

The Central Gujarat region includes the districts of Anand, Vadodara, Ahmadabad, Kheda, Nadiad, Gandhinagar, Panchmahal and Dahod. Majorly covering the area between Mahi and Narmada River. The total geographic area of the region comprises 34.13 lakh hectares. From this, we have selected six districts as Anand, Kheda-Nadiad, Vadodara, Panchmahal, Dahod and Narmada for sampling as shown in Plate 11.

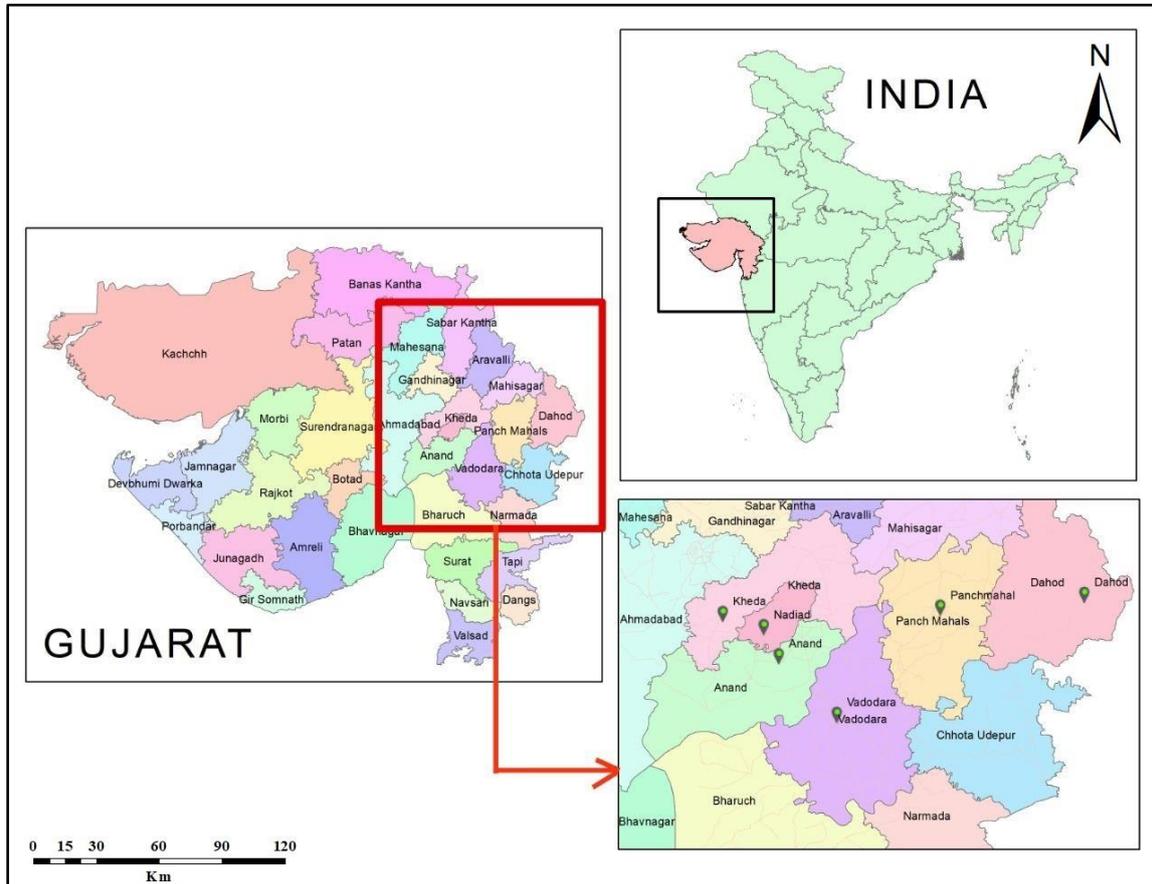


Plate 11. Map depicting Central Gujarat area

The Mahi and Narmada river give rise to many small tributaries flowing in the study area as mentioned in table 5. The study sites were surveyed for the data collection of ponds, lakes, dams or reservoirs, with their geolocations and area (in hectares) along with their Aquaculture status. For each districts, the total inland surface water area was calculated. The mapping of the study area along with the ponds and their aquaculture status were depicted. The ponds were then distinguished on two bases; Firstly, depending upon their sizes into small, medium or large and secondly on their aquaculture and non-aquaculture status.

Table 5. List of Major Rivers at the Central Gujarat

S. N.	Rivers name	Geo-Locations
1.	Mahi river	22.44397728588023, 73.07911165419361
2.	Vishwamitri river	22.307785754294326, 73.18809927543971
3.	Orsang river	22.06623594777591, 73.49123040317129
4.	Sukhi river	22.451800573574673, 73.88664319001742
5.	Vatrak river	22.75001497095855, 72.68044815689716
6.	Panam river	23.094235059218153, 73.65149742676594
7.	Narmada river	21.916117171670056, 73.3921797410586

Table 6. List of Major Waterbodies

City	Name of Dams/ Reservoirs/ Major ponds	Geo-Locations
Vadodara	Ajwa lake	22.394878421371672, 73.40056426449735
	Timbi lake	22.315290340294226, 73.28615695664159
	Vadhvana Lake	22.173400542000653, 73.48633834207506
	Vadatalav/Pavagadh lake	22.490312137664546, 73.55499901200231
	Vadadala lake	22.477899934547192, 73.31725396979779
	Hanuman lake/ Bodidra	22.471336540384016, 73.3593703154953
	Javla	22.556416193395904, 73.24100741352088
	Muval	22.582943573565025, 73.25850012903855
Panchmahal	Dev reservoir	22.385869393858272, 73.55692310970585
	Sukhi dam reservoir	22.447004064495825, 73.89480823415731
	Hadaf dam reservoir	22.897385687035374, 73.89202353934965
	Kanelav lake	22.781515284110203, 73.64224916431819
	Panam reservoir	23.02608529729007, 73.771720858751
Dahod	Karad dam	22.559083368691, 73.70252602539222
	Pata Dungri dam	22.709231636931623, 74.28029752114543
	Adalwada dam	22.71269025846032, 73.99186996958684
	Ramsagar lake	22.777406769783894, 73.61801321883992
	Kali reservoir	22.903837204882738, 74.23944129183525
	Muvalia dam	22.809150327035148, 74.23751695640405

Then the geolocations were used to locate the exact point on the map. The map depicts the number of ponds and their aquaculture status. The central Gujarat area shows a great number of Watersheets consisting of Dams, reservoirs, Lakes, Wetlands and Ponds. Some of the major reservoirs, lakes and dams are mentioned in Table 6.

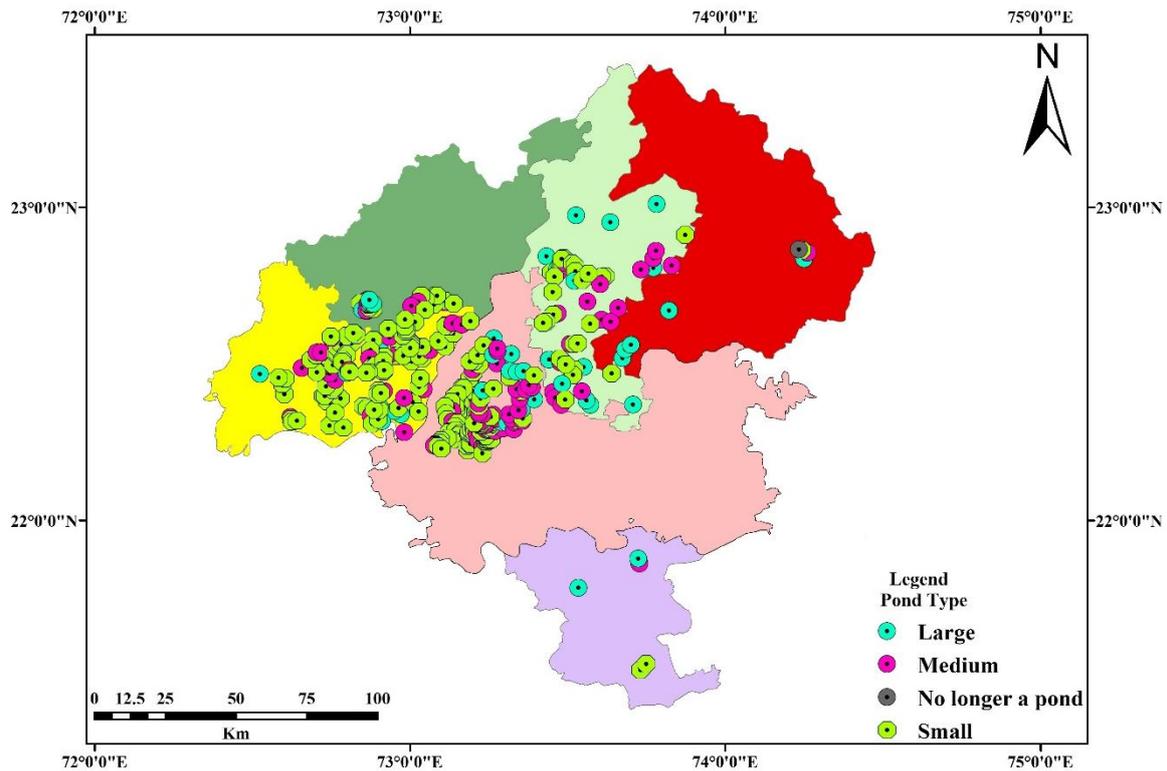


Fig. 2. Depicting the cumulative maps

The Fig. 2. Shows the total number of ponds surveyed during in the study period. It also shows the classification of ponds based on their area in Hectares, into Small, Medium and Large sized ponds. Some of the ponds were utilized by the municipal corporation under the city development projects and were beautified for the entertainment purposes. Hereafter, the district wise Maps with the data are described as follows.

#### 4.1 Anand

Here, the study site for the Anand district is shown. Anand is located at 22.57°N 72.93°E. It has an average elevation of 39 m (128 ft.). The district consists of 145 ponds of different sizes, belonging to large, medium, and small categories. 94 ponds are continuing with the aquaculture activity of IMC (Indian Major Carp) fishes, whereas the remaining 51 ponds didn't have any aquaculture activity and some were beautified by the state central government under the smart city Project.

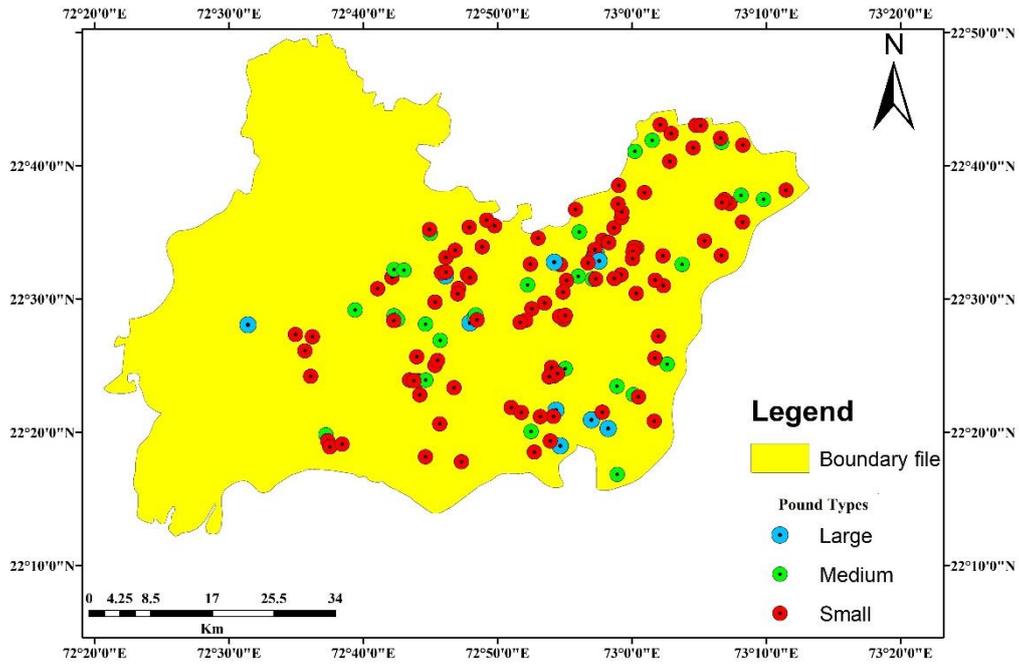


Fig. 3. Map depicting Classification of ponds based on Area (Anand City)

