78.7 mm.

4.3.1.1 Meristic and Morphometric measurement of male *Garra langlungensis*

The morphological study of male *Garra langlungensis* was carried out with 30 randomly selected samples ranging between 53.4 – 73.7 mm in total length. The morphometric measurements of 30 randomly selected males of *Garra langlungensis,* along with the minimum (min) and maximum (max) limit and their mean and standard deviation, is given in Table 4.1.

The standard length ranged between 41.7 – 59.6 mm with an average of 49.5 mm ± 3.7, and twenty-one characters were studied in percentage of standard length. The head length ranged between 11.2 – 14.5 mm with an average of 12.9 mm ± 0.9, and eight characters were studied in percentage of head length. The caudal peduncle length ranged between 6.1 – 9.8 mm with an average of 8.1 mm ± 1 and caudal peduncle depth was studied in percentage of caudal peduncle length. Morphological parameters expressed in percentage of standard length, head length and caudal peduncle length of male *Garra langlungensis* are depicted in Table 4.2.

The meristic characters in the present study include the dorsal fin with 2 simple and 8½ branched rays, pectoral fin 1 simple and 11-13 branched rays, pelvic fin with 1 simple and 7½ branched rays, anal fin with 2 simple and 5½ branched rays and caudal fin with 10+9 principal caudal rays. The lateral line scales along the lateral line ranged between 30-32 scales. The lateral transverse scale row was counted 3½ above the lateral line scale and 3 between the lateral line and pelvic fin origin. The fin formula for male

Garra langlungensis can be written as D ii 8½; P i 11-13; V i 7½; A ii 5½; C 10+9; LI 30-32; Ltr 3½|1|3.

Table 4.1: Morphometric measurements of 30 randomly selected males of *Garra langlungensis*

Parameters (measurements in mm)	**Min**	**Max**	**Mean**	**S.D**
Total Length (TL)	53.4	73.7	62.7	4.5
Standard Length (SL)	41.7	59.6	49.5	3.7
Head Length (HL)	11.2	14.5	12.9	0.9
Head Width (HW)	8	11.4	9.5	0.8
Body Depth (BD)	9.6	14.2	11.3	1.1
Snout Length (SnL)	5.1	7.6	6.3	0.6
Pre-Dorsal Length (PDL)	19.1	28.1	23.5	2.1
Pre-Pectoral Length (PPL)	8.9	13.1	11.1	1
Pre-Pelvic Length (PVL)	22.5	30.1	26.7	1.7
Pre-Anal Length (PAL)	25.9	43.4	37.2	3.6
Pelvic and Anal Fin Origin Distance (VAFO)	8.1	13.5	11.6	1.2
Vent and Anal Fin Origin Distance (VtAFO)	2.3	3.5	2.8	0.3
Length of Dorsal Fin Base (LDFB)	7.3	10.8	8.3	0.8
Height of Dorsal Fin (HDF)	8.7	13.6	11.8	1.1
Length of Pectoral Fin (LPF)	9.7	12.7	11.1	0.9
Length of Pelvic Fin (LVF)	8.1	11.1	9.6	0.9

Height of Anal Fin (HAF)	8	11.7	10	0.8
Length of Caudal Fin (LCF)	10.5	14.4	12.3	1.1
Length of Peduncle (LP)	6.1	9.8	8.1	1
Depth of Caudal Peduncle (DCP)	5.8	8.7	6.9	0.7
Height of Head at Occiput (HHO)	7.6	10.6	9	0.7
Eye Diameter (ED)	3.6	4.1	3.8	0.1
Inter – Orbital Space (IOS)	4.9	6.5	5.5	0.4
Length of Disc (LD)	3.6	4.9	4.4	0.3
Width of Disc (WD)	5.4	7.9	6.4	0.6
Length of Pulvinus (LPl)	2.1	3.4	2.7	0.3
Width of Pulvinus (WPl)	3.3	5.3	4.2	0.4
Mouth Width (MW)	6.4	8.8	7.6	0.6
Pre-Anus Length (PAsL)	29.2	40.3	34.4	2.7
Anal Fin Base Length (AFBL)	3	4.5	3.6	0.3

Table 4.2: Morphological parameters expressed in percentage of standard length, head length and caudal peduncle length of male *Garra langlungensis*

Parameters (measurements in mm)	**Ranges**	**Mean**	**S.D**
Percentage of standard length (% SL)			
Head length	23.8 – 28.4	26.3	1.1
Body depth at dorsal fin origin	21.2 – 25.2	23.1	1.3
Pre-dorsal length	45.4 – 49.9	47.3	1.5
Pre-anus length	66.2 – 75.1	70.5	2.2

Pre-anal length	72.8 – 80.6	76.6	2.3
Pre-pectoral length	21.2 – 25.6	22.8	1.1
Pre-pelvic length	50.5 – 57.5	53.8	1.9
Dorsal fin base length	15.1 – 18.1	16.7	0.9
Dorsal fin length	19.5 – 27.1	23.4	1.7
Pectoral fin length	20.4 – 25	22.1	1.3
Pelvic fin length	17.4 – 22.3	19.2	1.2
Anal fin base length	6.4 – 8.3	7.3	0.5
Anal fin length	18.7 – 21.9	20.0	0.9
Vent to anal distance	4.8 – 7.1	5.5	0.6
Caudal peduncle length	13.6 – 18.3	16.6	1.4
Caudal peduncle depth	12.5 – 15	14.0	0.8
Caudal fin length	23.2 – 28.1	25.0	1.5
Disc length	7.7 – 9.5	8.7	0.5
Disc width	11.4 – 14.9	13.1	1.0
Pulvinus length	4.8 – 6.8	5.6	0.6
Pulvinus width	8 – 9.8	8.6	0.5
Percentage of head length (% HL)			
Head depth at occiput	62.8 – 74.6	68.9	3.0
Snout length	44.6 – 53.4	49.3	2.3
Interorbital width	38.3 – 47.6	42.2	2.5
Eye diameter	26.8 – 32.2	29.0	1.5
Disc length	30.2 – 37	33.3	1.9

Disc width	46.2 – 57.2	49.9	3.0
Pulvinus length	18 – 25	21.4	2.2
Pulvinus width	30.3 – 36.5	32.7	1.6
Percentage of caudal peduncle length (% caudal peduncle length)			
Caudal peduncle depth	78.2 – 96.6	84.7	5.8

4.3.1.2 Meristic and Morphometric measurement of female *Garra langlungensis*

The morphological study of female *Garra langlungensis* was carried with 30 randomly selected samples ranging between 55 – 78.7 mm in total length. The morphometric measurements of 30 randomly selected females of *Garra langlungensis* along with the minimum (min) and maximum (max) limit and their mean and standard deviation is given in Table 4.3.

The standard length ranged between 42.4 – 60.8 mm with an average of 50.4 mm ± 5.3 and twenty one characters were studied in percentage of standard length. The head length ranged between 11.5 – 15.9 mm with an average of 13.3 mm ± 1.3 and eight characters were studied in percentage of head length. The caudal peduncle length ranged between 6.5 – 9.8 mm with an average of 8.2 mm ± 0.9 and caudal peduncle depth was studied in the percentage of caudal peduncle length. Morphological parameters expressed in percentage of standard length, head length and caudal peduncle length of female *Garra langlungensis* are depicted in Table 4.4.

The meristic characters in the present study include dorsal fin with 2 simple and 8½ branched rays, pectoral fin 1 simple and 11-13 branched rays, pelvic fin with 1 simple and 7½ branched rays, anal fin with 2 simple and 5½ branched rays and caudal fin with

10+9 principal caudal rays. The lateral line scales along the lateral line ranged between 30-32 scales. The lateral transverse scale row was counted 3½ above the lateral line scale and 3 between the lateral line and pelvic fin origin. The fin formula for female *Garra langlungensis* can be written as D ii 8½; P i 11-13; V i 7½; A ii 5½; C 10+9; LI 30-32; Ltr 3½|1|3.

Table 4.3: Morphometric measurements of 30 randomly selected females of *Garra langlungensis*

Parameters (measurements in mm)	**Min**	**Max**	**Mean**	**S.D**
Total Length (TL)	55	78.7	63.7	6.9
Standard Length (SL)	42.4	60.8	50.4	5.3
Head Length (HL)	11.5	15.9	13.3	1.3
Head Width (HW)	8	12.1	9.8	1.3
Body Depth (BD)	9.2	13.1	11.3	1.1
Snout Length (SnL)	5.2	8.1	6.5	0.8
Pre-Dorsal Length (PDL)	19.7	29.8	23.8	2.8
Pre-Pectoral Length (PPL)	9	14	11.3	1.4
Pre-Pelvic Length (PVL)	2.3	32.9	26.5	6.0
Pre-Anal Length (PAL)	33.8	47.3	38.8	3.9
Pelvic and Anal Fin Origin Distance (VAFO)	9.4	15.5	11.7	1.5
Vent and Anal Fin Origin Distance (VtAFO)	2.2	4.4	2.8	0.5
Length of Dorsal Fin Base (LDFB)	6.6	10	8.3	0.8
Height of Dorsal Fin (HDF)	9.4	15.1	12	1.6

Length of Pectoral Fin (LPF)	9	13.6	11.2	1.3
Length of Pelvic Fin (LVF)	7.8	11.9	9.5	1.1
Height of Anal Fin (HAF)	9	12.8	10.2	1.2
Length of Caudal Fin (LCF)	9.7	16.9	12.8	1.8
Length of Peduncle (LP)	6.5	9.8	8.2	0.9
Depth of Caudal Peduncle (DCP)	5.9	8.9	7.1	1
Height of Head at Occiput (HHO)	7.3	11.1	9.1	0.9
Eye Diameter (ED)	3.6	4.2	3.9	0.1
Inter – Orbital Space (IOS)	5	6.9	5.8	0.6
Length of Disc (LD	3.9	5.5	4.5	0.4
Width of Disc (WD)	5.6	8.5	6.6	0.8
Length of Pulvinus (LPl)	2.3	3.8	2.9	0.4
Width of Pulvinus (WPl)	3.7	5.2	4.3	0.5
Mouth Width (MW)	6.7	9.8	7.9	0.9
Pre-Anus Length (PAsL)	31.7	43.9	35.8	3.5
Anal Fin Base Length (AFBL)	3.1	4.4	3.6	0.4

Table 4.4: Morphological parameters expressed in percentage of standard length, head length and caudal peduncle length of female *Garra langlungensis*

Parameters (measurements in mm)	**Ranges**	**Mean**	**S.D**
Percentage of standard length (%SL)			
Head length	25.3 – 27.7	26.4	0.6

Body depth at dorsal fin origin	20.9 – 24.1	22.5	0.9
Pre-dorsal length	45.4 – 49.9	47.5	1.2
Pre-anus length	66.8 – 73.5	70.8	1.8
Pre-anal length	72.4 – 80.9	76.7	1.9
Pre-pectoral length	21.1 – 24.8	22.6	1.1
Pre-pelvic length	51.4 – 56.1	53.9	1.3
Dorsal fin base length	15.5 – 17.3	16.4	0.5
Dorsal fin length	21.7 – 26.1	23.7	1.5
Pectoral fin length	20.5 – 23.8	22.4	0.8
Pelvic fin length	18.1 – 19.8	19.0	0.5
Anal fin base length	6.2 – 8.2	7.1	0.6
Anal fin length	18.1 – 21.4	19.8	1.0
Vent to anal distance	4.6 – 8.7	5.7	1.1
Caudal peduncle length	14.7 – 17.9	16.3	1.1
Caudal peduncle depth	13.1 – 15.7	14.2	0.7
Caudal fin length	24.6 – 28.1	25.9	1.2
Disc length	7.7 – 9.9	8.9	0.7
Disc width	11.2 – 14.5	13.1	1.0
Pulvinus length	5.4 – 6.5	5.8	0.3
Pulvinus width	7.6 – 9.4	8.6	0.4
Percentage of head length (%HL)			
Head depth at occiput	62.1 – 75	68.6	3.4
Snout length	43.3 – 52.3	49.2	2.7

Interorbital width	38.9 – 47.2	42.8	2.2
Eye diameter	24.5 – 31.4	28.2	2.0
Disc length	28.5 – 36.9	33.8	2.5
Disc width	42.4 – 56.2	49.9	3.9
Pulvinus length	20.4 – 26	22.2	1.5
Pulvinus width	28.7 – 35.1	32.8	1.8
Percentage of caudal peduncle length (% caudal peduncle length)			
Caudal peduncle depth	79.5 – 94.5	87.6	5.1

Fin formula: D ii 8½; P i 11-13; V i 7½; A ii 5½; C 10+9; LI 30-32; Ltr 3½|1|3

4.3.2 Length – weight relationship of *Garra langlungensis*

The length-weight relationship study in *Garra langlungensis* has been estimated for male, female and pooled population based on 213 specimens ranging from 45.8 to 82.4 mm, respectively, in total length and weights ranging from 1.2 to 6.43 gm.

4.3.2.1 Length – weight relationship of the male population of *Garra langlungensis*

The male population of *Garra langlungensis* consists of 149 specimens ranging from 45.8 to 73.4 mm in total length and 1.2 to 5.84 gm in weight. The regression equation for the male population can be expressed as follows:

$$W = 0.0078\ L^{2.965}$$

$$\text{Log } W = -4.836 + 2.965 \text{ Log } L$$

The correlation coefficient 'r^{2}' between log length and log weight was 0.911. The scatter diagram for the logarithmic relationship between length and weight of the male

population of *Garra langlungensis* along with its correlation coefficient, is represented in Figure 3.3.

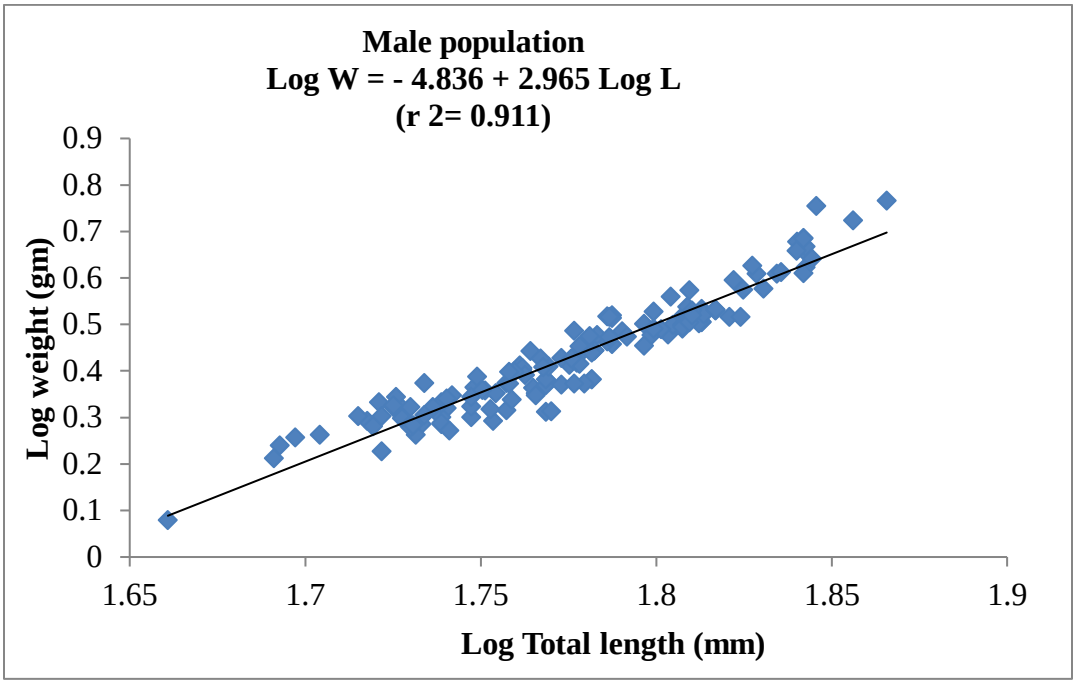


Figure 4.1: Length weight relationship in the male population of *Garra langlungensis*

4.3.2.2 Length – weight relationship of the female population of *Garra langlungensis*

The female population of *Garra langlungensis* consists of 64 specimens ranging from 49.3 to 82.4 mm in total length and 1.61 to 6.43 gm in weight. The regression equation for the female population can be expressed as:

$$W = 0.0126\ L^{2.710}$$

$$\text{Log } W = -4.371 + 2.710 \text{ Log } L$$

The correlation coefficient 'r' between log length and log weight was 0.938. The scatter diagram for the logarithmic relationship between length and weight of female

population of *Garra langlungensis* along with its correlation coefficient is represented in Figure 3.3.

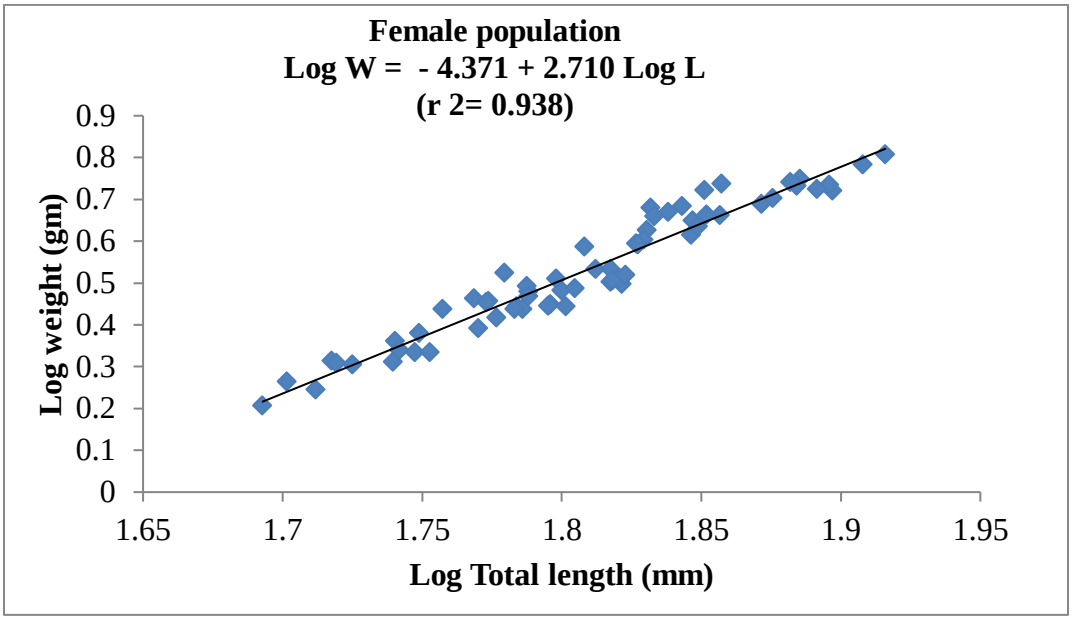


Figure 4.2: Length weight relationship in the female population of *Garra langlungensis*

4.3.2.3 Length – weight relationship of the pooled population of *Garra langlungensis*

Pooled population of *Garra langlungensis* consists of 213 specimens ranging from 45.8 to 82.4 mm in total length and 1.2 to 6.43 gm in weights. The regression equation of pooled population can be expressed as follows:

$$W = 0.0093\ L^{2.870}$$

$$\text{Log } W = -4.666 + 2.870 \text{ Log } L$$

The correlation coefficient 'r' between log length and log weight was 0.928. The scatter diagram for the logarithmic relationship between length and weight of the female

population of *Garra langlungensis*, along with its correlation coefficient, is represented in Figure 3.3.

