2.1 INTRODUCTION

2.1.1 Mullets

The grey mullet, a member of the Mugilidae Jarocki, 1822 family, is extremely adaptable to a wide range of coastal environments with varying characteristics such as temperature, salinity, turbidity, sedimentary composition, and dissolved oxygen levels (Wallace 1975; Whitfield *et al.* 2012). These fish are known to live in a variety of environments, including estuaries, lagoons, rivers, lakes, and hypersaline environments. Their ability to thrive in these environments is due to their adaptable physiology and critical role as fundamental members of the aquatic food web.

The mullet species plays a crucial role in these ecosystems by significantly adding to the number and biomass of fish populations, especially in estuary settings (Carpentier *et al.* 2013). Their eating habits highlight their versatility, since they are known to consume a diverse array of food sources such as microphytobenthos, particulate organic matter, and meiofauna etc. Mullet have been characterised in various studies as 'interface feeders,' 'benthic microphagous omnivores,' 'microbenthos and meiobenthos feeders,' 'iliophagous,' and 'limno-benthophagous,' indicating their wide range of feeding behaviours (Cardona 2015; Bhatt *et al.* 2022). The range of their trophic positions, which can vary from herbivorous to omnivorous to planktivorous, is determined by factors such as the availability of food supplies and varying interpretations by different writers. Mugilid species demonstrate ecological success worldwide, flourishing in tropical, subtropical, and warm temperate coastal environments in both the northern and southern hemispheres. Mullets are a prominent component of fish populations in many different places, and certain estuary environments exhibit remarkable variety. In the Koycegiz estuarine lagoon in Turkey, multiple mugilid species make a significant contribution to the overall fish capture (Akin *et al.* 2005). Furthermore, the estuary Lake St. Lucia in South Africa sustains a wide range of mullet species, as documented by Blaber in 1977. In habitats with lower abundance of mullet species, they still exhibit a significant influence on the fish community, as evidenced in the Berg Estuary where specific mullet species are particularly abundant relative to other marine fish taxa (Clark *et al.* 2009).

Although mugilids can thrive in various coastal habitats, they have a particular preference for certain situations. In the salt marsh creeks of Mont Saint-Michel Bay, France, the thin lip mullet [*Chelon ramada* (Risso, 1827)] made up a large part, accounting for 87% of the total fish biomass (Laffaille *et al.* 2000). Furthermore, in the salt marsh creeks of the Kariega Estuary in South Africa, mugilids constituted more than 75% of the total fish biomass, as reported by Paterson & Whitfield (2003). Furthermore, the red lip mullet [*Planiliza haematocheilus* (Temminck & Schlegel 1845) - Froese & Pauly 2024] has been identified as the most prevalent marine fish species in the salt marsh creeks of the Yangtze Estuary, China (Jin *et al.* 2007).

Several species of mullet display traits of marine estuarine-opportunists (Potter et al., 2015) or cycle migrants (Mariani 2001), frequently moving between estuaries, lagoons, and coastal catchments largely for the purpose of using them as nurseries, before returning to the sea once they reach maturity (Blaber 1987). Blaber (1987) found that 80% of the 15 species of mugilids in southern Africa regularly use estuaries during their life cycle. This pattern appears to be common worldwide. Nevertheless, if the link between an estuary and the marine environment is disrupted, it can result in a significant reduction in both the variety and quantity of mugilids within that system (Vivier *et al.* 2010).

2.1.2 Physico-chemical Parameters of Water

Fish depend on a combination of internal elements (such as nervous, endocrinological, and neuroendocrinological factors) and external factors (ecological) to control several physiological activities, including growth capacity. The ecological factors can be categorised into two main groups: determining factors, including temperature, salinity, and photoperiod, which directly impact growth by affecting receptors, and limiting factors, such as ammonia, oxygen, and pH, which function within specific thresholds or tolerance ranges (Boeuf & Payan 2001).

The physico-chemical properties of water, including temperature, salinity, pH, and total dissolved solids have an impact on the abundance of organisms and their seasonal fluctuations (Cyrus & Blaber 1987a, b; Barletta *et al.* 2017).

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

Temperature is a crucial factor in an aquatic system. According to Kastooribai (1991), there is a strong possibility that no other element has significant impact on the aquatic animals as well as it causes direct and indirect consequences on aquatic organisms. Dissolved oxygen levels are crucial in assessing the aerobic capability and physiological performance of grey mullet populations. The presence of oxygen affects where they choose to live, how they eat, and how fast their bodies work. When there is not enough oxygen, it can cause them to experience physical stress and have lower overall health (Chapman & McKenzie 2009). The pH level of natural waters is a significant environmental component that is associated with the species composition and life processes of animal and plant communities inhabiting them (Jhingran 1982).

The grey mullet, scientifically known as *Mugil cephalus* Linnaeus, 1758, is an ideal organism for investigating the impact of physico-chemical water factors on fish ecology and population dynamics due to its remarkable capacity to thrive in many aquatic environments. Physico-chemical factors are essential for determining the distribution of fish species in estuaries and coastal areas. Salinity and temperature are two important hydrobiological elements that have a substantial impact on the presence, concentration, and development of fish eggs in estuarine areas (Romos *et al.* 2006). Fluctuations in these factors can have a substantial effect on the spread, number, and actions of grey mullet populations, eventually influencing their ecological role and population patterns.

By conducting an extensive analysis of existing literature, it has been noted that changes in light, temperature, and other environmental elements over different seasons have a notable impact on the functioning of endocrine organs, including those in fish. This, in turn, plays a crucial role in controlling the timing of reproduction (Hoar 1957). Chacko & Ganapathi (1949) were early researchers that studied the hydrobiological parameters of the Adyar estuary specifically for the purpose of fish farming. Jayaram (1954) conducted a study on the seasonal fluctuations in salinity and dissolved oxygen in the nearshore waters of the Gulf of Mannar and Palk Bay near Mandapam. The study specifically examined how these fluctuations affect fisheries resources. In 1959, Tampi conducted a study specifically focused on examining the impact of salinity and temperature on the

characteristics of fisheries in a saltwater lagoon located near Mandapam. Ramamirtham & Jayaraman (1963) observed and recorded the levels of saltiness, rainfall, and temperature in the Cochin backwaters near Wellington Island. They studied how these elements affected the fishing in the area. Previous researchers have conducted similar studies on various marine and brackish water environments in Indian waters (Subrahmanyam 1963; Pandian 1966; Evangeline & Subbaiah (1969), Sankaranarayanan & Qasim 1969; Raman *et al.* 1977; Sarkar 1979; Benakappa *et al.* 1980; Gokhale & Patil 1989; Mishra *et al.* 1993; Soundarapandian *et al.* 2009; Solai *et al.* 2010; Kannappan & Karthikeyan 2013; Panchakshari *et al.* 2015).

It is widely accepted that the respiratory metabolism of teleost fishes operates at significantly varying rates depending on the temperature of their surroundings (Ege & Krogh 1914; Winberg 1956; Fry 1957, 1964; Wohlschlag 1964). A study conducted by Cameron in 1970 found that the hypoxic condition was the cause of the increase in blood concentration. In a similar manner, Cech Jr. & Wohlschlag (1971) observed an increase in the rate of metabolism (measured in milligrams of oxygen per kilogramme of body weight per hour), volume of ventilation (measured in millilitres of water per minute), *VR* (ventilation rate, measured in closures per minute), V_{sv} (opercular stroke volume, measured in millilitres of water per closure), and *% U.* (percentage utilisation of oxygen) during hypoxic conditions.

Moon *et al.* (2017) investigated the physiological reactions of grey mullet (*M. cephalus*) when exposed to saltwater with high acidity. The results of this study emphasise the substantial influence of declining pH values in saltwater on the physiological reactions and overall well-being of *M. cephalus*. As the pH fell, multiple metrics including as survival rate, body weight, aminotransferase levels, total protein levels, triglyceride levels, osmotic pressure, and antioxidant enzyme levels were impacted. The discovered alterations indicate that when fish are exposed to acidic water, it causes stress in them, which hampers their capacity to control haematological and osmotic processes. The activity of antioxidant enzymes was notably affected by pH values, demonstrating the susceptibility of these enzymes to osmotic stress.

The temperature range for the distribution of *M. cephalus* is documented to be between 12°C and 25°C. However, it appears that the lower limit of temperature that influences the natural distribution of this species is estimated to be between 16°C and 18°C. Their temperature tolerance was adaptable, contingent upon the ambient temperature in their specific geographical regions (Oren 1981). According to Prakoso *et al.* (2015), lower temperatures have been found to reduce operculum movement, haematocrit, and haemoglobin levels, while increasing blood cortisol levels. The fish acclimated to a temperature of 14°C and a salinity of 0.5 psu had 55% reduced total fatty acid content compared to fish acclimated to saltwater at the same temperature (Kheriji *et al.* 2003).

Salinity is an important component in aquaculture because it affects the growth, survival, and production efficiency of different species. Optimal salinity levels vary among species and are crucial for their well-being. Although certain species demonstrate tolerance to higher salinities, only a handful can withstand extended periods of exposure to water with salinities over 120‰ (Nordlie 1985; Nordlie *et al.* 1992; Nordlie & Haney 1998). In a laboratory experiment conducted by Cardona in 2000, it was found that excessive salinity levels had a negative impact on the metabolic rate of juvenile specimens. Additionally, it was seen that better growth performance was attained in fresh water and oligohaline water. Faizo *et al.* (2013) found that salinity levels had a notable impact on erythrocyte, haemoglobin, haematocrit, leucocytes, and thrombocytes, as well as on all biochemical parameters except for osmolarity. Furthermore, the breeding season of the annual breeder *M. cephalus* is marked by the lack of or restricted rainfall, which leads to an increase in salinity and a rise in water temperature. Spawning activity in this population seems to be triggered by a quick rise in temperature and salinity (Baskaran 1993).

In coastal environments, the salinity levels can undergo fast fluctuations across small distances. This presents a difficulty for aquatic organisms. In order to function well, marine creatures must maintain a consistent set of chemical conditions within their cells by utilising particular metabolic reactions. Any activity that induces substantial alterations in cellular chemistry will have detrimental effects on marine organisms. Substantial alterations in the levels of

potassium and sodium within cells will have a profound impact on the functioning of crucial proteins. The concentration of salts influences both the catalytic rate and the interaction between substrates and enzymes. The control of inorganic elements, such as potassium, is crucial for membrane transport (Levinton 2017). The productivity of fish and fisheries in any aquatic environment is strongly influenced by the physico-chemical parameters of that environment. Changes in fishery production are caused by variations in biological success, where economically significant fish species thrive most effectively under ideal physico-chemical circumstances. Industrial activities and other human interventions have caused changes in these conditions, which require investigations into physico-chemical parameters such as temperature, pH, dissolved oxygen, salinity, nutrients, as well as biological factors like food and feeding habits and reproductive biology of different fish species.

2.1.3 Length-Weight Relationship and Condition Factor

The historical account about condition factor and weight-length correlations is deeply interconnected. The concept can be traced back to Galileo Galilei's (1564–1642) 'square cube law,' which states that volume increases in proportion to the cube of linear dimensions, whereas strength, such as the diameter of legs, increases solely in proportion to the square. In his work Principles of Biology (1864–1867), Herbert Spencer reiterated Galileo's theory, asserting that "*In bodies with similar shapes, the masses, and consequently the weights, vary in proportion to the cubes of their dimensions.*" The principle was later referred to as the 'cube law.' According to this law, when a fish's length doubles, its weight increases by a factor of eight. Nevertheless, Fulton's 1904 study, which utilised the cube law on 5675 specimens of 19 fish species from the Scottish North Sea, demonstrated that it is not directly applicable to fish. He noticed that the majority of species have a greater rise in weight compared to what would be expected based solely on their length. Fulton observed considerable variation in weight relative to length among several species, as well as within species across different regions and seasons. He noticed clear patterns, such as a significant decrease in weight right after reproducing, which is quickly restored. Fulton emphasised that fish display differential growth rates during their first stages in

contrast to larger individuals, resulting in variances in length-to-weight ratios. Furthermore, he observed that the degree of weight variation among individuals of the same species increases substantially as the fish's length increases. Fulton's observations established the fundamental principles of allometric growth, form factor, regional and seasonal variation in condition, reproductive variation in condition, growth rate in different life stages, changes in condition with size, and the exponential relationship between weight and length data variation.

Extensive worldwide study has been undertaken on the mullet fishery, focusing on age, growth, mortality, migration, recruitment, and stock evaluation in different locales. Previous research has thoroughly examined the correlation between the length and weight of *M. cephalus* populations in different geographical areas, as well as their condition factor. The research conducted by Thomson (1951), Kesteven (1953), Chubb *et al.* (1981), Smith & Deguara (2002), and Bell *et al.* (2005) has yielded useful knowledge about the mullet populations in Australian seas. The research conducted by Aleleye-Wokoma *et al.* (2001) in the Bonney Estuary (Nigeria), Hwang *et al.* (1990), Chang & Tzeng (2000, 2004), and Hung & Shaw (2006) in Taiwan waters, Glamuzina *et al.* (2007) in Croatia, and Hakimelahi *et al.* (2010) in Iran has enhanced our knowledge of mullet ecology and the management of fisheries.

Studies conducted in India have predominantly concentrated on the diet and feeding habits, breeding biology, and fisheries of mullets. Notable contributions in this field include the work of Thakur (1970) in the Hooghly and Mahanadi estuary systems, and Luther (1963, 1973) in Mandapam waters on the southeast coast. In addition to the aforementioned sources, other notable works include Devasundaram (1954), Jones & Sujansingani (1954), Jhingran (1958, 1959, 1963), Jhingran & Mishra (1962), Jhingran & Natarajan (1966, 1969, 1973), Patnaik (1966), Murugan *et al.* (2012), Sahoo *et al.* (2012). Rao & Babu (2013), Murugan *et al.* (2014), Pramanik & Mohanty (2015), Rao & Babu (2016), Vaitheeswaran *et al.* (2016), Panda *et al.* (2018) and Durairaja *et al.* (2020). Several scientists, including myself, have conducted research on the LWR (length-weight relationship) and CF (condition factor) of *M. cephalus*, a fish species from India. The majority of this research was conducted along the east coast of India. In

their work, Panda *et al.* (2018) examined the grey mullet populations in Chilika Lake, India and observed a negative allometric growth pattern in *M. cephalus*. Murugan *et al.* (2012) conducted a study in the Vellar estuary, located on the South Coast of India. He discovered the presence of negative allometric growth in both the male and female populations. Vaitheeswaran *et al.* (2016) observed a significant negative relationship between the size of *M. cephalus* and its growth rate.

Although there have been gains, additional research is necessary for a complete understanding the elements that affect the growth patterns of *M. cephalus* populations, especially in relation to human-caused disturbances and changes in the environment. Hence, the study was conducted with the objective of finding crucial environmental factors that influence variations in growth. This research aims to provide valuable insights into the population dynamics and ecological resilience of grey mullet populations by combining field surveys, laboratory analyses, and statistical modelling approaches.

2.1.4 Gonadosomatic Index and Hepatosomatic Index

The relationship between reproduction and growth is a crucial trade-off in fish, as most reproductive characteristics, particularly female fecundity, and growth are influenced by body size. In addition, animals with uncertain development, such as fish, need to consider the expenses associated with survival and the quantity of energy that is available for reproduction. They must then adopt on how to distribute resources between current and future reproduction as a way to adapt to the changing environmental conditions. For example, if there is an upsurge in growth during the current spawning period, it would lead to a decrease in the number of offspring produced during that period. However, the larger size of the fish resulting from the growth surge could provide an advantage of increased fertility in the next reproductive season (Heino & Kaitala 1999).

The gonadosomatic index (GSI/ I_g) is a commonly used measure to compare the reproductive state of individuals or groups. It estimates the ratio between the weight of the gonads and the total or gutted body weight (De Vlaming

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

et al. 1982). The I_g has been widely employed to characterise the timing and duration of the spawning season (West 1990). Multiple research has effectively utilised I_g values to enhance the precision of predicting the maturity stage (McQuinn 1989; Hinton *et al.* 1997; Vitale *et al.* 2006; Ganias *et al.* 2007). McPherson *et al.* (2011) and Skjaeraasen *et al.* (2012) examined the suitability of I_g for evaluating the precision of maturity stage assignments.

Similarly, the mullet gonadosomatic index is employed to determine the spawning season. The term "GSI" was introduced by Meien in 1927, and since then, numerous researches have employed I_g as a reliable measure of gonad growth. The I_g offers valuable insights into the monthly variations in gonadal development of a species, making it a valuable tool for the management of the fishing and aquaculture sectors. The hepatosomatic index is calculated in relation to gonadal development. There is an inverse relationship between the size of the gonad and the size of the liver, meaning that as the gonad size increases, the liver size decreases. The hepatosomatic index has a substantial correlation with the gonadosomatic index, indicating that vitellogenesis utilises hepatic energy during reproduction (Albieri & Araújo 2010).

The hepatosomatic index (I_h) is a quantitative measure employed in the field of fisheries biology to evaluate the proportional size of a fish's liver in relation to its overall body weight. It offers significant data regarding the state and vitality of the liver, indicating several elements of the fish's general health and metabolic function. Although the detailed history of the hepatosomatic index may not be as extensively recorded as that of some other indices, its origin and application may be followed through significant milestones in fisheries research. The significance of the liver in fish physiology was acknowledged based on initial observations, in which researchers documented variations in liver size across several fish species (French *et al.* 1983). Nevertheless, specific definitions for formal indicators such as the hepatosomatic index were not established during this particular period. During the mid-20th century, advancements in biochemical and physiological studies in fisheries biology led researchers to acknowledge the liver's function in energy storage and metabolism (Jobling 2012). During this time, indices such as the hepatosomatic index were developed as instruments to

measure the size of the liver in relation to the general condition of the body. In the late 20th century, researchers began employing the hepatosomatic index in fisheries investigations. The researchers intended to confirm the usefulness of the index in evaluating the nutritional level, health, and reproductive state of fish populations. Validation studies frequently entailed the comparison of I_h values with alternative indices of fish health and reproductive status (Mommsen *et al.* 1992). The hepatosomatic index has gained significance as a useful tool in fishery management in recent years (French *et al.* 1983). The application of this method has expanded to include the monitoring of fish populations' health in response to human-caused and environmental influences. The hepatosomatic index's history mirrors the development of fisheries biology and the continuous pursuit of creating measurements that helps to understand the well-being, state, and ecological relationships of fish populations (Jobling 2012).

Several international research, including those by Aguirre & Cabello (2004) and Titouah *et al.* (2022), have investigated the habitat suitability index (I_h) of *M. cephalus*. In India, the majority of studies on I_h were conducted on the east coast by researchers such as Kumar *et al.* (2015), Pramanik & Mohanty (2016), Ali *et al.* (2017), and Kumar *et al.* (2022). Hence, this study was conducted to detect the alterations in the liver due to gonadal maturation.

2.2 METHODOLOGY

2.2.1 Study Area

The Gujarat coast, located on the Arabian Sea in western India, is known for its diverse landscape and abundant biodiversity. The Gujarat coast is predominantly comprised of coastal plains that span from the Gulf of Kutch in the north to the Gulf of Khambhat (previously known as the Gulf of Cambay) in the south. The topography of these plains is characterised by their relatively low elevation and includes sandy beaches, estuaries, mudflats, and marshy areas. The coastline is marked by numerous islands and peninsulas, including as the Kathiawar Peninsula and the islands of Pirotan and Bet Dwarka. These geographical features enhance the variety of environments found along the coastline. The Gujarat coast harbours a multitude of estuaries created by the merging of rivers with the sea. The estuaries in regions such as the Gulf of Khambhat and the Gulf of Kutch are home to vast mangrove forests. Mangroves are essential ecosystems that offer home for diverse species and function as natural barriers against coastal erosion and storm surges. These many habitats are crucial for the development, growth, and assimilation of nutrients by the organisms that inhabit them. The coastal waters of Gujarat are renowned for their bountiful populations of mullet fish (family Mugilidae). Mullet fishery make a significant contribution to the overall marine fish productivity of the state. These fish have significant commercial value and are caught for both local consumption and export markets.

Hence, the objective of the study was to assess the physico-chemical parameters (dissolved oxygen, pH, salinity, temperature, and total dissolved solids) as well as biological aspects including Length-Weight Relationship (LWR), Fulton's Condition Factor (CF/K), Gonadosomatic Index (GSI/I_g), and Hepatosomatic Index (HSI/I_h) of *Mugil cephalus* (Linnaeus 1758) in the coastal area of Gujarat. Therefore, a comparison was made between two distinct coastal environments, namely the Narmada estuary and Diu lagoon (20° 43' 46"N 70° 59' 13"E). Three stations were chosen in the Narmada estuary for the purpose of comparing the differences in growth and nutrient profile based on their microhabitat. These stations are Bharuch (22°25'78''N, 73O53'91''E), Bhadbhut

(21°40'70''N, 72°50'69''E), and Ambetha (21°40'81''N, 72°35'70''E). The methodology will primarily be classified into two basic categories (Fig. 2.1, 2.2).
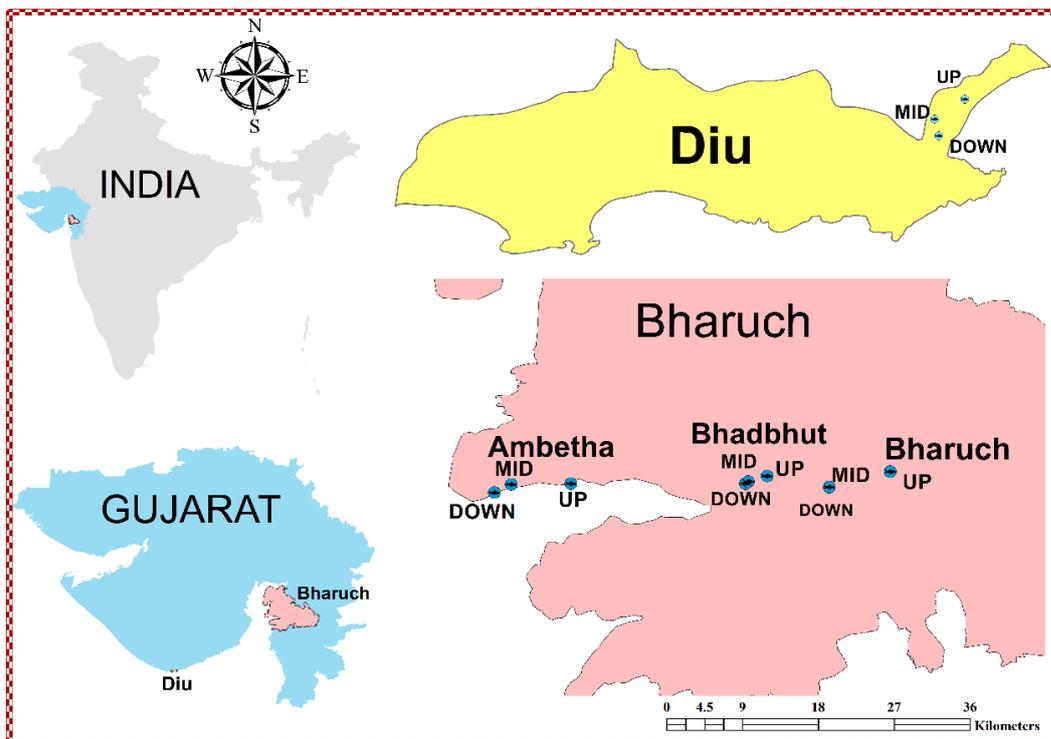

Figure 2.1: Study Site Location on GIS Map – Map showcasing the geographical locations of the collection points (upstream, midstream and downstream) of the selected study sites from Diu Lagoon and Narmada Estuary of Gujarat, India
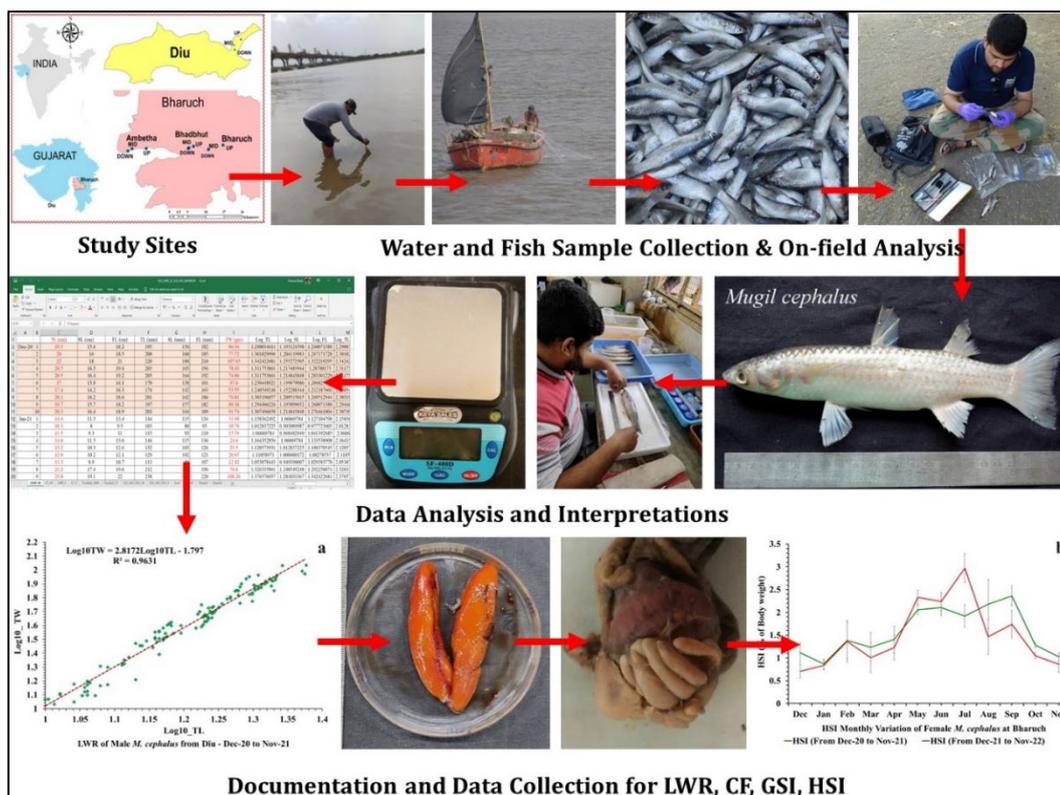
Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

Figure 2.2: Illustration of the sample collection and methodology of sample collection for physico-chemical parameters of water and length-weight relationship, condition factor, gonadosomatic index and hepatosomatic index of *M. cephalus* from Diu lagoon and Narmada estuary

2.2.2 Environmental (Water) parameters – Physico-Chemical Parameters

Water samples were collected from a designated study site at different points, including upstream, middle, and downstream, in order to assess the variability in physico-chemical parameters. Each site was separated by an estimated distance of 100 metres. Data was collected on monthly basis during spring tides over a span of two years, from December 2020 to November 2022 for the current study. The triplet measurements have been carried out to validate the empirical data or observed results.

2.2.2.1 pH

The pH levels at the upstream, midstream, and downstream locations of the selected study sites were carefully measured using an Aquasol Digital Handheld pH Metre (AM-PH-01) during a thorough two-year duration. The systematic and

thorough monitoring strategy yielded useful insights into the temporal fluctuations of pH within the studied aquatic habitat.

2.2.2.2 Temperature

The temperature at the selected study locations was monitored using a digital thermometer (MEXTECH – ST9264) between 08:00 AM and 11:00 AM at all sampling sites. This instrument enabled precise measurement of temperature fluctuations in the aquatic environment, providing crucial data for comprehending seasonal changes in specific habitats.

2.2.2.3 Salinity

The salinity of the selected study locations was evaluated using an ERMA Hand Refractometer, Model-MASTER-S10a, which is specifically developed for measuring salinity within a range of 0-100‰. This gadget utilises the phenomenon of refraction to measure the concentration of dissolved salts in water, thereby giving an indication of salinity. The refractometer determines the salinity level in parts per thousand (ppt/‰) by measuring the degree of light refraction when it passes through a sample. This method provides a rapid and easily transportable way to evaluate the salt content in different aquatic habitats, thereby offering vital information to the investigation of water quality and the health of ecosystems.

2.2.2.4 Dissolved Oxygen (Winkler method)

The reagents used in the Winkler method consist of Winkler-A (WA), which is made by dissolving 400 grammes of manganous chloride in 1000 ml of distilled water (DW) and keeping it in a polyethylene bottle. Winkler-B (WB) is made by dissolving and combining 360 grammes of potassium iodide and 100 grammes of sodium hydroxide in 1000 ml of distilled water (DW), which is then stored in a polyethylene bottle. Hydrochloric acid (HCl 50%) is produced by precisely combining 50 ml of concentrated HCl with 50 ml of distilled water (DW), and thereafter stored in a glass bottle covered with a ground cork. The preparation of a 0.02N sodium thiosulphate solution involves dissolving 5 grammes of sodium thiosulphate in distilled water and adjusting the volume to 1000 ml in a volumetric flask. The solution is then stored in a glass container with a ground cork. To

produce an indicator called starch solution, dissolve 1 g of starch in 100 ml of hot distilled water. Heat the mixture until it boils to guarantee that the starch is completely dissolved. Finally, let the solution to cool down. It is recommended to store this solution in a glass bottle with a stopper and use it within a week. In addition, a 0.02N standard iodate solution is made by dissolving 0.3567 g of potassium iodate (AR) in 500 ml of distilled water (DW) in a volumetric flask.

The necessary equipment for the technique consists of a 25 ml burette with a precision of 0.05 ml, 100 ml conical flasks, a magnetic stirrer, graded pipettes of 1 ml and 5 ml, and bulb pipettes of 10 ml and 50 ml. The utilisation of these specific reagents and apparatus is crucial for the precise and effective execution of the Winkler method, which is employed to detect the quantities of dissolved oxygen in water samples. The Winkler method for dissolved oxygen (DO) analysis consists of two primary stages: the preparation of a reagent blank and the examination of the sample. To prepare the reagent blank, transfer 50 ml of distilled water (DW) into a 100 ml conical flask. Then, add 3 ml of 50% hydrochloric acid and stir thoroughly. Subsequently, 1 ml of Winkler-B (WB) reagent and 1 ml of Winkler-A (WA) reagent are added one after the other, with thorough mixing after each addition. A blue colour indicator, namely starch solution, is introduced into the solution. The resulting solution is then subjected to titration using sodium thiosulphate from the burette until the blue colour completely vanishes. The burette reading at this phase is documented, and the experiment is repeated three times to ascertain the average burette reading (BR (b)).

To conduct a sample analysis, a 300-milliliter glass bottle specifically designed for Biological Oxygen Demand (BOD) is filled with the water sample. Then, 2 millilitres of manganese sulphate and alkali-iodide-azide reagents are added to the container with caution. The bottle is sealed with a cork to prevent the introduction of air and mixed by inverting it numerous times. Once the mixture has come to rest, 3 millilitres of hydrochloric acid with a concentration of 50% is introduced. The bottle is then aggressively agitated until all of the solid particles have completely dissolved. Next, transfer 50 millilitres of the sample into a 100 ml conical flask, and then add 10 millilitres of a potassium iodate solution with a concentration of 0.02N. the mixture were mixed thoroughly and then placed in a

dark environment for a duration of 5 minutes to facilitate the release of iodine. The iodine that has been freed is measured using a burette filled with sodium thiosulphate solution until the solution changes to a light yellow colour. One millilitre of starch indicator is introduced, and the titration proceeds progressively until the colour transitions from blue to colourless and remains colourless for a minimum of 30 seconds. The experiment is repeated thrice to get the average value of the burette reading (BR (ss)).

The normalcy (N) of sodium thiosulphate can be determined by applying the formula:

$$N = {10 \times 0.02}/{(BR\,(ss) - BR\,(b))}$$

The calculation of dissolved oxygen (DO) in one litre of the sample is determined using the formula:

$$DO\left(\frac{mg}{l}\right) = \frac{5.6 \times N \times (BR\,(ss) - BR\,(b)) \times V}{(V - 1)} \times \frac{1000}{a}$$

where,

BR (s) - represents the average titer value of the sample,

V - denotes the volume of the sample bottle (125 ml), and

a - represents the volume of the sample titrated (50 ml).

The dissolved oxygen (DO) concentration, measured in milligrammes per litre (mg/l), is determined by dividing the DO value (measured in millilitres per litre, ml/l) by 0.7, as described by Winkler in 1888.

2.2.2.5 TDS

Furthermore, to validate the findings, an alternative approach was employed alongside the use of the Aquasol Digital Handheld TDS Metre (AM-TDS-01; 0 to 19990 PPT) for the measurement of Total Dissolved Solids (TDS). For that gravimetric analysis method was opted, which involves evaporating a measured volume of a water sample until it is completely dry, and then measuring the weight of the remaining residue to calculate the concentration of total dissolved solids (TDS).

The gravimetric method was used to determine the concentration of Total Dissolved Solids (TDS). The following protocol was followed. At first, the water sample underwent filtration using Whatman Filter paper to eliminate any solid particles. The resultant filtrate, together with the rinse water, was then tranfered in a flask. Afterwards, a vacant, desiccated receptacle such as a porcelain or evaporating dish was measured to determine its initial mass. The liquid filtrate was thereafter transferred to the container, and the sample was kept in an oven at a temperature of 105°C for a duration of 24 hours in order to remove the liquid component through evaporation. After the drying process was finished, the container was taken out of the oven and left to cool in a desiccator for at least 1 hour until it reached the surrounding temperature in a dry air environment. Following the cooling process, the container was subsequently weighed.

To determine the concentration of TDS, the change in weight (B - A) of the container containing the dried residue was measured, where A represents the starting weight of the empty container and B indicates the weight of the container with the residue. The initial volume of the water sample (C) was also recorded. The concentration of Total Dissolved Solids (TDS) in milligrammes per litre (mg/L) was calculated using the following formula:

$$\text{Concentration} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{B - A}{C} \times 1000 \text{ mg/g} \times 1000 \text{ ml/L}$$

This technique aids accurate quantification of Total Dissolved Solids (TDS) content in the water sample, hence facilitating a full evaluation of its chemical composition.