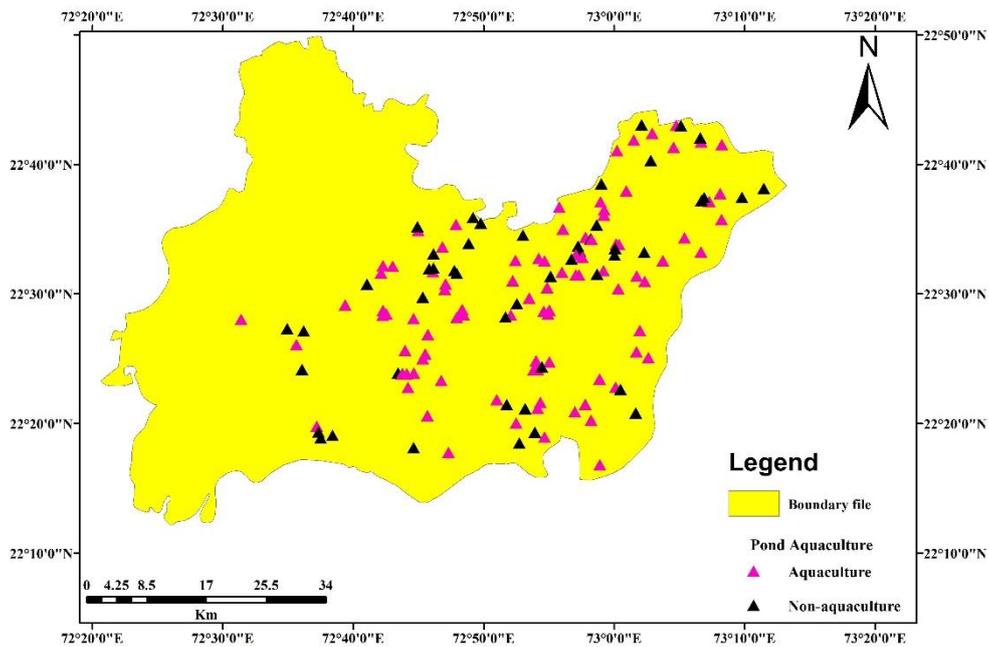


Fig. 4. Map showing the Aquaculture status (Anand District)

This city has more number of small ponds. The decrease in the aquaculture activity is highly related or directly proportional to the decreasing number of ponds due to urbanization and development in the cities. Which leads to the loss of biodiversity. Many ponds in Anand were used as a dumping waste site.

**4.2 KHEDA- NADIAD**

Kheda is one of the oldest districts in Gujarat. The district covers 3958.84 square kilometers. The district is bounded by two important Gujarati rivers: Sabaramati on the west and Mahisagar on the east. The district has nine important rivers: Mahi, Sabarmati, Meshwo, Khari, Luni, Varasi, Sehar, Vatrak, and Shedhi. Nadiad is located at 22.74 °N, 72.86 °E. The city is the administrative center of the Kheda district.

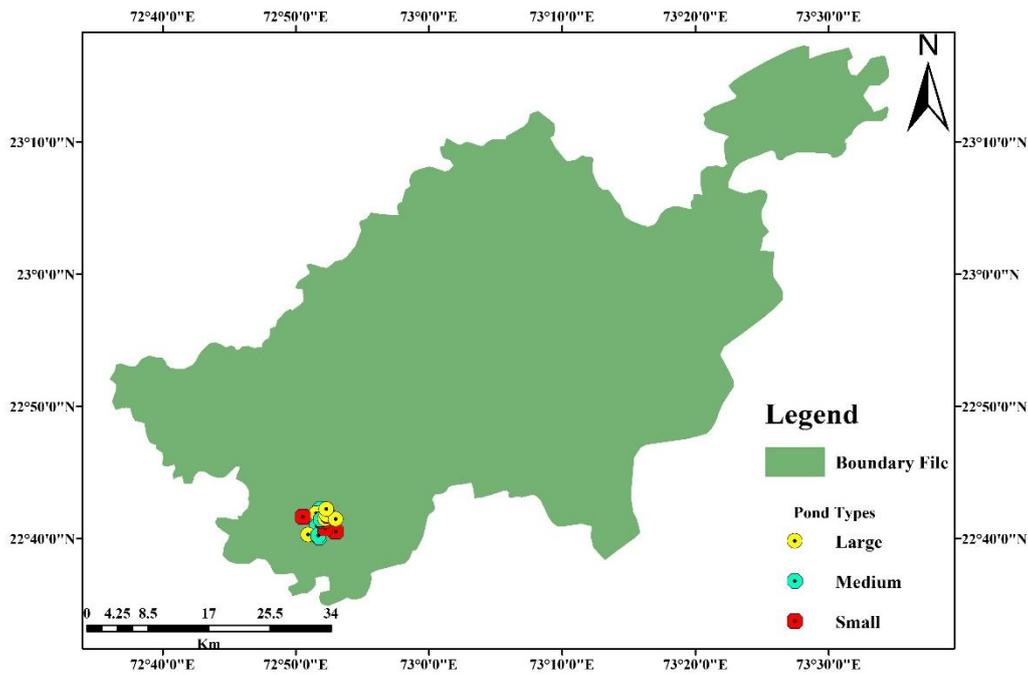


Fig. 5. Shows the classification of Ponds based on Area (Nadiad district)

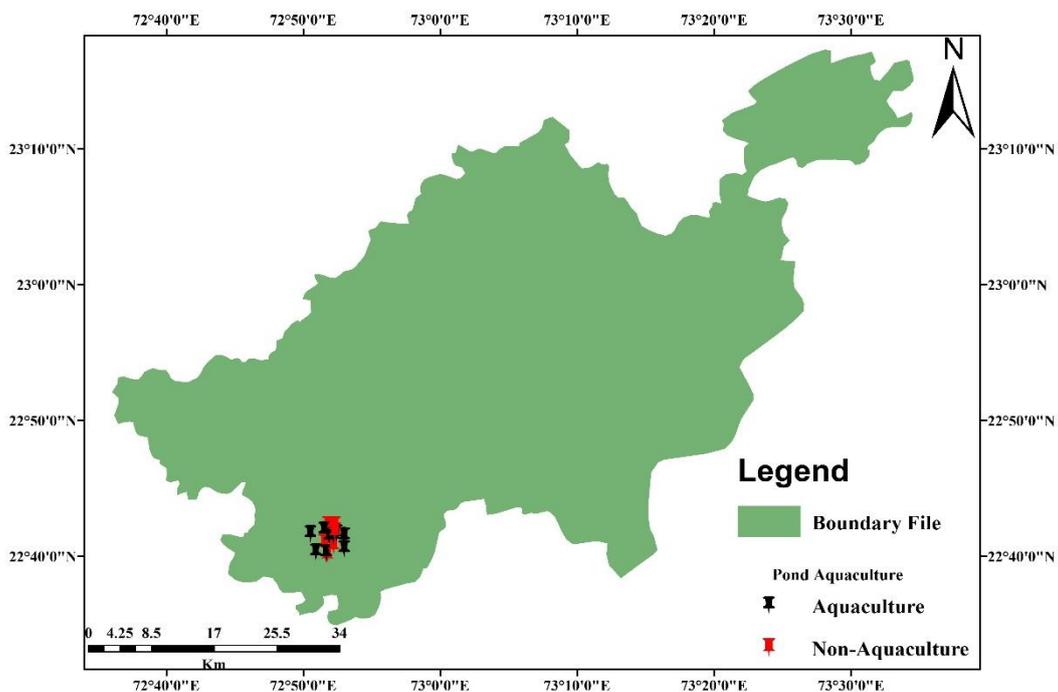


Fig. 6. Shows the Pond having Aquaculture status (Nadiad district)

The Kheda district has less number of ponds so to compensate it Nadiad was taken into consideration. Where a total of 16 water sheets were surveyed out of which 10 watersheets had Aquaculture practices. As the city is still developing, proper management practices can be developed.

### **4.3 VADODARA**

Vadodara, also known as Baroda, is a major city of Gujarat. It is located at 22.30 °N, 73.19 °E on the banks of the Vishwamitri River.

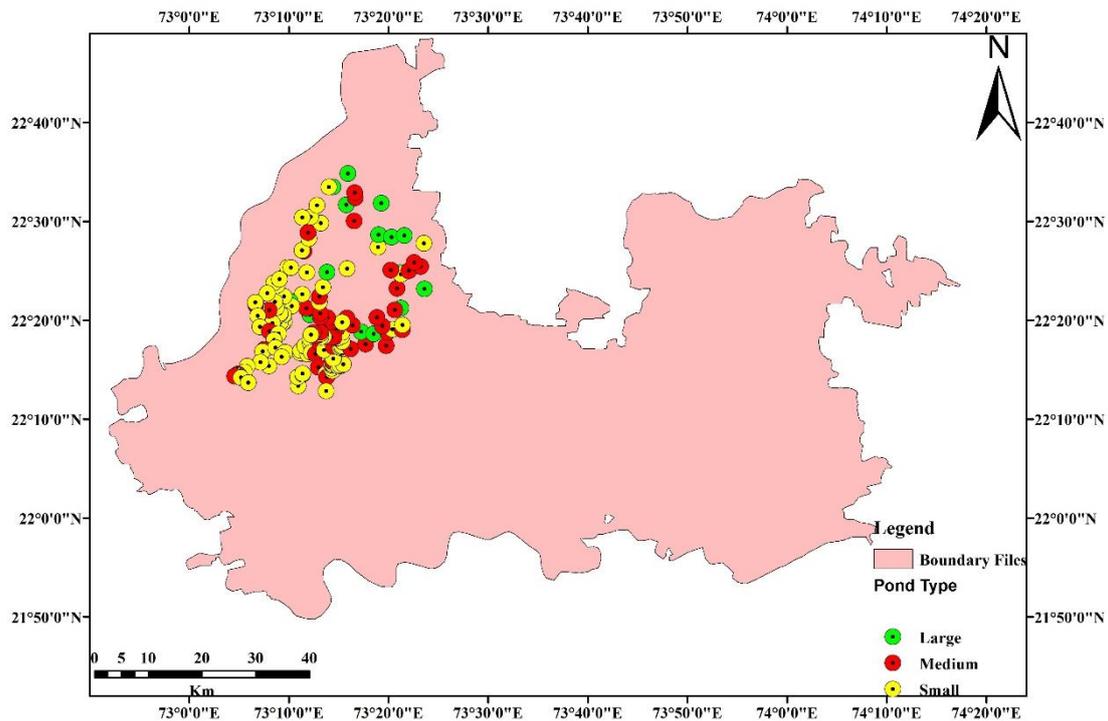


Fig. 7. Shows the classification of Ponds based on Area (Vadodara district)

The district has good amount of watersheets. A total of 156 surface watersheets were surveyed out of which only 94 watersheets showed Aquaculture practices. The district is surrounded by Major reservoirs as Timbi, Ajwa, Javla, Muval, Vadadla, etc. The ponds were further classified according to their area in hectares as in plate 7 and were also looked for Aquaculture activity as seen in Plate 8. The number of ponds have been decreased since last decade due to urbanization and also the beautification of ponds, which has certainly lead to the Habitat destruction and biodiversity loss.

