
REVIEW OF LITERATURE

1. Gut microbiota

Metchnikoff (1910), 110 years ago, remarked that ‘the activity of the intestinal microbiota is correlated with the longevity of host’. This observation has since instigated continuous research into the intestinal microbiota of both animals and humans. It was not until the 1970s that concrete evidence supporting the presence of a balanced aboriginal microbiota in aquatic animals became available. Before this, it was commonly held that the microbiotas in fish were less intricate and less diverse compared to those in warm-blooded animals. Fish, constituting a substantial and diverse group consisting of approximately 28,000 species, comprise nearly half of all vertebrate species. They manifest a wide variety of physiological, ecological, and natural characteristics (Wong and Rawls, 2012). Consequently, fish serve as a crucial subject for elucidating the diversity and characteristics of symbiotic relationships within microbial communities residing in the gastrointestinal tracts of vertebrates (Nayak, 2010).

The bacterial community residing in the gastrointestinal tract (GIT) of animals is a complex assembly consisting of both obligate and facultative aerobic and anaerobic bacterial populations. In the case of fish, this intestinal microbial community is notably dense in comparison to the surrounding aquatic environment, indicating that the GIT provides a favourable ecological niche for bacterial survival. The colonization of the fish gut occurs rapidly following hatching, primarily influenced by the microbial communities in the surrounding water (Reitan *et al.*, 1998). The significance of the feed microbiota becomes more pronounced once active feeding commences (Ringø *et al.*, 1999). This dynamic microbial community within the fish gut is highly responsive to changes related to the developmental stage of the host and continually adapts to fluctuations in nutritional and environmental conditions (Sullam *et al.*, 2012).

The composition of fish gut microbiota is subjected to variation due to factors such as season, salinity, pH, temperature, and trophic level (Sullam *et al.*, 2012). In healthy fish, a harmonious balance exists among the endogenous microbiota within the intestine, referred to as a symbiosis of gut microbiota. However, if this balance is disrupted, leading to dysbiosis, it can render the transient pathogens present in the GIT highly pathogenic, thereby potentially causing severe and even lethal infections in the host (Medina-Félix *et al.*, 2023).

2. Role of gut bacteria in fish physiology

The intestinal bacterial population of fish is recognized as a pivotal component of fish physiology. This bacterial community can be broadly categorized into two primary groups; autochthonous which

exhibit the capacity to colonize in the mucosal surfaces of host cell linings and allochthonous, which are non-colonizers. Notably, the autochthonous bacterial population assumes a more prominent and indispensable role in fish physiology when compared to their allochthonous counterparts. This heightened significance arises from their multifaceted contributions, encompassing enhanced efficacy in digestion and assimilation processes, active involvement in metabolic functions, facilitation of immune system development, responsiveness to pathogens, and the maintenance of overall host health (Banerjee and Ray, 2017).

The gastrointestinal bacteria play a crucial role in aiding fish in the breakdown of complex compounds that would otherwise remain indigestible to the host organism. This bacterial community produces a repertoire of enzymes essential for the digestion of intricate carbohydrates, proteins, and lipids, thereby augmenting the availability of vital nutrients for the fish. Simultaneously, the fish reciprocates by providing a favourable environment and a consistent supply of nutrients to sustain the gut bacteria (Banerjee and Ray, 2017). Furthermore, gut bacteria actively participate in a spectrum of metabolic functions, including production of the short-chain fatty acids (SCFAs) through the fermentation of dietary fibres. These SCFAs serve dual roles, functioning as an energy source for the fish and exerting beneficial effects on the overall health of the GIT (Hao *et al.*, 2017). Additionally, certain bacterial taxa within the gut are known to synthesize vitamin B12, an essential nutrient crucial for the health of animals (Walhe *et al.*, 2021).

In fish, gut is the prime site for attachment pathogenic bacteria that get inserted mainly through feeding. Within the GIT, gut-associated lymphoid tissues (GALT) are considered as one of the key regulators of host immunity (Lazado and Caipang, 2014). The symbiosis of gut microbiota controls the expression of various genes related to innate immunity. This bacterial colonization during early life stages is crucial for immune system education and the establishment of immune tolerance (Banerjee and Ray, 2017). The presence of beneficial bacteria helps stimulate the maturation of immune cells and the production of immune factors. The beneficial gut bacteria compete with potential pathogens for resources and space within the gut. By occupying attachment sites, consuming available nutrients, and producing antimicrobial substances, beneficial bacteria can limit the colonization and growth of harmful bacteria (Kelly *et al.*, 2017).