2.2.3 Biological Aspects of Mugil cephalus (Linnaeus, 1758)

2.2.3.1 Length-weight relationship

The collection of *M. cephalus* specimens carried out on a monthly basis, coinciding with spring tides, for a continuous period of two years from December 2020 to November 2022. For LWR, total length (TL) was measured to the nearest 0.1 mm and total body weight (BW) to the nearest 0.1 g (Fig. 2.3). Total length was measured from the anterior-most tip of the snout to the tip of the upper lobe of the caudal fin. LWR were expressed by the equation

$$W = aL^b \text{ (Le Cren, 1951; Froese, 2006)}$$

where W and L represent weight and length, respectively. The parameters a and b in the formula were calculated using the logarithmic form of the LWR equation:

$$Log(BW) = Log(a) + b\, Log(TL) \text{ (Le Cren, 1951; Froese, 2006)}$$

In this relationship, 'a' indicates the rate of change with regards to length and 'b' represents the weight at unit length. Based on the slope (b) of the relation between weight and length, one can check whether the growth of a fish species is isometric (b = 3, all fish dimensions increase at the same rate), hypoallometric (b < 3, a fish increases less in weight than predicted by its increase in length, i.e., it becomes more elongated as it grows; also termed negative allometric) or hyperallometric (b > 3, a fish increases more in weight than predicted by its increase in length, i.e., it becomes less elongated or more roundish as it grows; also termed positive allometric) (Morey *et al.* 2003; Froese *et al.* 2006). Further, 95% confidence limits for a, b and the coefficient of determination were estimated. Student's t-test was performed for the null hypothesis of isometric growth (H_0: b = 3) using the equation $t_b = (b - 3)/s_b$, where S_b is the standard error of the slope (p = 0.05) (Morey *et al.* 2003). The LWR was assessed across study sites, both for distinct male and female populations and for a pooled sample (population), in order to determine whether the growth of each population is isometric or not.
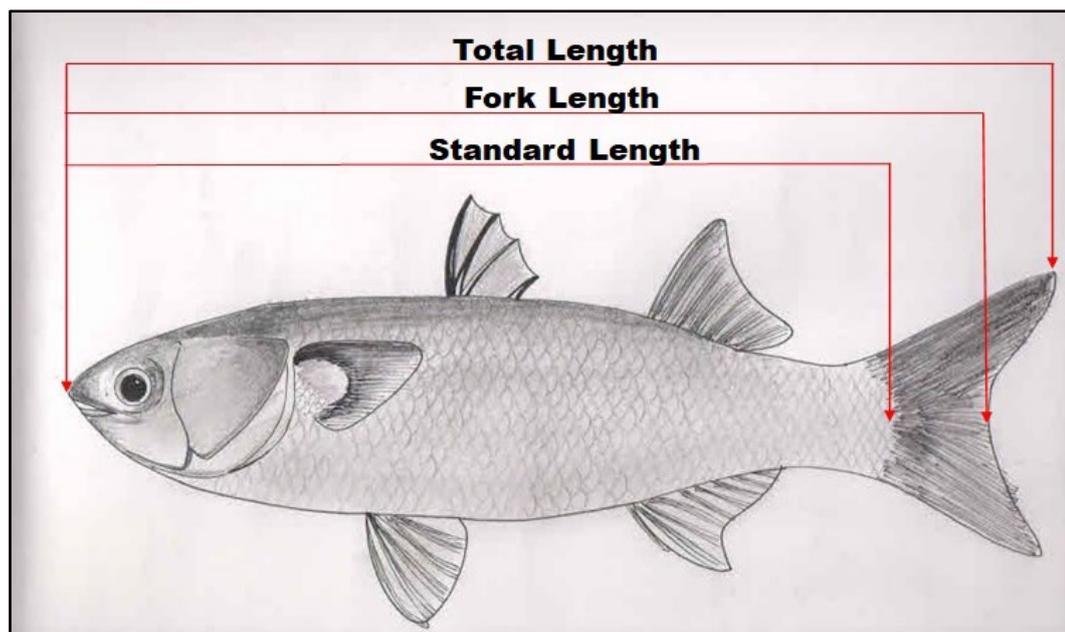
Figure 2.3: Representative image of *M. cephalus* (Linnaeus, 1758) showing morphometric measurements taken for biological aspects study

2.2.3.2 Condition factor

The 'coefficient of condition' or 'ponderal index' more popularly known as K factor indicate the well-being or reactive robustness of fish. Condition factor has been used as an indication of the 'fatness' or 'well-being' of fish, based on the assumption that heavier fish of a given length are in better condition (Bolger & Connolly 1989). For Fulton's *K*, *M. cephalus* specimens were collected every month from December 2020 to November 2022. Total length (TL) was measured to the nearest 0.1 mm and total body weight (BW) to the nearest 0.1 g. Total length was measured from the anterior-most tip of the snout to the tip of the upper lobe of the caudal fin. The *K* was estimated to evaluate the condition of each individual sampled in each month using the equation:

$$K = 100 \times (BW/TL^3)$$

where BW = total body weight in g and TL = total length in mm (Fulton 1904). An overall robustness or well-being for fish species is assumed when *K* values are equal or close to 1 (Jisr *et al.* 2018). Condition factor was compared among estuaries, both as separate male and female populations and as a pooled sample.

2.2.3.3 Gonadosomatic Index and Hepatosomatic Index of Mugil cephalus (Linnaeus, 1758).

In mullets, sexual dimorphism is not observed thus on-field identification of sex performed by slightly squeezing from belly towards anal pore. If golden yellow substance is observed then it is considered as female and leaking milt represent male sperms (McDonough *et al.* 2003) (Fig. 2.4). The mean I_g and I_h of the specimens were computed monthly to assess reproductive and metabolic conditions, respectively. The I_g, a key indicator of reproductive investment, was calculated using the formula:

$$I_g = \frac{BW - GW}{BW} \times 100$$

where GW denotes gonad weight and BW represents total body weight (Render *et al.* 1995). This index reflects the proportion of the organism's body weight allocated to its gonads, providing insights into reproductive activity and energy allocation strategies. Similarly, I_h, an important metric for assessing liver health and metabolic processes, was determined using the equation:

$$I_g = \frac{BW - LW}{BW} \times 100$$

where LW signifies liver weight and BW denotes total body weight (Albieri & Araújo 2010). I_h quantifies the proportion of the organism's body weight represented by the liver, serving as an indicator of metabolic investment and physiological condition.
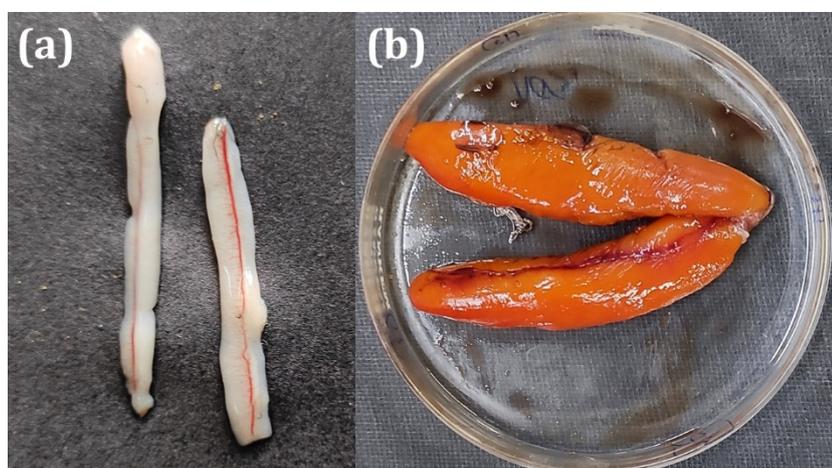

Figure 2.4: Gonads of *M. cephalus* (a) Male (b)Female

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

2.3 RESULTS

2.3.1 Environmental (Water) Parameters Analysis of Diu Lagoon and Narmada Estuary

2.3.1.1 pH

The pH data findings for the two successive years, December 2020 and November 2022, are displayed in Fig. 2.5a to 2.5h. The average pH values recorded in Diu were 8.47±0.19 and 8.39±0.15, in Bharuch were 8.79±0.31 and 8.77±0.26, in Bhadbhut were 8.66±0.21 and 8.63±0.21, and in Ambetha were 8.51±0.15 and 8.08±0.19. These values were obtained for two consecutive years, from December 2020 to November 2021 and from December 2021 to November 2022, respectively. In August 2022, the pH level was at its lowest point (7.77) in the upstream area of Ambetha. Conversely, in January 2021, the highest pH level (9.3) was recorded in the upstream area of Bharuch. The pH values recorded from the sampling sites in the upstream, midstream, and downstream locations are provided in Table 2.1, including the minimum and maximum values.

Table 2.1: Minimum and maximum pH values recorded from up, mid and down streams of Diu lagoon and Narmada estuary: Bharuch, Bhadbhut and Ambetha, Gujarat, India from December-2020 to November-2022

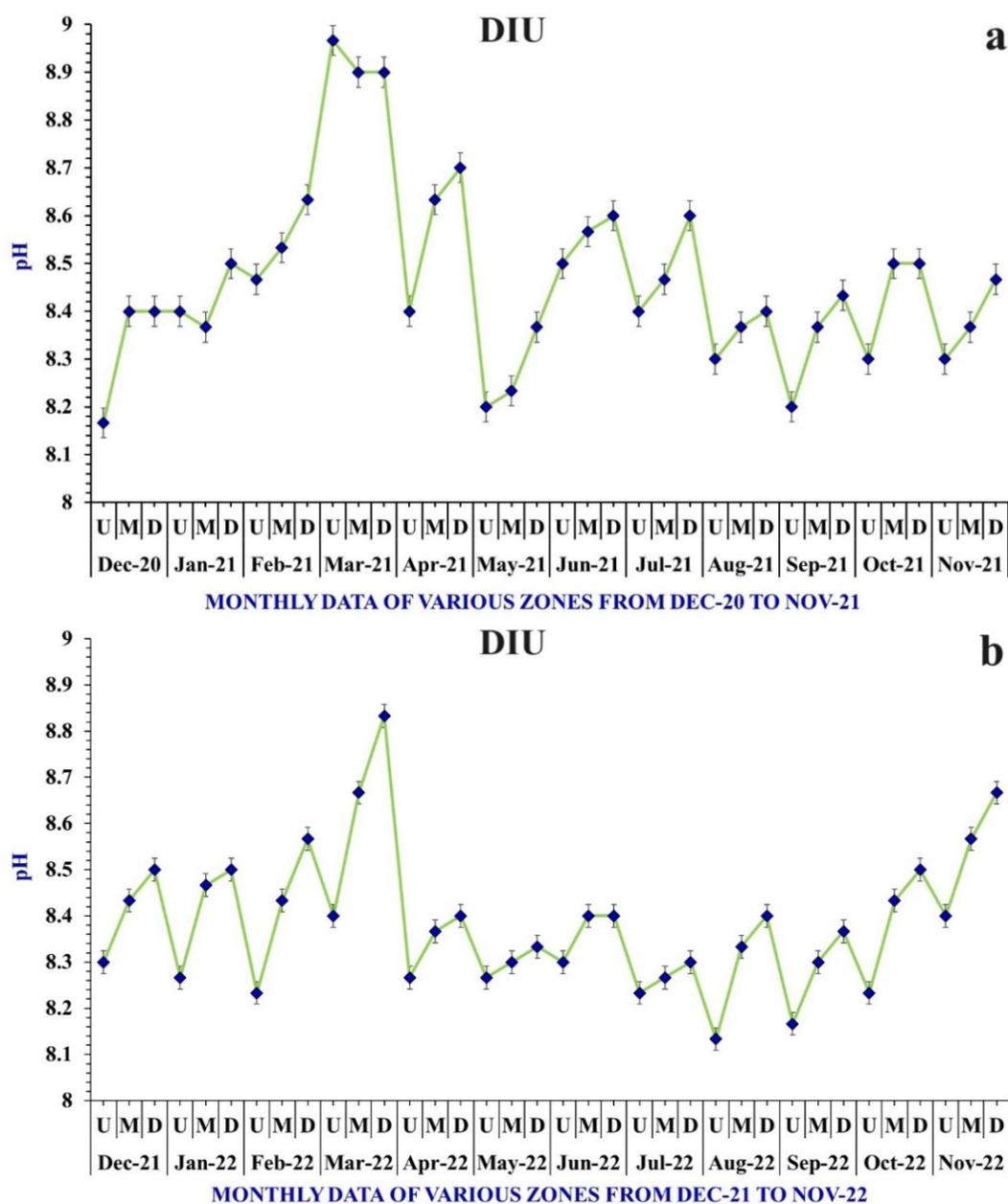| pH - 2020-2021				
	Diu	**Bharuch**	**Bhadbhut**	**Ambetha**
Min	8.17 U-Dec20	8.17 D-Aug21	8.23 U-Oct21	8.27 U-Dec20
Max	8.97 U-Mar21	9.30 U-Jan21	8.97 U/D-Apr21	8.87 U-Mar21
pH - 2021-2022				
Min	8.13 U-Aug22	8.27 M-Aug22	8.23 U-Aug/Sep22	7.77 U-Aug22
Max	8.83 D-Mar22	9.23 U-Jan22	8.90 M-Dec/Nov22 U-Mar/Apr22	8.70 D-Nov22

Figure 2.5 a & b: A graphical representation illustrating the dynamic changes in pH over the course of each month at Diu Lagoon: (a) from December-2020 to November-2021, (b) from December-2021 to November-2022
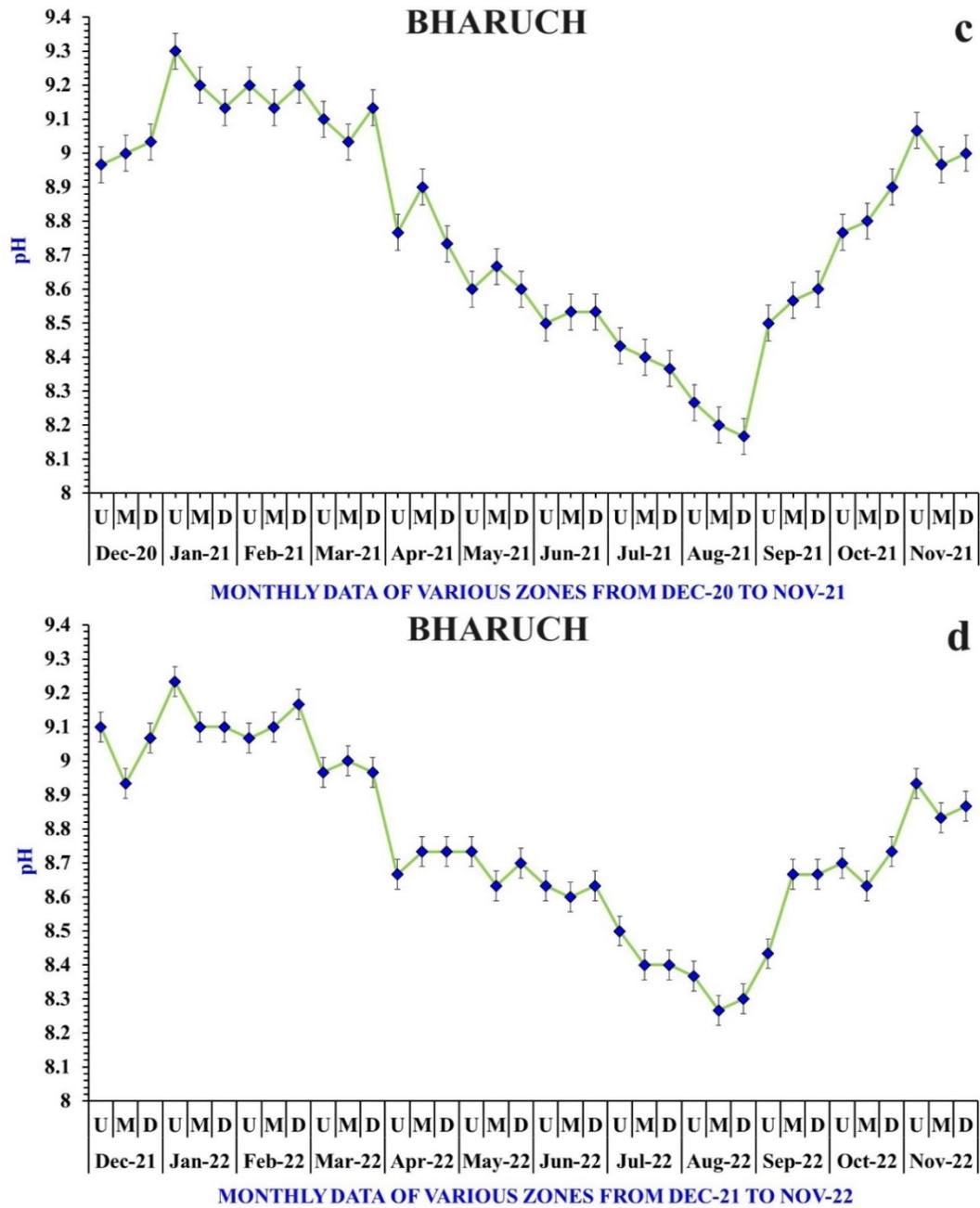
Figure 2.5 c & d: A graphical representation illustrating the dynamic changes in pH over the course of each month at Narmada Estuary – Bharuch Site: (c) from December-2020 to November-2021, (d) from December-2021 to November-2022
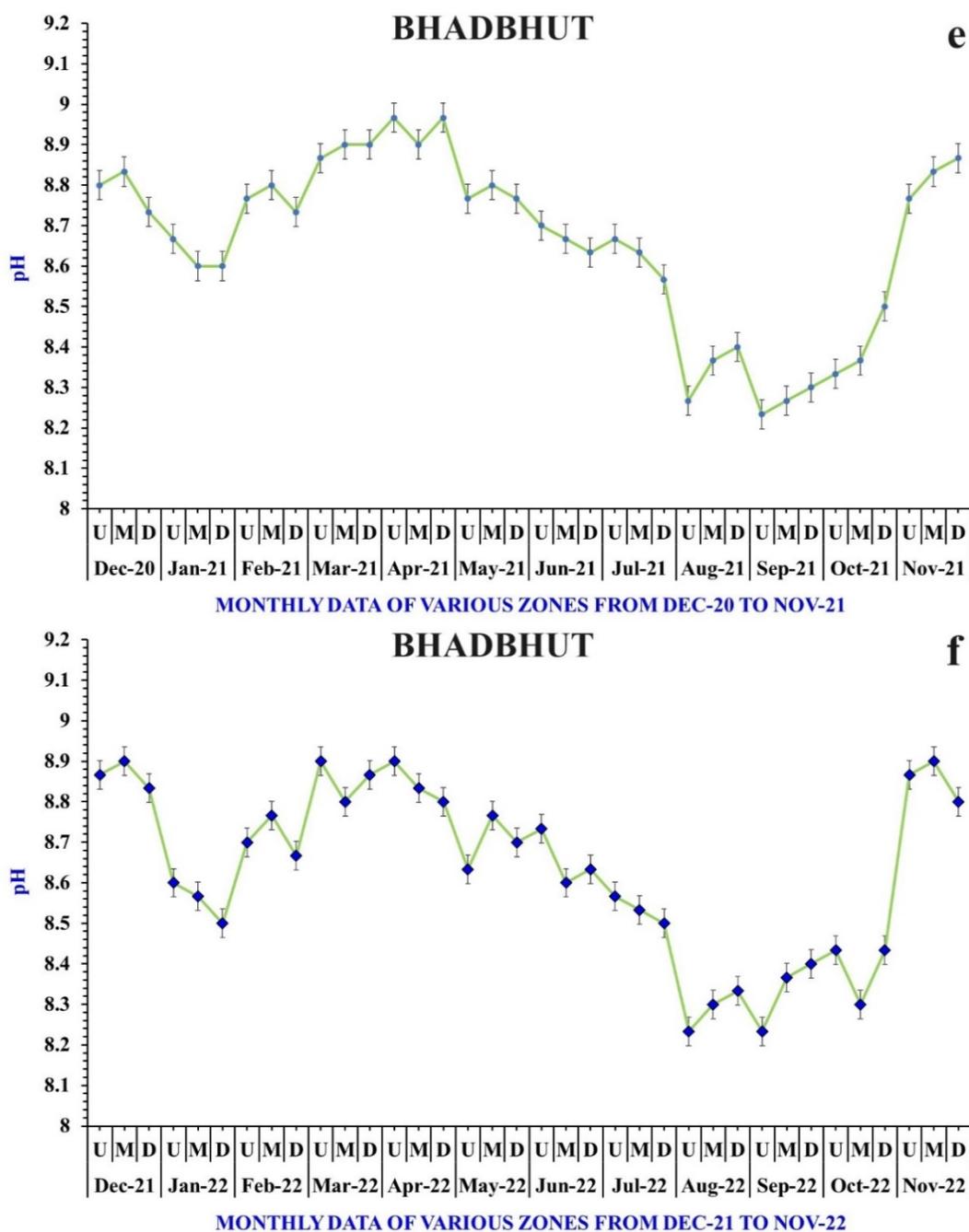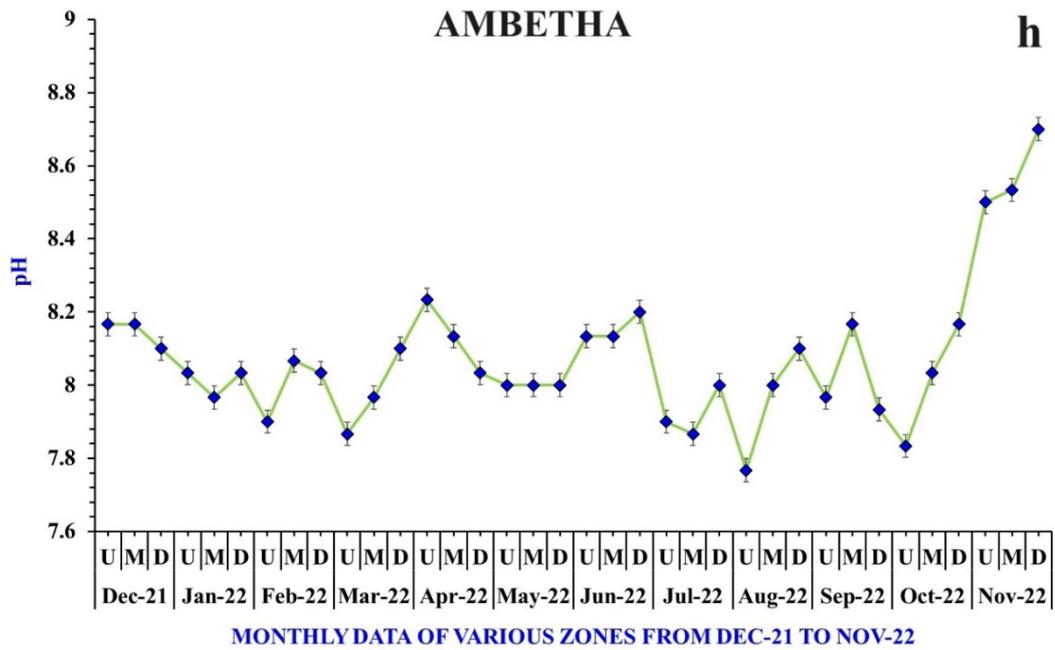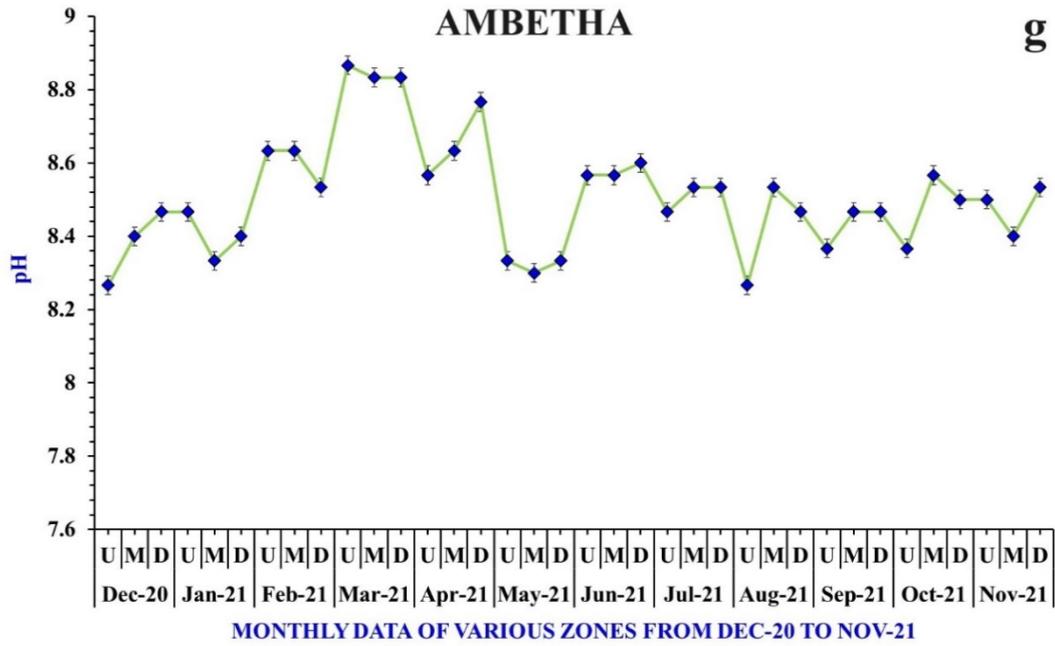
Figure 2.5 e & f: A graphical representation illustrating the dynamic changes in pH over the course of each month at Narmada Estuary – Bhadbhut Site: (e) from December-2020 to November-2021, (f) from December-2021 to November-2022

Figure 2.5 g & h: A graphical representation illustrating the dynamic changes in pH over the course of each month at Narmada Estuary – Ambetha Site: (g) from December-2020 to November-2021, (h) from December-2021 to November-2022

2.3.1.2 Temperature

Surface water temperature data is displayed in Fig. 2.6a to 2.6h for each of the following years. The average temperature values for Diu, Bharuch, Bhadbhut, and Ambetha were recorded for two consecutive years i.e., from December 2020 to November 2021 and from December 2021 to November 2022. The average temperature in Diu ranged from 28.10±3.03°C to 28.47±2.82°C, in Bharuch from 28.86±2.96°C to 28.90±2.75°C, in Bhadbhut from 28.82±3.39°C to 29.04±3.32°C, and in Ambetha from 28.63±3.01°C to 28.83±2.83°C. In December 2020, the coldest temperature of 21.9°C was observed upstream of Diu. Conversely, in June 2022, the hottest temperature of 33.9°C was recorded upstream of Bhadbhut. The table 2.2 provides the minimum and maximum temperature values recorded from the sample locations located upstream, midstream, and downstream.

Table 2.2: Minimum and maximum Temperature values recorded from up, mid and down streams of Diu lagoon and Narmada estuary: Bharuch, Bhadbhut and Ambetha, Gujarat, India from December-2020 to November-2022

Temperature - 2020-2021			
	Diu	**Bharuch**	**Bhadbhut**	**Ambetha**
Min	21.90°C U-Dec20	23.77°C D-Dec21	23.53°C U-Dec20	22.5°C D-Dec20
Max	32.67°C U-Apr21	32.8°C U-May21	33.73°C U-Jun21	32.77°C U-Apr21
Temperature - 2021-2022			
Min	23.83°C D-Dec21	24.53°C M-Nov22	24.30°C U-Jan22	24.20°C D-Feb22
Max	32.80°C U-Apr22	33.3°C D-May22	33.9°C U-Jun22	33.57°C U-Apr22

Figure 2.6 a & b: A graphical representation illustrating the dynamic changes in Temperature (°C) over the course of each month at Diu Lagoon: (a) from December-2020 to November-2021, (b) from December-2021 to November-2022
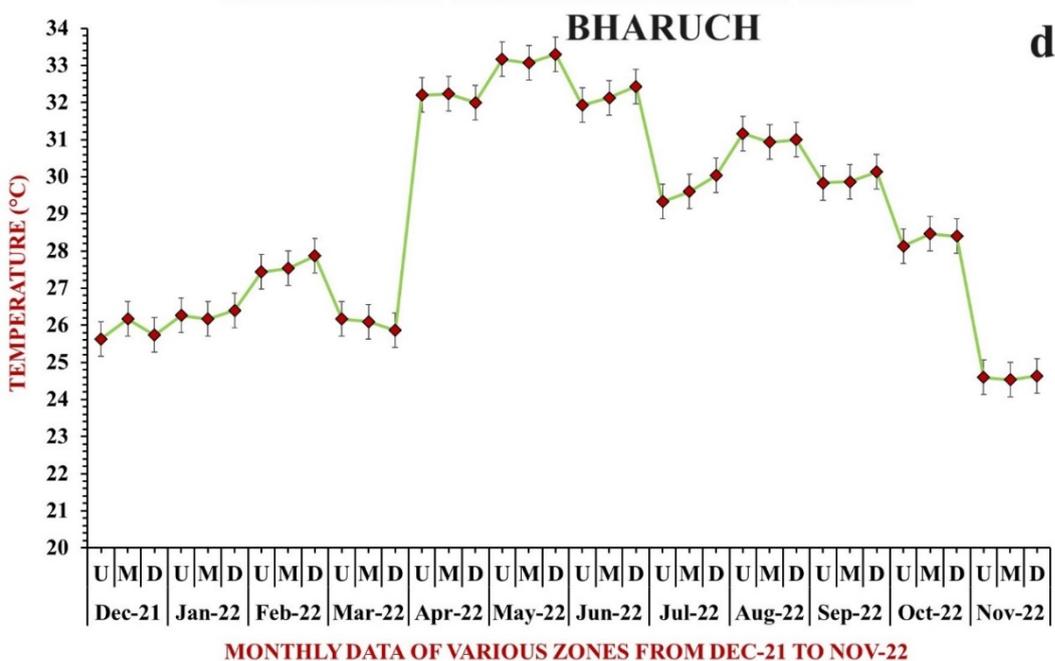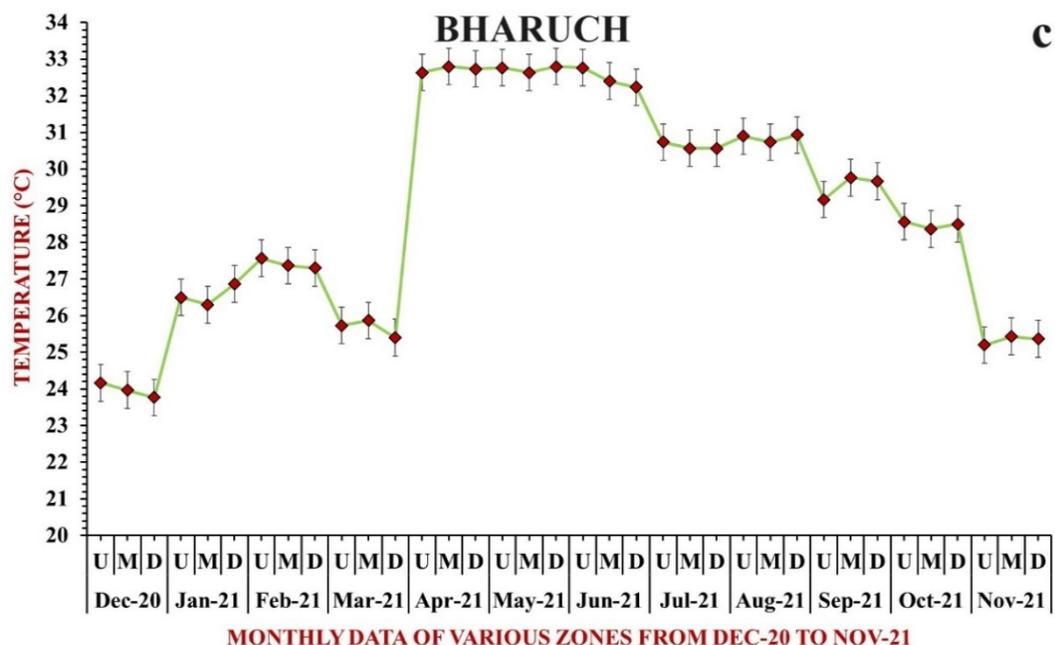
Figure 2.6 c & d: A graphical representation illustrating the dynamic changes in Temperature (°C) over the course of each month at Narmada Estuary – Bharuch Site: (c) from December-2020 to November-2021, (d) from December-2021 to November-2022

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)
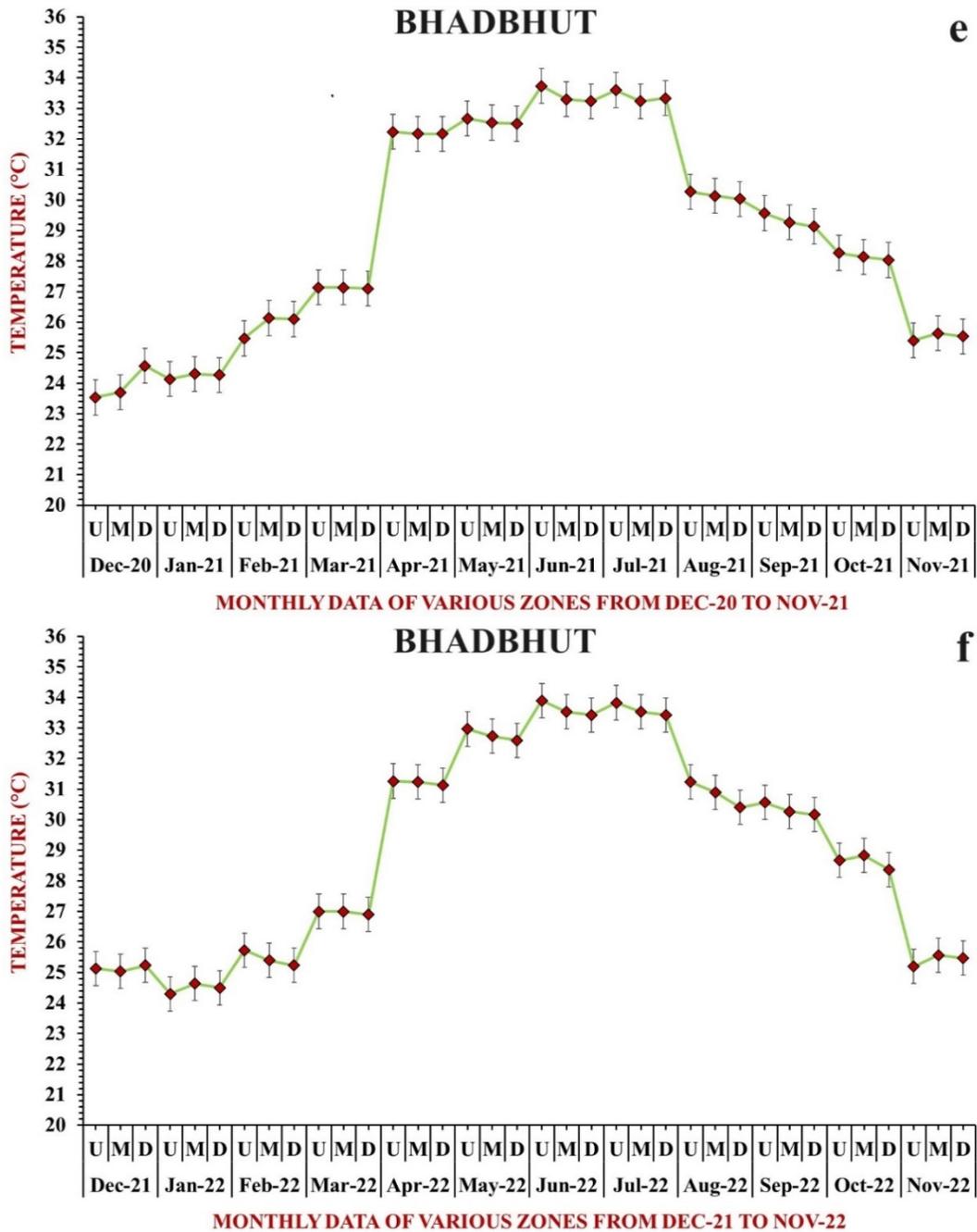
Figure 2.6 e & f: A graphical representation illustrating the dynamic changes in Temperature (°C) over the course of each month at Narmada Estuary – Bhadbhut Site: (e) from December-2020 to November-2021, (f) from December-2021 to November-2022
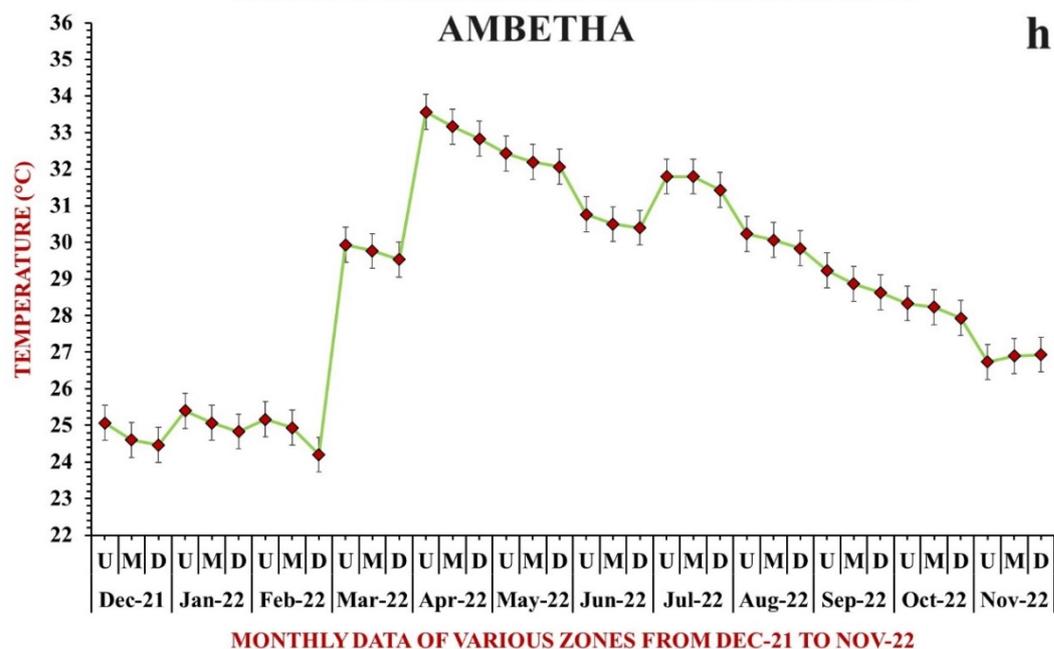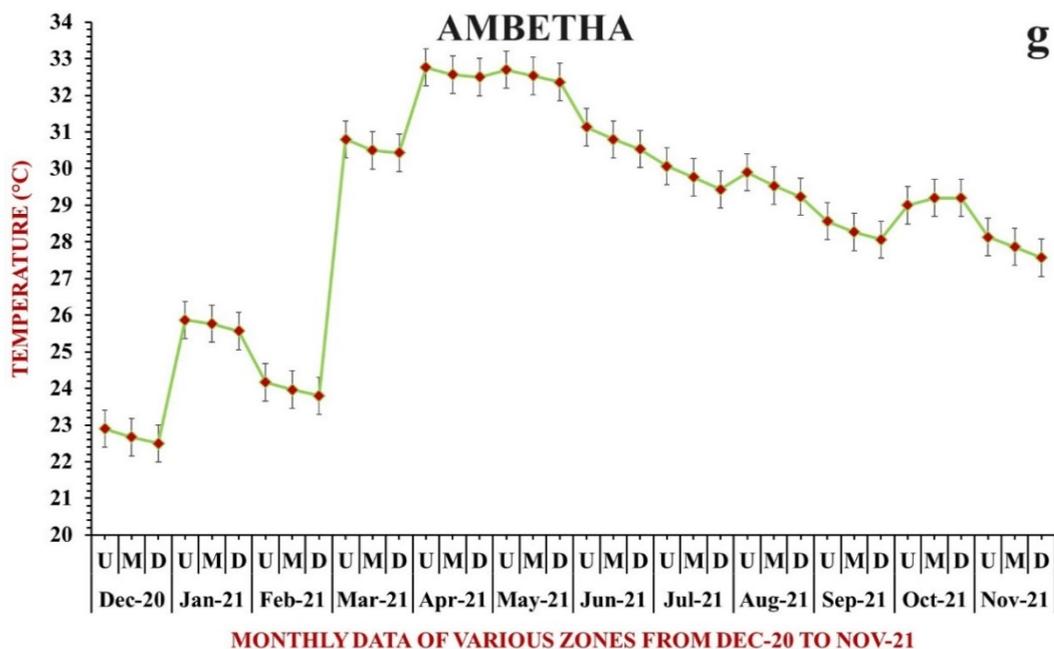
Figure 2.6 g & h: A graphical representation illustrating the dynamic changes in Temperature (°C) over the course of each month at Narmada Estuary – Ambetha Site: (g) from December-2020 to November-2021, (h) from December-2021 to November-2022

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

2.3.1.3 Salinity

The salinity data is displayed in Fig. 2.7a to 2.7h for both consecutive years of the research. The average salinity values reported in Diu were 39.44±1.00 ppt and 39.83±1.19 ppt, in Bharuch they were 12.71±4.04 ppt and 12.71±3.74 ppt, in Bhadbhut they were 17.01±4.93 ppt and 17.11±4.98 ppt, and in Ambetha they were 36.95±1.11 ppt and 36.65±1.24 ppt. In January 2021, the lowest salinity of 7.83 parts per thousand (ppt) was measured at the mid-stream of Bharuch. Conversely, in February 2022, the highest salinity of 42 ppt was recorded at the mid-stream of Diu. Table 2.3 provides the minimum and maximum salinity values obtained from the sampling locations located upstream, midstream, and downstream.