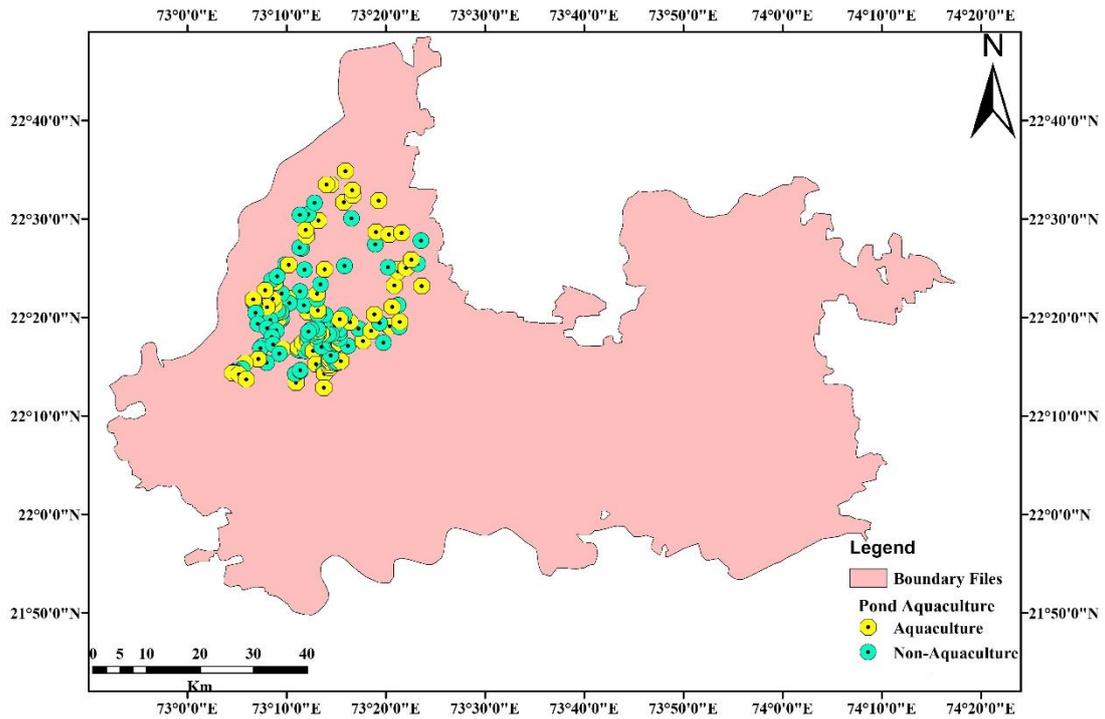


Fig. 8. Shows the Pond having Aquaculture status (Vadodara district)

#### 4.4 DAHOD

Dahod is a city on the banks of the Dudhimati River at 22.83 °N, 74.26 °E, covering an area of 3642 sq km. The city features a large pond known as Chab Talav, which has recently been beautified and developed by the Municipal Corporation for public entertainment purposes.

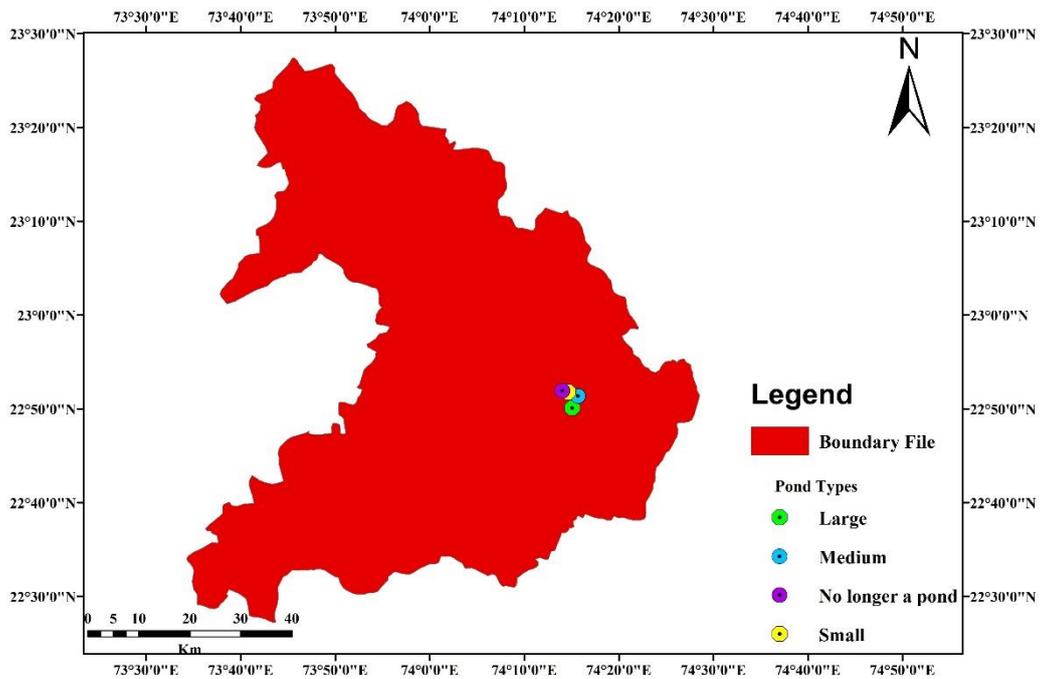


Fig. 9. Shows the classification of Ponds based on Area (Dahod district)

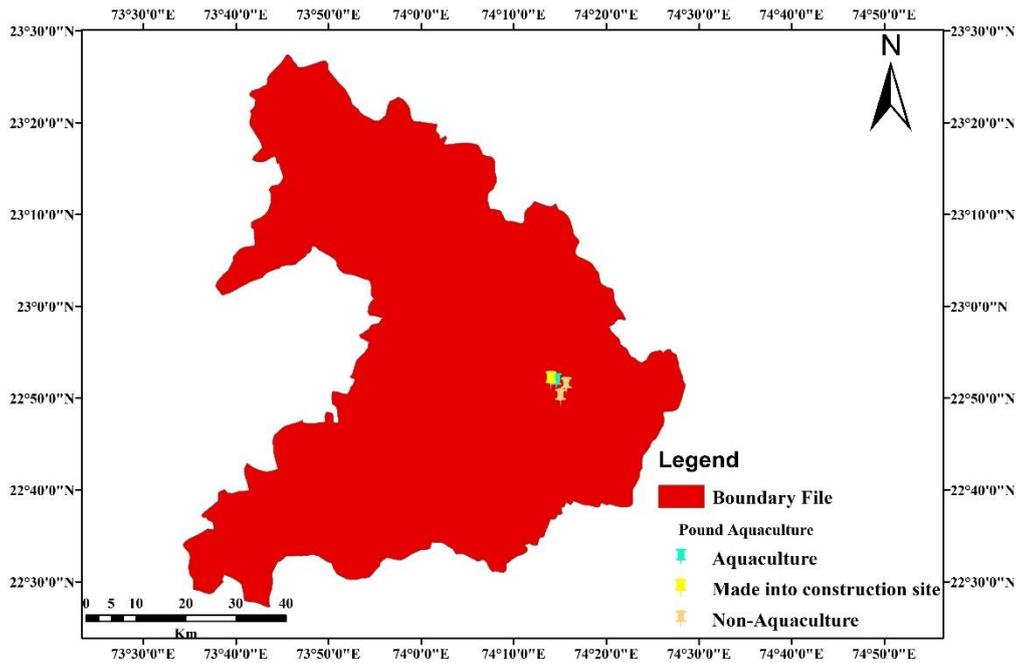


Fig. 10. Shows the Pond having Aquaculture status (Dahod district)

The Dahod district is the city that shares borders with two states, Rajasthan and Madhya Pradesh. The city has less number of ponds, surveyed were 5 as seen in Plate 9. The aquaculture practice was ongoing only in the ponds at village. Very less number of ponds had on going aquaculture activity as seen in Plate 10. City also has some large ponds with beautification projects for entertainment purpose famously known as Chab talav. Also the district has some major reservoirs as Kali dam, Dungri dam, Muvalia dam, etc.

#### 4.5 PANCHMAHAL

The city is located at 22.80 °N, 73.60 °E in the Western Gujarat. The term "Panchmahal" refers to the five sub-divisions handed to the British by Maharaja Jivajirao Scindia of Gwalior State: Godhra, Dahod, Halol, Kalol, and Jhalod, Devgadhi Baria. Dahod was formerly part of Panchmahal, but it is now an independent district.

A total of 65 ponds were surveyed for sampling. Out of 65 only 52 watersheets showed the active aquaculture practices. The species diversity was also good in this area. The main reason could be the number of Reservoirs and Dams, surrounding the district along with the flowing river. The Major Dams are Panam Dam, Dev reservoir, Wankleshwar dam, Hadaf dam, Kadana Dam, etc. And as the previous literature suggested that the small reservoir has a higher estimated average fish yield per hectare (49.9 kg/ha), followed by the medium reservoir (12.3 kg/ha) and the bigger reservoir by Sugunan and FAO, (1995). Similar results are observed. Fig.

11 Shows the classification of ponds based on Area (Hectares) and Fig. 12 shows the Aquaculture status of the district watersheets.

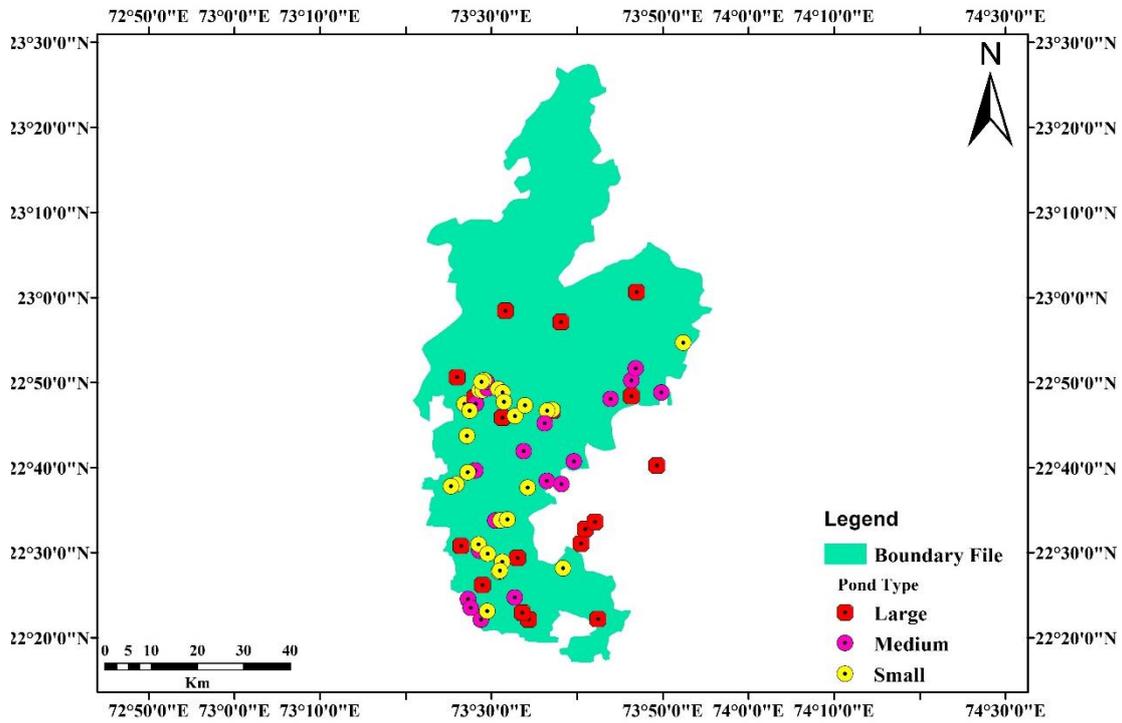


Fig. 11. Shows the classification of Ponds based on Area (Panchmahal district)

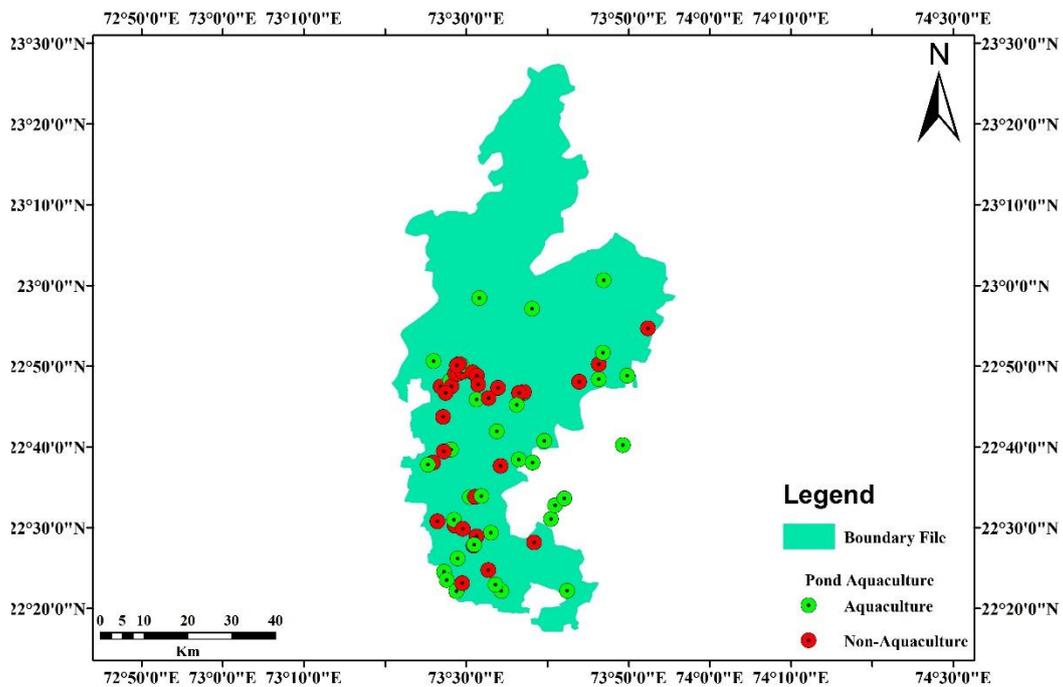


Fig. 12. Shows the Pond having Aquaculture status (Panchmahal district)

### 4.6 NARMADA

The district is situated on the eastern corner of Gujarat state at 21.76 °N, 73.65 °E, on the banks of Narmada. District now has 5 talukas and 1 municipality.