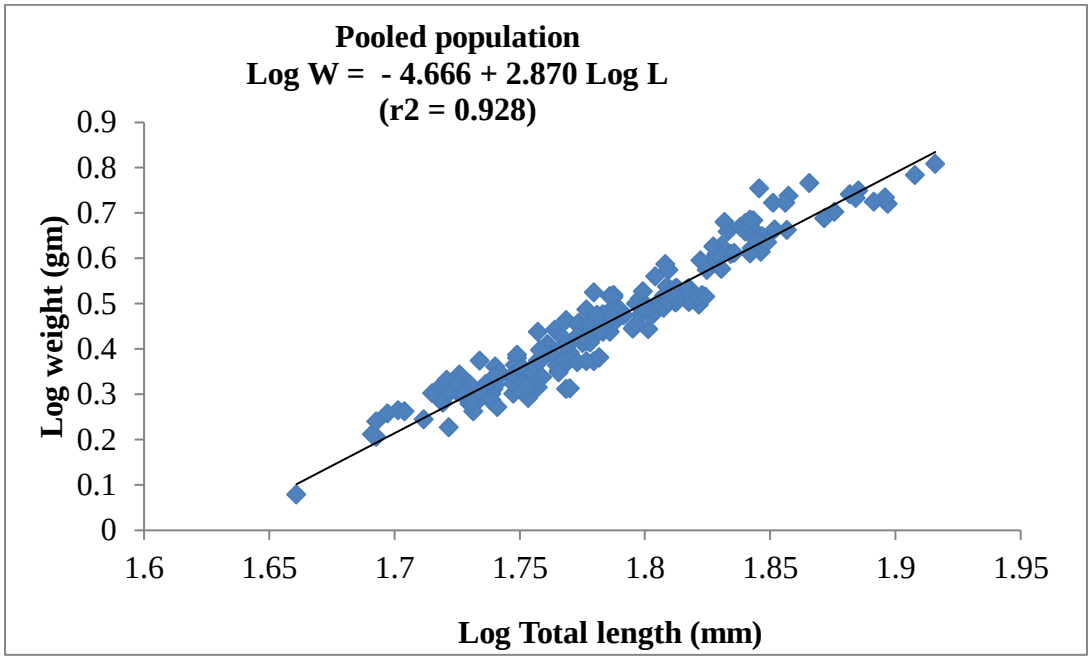


Figure 4.3: Length weight relationship in the pooled population of *Garra langlungensis*

4.3.2.4 Condition factor

Relative condition factor (Kn) was estimated for male, female and pooled populations of *Garra langlungensis* separately. The estimated value with average and range are shown below.

Male population : Kn = 1.825 (2.267-1.445)

Female population : Kn = 1.738 (2.116-1.484)

Pooled population : Kn = 1.720 (2.267-1.445)

The data show that the male population exhibit relatively better well-being than the female and the pooled population.

4.4 Discussion

The meristic and morphological measurement of male and female *Garra langlungensis* shows that there are wider ranges in some of the morphological characters and meristic count of this species. Morphological measurements such as pre-anus length 66.2 – 75.1 (male) and 66.8 – 73.5 (female) in relation to SL (*versus* 66.6 – 69.6 Ezung *et al*., 2021); pre-anal length 72.8 – 80.6 (male) and 72.4 – 80.9 (female) in relation to SL (*versus* 74.1 – 77.4); pre-pectoral length 21.2 – 25.6 (male) and 21.1 – 24.8 (female) in relation to SL (*versus* 21.4 – 22.6); pre-pelvic length 50.5 – 57.5 (male) and 51.4 – 56.1 (female) in relation to SL (*versus* 50.9 – 53.9); dorsal fin length 19.5 – 27.1 (male) and 21.7 – 26.1 (female) in relation to SL (*versus* 23.2 – 25.4); anal fin base length 6.4 – 8.3 (male) and 6.2 – 8.2 (female) in relation to SL (*versus* 6.4 – 7.6); disc length 7.7 – 9.5 (male) and 7.7 – 9.9 (female) in relation to SL (*versus* 8.5 – 9.8); pulvinus length 4.8 – 6.8 (male) and 5.4 – 6.5 (female) in relation to SL (*versus* 5.7 – 6.6); pulvinus width 8 – 9.8 (male) and 8 – 9.4 (female) in relation to SL (*versus* 8.6 – 9.5); disc width 46.2 – 57.2 (male) and 42.4 – 56.2 (female) in relation to HL (*versus* 46 – 54); pulvinus length 18 – 25 (male) and 20.4 – 26 (female) in relation to HL (*versus* 21 – 25). The meristic count showed more unbranched pectoral fin in both males and females 11 – 13 (*versus* 11 – 12 Ezung *et al*., 2021).

Length-weight relationship in the present study shows that the value of exponent 'b' for the male population was 2.965, which is the highest value, followed by the pooled population of 2.870 and the female population with the lowest value of 2.710. The

highest 'b' value indicates that males are in better condition and gain weight faster in relation to length than the females and pooled population. The exponent 'b' was found to fall under normal distribution frequency within the expected range of 2.5 to 3.5 (Froese, 2006). According to the general cube law, as supported by Allen (1938), Beverton & Holt, (1957), an ideal fish should exhibit an isometric growth having the value of regression coefficient not different from 3. The exponential value of 2.965 in male indicates that it is close to having an isometric growth, however it is not equal to 3 and the exponential value of 2.870 and 2.710 in pooled and female implies that the length-weight relationship of *Garra langlungensis* deviated from the cube law having a negative allometric growth. The deviation from cube law could be due to some general condition of the fish such as feeding and reproductive activities and certain environmental factors as suggested by Le Cren (1951).

Previous reports on length-weight relationship show that many cyprinids fishes did not follow and deviated from the cube law and tended to become more slender and lighter with increasing length. Negative allometric growth have been reported in *Neolissochilus hexagonolepis* (Kar *et al*., 2005); *Garra gotyla stenorhynchus* (Baby *et al*., 2011); *Schizopyge curvifrons* (Mir *et al*., 2012); *Puntius chola* and *Danio dangila* (Angami, 2012); *Puntius conchonius* (Amenla, 2014); *Garra birostris*, *Garra annandalei*, and *Raiamas bola* (Basumatary *et al*., 2017); *Salmostoma bacaila, Labeo boga* (Baitha *et al*., 2018). All these reports are in agreement with the present findings on the length-weight relationship of *Garra langlungensis* in male, female and pooled populations in which the ‘b’ value is below 3 having negative allometric growth.

Condition factor is a useful index for monitoring and evaluating feeding intensity, growth rates and overall population in fish (Oni *et al*., 1983, Brian *et al*., 2000). Le Cren (1951) stated that Kn values greater than 1 indicate good general condition of the fish. According to Jisr *et al*. (2018), an overall fitness for fish species is assumed when Kn values are equal or close to 1. The present study showed the highest value of condition factor in the male population, indicating better growth rate in males than females and pooled population. In the case of females and pooled population the female population showed slightly better growth rate than the pooled population. The Kn value was found to be greater than one in all three populations, which showed that the fish specimens are in good condition. The relative condition factor (Kn) ranging from 2.267 – 1.445 with an average value of 1.720 in the present study indicates a healthy condition and Langlung river as a suitable habitat for *Garra langlungensis.*

Chapter 5

Reproductive Biology

Contents

5.1 Introduction

Reproduction is one of the important physiological systems that are crucial in the life cycle of living organisms, including fish (Muchlisin, 2014). Knowledge of fish reproductive biology is critical for successful aquaculture and scientifically based fishery management in any water body (Jacob, 2013). 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). The study of fish reproductive biology is essential for determining the spawning season and stock as well as developing captive breeding techniques and conservation strategies. Reproductive biology, which includes fecundity, spawning, and sex ratio etc., are among the important aspects of the biology of fishes, which must be understood to explain the variations in the level of populations as well as to make efforts to increase the amount of harvest (Azadi & Mamun, 2004).

The primary step in developing a captive breeding program for a fish species is determining the sex. Fishes exhibit a wide range of sexual dimorphic characteristics; some species have highly remarkable differences between males and females, while some are incredibly identical in morphological features (Jacob, 2013). It is critical to determine sexual dimorphism because it exhibits morphological characteristics that differentiate them sex-wise. The sex ratio provides basic information to ensure proportional fishing of two sexes, estimate the stock size and assess the fish population's reproductive potential (Vazzoler, 1996; Stratoudakis *et al*., 2006). Information on the sex ratio of fish is essential to determine female spawning biomass and understand the status of fish stock in relation to a selected point of biological reference (Stratoudakis *et al*., 2006; Morgan, 2008; Adebiyi, 2013).

The fecundity of a fish is the number of eggs that are likely to be laid during a spawning season (Chondar, 1977; Bagenal, 1978). Fecundity varies between fish populations and from species to species, depending on numerous factors such as stock of fish, age, size, body and gonad weight, environmental conditions, nutritional status, time of sampling and maturation stages. (Simpson, 1951; Lagler, 1956; Gupta, 1967; Bhuiyan *et al*., 2006). Fecundity studies are an important part of fishery science because they directly affect fish production and exploitation (Shafi *et al*., 2012). Knowledge of fecundity is essential for studies of abundance, population dynamics, reproductive potential and fish life history (Gupta, 1967; Bruch *et al*., 2006). Assessment of fecundity is of paramount importance in fisheries management as it provides knowledge about the number of offspring produced in a season and the reproductive capacity of the species (Qasim & Qayyum, 1963).The ratio between the gonad weight and the fish weight is known as the Gonado-somatic index. The gonado-somatic index is a helpful measure for determining reproductive periodicity in fishes. The gonado-somatic index is one of the important parameters of fish biology, which gives a clear idea regarding fish reproduction and the reproductive status of the species and helps in ascertaining the breeding period of fish (Gupta & Srivastava, 2001; Shankar *et al*., 2005).

This study aims to understand the life history, reproductive potential, and spawning season of *Garra langlungensis*. This is a pioneer study that would assist in the development of the captive breeding program for this species. The work would also contribute to identifying the fish stock in its natural habitat and developing appropriate conservation measures.

5.2 Materials and methods

Monthly samplings of the fish were done from Langlung river at uniform intervals during the period from February 2017 to January 2018. The study for different aspects of reproductive biology was based on 213 specimens of *Garra langlungensis,* 149 males and 64 females ranging in total length from 45.8 mm to 73.7 mm and 50.3 to 88.2 mm, respectively and weight between 1.20 to 6.07 gm and, 1.67 to 8.22 gm in males and females respectively. Collected fish specimens were preserved in 10% formaldehyde and brought to the laboratory for further investigation. Excess water was removed by filter paper, and lengths (to the nearest mm) and weights (to the nearest 0.01 gm) for each fish were recorded. Fishes were then dissected out to identify the sex, gonads were removed, and the length and weight were recorded to the nearest millimeter and milligram, respectively. The ovary was preserved in 4% formaldehyde for fecundity studies.

Sexual dimorphism was observed during breeding as well as non-breeding seasons. Male and female were identified based on different morphological characteristics such as overall body shape and colouration, bulginess of stomach and fins.

The sex ratio was analyzed on the monthly percentage of males and females. Homogeneity of the sex ratio was estimated using the Chi-square test (Snedecor & Cochran, 1967) to observe whether the ratio between males and females deviated from the expected 1:1 ratio. The Chi-square test was calculated using the formula.

$$X^2 = \frac{(O - E)^2}{E}$$

Where, O = Observed value

E = Expected value

Fecundity in *Garra langlungensis* was estimated from 16 ripe females of size ranging from 55.2 mm to 78.7 mm. Three sub-samples of the ovary, i.e., anterior, middle and posterior regions, were weighed separately. The number of ova in each sub-sample was counted under the Huvitz stereo zoom microscope (HSZ-ILST6). Fecundity was estimated by applying the gravimetric method adopted by Hunter *et al*. (1992).

$$Fecundity = \frac{Weight\ of\ the\ ovary\ X\ Average\ number\ of\ eggs\ per\ sub\ sample}{Average\ weight\ of\ the\ sub-sample}$$

Regression analysis was used to determine the relationship between computed fecundity and various parameters such as total body length, total body weight, ovary length and ovary weight and between ovary weight and parameters such as total body length and total body weight (Joshi & Khanna, 1980).

Relative fecundity was estimated using the mathematical equation expressed below:

$$Relative\ fecundity\ (Fr) = \frac{Absolute\ fecundity}{Total\ length\ (cm)\ or\ body\ weight\ (gm)\ or\ ovary\ weight\ (gm)}$$

Where absolute fecundity was calculated and expressed in terms of unit body weight, unit total length and unit ovary weight.

The Gonado-somatic index (GSI) was determined to understand the spawning season and general reproductive status of the fish. GSI was calculated on a monthly basis, by applying the standard formula of Nikolsky (1963).

$$GSI = \frac{Weight\ of\ the\ gonads}{Weight\ of\ the\ fish}\ x\ 100$$

All the statistical analysis was calculated using Microsoft office excel 2007.

5.3 Results

5.3.1 Sexual dimorphism

Generally, no sexual dimorphism is observed in *Garra langlungensis*. However, secondary external morphology characters were observed during the breeding season in both male and female *Garra langlungensis*, as shown in Table 5.1. Anal opening in male and female *Garra langlungensis* is shown in Figure 5.1.

Table 5.1: Sexual dimorphism in males and females of *Garra langlungensis*

S. No	Parameters	Female	Male
1	Body size	Larger in size than the male, having a bulging belly, and egg oozed out from the anal opening when pressed slightly in the abdomen	Smaller in size than the female with a slender body, and when pressed slightly in the abdomen, a whitish-coloured milt oozed out from the anal opening
2	Body shape	Higher body depth than the male	Lower in body depth than the female
3	Fin characters	Smooth pectoral fin	Rough pectoral fin
4	Anal opening	Oval and raised	Elongated and pointed

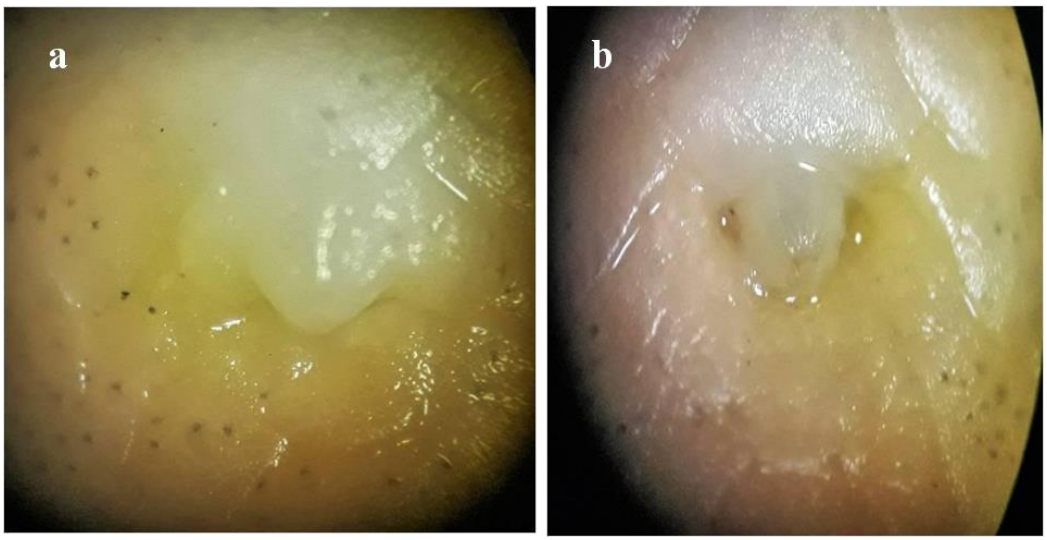


Figure 5.1: Anal opening in *Garra langlungensis*: (a) male and (b) female

5.3.2 Sex ratio

The sex ratio was ascertained by examining a total of 213 specimens of *Garra langlungensis* in the laboratory. The fishes were sexed by internal examination owing to the absence of sexual dimorphism in *Garra langlungensis*. Out of the total 213 specimens, 149 were males and 64 were females. The sex ratio of male to female was observed to be 1:0.43. The percentage occurrence of males was found to be 69.95, while that of females was 30.04. The Chi-square value of 33.92 showed that the variation in the sex ratio of *Garra langlungensis* was highly significant at 0.05. The sex ratio month-wise distribution of males and females of *Garra langlungensis* depicted in Table 5.2 showed that males outnumbered females in all the months. The sex ratio of various group sizes shows males outnumbering females in almost all the length groups except in the higher length groups. The sex ratio of *Garra langlungensis* in different length groups is represented in Table 5.3.