Table 2.3: Minimum and maximum Salinity values recorded from up, mid and down streams of Diu lagoon and Narmada estuary: Bharuch, Bhadbhut and Ambetha, Gujarat, India from December-2020 to November-2022

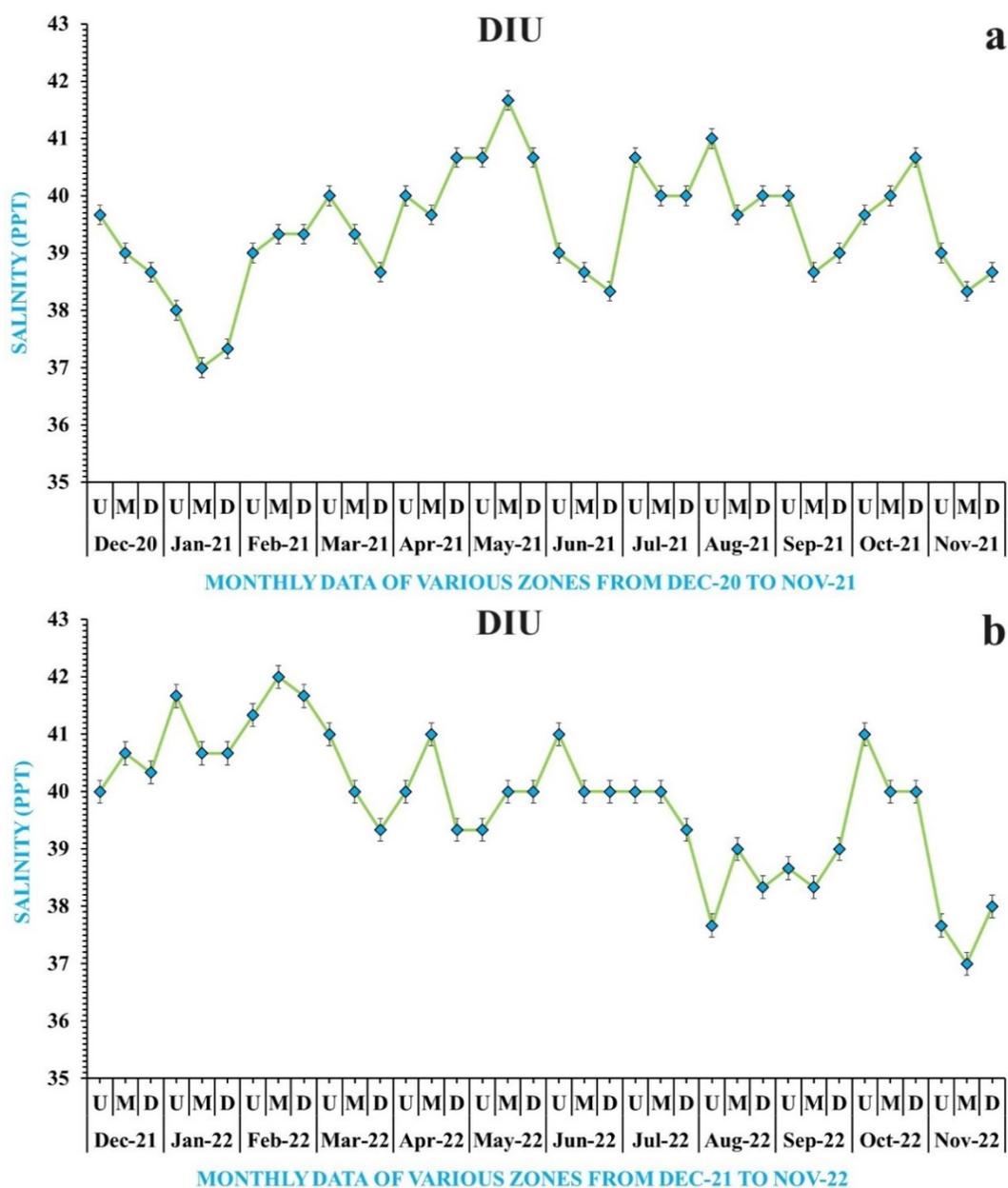|	Diu	Bharuch	Bhadbhut	Ambetha
Salinity - 2020-2021				
Min	37.00 ppt M-Jan21	7.83 ppt M-Jan21	11.33 ppt U-Nov21	34.33 ppt U-Oct21
Max	41.67 ppt M-May21	19.00 ppt M-Jun21/ D-Aug21	24.67 ppt D-Aug21	39.00 ppt D-Apr21
Salinity - 2021-2022				
Min	37.00 ppt M-Nov22	8.33 ppt M-Jan22	10.33 ppt U-Nov22	34.33 ppt M-Oct22/ U-Jul22
Max	42.00 ppt M-Feb22	19.67 ppt D-Aug22	24.67 ppt D-Aug/ D-Sep22	38.67 ppt D-Mar22/ D-Jan22

Figure 2.7 a & b: A graphical representation illustrating the dynamic changes in Salinity (PPT) over the course of each month at Diu Lagoon: (a) from December-2020 to November-2021, (b) from December-2021 to November-2022
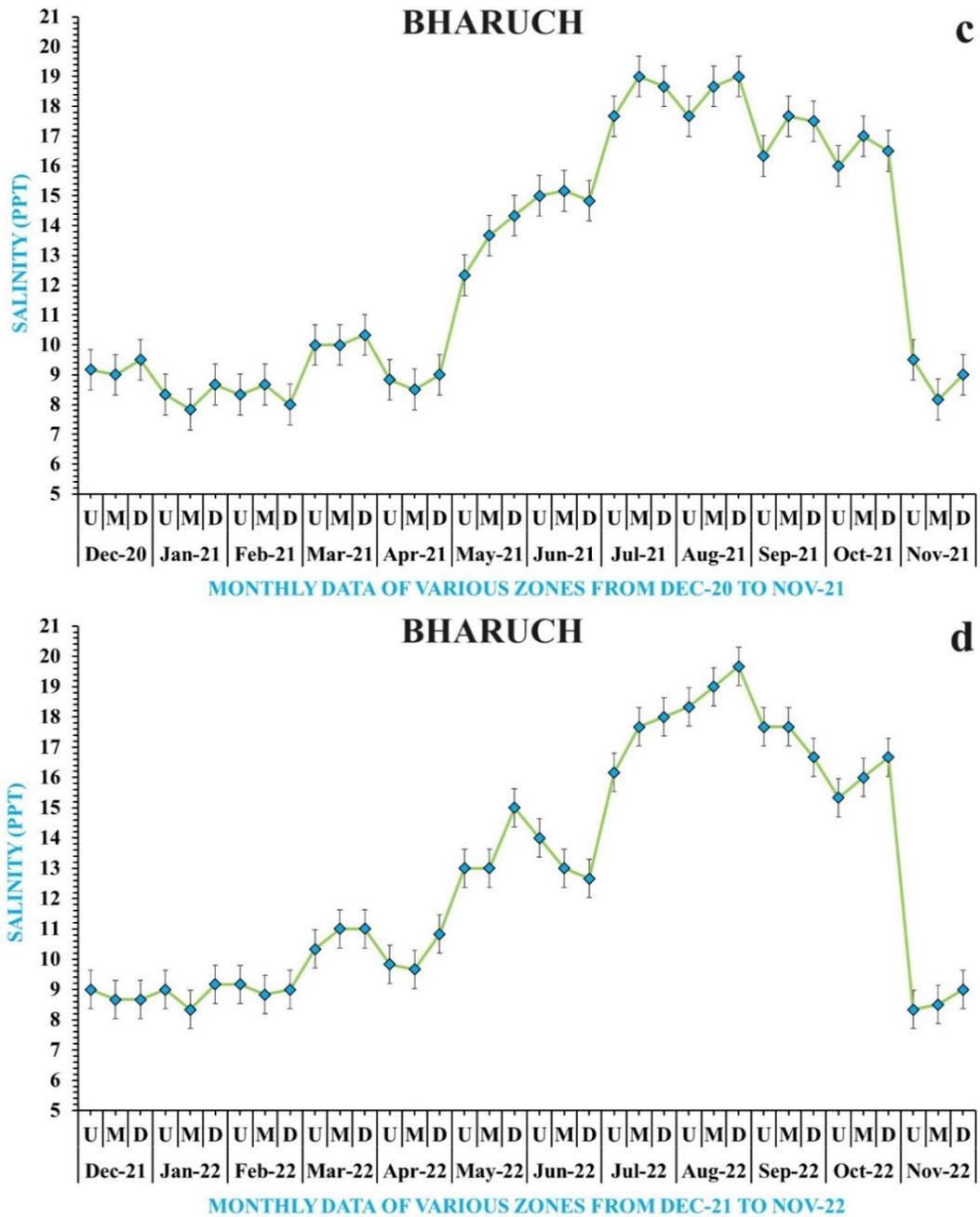
Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

Figure 2.7 c & d: A graphical representation illustrating the dynamic changes in Salinity (PPT) over the course of each month at Narmada Estuary – Bharuch Site: (c) from December-2020 to November-2021, (d) from December-2021 to November-2022

Figure 2.7 e & f: A graphical representation illustrating the dynamic changes in Salinity (PPT) over the course of each month at Narmada Estuary – Bhadbhut Site: (e) from December-2020 to November-2021, (f) from December-2021 to November-2022
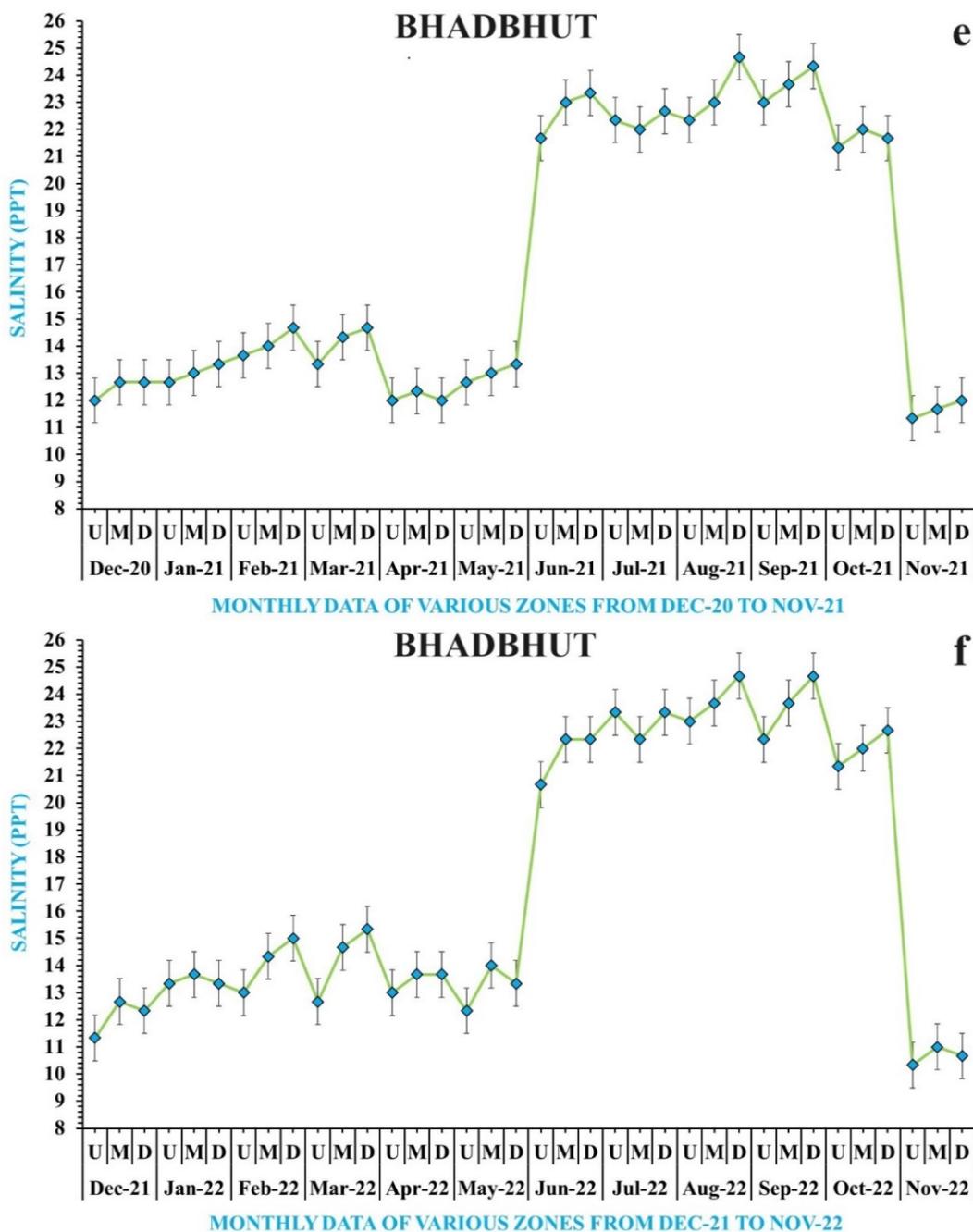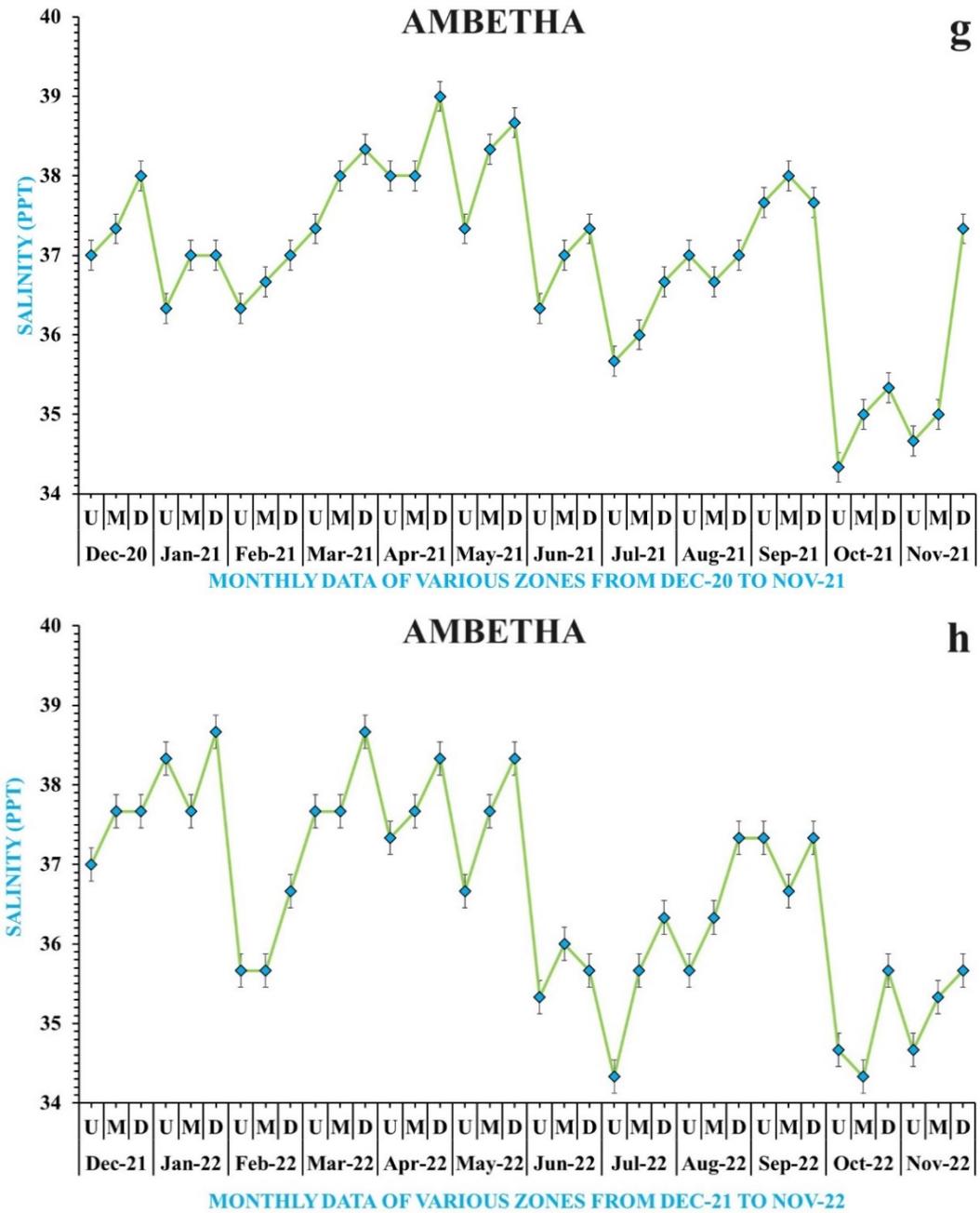
Figure 2.7 g & h: A graphical representation illustrating the dynamic changes in Salinity (PPT) over the course of each month at Narmada Estuary – Ambetha Site: (g) from December-2020 to November-2021, (h) from December-2021 to November-2022

2.3.1.4 Dissolved Oxygen

The Dissolved Oxygen (DO) data is displayed in Fig. 2.8a to 2.8h for both consecutive years of the investigation. The average Dissolved Oxygen values for Diu were 7.84±0.22mg/l and 7.86±0.21mg/l, for Bharuch they were 4.94±0.21mg/l and 4.92±0.24mg/l, for Bhadbhut they were 5.90±0.24mg/l and 6.00±0.14mg/l, and for Ambetha they were 6.63±0.22mg/l and 6.64±0.21mg/l. In May 2022, the lowest level of Dissolved Oxygen was measured upstream region of Bharuch at 4.56mg/l. Conversely, in January 2021, the highest level of Dissolved Oxygen was recorded from upstream of Diu at 8.3mg/l. The table 2.4 provides the minimum and maximum Dissolved Oxygen values obtained from the sampling locations located upstream, midstream, and downstream.

Table 2.4: Minimum and maximum Dissolved Oxygen (mg/l) values recorded from up, mid and down streams of Diu lagoon and Narmada estuary: Bharuch, Bhadbhut and Ambetha, Gujarat, India from December-2020 to November-2022

	Dissolved Oxygen - 2020-2021			
	Diu	**Bharuch**	**Bhadbhut**	**Ambetha**
Min	7.57 mg/l	4.57 mg/l	5.51 mg/l	6.32 mg/l
	U-Jun21	U-Apr21	D-Apr21	U-Jun21
Max	8.30 mg/l	5.31 mg/l	6.54 mg/l	7.09 mg/l
	U-Jan21	D-Nov21	U-Feb21	U-Jan21
	Dissolved Oxygen - 2021-2022			
Min	7.51 mg/l	4.56 mg/l	5.71 mg/l	6.30 mg/l
	D-Aug22	U-May22	U-Jun22	D-Aug22
Max	8.23 mg/l	5.63 mg/l	6.20 mg/l	7.00 mg/l
	D-Jan22	D-Oct22	U-Mar22	D-Jan22

Figure 2.8 a & b: A graphical representation illustrating the dynamic changes in Dissolved Oxygen (mg/l) concentrations over the course of each month at Diu Lagoon: (a) from December-2020 to November-2021, (b) from December-2021 to November-2022

Figure 2.8 c & d: A graphical representation illustrating the dynamic changes in Dissolved Oxygen (mg/l) concentrations over the course of each month at Narmada Estuary – Bharuch Site: (c) from December-2020 to November-2021, (d) from December-2021 to November-2022

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)
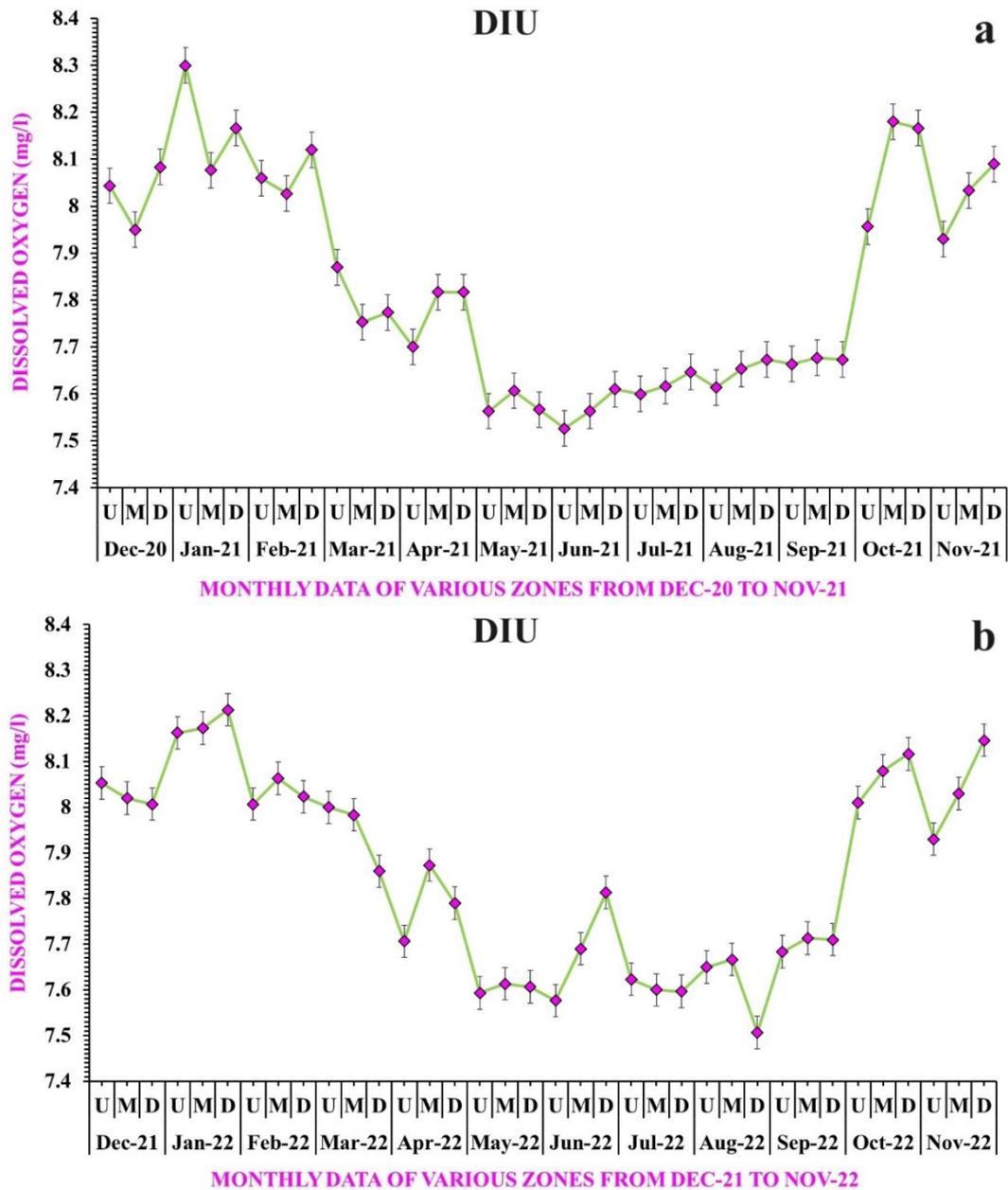
Figure 2.8 e & f: A graphical representation illustrating the dynamic changes in Dissolved Oxygen (mg/l) concentrations over the course of each month at Narmada Estuary – Bhadbhut Site: (e) from December-2020 to November-2021, (f) from December-2021 to November-2022
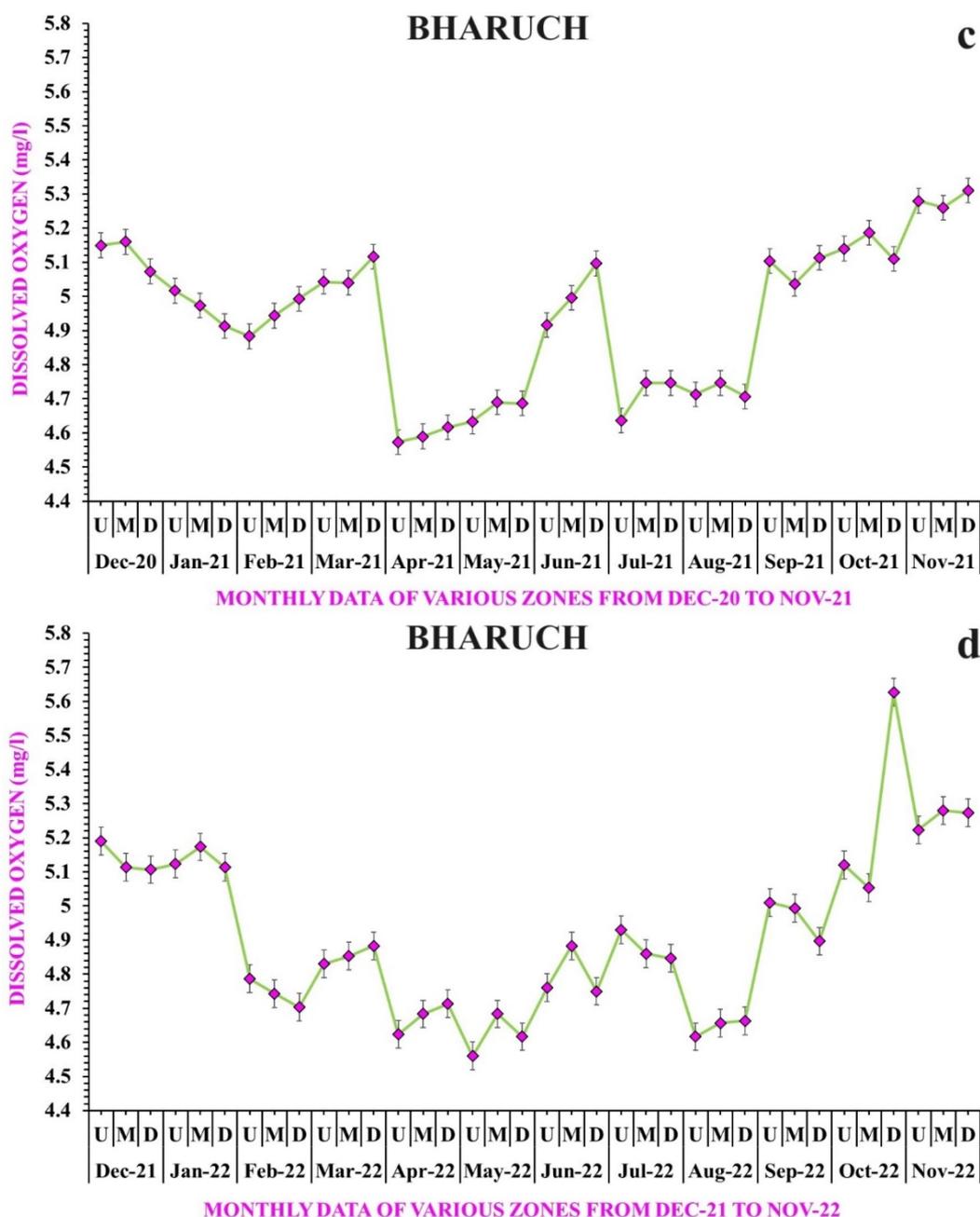
Figure 2.8 g & h: A graphical representation illustrating the dynamic changes in Dissolved Oxygen (mg/l) concentrations over the course of each month at Narmada Estuary – Ambetha Site: (g) from December-2020 to November-2021, (h) from December-2021 to November-2022
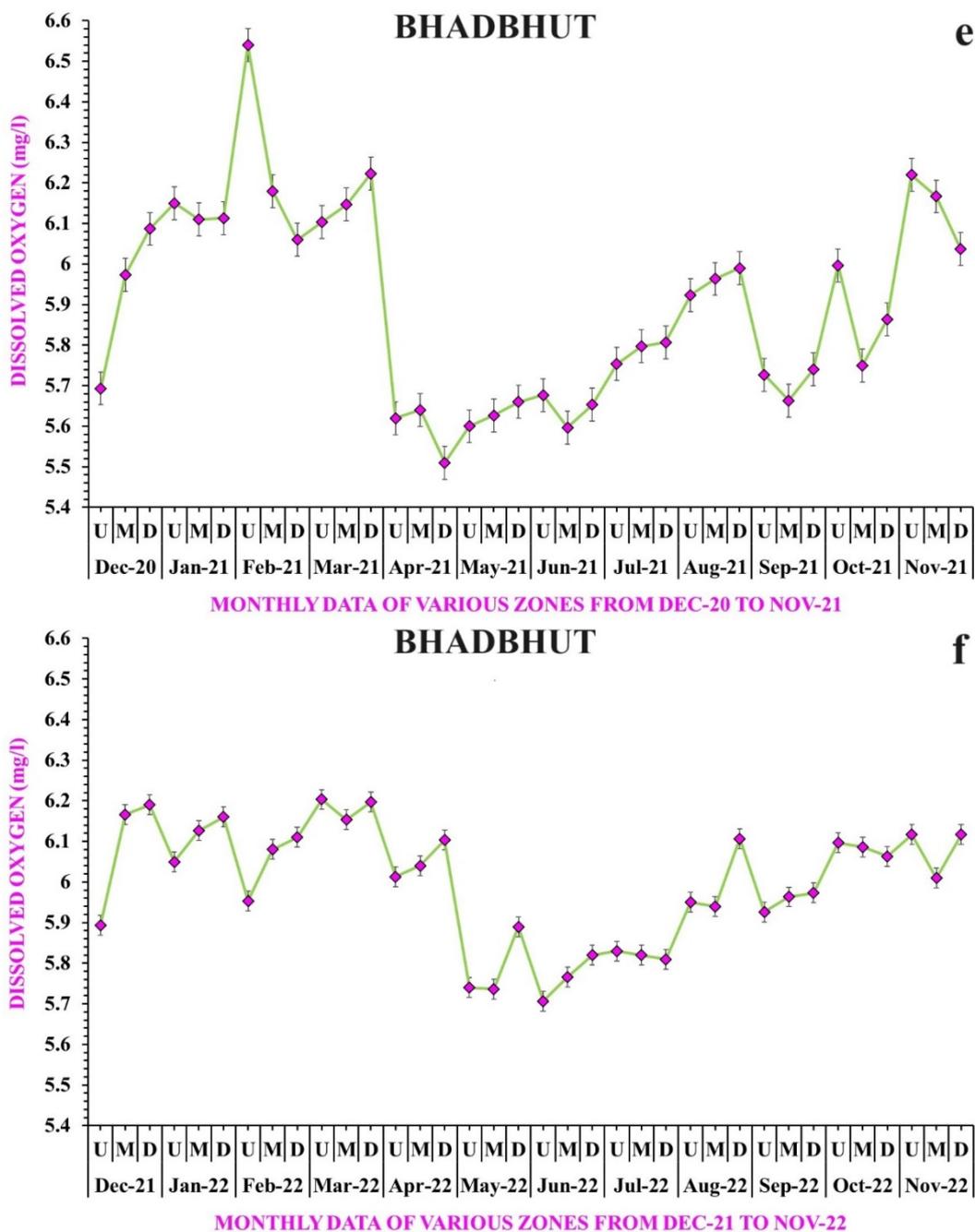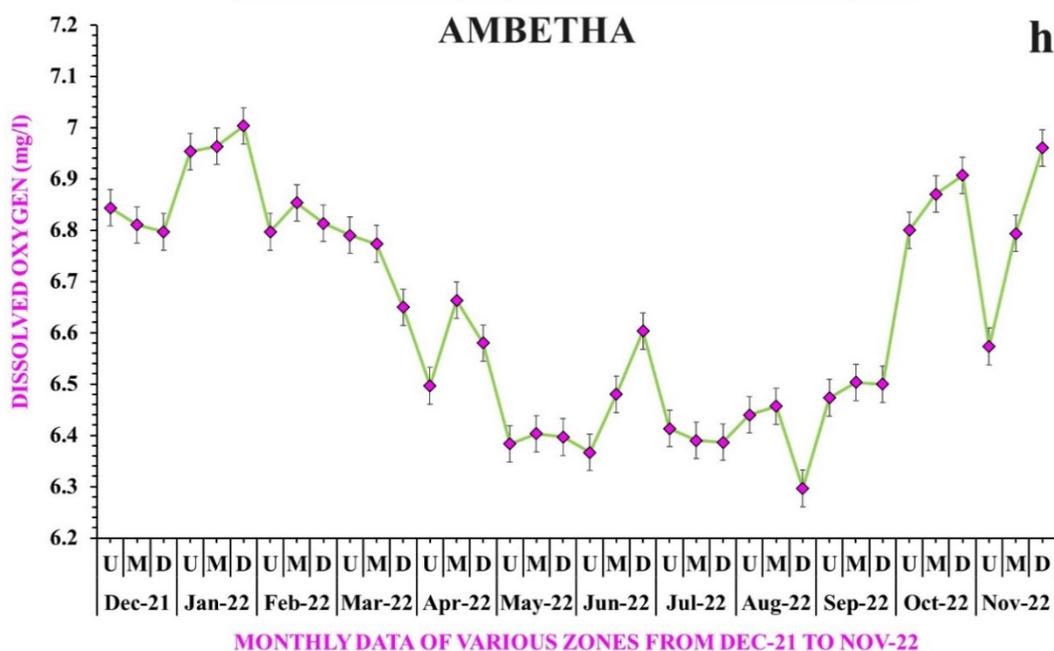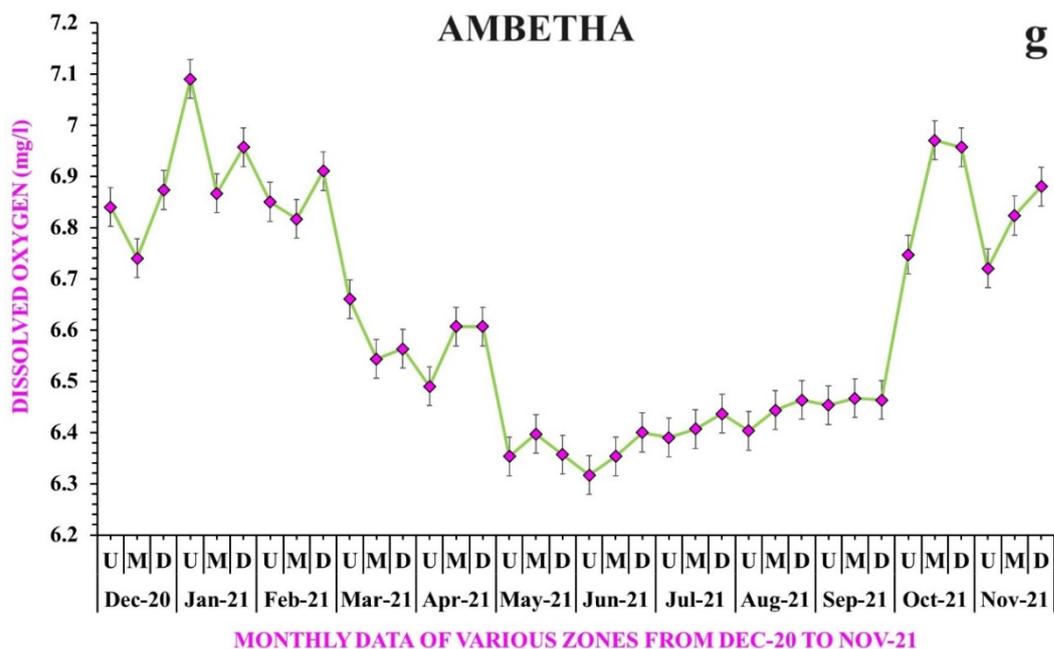
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2.3.1.5 Total Dissolved Solids