It is noteworthy to inscribe that the gut bacterial composition and function can be influenced by various factors and disruptions in the gut bacteria can have negative effects on fish physiology and health. Therefore, maintaining healthy and diverse gut bacteria is crucial for optimal fish physiology and overall well-being.

3. Review of research on loaches

Several researches on loach fish were carried out in various disciplines, including taxonomy and systematics, conservation, physiology, animal behaviour, ecology, genetics, reproductive biology, microbiology, aquaculture, and others.

3.1. Conservation and Threats

Depending upon species, loaches are categorized into different categories starting from “extinct” to ‘least concerned’ as designated by IUCN (Abdoli *et al.*, 2011; Eros *et al.*, 2003). Research has focused on assessing the population status, conservation genetics (Liu *et al.*, 2012), and developing conservation strategies to protect and manage threatened loach species (Nunn *et al.*, 2014). In an implication for conservation, mitochondrial DNA analysis indicates limited population differentiation in the endemic Chinese species, *Leptobotia elongata* (Bleeker), commonly known as the elongated loach. A comparative study of the mitochondrial DNA control (D- loop) region was conducted to assess the wild population structure and genetic diversity. The analysis revealed 49 polymorphic sites and 45 haplotypes, reflecting high diversity of haplotypes but low diversity of nucleotides, similar to many other fish species. Notably, there was absence of any notable population structure, and gene flow estimates among groups were exceptionally high, indicating minimal genetic divergence among populations of this species. The reduced genetic differentiation among these groups likely results from combined gene flow, facilitated by the downstream transport of eggs as well as larvae and the unidirectional migration of adults across their distribution. Consequently, this study recommended that these *Leptobotia elongata* populations, located in the upper reaches of the Yangtze River, be treated as a single management unit for conservation purposes (Liu *et al.*, 2012). In another study of conservation and condition assessment, the role of temporal as well as spatial variations was evaluated on the population structure of the spined loach (*Cobitis taenia*). This study concluded that accurate population assessments are essential for successful conservation of species. Also, a set of sampling protocols should be developed to evaluate the status of designated species in relation to predefined conservation objectives. This process, known as 'condition assessment,' entails comparing various parameters, typically including population distribution, with criteria that indicate the viability of populations (Nunn *et al.*, 2014).

3.2. Ecology and behavioural studies

Besides the taxonomy, systematics and conservation, studies were conducted to investigate the ecological preferences, feeding behaviour and adaptations of different loach species. In an experiment feeding behaviour study, the loach *Misgurnus anguillicaudatus* was experimentally analysed. Results showed that the feeding nature of this fish is chemically induced and the standard food intake patterns of *Misgurnus anguillicaudatus* are quick engulfing of organic particles together with substrate. Thus this fish was regarded as a typical “detritus feeder” (Watanabe and Hidaka, 1983). Another study investigated the genetic mechanisms of well adaptation of *Triplophysa* to the conditions on the Tibetan Plateau at the genome-scale. To identify genetic mechanisms of this adaptation, a comprehensive analysis of transcriptome and genome sequences of three species of *Triplophysa* namely *T. siluroides*, *T. scleroptera*, and *T. dalaica* living in high altitude, cave fish *Astyanax mexicanus*, zebrafish *Danio rerio*, large-scale loach *Paramisgurnus dabryanus* living at low altitudes were analyzed. Analysis revealed that each of the terminal branches of the *Triplophysa* fishes exhibited a significantly elevated ratio of nonsynonymous to synonymous substitutions compared to the branches of low-altitude-dwelling fishes. Particularly noteworthy was the identification of accelerated evolution, candidate genes implicated in this process, and *Triplophysa*-specific genetic variations associated with energy metabolism and responses to hypoxia (Wang *et al.*, 2015).

