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Niche occupancy and dietary profiling of *Polypedates maculates* tadpoles in temporary ponds of Northen Odisha

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Abstract

All living creatures, whether plants or animals depend on the food available in the system for nourishment and energy necessary for the completion of their life cycle. Feeding constituents are always been the important aspect of biology of tadpoles, which is the main target of this study. Available dietary resources in the ecosystem is especially important in tadpoles because they need to attend the early stage in very short-lived aquatic environments i.e. temporal ponds and tadpoles need to consume food that will ensure their metamorphosis prior to drying up the pond. Tadpoles of *Polypedates maculatus* were collected from temporary ponds of northern Odisha. The guts of tadpoles were dissected out and analysed for the qualitative and quantitative analysis of food consumed. Diet is basically composed of microalgae and relatively low amount of detritus. The algae belonging to class Chlorophyceae, Bacillariophyceae, Cyanophyceae and Euglenophyceae were recorded. The numeric frequency (NF%) and frequency of occurrence (FO%) of different food items show the species richness and abundance, which is consumed by tadpole. Huge diversity of algal flora as tadpole's food items are determined by the two diversity indices i.e. Simposon and Shannon-Weiner. Niche breadth of the tadpole was analysed through Levin's measure. The physico-chemical parameters of water signifies the pollution free tadpole's habitat, which the support the growth and metamorphosis of tadpoles. The diet preference and choice of algae as food indicates that the conservation of habitat in terms of algal diversity is essential for survival and completion of their life cycle of the tadpoles for successful survival of anurans. .

Keywords: Anuran, Niche, Oral disc, Food spectrum, Numeric frequency

1. *Introduction*

Amphibians were a class of vertebrates, who could lead a terrestrial existence for first time, as prompted by the nature to advance over their aquatic ancestors and carry forward the evolutionary rules, creating a remarkable change in habitat at some point of their lives. Amphibians are classified into three living orders i.e. Anura - frogs and toads; Caudate-salamanders and newt; and Gymnophiona- caecilians and three extinct orders i.e. Ichthyostegalia- tiktaalik, acanthostega, icthyostega; Temnospondyli- eryops, cacops, buettneria; and Anthracosauria- seymoria. Among the three living groups, the anurans have undergone large adaptive radiation, caudate amphibians exhibit aquatic adaptations, whereas anuran amphibians diverged towards different modes of life i.e. cave dwelling , burrowing, Volant and arboreal habitats. Anurans constitute about 85% of amphibians, have the broadest geographical distribution, and hence, significantly the largest group. Feeding constituent is an important aspect of biology of tadpoles. Tadpoles are essential parts of aquatic ecosystems and depend on resources available for successful completion of their life cycle. Anuran larvae are mostly grazers, feeding from substrates in aquatic systems or suspension feeders whereas adult anurans are largely carnivorous. Diet is specifically important in tadpoles because they complete their life cycles in short- lived aquatic environment i.e. ephemeral ponds and tadpoles need to consume food, that will ensure their metamorphosis prior to the drying up of the pond. The ecological niche of amphibian species is the result of interaction of three types of resources: space, food availability and time(Schooner,1974). Predation can also influence community organization (De Benedicts, 1974; Heyer et al., 1975; Heyer, 1976; Morin, 1981,1983; Wilbur et.al, 1983) although according to Heyer (1973,1976) food plays a less important role in niche segregation and habitat is mainly partitioned in space and time. In larval amphibian communities an almost complete overlap in the use of feeding resources is frequently found (Diaz-Paniagua 1985). Broadly, a life of an anuran can be described by the following three