The Fig. 2.9a to 2.9h display the Total Dissolved Solids (TDS) for the consecutive years spanning from December 2020 to November 2022. The average total dissolved solids (TDS) values from Diu were 53334±7073.94mg/l and 52110.77±7703.24mg/l for the consecutive years Dec-20 to Nov-21 and Dec-21 to Nov-22, respectively. Similarly, Bharuch recorded TDS values of 4732.27±2721.69mg/l and 4971.20±2581.86mg/l, while Bhadbhut had TDS values of 10351.57±3856.38mg/l and 9581.68±4030.79mg/l. Ambetha had the highest TDS values, with 52980.46±6209.78mg/l and 52863.80±6038.36mg/l for the same time periods. In December 2021, the TDS level was lowest at 2950mg/l in the mid-stream of Bhadbhut. Conversely, in August 2022, the maximum TDS level of 68730mg/l was recorded in the down-stream of Ambetha. The table 2.5 provides the minimum and maximum Total Dissolved Solids (TDS) values obtained from the sampling sites located upstream, midstream, and downstream.

Table 2.5: Minimum and maximum Total Dissolved Solids (mg/l) values recorded from up, mid and down streams of Diu lagoon and Narmada estuary: Bharuch, Bhadbhut and Ambetha, Gujarat, India from December-2020 to November-2022

	Total Dissolved Solids - 2020-2021			
	Diu	**Bharuch**	**Bhadbhut**	**Ambetha**
Min	42550 mg/l	1366.67 mg/l	4050 mg/l	44350 mg/l
	D-Jan21	U-Jan21	U-Apr21	D-Jan21
Max	68350 mg/l	9826.67 mg/l	15100 mg/l	68510 mg/l
	D-Aug21	D-Aug21	M-Sep21	D-Aug21
	Total Dissolved Solids - 2021-2022			
Min	39283.33 mg/l	1866.67 mg/l	2950 mg/l	45540 mg/l
	D-Dec21	U-Jan22	M-Dec21	D-Jan22
Max	64666.67 mg/l	9803.33 mg/l	14693.33 mg/l	68730 mg/l
	M-Aug22	M-Aug22	M-Oct22	D-Aug22

Figure 2.9 a & b: A graphical representation illustrating the dynamic changes in Total Dissolved Solids (mg/l) over the course of each month at Diu Lagoon: (a) from December-2020 to November-2021, (b) from December-2021 to November-2022

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

Figure 2.9 c & d: A graphical representation illustrating the dynamic changes in Total Dissolved Solids (mg/l) over the course of each month at Narmada Estuary – Bharuch Site: (c) from December-2020 to November-2021, (d) from December-2021 to November-2022

Figure 2.9 e & f: A graphical representation illustrating the dynamic changes in Total Dissolved Solids (mg/l) over the course of each month at Narmada Estuary – Bhadbhut Site: (e) from December-2020 to November-2021, (f) from December-2021 to November-2022
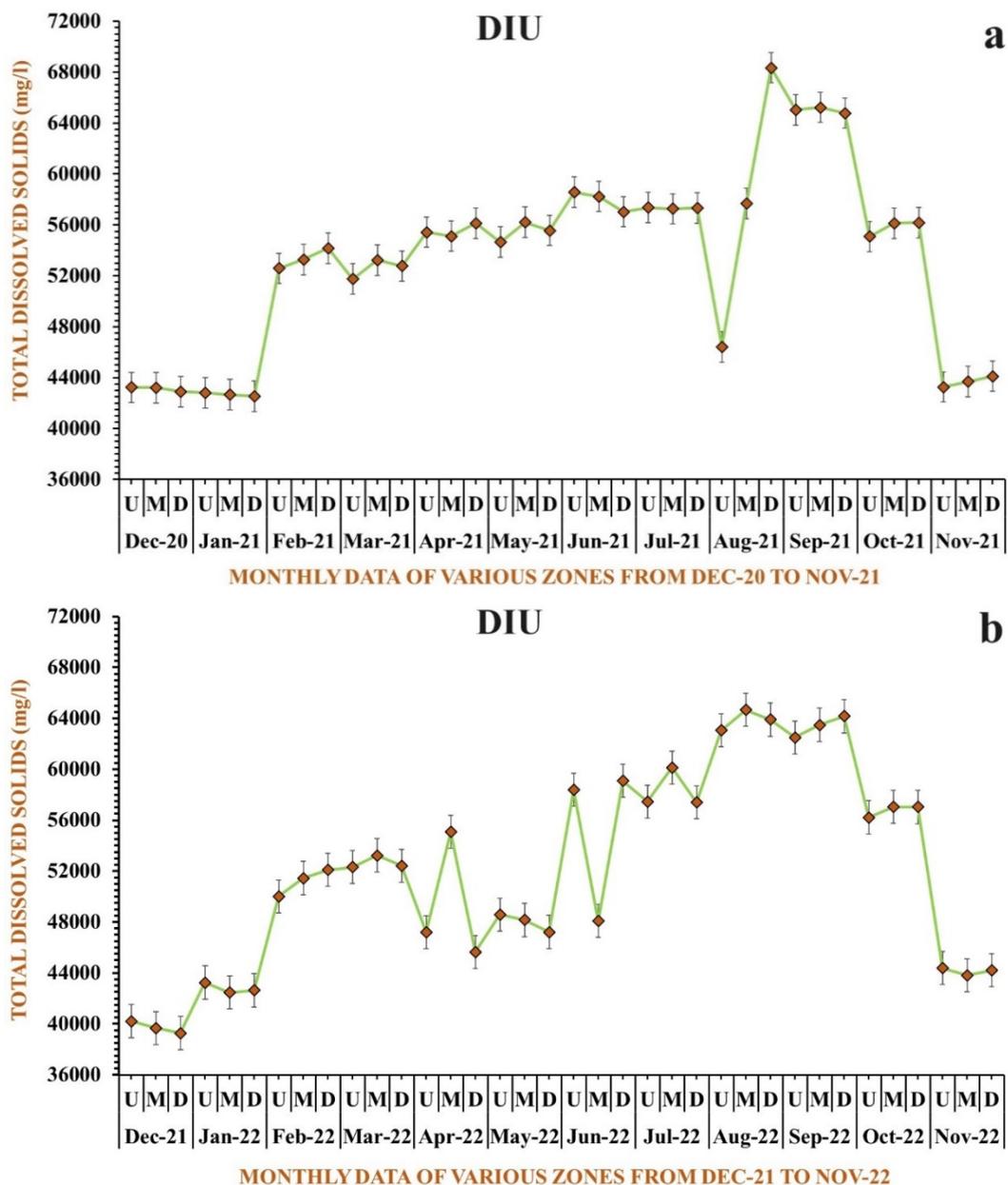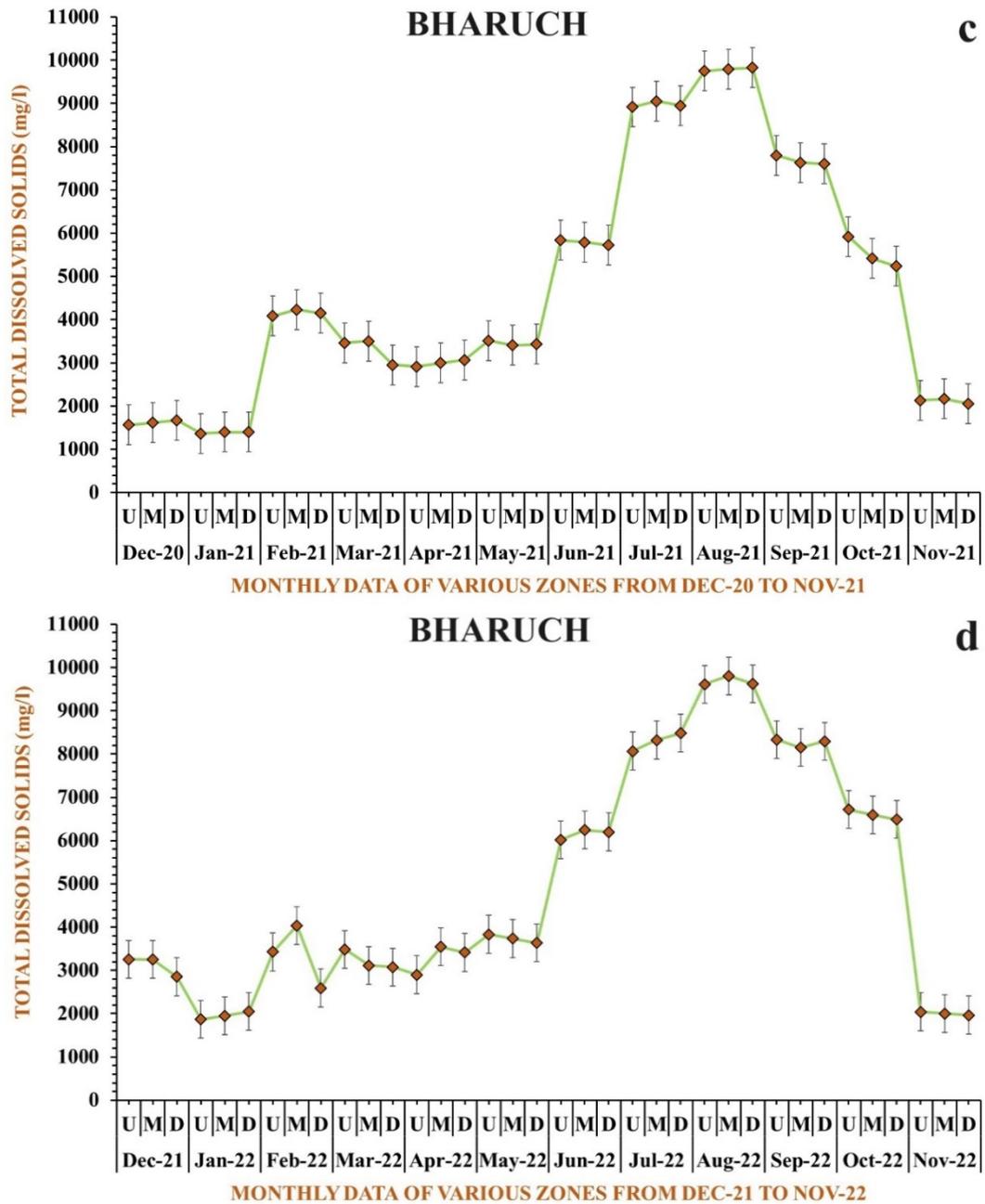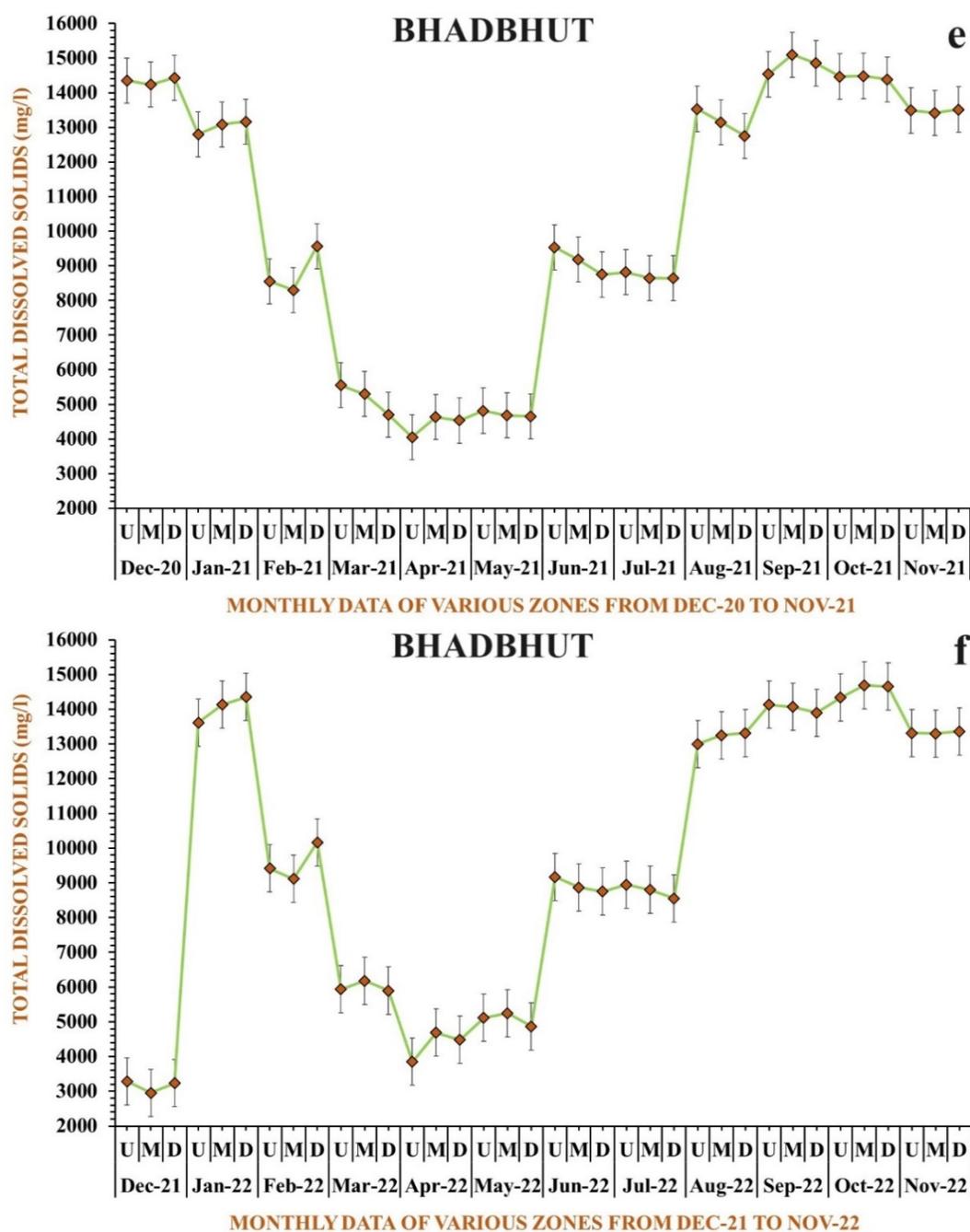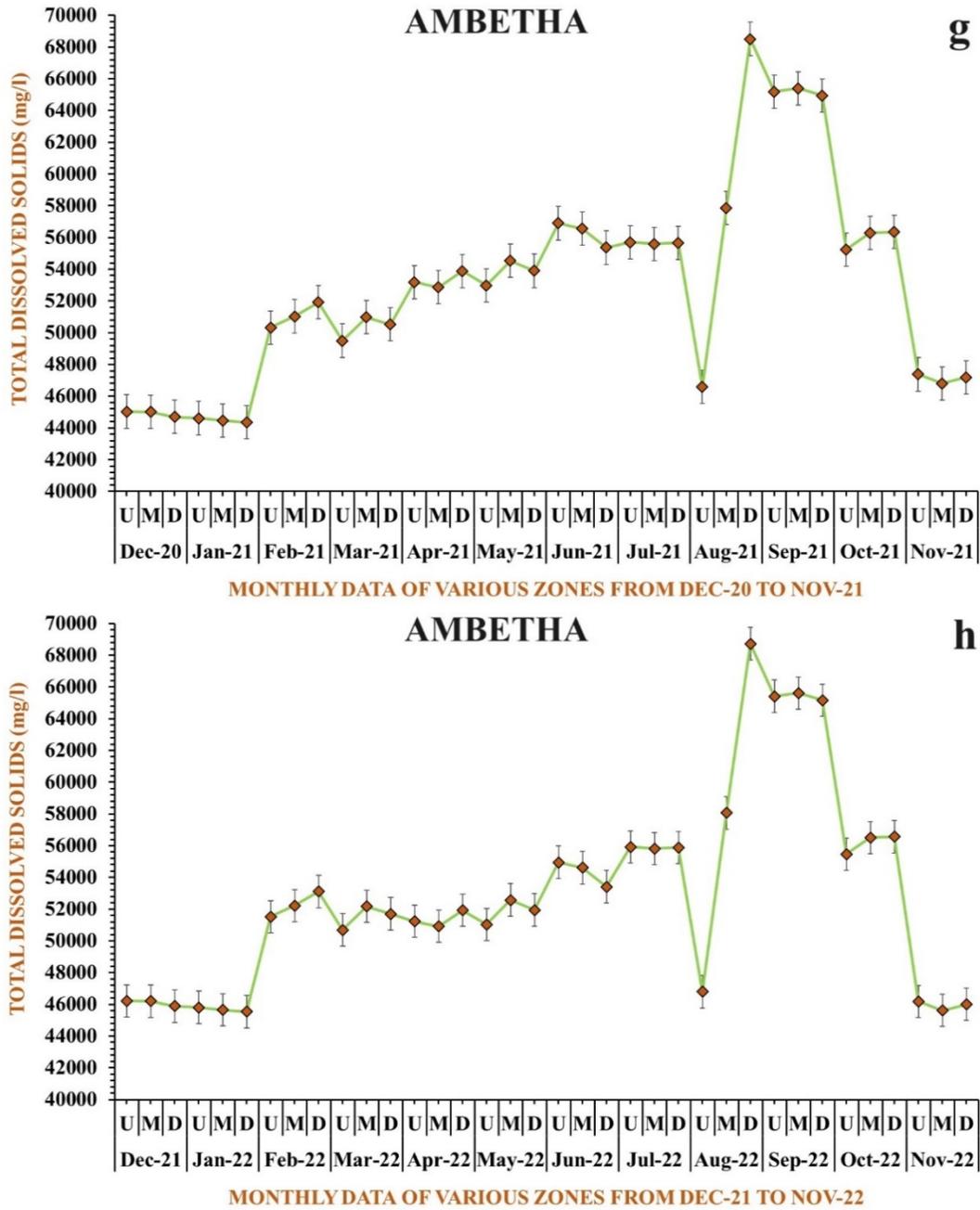
Figure 2.9 g & h: A graphical representation illustrating the dynamic changes in Total Dissolved Solids (mg/l) over the course of each month at Narmada Estuary – Ambetha Site: (g) from December-2020 to November-2021, (h) from December-2021 to November-2022

A correlation matrix shows correlation coefficients between physico-chemical parameters, where each cell represents the correlation between two variables (Table 2.6 to 2.9). Correlation coefficients range from -1 to 1: 1 indicates a perfect positive correlation, -1 indicates a perfect negative correlation, and 0 indicates no correlation. Each table shows the correlation coefficients between various parameters: pH, Temperature (Temp.), Salinity, Dissolved Oxygen (DO), and Total Dissolved Solids (TDS). The correlation coefficients are denoted by values ranging from -1 to 1, with ** indicating significance at the 0.01 level (2-tailed).

At Diu lagoon, pH has a significant positive correlation with DO (0.201**) and a significant negative correlation with Salinity (-0.128**). Temperature shows a significant negative correlation with DO (-0.728**) and a significant positive correlation with TDS (0.406**). DO exhibits a significant negative correlation with Temperature (-0.728**) and a significant positive correlation with pH (0.201**).

Whereas at different sites of Narmada Estuary were showing following correlation: (i) at Bharuch - pH has a strong negative correlation with Salinity (-0.855**), indicating that as pH decreases, Salinity increases significantly. Temperature shows a strong negative correlation with pH (-0.681**) and a significant positive correlation with Salinity (0.529**). DO has a significant positive correlation with Temperature (0.389**) and a significant negative correlation with Salinity (-0.694**). (ii) Bhadbhut - pH exhibits a strong negative correlation with Salinity (-0.787**). Temperature shows a significant negative correlation with pH (-0.209**) and a significant positive correlation with Salinity (0.546**). DO demonstrates a significant negative correlation with Temperature (-0.680**) and a significant positive correlation with pH (0.123**). (iii) Ambetha - pH has a weak but significant positive correlation with Salinity (0.077**). Temperature exhibits a significant negative correlation with pH (-0.026**) and a significant negative correlation with DO (-0.703**). DO shows a significant negative correlation with Temperature (-0.703**) and a significant negative correlation with TDS (-0.431**).

Table 2.6: Correlation matrix among physico-chemical parameters of water from Diu Lagoon region, Gujarat, India

	pH	Temp.	Salinity (ppt)	DO	TDS
pH	1				
Temp. (°C)	-0.039**	1			
Salinity (ppt)	-0.128**	0.107**	1		
DO (mg/l)	0.201**	-0.728**	-0.019**	1	
TDS (mg/l)	-0.071**	0.406**	0.039**	-0.525**	1

** Correlation is significant at the 0.01 level (2-tailed).

Table 2.7: Correlation matrix among physico-chemical parameters of water from Bharuch, Narmada Estuary, Gujarat, India

	pH	Temp.	Salinity (ppt)	DO	TDS
pH	1				
Temp. (°C)	-0.681**	1			
Salinity (ppt)	-0.855**	0.529**	1		
DO (mg/l)	0.389**	-0.694**	-0.207**	1	
TDS (mg/l)	-0.838**	0.497**	0.919**	-0.268**	1

** Correlation is significant at the 0.01 level (2-tailed).

Table 2.8: Correlation matrix among physico-chemical parameters of water from Bhadbhut, Narmada Estuary, Gujarat, India

	pH	Temp.	Salinity (ppt)	DO	TDS
pH	1				
Temp. (°C)	-0.209**	1			
Salinity (ppt)	-0.787**	0.546**	1		
DO (mg/l)	0.123**	-0.680**	-0.307**	1	
TDS (mg/l)	-0.653**	-0.334**	0.389**	0.142**	1

** Correlation is significant at the 0.01 level (2-tailed).

Table 2.9: Correlation matrix among physico-chemical parameters of water from Ambetha, Narmada Estuary, Gujarat, India

	pH	Temp.	Salinity (ppt)	DO	TDS
pH	1				
Temp. (°C)	-0.026**	1			
Salinity (ppt)	0.077**	0.110**	1		
DO (mg/l)	0.044**	-0.703**	-0.121**	1	
TDS (mg/l)	-0.065**	0.309**	0.005**	-0.431**	1

** Correlation is significant at the 0.01 level (2-tailed).

2.3.2 Length-Weight Relationship

This chapter provides the latest comprehensive evaluation of the LWR, Fulton's K, I_g, and I_h of *M. cephalus* (Linnaeus, 1758) in two distinct habitats of the Gujarat Coast: Diu lagoon and Narmada estuary. The important findings of the LWR, such as the sample size (N), the ranges of total length (mm) and weight (g), the parameters "a" and "b" with their respective 95% confidence intervals, the coefficient of determination (R^2), and the parabolic equation $W = aL^b$, are given in Table 2.10 to 2.13. The linear regression analysis conducted on the log-transformed data of all populations from all research sites yielded highly significant results ($p < 0.01$), with the exception of the male population (Dec-21 to Nov-22), which showed significant results ($p \leq 0.05$). The 'b' values for males in the Diu lagoon area (from Dec-20 to Nov-21: $t_b = -3.60$, df = 119, p = 0.005; from Dec-21 to Nov-22: $t_b = -0.27$, df = 119, p = 0.006), females in the Diu lagoon area (from Dec-20 to Nov-21: $t_b = -4.26$, df = 119, p = 0.006; from Dec-21 to Nov-22: $t_b = -5.09$, df = 119, p = 0.007), and males in the Bharuch, Narmada estuary area (from Dec-20 to Nov-21: $t_b = -0.65$, df = 119, p = 0.005; from Dec-21 to Nov-22: $t_b = -2.02$, df = 119, p = 0.007) were not significantly different from the cubic value as accepted by isometry ($H_0 = 3$). This indicates negligible negative allometric growth during both consecutive years of sampling (Fig. 2.10a-f to 2.13a-f). However, the 'b' value for females in Bharuch (from Dec-20 to Nov-21: $t_b = -6.99$, df = 119, p = 0.01; from Dec-21 to Nov-22: $t_b = -5.26$, df = 119, p = 0.01), males in Bharuch (from Dec-20 to Nov-21: $t_b = -4.09$, df = 119, p = 0.01; from Dec-21 to Nov-22: $t_b = -6.15$, df = 119, p = 0.02), females in Bhadbhut (from Dec-20 to Nov-21: $t_b = -7.63$, df = 119, p = 0.01; from Dec-21 to Nov-22: $t_b = -6.72$, df = 119, p = 0.01), and males in Ambetha (from Dec-20 to Nov-21: $t_b = -7.16$, df = 119, p = 0.01; from Dec-21 to Nov-22: $t_b = -3.66$, df = 119, p = 0.009), as well as females in Ambetha (from Dec-20 to Nov-21: $t_b = -5.44$, df = 119, p = 0.01; from Dec-21 to Nov-22: $t_b = -6.77$, df = 119, p = 0.009), in the Narmada estuary, were significantly different from the expected cubic value that represents negative allometric ($H0 \neq 3$) growth. The negative allometric growth suggests that the body weight of an individual does not increase in proportion to its body length. In fact, as it grows, the body becomes more elongated.

The combined populations of the Diu lagoon area, collected from December 2020 to November 2021 (t_b = -4.12, df = 239, p = 0.005), and from December 2021 to November 2022 (t_b = -3.95, df = 239, p = 0.005), did not differ significantly from the expected cubic value according to isometry (H_0 = 3). This indicates that there was minimal negative allometric growth observed during both years of sampling (Fig. 2.10 e & f). The pooled populations of Bharuch, Bhadbhut, and Ambetha in the Narmada estuary showed a significant difference from the expected cubic value, indicating negative allometric growth. This means that there was a decrease in weight at a particular length.

The growth conditions were altered due to multiple parameters including gonad development, sex, stomach fullness, health, season, habitat, temperature, salinity, and nutrition (Ricker 1975; Bagenal & Tesch 1978). The results obtained suggest that the slope (b) of the LWR of *M. cephalus* in the study area was consistent with the expected range of 2.5–3.5 proposed by Froese (2006), except for the female population in Bharuch. In the Bhadbhut area of the Narmada estuary, both males and females, as well as the pooled populations, exhibited allometric growth with a slope (b) of less than or equal to 2.5-3.5, indicating highly negative growth. The coefficient of determination (R^2) determines the significance of the link between length and weight. It can have values ranging from 0 to 1. The value of (R^2) greater than 0.5 indicates a favourable correlation in the LWR (Mon *et al.* 2020). The R^2 values in the current study varied between 0.93 and 0.97 in Diu lagoon, 0.60 and 0.85 in Bharuch area, 0.73 and 0.92 in Bhadbhut area, and 0.86 and 0.95 in Ambetha area of Narmada estuary for male, female, and combined populations of *M. cephalus*. The relationship between the length and weight of *M. cephalus* was found to be strongly associated and very significant at Diu lagoon, followed by Ambetha, Bhadbhut, and Bharuch.

Table 2.10: Statistical analysis of Length-Weight Relationship (LWR) of *M. cephalus* from Diu Lagoon

Study Site	Sex	N	TL (mm) Min	Max	TW (g) Min	Max	'a'	95% CI of a	'b'	95% CI of b	R^2	Growth (t-test)	p	Parabolic Equation $W = aL^b$
	♂	120	10	23.8	10.76	108.26	0.16	0.14-0.18	2.81	2.72-2.92	0.96	b<3	HS	$W = 0.16L^{2.81}$
	♀	120	12.5	29.4	21.37	251.73	0.18	0.15-0.20	2.77	2.66-2.88	0.93	b<3	HS	$W = 0.18L^{2.77}$
Diu Lagoon	♂ + ♀	240	10	29.4	10.76	251.73	0.16	0.15-0.17	2.86	2.79-2.93	0.97	b<3	HS	$W = 0.16L^{2.86}$
	♂	120	11	24	12.76	112.16	0.17	0.15-0.20	2.78	2.63-2.90	0.95	b<3	HS	$W = 0.17L^{2.78}$
	♀	120	13	29.2	28.33	258.39	0.19	0.16-0.21	2.75	2.57-2.85	0.96	b<3	HS	$W = 0.19L^{2.75}$
	♂ + ♀	240	11	29.2	12.76	258.39	0.17	0.15-0.18	2.85	2.77-2.92	0.96	b<3	HS	$W = 0.17L^{2.85}$

N number of individuals, *TL* total length, *TW* total weight, *CI* confidence interval, *S* Significant (p 0.05), *NS* non-significant (p > 0.05), *HS* highly significant (p ≤ 0.01).

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

$Log10TW = 2.8172 Log10TL - 1.797$
$R^2 = 0.9631$

a

LWR of Male *M. cephalus* from Diu - Dec-20 to Nov-21

$Log10TW = 2.786 Log10TL - 1.7371$
$R^2 = 0.9526$

b

LWR of Male *M. cephalus* from Diu - Dec-21 to Nov-22

Figure 2.10 a & b: Logarithmic Representation of Length-Weight Relationship in Male *M. cephalus* from Diu Lagoon: (a) Dec 2020 - Nov 2021, and (b) Dec 2021 - Nov 2022

The chart (c) shows:

$$\text{Log10TW} = 2.7725\text{Log10TL} - 1.7126$$
$$R^2 = 0.9581$$

with axes Log10_TW (vertical) and Log10_TL (horizontal).

LWR of Female *M. cephalus* from Diu - Dec-20 to Nov-21

The chart (d) shows:

$$\text{Log10TW} = 2.7526\text{Log10TL} - 1.6545$$
$$R^2 = 0.9645$$

with axes Log10_TW (vertical) and Log10_TL (horizontal).