Table 5.2: Sex ratio of *Garra langlungensis* during the period February 2017 - January 2018

Months	Total	Male	% of male	Female	% of female	Ratio of M:F	Chi-square	D.F	Significance at 5% level
February	22	15	68.18	7	31.81	1:0.47	1.92	1	N.S
March	21	12	57.14	9	42.85	1:0.75	2.9	1	N.S
April	44	30	68.18	14	31.81	1:0.47	0.42	1	N.S
May	29	22	75.86	7	24.13	1:0.32	5.82	1	S
June	17	12	70.58	5	29.411	1:0.42	7.76	1	S
July	8	6	75	2	25	1:0.33	2.88	1	N.S
August	11	8	72.72	3	27.27	1:0.38	2	1	N.S
September	9	7	77.77	2	22.22	1:0.29	2.28	1	N.S
October	14	10	71.42	4	28.57	1:0.40	2.78	1	N.S
November	13	9	69.23	4	30.76	1:0.44	2.58	1	N.S
December	12	9	75	3	25	1:0.33	1.92	1	N.S
January	13	9	69.23	4	30.76	1:0.44	3	1	N.S
Total	213	149	69.95	64	30.04	1:0.43	33.92	11	S

Table 5.3: Sex ratio of *Garra langlungensis* in different length groups (each ≈ 5mm).

Length groups (mm)	Total	Male	% of male	Female	% of female	Ratio of M:F	Chi-square	Significance at 5% level
45-50	7	5	71.43	2	28.57	1:0.4	1.28	N.S
51-55	41	33	80.49	8	19.51	1:0.24	15.24	S
56-60	63	52	82.54	11	17.46	1:10.21	26.68	S
61-65	53	38	71.70	15	28.30	1:0.39	9.98	S
66-70	33	19	57.58	14	42.42	1:0.74	0.76	N.S
71-75	8	2	25.00	6	75	1:3	2	N.S
76-80	7	0		7				
81-85	1	0		1				

5.3.3 Fecundity

The fecundity of *Garra langlungensis* in the present study ranged from 319 – 844 in fishes of size ranging from 55.2 – 78.7 mm TL. The highest fecundity value, 844 was observed in species weighing 5.31 gm. The average fecundity was worked out to be 565. The values of fecundity count are given in Table 5.4.

Relative fecundity or a number of ova produced per gram body weight, values ranged from 5.79 – 11.23 per mm in total length, 95.34 – 215.65 per gm body weight

and 1175.47 – 1765.52 per gm ovary weight. The values of relative fecundity in *Garra langlungensis* are given in Table 5.5.

The regression analysis was carried out to find the relationship between fecundity and the total length, body weight, ovary weight and ovary length and also between ovary weight and total length and body weight which are illustrated in Figure 5.1 – 5.6. The scatter diagram revealed a linear relationship between fecundity and body parameters. The value of correlation coefficient between fecundity and body parameters showed that fecundity is significantly correlated with ovary weight. The regression equations of the variables after logarithmic transformation are given in Table 5.6.

Table 5.4: Absolute fecundity in the spawners of *Garra langlungensis*

S. No	Total length (mm)	Body weight (gm)	Ovary weight (gm)	GSI	Fecundity
1	78.7	5.43	0.57	10.49	811
2	67.7	4.24	0.33	7.78	404
3	70.6	4.32	0.47	10.87	673
4	61.1	3.09	0.45	14.56	528
5	62.5	2.82	0.24	8.51	411
6	75.1	5.05	0.48	9.50	644
7	64.9	3.42	0.3	8.77	421
8	59.3	2.52	0.28	11.11	427
9	60.7	2.74	0.35	12.77	590
10	63.1	3.04	0.29	9.53	512

11	67.9	4.79	0.52	10.85	762
12	55.2	2.19	0.23	10.50	319
13	58.7	2.91	0.41	14.08	568
14	74.4	4.89	0.42	8.58	650
15	66.8	4.07	0.39	9.58	480
16	77.9	5.31	0.56	10.54	844

Table 5.5: Relative fecundity in the spawners of *Garra langlungensis*

S. No	Fecundity	Relative fecundity (per mm total length)	Relative fecundity (per gram body weight)	Relative fecundity (per gram ovary weight)
1	811	10.31	149.44	1423.64
2	404	5.97	95.34	1225
3	673	9.53	155.79	1431.91
4	528	8.66	171.19	1175.47
5	411	6.57	145.79	1713.04
6	644	8.58	127.61	1342.55
7	421	6.49	123.11	1403.45
8	427	7.20	169.44	1525
9	590	9.73	215.65	1688.24
10	512	8.11	168.42	1765.52

11	762	11.23	159.22	1466.67
12	319	5.80	146.08	1390.91
13	568	9.69	195.49	1387.5
14	650	8.74	133.02	1548.78
15	480	7.19	117.94	1230.77
16	844	10.84	158.97	1507.41

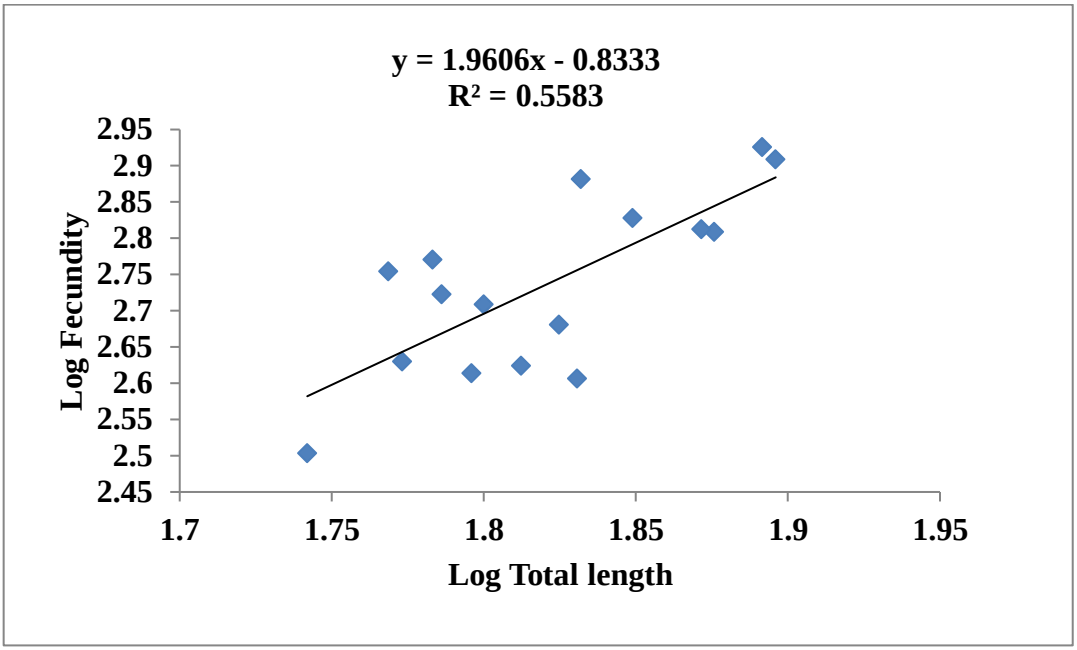


Figure 5.2: Relationship between fecundity and total length

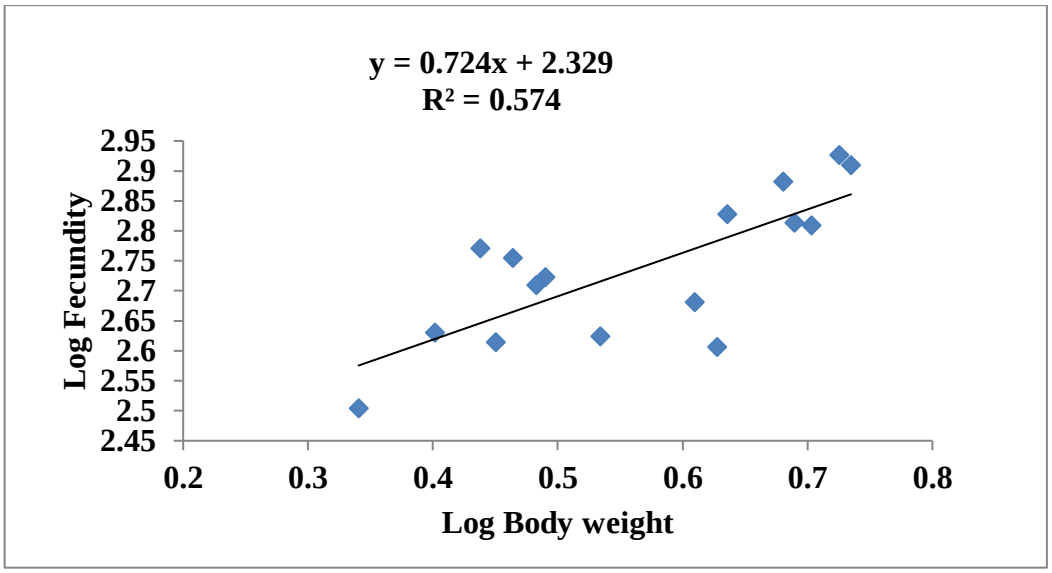


Figure 5.3: Relationship between fecundity and body weight

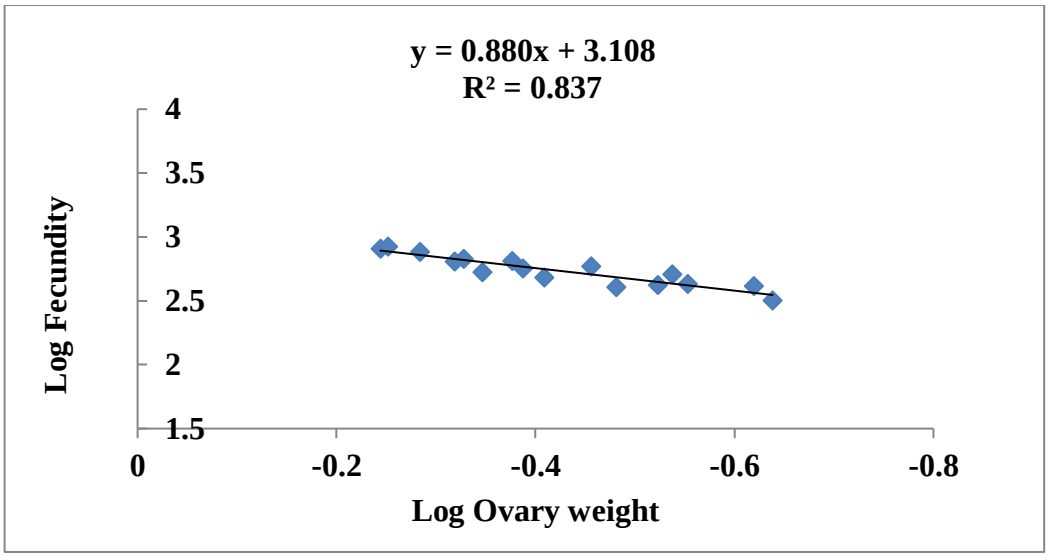


Figure 5.4: Relationship between fecundity and ovary weight

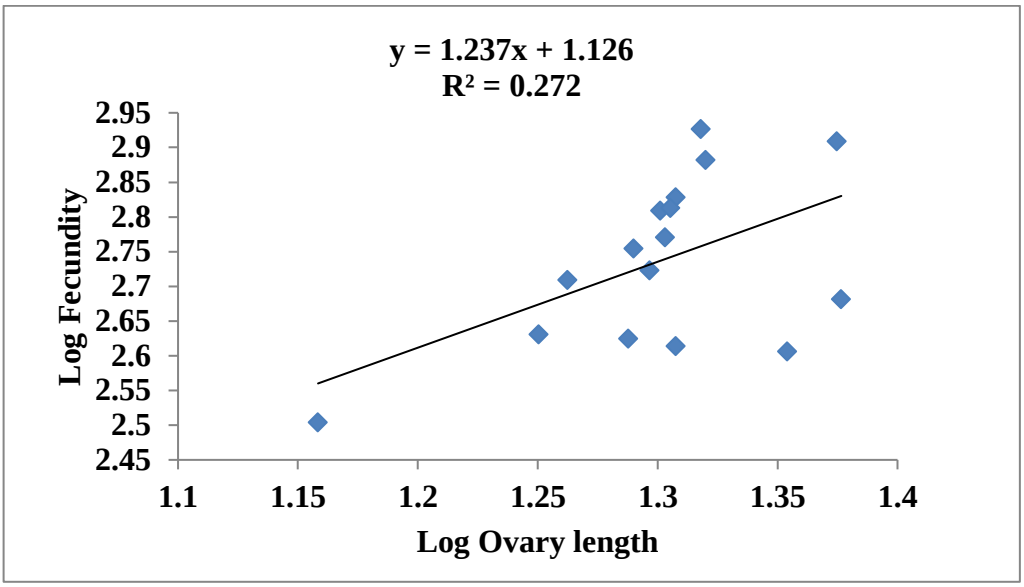


Figure 5.5: Relationship between fecundity and ovary length

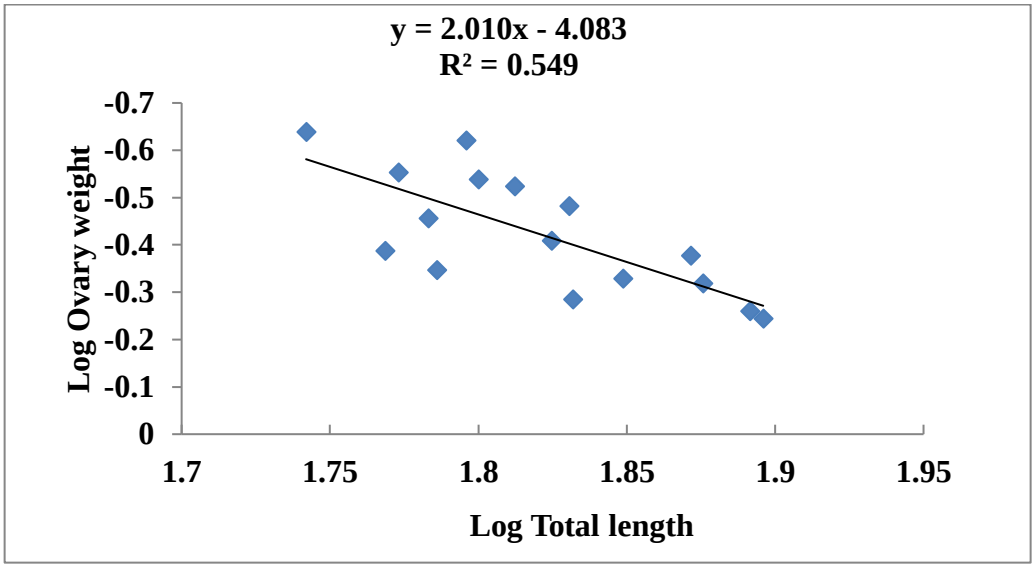


Figure 5.6: Relationship between ovary weight and total length

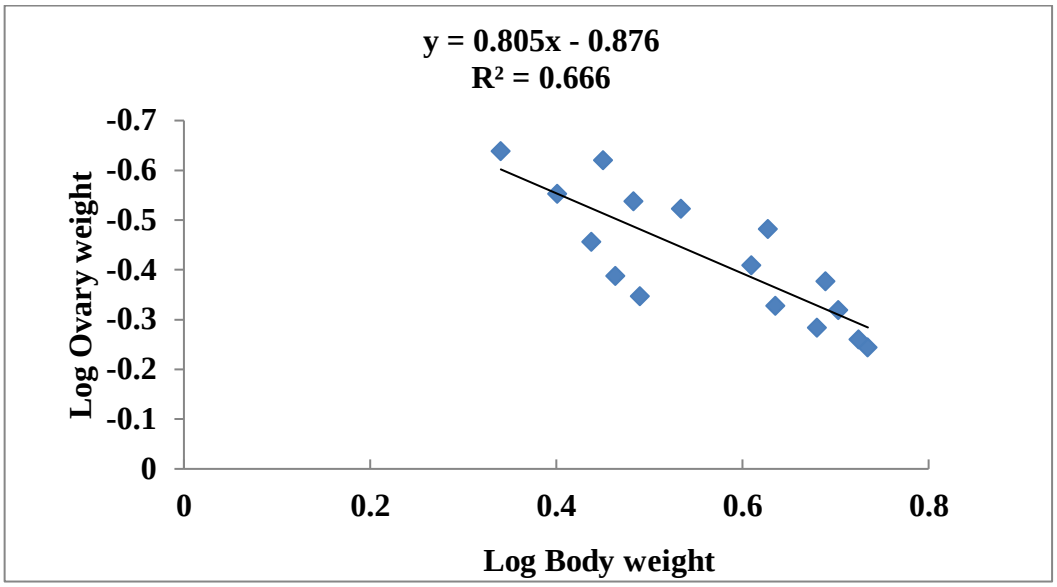


Figure 5.7: Relationship between ovary weight and body weight

Table 5.6: Regression equation between fecundity and body parameters; ovary weight and total length and body weight.

S. No	Variant (x)	Equation Log Y = Log a + b Log X	Correlation coefficient (r)
1	Total length (mm)	Log F = - 0.833 + 1.960 Log TL	0.747
2	Body weight (gm)	Log F = 2.329 + 0.724 Log BW	0.758
3	Ovary weight (gm)	Log F = 3.108 + 0.880 Log OW	0.915
4	Ovary length (gm)	Log F = 1.237 + 1.126 Log OL	0.522
5	Total length (mm)	Log OW = - 4.083 + 2.010 Log TL	0.741
6	Body weight (gm)	Log OW = - 0.876 + 0.805 Log BW	0.816

5.3.4 Gonado-somatic index

The breeding season of the fish extends from February to May. The gonado-somatic index (GSI) ranged between 0.3 to 8.1 in males and between 0.5 to 11.5 in females. The GSI was found to be higher during the month of February to May and lowest during the month of September to November in both the male and female populations. The peak in GSI was observed during the month of March and April in the male population, while in the case of females, the peak was in April. A declining trend was observed right after the peak point in both the male and female populations. The monthly trend of the gonado-somatic index value for male and female are presented in Figure 5.7.