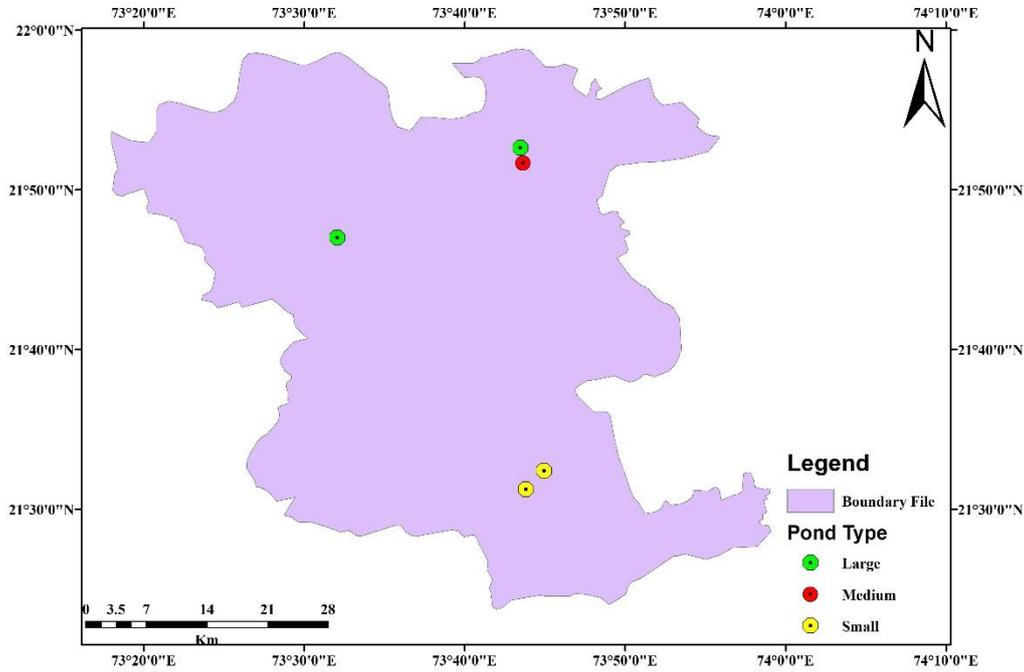


Fig. 13. Shows the classification of Ponds based on Area (Narmada district)

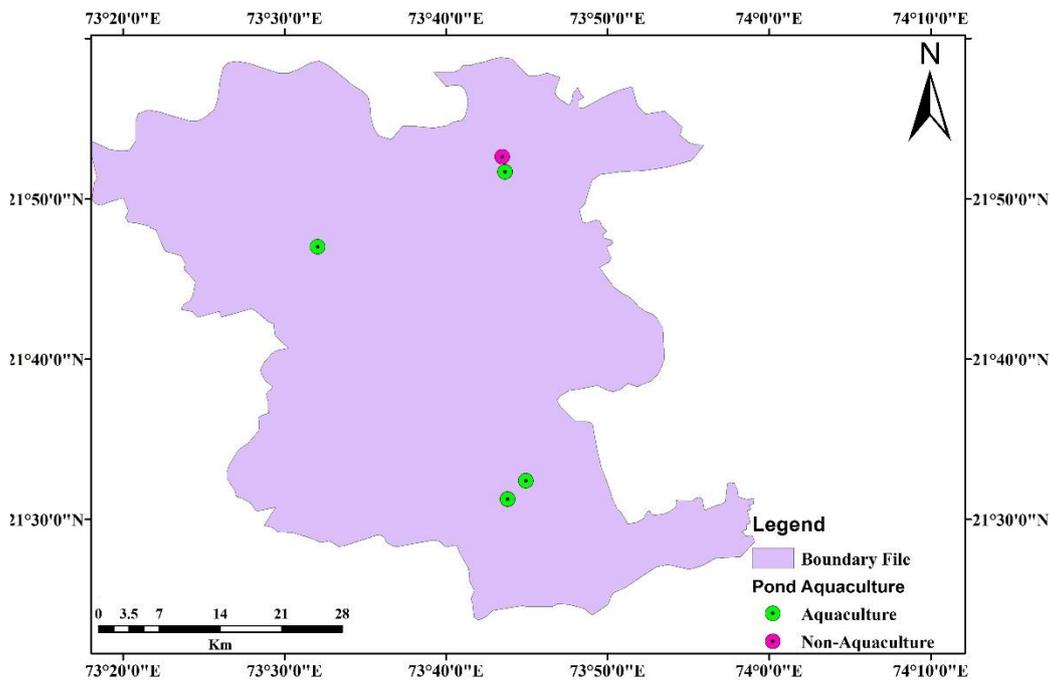


Fig. 14. Shows the Pond having Aquaculture status (Narmada district)

Total five watersheets were surveyed for this district, out of which three were reservoirs with good species diversity, whereas one was Non-Aquaculture and other one was medium

sized pond, with a regular ongoing fishing activity as marked in Fig. 13 and 14. The Narmada city being favoured by River Narmada showed the higher species diversity added by the migratory fishes mainly.

**4.7 The Aquaculture status**

The table 11 is a datasheet having data about the total of surface watersheets in the study area within each district out numbering the number of aquaculture watersheets.

Table 11. The datasheet about Aquaculture status of water sheets from all districts.

District	Total Number of Water sheets	No. of Water sheets with Aquaculture status
Anand	145	94
Kheda-Nadiad	16	10
Vadodara	156	96
Dahod	12	9
Panchmahal	65	52
Narmada	5	3
<b>Total</b>	<b>404</b>	<b>264</b>

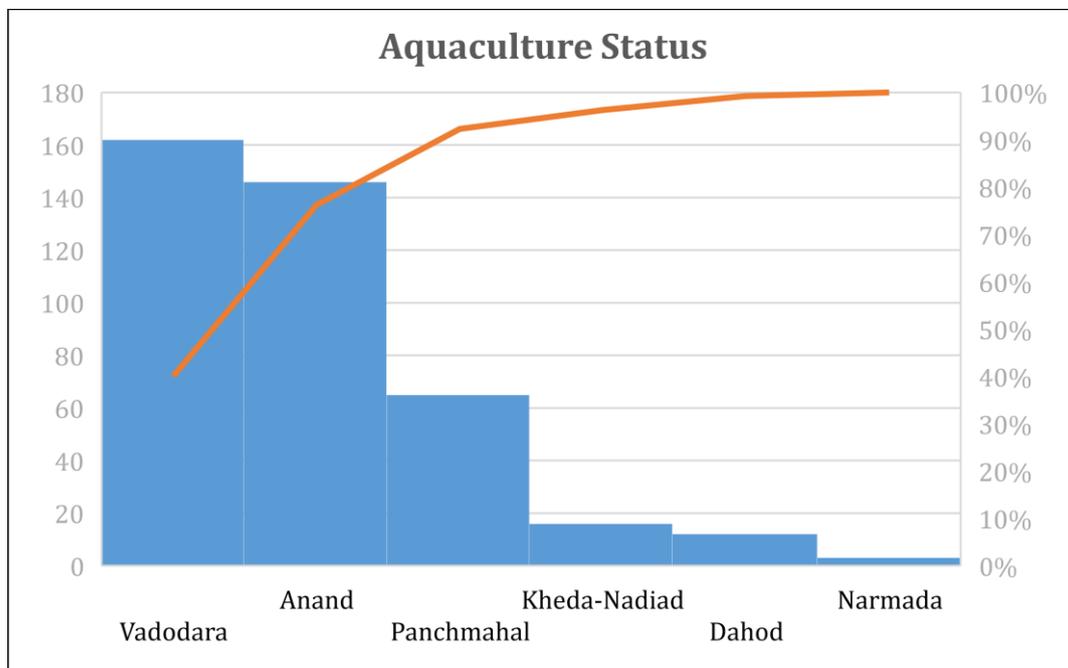


Fig. 15. Depicts the overall Aquaculture status

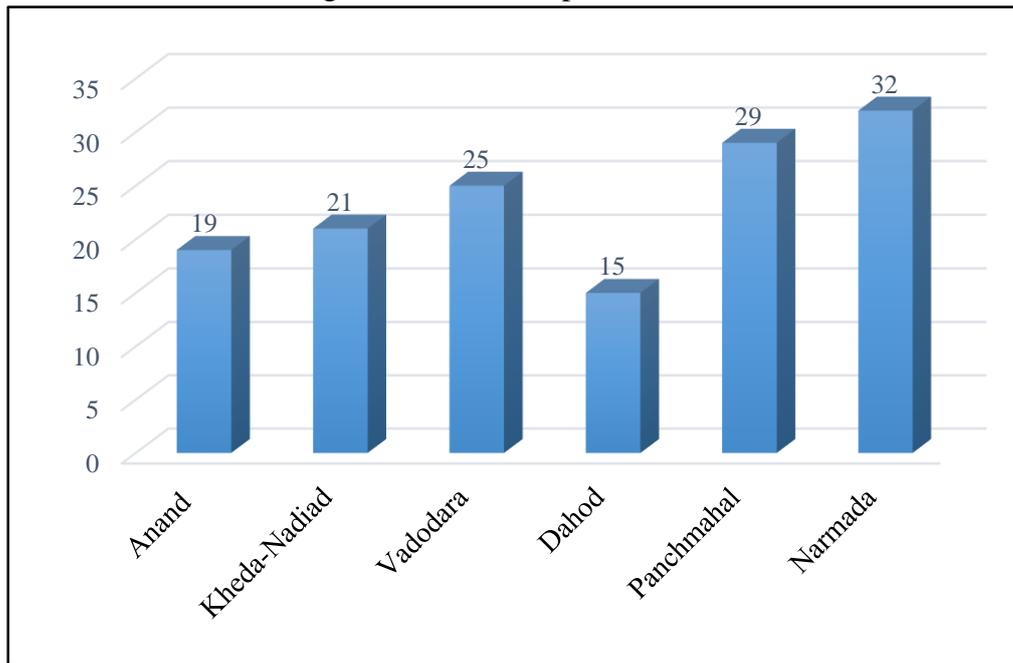
A total of 404 Freshwater water sheets were surveyed for the study, out of which the Aquaculture activity is continuing only in 264 water sheets. The Aquaculture status of the

Districts with percentage depending upon the number is depicted in Fig.15, where the Narmada district has 100% Aquaculture activity among less number of pond i.e, contributed by rivers, followed by Dahod with 95%, as the Dahod district is surrounded by most of the Reservoirs, they contribute to the fisheries data. Similar result is with Panchmahal district as it has contribution of large number of Dams, i.e. Kadana, Panam, Sukhi, etc. Then the aquaculture activity is decreasing as we move towards cities Kheda- Nadiad, Anand, Vadodara. Ponds are highly vulnerable to mounting land-use pressures (e.g., urban expansion, and agriculture intensification) and environmental changes, leading to degradation and loss of the pond ecosystem (Yadav and Goyal, 2022).