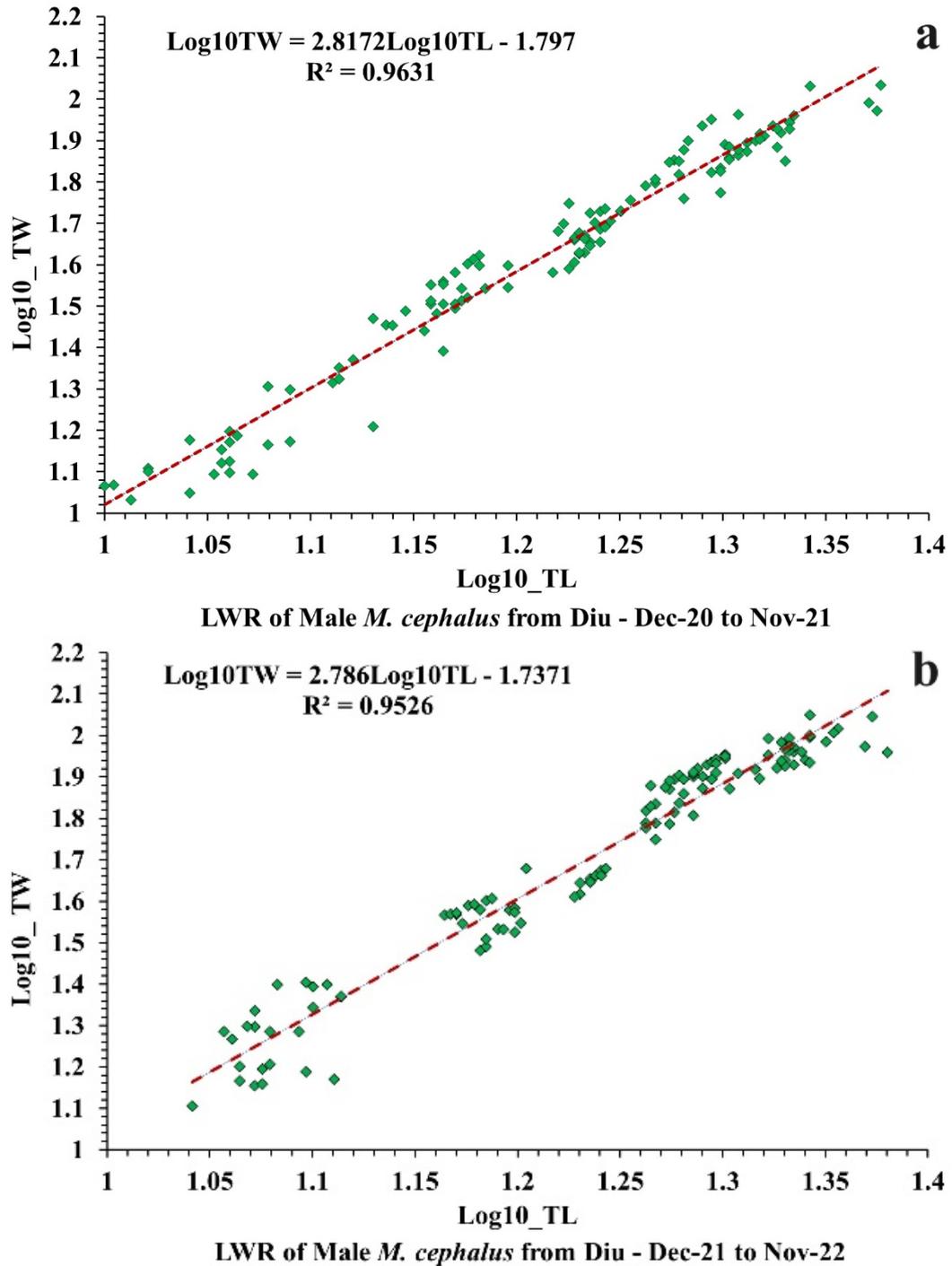**LWR of Female *M. cephalus* from Diu - Dec-21 to Nov-22**

Figure 2.10 c & d: Logarithmic Representation of Length-Weight Relationship in Female *M. cephalus* from Diu Lagoon: (c) Dec 2020 - Nov 2021, and (d) Dec 2021 - Nov 2022

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

Figure 2.10 e & f: Logarithmic Representation of Length-Weight Relationship in *M. cephalus* Population from Diu Lagoon: (e) Dec 2020 - Nov 2021, and (f) Dec 2021 - Nov 2022
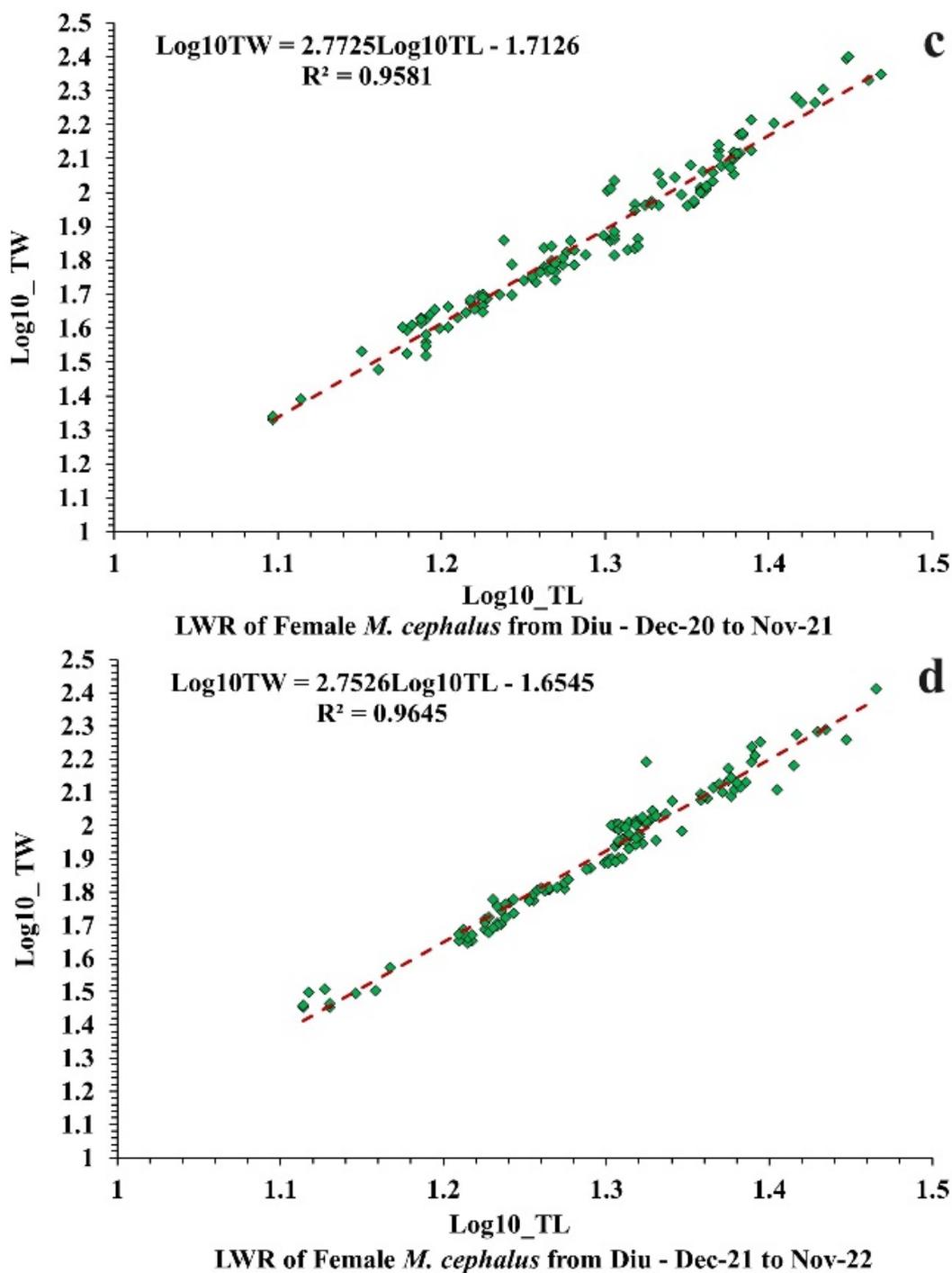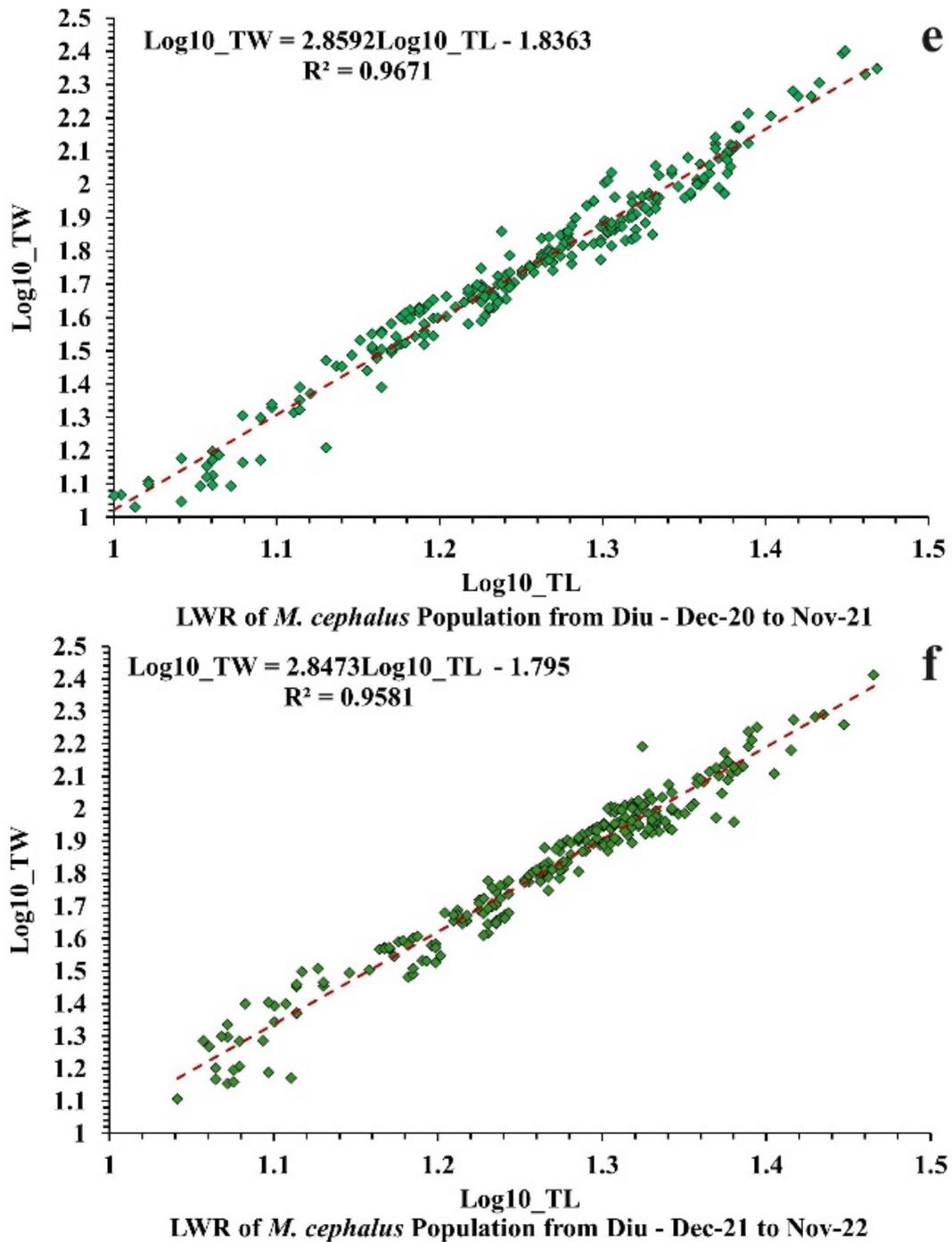
Table 2.11: Statistical analysis of Length-Weight Relationship (LWR) of *M. cephalus* from Bharuch, Narmada Estuary, Gujarat, India

Study Site	Sex	N	TL (mm)		TW (g)		Regression Parameters					Growth (t-test)	p	Parabolic Equation
			Min	Max	Min	Max	'a'	95% CI of a	'b'	95% CI of b	R^2			$W = aL^b$
Bharuch, Narmada Estuary	♂	120	13.8	24.7	25.64	120.46	0.16	0.09-0.28	2.86	2.43-3.28	0.6	b<3	HS	$W = 0.16L^{2.86}$
	♀	120	15.8	26.7	48.46	216.92	0.32	0.25-0.41	2.37	2.19-2.54	0.85	b<3	HS	$W = 0.32L^{2.37}$
	♂ + ♀	240	13.8	26.7	25.64	216.92	0.19	0.15-0.26	2.73	2.53-2.93	0.75	b<3	HS	$W = 0.19L^{2.73}$
	♂	120	13.2	25.4	25.86	128.09	0.19	0.13-0.26	2.73	2.47-2.99	0.78	b<3	HS	$W = 0.19L^{2.73}$
	♀	120	15.3	30.2	41.98	243.11	0.29	0.22-0.39	2.42	2.20-2.64	0.8	b<3	HS	$W = 0.29L^{2.42}$
	♂ + ♀	240	13.2	30.2	25.86	243.11	0.22	0.18-0.27	2.65	2.49-2.80	0.82	b<3	HS	$W = 0.22L^{2.65}$

N number of individuals, *TL* total length, *TW* total weight, *CI* confidence interval, *S* Significant (p 0.05), *NS* non-significant (p > 0.05), *HS* highly significant (p ≤ 0.01).

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

Figure 2.11 a & b: "Logarithmic Representation of Length-Weight Relationship in Male *M. cephalus* from Bharuch, Narmada Estuary: (a) Dec 2020 - Nov 2021, and (b) Dec 2021 - Nov 2022
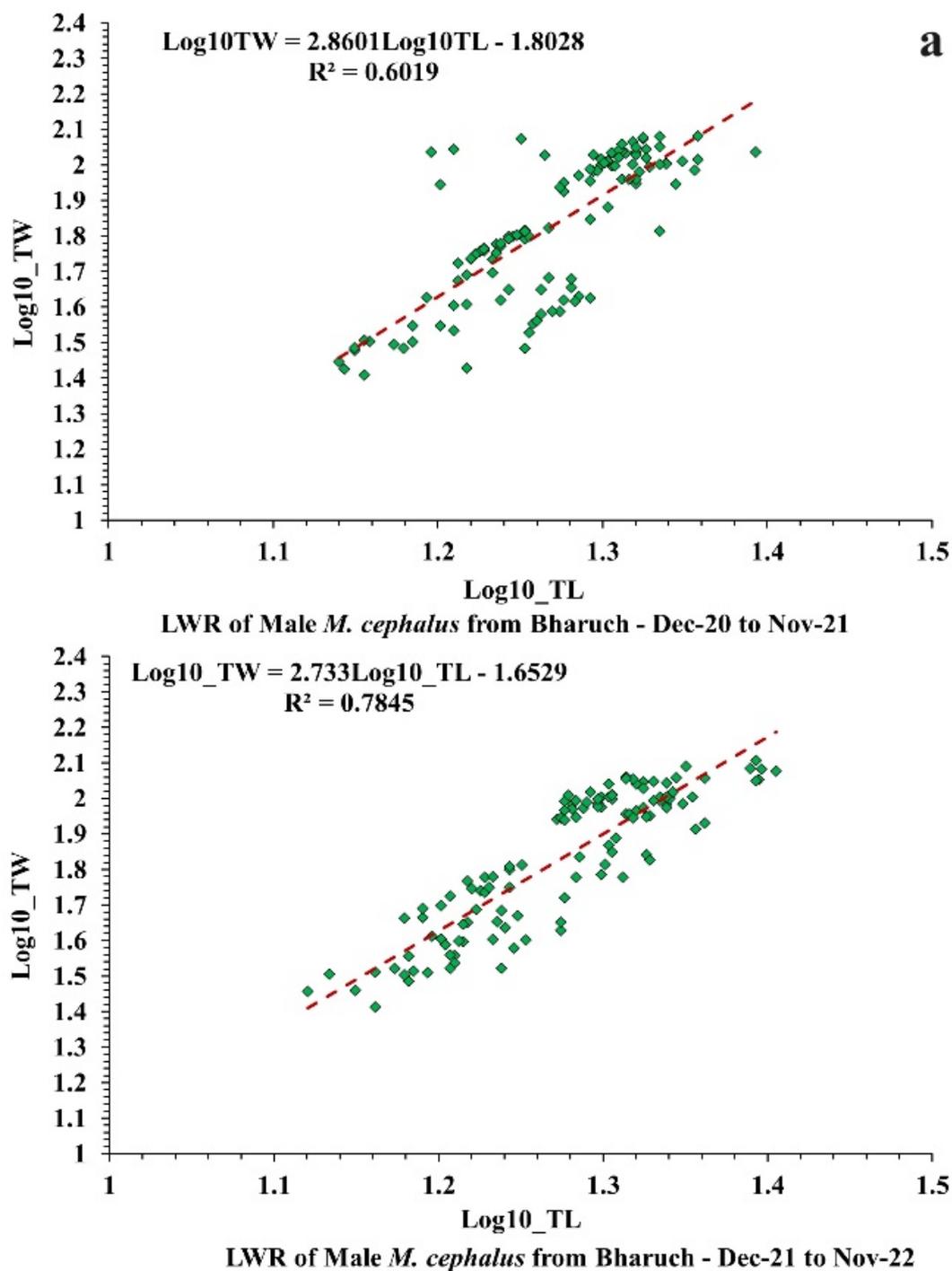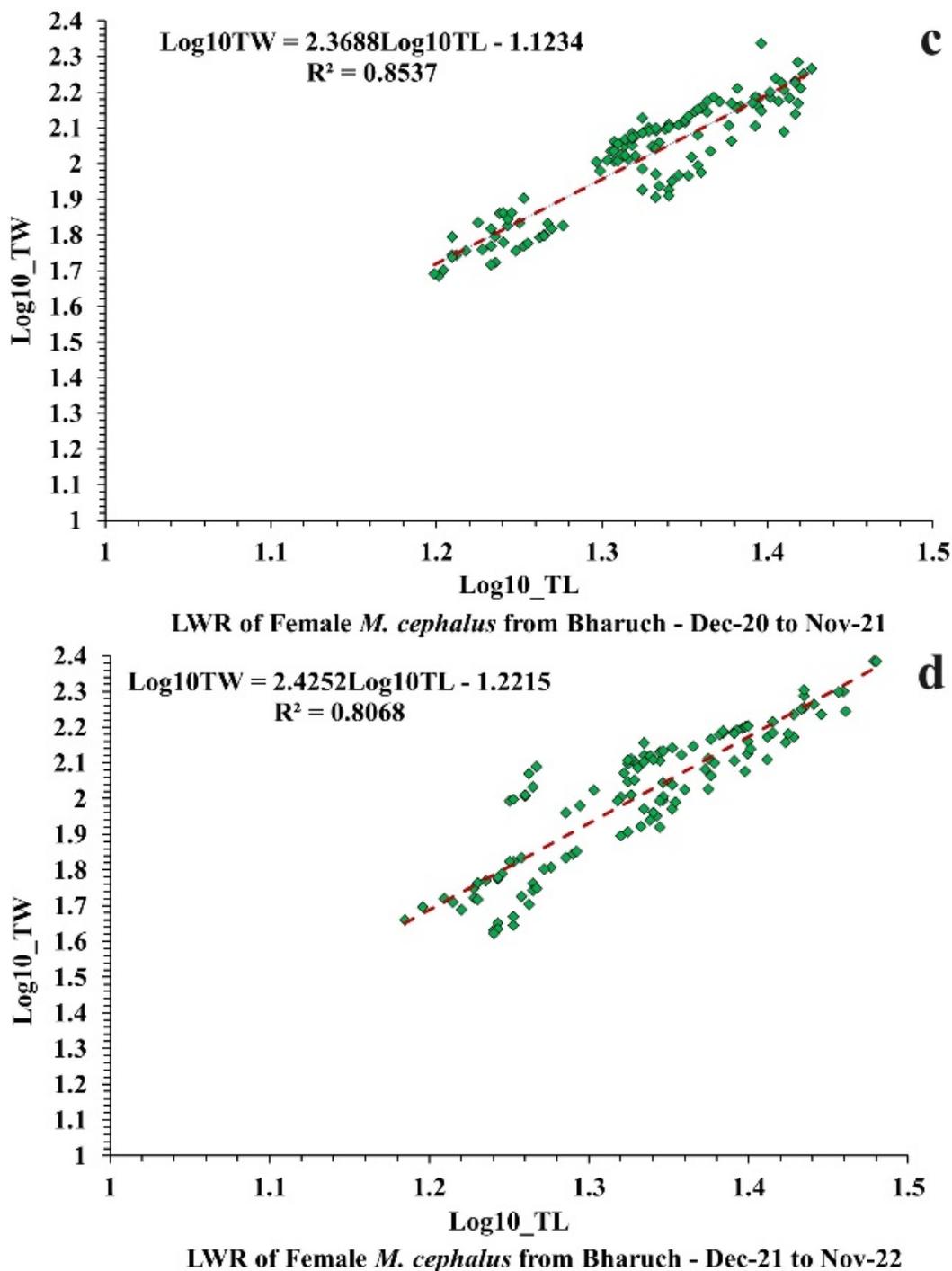
Figure 2.11 c & d: Logarithmic Representation of Length-Weight Relationship in Female *M. cephalus* from Bharuch, Narmada Estuary: (c) Dec 2020 - Nov 2021, and (d) Dec 2021 - Nov 2022
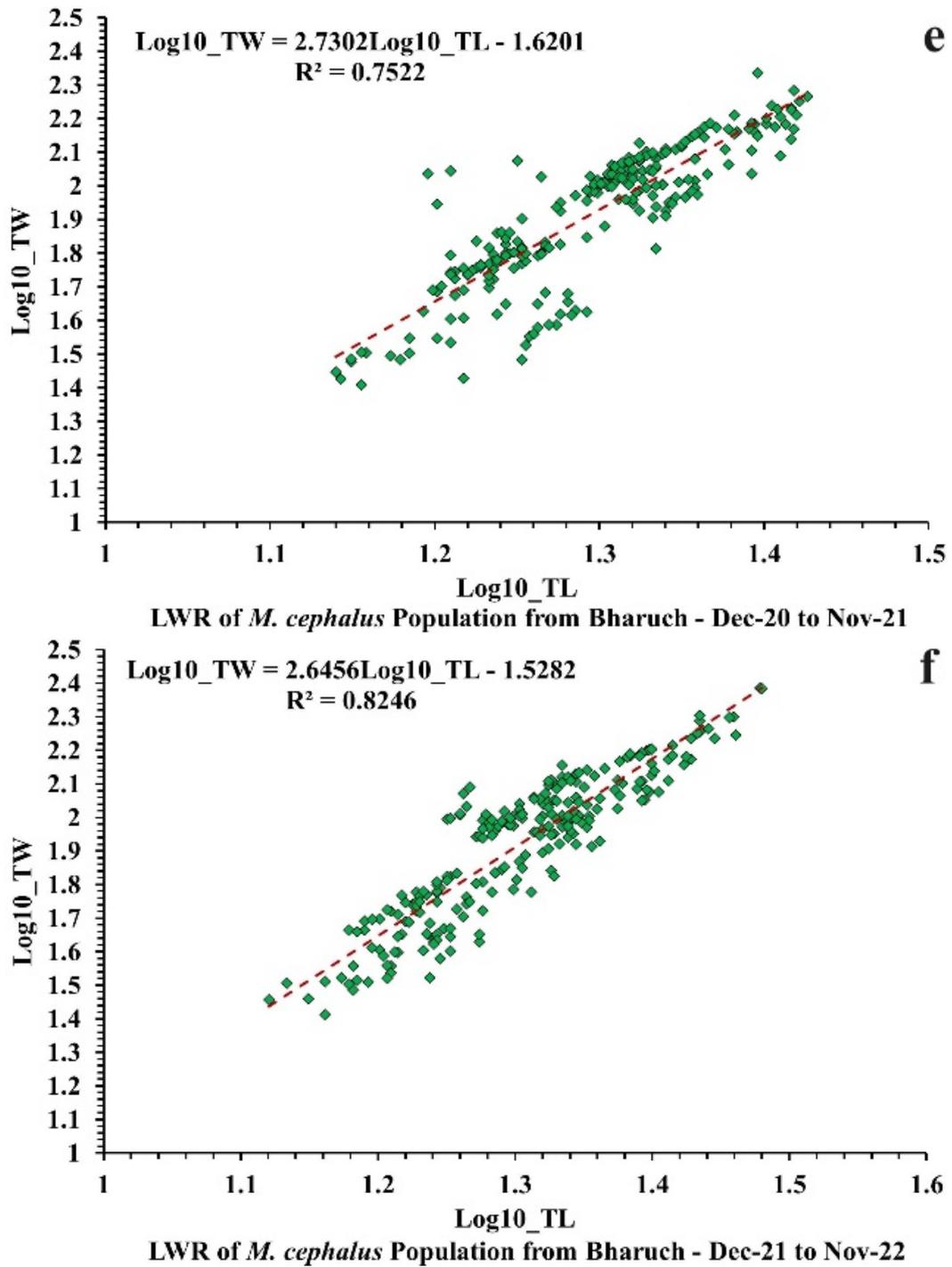
Figure 2.11 e & f: Logarithmic Representation of Length-Weight Relationship in *M. cephalus* Population from Bharuch, Narmada Estuary: (e) Dec 2020 - Nov 2021, and (f) Dec 2021 - Nov 2022

Table 2.12: Statistical analysis of Length-Weight Relationship (LWR) of *M. cephalus* from Bhadbhut, Narmada Estuary, Gujarat, India

Study Site	Sex	N	TL (mm)		TW (g)		Regression Parameters					Growth (t-test)	p	Parabolic Equation
			Min	Max	Min	Max	'a'	95% CI of a	'b'	95% CI of b	R^2			$W = aL^b$
Bhadbhut, Narmada Estuary	♂	120	11.6	24.8	25.64	12.3.43	0.28	0.19-0.39	2.45	2.19-2.72	0.74	b<3	HS	$W = 0.28L^{2.45}$
	♀	120	14.5	28.2	39.38	238.9	0.3	0.24-0.36	2.41	2.25-2.56	0.89	b<3	HS	$W = 0.30L^{2.41}$
	♂ + ♀	240	11.6	28.2	25.64	238.9	0.28	0.23-0.33	2.47	2.32-2.62	0.82	b<3	HS	$W = 0.28L^{2.47}$
	♂	120	12.6	25.6	29.26	130.27	0.37	0.27-0.50	2.24	1.99-2.48	0.73	b<3	S	$W = 0.37L^{2.24}$
	♀	120	13.6	27.3	35.74	233.48	0.25	0.21-0.30	2.55	2.41-2.68	0.92	b<3	HS	$W = 0.25L^{2.55}$
	♂ + ♀	240	12.6	27.3	29.26	233.48	0.3	0.25-0.37	2.40	2.25-2.54	0.82	b<3	HS	$W = 0.30L^{2.40}$

N number of individuals, *TL* total length, *TW* total weight, *CI* confidence interval, *S* Significant (p 0.05), *NS* non-significant (p > 0.05), *HS* highly significant (p ≤ 0.01).

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

$$Log10TW = 2.4508 Log10TL - 1.2733$$
$$R^2 = 0.7387$$

a

LWR of Male *M. cephalus* from Bhadbhut - Dec-20 to Nov-21

$$Log10TW = 2.2379 Log10TL - 1.0001$$
$$R^2 = 0.7341$$

b

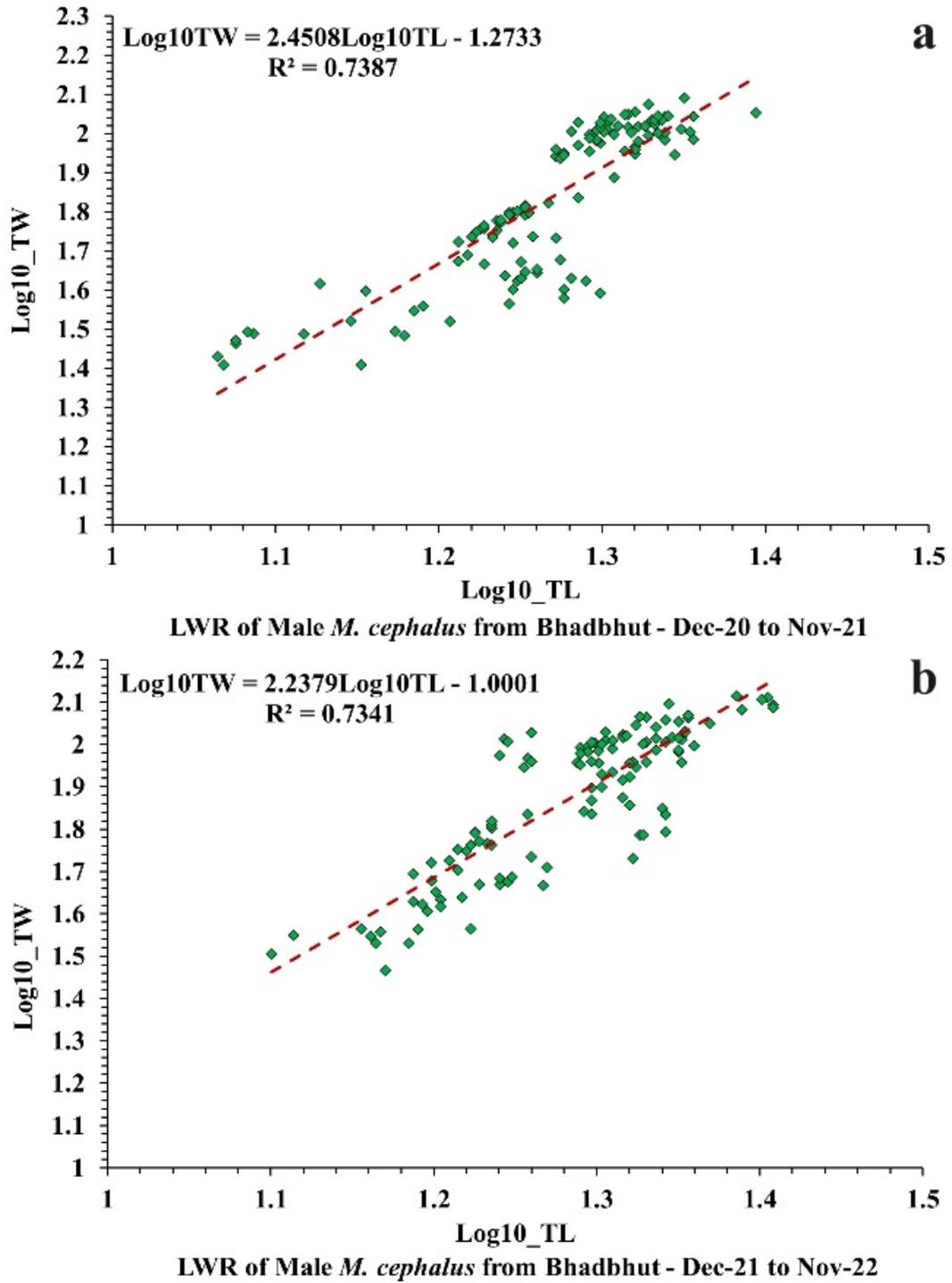LWR of Male *M. cephalus* from Bhadbhut - Dec-21 to Nov-22

Figure 2.12 a & b: Logarithmic Representation of Length-Weight Relationship in Male *M. cephalus* from Bhadbhut, Narmada Estuary: (a) Dec 2020 - Nov 2021, and (b) Dec 2021 - Nov 2022

Log10TW = 2.4105Log10TL - 1.197
R² = 0.8919

LWR of Female *M. cephalus* from Bhadbhut - Dec-20 to Nov-21

Log10TW = 2.5487Log10TL - 1.3704
R² = 0.9243

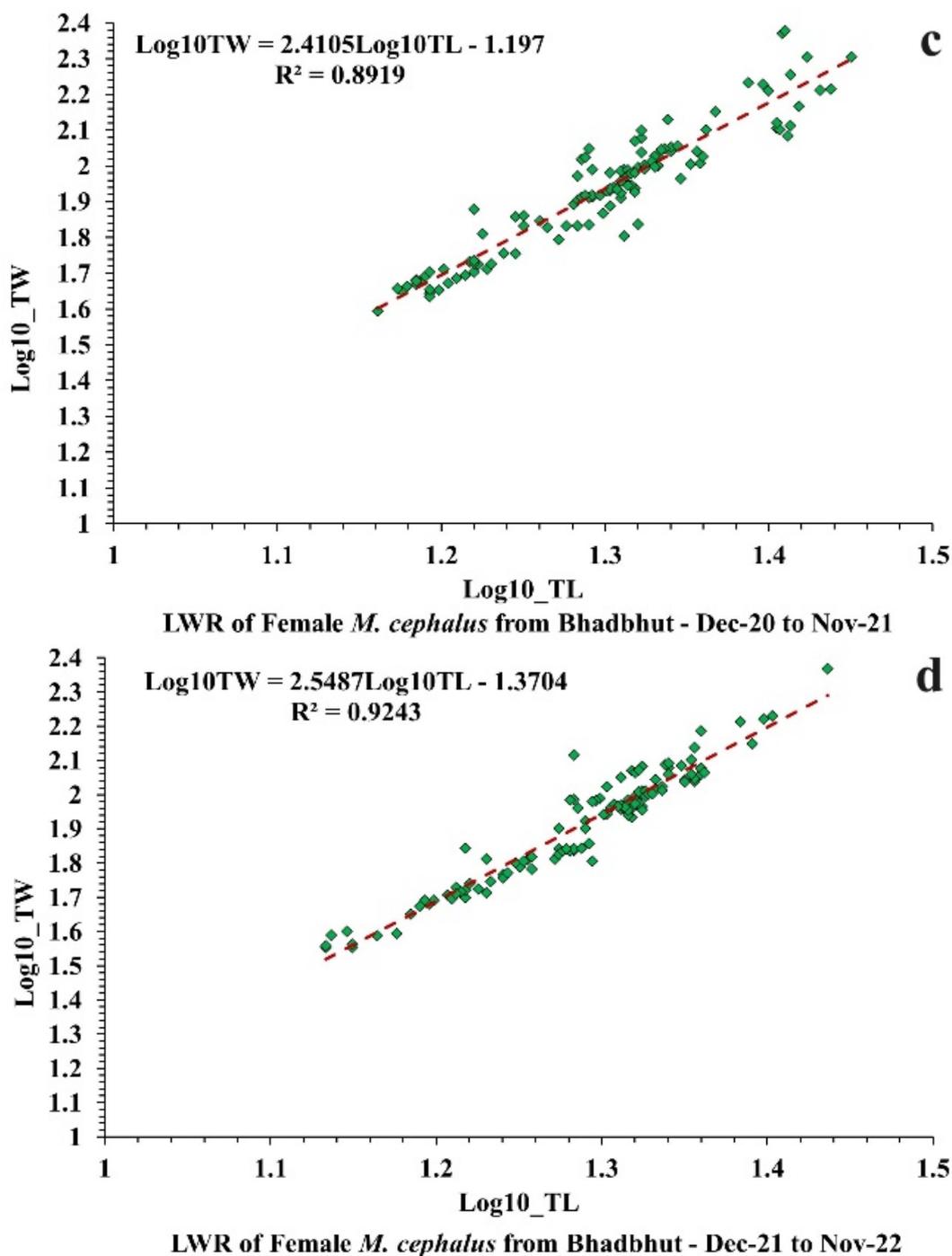**LWR of Female *M. cephalus* from Bhadbhut - Dec-21 to Nov-22**

Figure 2.12 c & d: Logarithmic Representation of Length-Weight Relationship in Female *M. cephalus* from Bhadbhut, Narmada Estuary: (c) Dec 2020 - Nov 2021, and (d) Dec 2021 - Nov 2022

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)
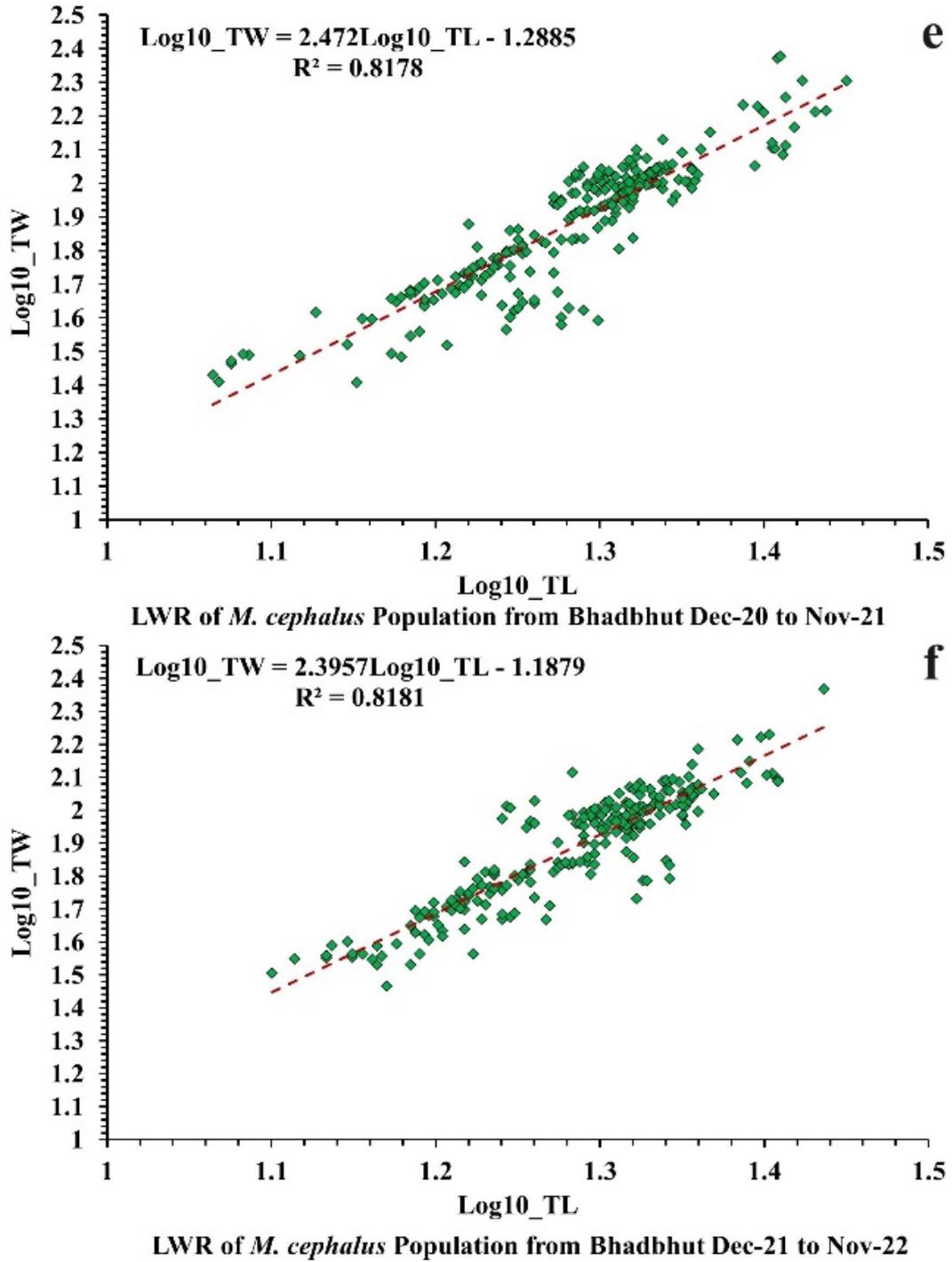
Figure 2.12 e & f: Logarithmic Representation of Length-Weight Relationship in *M. cephalus* Population from Bhadbhut, Narmada Estuary: (e) Dec 2020 - Nov 2021, and (f) Dec 2021 - Nov 2022

Table 2.13: Statistical analysis of Length-Weight Relationship (LWR) of *M. cephalus* from Ambetha, Narmada Estuary, Gujarat, India

Study Site	Sex	N	TL (mm) Min	TL (mm) Max	TW (g) Min	TW (g) Max	'a'	95% CI of a	'b'	95% CI of b	R^2	Growth (t-test)	p	Parabolic Equation $W = aL^b$
	♂	120	10.3	23.9	12.8	106	0.24	0.20-0.28	2.53	2.40-2.66	0.93	b<3	HS	$W = 0.24L^{2.53}$
	♀	120	14.6	29.1	38.21	249.72	0.26	0.20-0.33	2.50	0.32-2.68	0.86	b<3	HS	$W = 0.26L^{2.50}$
Ambetha, Narmada Estuary	♂ + ♀	240	10.3	29.1	12.8	249.72	0.21	0.18-0.25	2.63	2.52-2.75	0.9	b<3	HS	$W = 0.21L^{2.63}$
	♂	120	12.2	24.5	19.11	141.46	0.21	0.17-0.27	2.65	2.46-2.84	0.87	b<3	HS	$W = 0.21L^{2.65}$
	♀	120	13.4	29.6	34.45	269.17	0.22	0.19-0.26	2.64	2.53-2.75	0.95	b<3	HS	$W = 0.22L^{2.64}$
	♂ + ♀	240	12.2	29.6	19.11	269.17	0.20	0.17-0.23	2.71	2.60-2.82	0.91	b<3	HS	$W = 0.20L^{2.71}$

N number of individuals, *TL* total length, *TW* total weight, *CI* confidence interval, *S* Significant (p 0.05), *NS* non-significant (p > 0.05), *HS* highly significant (p ≤ 0.01).