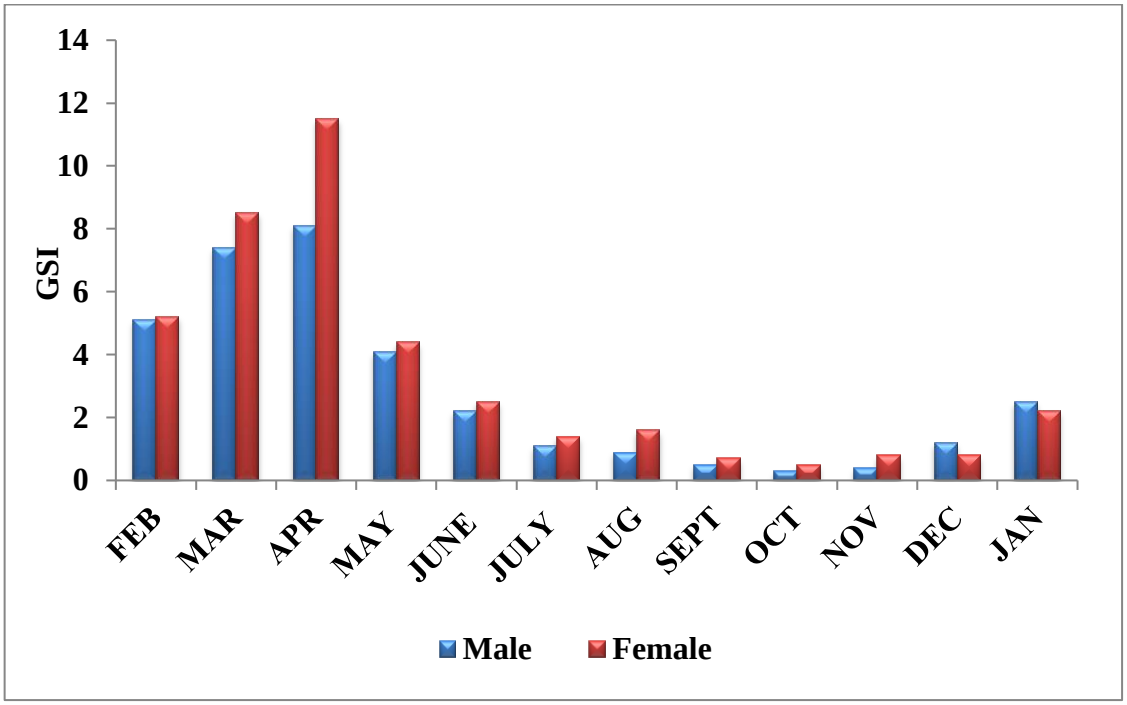


Figure 5.8: Monthly variations of gonado-somatic index in *Garra langlungensis* February 2017 – January 2018

5.4 Discussion

In the present study, females are generally larger in size than males, and *Garra langlungensis* exhibited secondary sexual character during the onset of the spawning season. Observation of secondary sexual characters has also been reported in *Garra surendranathanii* by Thampy (2009) and Kanwal (2017) in *Garra lamta*.

Knowledge of the estimation of the sex ratio in a fish population is important to understand the abundance of the sexes in a natural habitat in different seasons or particular time. According to Nikolsky (1980), the ideal sex ratio is having close to 1:1 in a natural population; however, drastic deviations from the optimum sex ratio can occur due to various factors. Beevi & Ramachandran (2005) stated that a rising temperature and moderate water velocity, vulnerability of females to their predators and other natural hazards, and the migratory phase in the brooder population are some of the reasons for the changes in the sex ratio in fish.

In our present finding, the mean sex ratio of males to females was 1:0.43 (χ^2=33.92), where the female was male-dominated in all the months during the study period. Even though males outnumbered females, variations were found to be non-significant in almost all the months. However, considerable variations were observed during the months of April and May, which is the peak spawning season for this fish. The length group variation of this species indicated considerable differences in the sex ratio at various phases of its life history. When examining the length groups of this species, it was found that while males dominated almost all of the length groups, only females were observed in the higher length group, implying that females are generally larger than males. Siddiqui *et al.* (1976) stated that the predominance of females in

higher groups might be due to the heavy mortality of males in smaller size groups due to either natural death or fishing pressure as they were more active and caught more easily. However, based on the current finding, we can presume that the female is more easily exploited and caught for food due to its larger size than the male, particularly during the spawning season.

A significant variation in sex ratio, with males outnumbering females, has been reported in *Garra surendranathanii* with a sex ratio of 1: 0.34 by Thampy (2009). Hossain *et al.* (2013) reported a sex ratio of 1: 0.79 in *Cirrhinus reba*. Sex ratio of 1: 0.8 was reported by Bindu & Padmakumar (2014) in *Etroplus suratensis*. Hossain *et al.* (2019a) also reported a sex ratio of 1: 0.90 in *Clupisoma garua*. In the case of fish, a sex ratio greater than one between males and females may indicate a higher possibility of fertilization, according to Nikolsky (1999). The present study resulted in a significant deviation in sex ratio from the ideal 1:1 ratio having male dominance over the female, which indicates a low chance of fertilization in *Garra langlungensis*.

Knowledge of fecundity is essential to assess the productive potential, life history, the commercial potentialities of a fish stock, and efficient fish culture (Lagler, 1956; Mian & Dewan, 1984; Das *et al*., 1989). Fecundity in *Garra langlungensis* was found to vary from 319 – 844 with an average value of 565 ranging from 55.2 – 78.7 mm total length and 2.19 – 5.43 g body weight. In teleosts, fecundity ranges from a few hundred to several lakhs, such as *Cirrhinus reba* with 19549 – 265042 ova (Jewel *et al*., 2019) and *Cyprinus carpio* with 18280 – 390600 ova (Bakht *et al*., 2020). The range of fecundity observed in the present study was low compared to other high fecund fish with thousand to several lakhs ova. Similar findings with low fecund were reported in *Garra*

surendranathanii by Thampy (2009); *Garra rufa* by Abedi *et al*. (2011); *Garra regressus* and *Garra tana* by Geremew *et al*. (2015); *Xenontedon cancila* by Borthakur (2018); *Puntius ticto* by Bahuguna *et al*. (2021). The findings of this study also show that the number of ova varies within the same size group of this species.

The present findings showed that the mean relative fecundity of *Garra langlungensis* is 152 eggs per gram body. It is quite high when compared with relative fecundity of 32 in *Garra surendranathanii* (Thampy, 2009), 63 in *Garra regressus*, 102 in *Garra tana* (Geremew *et al*., 2015). This finding indicates that *Garra langlungensis* is abundant in its natural habitat despite its small size and low fecundity.

The statistical analysis shows that fecundity was found to have a linear and positive relationship with the total length, body weight, ovary weight and ovary length. Similar observations were reported by Rahman & Miah (2009) in *Mastacembelus pancalus*, Marimuthu *et al*. *(*2009) in *Anabas testudineus*, Angami (2012) in *Danio dangila* and *Puntius chola*, Kant *et al*. (2016) in *Puntius sophore*, Borthakur (2018) in *Xenontedon cancila*, Hossian *et al*. (2019b) in *Neotropius atherinoides,* Bahuguna *et al*. *(*2021) in *Puntius ticto*. The exponential value of *Garra langlungensis* was observed to be 1.960. Wooton (1979) stated 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. According to Bagenal & Braum (1978), in many fish species, 'b' value is usually around three when fecundity is related to length and about one when related to weight. The present study revealed that the 'b' value deviates significantly from '3', but it remains close to 1, indicating that fecundity is correlated to weight.

The value of correlation coefficient shows that fecundity is significantly correlated with total length (r = 0.747), body weight (r = 0.758), ovary weight (r = 0.915) and moderately with ovary length (r = 0.522). It was also observed that ovary weight is significantly correlated with total length (r = 0.741) and body weight (r = 0.817). The present observation revealed that fecundity has a significant correlation with body parameters. However, the highest degree of correlation was observed between fecundity and ovary weight. This finding is in agreement with observations reported by Sivashanthini *et al*. (2008) in *Gerres abbreviatus*, Abedi *et al*. (2011) in *Garra rufa*, Kant *et al*. (2016) in *Puntius sophore*, Kanwal (2017) in *Garra lamta*, Jewel *et al*. (2019) in *Cirrhinus reba*. Fecundity in *Garra langlungensis* is thus directly proportional to the weight of the ovary in the present study.

The gonado-somatic index (GSI) is a reliable indicator of gonadal maturity; since the weight of the gonad increases with maturity, and when it spawns, there is a reduction in the weight of the gonad on account of the release of gametes (de Vlaming *et al.*,1982). The maximum GSI value indicates the breeding season of the fish in its natural habitat (Rao, 1993; Hamza, 1980; Sharma, 1987). The highest GSI value for this fish was recorded for males during the month of March and April, and for females, it was recorded in April, while the lowest were recorded in October for both males and females. The GSI typically increases with the maturation of fish, attaining maximum during the period of peak maturity and then declining abruptly after that (Borthakur, 2018). It was observed that the mean GSI value gradually increased from November, gaining higher from February, reaching the peak in April, and subsequently declining to its lowest in October.

The gonado-somatic index differs by species; however, similar findings with one peak have been reported by Patimar *et al*. (2010) in *Garra rufa*, Kanwal, (2017) in *Garra lamta*, Borthakur, (2018) in *Xenontedon cancila*. The present study shows that *Garra langlungensis* breed once a year, indicating its peak breeding period in April, with a short duration extending from February to May.

Chapter **6**

Captive breeding and breeding behaviour

Contents

6.1 Introduction

Many factors, including overexploitation, various anthropogenic activities and natural hazards in the aquatic ecosystem, severely reduce the population size of many species. Supplemental breeding in stocking natural waters is an intense population management strategy where in adults are captured from the wild and spawned in controlled environments. The resulting offspring are released back into the wild. (Fiumera *et al*., 2004). Seed production by the process of captive breeding is important and one of the major steps in the restocking and conservation programs of a species. Captive breeding also provides crucial life history information while supplementing or restoring extirpated populations and allows the discovery of important behavioural or life history characteristics that may limit the reproduction of rare species in altered natural habitats. (Rakes *et al*., 1999).

Spawning is the external release of sexual products, such as ova in the case of females and milt in the case of males (Basaran *et al*., 2008). Induced breeding is a method of stimulating breeding by injecting exogenous hormones into the bodies of mature parent fish (Heggberget, 1996). GnRH is the most significant hormone that regulates gonadotropin-releasing hormone (GtH) (Peter & Yu, 1997). The hypothalamus produces GnRH under specific conditions, which prompts the pituitary gland to release gonadotropins. In fish, environmental factors influence the release and management of hormones essential for spawning. In stressful conditions, the hypothalamus also produces dopamine to suppress the production of gonadotropins (Chang & Peter, 1983). The discovery that dopamine works as an inhibitory factor for synthesising gonadotropins was

a major breakthrough in fish breeding research (Peter *et al*., 1988). The finding of the dopamine inhibitory action has substantial aquaculture implications because environmental circumstances in captivity often impede egg development and ovulation (Dufour *et al*., 2005). Several hormones containing GnRH and dopamine antagonists, such as Ovaprim, have been successfully used to induce ovulation in *Garra surendranathanii* (Thampy, 2009), *Puntius chola*, and *Danio dangila* (Angami, 2012), *Pangasianodon hypophthalmus* (Chaturvedi *et al.*, 2014), *Sahyadria denisonii* (Peter *et al*., 1988). Since gonadal maturation depends on the endocrine system and in captivity, it can be achieved by applying hormones. Therefore, hormonal manipulation appears to be the most direct approach for brood stock development.

Garra langlungensis is an endemic, indigenous hill stream fish of Nagaland which is relished for its food value. Since captive breeding is considered a major step in conservation programs, there is no knowledge of the breeding and early developmental stages of this fish. Therefore, the present work aimed to breed this fish using Ovaprim as the hormonal agent, to study the breeding behaviour, and to analyze the effects of different doses on its breeding performance to obtain an optimal dose for effective induced breeding of this species.

6.2 Materials and methods

6.2.1 Spawning habitat

At the collection site, observations were made to ascertain the spawning habitat and chances of spawning migration in *Garra langlungensis*. Physicochemical parameters of the spawning habitat were estimated.

Water temperature was measured using a mercury thermometer graduated from 0 to 100 ˚C.

Water depth was recorded with the help of a graduated rope tied with a weight at the bottom.

Transparency was determined using a Secchi disc. The transparency of the water was computed as follows:

$$Secchi\ disc\ light\ penetration = \frac{A+B}{2}\ (cm)$$

Where,

A= depth at which Secchi disc disappears

B= depth at which Secchi disc reappears

Water velocity was measured using afloat, a stop clock and a measuring tape. Time taken by the drifted float to cover a particular distance represents the velocity of running water. The velocity of water was determined after Saha (2010) following the empirical formula:

$$V = \frac{d}{1.2t}$$

Where,

V = velocity (m/sec)

d = distance between the two poles (meter)

1.2 = a constant

t = time required (sec)

Dissolved oxygen was estimated using a compact water analysis kit Aquamerck 1.11151.0001.

The **pH** of water samples was measured using a portable digital pH meter.

6.2.2 Collection, transportation and acclimatization

Live specimens of *Garra langlungensis* were collected using cast net from the upstreams of Langlung river near Zutovi village, a tributary of Dhansiri river (25˚42'N and 93˚39'E), Dimapur, Nagaland, during the evening and late evening hours. Captured live fishes were packed in Low-Density Polyethylene (LDPE) oxygenated bags, placed in loft tanks, filled with river water and brought to the laboratory, Department of Zoology, Nagaland University, Lumami. The fishes were disinfected by dipping them in 10 ppm potassium permanganate solution containing de-chlorinated water. After the treatment, the fishes were released into glass storage tanks with aeration. Each tank was provided with sufficient numbers of stones and boulders for fish to rest and hide.

6.2.3 Maintenance of brood stock

Water quality assessment of the brood stock such as D.O (6.22 ± 0.25 mg/1), temperature (27.94 ± 0.73 ˚C), alkalinity (49.89 ± 6.61 mg/l), and pH (7.27 ± 0.11) were recorded periodically. Maintenance of brooders and feeding was made after Sundarabarathy *et al.* (2005) and Thampy (2009).

6.2.4 Induced breeding

The selection of breeders was based on the secondary morphology of external characters. Female and male brooders of *Garra langlungensis* are depicted in Figure 6.1.

Induced breeding experiments were carried out based on the hormonal method following the Linpe method (Peter *et al.,* 1988) using synthetic hormone (Ovaprim). Three doses *viz*., low dose (0.02 ml/gm of body weight), medium dose (0.03 ml/gm of body weight) and high dose (0.04 ml/gm of body weight) was administered intramuscularly using 5 ml insulin syringe at the ratio of 2:1. The experimental design is shown in Table 6.1. Fishes were placed in a wet cloth, and the hormones were intramuscularly injected at the base of the dorsal fin. The process of intramuscular injection of *Garra langlungensis* is shown in Figure 6.2. Female and male brooders were kept separately prior to the breeding experiment. The weight and length of the brooders were measured before injection. After careful administration of hormone, brooders were immediately released to the breeding tank provided with high aeration and stones and boulders as the substratum for hiding purposes.

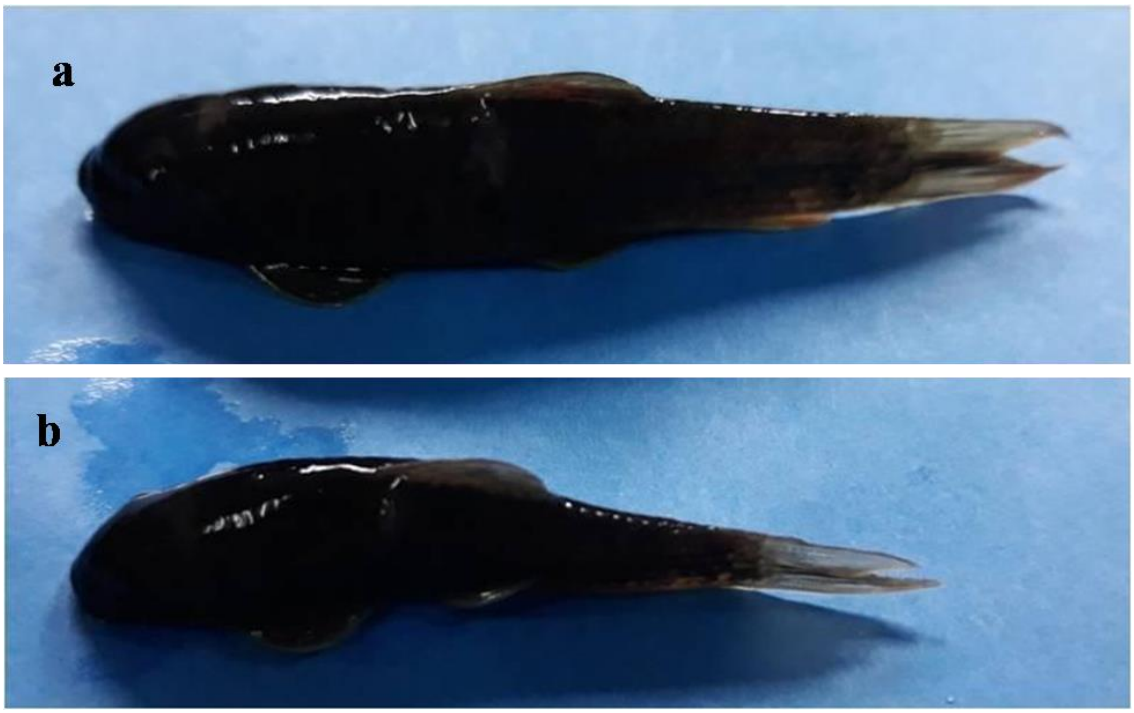


Figure 6.1: *Garra langlungensis* (a) Female (b) Male

Figure 6.2: Intramuscular injection in *Garra langlungensis*

Table 6.1: Different doses of synthetic hormone used for induced breeding of *Garra langlungensis*

Composition	**Hormone**	**Doses**		**Time of injection**
2:1	Ovaprim	Low dose (LD)	0.02 ml	Evening
		Medium dose (MD)	0.03 ml	
		High dose (HD)	0.04 ml	

6.2.5 Breeding behaviour

After hormonal administration, breeding behaviour was recorded at every one-hour interval following Thampy (2009). The behaviour was captured using a Canon EOS 1300D DSLR camera. The documented video footage was analysed frame by frame to study the behavioural pattern of *Garra langlungensis*.