3.3. Molecular biology and genetics

Molecular techniques have been employed to study genetic diversity, population structure, and phylogenetic relationships among different loach species. These studies contribute to understanding the evolutionary history and genetic conservation of loach fish populations. In a study of genetic marker-based biodiversity, to understand the origin of tetraploid Japanese *Cobitis* loaches, an analysis of mitochondrial in addition to four nuclear loci in two tetraploids and various diploid populations of *Cobitis biwae*, *Cobitis striata*, and related species was performed. The mitochondrial gene tree showed a monophyletic *C. striata* and a paraphyletic *C. biwae* relative to *C. striata*, while the nuclear sequence analysis indicated monophyletic diploid *C. biwae* and *C. striata*. This disagreement suggests a primitive hybridization episode and ingression of mitochondrial genes. Tetraploid *C. biwae* exhibited both mitochondrial and nuclear sequences evolutionary related to *C. biwae*, suggesting autotetraploidy. Being tetraploid, *C. striata* inherited nuclear alleles from both *C. biwae* and *C. striata* and mtDNA from *C. biwae*, indicating that there is an event of allotetraploidy and *C. biwae* was the maternal contributor. This study also revealed genome reshaping following allotetraploidization (Saitoh *et al.*, 2010).

3.4. Reproductive biology

Reproductive biology of loach is mainly focused on the fecundity of fish, spawning behaviour and strategies, genomic constitutions, sexual dimorphism and gene expression, introduction of triploidy and its effect on gonad development and other factors regulating reproductive success. Understanding the reproductive biology of loaches is essential for conservation and sustainable management practices. In an experiment, the genetic makeup and atypical reproduction in unisexual polyploid lineages of the *Misgurnus* loach were explored. *Misgurnus anguillicaudatus* serves as an ideal animal model for studying the biological origins and evolutionary importance duplication of genome and unisexual reproduction. This is due to the fact that artificially induced fishes, naturally occurring polyploids, as well as parthenogenetic animals of this species, can be compared (Arai and Fujimoto, 2013). In a separate experiment, researchers induced triploidy in *Misgurnus mizolepis*, a mud loach and studied its effects on development and growth of gonads. Triploidy was induced by exposing fertilized eggs to cold shock, at 2 °C, for 15 to 60 min, just 5 min after fertilization. Histological analysis of 9-month-old triploid ovaries revealed a notable presence of oocytes with significant interstitial tissue, whereas diploids showed well-developed oocytes. Diploid males had normal spermatids with spermatozoa, but triploid males had fewer of these. Growth rates were assessed over a 9-month growth trial, but the growth rates of triploids did not show significant differences when compared to their diploid counterparts (Kim *et al.*, 1994).

3.5. Microbiology and pathology

Besides these research areas, another broad area of research includes the isolation, identification and characterization of fish pathogens from diseased loaches. In June 2012, a disease outbreak hit mud loach farms in Korea, causing up to 1.2% daily mortality rates. Typical symptoms included bleeding ulcers on the head and hemorrhagic erosion of the operculum. The culprit, identified as *Aeromonas sobria* due to its biochemical traits and the presence of haemolytic genes (*sob* and *asa1*), was tested on a healthy mud loach. At a 10^6 cfu fish⁻¹ infectious dose, a 10.3% mortality rate was observed within 7 days. With a 10^7 cfu fish⁻¹ dose, the rate of mortality soared to 60.9% within 2 days, while all fish died in single day when dosed with 10^8 cfu fish⁻¹ (Yu *et al.*, 2015). In 2020, five bacterial isolates from diseased loach *Misgurnus anguillicaudatus* were characterized in a fish culture farm in China. The symptoms include shedding of skin mucus followed by petechial haemorrhages all over the body. Those bacterial isolates were later taxonomically identified as *Acinetobacter*, the first time reported to cause fish mortality. Experiments were conducted to confirm the pathogenicity of *Acinetobacter* in zebrafish and nematode which was proved to be pathogenic and highly virulent to those organisms also (Wang *et al.*, 2020). Another focus of loach research was to investigate the antibiotic-resistant bacterial population (Roy *et al.*, 2014) and the

influence of water quality in these resistant bacterial populations (Roy and Barat, 2011). In another disease outbreak in *Misgurnus anguillicaudatus* in China displayed symptoms like loss of energy and appetite, black coloured body, anasarca, and skin redness. Clinical signs included discoloured liver, swollen spleen, and intestinal and peritoneal membrane bleeding. Analysis, including physiological, and biochemical tests, and 16S rRNA sequence, identified *Shewanella* sp. and *Listonella* sp. as the causative agents. Antibiotic susceptibility testing revealed susceptibility of *Shewanella putrefaciens* and *Listonella anguillarum* to levofloxacin, enrofloxacin, pipemidic acid, ciprofloxacin, and norfloxacin (Qin *et al.*, 2014). A different study showed antibiotic-resistant *Aeromonas hydrophila* isolated from *Misgurnus anguillicaudatus* causing a mass mortality in a fish farm in Korea. The lethal dose 50 (LD50) of the pathogen was found to be 6.0×10^7 cfu fish⁻¹. Genetic analysis showed that the presence of tetracycline resistance gene (*tet E*) in *A. hydrophila* helped it to survive even in the presence of tetracycline (Jun *et al.*, 2010).