## 4.8 Species Diversity

A total of 42 species were found in the different water sheets of the study area. The diversity among the freshwater ponds was mostly the same as having one or two other species due to the water entering the pond or the region and favorable conditions. Below provided is the list of species diversity along with its classification and frequency of occurrence in Table 7.

Fig. 16. Shows the Species Richness



The Fig.16 gives a detailed account on the species richness of each study site. The Narmada district has the highest species richness with 32 different species and a major reason behind it is the Narmada riverine ecosystem, followed by Panchmahal district having the species richness of 29 due to Major reservoir fisheries. The species richness of Vadodara, Kheda-Nadiad, Anand and Dahod was 25, 21, 19 and 15 respectively. The decrease in the species diversity shows the negative impacts of Urbanization, which has led to Habitat loss, Pollution, Resource competition, etc gradually leading to reduction in ecosystem health and resilience.

Table 12. List of freshwater fishes with their Order, Family, Scientific name, Common name, IUCN status and level of abundance

SN	Family	Scientific Name	Common Name	IUCN Status	Level of Abundance
<b>Order: Cypriniformes</b>					
1.	Cyprinidae	<i>Labeo catla</i> (Hamilton, 1822)	Catla	LC	+++
2.		<i>Labeo rohita</i> (Hamilton, 1822)	Rohu	LC	+++
3.		<i>Cirrhinus mrigala</i> (Hamilton, 1822)	Mrigal carp	LC	+++
4.		<i>Hypophthalmichthys molitrix</i> (Valenciennes, 1844)	Silver carp	NT	++
5.		<i>Barbonymus gonionotus</i> (Bleeker, 1849)	Silver barb	LC	++
6.		<i>Puntius sophore</i> (Hamilton, 1822)	Pool barb	LC	+++
7.		<i>Osteobrama cotio</i> (Hamilton, 1822)	-	LC	+++
8.		<i>Vimba vimba</i> (Linnaeus, 1758)	Vimba bream	LC	+++
9.		<i>Tor tor</i> (Hamilton, 1822)	Tor barb	DD	-
10.		<i>Pethia ticto</i> (Hamilton, 1822)	Ticto barb	LC	-
11.		<i>Labeo calbasu</i> (Hamilton, 1822)	Orangefin labeo	LC	-
12.	Danionidae	<i>Rasbora daniconius</i> (Hamilton, 1822)	Slender rasbora	LC	+
13.		<i>Salmostoma phulo</i> (Hamilton, 1822)	Finescale razorbelly minnow	LC	++
14.		<i>Salmostoma bacaila</i> (Hamilton, 1822)	Large razorbelly minnow	LC	++
15.	Leuciscidae	<i>Alburnus alburnus</i> (Linnaeus, 1758)	Bleak	LC	++
16.		<i>Cyprinella venusta</i> (Girard, 1856)	Blacktail shiner	LC	-
17.		<i>Leucos panosi</i> (Bogutskaya & Iliadou, 2006)	Acheloos roach	VU	-
18.	Xenocyprididae	<i>Ctenopharyngodon idella</i> (Valenciennes, 1844)	Grass carp	NE	+

19.		<i>Hypophthalmichthys nobilis</i> (Valenciennes, 1844)	Bighead carp	DD	+
20.		<i>Culter alburnus</i> (Basilewsky, 185)	-	NE	++
<b>Order: Siluriformes</b>					
21.	Bagridae	<i>Mystus cavasius</i> (Hamilton, 1822)	Gangetic mystus	LC	-
22.		<i>Sperata seenghala</i> (Sykes, 1839)	Giant river-catfish	LC	+
23.	Siluridae	<i>Wallago attu</i> (Bloch & Schneider, 1801)	Wallago	VU	+
24.		<i>Ompok bimaculatus</i> (Bloch, 1794)	Butter catfish	NT	+++
25.	Pangasiidae	<i>Pangasius pangasius</i> (Hamilton, 1822)	Pangus catfish	LC	++
26.	Loricariidae	<i>Pterygoplichthys pardalis</i> (Castelnau, 1855)	Amazon sailfin catfish or Suckermouth catfish	NE	-
<b>Order: Cichliformes</b>					
27.	Cichlidae	<i>Oreochromis mossambicus</i> (Peters, 1852)	Mozambique tilapia	VU	+++
28.		<i>Oreochromis niloticus</i> (Linnaeus, 1758)	Nile tilapia	LC	-
<b>Order: Ovalentaria</b>					
29.	Ambassidae	<i>Chanda nama</i> (Hamilton, 1822)	Elongate glass- perchlet	LC	+++
30.		<i>Parambassis ranga</i> (Hamilton, 1822)	Indian glassy fish	LC	+++
31.		<i>Parambassis lala</i> (Hamilton, 1822)	Highfin glassy perchlet	NT	-
<b>Order: Anabantiformes</b>					
32.	Channidae	<i>Channa striata</i> (Bloch, 1793)	Striped snakehead	LC	+++
33.		<i>Channa marulius</i> (Hamilton, 1822)	Great snakehead	LC	+
34.	Osphronemidae	<i>Trichogaster lalius</i> (Hamilton, 1822)	Dwarf gourami	LC	-
35.	Nandidae	<i>Nandus nandus</i> (Hamilton, 1822)	Gangetic leaf fish	LC	+

<b>Order: Gobiiformes</b>					
36.	Gobiidae	<i>Glossogobius giuris</i> (Hamilton, 1822)	Tank goby	LC	+
<b>Order: Beloniformes</b>					
37.	Belonidae	<i>Xenentodon cancila</i> (Hamilton, 1822)	Freshwater garfish	LC	+++
<b>Order: Synbranchiformes</b>					
38.	Mastacembeleidae	<i>Macrogathus pancalus</i> (Bloch, 1786)	Lesser spiny eel	NE	++
<b>Order: Osteoglossiformes</b>					
39.	Notopteridae	<i>Notopterus notopterus</i> (Pallas, 1769)	Bronze featherback	LC	++
40.		<i>Chitala chitala</i> (Hamilton, 1822)	Clown knife fish	NT	-
<b>Order: Characiformes</b>					
41.	Serrasalminidae	<i>Piaractus brachypomus</i> (Cuvier, 1818)	Red Piranha	NE	+
<b>Order: Carangaria/misc</b>					
42.	Latidae	<i>Lates calcarifer</i> (Bloch, 1790)	Barramundi	LC	-

+ = Present, ++ = Common, +++ = Abundant, - = Rare

IUCN - Threatened list status: VU - Vulnerable; LC - Least concern; DD - Data Deficit; NE - Not evaluated, NT - Near threaten

A total of 42 species are recorded from the different freshwater resources belonging to 11 orders, 19 families, and 35 genera. Among them, Cypriniformes was dominated by 4 families and 20 species, Siluriformes, which consisted of 4 families and 6 species, and Anabantiformes, with three families and four species. Ovalentaria, Cichliformes, and Osteoglossiformes orders were represented with one family each and 3, 2, and 2 species respectively. Order Gobiiformes, Beloniformes, Synbranchiformes, Characiformes, and Carangaria represent one family with one species each as represented in Fig. 2.

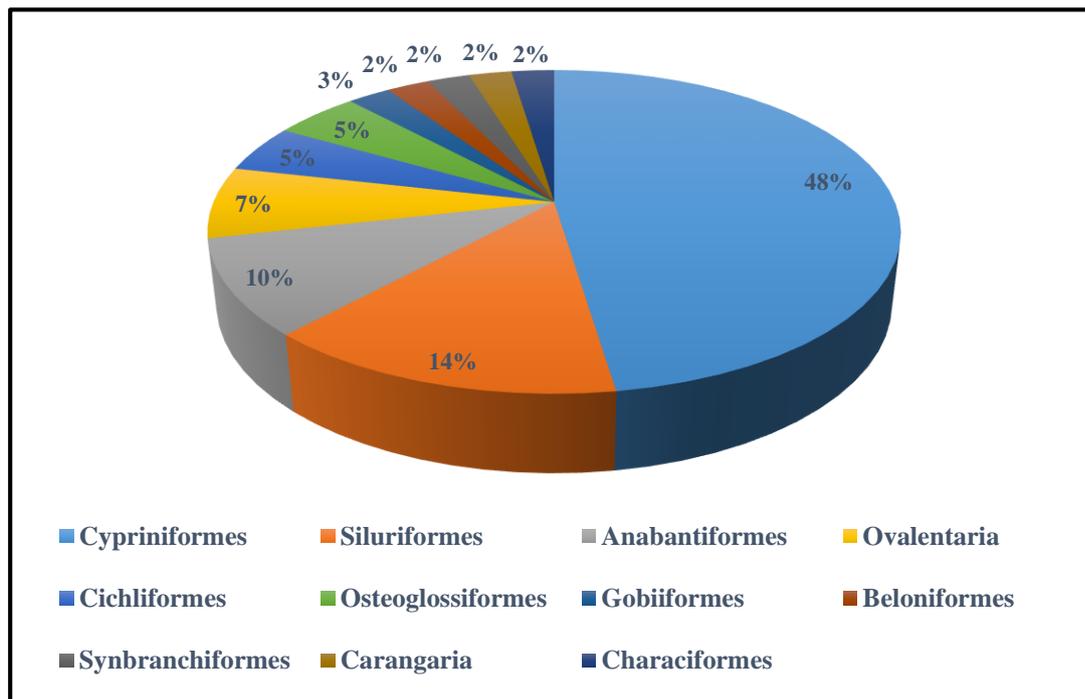


Fig. 17. The graph displays the percentage of species in various orders

The above graph explains the species richness familywise, giving an insight into diversity pattern. Amongst these Cypriniformes species are highest at 48%, followed by Siluriformes at 14%, Anabantiformes at 10%, Ovalentaria at 7%, Cichliformes and Osteoglossiformes at 5%, Gobiiformes at 3% whereas Beloniformes, Synbranchiformes, Carangaria and Characiformes are at 2% (Fig. 17).

The Graph shown in fig. 3 depicts the number of Families in each orders. Order Cyprinidae and Siluriformes had equal number of four families, followed by three in Anabantiformes, and all other orders had species belonging to only one family (Fig. 18).

The above results highlight the ecological dominance of specific orders such as Cypriniformes, and an extent of varying difference between the families and orders. It also provides a comprehensive data about the taxonomic distribution and richness in studies area.

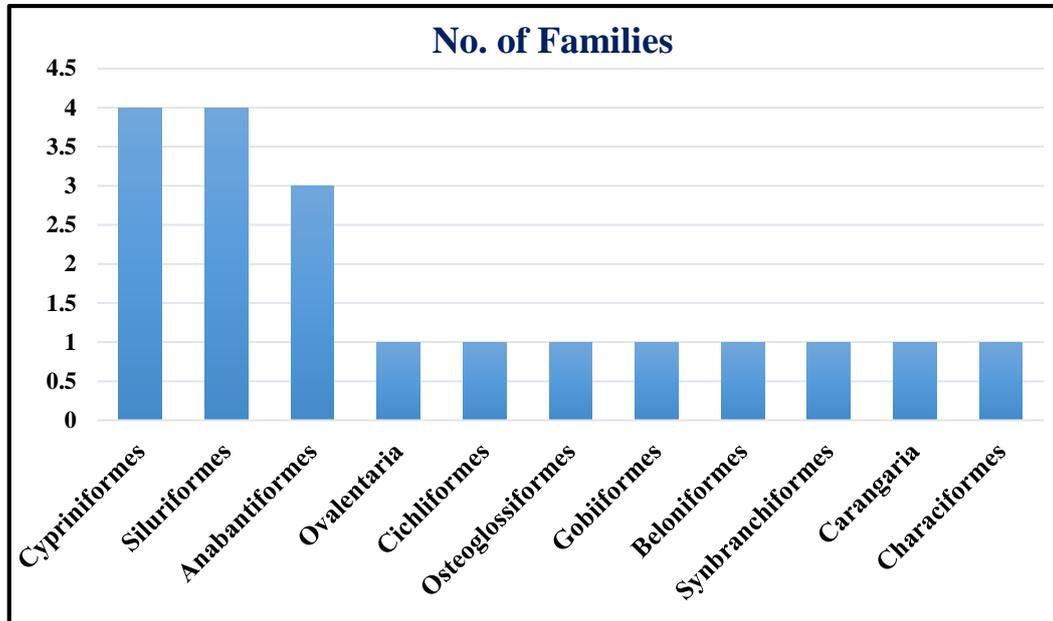


Fig 18. Graph showing the number of Families belonging to different orders

In addition to morphological identification, the species were also considered for molecular confirmation to ensure taxonomic accuracy. DNA extraction was performed using the manual Phenol Chloroform method. Subsequently, the extracted DNA underwent PCR amplification, and the resulting product was sequenced using the Sanger sequencing method. Here the sequence data of 11 species is obtained, which was further uploaded on NCBI and the accession numbers are generated, as shown in Table 8.