6.2.6 Breeding response and collection of eggs

Brooders response to hormonal administration was monitored every half an hour. About a hundred fertilised eggs were set aside to hatch and grow alongside the parent in order to study the parental care behaviour of the brooders toward the eggs. After spawning, about 100 eggs were collected from each tank, and the percentage of fertilisation, hatchling and survival were estimated following Muir & Robert (1985) using the formula:

$$Fertilisation\ rate\ (\%) = \frac{Number\ of\ fertilised\ eggs}{Total\ number\ of\ eggs} \times 100$$

$$Hatchling\ rate\ (\%) = \frac{Number\ of\ hatchlings}{Total\ number\ of\ fertilised\ eggs} \times 100$$

$$Survival\ rate\ (\%) = \frac{Number\ of\ survivals}{Total\ number\ of\ fertilised\ eggs} \times 100$$

6.2.7 Statistical analysis

The collected data was analysed in GraphPad Prism 8 software. One-way ANOVA with Tukey's multiple comparison tests at the significant level, $p<0.05$ was performed to study the effect of differences between doses of Ovaprim.

6.3 Results

6.3.1 Spawning habitat

During the breeding season, i.e., February to May, a field survey was carried out every month to ascertain the natural spawning ground of *Garra langlungensis*. An account of the physicochemical parameters of the spawning habitat estimated during the breeding season is shown in Table 6.2. Monthly visitation of the spawning habitat during the breeding season revealed that *Garra langlungensis* showed breeding migration. The brooders were observed to swim upstream towards the canal against the water current. The habitat observed in these canals was shallow water with the presence of boulders, stones, sand and substratum with green moss boulders and rocks. The spawning habitat of *Garra langlungensis* is shown in Figure 6.3.

Figure 6.3: Spawning ground of *Garra langlungensis*

Table 6.2: Physicochemical parameters of spawning habitat of *Garra langlungensis*

Physicochemical parameters	**Months**			
	February	**March**	**April**	**May**
Water temperature (˚C)	26.5	27.1	28.65	30
Water colour	Light and transparent	Light and transparent	Light and transparent	Lightly muddy and turbid
Water depth (cm)	13	10	15	20
Transparency (cm)	13.5	8	5.25	3.25
Water velocity (m/sec)	1.04	0.9	1.2	2.52

Dissolved oxygen (mg/l)	9.5	8.1	6.4	5.7
pH	7.8	7.6	7.5	7.3

6.3.2 Food and feeding

Mosquito larvae, pellet feeds and frozen tubifex worms were the major components in feeding *Garra langlungensis*. The female fishes were fed with egg yolk prior to the breeding experiment. Fishes were fed twice a day. It was observed that *Garra langlungensis* adapted well to the feeds in one week. During the stocking period, no mortality was observed. The feeding of *Garra langlungensis* in the aquarium is shown in Figure 6.4.

Figure 6.4: Feeding in *Garra langlungensis*

6.3.3 Behaviour in the aquarium

When placed in the aquarium, *Garra langlungensis* displayed calm behaviour, mostly hiding behind or inside the boulders and stones. One peculiar behaviour of this species was sticking to the glass aquarium with the help of the gular disc, i.e., its modified lower lip.

6.3.4 Captive breeding of the fish

Captive breeding was carried out in three sets for each dose. The female spawners ranged from 60.2 – 78.5 mm in total length and 2.16 – 5.62 gm in body weight. The male spawners ranged from 53.5 – 65 mm in total length and 1.93 – 3.20 gm in body weight. The male and female brooders were given a single dose of Ovaprim for inducing breeding. After the administration of the hormone Ovaprim, spawning occurred at 5 hours 15 minutes in the late evening. Complete spawning occurred in all three different doses of Ovaprim. Breeding of *Garra langlungensis* was successful using the synthetic hormone Ovaprim in captivity. The breeding set up of *Garra langlungensis* is shown in Figure 6.5.

Figure 6.5: Breeding tank of *Garra langlungensis*

6.3.5 Breeding behaviour

Initially, after the administration of Ovaprim, all the 3 fishes were resting at the bottom separately and were inactive. However, after an hour, the fish started to perform their usual activity. The female performed swimming more actively than the male; no courtship behaviour is observed at this hour. At 2 hrs 30 minutes, the courtship behaviour is observed among the fishes. The males start chasing the female fish. At 3hrs, the female begins to chase the male; subsequently, the male fish starts to touch the vent region of the female with its snout. The males began to swim around the female in a synchronised manner. All three fishes move to the bottom between the rocks and the surface. The activities of the males and females were observed several times, and by 4 hrs 30 minutes, the males continued chasing the female in a sandwich manner. Both the male at the side and the female at the middle, the males start touching the female genital region with their

snout and head region showing nipping behaviour. The males were observed to tap the female genital region with its caudal fin gradually.

By 5 hrs, the activity of the fishes increased. At every short interval the fish jumped out of the water and the male began to shake the female and hit its vent region with its head. The female responds by either sinking at the bottom, moving continuously or jumping out of the water. At this point, both males followed the female and the frequency of males hitting the female increased. At the end of 5 hours 15 minutes, the male kept their body laterally compressed against the female, and spawning occurred when a male nipped the female's vent region pressed against the male. After 2-3 seconds, the female released a batch of eggs with a quivering movement. This activity continued till spawning was completed, which took about 1 hr. The fertilised eggs were scattered and non-adhesive. The fertilised eggs collected from the same brood fish were observed to show different developmental stages. This finding indicated that the female releases the eggs in batches during spawning. It was also observed that the male did not show any sign of aggression, and the female spawned simultaneously with the male. Breeding behaviours in *Garra langlungensis* is shown in Figure 6.6.

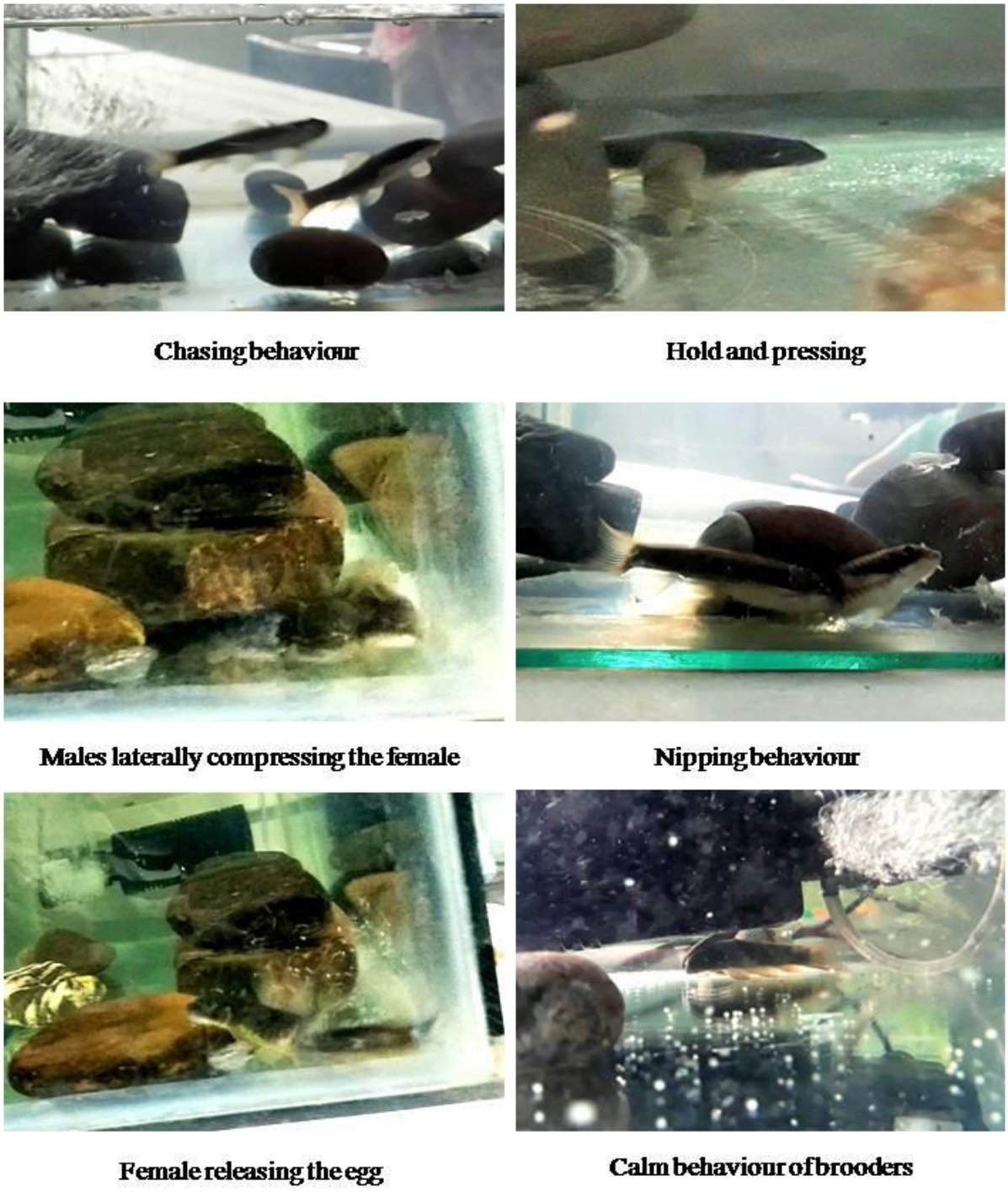


Figure 6.6: Breeding behaviour of *Garra langlungensis*

6.3.6 Parental care

No parental care was observed in either male or female breeders of *Garra langlungensis*. After spawning, both the male and female were active, showed no sign of hostility and remained at the corner of the breeding tank.

6.3.7 Effect of doses of Ovaprim on induced breeding

Different doses of Ovaprim resulted in varied effects on the latency period, hatchling period, fertilisation rate, hatchling rate and survival rate of *Garra langlungensis*. The variation of induced spawning towards different doses is presented in Table 6.3.

Table 6.3 Effects of different doses of Ovaprim on induced breeding of *Garra langlungensis*

Hormone	Dose (ml)	Weight of spawners (gm)		Latency period (hrs)	Hatchling period (hrs)	Fertilization rate (%)	Hatchling rate (%)	Survival (%)
		Male range	Female					
Ovaprim	0.02 low dose (L.D)	1.94 – 2.78	3.84 – 5.26	5.13 ± 0.02	36.4 ± 0.02	95.3 ± 0.3	92.3 ± 0.7	91.7 ± 0.4
	0.03 medium dose (M.D)	1.99 – 3.20	3.37 – 5.47	4.48 ± 0.02	36.1 ± 0.02	88.7 ± 1.2	85.7 ± 0.8	83.1 ± 0.6
	0.04 high dose (H.D)	1.93 – 3.18	3.78 – 5.62	4.18 ± 0.02	35.5 ± 0.02	85.7 ± 1.2	80.2 ± 1.9	79.0 ± 1.1

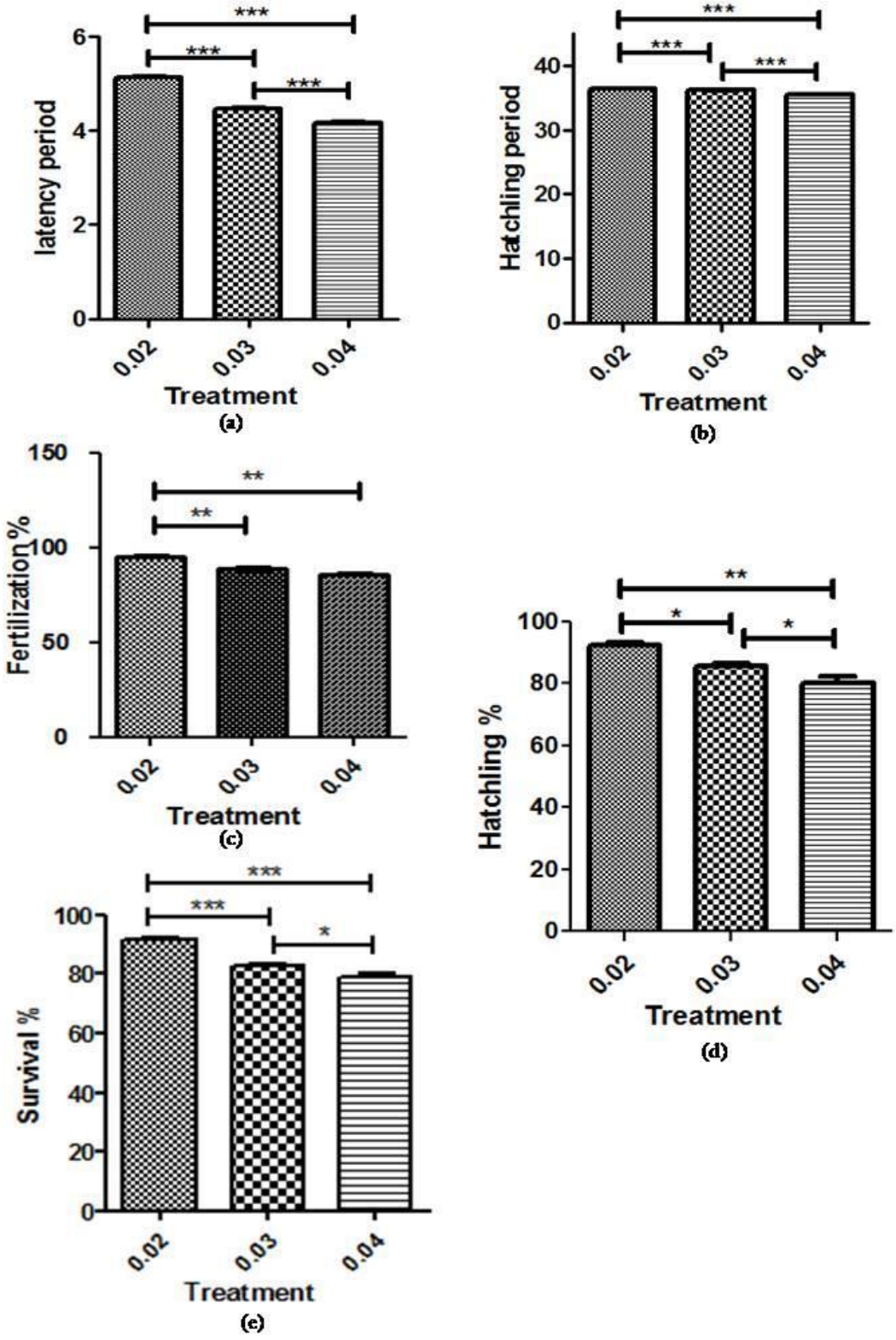

latency period
Treatment
(a)
Hatchling period
Treatment
(b)
Fertilization %
Treatment
(c)
Hatchling %
Treatment
(d)
Survival %
Treatment
(e)
0.02
0.03
0.04
***
**
*

Figure 6.7: Effect of different doses of Ovaprim on (a) latency period, (b) hatchling period, (c) fertilization rate (%), (d) hatchling rate (%) and (e) survival rate (%)with a significant level ($p < 0.05$) between the doses on *Garra langlungensis*

The latency period (5.13 ± 0.02 hrs) and hatchling period (36.4 ± 0.02 hrs) were recorded highest on the treatment with a low dose of Ovaprim (0.02ml/gm bw), while treatment with a high dose (0.04 ml/gm bw) resulted in low latency period (4.18 ± 0.02 hrs) and hatchling period (35.5 ± 0.02 hrs). A reverse trend was observed between the different doses and the latency and hatchling period i.e., with the increasing dose of Ovaprim, the time taken for latency and hatchling period decreased. The difference in latency and hatchling period were statistically significant ($p < 0.05$) compared to three different doses of Ovaprim. The difference in latency and hatchling period with three different doses of Ovaprim is depicted in Table 6.4 - 6.7.

The fertilisation rate (95.3 ± 0.3 %), hatchling rate (92.3 ± 0.7 %) and survival rate (91.7 ± 0.4 %) were found to be high in individuals injected with a low dose of Ovaprim. However, in fishes induced with high doses, lower percentages of F.R (85.7 ± 1.2 %), H.R (80.2 ± 1.9 %) and S.R (79.0 ± 1.1 %) were observed. A significant difference ($p < 0.05$) was observed in the fertilisation rate between low doses with medium and high doses. However, no significant difference was observed between medium and high doses. The difference in fertilisation rate with three different doses of Ovaprim is depicted in Tables 6.8 and 6.9. The hatchling rate and survival rate indicate a significant difference ($p < 0.05$) between all the pairs of doses. The difference in

hatchling and survival rate with three different doses of Ovaprim is depicted in Table 6.10 - 6.13.