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a

Log10TW = 2.5325Log10TL - 1.4444
R² = 0.9273

LWR of Male *M. cephalus* from Ambetha - Dec-20 to Nov-21

b

Log10TW = 2.6484Log10TL - 1.5473
R² = 0.8658

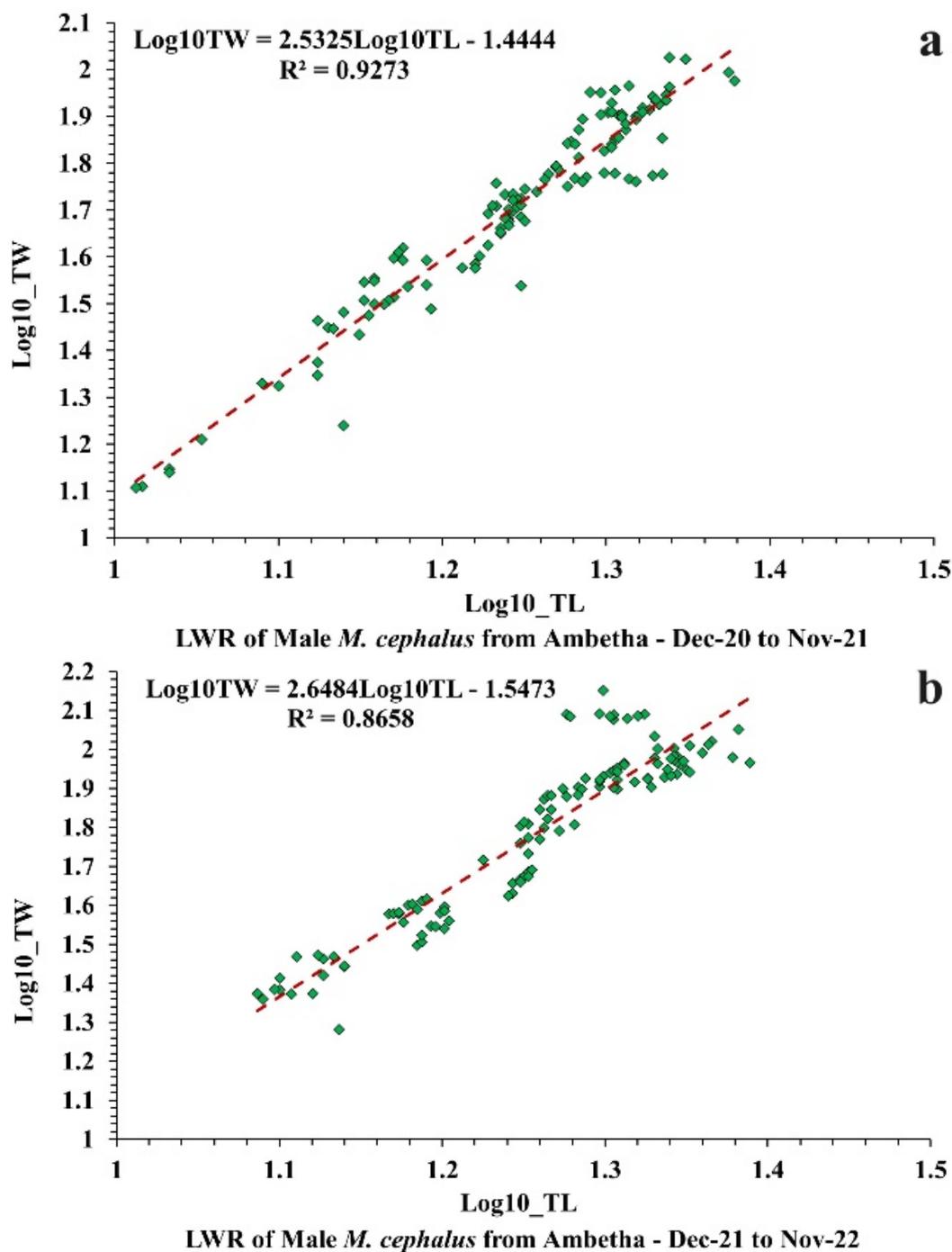LWR of Male *M. cephalus* from Ambetha - Dec-21 to Nov-22

Figure 2.13 a & b: Logarithmic Representation of Length-Weight Relationship in Male *M. cephalus* from Ambetha, Narmada Estuary: (a) Dec 2020 - Nov 2021, and (b) Dec 2021 - Nov 2022
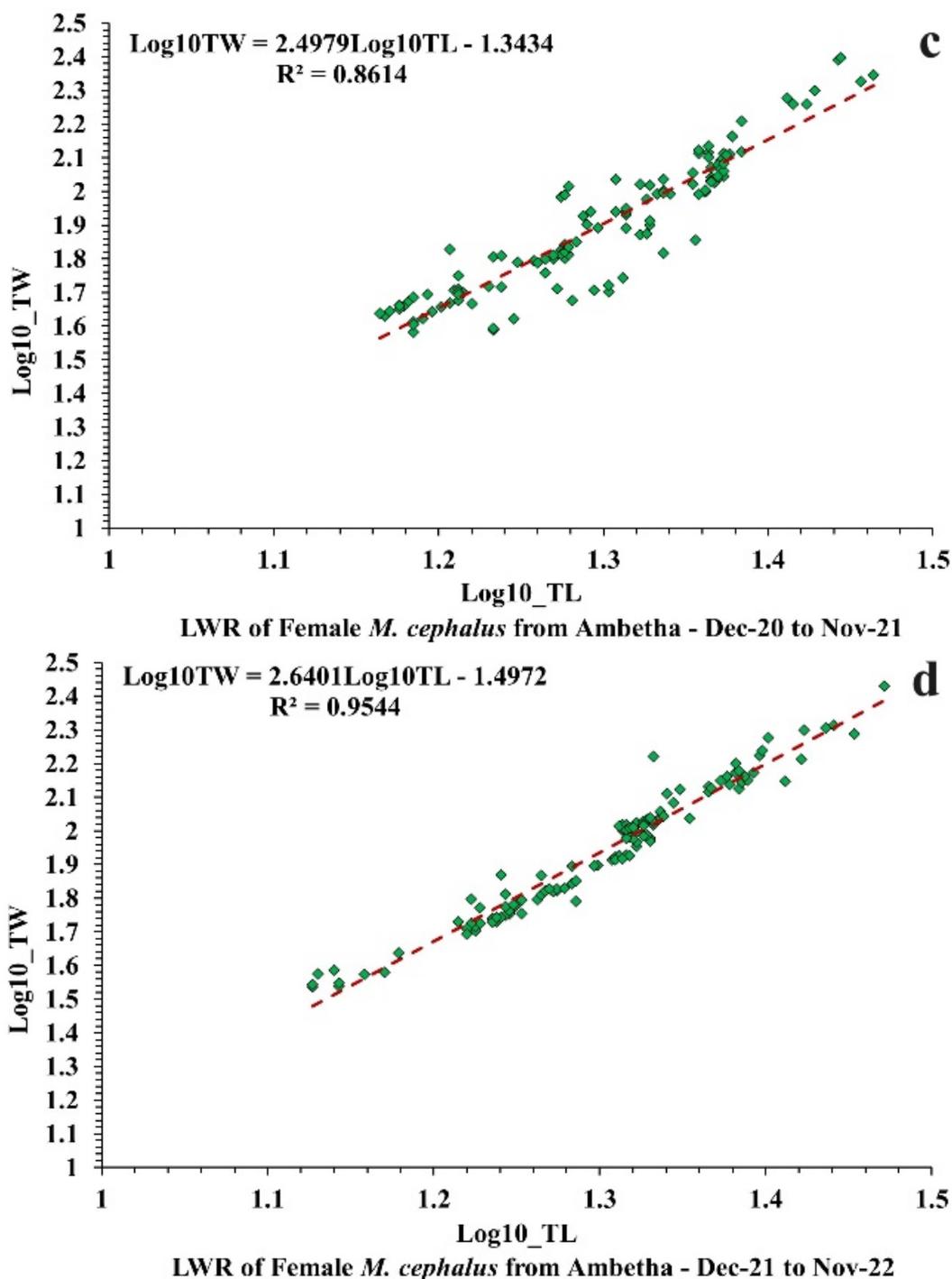
Figure 2.13 c & d: Logarithmic Representation of Length-Weight Relationship in Female *M. cephalus* from Ambetha, Narmada Estuary: (c) Dec 2020 - Nov 2021, and (d) Dec 2021 - Nov 2022
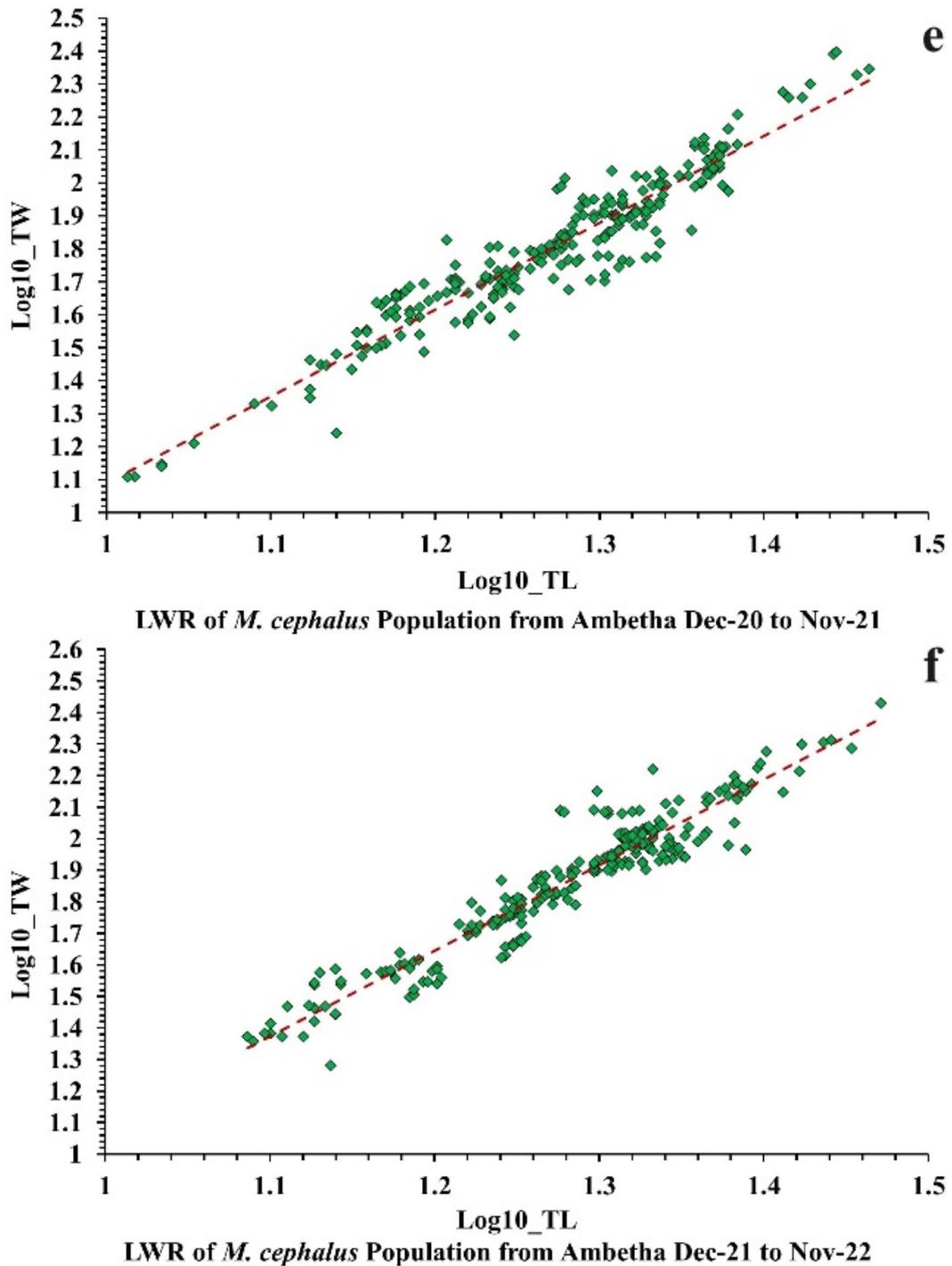
Figure 2.13 e & f: Logarithmic Representation of Length-Weight Relationship in *M. cephalus* Populations from Ambetha, Narmada Estuary: (e) Dec 2020 - Nov 2021, and (f) Dec 2021 - Nov 2022

2.3.3 Condition Factor

In this study, the Fulton's *K* has been calculated and the monthly changes in condition were compared among male, female, and the overall population of *M. cephalus* (Linnaeus, 1758) from Diu lagoon and Narmada Estuary (Bharuch, Bhadbhut, and Ambetha) (Fig. 2.14a-c to 2.17a-c). The *K* values indicate the state of the species' and specific sex's wellness in their respective habitat (Froese 2006). The mean *K* value of male *M. cephalus* was reported as 0.97±0.09 in Diu lagoon, 1.01±0.11 in Bharuch, 1.09±0.28 in Bhadbhut, and 0.95±0.10 in Ambetha during the period from December 2020 to November 2021. Similarly, during the period from December 2021 to November 2022, the mean *K* value was recorded as 1.01±0.34 in Diu lagoon, 1.04±0.17 in Bharuch, 1.08±0.21 in Bhadbhut, and 1.04±0.19 in Ambetha.

The fitness or well-being of fish is maximised when the value of *K* is equal to or in close proximity to 1 (De Vries *et al.* 2020). Therefore, the well-being of male *M. cephalus* was found to be superior in Diu, followed by Bhadbhut, Ambetha, and Bharuch. This is indicated by values close to 1, which signify robustness and better condition of the fish. The average *K* value for female *M. cephalus* was reported as 0.99±0.10 in Diu lagoon, 1.07±0.07 in Bharuch, 1.11±0.15 in Bhadbhut, and 1.03±0.18 in Ambetha during the period from December 2020 to November 2021. Similarly, during the period from December 2021 to November 2022, the average *K* value was recorded as 1.11±0.15 in Diu lagoon, 1.05±0.22 in Bharuch, 1.13±0.11 in Bhadbhut, and 1.09±0.10 in Ambetha.

Both males and females exhibited an increase in *K* values from November to March/April. Both genders experienced a slight increase in September. There was a significant decline in *K* values from May to August. The table 2.14 displays the lowest and highest values of *K* recorded for each month among males, while table 2.15 shows the same information for females. The highest *K* value was seen in females from Diu, followed by Ambetha, Bharuch, and Bhadbhut.

The Fulton's *K* has been calculated for the populations in order to assess their respective habitats' superior condition. Table 2.16 displays the minimum and greatest values of the population's *K* reported for each month. The average

values were measured over the periods of December 2020 to November 2021 and December 2021 to November 2022, respectively. The recorded values are as follows: The measurements obtained from Diu lagoon were 0.98±0.08 and 1.04±0.08. From Bharuch, the measurements were 1.10±0.18 and 1.05±0.16. From Bhadbhut, the measurements were 1.11±0.10 and 1.10±0.11. Lastly, from Ambetha, the measurements were 0.99±0.11 and 1.07±0.12. The majority of the population excelled in Diu followed by Ambetha, Bharuch, and Bhadbhut. The overall results indicate that males exhibited superior conditions compared to females. Furthermore, *M. cephalus* discovered in close proximity to coastal regions such as Diu and Ambetha had better conditions.

Table 2.14: The minimum and maximum Fulton's Condition Factor (K) values recorded of male *M. cephalus* from Diu lagoon and Narmada estuary, Gujarat, India

Male - Fulton's K - 2020-2021				
	Diu	**Bharuch**	**Bhadbhut**	**Ambetha**
Min	0.84±0.08	0.62±0.06	0.44±0.03	0.74±0.11
	Aug-21	Apr-21	Aug-21	Dec-20
Max	1.14±0.07	1.75±0.61	1.72±0.79	1.09±0.20
	May-21	Dec-20	Dec-20	May-21
Male - Fulton's K - 2021-2022				
Min	0.84±0.07	0.75±0.07	0.69±0.09	0.79±0.07
	Aug-22	Apr-22	Mar-22	Aug-22
Max	1.15±0.15	1.30±0.10	1.56±0.25	1.54±0.18
	Mar-22	Dec-21	Dec-21	Dec-21

Table 2.15: The minimum and maximum Fulton's Condition Factor (*K*) values recorded of female *M. cephalus* from Diu lagoon and Narmada estuary, Gujarat, India

Female - Fulton's *K* - 2020-2021			
	Diu	**Bharuch**	**Bhadbhut**	**Ambetha**
Min	0.84±0.05	0.85±0.07	0.79±0.04	0.70±0.07
	Aug-21	Apr-21	Aug-21	Jul-21
Max	1.18±0.12	1.31±0.08	1.37±0.14	1.35±0.02
	Mar-21	May-21	Mar-21	Apr-21
Female - Fulton's *K* - 2021-2022			
Min	0.97±0.12	0.81±0.06	0.97±0.02	0.98±0.02
	Dec-21	Dec-21	Nov-22	Aug-22
Max	1.21±0.12	1.61±0.28	1.31±0.14	1.32±0.14
	Apr-22	Sep-22	Apr-22	Apr-22

Table 2.16: The minimum and maximum Fulton's Condition Factor (*K*) values recorded of *M. cephalus* population from Diu lagoon and Narmada estuary, Gujarat, India

Population - Fulton's *K* - 2020-2021			
	Diu	**Bharuch**	**Bhadbhut**	**Ambetha**
Min	0.84±0.07	0.73±0.13	0.98±0.19	0.84±0.06
	Aug-21	Apr-21	Aug-21	Dec-21
Max	1.10±0.12	1.47±0.51	1.37±0.26	1.22±0.14
	Jul-21	Nov-21	May-21	Apr-21
Population - Fulton's *K* - 2021-2022			
Min	0.91±0.09	0.82±0.09	0.92±0.24	0.88±0.11
	Aug-22	Apr-22	Mar-22	Aug-22
Max	1.17±0.15	1.44±0.26	1.37±0.29	1.29±0.30
	Apr-22	Sep-22	Dec-21	Dec-21

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)
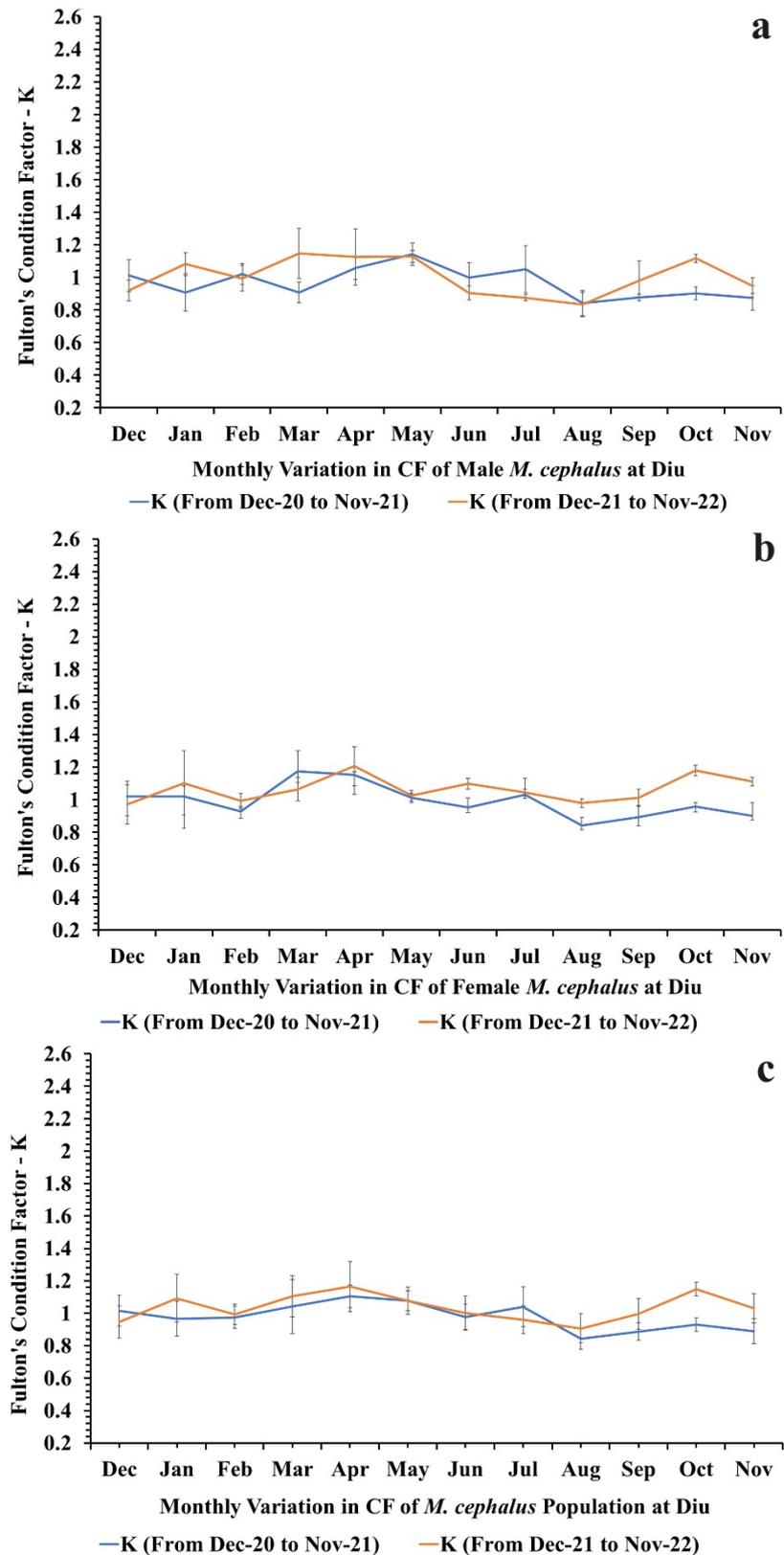
Figure 2.14 a, b & c: Fulton's Condition Factor (*K*) Analysis of *M. cephalus* in Diu Lagoon: (a) Male, (b) Female, and (c) Population from December 2020 to November 2021
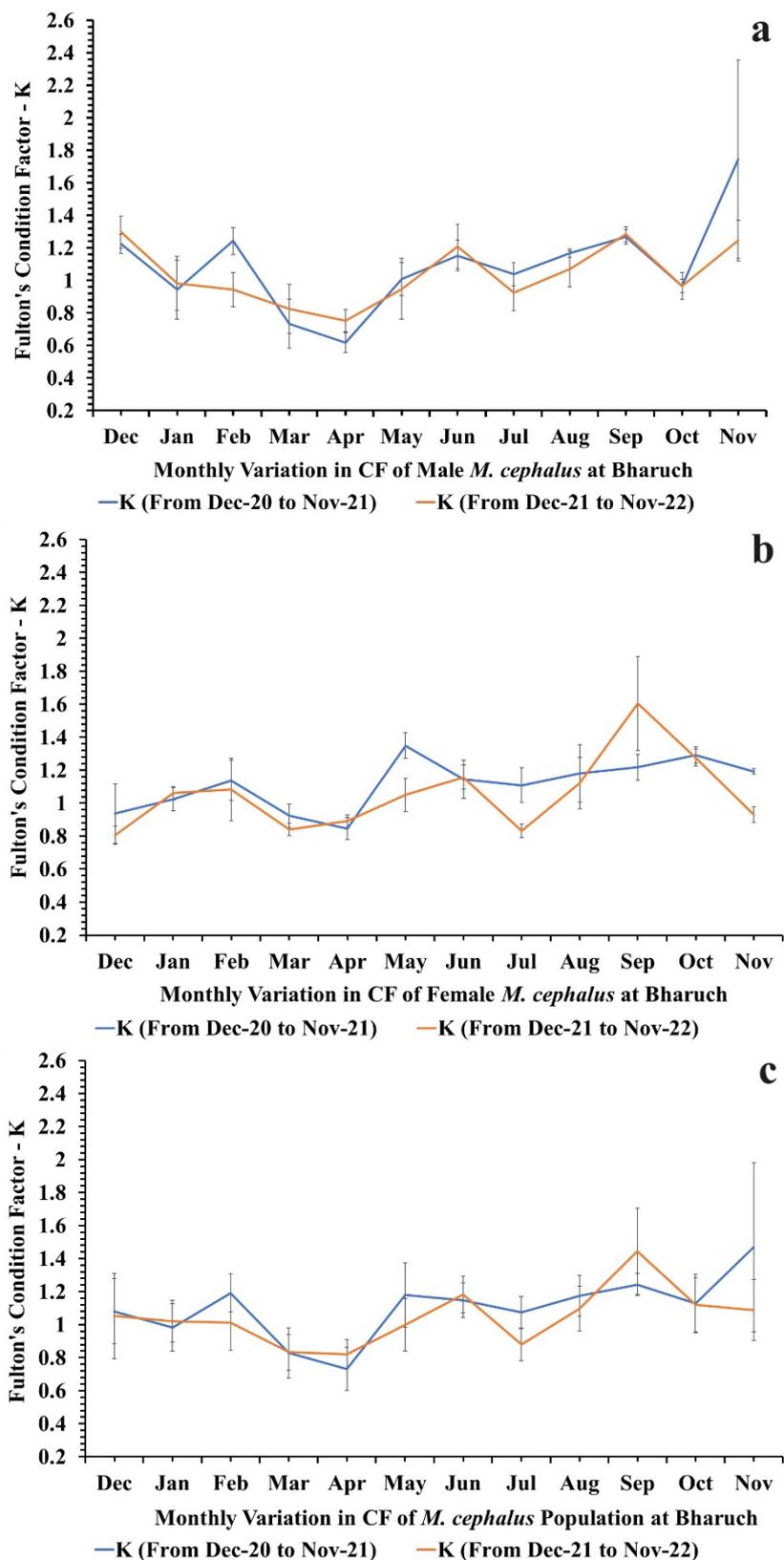
Figure 2.15 a, b & c: Fulton's Condition Factor (*K*) Analysis of *M. cephalus* in Bharuch, Narmada Estuary: (a) Male, (b) Female, and (c) Population from December 2020 to November 2021
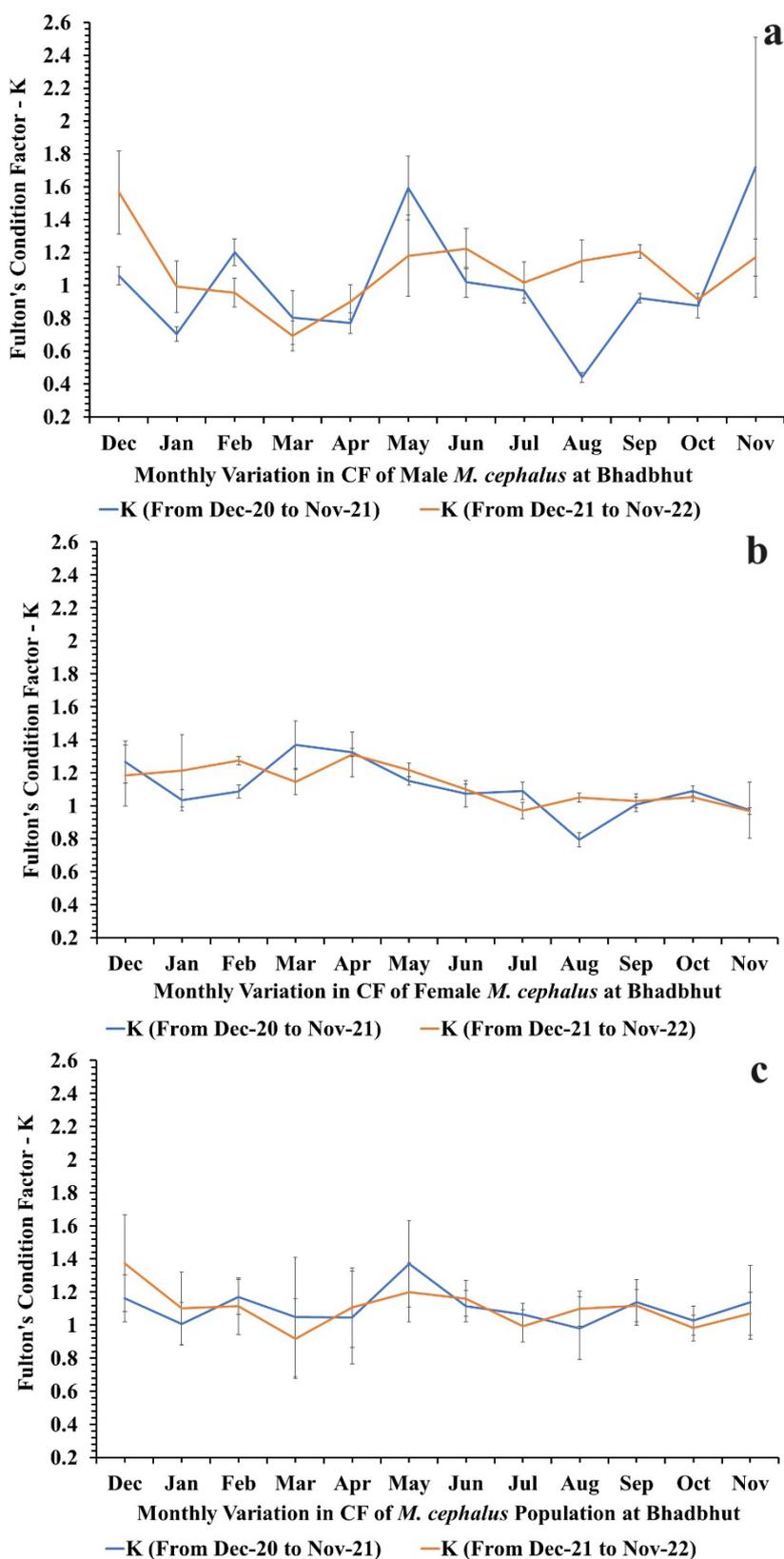
Figure 2.16 a, b & c: Fulton's Condition Factor (*K*) Analysis of *M. cephalus* in Bhadbhut, Narmada Estuary: (a) Male, (b) Female, and (c) Population from December 2020 to November 2021.
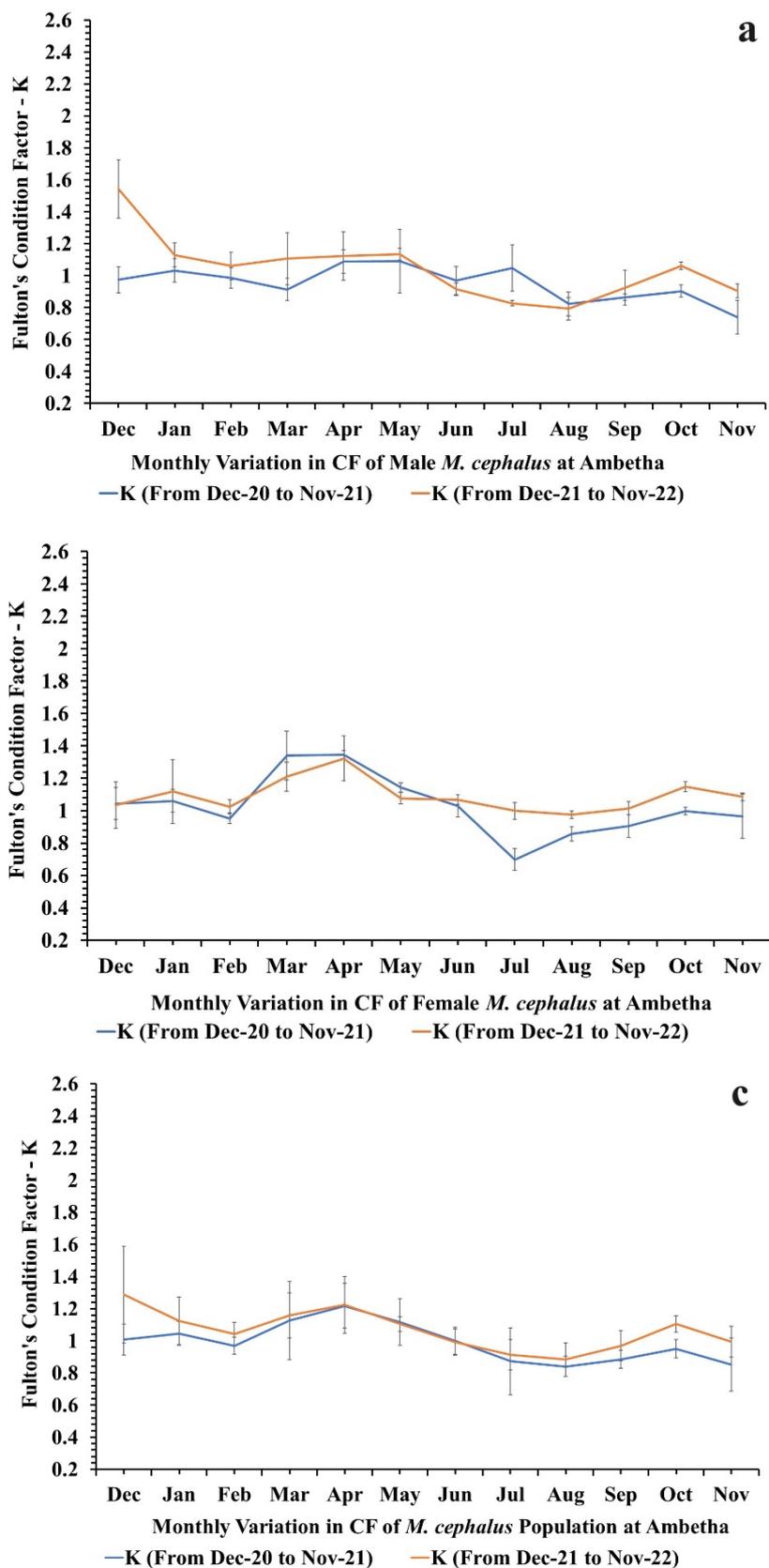
Figure 2.17 a, b & c: Fulton's Condition Factor (*K*) Analysis of *M. cephalus* in Ambetha, Narmada Estuary: (a) Male, (b) Female, and (c) Population from December 2020 to November 2021.