Table 13. Species Sequence Data with Accession Number

Sr. No.	Species Name	Accession number (NCBI)
1.	<i>Parambassis ranga</i>	PP811807
2.	<i>Trichogaster lalius</i>	PP748312
3.	<i>Macrogathus pancalus</i>	PP751492
4.	<i>Ctenopharyngodon idella</i>	PP812122
5.	<i>Ompok bimaculatus</i>	PP741824
6.	<i>Hypophthalmichthys nobilis</i>	PP751622
7.	<i>Labeo calbasu</i>	PP741866

8.	<i>Salmostoma phulo</i>	PP741868
9.	<i>Wallago attu</i>	PP741879
10.	<i>Salmostoma bacalia</i>	PP741880
11.	<i>Xenentodon cancila</i>	PP812196

The phylogenetic analysis is valuable for studying the genetic diversity, evolutionary biology, and ecological interactions among these fish species. the Phylogenetic related among the species was calculated using the Mega X software. Closely related sequences are grouped. For instance, *Ctenopharyngodon idella* and *Hypophthalmichthys nobilis* being carps share a branch with a 62% support value, suggesting a closer evolutionary relationship between these two species.

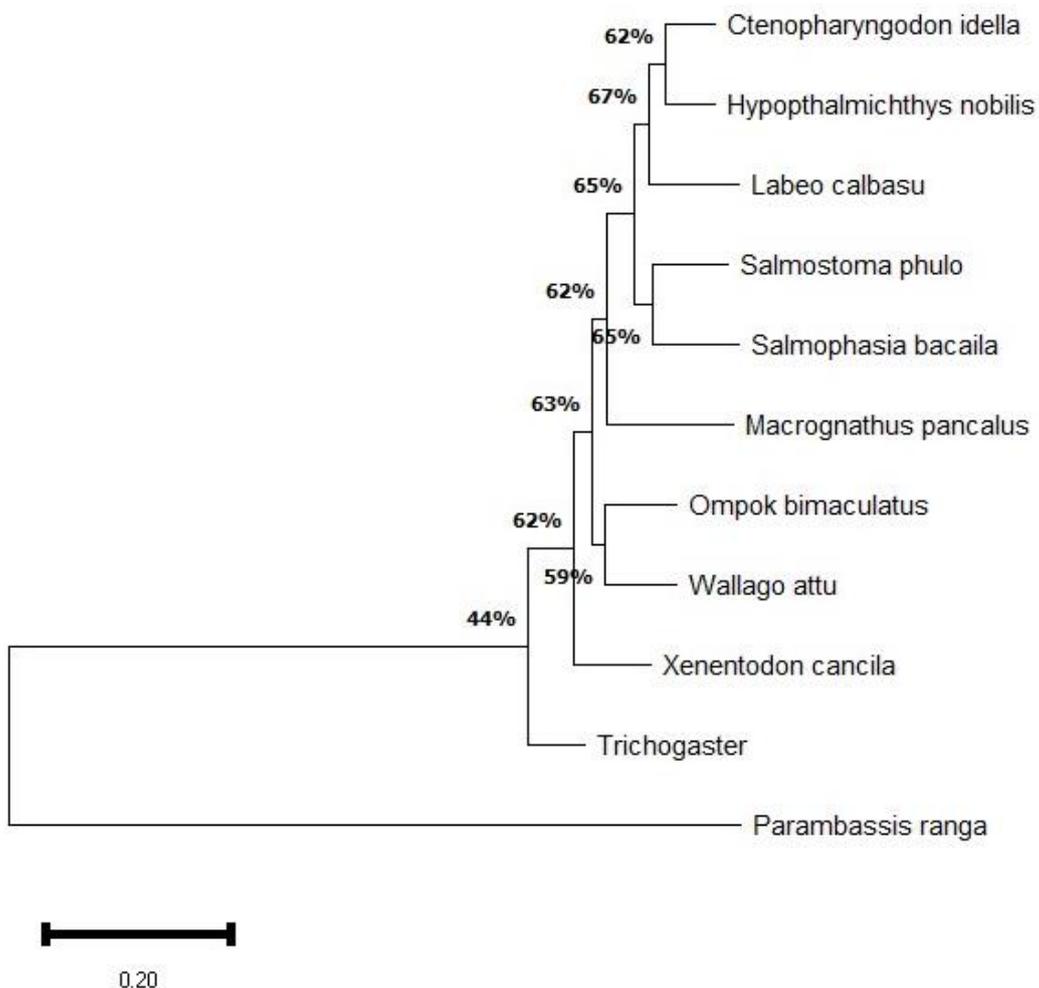


Fig. 19. The Phylogenetic tree for the sequence data

The molecular confirmation results are awaited for the other species, which will be incorporated in further publications. The Molecular confirmation of a species is still a task, and many fishes are left to deal with.

## **4.9 Parasitic Results**

Fish farming is a highly risky business for fish farmers mainly due to the disease problems because insidious diseases pose a major threat to the fish population. In general, it is believed that the parasite ecology of marine fishes is more diverse and abundant than that of freshwater species (Sindermann, 1990; Rohde, 1993). Fish production is often reduced due to diseases and parasites by affect the normal physiological condition of fish and it can result in mass mortalities (Kabata, 1985). A rise in intensification, which leads to greater stress levels in the cultured animal and their surroundings, is associated with the likelihood of aquatic animal disease propagation (Mohapatra et al., 2012). Death or mortality due to such diseases can account for 10-15% loss. Disease is one of the major constraints to aquaculture and a limiting factor for socio-economic development in India and as in many other countries of the world (Mishra et al., 2017). Fish diseases are classified based on causative agents as (a) Non-Parasitic infection by environmental stressors e.g. Gas diseases and (b) Parasitic infection by Fungi, Bacteria, Protozoa, Worms, and Crustaceans.

Fish in aquaculture ponds are generally affected by diseases of parasitic, fungal, and bacterial in origin. Above all, parasitic infestations are frequently encountered; however, they are studied to a limited extent in some regions of the world.

Listed below is an inventory of a couple of the fish diseases:

### **4.9.1 Branchiomycosis**

Unlike bacterial and parasitic infections, only a few fungal species are known to be harmful to fish. Fungal spores are routinely detected in fish culture water under normal circumstances and do not cause fish diseases. Healthy fish have a protective mucus layer that protects them from fungal spores. The protective mucus is degraded in a poor aquatic environment due to low water quality, rough handling, fighting, or physical injury, resulting in an epidemic of fungal diseases (Opiyo et al., 2020). Fungal infections are second to bacterial diseases of economic importance and are generally restricted from chronic to steady losses (Ramaiah, 2006). The most common fungal diseases of fish are Saprolegniasis, a disease caused by *Achlya*, Branchiomycosis, Epizootic Ulcerative Syndrome (EUS), and *Ichthyophoniasis* (Choudhury et al., 2014). Branchiomycosis is a fungal disease commonly known as 'Gill Rot' disease (majorly involving the gill tissues), affects freshwater fishes (Sen et al., 2018), manifested by necrosis of the gills, and high incidences of mortality (Adeshina et al., 2019). Branchiomycosis develops in eutrophic conditions with a high organic matter

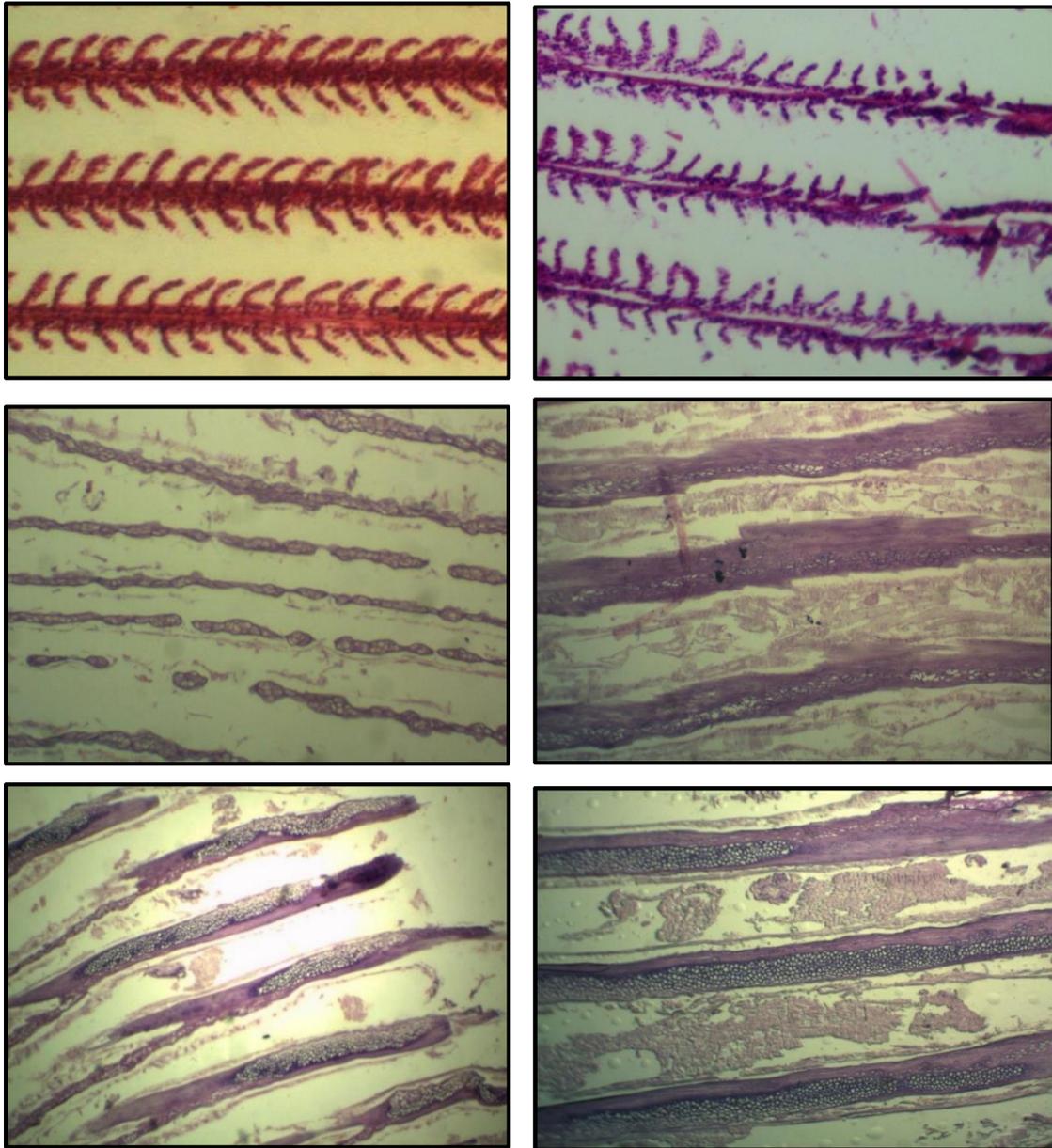
content and temperatures exceeding 20°C. Infection is usually confined to the gills and leads to damage by colonizing gill vessels and capillaries (Opiyo *et al.*, 2020). Showing the clinical signs of the acute respiratory disorder leading to high mortality, because of anoxia that has been similar as reported in previous works of literature (Paperna, & Smirnova, 1997; Durborow, et al., 2003; Ibrahim K. S., 2011; Adeshina et al., 2019). There are primarily two species of *Branchiomyces* that are responsible for this disease and include *B. sanguinis* and *B. demigrans*. Both species produce branched, non-septate hyphae (Bright Singh & Sreedharan, 2009; Smith, 2019). Both *Branchiomyces* species cause similar pathology, except that *B. demigrans* affects the entire gill, with hyphae penetrating through blood vessel walls into the lumen, while *B. sanguinis* is restricted to gill blood vessels (Choudhury *et al.*, 2014). *Branchiomycosis* is caused by the fungi *Branchiomyces sanguinis* in carps and tench (Plehn, 1912) and by *Branchiomyces demigrans* in pike, largemouth bass, striped bass, and tench (Choudhury et al., 2014). It has been reported several times in carps (Hora and Pillai 1962; Pillai et al., 1983). High mortalities are often associated with this infection (Paperna, 1996; Florio et al., 2009; Sharma et al., 2012).