3.6. Physiology

Loaches are known for their unique ability to respire through their skin and posterior intestine. However, this air-breathing capacity of the intestine may affect its ability to absorb nutrients. To understand this compromise, a transcriptomic analysis was conducted on the posterior intestine of *Misgurnus anguillicaudatus*, focusing on the relationship between the air-breathing activity and uptake of nutrients. The results indicate an overlap between the functions of air-breathing and nutrient uptake in the posterior intestine of loaches. Inhibiting intestinal air-breathing in loaches could impact both their nutrient absorption capacity and the stability of endothelial capillary structures (Huang *et al.*, 2016).

Explaining the process and patterns of research in loach is essential in understanding the advancement in scientific knowledge and its perspective. Loaches have served as valuable subjects in diverse areas of scientific investigation. However, a conspicuous gap existed in our understanding of the composition and functional aspects of gut bacterial communities in facultative air-breathing loaches. The present research endeavour seeks to address this deficiency by employing *Lepidocephalichthys guntea* as a model fish species.

4. Introduction to *Lepidocephalichthys guntea*

Loaches belong to one of the ancient world freshwater fish families that consist of at least 260 species, covering parts of Europe, Asia and Morocco's riverine ecosystem. These 260 species under the family *Cobitidae* (Phylum: *Chordata*, Class: *Actinopterygii*, Order: *Cypriniformes*) are distributed in 18 different genera, among which genus *Lepidocephalichthys* currently contains 18 recognizable

species (Kottelat, 2012). Taxonomically identified by F. Hamilton in 1822, *Lepidocephalichthys* (derived from Greek, *lepas* = limpet + Greek, *ichtys* = fish) *guntea* is also known as the guntea loach, scavenger loach, or peppered loach. It has a broad distribution encompassing Northern India, Nepal, Bangladesh, Myanmar, and Thailand (Talwar, 1991). As a member of the *Cobitidae* family, it possesses both commercial and aesthetic values.

4.1. Morphological remarks

L. guntea is a freshwater, brackish, demersal, potamodromous fish that prefers sandy or fine gravelly bottom into which they can burrow themselves and disappear quickly due to their self-effacing nature. These fish are scavengers by nature, predominantly consuming small invertebrates such as insect larvae, crustaceans, and other bottom-dwelling organic matter. In eutrophic water systems, they particularly favour tubifex worms and associated benthic organisms as their dietary choices (Kottelat, 1989). The body of *L. guntea* is slender and compressed slightly from the anterior side but strongly from the posterior side. The maximum recorded length of the fish is 15 cm (Talwar and Jhingran, 1991). The lateral line is absent generally, but a light black coloured band extends from the snout to the caudal fin. The dorsal and ventral profile is almost straight and parallel. Barbels are 3 pairs including rostral (2 pairs) and maxillary barbels (1 pair). The caudal fin is rounded and blackish in appearance with multiple deep black stripes. The pectoral fin is with an osseous spine in males (Rahman, 1989). A black ocellus is present on the upper half of the caudal base. Although their colour varies, the ground colour appears to be dirty yellowish, which is a signature mark of them. The body is covered with scale and notably, a patch of scale extends from below the eye to the upper part of the operculum. The fish has eight soft dorsal rays and seven soft anal rays, with the fin formula being D. 8 (2/6); P1. 8-9; P2. 7-8; A. 7 (2/5). Breeding time for this species is reported from July to September. The local names of *L. guntea* include gutum, gutia, gorkun, poa, puiya and others (Kottelat, 2012).