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

2.3.4 Gonadosomatic Index and Hepatosomatic Index

2.3.4.1 Gonadosomatic Index

The I_g of *M. cephalus* was examined in the Diu lagoon and Narmada estuary to assess the physiological condition of the gonads (Fig. 2.18a, 2.19a, 2.20a, 2.21a, 2.22a, 2.23a, 2.24a, 2.25a). The average I_g levels in males were recorded as follows during the period from December 2020 to November 2021: 2.71±2.41 in Diu, 3.88±1.83 in Bharuch, 4.04±2.40 in Bhadbhut, and 4.02±2.91 in Ambetha. From December 2021 to November 2022, the average I_g levels were 3.27±2.49 in Diu, 2.73±1.51 in Bharuch, 2.96±2.03 in Bhadbhut, and 3.01±2.39 in Ambetha. The average I_g levels in females were recorded as follows during the period from December 2020 to November 2021: 8.85±6.21 in Diu, 8.43±4.18 in Bharuch, 8.80±5.39 in Bhadbhut, and 8.31±5.40 in Ambetha. From December 2021 to November 2022, the average I_g levels were 7.87±5.29 in Diu, 8.86±3.51 in Bharuch, 9.15±5.50 in Bhadbhut, and 7.82±5.08 in Ambetha.

In November 2021, the lowest I_g levels in males were seen in Diu. In May 2021, during the period from December 2020 to November 2021, Ambetha, Bhadbhut, and Bharuch also had low I_g levels. In May 2022, the lowest I_g level in male was seen across all sampling sites throughout the period from December 2021 to November 2022 (Table 2.17). The highest I_g levels in male were observed in Ambetha (Dec-20), followed by Bhadbhut (Jan-21), Diu (Dec-20), and Bharuch (Jan-21) between December 2020 and November 2021. During the period from December 2021 to November 2022, the highest I_g levels for male were recorded in the following order: Ambetha in January 2021, Diu in January 2022, Bhadbhut in December 2021, and Bharuch in January 2022 (Table 2.17).

The lowest I_g levels in females were seen in Bharuch (Jun-21), Ambetha (Jul-21), Bhadbhut (May-21), and Diu (Jul-21) between December 2020 and November 2021. During the period from December 2021 to November 2022, the lowest I_g levels in females were seen in Bharuch (May-22), followed by Ambetha (Jun-22), Bhadbhut (May-22), and Diu (Jul-22) as shown in Table 2.18. The highest I_g levels in females were observed in Bhadbhut (Nov-21), followed by Diu (Mar-21), Ambetha (Feb-21), and Bharuch (Feb-21) between December 2020 and November 2021. During the period from December 2021 to November 2022, the

highest I_g values for females were recorded in the following order: Bhadbhut (Dec-21), Du (Dec-21), Ambetha (Feb-22), and Bharuch (Jan-22) (Table 2.18).

Table 2.17: The minimum and maximum Gonadosomatic Index (I_g) values recorded of male *M. cephalus* population from Diu lagoon and Narmada estuary, Gujarat, India

Male – Gonadosomatic Index (I_g) - 2020-2021			
	Diu	**Bharuch**	**Bhadbhut**	**Ambetha**
Min	0.79±0.07	1.30±1.05	1.03±1.02	1.00±0.88
	Nov-21	May-21	May-21	May-21
Max	8.97±0.84	7.47±1.42	9.02±1.42	9.61±2.20
	Dec-20	Jan-21	Jan-21	Dec-20
Male – Gonadosomatic Index (I_g) - 2021-2022			
Min	0.58±0.17	0.40±0.18	0.40±0.17	0.49±0.17
	May-22	May-22	May-22	May-22
Max	9.66±3.09	5.40±0.56	7.06±0.62	9.88±3.31
	Jan-22	Jan-22	Dec-21	Jan-22

Table 2.18: The minimum and maximum Gonadosomatic Index (I_g) values recorded of female *M. cephalus* population from Diu lagoon and Narmada estuary, Gujarat, India.

Female – Gonadosomatic Index (I_g) - 2020-2021			
	Diu	**Bharuch**	**Bhadbhut**	**Ambetha**
Min	2.53±0.30	2.28±0.28	2.44±1.15	2.34±0.17
	Jul-21	Jun-21	May-21	Jul-21
Max	18.60±2.71	13.34±1.24	20.74±1.92	15.52±1.74
	Mar-21	Feb-21	Nov-21	Feb-21
Male – Gonadosomatic Index (I_g) - 2021-2022			
Min	2.32±0.37	2.06±0.56	2.18±0.92	2.08±0.22
	Jul-22	May-22	May-22	Jun-22
Max	16.46±2.71	13.73±0.45	21.97±4.27	15.14±0.23
	Dec-21	Jan-22	Dec-21	Feb-22

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

2.3.4.2 Hepatosomatic Index

A study was conducted to analyse the I_h of *M. cephalus* in the Diu lagoon and the Narmada estuary, focusing on specific areas such Bharuch, Bhadbhut, and Ambetha (Fig. 2.18b, 2.19b, 2.20b, 2.21b, 2.22b, 2.23b, 2.24b, 2.25b). The objective of this study was to evaluate the hepatic physiological condition in these fish populations. The average I_h measurements for males were recorded as follows during the period from December 2020 to November 2021: 1.90±0.47 in Diu, 1.66±0.59 in Bharuch, 1.62±0.50 in Bhadbhut, and 2.22±0.70 in Ambetha. From December 2021 to November 2022, the average I_h measurements were 2.10±0.60 in Diu, 1.67±0.47 in Bharuch, 1.64±0.52 in Bhadbhut, and 1.88±0.54 in Ambetha. The average I_h values for females were recorded as follows during the period from December 2020 to November 2021: 2.07±0.87 in Diu, 1.58±0.49 in Bharuch, 1.83±0.75 in Bhadbhut, and 1.93±0.82 in Ambetha. For the period from December 2021 to November 2022, the average I_h values were 1.74±0.73 in Diu, 1.48±0.68 in Bharuch, 1.71±0.63 in Bhadbhut, and 1.62±0.67 in Ambetha.

The lowest I_h value in male was seen in Diu in January 2021, followed by Bharuch (Nov-21), Bhadbhut (Jan-21), and Ambetha (Jan-21), between December 2020 and November 2021. The lowest I_h value in male was seen in Bhadbhut (Feb-22), Bharuch (Dec-21), Diu (Feb-22), and Ambetha (Jan-22) between December 2021 and November 2022 (Table 2.19). The highest I_h value in male was seen in Ambetha (Jul-21), followed by Bharuch (Apr-21), Diu (May-21), and Bhadbhut (Aug-21) between December 2020 and November 2021. During the period from December 2021 to November 2022, the highest I_h values for male were recorded in Diu in December 2021, followed by Ambetha and Bharuch in August 2022, and Bhadbhut in March 2022 (Table 2.19).

The lowest I_h value among females was documented in January 2021 in Ambetha, followed by Diu, Bhadbhut, and Bharuch from December 2020 to November 2021. During the period from December 2021 to November 2022, the lowest I_h value in females was seen in Ambetha on January 22nd, followed by Bharuch on December 21st, Diu on January 22nd, and Bhadbhut on January 22nd, as shown in Table 2.20. The highest I_h value in females was nearly identical in Ambetha and Diu in May 2021, followed by Bhadbhut (May 2021) and Bharuch

(September 2021) between December 2020 and November 2021. During the period from December 2021 to November 2022, the highest I_h values for females were recorded in Diu in May 2022, followed by Bharuch in July 2022, Bhadbhut in May 2022, and Ambetha in May 2022 (Table 2.20).

Table 2.19: The minimum and maximum Hepatosomatic Index (I_h) values recorded of male *M. cephalus* population from Diu lagoon and Narmada estuary, Gujarat, India

	Male – Hepatosomatic Index (I_h) - 2020-2021			
	Diu	**Bharuch**	**Bhadbhut**	**Ambetha**
Min	0.72±0.53	0.88±0.10	0.94±0.18	1.01±0.33
	Jan-21	Nov-21	Jan-21	Jan-21
Max	2.71±0.06	2.76±0.46	2.52±0.48	4.10±0.41
	May-21	Apr-21	Aug-21	Jul-21
	Male – Hepatosomatic Index (I_h) - 2021-2022			
Min	1.09±0.32	0.96±0.07	0.87±0.30	1.17±0.29
	Feb-22	Dec-21	Feb-22	Jan-22
Max	3.20±0.55	2.61±0.31	2.40±0.60	2.72±0.27
	Dec-21	Aug-22	Mar-22	Aug-22

Table 2.20: The minimum and maximum Hepatosomatic Index (I_h) values recorded of female *M. cephalus* population from Diu lagoon and Narmada estuary, Gujarat, India

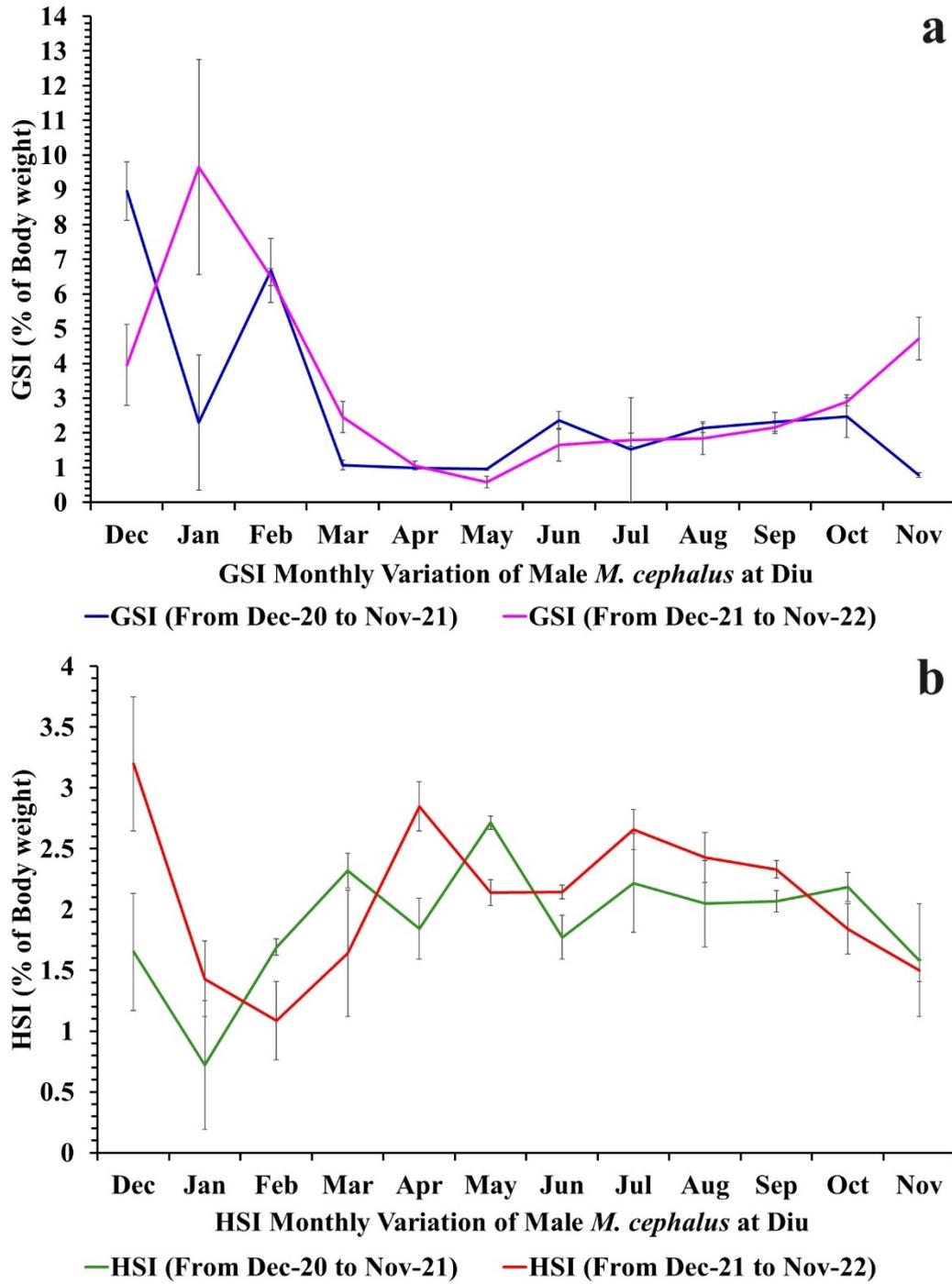|	Female – Hepatosomatic Index (I_h) - 2020-2021			
	Diu	**Bharuch**	**Bhadbhut**	**Ambetha**
Min	0.68±0.08	0.87±0.11	0.77±0.11	0.65±0.07
	Jan-21	Jan-21	Jan-21	Jan-21
Max	3.45±0.19	2.36±0.22	3.08±0.15	3.48±0.18
	May-21	Sep-21	May-21	May-21
	Female – Hepatosomatic Index (I_h) - 2021-2022			
Min	0.76±0.08	0.70±0.15	0.83±0.08	0.69±0.06
	Jan-22	Dec-21	Jan-22	Jan-22
Max	3.13±0.20	2.97±0.32	2.94±0.16	2.76±0.16
	May-22	Jul-22	May-22	May-22

Figure 2.18 a & b: (a) Gonadosomatic Index (I_g) and (b) Hepatosomatic Index (I_h) of Male *M. cephalus* in Diu lagoon from December 2020 to November 2022
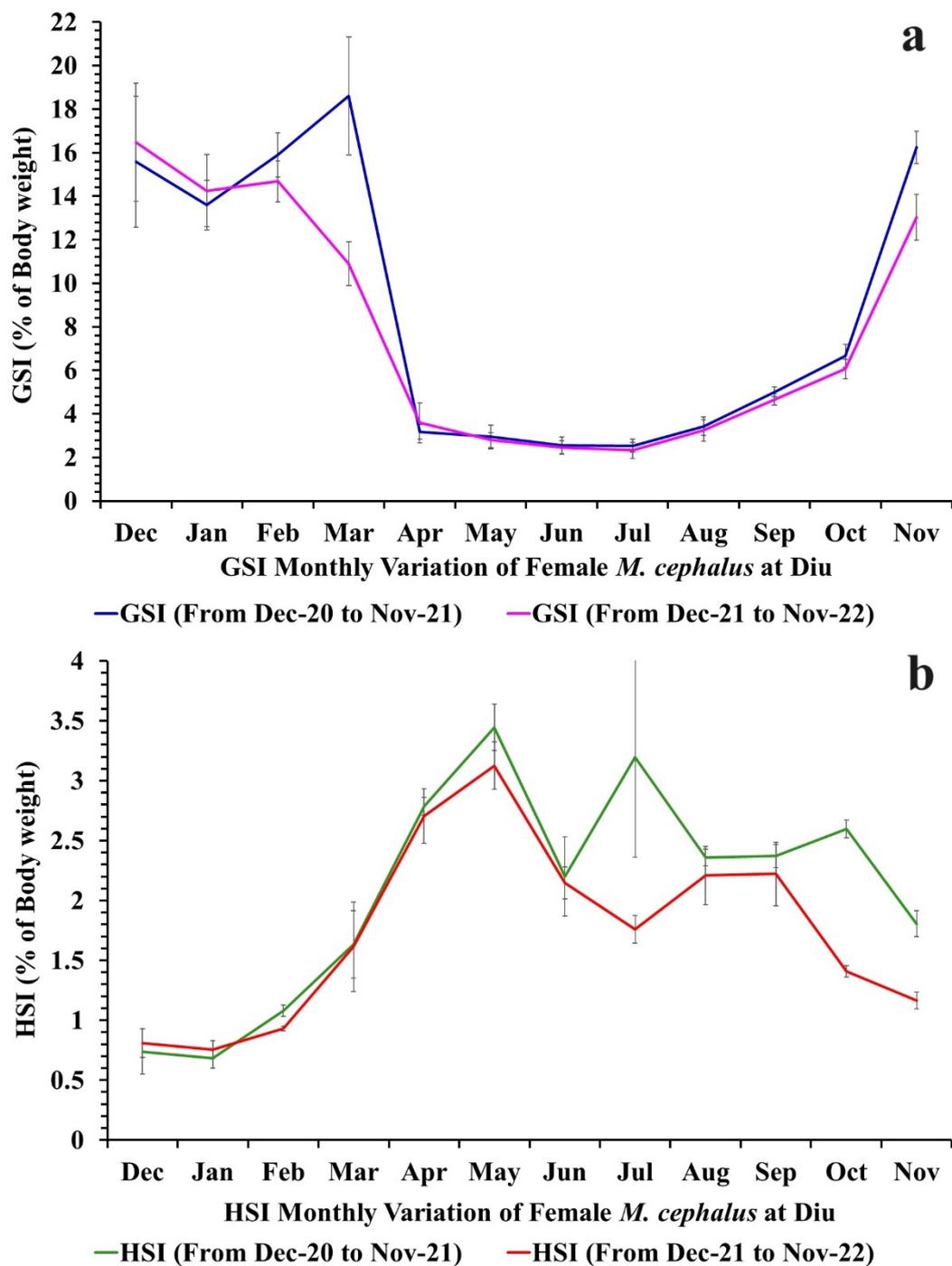
Figure 2.19 a & b: (a) Gonadosomatic Index (I_g) and (b) Hepatosomatic Index (I_h) of Female *M. cephalus* in Diu lagoon from December 2020 to November 2022
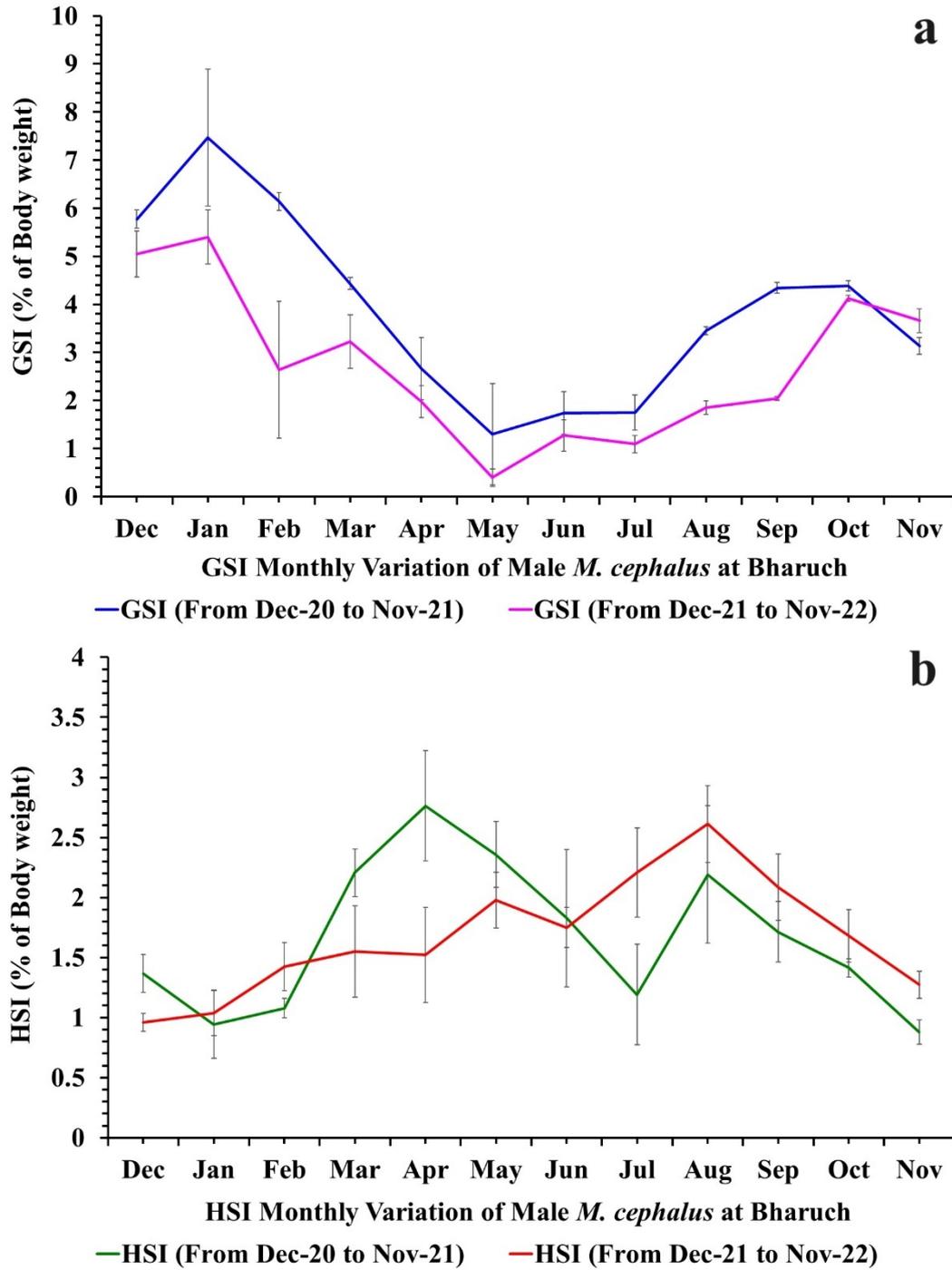
Figure 2.20 a & b: (a) Gonadosomatic Index (I_g) and (b) Hepatosomatic Index (I_h) of Male *M. cephalus* in Bharuch, Narmada Estuary from December 2020 to November 2022

Figure 2.21 a & b: (a) Gonadosomatic Index (I_g) and (b) Hepatosomatic Index (I_h) of Female *M. cephalus* in Bharuch, Narmada Estuary from December 2020 to November 2022

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

Figure 2.22 a & b: (a) Gonadosomatic Index (I_g) and (b) Hepatosomatic Index (I_h) of Male *M. cephalus* in Bhadbhut, Narmada Estuary from December 2020 to November 2022
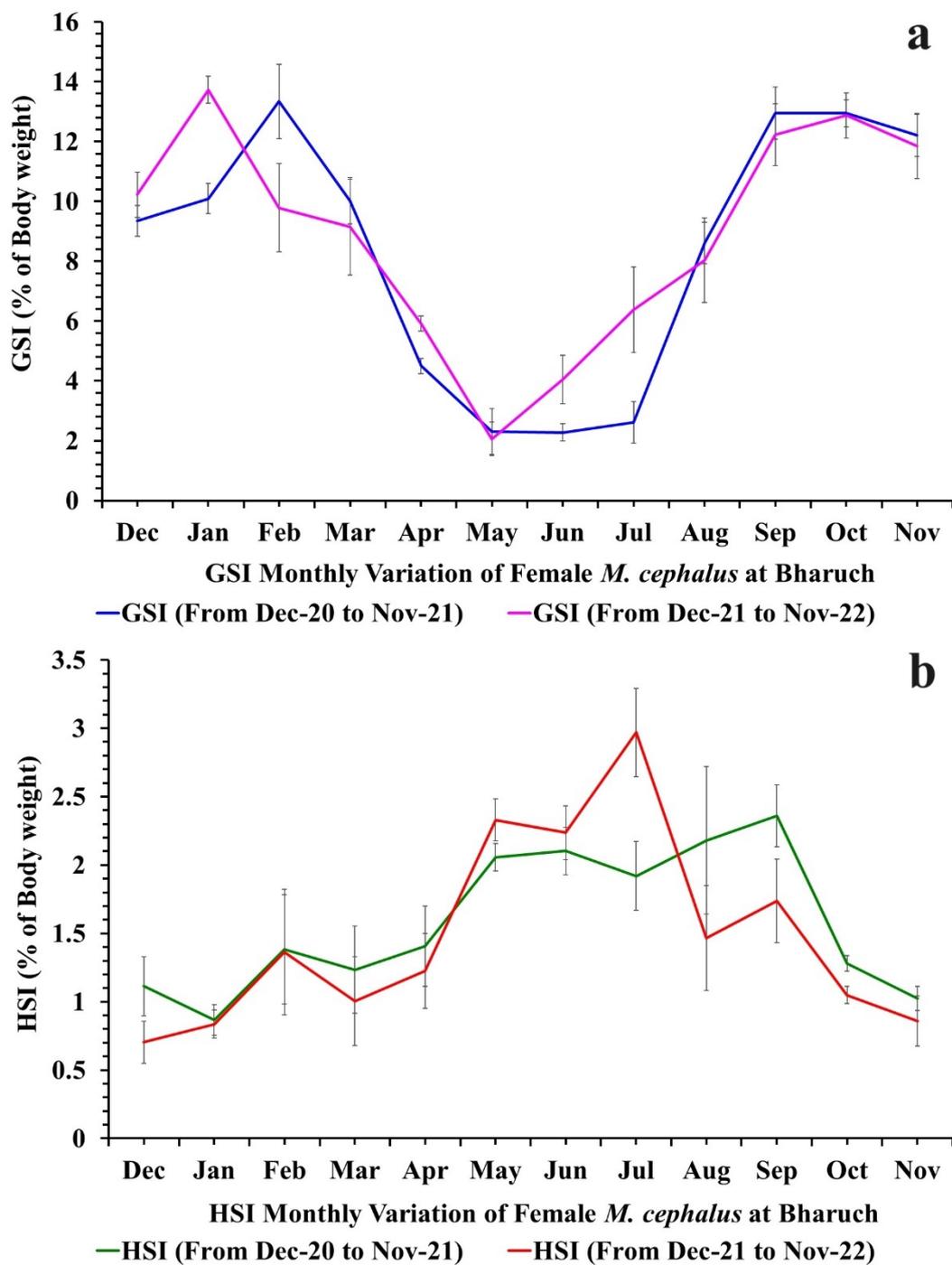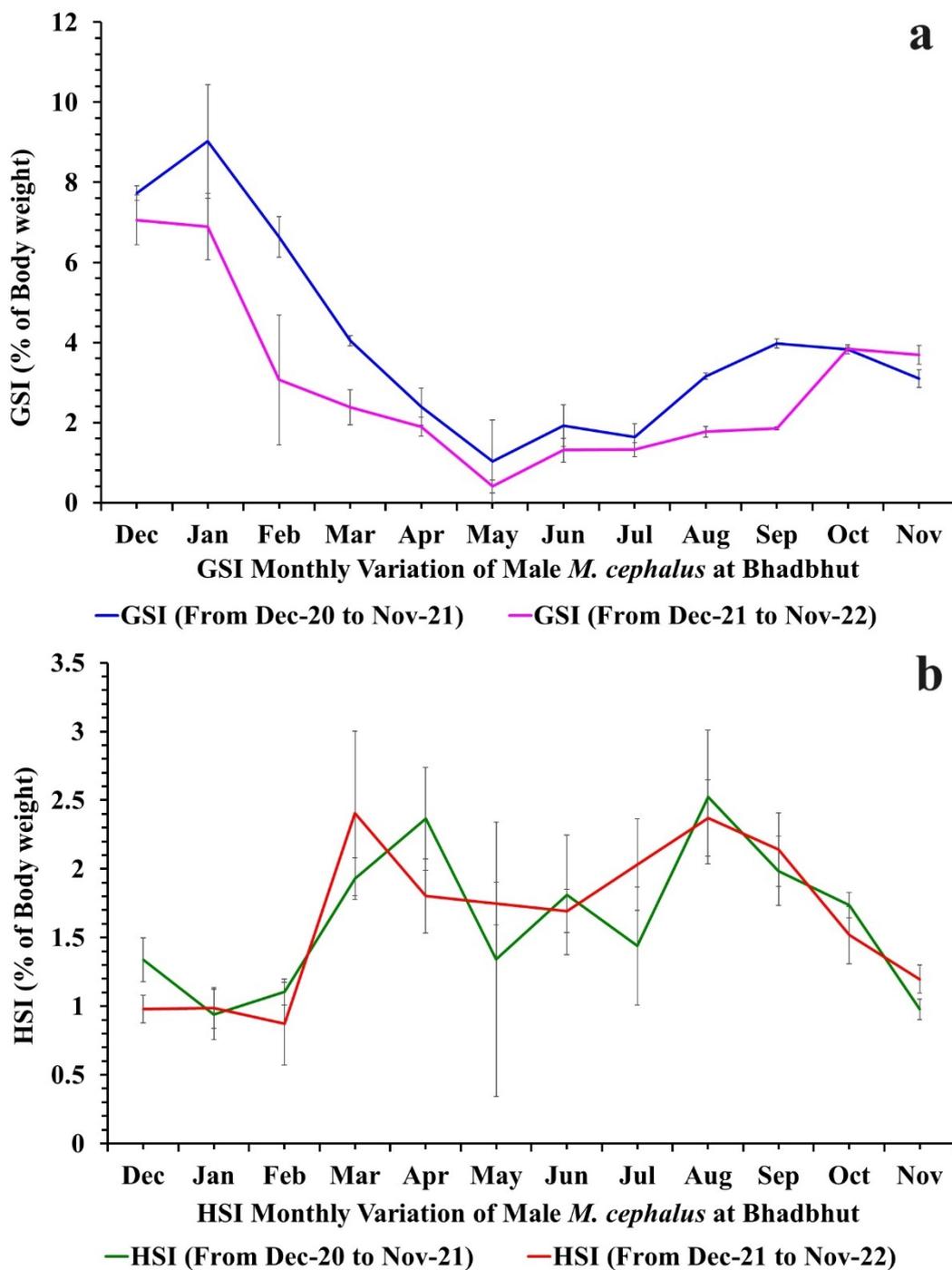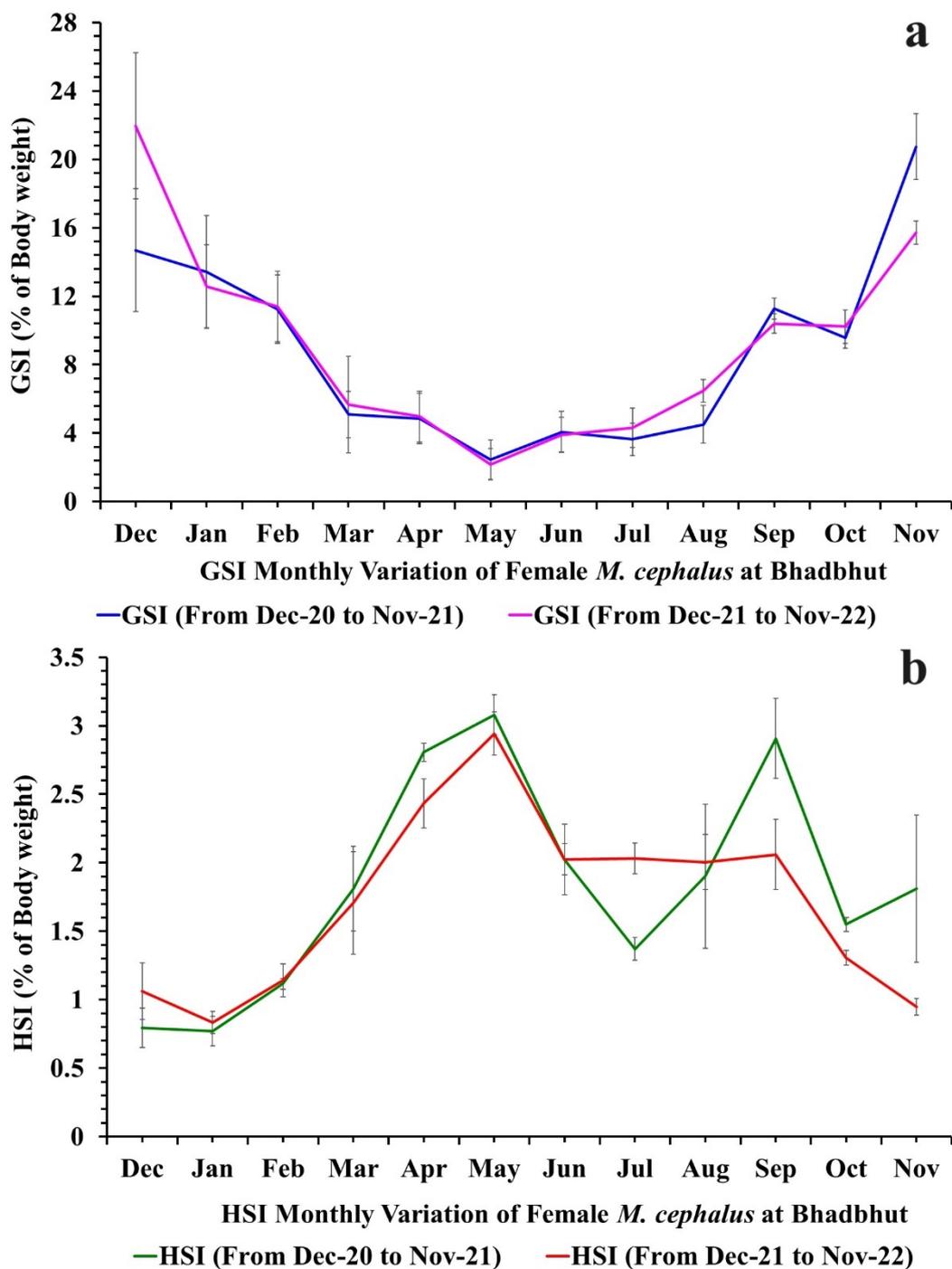
Figure 2.23 a & b: (a) Gonadosomatic Index (I_g) and (b) Hepatosomatic Index (I_h) of Female *M. cephalus* in Bhadbhut, Narmada Estuary from December 2020 to November 2022