Table 6.4: One-way ANOVA table showing the effect of different doses of Ovaprim on the latency period

Source of variation	Sum of squares	df	Mean squares	F - value	p - value
Dose	1.415	2	0.708	849.0	0.000
Error	0.005	6	0.001		
Total	1.42	8			

Table 6.5: Tukey's multiple comparisons test between the different doses of Ovaprim on the latency period

Comparison between doses	Mean difference	Standard error of difference	p - value	Conclusion
L.D vs. M.D	0.65	0.024	0.000	Significant
L.D vs. H.D	0.95	0.024	0.000	Significant
M.D vs. H.D	0.3	0.024	0.000	Significant

Table 6.6: One-way ANOVA table showing the effect of different doses of Ovaprim on the hatchling period

Source of variation	Sum of squares	df	Mean squares	F - value	p - value
Dose	1.347	2	0.674	808.3	0.000
Error	0.005	6	0.001		

Total	1.352	8

Table 6.7: Tukey's multiple comparisons test between the different doses of Ovaprim on the hatchling period

Comparison between doses	Mean difference	Standard error of difference	p - value	Conclusion
L.D vs. M.D	0.25	0.024	0.000	Significant
L.D vs. H.D	0.917	0.024	0.000	Significant
M.D vs. H.D	0.667	0.024	0.000	Significant

Table 6.8: One-way ANOVA table showing the effect of different doses of Ovaprim on the fertilization rate

Source of variation	Sum of squares	df	Mean squares	F - value	p - value
Dose	146.9	2	73.44	24.48	0.001
Error	18	6	3		
Total	164.9	8			

Table 6.9: Tukey's multiple comparisons test between the different doses of Ovaprim on the fertilization rate

Comparison between doses	Mean difference	Standard error of difference	p - value	Conclusion
L.D vs. M.D	6.667	1.414	0.008	Significant
L.D vs. H.D	9.667	1.414	0.001	Significant
M.D vs. H.D	3	1.414	0.166	Not significant

Table 6.10: One-way ANOVA table showing the effect of different doses of Ovaprim on the hatchling rate

Source of variation	Sum of squares	df	Mean squares	F - value	p - value
Dose	222.6	2	111.3	23.28	0.002
Error	28.68	6	4.78		
Total	251.3	8			

Table 6.11: Tukey's multiple comparisons test between the different doses of Ovaprim on the hatchling rate

Comparison between doses	Mean difference	Standard error of difference	p - value	Conclusion
L.D vs. M.D	6.6	1.785	0.024	Significant
L.D vs. H.D	12.17	1.785	0.001	Significant
M.D vs. H.D	5.567	1.785	0.047	Significant

Table 6.12: One-way ANOVA table showing the effect of different doses of Ovaprim on the survival rate

Source of variation	Sum of squares	df	Mean squares	F - value	p - value
Between groups	249.5	2	124.8	68.47	0.000
Within groups	10.93	6	1.822		
Total	260.5	8			

Table 6.13: Tukey's multiple comparisons test between the different doses of Ovaprim on the survival rate

Comparison between doses	Mean difference	Standard error of difference	p - value	Conclusion
L.D vs. M.D	8.567	1.102	0.001	Significant
L.D vs. H.D	12.63	1.102	0.000	Significant
M.D vs. H.D	4.067	1.102	0.024	Significant

6.4 Discussion

Many hill stream fish species exhibit upstream spawning migrations. Such migrations may help to offset downstream drift or translocation of young life stages to some degree or enhance their dispersal over a range of appropriate habitats (Northcote, 1978, 1984; Linfield, 1985). Spawning migration coincides during the rainy season with increased movement of adult fish upstream, as Thampy (2009) reported in *Garra surendranathanii*. Acharjee & Barat (2014) also reported that the increasing rainfall coincided with the increased movement and upstream spawning migration of many adult fishes, such as *Schizothorax richardsonii*, *Schizothorax progastus, Neolissochilus hexagonolepis*, *Neolissochilus hexastichus, Garra* spp., and *Cyprinion semiplotum.* In the present study, during induced breeding it was observed that *Garra langlungensis* tends to actively swim through the water bubble provided by the aerator and prefers a habitat with boulders and rock. Hence, the presence of clear water with high oxygen and velocity during the rainy season may be another essential habitat for its breeding.

Garra langlungensis is moderately small in size, dark-coloured and easily adaptable when introduced to aquarium life. According to the diversified ornamental criteria recorded by Mahapatra *et al.* (2007) for many species based on their ornamental value, *Garra langlungensis* can be categorised under the ornamental character sucker. Hence, *Garra langlungensis* may be considered an ornamental fish after assuming these characteristics.

This chapter describes a study on the captive breeding and behaviour pattern of *Garra langlungensis* for the first time. This information presents a practical approach for breeding this fish species in captivity. Generally, cyprinid fishes exhibit some common patterns in their courtship and reproductive behaviour (Turner, 1993; Mercy *et al*., 2003). It was observed that the general activity of fishes gradually increased on the onset of spawning, which is also described in other cyprinid species such as *Garra surendranathanii* (Thampy, 2009), *Puntius chola* (Vincent & Thomas, 2008) and *Devario aequipinnatus* (Dey *et al*., 2014). The males of *Garra langlungensis* do not show territoriality; hence, there is no agonistic behaviour in male-male interactions. Similar observations have been observed in *Garra surendranathanii* (Thampy, 2009), *Danio dangila* (Angami, 2012) and *Esomus danricus* (Amenla, 2014). In *Garra langlungensis*, the male initiates the courtship behaviour by chasing and touching the vent region of the female with its snout. Courtship behaviour lasted for 2hr 45 minutes, and spawning took place where the female was mated simultaneously with both the males. The fishes show polygamy i.e., each male can mate successively with several females, and each female can mate simultaneously and successively with several males (Turner, 1986). This

polygamous character is seen in the present study during the breeding season of *Garra surendranathanii*. Studies have shown that *Garra surendranathanii* migrates to small streams, and in the migrating population, only 10% of the fish were females, and the rest 90% were males (Thampy, 2009). In the present study, parental care for eggs and hatchlings was not observed in both male and female brooders of *Garra langlungensis*, which is consistent with cyprinid fish behaviour in general.

Captive breeding and reintroduction initiatives have become one of the principal tools, as this approach is a means of preserving the declining fish population for the long term maintenance of biodiversity (Tear *et al.*, 1993; Fleming, 1994; Huntley & Langton, 1994). Observations made on the captive breeding in the present study showed the successful use of the synthetic hormone, Ovaprim for induced spawning of *Garra langlungensis* in laboratory conditions. Different doses of Ovaprim led to changes in the spawning behaviour, latency period, hatchling period, fertilisation rate, hatchling rate and survival rate.

Brooders injected with a low dose of Ovaprim showed equal participation of both male and female brooders in the spawning activity. In contrast, partial participation of males was found in breeders injected with medium and high doses. The latency and hatchling periods were shown to be related to the various hormone levels. It was observed that with the increasing dose administration, the latency and hatchling period decreased. In other words, the higher the dose, the shorter the latency and hatchling period. Similar observations were reported by Thampy (2009) in *Garra surendranathanii*, Banik *et al*. (2011) in *Ompok bimaculatus* and Rajbongshi *et al*. (2020)

in *Clarias batrachus*. This study revealed that the fertilisation rate, hatchling rate and survival rate showed a declining trend with the increasing dose. The highest fertilisation rate (95.3 ± 0.3), hatchling rate (92.3 ± 0.7) and survival rate (91.7 ± 0.4) was observed in low dose. Several other researchers examined that different dose of Ovaprim affected fertilisation, hatchling and survival rates in order to determine the optimal dose for the fish, such as *Mystus gulio* (Alam *et al*., 2006), *Labeo parvus* (Montchowui *et al*., 2011), *Clarias gariepinus* (Kasi *et al*., 2015), *Osteobrama belangeri* (Das *et al*., 2016), *Notopterus notopterus* (Yulindra *et al*., 2017), *Neolissochilus tamiraparaniensis* (Vijayakumar *et al*., 2020) and *Clarias batrachus* (Rajbongshi *et al*., 2020).

Ovaprim has been successfully used in various ornamental fishes, including members of the family Cyprinidae, Characidae, Cobitiidae, different species of catfish, and Helostomatidae, in addition to other fish families and species (Yanong *et al*., 2010). Earlier reports on successful and complete spawning using synthetic hormone Ovaprim include the following, carps (Nandeesha *et al*., 1990), *Cirrhinus reba* (Sarkar *et al*., 2004), *Clarias gariepinus* and *Haterobranchus longifilis* (Olubiyi *et al*., 2005), *Garra surendranathanii* (Thampy and Ramachandran, 2009), *Heteropneustes fossilis* (Christopher *et al*., 2009), *Ompok bimaculatus* (Banik *et al*., 2011), *Hampala macrolepidota* (Intan *et al*., 2013), *Garra rufa* (Vazirzadeh *et al*., 2015), *Mastacembelus pancalus* (Hasan *et al*., 2016) and *Clarias batrachus* (Rajbongshi *et al*., 2020).

In the present study, equal participation of breeders during the spawning activity, high fertilisation rate, hatchling rate and survival rate was attained on brooders induced with a low dose. From the above discussion, it can be considered that the dose of 0.02

ml/gm body weight of Ovaprim is recommended for the captive breeding of *Garra langlungensis*.

Chapter 7

Embryonic and larval development

Contents

7.1 Introduction

Embryonic development is a complex process that occurs during the early stages of life in which cellular differentiation and proliferation occur simultaneously but at different rates (Hall, 1992). All biological systems and organs of the fish develop during the embryonic and larval stages (Gupta & Banerjee, 2016). Embryonic development commences with the formation of the zygote by the fusion of male and female gametes. A zygote undergoes a series of developmental stages and becomes free-swimming larvae. The larval development begins with the absorption of yolk sac feeding externally, which goes through organogenesis and emerges as their parents, consequently ending the larval stages. Embryonic and larval developmental studies provide sufficient information regarding the successful rearing of larvae (Mathew *et al*., 1996). Good numbers of literature are available on the various stages of embryonic and larval development. Kendall *et al*. (1984) described the early life stages of fishes and their characters. Kimmel *et al*. (1995) worked on a detailed description of a series of stages of development of the embryo of the *Danio rerio*. Arockiaraj *et al*. (2003) investigated the early development of *Mystus montanus*. Rahman *et al*. (2009) also investigated the embryonic and larval development of *Mastacembelus pancalus.* Bhattacharya *et al*. (2005) gave a detailed description of the embryonic development of *Puntius conchonius*. Dey *et al*. (2014) investigated the embryonic and larval development of *Devario aequipinnatus*.

The seed production success rate for freshwater fish mainly depends on larval rearing. This stage is the most crucial phase because the nutrient requirement of the fish

larva changes rapidly with its increasing growth. Knowing early life history information is essential for optimizing mass seed production, culture, and management, which is very important to optimize larval growth and survival (Khan & Mollah, 1998). Breeding of *Garra langlungensis* is a pioneering effort. With the success of induced breeding captivity (chapter 6), it is crucial to understand the developmental biology and cultural techniques of this species, which is a prerequisite for successful rearing and hence taken up.

In the present chapter, efforts have been made to explain the various stages of embryonic and larval development of *Garra langlungensis*. The whole developmental process has been grouped into three phases: (a) Embryonic phase, (b) Pre-larval phase, and (c) Post-larval phase.

7.2 Materials and methods

After breeding, the fertilized eggs were collected with a dropper from the breeding tank and transferred to a smaller aerated tank of 20 litre capacity (temperature (27 ± 0.1 ˚C), dissolved oxygen (6.5 ± 0.25 mg/1) and pH (7.1 – 7.4). The developing stages were based on examining live specimens under a Huvitz stereo zoom microscope (HSZ-ILST6). Microphotographs and measurements of the developmental stages of eggs and larvae were taken with an SLR camera (Canon EOS 1300D) and digital calipers. The developing egg samples were taken every 10-15 minutes interval till hatching and every 2-3 hours for the next 3 days and thereafter once a day at 9-10 AM. The larvae were

observed for 2 months until they attained the adult's character and shape. For further observation, the sampled eggs and larvae were fixed in 4 % formaldehyde.

7.3 Results

7.3.1 Description of fertilized eggs

Fertilized eggs of *Garra langlungensis* are spherical, translucent, non-adhesive and demersal, containing a large amount of yolk. The embryo condenses in the middle, becoming smaller in size after 15 minutes of fertilization. The fertilized egg is pale yellowish in colour. The fully swollen fertilized eggs range in diameter from 1.3 – 1.5 mm, having an average diameter of 1.4 ± 0.08 mm.

7.3.2 Embryonic development

The first cleavage begins 30 minutes after fertilization. The two-celled stage was then observed at 45 minutes which transformed into a four-celled stage at 55 minutes, and at 1.05 hours, an 8-celled stage was observed. The 16-celled stage was attained at 1.15 hours after fertilization. Within 1.30 hours, the embryo became a 32-celled stage and transformed into a multi-celled stage till it reached the morula stage at 3.30 hours. At this stage, the dividing cell is dense and looks like a cap placed on the animal pole. At 5 hours, the dividing cell is much reduced, and the periphery of the animal pole begins to epibolize over the yolk; it seems like a sac about to cover a sphere. This stage is called the yolk plug stage.

At 7.10 hours, the yolk part of the embryo begins to shrink, and an embryonic shield or germ ring is observed as the whole embryo begins to elongate. Organogenesis

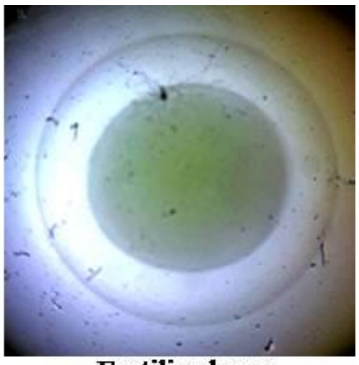
commences with the elongation of yolk mass about 7.45 hours after fertilization. By 10.30 hours, the embryo shows the appearance of the embryonic rudiment. At 11.50 hours, somite is visible, and the embryo is elongated. After 13.30 hours, the tail of the embryo started developing sideways and was found to be much more elongated. The development of the eyes and the appearance of a rudimentary heart is well noticeable during this time. At 15.55 hours, the whole embryo became more elongated, and the yolk appeared as a curved pear-shaped. At 17 hours, the tail region becomes free from the yolk mass and the twitching movement of the embryo begins. At this stage, the shape of the yolk sac has changed almost into a spherical shape with a narrow bud. At 19 hours, the embryo was found to be more active, moving rapidly and constantly in motion. At 20 hours, a rudimentary pectoral fin in the embryo was observed, and the yolk had the shape of the bulbous anterior region with a narrow and elongated posterior region. The embryo continued twitching movement within the egg and was found to be more elongated and transparent, having both ends irregularly rounded. This stage was achieved at 26 hours after fertilization. By 30 hours the posterior part of the yolk also started to get elongated and the shape of the egg also slightly changed from round to elliptical. The embryo continued to elongate further, changing the shape of the egg membrane with rapid twitching movement and was found to attach its head to the wall of the egg membrane at 36 hours. During this period, the heartbeat of the embryo was clearly visible. The embryos were found to hatch out from the egg at 36.45 hours after fertilization. The major developmental features alongside the respective time period of *Garra* is given in

Table 7.1. The embryonic developmental stages of *Garra langlungensis* are represented in Figure 7.1.

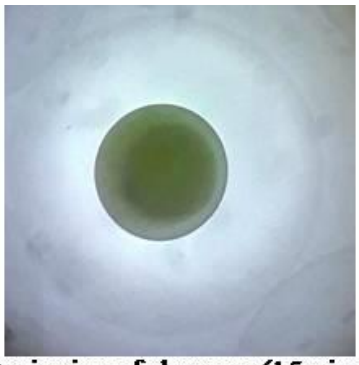
Table 7.1: Developmental stages of *Garra langlungensis*

Developmental features	Hours after fertilization
1-celled stage	0.30
2-celled stage	0.45
4-celled stage	0.55
8-celled stage	1.05
16-celled stage	1.15
32-celled stage	1.30
Morula stage	3.30
Yolk plug stage	5.00
Appearance of embryonic shield/germ ring	7.10
Elongated yolk mass	7.45
Appearance of embryonic rudiment	10.30
6-somite stage	11.10
8-somite stage	11.50
12-somite stage	12.40
Elongation of the tail	13.30
Eyes developed, appearance of rudimentary heart	14.10
Appearance of gill segment, yolk is bean-shaped	15.55

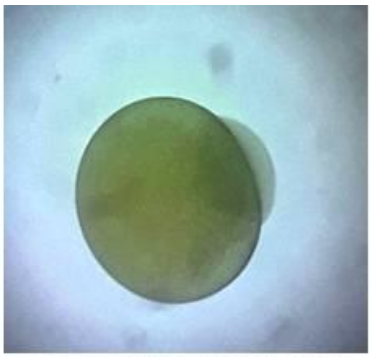
Appearance of pectoral fin bud	16.10
Tail separation from the embryo & twitching movement	17.05
Heart beat visible & head attached to the wall of chorion	36.00
Larvae hatched from chorion	36.45



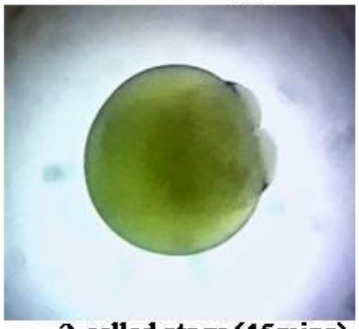
Fertilized egg



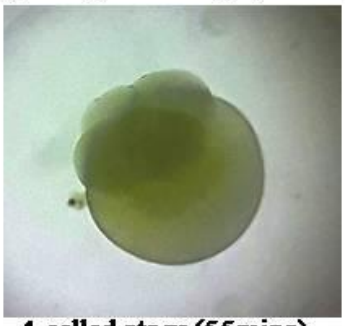
Beginning of cleavage (15mins)



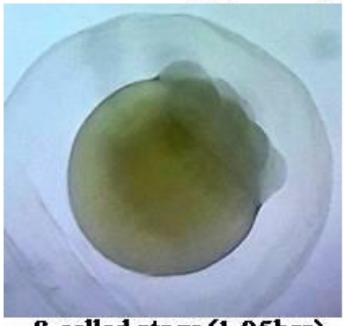
1-celled stage (30mins)



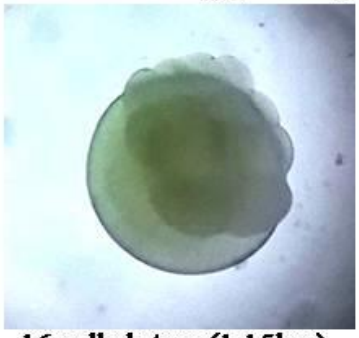
2-celled stage (45mins)



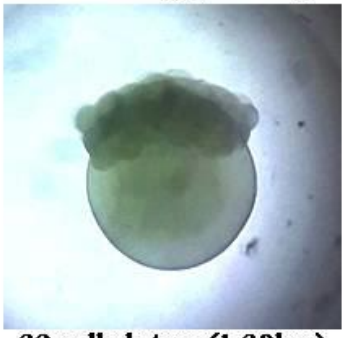
4-celled stage (55mins)



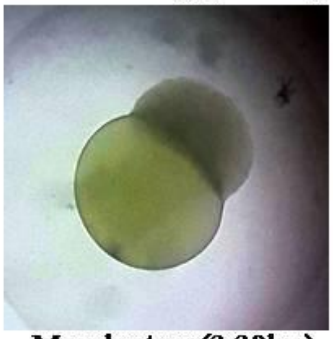
8-celled stage (1.05hrs)