4.2. Anatomical remarks

The anatomy and cytology of the gastro-respiratory structure of the intestine in relation to the respiratory function showed *L. guntea* shows a remarkable feature of additional respiration which is performed by the intestine (Ghosh *et al.*, 2011). In environments characterized by muddy, eutrophic conditions, where the dissolved oxygen levels drop below the normal, this fish comes across the water surface and engulfs oxygen from the air. The intestine of *L. guntea* is straight and uncoiled in a structure which is significantly different from the structure of carp's intestine (Ghosh *et al.*, 2011). Detailed examination of the micro-architectural pattern and cellular composition of the anterior and

middle sections of *L. guntea*'s intestine strongly suggests that these regions are specialized for the processes of digestion and nutrient absorption. However, notable changes occur in the posterior section of the intestine. Here, there is a substantial reduction in the absorptive surface area, and blood capillaries penetrate between the intestinal epithelial cells (Ghosh *et al.*, 2011). These adaptations in the posterior intestine of *L. guntea* likely serve the specific purpose of facilitating aerial respiration when needed in low-oxygen environments.

5. Review on research concerning *Lepidocephalichthys guntea*

The study of *L. guntea* is limited to selected fields like toxicity study (Mondal *et al.*, 2007), length-weight relationship (Dhakal and Subba, 2003), isolation and antibiotic resistance profile of pathogenic bacteria present in *L. guntea* (Roy *et al.*, 2014), influence of quality of water on the bacterial contamination of *L. guntea* (Roy and Barat, 2011), life history traits (Saha *et al.*, 2021), gross structure and dimension of gill (Singh *et al.*, 1981), comparison of gut bacterial population in gill breather vs facultative air breather fish (Roy *et al.*, 2018), and reproductive biology (Sayeed *et al.*, 2009; Mandal and Mandal, 2022).

5.1. Life history traits

In 2021, Saha *et al.* first reported a study on life-history traits of *L. guntea* that includes length-frequency distribution (LFD), length-weight relationship (LWR) and length-length (LLR) relationship, form factor ($a_{3.0}$), best condition factor (allometric, KA; Fulton, KF; relative, KR; and relative weight, WR), length at first maturity (Lm), natural mortality (Mw) and optimum catchable length (Lopt). Between February 2019 and January 2020, 470 fish were randomly gathered using a variety of traditional fishing techniques. The total body length (TL), standard body length (SL), and body weight (BW) of each fish were measured. The two TL size classes (6.00–6.99 cm and 7.00–7.99 cm) made up the majority of the population of fish in terms of numbers. Positive allometric growth was shown by exponential b values of LWR (TL vs. BW). A similar growth pattern was also indicated by the LLR's b value. The fish has an elongated body shape, as indicated by the estimated $a_{3.0}$ value of 0.007. KF was the instrument that is best suitable for assessing the health of *L. guntea* in the Payra River out of the four different condition factor kinds. For *L. guntea*, the computed Lm, Mw, and Lopt values were 6.54 (6.5 cm) in TL, 1.16/year, and 7.46 cm TL, respectively. These findings could thus be applied in the future to link ecosystems and enhance the micro-management of this species.

5.2. Reproductive biology

On the *L. guntea* population of the Kangsabati River, a study was done to assess their fecundity, sex ratio, sexual dimorphism, gonadosomatic index, and initial maturity length. Male of the species has

distinct pectoral fin modifications, with the seventh and eighth pectoral rays combining to form the laminar circuli, a thicker ray that distinguishes males from females of the species. The ratio of male to female overall was 1.61:1. The highest GSI values for both sexes were recorded in June. The mean absolute fecundity of females in *L. guntea* ranged from 2832.19 ± 40.88 to a high of 9951.28 ± 142.92 for females with a mean body weight of 2.01 g to 9.73 g. June and July were found to be ideal for its reproductive success, according to the GSI and fecundity statistics. The findings of this study will facilitate the breeding and economic expansion of this species (Mandal and Mandal, 2022). In another study, the pituitary gland of carp was used in the artificial breeding of the *L. guntea* (Hamilton, 1822). After raising, the fully grown female fishes were given two injections, of 20, 15, 10, and 5 PG/kg body weight, separated by a 4-hour interval. The dose of female fish was first applied at one-third and later at two-thirds. Only the second injectable dose was given to the males as treatment. In the highest dose, all fish naturally spawned without being stripped, while in lower doses, natural spawning only partially happened before being finished by hand stripping. In the lower two doses, the rates of fertilization and hatching were much higher. The survival rate of larvae was up to 50%, and their average weight was 65 mg. The optimal timing for injection was between 8 and 12 PM, and the effective dose for the induction of gutum breeding was 5-10 mg PG/kg body weight (Sayeed *et al.*, 2009).