Figure 2.24 a & b: (a) Gonadosomatic Index (I_g) and (b) Hepatosomatic Index (I_h) of Male *M. cephalus* in Ambetha, Narmada Estuary from December 2020 to November 2022
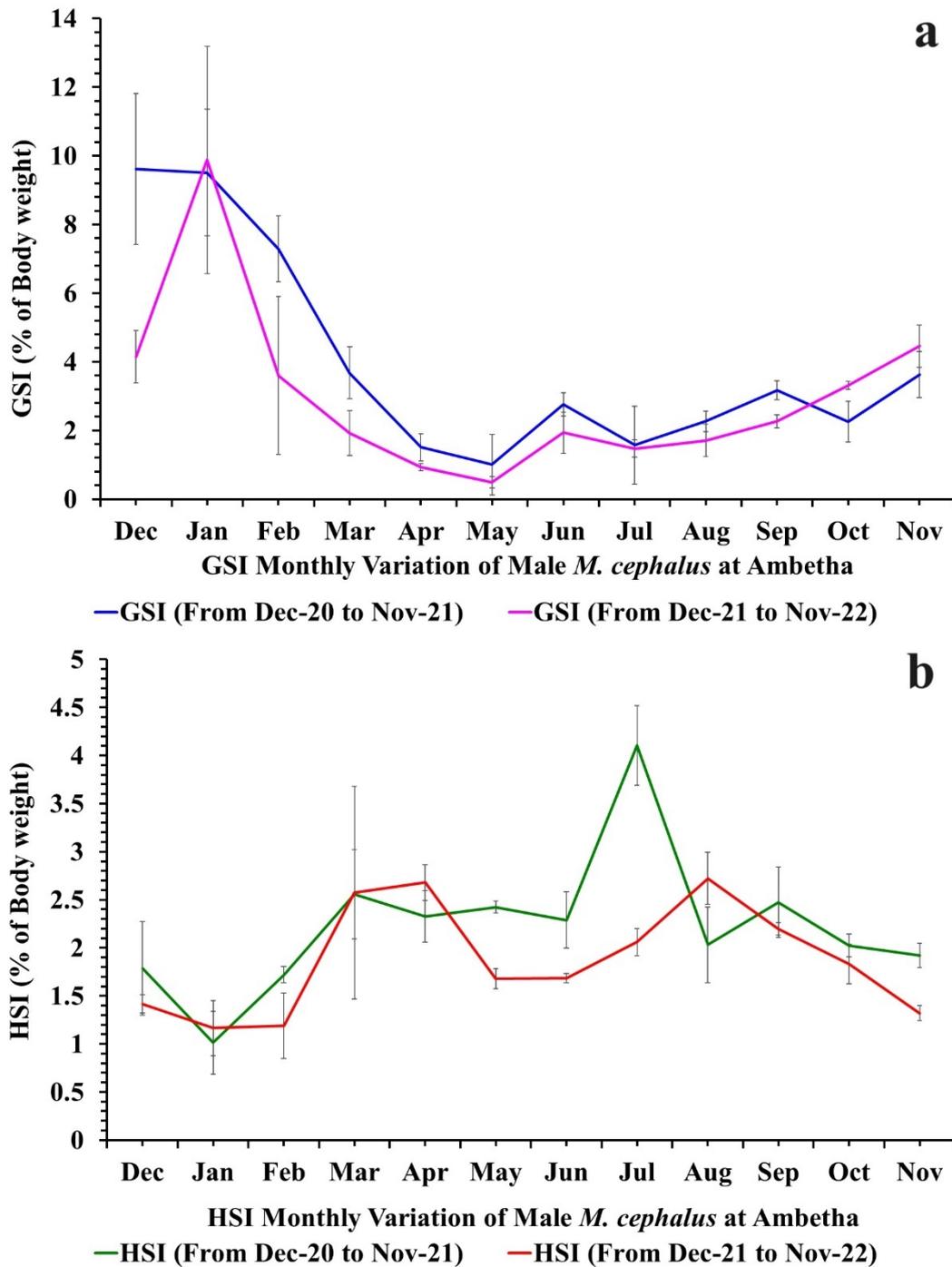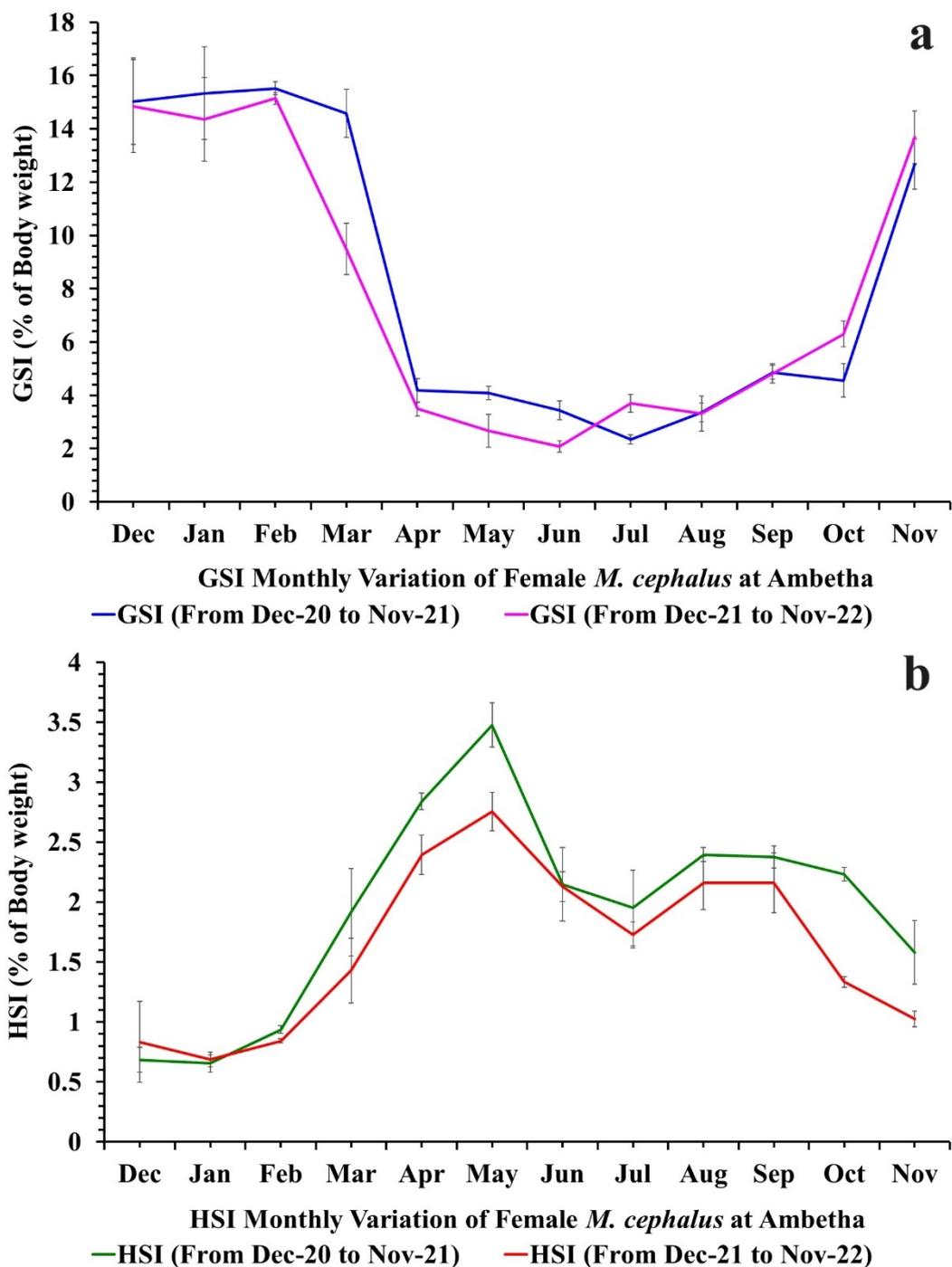
Figure 2.25 a & b: (a) Gonadosomatic Index (I_g) and (b) Hepatosomatic Index (I_h) of Female *M. cephalus* in Ambetha, Narmada Estuary from December 2020 to November 2022

2.4 DISCUSSION

2.4.1 Physico-chemical Parameters of Water

The Diu lagoon and Narmada estuary are significant coastal habitats located on the North-Western Coast of India. The environmental circumstances that influence the fluctuating characteristics of the water in these habitats are primarily determined by two main factors: (1) short-term variations caused by tides and (2) significant seasonal fluctuations brought about by the monsoon cycle (Chandran & Ramamoorthy 1984). The current study indicates that the hydrographic conditions at all the research sites undergo significant variations across the seasons, primarily due to the impact of monsoon precipitation, which results in a substantial input of freshwater.

Abowei (2010) states that a pH level greater than 7.0, but less than 9.0, is optimal for biological productivity. The pH readings were consistently similar across all the study sites and consistently stayed slightly alkaline during the whole study period. In August 2022, the pH of water sample collected from upstream of Ambetha was found to be the lowest. The lower pH values may be attributed to the insufficient freshwater input from upstream areas and influent water. In January 2021, the pH level reached its peak in the upstream area of Bharuch. The post-monsoon freshwater influx in the Narmada river could potentially be the cause. Throughout the year, the water at the Bharuch research location exhibited consistently higher alkalinity compared to other sites. The pH parameters of Diu and Bhadbhut waters are nearly same in consecutive years. The pH values exhibited a downward trend during the summer months and then increased, attaining elevated levels during the monsoon and post-monsoon periods. The alkaline quality of the water has been seen in both the Kadinamkulam backwater and the Vellar estuary, as reported by Nair *et al.* (1984) and Chandran & Ramamurthy (1984) respectively.

The water temperature remained reasonably stable among the different stations, showing negligible fluctuation. The values of the variable tended to decrease throughout the winter months and then progressively increase, reaching elevated levels during the summer season. Throughout both years, the temperature of the water at Diu lagoon was consistently lower compared to the

other study sites. Gupta *et al.* (2018) and Patale & Tank (2022) conducted assessments of the water characteristics of the Diu coast and Narmada estuary, respectively, and documented comparable findings. Temperature fluctuations can impair physiological performance by disrupting physiological integration. Elevated temperatures have an impact on the functioning of proteins, particularly the efficiency of enzymes. Enzymes necessitate accurate substrate binding, catalysis, and release of the product. At elevated temperatures, the binding sites expand, leading to a decrease in the effectiveness of the enzyme (Hoffman & Somero 1996). The decrease in water temperature observed during the monsoon period can be attributed to reduced solar radiation caused by cloudy skies and increased precipitation. Azis & Nair (1976) observed a similar influence of the monsoon on temperature in the Edava-Nadayara-Paravur backwater system, while Nair *et al.* (1984) made a similar observation in the Kadinamkulam backwater system. Thangaraj (1985), Mani (1989), Vasantha (1989), Kaliyaperumal (1992), Gothandaraman (1993), and Karuppasamy (1997) have all made similar observations. Additionally, Mohan Raj *et al.* (2013), Santhosh Kumar & Ashok Prabhu (2014), and Panchakshari *et al.* (2015) have all reported similar findings.

Salinity is a critical chemical element that significantly impacts the distribution and abundance of species in aquatic environments. The salinity exhibited significant variation and adhered to a distinct spatial pattern throughout all the experimental sites, mostly due to the contrasting habitat types, namely estuary and lagoon. Overall, there was a consistent decline in the estuary's characteristics as one moved from the mouth to the head. The water in the Diu lagoon tends to have a higher salinity due to the absence of freshwater inflow. The salinity exhibited a significant elevation throughout the summer months, although it remained low during the wet or winter season. Deshkar *et al.* (2014) and Pandey *et al.* (2022) recorded similar results from Diu. The Narmada estuary experiences a prevalence of marine water throughout the summer months. Upon the arrival of the south-west monsoon, there was a significant decrease in the salinity levels, and it continues to be more brackish from April to June. Subsequently, an increase in salinity was noted. Gajbhiye *et al.* (1981), Deshkar *et al.* (2012), and Deshkar *et al.* (2014) documented comparable salinity ranges in the Narmada estuary.

Dissolved oxygen (DO) is a crucial abiotic element that significantly impacts life in coastal environments. Typically, places with significant levels of pollution tend to have elevated levels of dissolved oxygen. The most crucial consequence of pollution is the further reduction of dissolved oxygen to the point of becoming anaerobic, as stated by Lester (1975). The highest concentration of dissolved oxygen was measured at the upstream of Diu lagoon in January 2021, while the lowest concentration was recorded in the upstream of Bharuch in May 2022. The dissolved oxygen (DO) concentrations exceed 5 mg/l, which is sufficient to sustain aquatic life (Mishra *et al.* 2008; Panchakshari *et al.* 2015). The results of dissolved oxygen (DO) measurements from all research sites indicate higher concentrations than the desired amount, except for the Bharuch site of the Narmada estuary during the summer season. This may be due to the minimal inflow of freshwater and changes in tide patterns. In a research conducted by Bhadja & Kundu (2012) near the Diu coast, the dissolved oxygen (DO) content was found to be in the range of 5.17-6.08 mg/l, which is slightly lower than the values observed in the current inquiry. Raghunath *et al.* (2004) observed dissolved oxygen (DO) levels from the Diu shore and reported similar concentrations to those obtained in the current study. Similar phenomena were recorded for Narmada estuary by Gajbhiye *et al.* (1981), Deshkar *et al.* (2012), and Deshkar *et al.* (2014).

Increased salinity, alterations in water's ionic composition, and the toxicity of specific ions are all consequences of total dissolved solids. TDS measurement encompasses all anions and cations present in the sample, with certain ions or combinations of ions being significantly more poisonous than others. Some species may exhibit increased susceptibility to toxicity from Total Dissolved Solids (TDS) at specific life stages, such as during fertilisation, as observed in many fish species (Weber-Scannell & Duffy 2007). The current findings indicate that the lowest Total Dissolved Solids (TDS) values were recorded from midstream area of Bhadbhut in December 2021, while the highest values were reported in the downstream area of Ambetha in August 2022. Out of all the locations, Ambetha had the highest Total Dissolved Solids (TDS) values, followed by Bharuch, Diu, and Ambetha. This is possible because Ambetha is situated at the mouth of an estuary that receives influents from the area around the mouth and is connected to the

Gulf of Khambhat. Bhadja & Kundu (2012) and Patale *et al.* (2022) conducted a physico-chemical analysis of Diu water and documented total dissolved solids (TDS) statistics that align with the findings of the current study. No investigations were conducted on the Narmada estuary, except for the study conducted by Kumar *et al.* (2010).

2.4.2 Length-Weight Relationship and Condition Factor

The LWR of several fish species reveals that some strictly adhere to the Cube law, while others exhibit either an increase or reduction in weight that is proportional to the cube of their length. Length-weight connection studies of fish are crucial in fisheries research for fish production and estimating biomass (Sunil 2000). The LWR is a scientific tool used to assess the survival, growth, maturity, reproduction, and overall well-being of fishes (Le Cren 1951). Le Cren (1951) suggested that deviations from the cube law could be attributed to factors such as the fish's health, reproductive habits, taxonomic differences, or environmental circumstances.

The equation $W = aL^b$ is applied individually to the male, female, and population data for *M. cephalus* (Table 10-13) to determine any potential differences. The data is then subjected to analysis of variance (ANOVA). The analysis of variance reveals that there is a substantial association between males and females in *M. cephalus*, at a significance level of 5%. Therefore, the data is analysed individually for males, females, and populations, resulting in different equations for *M. cephalus*. For males, the equation is $W=0.16L^{0.81}$ with an R^2 value of 0.96. For females, the equation is $W=0.18L^{2.77}$ with an R^2 value of 0.93. For the population as a whole, the equation is $W=0.16L^{2.86}$ with an R^2 value of 0.97. During December 2021 to November 2022 at DIU, the weight (W) of males can be determined by calculated using the formula $W = 0.17L^{2.78}$, with an R-squared value of 0.95. Similarly, the weight of females can be determined using the formula $W = 0.19L^{2.75}$, with an R-squared value of 0.96. For the population as a whole, the weight can be calculated using the formula $W = 0.17L^{2.85}$, with an R-squared value of 0.96.

In Bharuch during the year 2020-21, the relationship between weight (W) and height (L) for males may be described by the equation $W=0.16L^{2.86}$ with an R-squared value of 0.60. For females, the relationship is given by $W=0.32L^{2.37}$ with an R-squared value of 0.85. The equation that represents the relationship between weight and height for the population as a whole is $W=0.19L^{2.73}$ with an R-squared value of 0.75. In Bharuch for the year 2021-22, the weight of males can be estimated using the equation $W=0.19L^{2.73}$ with an R-squared value of 0.78. Similarly, the weight of females can be estimated using the equation $W=0.29L^{2.42}$ with an R-squared value of 0.80. For the population as a whole, the weight can be estimated using the equation $W=0.22L^{2.65}$ with an R-squared value of 0.82.

In Bhadbhut during 2020-21, for males, weight (W) is determined by the formula $W=0.28L^{2.45}$ with a coefficient of determination (R^2) of 0.74. For females, weight (W) was determined by the formula $W=0.30L^{2.41}$ with an R^2 of 0.89. For the population as a whole, weight (W) was determined by the formula $W=0.28L^{2.47}$ with an R^2 of 0.82. Whereas in Bhadbhut during 2021-22 weight (W) of the males may be estimated using the formula $W=0.37L^{2.24}$ with an R-squared value of 0.73. For females, weight (W) can be estimated using the formula $W=0.25L^{2.55}$ with an R-squared value of 0.92. For the population as a whole, weight (W) can be estimated using the formula $W=0.30L^{2.40}$ with an R-squared value of 0.82.

For males, weight (W) is determined by the formula $W=0.24L^{2.53}$ with an R-squared value of 0.96 whereas, for females, weight (W) was determined by the formula $W=0.26L^{2.50}$ with an R-squared value of 0.96 in Ambetha during 2020-22. For the population as a whole, weight (W) was determined by the formula $W=0.21L^{2.63}$ with an R-squared value of 0.96 in Ambetha during 2020-22. In Ambetha during 2021-22, male individual's weight (W) was calculated using the formula $W=0.21L^{2.65}$ with a coefficient of determination (R^2) of 0.96. For females, weight (W) was calculated using the formula $W=0.22L^{2.64}$ with an R^2 of 0.96. For the population as a whole, weight (W) was calculated using the formula $W=0.20L^{2.71}$ with an R^2 of 0.96.

According to Wootton (1990), if a fish grows isometrically, its body shape remains the same and its specific gravity remains constant throughout its lifetime. In such instances, the b-value must be equal to 3.0. Therefore, the growth pattern

in fish will adhere to the cube law. However, in natural conditions, the cube law is not typically observed in most fish due to their ability to alter their body form as they grow or increase in size. This allows them to become heavier during one season and lighter during another season. Therefore, Le Cren (1951) found that the observed correlation between the length and weight of fish may deviate from the expected value of 3.0. This deviation could be attributed to specific environmental factors or the health of the fish. Therefore, the b-value for each fish species may deviate greatly from the optimum value of 3.0, suggesting that the growth pattern is allometric (Gayanilo & Pauly 1997). Therefore, if the b-value is precisely 3.0, then the growth is isometric. However, if the b-value is less than 3.0, the fish will become more slender as its length increases. This indicates a negative allometric growth, which could be attributed to unsuitable environmental conditions. Conversely, when the b-value exceeds 3.0, the fish's weight increases and exhibits a positive allometric development pattern in relation to their individual lengths. This could be attributed to optimal conditions (Wootton 1998; Abowei 2010).

Le Cren (1951) proposed that the females exhibited more weight compared to males of the same length, perhaps due to disparities in fat composition and gonadal growth. Hossain (2010) found that various factors, such as habitat, spawning season, environmental conditions (such as temperature, salinity, and seasonality), food availability, sex, maturity stages, appetite, and gonadal content, can influence the length and weight relationships in fishes. These factors can also affect the b-values, even within the same species. The examination of length-weight connections, condition, and relative condition parameters has proven to be highly beneficial tools in the investigation of fishery biology. The text discusses three main topics: fisheries management, the estimation of fish health and growth patterns, and the assessment of fish stock. These topics are covered in the works of Froese (2006), Gomiero *et al.* (2008), and Zubia *et al.* (2014). Based on a review of the literature, weight-length statistics for mullets usually show stable values within a species up until they reach the length where they undergo first or second maturation. After this time, the discrepancies become more noticeable (Kesteven 1942; Pillay 1954; Erman 1961; Cassifour 1975). Nevertheless, there is frequently a significant difference between females that are

capable of reproducing and those who are not (Erman 1961; Farrugio & Quignard 1974). In fact, the regression coefficient for females tends to be larger than that of male (Morovic 1957; Erman 1959; Hendricks 1961; Kennedy & Fitzmaurice 1969). According to literature, research indicates that during the early stages of a grey mullet's life, there is a relatively low weight-to-length ratio (Thomson 1951; Broadhead 1953; Erman 1959; Ezzat 1965; Farrugio 1975). However, once the fish reaches maturity for the first time, its weight increases rapidly and this trend continues as the fish ages.

It is noteworthy that the weight of grey mullets of the same age, residing in lakes (lagoons) and estuaries, is often greater (Morovic 1957; Denizci 1958) than that of the fish inhabiting the sea (excluding mature females). Luther (1963) documented the findings (b = 3.02) obtained from the saltwater lagoon located in Mandapam, India. Rangaswamy (1976) found that there was no significant difference in the LWR of grey mullet between different years and habitats in the Ennore and Adyar estuaries. In a study conducted by Murugan *et al.* (2012), they observed negative allometric growth in *M. cephalus* of the Vellar estuary, located on the South East Coast of India. The study found that the male *M. cephalus* had a growth rate of b = 2.7658 (R^2 = 0.99), while the female *M. cephalus* had a growth rate of b = 2.8586 (R^2 = 0.99). Sahoo *et al.* (2012) investigated the LWR of *M. cephalus* in Chilika lagoon, located in Odisha, India. The researchers observed that males exhibited isometric growth with a coefficient of 3.004 (R^2 = 0.976), whereas females and the overall population showed negative allometric growth with coefficients of 2.950 (R^2 = 0.988) and 2.977 (R^2 = 0.982), respectively. A study conducted by Panda *et al.* (2018) found evidence of negative allometric growth [2.96 (R^2 = 0.95)] in the population of *M. cephalus* in Chilika lake, India. In their study done in Pulicat lake, Tiruvallur (dt), Tamil Nadu, Durairaja *et al.* (2020) found that the slope value (b) was 2.7638 (R^2 = 0.89) for males, 2.7624 (R^2 = 0.93) for females, and 2.7653 (R^2 = 0.90) for both sexes combined. Rekha *et al.* (2020) did a study in Pulicat lake, Tamil Nadu, and Cochin backwaters, Kerala, which are located on the east and west coast of India, respectively. Positive allometric growth was observed in females, with females of west coast exhibiting a growth rate of 3.081 (R^2 = 0.89) and females of east coast exhibiting a growth rate of 3.322 (R^2 = 0.79). On the other hand, male exhibited negative allometric growth, with a

growth rate of 2.876 (R^2 = 0.88) and growth rate of 2.606 (R^2 = 0.82) respectively for males of west and east coast. Only a limited number of research have been conducted on the west coast of India about the LWR of *M. cephalus*. A study was undertaken by Bhatt *et al.* (2022) in Gujarat to investigate the LWR of *M. cephalus* in the Sabarmati, Mahi, and Tapi estuaries. The study yielded the following results: The average length of males in the Sabarmati estuary is 2.6, with a correlation coefficient of 0.6. The average length of females is 2.7, also with a correlation coefficient of 0.6. The average length of the population is 2.7, with a correlation coefficient of 0.6. In the Mahi estuary, the average length of males is 3.2, with a high correlation coefficient of 0.976. The average length of females is 2.6, with a perfect correlation coefficient of 1.0. The average length of the population is 2.8, with a correlation coefficient of 0.8. In the Tapi estuary, the average length of males is 3.2, with a correlation coefficient of 0.8. The average length of females is 2.5, with a perfect correlation coefficient of 1.0. The average length of the population is 2.7, with a correlation coefficient of 0.9.

2.4.3 Condition Factor

The variation in body weight in respect to the overall length was not solely determined by specific gravity, but rather by changes in volume. Le Cren (1951) suggests that the condition factor (*K*) or "Pondered index" can be used to assess all of these changes. The LWR data can be used to assess the condition of any fish species (Offem *et al.* 2009). According to Wootton (1998), if the b value is 3.0, then the *K* value remains constant. However, if the weight of the fish increases at a faster rate than the cube of its length (b>3.0), then its *K* value will increase as the length of the fish increases. If the weight of a fish increases by less than the cube of its length (b<3.0), its *K* value will fall (Zebia & Rahan 2011). Therefore, the condition factor (*K*) varies as the size or weight of the fish increases.

Offem *et al.* (2009) found that the condition factor is highly dependent on the length and weight of fish. Therefore, any factors that might influence the link between length and weight of fish can also affect the condition factor. Thus, the *K* values of this species would be expected to grow as the length of the fish increases, which is consistent with the findings of Ali *et al.* (2002). The study conducted by Lawson *et al.* (2010) found that a low *K* value in fish indicated a period when

stored fat was used for spawning. Conversely, a high K value indicated a period of maximum feeding, during which fat gradually accumulated in preparation for a new reproductive season. Therefore, the differences in K values of the mullet species *M. cephalus* observed in this study may be attributed to variations in gonad maturation, changes in feeding behaviour, fluctuations in fat content, or alterations in population size resulting from shifts in food availability, as reported by Akombo *et al.* (2011) in relation to other fish species.

The average relative condition factor is determined for both male and female populations of *M. cephalus*. The results indicate that the female population is in better condition compared to the male population. These data also indicate sex-specific variations between male and female populations, with females showing slightly better condition than males. The condition factor is a metric used to assess alterations in food reserves, food intake, and the overall well-being of fish. Conversely, the seasonal data on the condition factor provide information about the reproductive cycle of the fish (Hossain 2010). One can analyse the condition factor using seasonal data to understand the reproductive seasonality and gonadal cycle of this species. This study presents the initial data on the length-weight connection of *M. cephalus* in the Narmada estuary and Diu lagoon. This information will be valuable for comprehending the health and fishery condition of fish in Gujarat.

The condition factor (K) of this mullet species indicates that the growth of the fish is most favourable in Diu lagoon, followed by Bhadbhut, Ambetha, and Bharuch of Narmada estuary. Anene (2005) said that the condition factor of fish in an aquatic ecosystem is significantly influenced by specific abiotic and biotic elements. Therefore, it can be utilised as an indicator to evaluate the overall health of the fish population and their surroundings. There is a limited amount of research on the condition factor of *M. cephalus* from the Indian coast. Luther (1963) made an extensive examination on *M. cephalus* in the vicinity of Mandapam, India. He noted that the condition of *M. cephalus* varied from 0.87 to 1.10 across the entire population. Bhatt *et al.* (2022) conducted a comprehensive study on the LWR and condition factor (CF) of *M. cephalus* in the three primary estuaries (Sabarmati, Mahi, and Tapi) in Gujarat. It was noted that the condition

factor is typically at its lowest following the spawning season, since the majority of energy is used for migration and reproduction (Lemos *et al.* 2014). The findings indicate that, with the exception of the Tapi estuary, the average condition of females was significantly superior than that of males. Their observations align with current findings in the Narmada estuary, indicating the lowest levels of potassium (K) near the beginning or end of the spawning season.

A study conducted in the Tidal stream in the Niger Delta, Nigeria, examined *M. cephalus* and observed that the *K*-values of the fish remained rather stable throughout the study period. This suggests that *M. cephalus* was in a healthy state (Moslen & Miebaka 2017). A research conducted by Ajah & Udoh (2012) examined *M. cephalus* in the Cross-River estuary, Nigeria, and discovered a value of *K* is 0.946. Similarly, Zubia *et al.* (2014) documented the condition factor as 1.08, 1.11, and 1.05 for the combined population, females, and males from the Karachi coast, Pakistan. The study found that males of these mullet species had a higher mean condition factor (*K*) than females. This suggests that males of the same length were heavier than females of equal length, which aligns with the present study's findings. This could be attributed to the presence of testes that exhibited more weight than the ovaries in females. Thus, the highest *K* values may align with the start of the spawning season. However, as fish reach the spent stage, the *K* values may decrease or reach a minimum due to a significant reduction in the weight of the fish's gonads.

2.4.4 Gonadosomatic Index and Hepatosomatic Index

A number of scientists (Qasim 1957; Sarojini 1958; Sobhana 1976; Das 1978) have used the gonadosomatic index technique to determine the spawning season in fish. This technique involves expressing the weight of the gonads as a percentage of the overall weight of the fish. As the mullets matured, the I_g showed a more significant increase in females compared to males. The I_g is rapidly increasing due to the enlargement of the ovaries caused by an accumulation of yolk, which serves as the nutritional reserve for the embryo, by the oocytes (Wiegand 1996). The study by Henderson *et al.* (1984) found that females allocate more energy for reproduction than males. This is consistent with the higher I_g values seen in females compared to males in the current study.

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

Brusle (1981) states that spawning is influenced by variables that might differ from year to year and from region to region. Among these elements, temperature appears to be the most significant in stimulating spawning activity. In Korea, Yang & Kim (1961) discovered that the ideal temperature range for *M. cephalus* is between 19.5 and 22°C. Yashouv & Berner Samsonov (1970) have demonstrated that exposing *M. cephalus* eggs to temperatures below 22 °C in controlled laboratory conditions halts their development and leads to their demise. Linder *et al.* (1975) provided evidence that the maturity of pond grown *M. cephalus* can be influenced by temperature. They observed that when the fish received heated effluents from a power station, their gonads were fully matured by the age of one year (160 mm SL).

In his 1954 publication, Thomson noted that several geographical variations of mullet exhibit distinct spawning seasons. Jhingran & Natarajan (1969) documented that the reproductive period of *M. cephalus* in Chilka lake spans from September to December, but Rangaswamy (1972) and Das (1978) said that it reproduces from September to February in Pulicat lake and Goa waters, respectively. Bromhall (1954) reported that *M. cephalus* in Hongkong has the potential to undergo multiple spawning events within a single year. According to Moore (1974), based on the observation of partially spawned out females, it is suggested that individual mullet (*M. cephalus* in Texas) have the ability to spawn many times over their extended spawning season.

Rekha *et al.* (2020) conducted an extensive study along the west coast and discovered that the mean I_g (gonadosomatic index) of female fishes exhibited a significant increase during the months of May (8.93±1.96) and June (8.32±1.87). Conversely, the lowest average I_g value was observed in August (1.10±0.27), suggesting that *M. cephalus* engages in spawning activities between the months of May and July in Cochin backwaters. On the east coast, the average values of female I_g were higher in December (18.58±1.14) and January (25.46±1.57), and then declined dramatically in the following months. Male individuals had higher average I_g values in December (0.65±0.11) and January (0.89±0.08), providing confirmation that *M. cephalus* reproduces between December to January on the east coast.

The study conducted by Rekha *et al.* (2020) found that the greatest I_g (gonadosomatic index) for both females and males was nearly identical in Diu lagoon and Bhadbhut in Narmada estuary over consecutive years. The current discovery verifies that *M. cephalus* reproduces between the months of December and March at all of the research locations. In their study, Bhatt *et al.* (2022) conducted a thorough examination of the I_g of *M. cephalus* in the main estuaries of Gujarat. They found that the maximum level of gonadal maturation occurred between November and February. The conclusions of these results are comparable to the current study conducted in Diu lagoon and Narmada estuary. Prior research conducted in Taiwan, Korea, and the USA about the reproductive cycles of *M. cephalus* indicated that the greatest average I_g of *M. cephalus* was seen in January (18), December (6.97), and November (17) respectively (Su & Kawasaki 1995; Kim *et al.* 2004; McDonough *et al.* 2005).

The liver, which is an essential storage organ in vertebrates like fish, is subject to comprehensive tests that include histological, histochemical, and biochemical examinations. The hepatosomatic index, which is calculated by dividing the weight of the liver by the weight of the body and expressing it as a percentage, is used to measure the animal's ability to store nutrients. The hepatic index fluctuates throughout the year, indicating seasonal changes in the animal's storage levels. The average hepatosomatic index of a fish population offers valuable information on its nutrient storage condition. An elevation in the average hepatosomatic index implies improved feeding, resulting in increased absorption and storage, whereas a decline may imply decreased feeding rates or utilisation for metabolic processes. Reproduction is a vital physiological process that requires the mobilisation of energy (Baskaran 1993). The liver, similar to the hepatopancreas in invertebrates, has a crucial function in mobilising energy necessary for reproduction. The present study aims to monitor the hepatic index of both male and female *M. cephalus* individuals for a duration of two consecutive years. When comparing the hepatosomatic index and the respective gonadosomatic index, two observations can be made. Firstly, the magnitude of fluctuations in males was higher in the hepatic index compared to the gonad index. Secondly, the decrease in the hepatic index consistently occurred before the increase in the gonad index (Baskaran 1993).

Bhatt, D. M., 2024. Mullet Fish Resources of Coastal Gujarat with Special Reference to Biological Aspects and Aquaculture Potential of Mugil cephalus (Linnaeus, 1758)

Titouah *et al.* (2022) conducted a study on *M. cephalus* in the Agarioun River Estuary and coastal sites located on the eastern shore of the Bejaia Sea, in the northeast region of Algeria. Their findings corroborate the current study's suggestion that males and females exhibit the highest hormone-induced spawning (I_h) during the summer months (April - pre-spawning season), followed by a gradual decrease to the lowest level in November and December. The present study found similar results to a previous study conducted on the Reproduction of *M. cephalus* off the Central Mexican Pacific Coast (Espino-Barr *et al.* 2016). In their study, Ferreira *et al.* (2004) examined the correlation between I_g and I_h. They discovered that I_h exhibited a comparable pattern, with elevated values occurring in January and February, which coincided with the peak development of ovaries and testes.