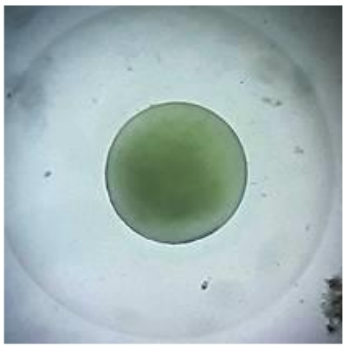
16-celled stage (1.15hrs)



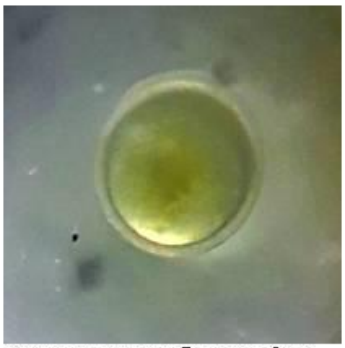
32-celled stage (1.30hrs)



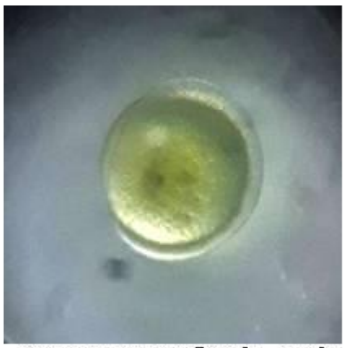
Morula stage (3.30hrs)



Yolk plug stage (5hrs)



Appearance of germ ring (7.10hrs)



Appearance of embryonic rudiment (10.30hrs)

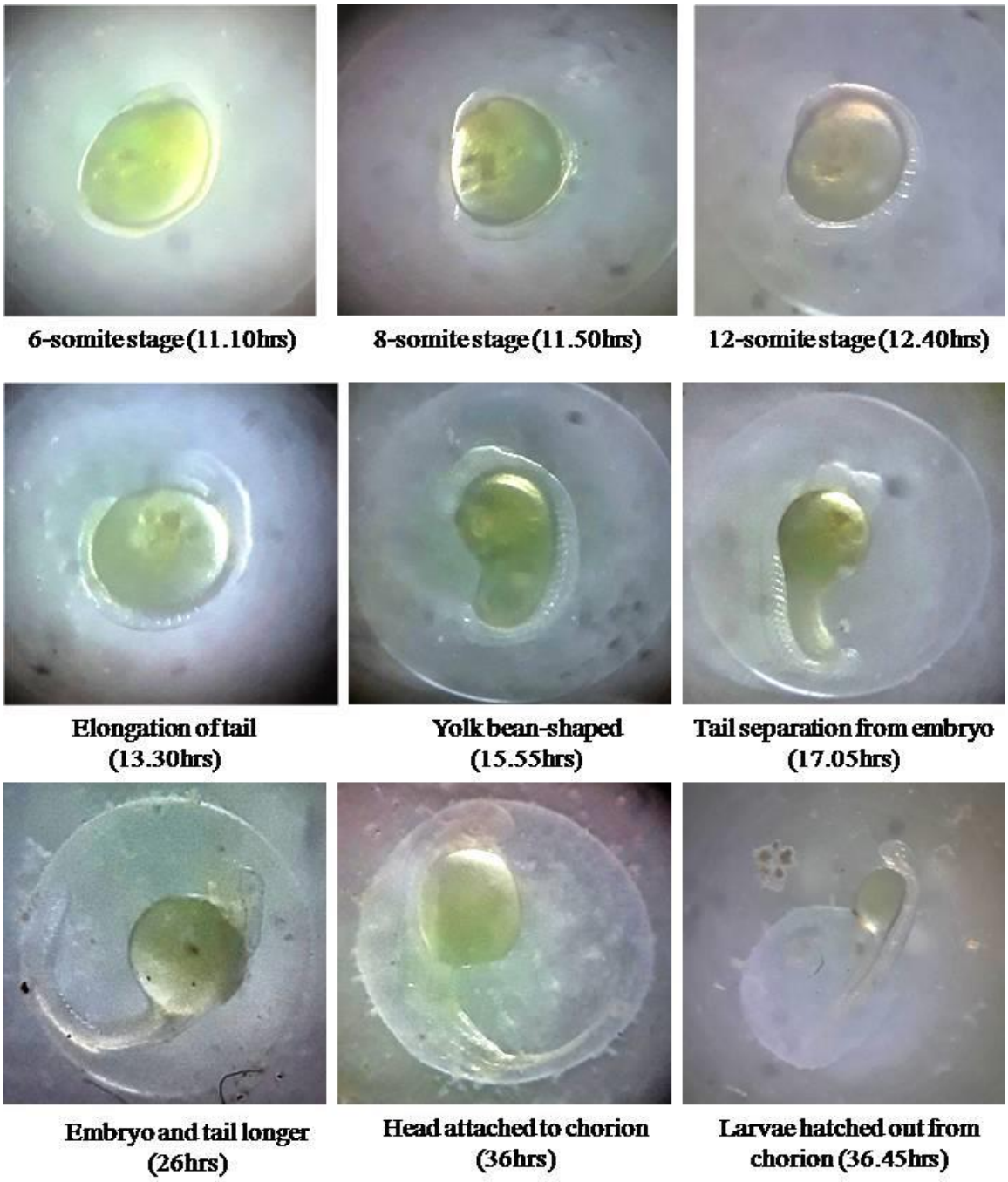


Figure 7.1: Embryonic developmental stages of *Garra langlungensis*

7.3.3 Pre-larval development

1. **Hatchling:**

   The newly hatched larvae ranged in total length between 3.9 – 4.3 mm, averaging 4.1 ± 0.1 mm. The hatchling had a slender, transparent and greenish body without any pigmentation. The yolk sac has a prominent bulbous anterior end with a narrow and elongated posterior that ends bluntly. The length of the yolk sac ranged between 0.9 – 1.2 mm, with an average of 1.1 ± 0.1 mm. The width of the yolk sac ranged between 0.6 – 0.8 mm, with an average of 0.7 ± 0.1 mm. The heart is situated forward to the yolk sac. The optic vesicles appear clear and visible.

2. **6 hours post hatchling:**

   In 6 hours, the larvae attained a total length ranging between 4 – 4.3 mm with an average of 4.2 ± 0.1 mm. The length of the yolk sac ranged between 0.9 – 1.1 mm, with an average of 0.9 ± 0.1 mm. The width of the yolk sac ranged between 0.4 – 0.7 mm, with an average of 0.5 ± 0.1 mm. The larvae at this stage have an unbalanced darting movement touching the bottom. The chromatophores started developing in the eyes, appearing as a black spot on the dorsal part of the body, particularly in the head region.

3. **12 hours post hatchling:**

   The larvae reached a total length ranging between 4.1 – 4.5 mm with an average of 4.3 ± 0.1 mm. The yolk sac has slightly reduced in size. The length of the yolk sac ranged between 0.6 – 0.9 mm, with an average of 0.7 ± 0.1 mm. The

width of the yolk sac ranged between 0.3 – 0.6 mm, with an average of 0.4 ± 0.1 mm. The chromatophores on the eyes and dorsal surface have become darker. Rudimentary pectoral fins started to appear at this stage. The appearance of the mouth cleft is clear.

4. **24 hours post hatchling:**

   In 24 hours, the larvae attained a total length ranging between 4.2 – 4.7 mm with an average of 4.5 ± 0.2 mm. The length of the yolk sac ranged between 0.5 – 0.8 mm, with an average of 0.6 ± 0.1 mm. The width of the yolk sac ranged between 0.2 – 0.5 mm, with an average of 0.3 ± 0.1 mm. The chromatophore is seen to develop in the heart, more on the dorsal side and head region. The pectoral fin bud appeared at this stage. The development of striations in the caudal fin started. The alimentary canal was observed to be straight.

5. **48 hours post hatchling:**

   The larvae at 48 hours attained a total length ranging between 4.4 – 5 mm with an average of 4.7 ± 0.2 mm. The yolk has started to diminish at this stage. The length of the yolk sac ranged between 0.5 – 0.7 mm, with an average of 0.6 ± 0.1mm. The width of the yolk sac ranged between 0.1 – 0.3 mm, with an average of 0.2 ± 0.1 mm. Eyes have become darker. Mouth well developed. Operculum and gill developed. Chromatophores have increased in the body. The alimentary canal clearly visible.

6. **72 hours post hatchling:**

The larvae reached a total length ranging between 4.9 – 5.6 mm with an average total length of 5.2 ± 0.2 mm. The larvae appear greenish-yellow in colour laterally and yellow-golden when viewed dorsally. The yolk sac has dramatically reduced and become slender. Dark chromatophores developed between the eyes, extending up to the auditory vesicles and 3 – 4 rows of black asterisk-shaped spread from the auditory vesicles to the tip of the notochord. The formation of the air bladder appears as a black round structure below the pectoral fin covering almost the entire dorsal view.

7. **96 hours post hatchling:**

The larvae attained a total length between 5.4 – 6 mm and an average total length of 5.7 ± 0.2 mm. Red pigmentation started to appear above the air bladder in the head region. Gills are clearly visible with slight pigmentation. Black asterisk-shaped chromatophores developed till the caudal fin. A rudimentary dorsal fold appears. The yolk sac diminished, and the caudal fin was clearly visible. The pre-larval stages of *Garra langlungensis* are represented in Figure 7.2.

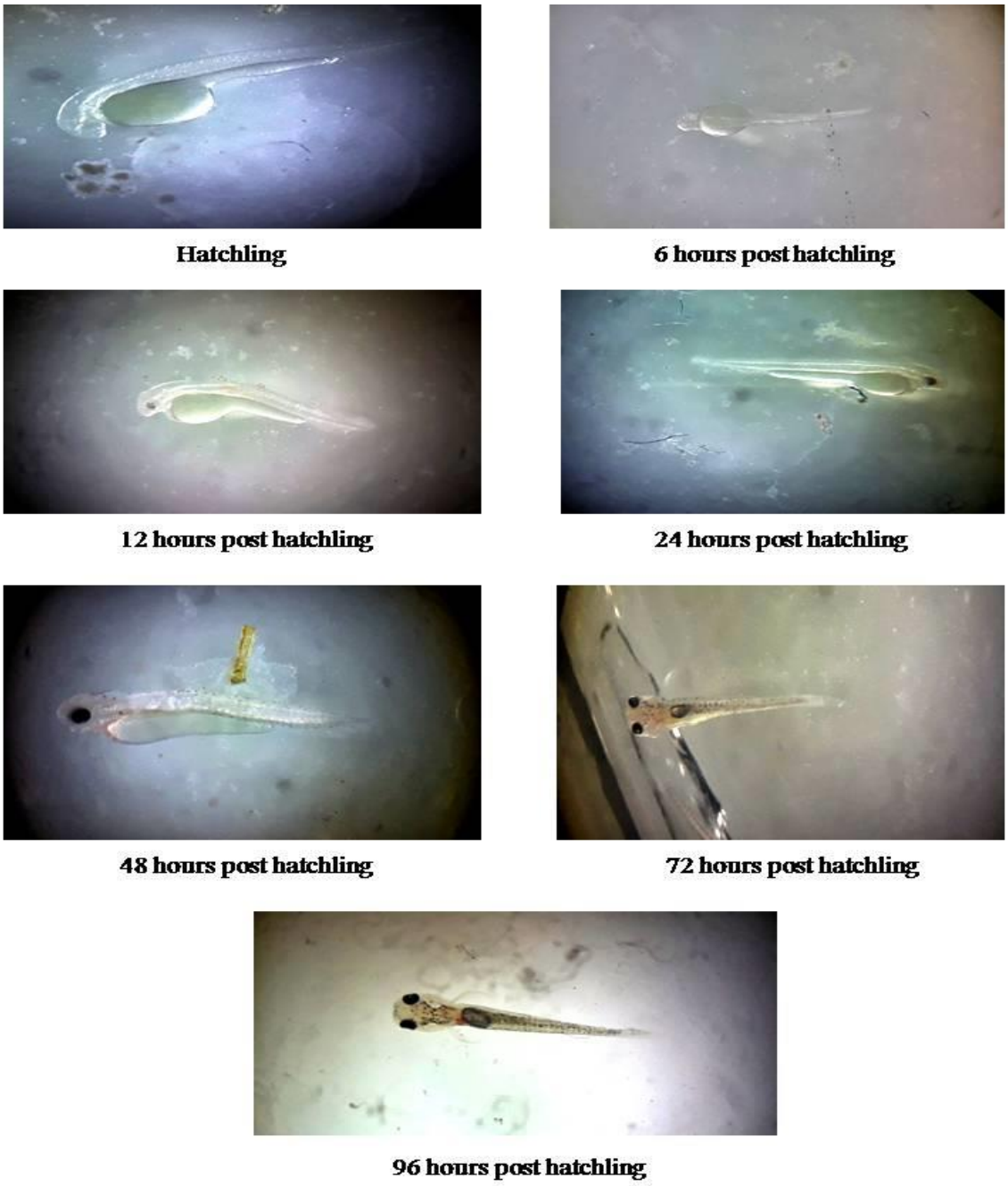


Figure 7.2: Pre-larval stages of *Garra langlungensis*

7.3.4 Post-larval development

1. **5 days old:**

   At this stage, the total length ranged between 6 – 6.5 mm with an average of 6.3 ± 0.1 mm. The yolk is almost absorbed. The dorsal profile became greenish yellowish in colour. Caudal fin rays are clearly visible. Rudimentary rays called streaks started to appear on the dorsal and caudal fin. The dorsal fins seem to be separated, however, slightly confluent with the caudal fin.

2. **7 days old:**

   At 7 days, the larvae attained a total length ranging between 6.3 – 6.8 mm with an average of 6.5 ± 0.2 mm. The dorsal fin is seen but still attached to the caudal fin. Black asterisk-shaped chromatophores have increased all around the body except the ventral side. The anal opening is visible.

3. **10 days old:**

   The larvae attained a total length ranging between 6.9 – 7.6 mm with an average of 7.2 ± 0.2 mm. The chromatophores distribution is more or less uneven, and the head region shows more brown chromatophores. Fin rays are visible at the caudal fin with the appearance of a rudimentary pelvic fin. The dorsal fin is clearly visible, but the fin rays are not distinct.

4. **15 days old:**

   The larvae attained a total length ranging between 7.9 – 8.9 mm, averaging 8.1 ± 0.3 mm. Dorsal fin was observed with 10 distinct fin rays. The chromatophores are more distinct in the head region. The caudal peduncle is

distinct. The caudal fin is starting to develop into a forked shape. Black spots developed in the dorsal and caudal fin ray region. Alimentary canal coiled.

5. **20 days old:**

The larvae attained a total length ranging between 10.9 – 13.2 mm with an average of 12.2 ± 0.7 mm. At this stage, the anal fin is well developed with 7-8 rays, the dorsal fin with 11 rays and the caudal fin with 18 rays. Pelvic fin rays are clearly visible. The lips are thick. One pair of small mandibular barbells appears. Chromatophores at the head region are prominent with dark brown colour. The black colour spot has developed throughout the body except on the ventral side.

6. **1 month old:**

At this stage, the larvae attained a total length ranging between 13.8 – 18.1 mm with an average of 15.9 ± 1.4 mm. All the fins are well developed with rays. Two pairs of barbells are clearly visible. Scales developed. Sucker started developing. The anus is clearly visible. The chromatophores are more developed on the dorsal and lateral sides and significantly less on the ventral side.

7. **2 month old:**

The larvae at this point resemble the adult fish having a total length ranged between 18.6 – 23.8 mm with an average of 21.1 ± 1.8 mm. Gular disc well developed. Lateral lines are clearly visible. All the fins were well developed, and the caudal fin was deeply forked. A black spot at the upper angle of the gill opening is visible, with six narrow black stripes on the lateral side, more

prominent toward the caudal peduncle. The post-larval stages of *Garra langlungensis* are represented in Figure 7.3.