**Histopathology:**

The gills were directly examined for the spores under the light microscope. *Branchiomyces* species caused lesions on the gills of infected fish. Branchiomycosis causes three phases of changes, according to the histological study of infected gills, the primary lamellae were less affected as compared to secondary lamellae where complete necrosis occurred. There was a widespread infiltration in the lamellae. Furthermore, moderate to mild lamellae hyperplasia with enhanced respiratory epithelium fusion and secondary gill lamellae disarray were observed (Flores and Thomas, 2011).



Plate 12. The image shows the infestation of the Fungi *Branchiomyces sp.* in the gill tissue of *Labeo Catla* species.



**Plates. 13 to 18.** **Plate. 13.** Normal Gill Tissue (45x). **Plate. 14.** Infected tissue showing the progressive necrosis in different areas of the primary and secondary lamella of gill filaments (45x). **Plate. 15.** Fungal mycelia (Arrow) in the gill tissue (10x). According to Flores and Thomas, 2011, **Plate. 16-18.** Shows Progressive degeneration of the Primary lamella in different stages as **Plate. 16.** Primary degradation (10x), **Plate. 17.** Secondary degradation (10x), **Plate. 18.** Tertiary level of tissue degradation (10x).

The pathological alterations identified in this histopathological investigation were comparable to those previously described, including epithelial hypertrophy, secondary lamella fusion, and hemorrhages in the original lamella (Grimaldi 1971; Ebrahimzadeh *et al.*, 2005; Ibrahim, 2011; Adeshina *et al.*, 2019; Hussain & Kadhim, 2020).

**Treatment:**

- If an epidemic arises, the fish should be stopped being fed, and dead fish should be taken from the ponds and buried in a lime pit.
- The pond should be emptied, dried up, and cleaned with quicklime to help prevent further breakouts (Leaño, 2001).
- Further research for preventing this disease may include the use of fungicides and improved diets, including more lipids which will eventually allow the fish's immune cells (especially the T cells) to function even in cold temperatures. FDA-approved Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as an anti-fungal for the treatment of fish eggs (Durborow, et al., 2003).
- Use of Potassium permanganate (KMnO<sub>4</sub>): Potassium permanganate (KMnO<sub>4</sub>) is an oxidizing agent that has been used for many years in aquaculture. It is also used in water conditioning systems as it works as an Oxidizer and is able to chemically "burns up" organic material disinfecting the external surfaces including bacteria, parasites and fungus (Francis-Floyd & Klinger, 1997). The proper use of potassium permanganate can successfully eradicate many bacterial, parasite, and fungal agents before systemic illnesses develop, often removing the need for antibiotic treatment. Other antibiotics created by ICAR-CIFA scientists, such as CIFAX and Aqua-Health, have been found to be very beneficial in controlling EUS and other illnesses that farmers can easily handle. This is the most commonly utilized strategy among local farmers.

#### **4.9.2 Argulosis**

The production from culture system is remarkably hampered by the infestation of various fish parasites. Argulus is an ectoparasite commonly known as Fish lice. About 129 species of Argulus have been reported worldwide (William 2008). It has a complex life cycle involving several metamorphic stages. Argulus species create skin lesions on the host using their suckers and proboscis when eating. These lesions frequently cause subsequent infections with bacteria and fungus. The infection also causes reduced appetite, weight loss, and anaemia in fish. Argulus serves as a vector of some viruses and parasitic nematode larvae (Walker et al., 2004; Sahoo et al., 2013).

**Classification:**

Phylum: Arthropoda

Subphylum: Crustacea

Subclass: Branchiura Thorell, 1864

Order: Arguloidea Wilson, 1932

Family: Argulidae Rafinesque, 1815

Genus: *Argulus* Müller, 1785



Plate 19. Image showing *Argulus* sp.

The collected *Argulus* sp. from carp were of different sizes, with females larger than males. Morphological identification of *Argulus* spp. is mostly based on distinguishing features of an adult male such as carapace and abdominal length or width, dorsal ridges of the carapace, respiratory areas, leg pigments, abdominal lobes and incision, and the presence of a small coxal at the swimming appendages (Sahoo et al., 2012; Soes et al., 2010). The challenge for species identification was amongst the most common two species *A. japonicas* and *A. foliaceus*, which was confirmed using the recent available literature. Based on the morphological characters such as dorsal ridges of the carapace, respiratory areas, teeth on the basal plate of 2nd maxillae, presence or absence of bilobed protuberances on 2nd swimming appendage in male and position, shape of peg and boot-shaped lobe on 4th swimming appendage in male and female, the parasites were identified as *Argulus japonicas* (Tandel et al., 2021).

As *Argulus* was found to be a highly occurring disease, the prevalence was calculated for the same. During the investigation period, cultivable fishes (*Labeo Catla*, *Labeo rohita*, *Cyprinus carpio*; random sampling; n=111) were collected from freshwater resources, whereas ornamental fishes (*Carassius auratus* and *Siamese splendensis*; convenient sampling; n=101) were collected from aquariums of the Vadodara district with the assistance of a local fisherman and owners. All the fish samples (N=212) were subjected to detailed physical examination to detect the presence of parasites and clinical symptoms associated with clinical infestation. *Argulus*, a crustacean parasite, was found on the skin's surface among fishes screened during the study period. Under a low-power binocular microscope, the parasites were carefully observed and photographic evidence was taken for proper identification, shown in plate 19. The *Argulus* parasites' permanent mounts were prepared per the National Wild Fish Health Survey (NWFHS) Laboratory Procedure Manual-2004. The specimens were stained with

acetocarmine, destained in 1% acid alcohol, dehydrated in a succession of increasing alcohols of 30%, 50%, 70%, 90%, and pure alcohol, and then cleaned in xylene before mounting with DPX mountant. The stereo microscope was used to observe the gross specimens. Identification and classification of *Argulus* was based on descriptions mentioned by Yamaguti (1963). The prevalence and abundance of parasites was observed and analysed among screened population of fishes by using formulae given by Parveen and Gaikwad (2018).

$$\text{Incidence \%} = \frac{\text{Infected Host} \times 100}{\text{Total Host examined}}$$

$$\text{Abundance} = \frac{\text{No. of Parasites}}{\text{No. of Host examined}}$$

Detailed analysis of sampling, incidence and abundance is shown in Table-14 and Fig 20. Parasitic infestation significantly affected the ventral side of the body as shown in Plate 20 and 21. Hence, the parasites were collected mostly from the ventral side of the body.

Table 14. Incidence, abundance and number of parasites collected from fresh water fishes

SN	Name of Fish	Sample site	Number of fishes		Number of parasites collected	Incidence	Abundance
			Examined	Infected			
1.	<i>Labeo Catla</i>	Freshwater reservoirs	38	11	53	25.22 %	1.59
2.	<i>Labeo rohita</i>	Freshwater reservoirs	35	09	88		
3.	<i>Cyprinus carpio</i>	Freshwater reservoirs	38	08	36		
<b>Total</b>			<b>111</b>	<b>28</b>	<b>177</b>		
4.	<i>Carassius auratus</i> (Gold fish)	Aquarium shop	39	21	126	49.50 %	2.50
5.	<i>Siamese splendensis</i> (Fighter fish)	Aquarium shop	24	09	25		
6	<i>Carassius auratus</i> (Black moor)	Aquarium shop	38	20	92		
<b>Total</b>			<b>101</b>	<b>50</b>	<b>243</b>		

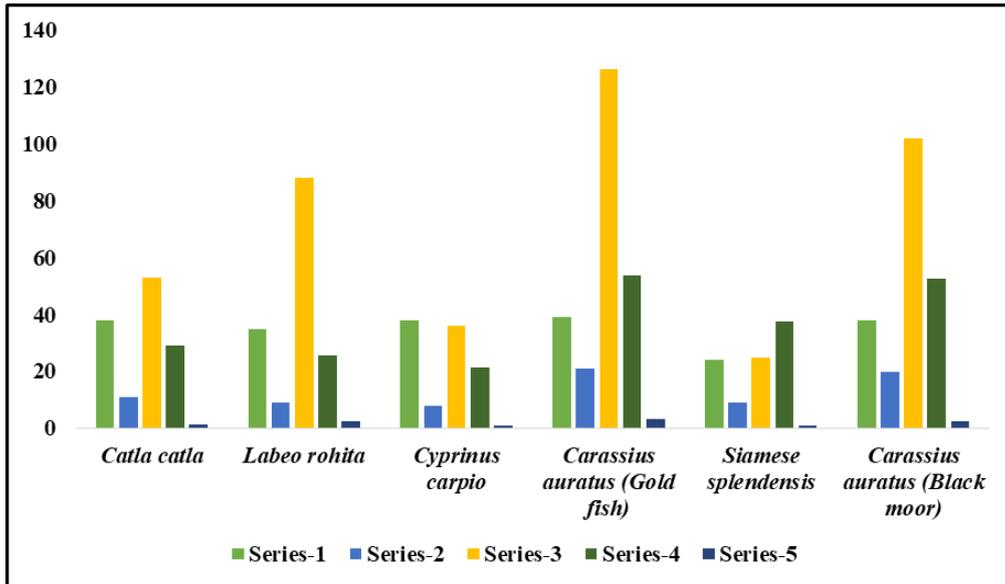


Fig. 20: Incidence and abundance *Argulus* in Ornamental and Cultivable fishes [Series-1: Number of Examined fishes; Series-2: Number of Infected fishes; Series-3: Number of Parasites collected; Series-4: Incidence %; Series-5: Abundance]



Plate 20. *Argulus* infestation in *Labeo rohita*



Plate 21. *Argulus* infestation in *Labeo Catla*

Out of 111 fishes screened from freshwater reservoirs, 28 were observed to be infested with the *Argulus* parasites with an overall incidence of 25.22%. In cultivable fishes, greater number parasites were found in *Labeo rohita* (88), followed by *Labeo Catla* (53) and the least number of *Argulus* was found in *Cyprinus carpio* (36). Whereas, out of 102 ornamental fishes screened at aquarium shops, 50 were observed to be infested with the *Argulus* parasites with an overall incidence of 49.50%. *Carassius auratus* (Gold fish) had the most parasites (126), followed by *Carassius auratus* (Black moor) (92) and the least number of parasites found in *Siamese splendensis* (Fighter fish) (25).

The incidence of infection in cultivable fishes from freshwater reservoirs (25.22%) with an abundance of infection at 1.59 was significantly less than ornamental fishes (49.50%) with an abundance of infection at 2.50. Ornamental fishes found maximum number of parasites mainly the gold fishes (*Carassius auratus*) and minimum in *Siamese splendensis*. Fresh water fishes showed maximum number of parasites in Rohu (*Labeo rohita*) and minimum *Cyprinus carpio*. *L. rohita* had the highest infection, while other species had lower than *L. rohita* which coincides with descriptions of Jarfri and Ahmed (1994) and Singh and Khan (2014). Carps were more suitable host for *Argulus*. Infestation was noticeable in Rohu. This result was similar to the observations made by Bakshi *et al.* (2006). In ornamental fish, major incidence and abundance (number of parasites) was higher in *Carassius auratus* (Gold fish), *Carassius auratus* (Black moor) and *Siamese splendensis* (Fighter fish), respectively. Researches carried out on goldfish and Koi confirms the *Argulus* as the most prevalent parasite (Noga, 2010). In both, black moor and fighter fish, the infection rate was low as compared to Goldfish which are similar to observations and descriptions given by Iqbal *et al.* (2013). These observations

indicate that *Argulus* is present in noticeable population of fish from freshwater reservoirs and aquarium shops. *Argulosis* disease is a serious threat in IMCs as well as ornamental fishes which can be extensively harmful to the yield of fishes. The observations recorded in the present study provides pioneer and baseline data on existing occurrence and abundance of *Argulus* which can be utilized for further research on disease investigation and pathological impacts of this parasite in fish. Chemical treatment of externally parasitized fish is possible (Hoffman, 2019) and concerned fishermen/shop owners can be advised accordingly to treat fishes and prevent economic losses when such parasites are identified in different regions.