5.3. Toxicity study

A study evaluated the acute toxicity of two widely used neem derived biopesticides, nimbecidine and neem gold, applied separately and in combination, on freshwater loach fingerlings of *L. guntea*. Notably, nimbecidine was found to be more effective than the neem Gold and the mixture of both biopesticides, based on their respective 96-hour LC50 values. Water quality analysis revealed that as the biopesticide doses increased, the dissolved oxygen levels decreased, while parameters such as acidic pH, alkalinity, free carbon dioxide, water hardness, and chloride ions increased. Fishes exposed to the biopesticides displayed abnormal behaviours including abnormal movements, imbalance, and surface floating, with the severity increasing with higher pesticide concentrations (Mondal *et al.*, 2007).

5.4. Influence of water quality on bacterial contamination

This investigation underscores a significant correlation between bacterial contamination observed within the healthy *L. guntea* fish and the microbial communities present in their immediate aquatic environment. However, noteworthy findings emerged concerning the abundance of heterotrophic bacteria present in the water samples, which ranged from $4.1 \times 10^6 \pm 0.8 \times 10^6$ cfu ml⁻¹ to $5.5 \times 10^6 \pm$

1×10^6 cfu ml⁻¹. Similarly, the gut of the *L. guntea* exhibited substantial quantities of bacteria, with counts recorded at $4.5 \times 10^6 \pm 1.8 \times 10^6$ cfu g⁻¹. The bacterial communities in both the fish and river water were characterized by the presence of dominant genera, which included *Aeromonas* spp, *Pseudomonas* spp, *Enterobacteriaceae* spp, *Streptococcus* spp, and *Salmonella* spp (Roy and Barat, 2011).

5.5. Antibiotic resistance profiling of the bacterial population of *Lepidocephalichthys guntea*

Currently antibiotic-resistant bacterial populations are frequent in various organisms, including aquatic animals and fish such as loaches. The development of resistance to antibiotics is a congenital evolutionary action that has been raised as a result of the overuse or misuse of antibiotics in the field of both human and veterinary medicine (Davies and Davies, 2010). While the study of antibiotic-resistant bacterial populations has been remarked in various fish species, studies focused on bacterial antibiotic resistance in loaches still appear to be limited. Few pieces of research were conducted to estimate the pathogenic bacterial population in the skin, gill and gut of *L. guntea* which showed that the population of *Aeromonas* spp. and *Salmonella* spp. are highest in the gut ($2.3 \times 10^6 \pm 2.4$ cfu g⁻¹) followed by gills ($2.1 \times 10^6 \pm 2.2$ cfu g⁻¹) and skin ($1.7 \times 10^6 \pm 1.8$ cfu g⁻¹). Antibiotic susceptibility tests of these bacterial isolates were performed against different antibiotics which showed maximum resistance was against penicillin-G, ampicillin, and cephalothin but none of the bacteria was resistant against ciprofloxacin and tetracycline (Roy *et al.*, 2013a). Similarly, another investigation was conducted to check the antibiotic resistance profile of *Pseudomonas* spp. isolated from the skin, gill and gut of *L. guntea*. A total of 40 *Pseudomonas* isolates were studied which showed maximum resistance against cephalothin and penicillin-G (100%), followed by ampicillin (>57%). Almost no resistance was reported against ciprofloxacin, gentamycin and streptomycin (Roy *et al.*, 2014). Another research work conducted to check the antibiotic resistance profile of *Enterobacteriaceae* isolated solely from the gut of *L. guntea* showed all gut isolates were resistant to erythromycin and kanamycin but sensitive to ciprofloxacin, moxifloxacin, tetracycline and gentamicin. All isolates were resistant to ampicillin, penicillin-G, and cephalothin (belonging to the β -lactam class). The gut isolates showed 60-100% resistance against streptomycin, kanamycin and erythromycin (Roy *et al.*, 2013b).