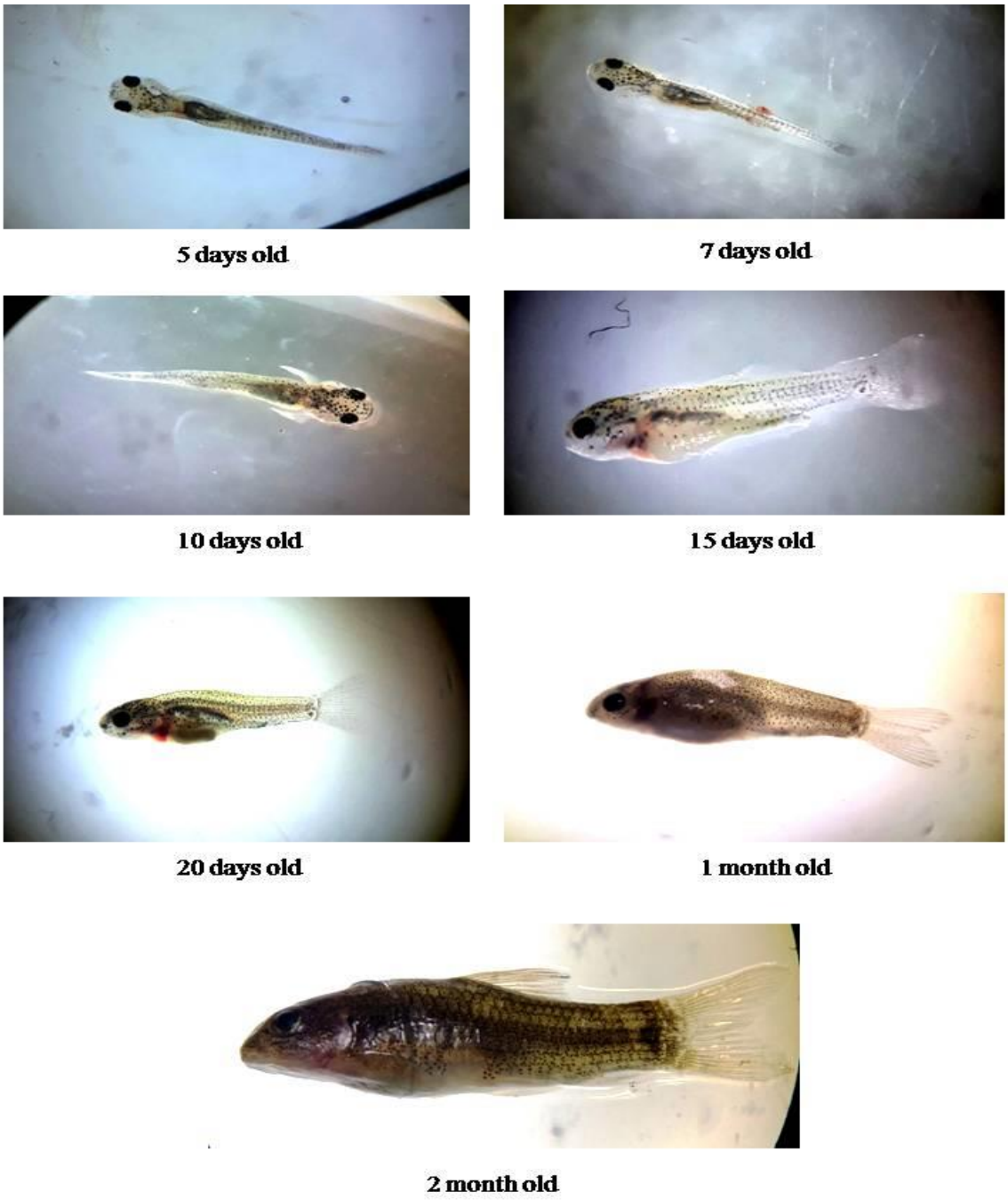


Figure 7.3: Post-larval stages of *Garra langlungensis*

7.4 Discussion

Knowledge of embryonic and larval development and organogenesis is critical for understanding a species basic biology (Borcato *et al*., 2004; Koumoundouros *et al*., 2001). In the present study, the fertilized eggs of *Garra langlungensis* are spherical, non-adhesive, and demersal. Similar observations of fertilized eggs have been reported by Thampy (2009) in *Garra surendranathanii*; Dey *et al*. (2014) in *Devario aequipinnatus* and Saxena *et al*. (2019) in *Barilius bendelisis.*

The embryo in *Garra langlungensis* attached to the chorion wall after 36 hours, and hatchling occurred 36.45 hours post fertilization. Similar hatchling periods have been reported by Sundarabarathy *et al*. (2005), having a hatchling period of 36 hours in *Garra cylonensis*. Thampy (2009) reported a hatchling period of 36 hours in *Garra surendranathanii*. Dey *et al.* (2014) also reported hatchling period of 36 hours in *Devario aequipinnatus*. Olaniyi & Omitogun (2014) stated that the hatching period depends on the fertilization period within oocytes; hence, the faster the fertilization shorter the hatching period and survival of the embryos. Several cyprinid fishes were reported to have a difference in the hatchling period. 24 hours hatchling period was reported in *Puntius pookodensis* by Jacob (2013). Swain *et al*. (2008) reported the hatchling period of 28-36 hours in *Puntious ticto.* The hatchling period for *Carassius auratus* was reported to be 75-80 hours by Rahaman *et al.* (2011). Sutin & Tina (2020) also reported that hatchling period of 12.58 hours in *Garra cambodgiensis*. Hatchling period with a long duration of 109 hours was reported by Zhu *et al*. (2018)

in *Pseudorasbora parva*. Saxena *et al.* (2019) also reported a hatchling period of 140-160 hours in *Barilius bendelisis.*

The chromatophores in *Garra langlungensis* started developing on the head region 6 hours post-hatching from the chorion. The initial appearance of chromatophores in the head region has been reported by Bhattacharya *et al.* (2005) in *Puntius conchonius*, where the pigmentation emerges at about 2 to 3 hours post fertilization in the eye. Jacob (2013) reported the appearance of melanophores in the optic rim and myotomes in 24 hours hatchling in *Puntius pookodensis*. Saxena *et al*. (2019) also reported the appearance of pigmentation in the cephalic region in 2 days old larvae of *Barilius bendelisis.*

In *Garra langlungensis*, the yolk absorption was completed by the 5th-day larvae. Complete absorption of yolk in 5 to 6 days of larvae of *Puntius gelius* was reported by Sarma (2008). Rahaman *et al*. (2011) reported the absorption of yolk within 2 to 4 days in larvae of *Carassius auratus*. Angami (2012) also reported the complete absorption of yolk by 2 days in larvae of *Puntius chola* and *Danio dangila*. The larvae started feeding 4 days before completely absorbing the yolk sac. By 30 days onward after hatching, the larvae started feeding on mosquito larvae, frozen tubifex worms, and pelleted feed.

The larvae of *Garra langlungensis* started developing the gular disc by 30 days after hatching and completed development between 60-65 days. With the development of the gular disc by 30 days onward, the post-larvae tend to move and feed all around the tank and start sticking to the tank wall. By 60 days, the larvae

attained a total length ranging between 18.6 – 23.8 mm with an average of 21.1 ± 1.8 mm and attained the external morphology and behaviour of the adult fish. At this stage, the larvae had a well-developed gular disc, distinct lateral lines, well-developed dorsal, pectoral, pelvic, anal, and caudal fins, a black spot at the upper angle of the gill opening, and six thin black stripes on the lateral side, with the latter being more pronounced toward the caudal peduncle. The larvae lingered more at the bottom of the tank and under the rocks like adults.

Chapter 8

Summary and Conclusion

The labeonine genus *Garra* Hamilton, 1822 are fishes inhabiting rapid flowing streams and rivers comprising river beds mainly of boulders, stones, rocks, and gravel. The members of this genus have a highly modified lower lip called the gular disc that enables them to adhere to the substratum in response to adapt to fast-flowing rivers and streams. *Garra* is a bottom dweller with an extraordinary snout morphology that allows them to adapt to their environment. *Garra langlungensis* is an indigenous fish reported only from the Langlung river, Nagaland. Considering its distribution report, this species is most likely an endemic fish, and given its recent description, there is no information available on the life history of this fish. Knowledge of the species' life history is crucial for the scientific exploration and conservation management of any fish. The work carried out for the present study emphasized the following objectives:

1. To study the habitat ecology of the *Garra langlungensis.*
2. To examine the length-weight relationship and condition factor to ascertain the relationship between length and weight and the general well-being of the fish.
3. To study the reproductive biology of *Garra langlungensis* to gain insights the sexual dimorphism, sex ratio, fecundity and gonado-somatic index.
4. To develop in-house breeding technology and propagation of *Garra langlungensis* for conservation.

For the present study, Langlung River (25˚ 43' 51.6947" N & 93˚ 39' 40.3186" E), near Zutovi village, Dimapur district, Nagaland, was selected for habitat study as well as specimen collection. Langlung river is a tributary of the Dhansiri river, originating near New Jalukie, Peren District and flowing through Zutovi Village, Dimapur. The river eventually joins with the Dhansiri river and confluences into the

Brahmaputra basin. The present work was carried out between March 2017 and February 2019.

The study on the water analysis of Langlung river was based on five selected study sites. In winter, spring, summer and autumn along the stretch of Zutovi village. Eight physicochemical parameters were analyzed to assess the river's water quality. The study revealed that air and water temperature ranged between 34.53 – 27.16 ˚C and 31.66 – 24.75 ˚C, which fall just about the ideal temperature for proper fish growth. Water velocity and water transparency ranged from 3.89 – 0.74 m/sec and 25.5 – 0.75 cm. The pH ranged from 7 – 8, indicating neutral to the alkaline water quality of the river, which is considered a good quality of water. Dissolved oxygen ranged from 11.6 – 5.2 mg/l showing rich dissolved oxygen in the river. Total alkalinity ranged from 70 – 35.9 mg/l indicating medium alkaline. Total hardness ranged from 112.5 – 30 mg/l, indicating soft to moderately hard water. The studies on the physicochemical parameters of the Langlung river showed seasonal variation in all the parameters, and the water quality of the river indicates a good and productive one favorable for aquatic organisms.

Garra langlungensis was diagnosed as a member of the snout with proboscis species group, having the combination of characters: weakly-developed unilobed proboscis, a distinct transverse lobe with 8–12 small sized unicuspid acanthoid tubercles, 8–9 pre-dorsal scales, 30–32 lateral line scales and 13–15 circumpeduncular scales. Vent closed to the anal-fin origin than pelvic-fin origin.

The morphometric and meristic characteristic was portrayed separately for both male and female. *Garra langlungensis* was found to have a broader range of standard

length, 45.8 to 73.4 (male) and 49.3 to 82.4 (female) mm than the original description. It was also found that *Garra langlungensis* has broader pre-anus length, pre-anal length, per-pectoral length, per-pelvic length, dorsal fin length, anal fin base length, disc length, pulvinus length, pulvinus width in relation to SL, disc width, pulvinus length in relation to HL and more unbranched pectoral fin than the original description.

A total of 213 specimens of *Garra langlungensis* (149 males and 64 females) ranging from 45.8 to 82.4 mm in total length and 1.2 to 6.43 g in weight were analyzed for the length-weight relationship. The exponent value 'b' was lower than 3 in males (2.965), females (2.710) as well as the pooled population (2.870). The 'b' value implies that the males gained weight faster than the females in *Garra langlungensis* and grew negative allometrically. The relative condition factor (Kn) equal to or close to 1 indicates the good condition of the fish. The Kn value in the present study ranged from 2.267-1.445, with an average value of 1.720, showing the healthy condition of the fish in the Langlung river during the study period.

The males and females of *Garra langlungensis* exhibit secondary sexual character during the breeding season. The sex ratio significantly deviated from the expected 1:1 ratio having a male-to-female 1:0.43, male-dominated sex ratio. The fecundity varied from 319 – 844 eggs with an average value of 565 ova ranging from 55.2 – 78.7 mm in total length. This shows that *Garra langlungensis* has low fecundity. The regression analysis showed that fecundity has a linear and positive relationship with total length, body weight, ovary weight and ovary length. The exponent value 'b' remained close to 1, indicating that fecundity is correlated to weight. The value of correlation coefficient between fecundity and total length (r = 0.747), body weight (r =

0.758), ovary weight (r = 0.915) and ovary length (r = 0.522) showed that fecundity is significantly correlated with ovary weight. The gonado-somatic index peaked during March and April in the male population, while in the case of the female population, the peak was observed in April. The data indicate that *Garra langlungensis* breed once a year.

Studies on the spawning habitat revealed that *Garra langlungensis* showed breeding migration prior to the breeding season by flowing upstream towards the canal against the water current. The physicochemical study on the spawning habitat showed that water temperature ranged between 26.5 – 30 ˚C. Water depth ranged between 10 – 20 cm. Water transparency ranged between 3.25 – 13.5 cm. Water velocity ranged between 0.9 – 2.52 m/sec. Dissolved oxygen ranged between 5.7 – 9.5 m/sec, and pH ranged between 7.3 – 7.8.

The breeding of *Garra langlungensis* was successfully done in captivity at a male-to-female ratio of 2:1 with Ovaprim. Courtship behaviour between males and females was observed at 2.30 – 3 hours after Ovaprim administration. Fertilization occurred at 5.15 hours after hormonal administration. The dose of 0.02 ml/gm body weight was determined to be the optimum among the three doses of Ovaprim treatment in the present research and is thus recommended for induced breeding of the fish.

The fertilized egg of *Garra langlungensis* was observed to be spherical, translucent, non-adhesive, and demersal in the current study. After fertilization, the eggs hatch out within 36.45 hours. The newly hatched larvae undergo a series of development, and by 60 days, the hatchlings resembled the adults in having the external morphology such as well developed gular disc, lateral lines, a black spot at the upper

angle of the gill opening and six narrow black stripes on the lateral side with more prominent toward the caudal peduncle as that in the adult fish.

Garra langlungensis is moderately small in size, dark-coloured, showing calm behaviour, with a peculiar movement of sticking to the glass aquarium wall with the gular disk, and they are easily adaptable to aquarium life. Assuming all the characteristics portrayed in the aquarium, *Garra langlungensis* may be categorized as ornamental fish.

Thus, the present work was carried out to bring insights into some of the eco-biological studies of the new species *Garra langlungensis*. However, only some aspects of the growth, bionomic and reproductive biology of *Garra langlungensis* are claimed to have been covered in this research work. Extensive studies on food and feeding, as well as the histological study of the organs, will throw more light on the subject, and hopefully, the present work will form the basis of future studies.

Recommendations

Based on the present work, the following measures are suggested for the sustainable growth and conservation of the fish species in Langlung river, Nagaland:

1. Awareness among the local masses to conserve the natural fish stock for which the scientific organization, academic institutions, village council and fish farmers should join.
2. Regulate human intervention in fish habitats, such as conduct of unethical fishing practices, sand mining and boulder digging, discharge of plastic waste, and irrigation of water for human needs.

3. Proper planning in constructing river dams to avoid the destruction of habitat, breeding ground and migration of fishes.
4. *Garra langlungensis* is a male-dominated population with low fecundity; therefore, local fishermen and the general public should be made aware of the need to prohibit indiscriminate capture of the brooder, particularly females, during the breeding season.
5. The State Fishery Department should take the initiative to declare and categorize the spawning ground of *Garra langlungensis* as Green Zone to prevent over-exploitation and habitat destruction of the species.
6. The captive breeding protocol for *Garra langlungensis* could be applied to plan a captive breeding program for related fish species.
7. Further identification and protection of native fish breeding grounds and minimizing disturbances of fish during the breeding season to facilitate the natural spawning ground.
8. Proper planning for developing and standardizing captive breeding programs for other indigenous fishes in Nagaland to reduce long-term and conservation pressure on the volume of wild catch.
9. Considering the prospects of aquatic resources of Nagaland, the State Fishery Department and similar organizations should take the lead role in exploring and documenting fish fauna from the remote, inaccessible and unexplored rivers and streams of Nagaland.

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Appendix

Seminars and workshop attended

1. Oral presentation on the topic "Captive breeding and larval rearing: A sustainable technique for the conservation of ornamental fishes" at National Seminar on "Climate change and sustainable development: With special focus on North East India" at Nagaland University, Lumami (17^{th}-18^{th} May, 2017).

2. Oral presentation on the topic "Ichthyofauna diversity of Nagaland, Northeast, India" at National Seminar on "Bio-Prospecting and Conservation of Biodiversity for Sustainable Agriculture and Health" at Nagaland University, Lumami (6^{th}-7^{th} February, 2020).

3. Oral presentation on the topic "Description of a new fish species of the genus *Garra* (Teleostei : Cyprinidae) from the Brahmaputra basin, Nagaland, India" at National Seminar on "Bioresources and Sustainable Livelihood of Rural India" at Nagaland University, Lumani (28^{th}-29^{th}, 2020).

4. Hands on training on "Integrated Taxonomy and Systematics in Freshwater Fishes" at National Bureau of Fish Genetic Resources, ICAR, Lucknow. (05^{th}-10^{th} February, 2018).

5. One day "Scientists-Officers-Farmers Interactive Meet" at Suteplenden, Lokongong Village, Mokokchung (25^{th} April 2018).

6. Sensitization workshop on "DST – Women Scientist Scheme" at SASRD, Medziphema (4^{th} -5^{th} March, 2019).

List of publications

1. **Ezung, S**., & Pankaj, P. P. (2019). Captive breeding and larval rearing: A sustainable technique for the conservation of ornamental fish species (pp 146-169). Purbayon publication, Guwahati.

2. **Ezung, S**., Kechu, M., Longkumer, S., Jamir, A., & Pankaj, P. P. (2020). A review on the ichthyofauna of Nagaland, North-East India. *World News of Natural Sciences*, 30(2).

3. **Ezung, S.,** Bungdon, S., & Pankaj, P. P. (2020). A new fish species of the genus *Garra* (Teleostei: Cyprinidae) from the Brahmaputra basin, Nagaland, India. *Journal of Experimental Zoology, India*, 23(2), 1333-1339.

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NAGALAND UNIVERSITY
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Certificate

This is to certify that Prof/Dr/Mr/Ms SOPHIYA EZUNG

of DEPTT. OF ZOOLOGY, NU has participated as PAPER PRESENTER

in the two day National Seminar on Climate Change and Sustainable Development with Special focus on North East India organised by NUTA, Nagaland University, held on 17th-18th May, 2017.

Organising Secretary
NSCCSD 2017

Convener
NSCCSD 2017

ST. JOSEPH UNIVERSITY

Virgin Town, Ikishe Model Village, Dimapur, Nagaland - 797 115

(A State Private University Established Under Nagaland Govt.Act No.6 of 2016: Recognized by UGC & AICTE)

DEPARTMENT OF ZOOLOGY

National Conference on Bio-Prospecting and Conservation of Biodiversity for Sustainable Agriculture and Health

Ministry of Environment, Forest and Climate Change

Certificate

This is to certify that

Dr/ Mr/ Ms. Sophiya Ezung *has presented a paper entitled "* Ichthyofaunal diversity of Nagaland, Northeast India. *" in the Two Day National Conference on "Bio-Prospecting and Conservation of Biodiversity for Sustainable Agriculture and Health (BCBSAH - 2020)" organized by the Department of Zoology, St. Joseph University, Dimapur, Nagaland on 6th & 7th February 2020. This Conference is Sponsored by the Defence Research and Development Organisation - DRDO, (Ministry of Defence) & National Biodiversity Authority - Chennai, (Ministry of Enviroment and Forests).*

Convernor & HOD **Registrar** **Vice Chancellor**

National e-Conference On
'Bioresources and Sustainable Livelihood of Rural India'
September 28-29, 2020

Organized by

Department of Botany, Nagaland University, Lumami-798627, Nagaland

Certificate of Participation

This is to certify that **Ms. SOPHIYA EZUNG** has participated virtually in the National e-Conference **'Bioresources and Sustainable Livelihood of Rural India'** held during September 28-29, 2020 in the Department of Botany, Nagaland University, Lumami-798627,Nagaland, India. She also presented a paper entitled **'Description of a New Fish Species of the Genus *Garra* (Teleostei: Cyprinidae) from the Brahmaputra Basin, Nagaland, India '** (Abstract No. 06) in the Technical Session.

(Sangyu Yaden)
Dean, School of Sciences
Nagaland University

(Chitta Ranjan Deb)
Head, Department of Botany &
Organizing Secretary