### **4.9.3 Lernaeosis**

Lernaeosis is a fish illness caused by Copepod ectoparasites of the genus *Lernaea* (Hua et al., 2019). These Ectoparasites attach to all exterior parts of the fish, including some internal parts such as the mouth, gills (Noga, 1986; Barson et al., 2008), gill filaments, or even the eyes (Woo, and Shariff, 2006; Nur et al., 2022). *Lernaea* is commonly associated with considerable mortality in aquaculture, and the impact is severe, as fish death can occur in large numbers. Due to the widespread introduction of tropical fishes such as cyprinids, *Lernaea cyprinacea* has become the most pervasive lernaeid (Soares et al., 2018; Zhu et al., 2021; Prastowo et al., 2023).

**Classification:**

Phylum: Arthropoda

Subphylum: Crustacea

Subclass: Copepoda Milne-Edwards, 1840

Order: Cyclopoida Burmeister, 1834

Family: Lernaeidae Cobbold, 1879

Genus: *Lernaea* Linnaeus, 1758



Plate 22. Image shows *Lernaea* sp.

Lernaeids commonly known as ‘anchor worms’ are crustacean copepod parasites infecting various wild-caught and pond-raised freshwater fishes. The microscopic image of *Lernaea* sp. is shown in Plate 22, with the naked eye and under stereo microscope. Approximately, 110 Lernaeids species have been reported under the genus (Ho, 1998). They mate during their final free-swimming stage of development. Following mating, the female burrows into a fish's flesh and turns into an unsegmented, wormlike shape, usually with a section hanging from the fish's body, leaving only the body and tail visible. Female Lernaeids are believed to be more parasitic as they attack the body surface of fish and penetrate deep into the tissues after consuming fish scales, causing deep lesions as seen in Plate. 23. A and B, which in turn invite secondary microbial infections (Baur, 1962; Dziridziul, 1973; Shariff et al., 1986; Jalali and Barzegar, 2006; Tasawar et al., 2012).

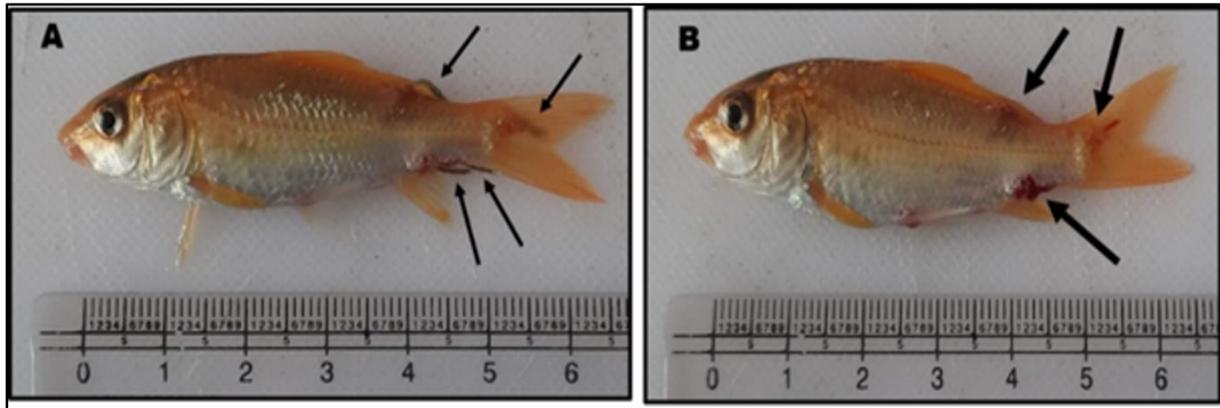


Plate 23. A. Shows the site of attachment of the *Lernaea sp.* B. Represents the Lesions

*L. cyprinacea* was found on the scales and tail fins of fish as seen in Plate 23. A., range from 10 to 13 millimeters. *Lernaea*'s morphology revealed a tiny, semi-spherical cephalothorax connected to the first swimming limb located in the center of the holdfast system. The second and fourth leg segments were located in the neck and abdomen, respectively. The body is not segmented. The holdfast is separated into two branches, dorsal and ventral. Ventral branches appeared smaller and unbranched, while dorsal branches had two additional branches. A 1-2 mm long egg sac was discovered in the posterior area of female *L. cyprinacea*. The morphology coincides with the description of *L. cyprinacea* in the previous studies (Kabata, 1985; Pallavi et al., 2017; Hall, 1999; Raja et al., 2020 and 2022; Tandel et al., 2021), which specifies that *L. cyprinacea* has a holdfast that branches into two pairs. *Lernaea sp.* Was the second most occurring parasite after *Argulus*. Hence, the prevalence data was calculated.

Table 15. Gives the Prevalence data about the *Lernaea* species

Sr. No.	Name of Fish	Number of fishes		Number of parasites Collected	Incidence	Abundance
		Examined	Infected			
1	Guppy ( <i>Poecilia reticulata</i> )	17	6	24	32.07	1.50
2	Gold Fish ( <i>Carassius auratus</i> )	10	4	18		
3	Gold koi fish ( <i>Cyprinus rubrofuscus</i> )	12	2	15		
4	Molly ( <i>Poecilia sphenops</i> )	14	5	23		
	<b>Total</b>	<b>53</b>	<b>17</b>	<b>80</b>		

A total of 53 fishes were screened which included 17 infected fish specimens from freshwater reservoirs and 80 *Lernaea sp.* collected. Detailed analysis of sampling, incidence and abundance is shown in Table 10. Parasitic infestation significantly affected the ventral side of the body as shown in Plate 11; hence, the parasites were collected mostly from the ventral side of the body. The host preference was directly proportional to the number of parasite collected from each species i.e. Guppy, Molly, Gold fish and Gold Koi fish. The incidence of infection came to be 32.07 with an abundance of 1.50, respectively.

#### **4.9.4 Spring Viremia in Carp (SVC)**

Spring viremia of carp (SVC) is a major disease of cyprinids. The disease is common in European carp culture, resulting in severe morbidity and death. The Office International des Epizooties has designated SVC as a notifiable disease. It is caused by a Rhabdovirus known as Spring Viremia of Carp Virus (SVCV). SVC primarily affects carp, goldfish, pike, roach, rudd, tench, and Wels catfish, causing numerous clinical indications such as belly enlargement, exophthalmos, petechial haemorrhages, and darkening of the skin. Affected fish display tissue death in the kidney, spleen, and liver, resulting in haemorrhage, loss of water-salt balance, and impaired immunological response. Water temperatures ranging from 10 to 17°C are associated with high mortality, particularly in the spring.



Plate 24. Infected fish sample of SVC

During the study, the SVC infection was recorded in *Hypophthalmichthys molitrix*, Silver carp. The reports of SVC are from America, China and East India, but no such record is from Gujarat, till date.

The previous studies on SVC are majorly the first record or pathogen species identification to list some Ahne et al., (2002); Goodwin, (2002); Dikkeboom et al., (2004);

Teng et al., (2007); Phelps et al., (2012). However, managing such viral diseases is challenging due to their high transmission rates.

#### **4.9.5 Tail and Fin rot disease**

Tail and fin rot disease occurred in Indian major carp, catla (*Labeo catla*). The injured fish has lesions and erosion on its tail and fins. Infected farms saw a mortality rate of approximately 40%. Bacterial tail and fin rot is a common issue in young fish, often linked to both polluted and clean conditions in hatcheries. Although bacteria are the primary cause of this disease, pathogenic protozoans and fungi may also contribute. The infection can extend to the body surface as well. *Aeromonas* bacteria are the most frequent pathogens responsible for this troublesome infectious disease in fish, and they are typically found in the normal microflora and hydrobionts of fish habitats (Kompanets et al., 1992; Satish et al., 2013). The diseased fish showed loss of lamellar structure of gills and caudal fin, as shown in plate 25. Swab samples of the gills and tails were also collected for the screening and isolation of causative agents.



Plate 25. Image depicts the Infected Fish with Tail and Fin rot

Tail and Fin rot disease was a very common disease occurring in Tilapia, Indian Major Carps, Snake headed fishes. Similar results were obtained in many of the previous studies, to list some El-Attar and Moustafa, (1996); Rahman et al., (2010); Satish et al., (2013); Timalisina et al., (2022).

#### **4.9.6 Ichthyophthirius (White Spot Disease)**

One of the most significant challenges in freshwater aquaculture is the parasite ciliate *Ichthyophthirius multifiliis* (Ich), which causes white spot disease. White spots (Ichthyophthiriasis) is a common ectoparasite disease that mostly affects farmed and aquarium fish. The morbidity rate owing to this disease can reach up to 100%, producing significant economic losses in fish farms. *Ichthyophthiriasis* has also been known as sand grain, gravel or Ich disease (Cross and Matthews, 1992; Richards and McDonald, 1998; Li and Buchmann, 2001; Xu et al., 2002; Eissa, 2004). This deadly ectoparasite mostly targets skin, fins, gills, and the buccal cavity, and is distinguished by the presence of white dots all over the external body surface. The clinical signs recorded in this study due to *Ich multifiliis* infection (white spot disease) are coincide with those recorded by most researchers as (Khalifa et al., 1983; Derwa, 2004; Osman, 2009).

It a very common disease occurring in the ornamental fish, *Mystus cavasius* (Also called Freshwater shark). This disease is similar to SVC, non- treatable. The infected fish die soon. Many research is being conducted for its treatment. Generally, the  $KMNO_4$  treatment is found effective but only for some time.

#### **4.9.7 Black Spot Diseases**

Blackspot disease in goldfish (*Carassius auratus*) is a common issue in aquariums. It can typically be treated effectively with antibiotics. One common cause of black spots on goldfish is high ammonia levels in the tank. Elevated ammonia levels can lead to chemical burns on the fish, which manifest as black spots. To prevent this, ensure the tank remains clean by regularly removing uneaten food, fish waste, and plant debris, and frequently check ammonia levels.



Plate 26. Image depicts infected fish (Black spot disease)

The Plate 26. depicts the Infected fish, showing the black patches on skin, which after the treatment gets disappeared as seen in Plate 27. Result in a widespread reddening, visible as hyperaemia of the fins and other non-pigmented areas of the body (Wildgoose, 1998). Many cases are caused by Gram-negative bacteria, including *Aeromonas*, *Pseudomonas*, and *Vibrio* species. Goldfish may also have brown pigmentation patches, which will fade after successful antibiotic therapy. The traditional way of preventing this disease is to add rock salt, but nowadays many Antibiotics are available.



Plate 27. Image taken after treatment (Black spot disease)

Hence, it is concluded that the Gold fishes are more susceptible to this disease compared to other aquarium fishes.

However, five factors namely age, diet, abundance of fishes, independent number of a parasite within the fish and season, directly influence the parasite fauna of fishes (Dogiel, 1964; Kabata, 1985; Srivastava, 1975; Ramudu and Dash, 2013). Fish with these parasites may exhibit a variety of symptoms, including respiratory distress, decreased appetite, weight loss, lethargy, skin discoloration, fin erosion, and increased susceptibility to other illnesses. Fish that are infected may display altered behaviour, such as more frequent scratching against objects or flashing actions. Fish health management techniques, such as good sanitation habits, regular monitoring, and suitable treatment options, can be put into place to avoid and manage parasite infestations. To reduce the parasite burden and enhance fish health in wetland environments, these techniques may involve the use of chemical treatments, biological control agents, or environmental alterations. It's worth noting that the specific prevalence and distribution of parasites can vary across different wetlands in India and may depend on factors such as water quality, fish species present, and environmental conditions.