5.6. Comparison of the gut bacterial population in gill breather vs facultative air breather fish

A comparison of gut bacteria of gill-intestinal breather *L. guntea* with sole gill breather fish *L. rohita* was performed. In fish, the additional respiratory system known as the accessory or supplemental respiratory system allows fish to obtain oxygen from environments with low oxygen levels, such as stagnant or oxygen-depleted water. The presence of an additional respiration system in the intestine allows air to pass through the intestine which creates a suitable environment for microaerophilic and aerobic bacteria to survive. While in the sole gill breather fish intestine must contain a higher number of anaerobic and microaerophilic bacterial populations. A contrasting study to estimate and compare the gut microbial population between *L. guntea* and *Labio rohita* by Roy *et al.* (2018), showed that in high dissolved oxygen concentrations the aerobic and micro-aerophilic bacterial population in *L. guntea* is 232 ± 33 and $125 \pm 9.7 (\times 10^5 \text{ cfu g}^{-1} \text{ of gut tissue})$ while in *L. rohita* it is 708 ± 11.8 and $565 \pm 29 (\times 10^5 \text{ cfu gm}^{-1} \text{ of gut tissue})$. On the other hand, under low dissolved oxygen concentration the aerobic and micro-aerophilic bacterial population in *L. guntea* is 272 ± 47 and $115 \pm 7 (\times 10^5 \text{ cfu gm}^{-1} \text{ of gut tissue})$ while in *L. rohita* it is 25 ± 4.4 and $40 \pm 7.9 (\times 10^5 \text{ cfu gm}^{-1} \text{ of gut tissue})$. These results confirmed that aerophilic bacterial density in the gut of *L. guntea* is independent of dissolved oxygen (DO_2) content as the air pockets present in the gut balance the deficit of oxygen obtained through gill respiration. The phenomenon was found to be reversed in gill-breathing fish like *L. rohita* because the additional respiration mechanism is absent (Roy *et al.*, 2018).

While focusing on the composition and function of gut bacteria in a fish, specific studies on the gut bacterial population in *L. guntea* are limited. Also, it's important to note that not all members of the fish gut bacteria are easily culturable using traditional laboratory techniques. The fundamental reason is inappropriate growth conditions in the laboratory environment which is unable to mimic the environmental condition from which they belong. Other reasons may be interruptions in intricate interactions with each other and the host fish, fastidious nature, overgrowth of dominant species etc. To deal with these limitations, advancements in molecular techniques, like metagenomics and high-throughput sequencing, have allowed researchers to study the unculturable fraction of fish gut bacteria. Overall, while the fish gut bacteria are complex and not all bacteria are easily culturable, scientists continue to employ innovative approaches to isolate and characterize the bacterial population present in the fish gut. But still, none of the approaches was carried forward to estimate the gut bacterial population of *L. guntea* and other loaches.

Henceforth, the present investigation is dedicated to an in-depth examination of the intestinal microbiota of *L. guntea*, with a primary emphasis on characterizing both the cultivable and non-cultivable constituents of the gut bacterial population. This research endeavors to elucidate the

physiological, biochemical, and genomic attributes of selected bacterial isolates originating from the intestinal milieu. Moreover, this study is oriented towards conducting a comparative analysis between the gut bacterial populations of *L. guntea*, a facultative air-breathing fish, and *C. mrigala*, exclusive gill-breathing fish. Furthermore, a significant facet of this research entails the scrutiny of the modulation of gut immunity within *L. guntea* in response to pathogenic bacterial infection.

❖ **ETHICAL APPROVAL FOR EXPERIMENTS ON ANIMALS**

All the experiments were performed under the guidance of “Committee for the purpose of control and supervision of experiments on animals (CPCSEA)” for experimentation on fishes (<http://cpcsea.nic.in/WriteReadData/LnPdf/GuidelinesofCPCSEAforExperimentationonFishes-2021.pdf>) through Institutional Animal Ethics Committee (Approval No. IAEC/NBU/2022/32).

OBJECTIVES OF THE STUDY

1. Enumeration of cultivable bacteria; both aerobic and facultatively anaerobic, with special emphasis on probiotic and putatively pathogenic strains in the gut of a scavenger loach *Lepidocephalichthys guntea*.
2. Genomic characterization of few gut bacteria and understanding their role in fish physiology
3. Comparison of gut bacteria of *Lepidocephalichthys guntea* with *Cirrhinus mrigala* to reveal differences in the gut bacterial community during oxygen stressed environmental condition by Meta-taxonomic study.
4. To establish an infection model in order to study gut immunity in *Lepidocephalichthys guntea*