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sentences, "Breed to free", "free to feed" and “feed more to be free". "Breed to free" means the egg laying in a mass and into the open area increases the possibility of eggs development and survival of tadpoles."Free to feed" means since the tadpoles are developed in an open area and without any parental care; hence they are free to feed adequately and free to compete with others. "Feed more to be free" means to eat more and more in a short time that will help developing the tadpoles, winning the competition of feeding to get matured, becoming the adult in QuickTime development and getting free as a juvenile, then adult and then ready for reproduction again. *Polypedatesmaculatus* is a common species of tree frog mainly distributed in south Asia. It is widespread throughout Bhutan, India, Nepal and Sri Lanka as well as western and southern Bangladesh. It has been recorded from a wide variety of habitat types including tropical dry and moist forests, grasslands, and agricultural lands and close to human habitats. It is largely arboreal, although it can be found on walls and hidden under rocks and leaves. It bears foam nest which is semi-globular in shape with a flat bottom attached to the substrate. Trees overhanging water tanks and pools are used as spawning sites. Tadpoles take 55 days for complete metamorphosis, feeding on algae and diatoms. There are at least 6 species of *polypedates* currently recognized in north-eastern India (Chakravarty et al. 2011). Instead of this, very inadequate knowledge have been obtained regarding larval biology, specially about feed and feeding ecology of these species from this area. The present study describes the external morphology, oral structure, feeding ecology and food diversity of *p. maculates tadpoles* from northern Odisha.

2. Materials and methods

A. Study Site:

The study was conducted in four temporary and permanent water bodies in different locations of Baripada (21º56’24.00”N-86º43’12.00”E) in Mayurbhanj district of Odisha. Different developmental stages (Gosner, 1960) of the tadpoles were collected during July, through October 2014 with dip net. The study began when egg masses were observed in different water bodies. A few tadpoles of each species were reared in seminatural experimental set up in an aquarium in the laboratory till emergence of hatchlings so that identification could be confirmed.

B. Gut content analysis:

Tadpole assemblage were collected from temporary ponds of Baripada, Mayurbhanj district, Odisha, India during July -September 2015 by means of dip net (mesh size 1mm) and preserved in 10% formaldehyde immediately after collection in order to prevent complete digestion of ingested food particles. In the laboratory, individuals of stages 35-38 (Gosner, 1960) were separated which were subsequently preserved in 4% formaldehyde. Measurements of total body length, body length, body width of each tadpole were made with the help of a digital vernier calliper to the nearest 0.1 mm.

Each tadpole gut was dissected out and gut length was recorded before flushing with distilled water. The gut contents (the first four centimeter) were then taken in a Sedgewick rafter chamber and quantitative and qualitative analyses were done under a binocular microscope. Photographs of the gut contents were taken with the help of a Sony cyber shot camera (5.1 megapixels, DCSW5) attached to a binocular microscope (Laboscopy, CMS-2). The food items were identified to the lowest possible taxonomic level and quantified. The identification of the food items was done by following standard literature (Smith 1994; Edmondson 1959). The items ingested by tadpoles were quantified based on the numeric frequency (NF%= total number of food items of a specific food group consumed/ total number of items of all food groups consumed x 100) and frequency of occurrence (FO%= number of guts in which the specific food item was present/ total number of guts with these food items x 100). The importance index was obtained by calculating the %NF plus the %FO divided by two, in accordance with Colli et al.,(2003).

Diet diversity was calculated for each species by the Shannon-Wiener index (H’) and the niche breadth for the food items ingested was calculated by Levins’ measure (B). The food items were identified up to genus level. These analyses were done using the software PAST (version 2.14). The water samples were collected from different temporary pools in water sampling bottles and taken to the lab for the estimation of various physico-chemical parameters.

The samples were collected between 9.00-10.30 am and the temperature was noted down. The physical parameters like pH, turbidity, conductivity and chemical parameters like DO, alkalinity were estimated following the standard method described by APHA et.al.,(1995).

Table 1: Characteristics of the sampling sites studied in Northern Odisha.

Ecological Parameters	**Bangiriposi**	**Kostha**	**Astia**
Date of collection	04.07.2015	22.07.2015	09.08.2015
Shape	Irregular	Irregular	Oval
Margin type	50% flat 50% ravine	60% flat 40% ravine	50% flat 50% ravine
Coordinate	21º58’.353”N- 086º46’.978”E	21º56’.759”N- 086º47’.517”E	21º58’.949”N- 086º46’.978”E

C. Water Sampling:

The water sample were collected from the different lakes in water sampling bottles and taken to the labs for the estimation of various physical and chemical parameters. The samples were collected between 9 - 10.30 am and the temp was noted down. The Physical parameter such as pH and turbidity and chemical parameters such as COD, BOD and DO were estimated following the standard methods prescribed by APHA *et al.,* (1995).

Water sample Analysis:
Physico- Chemical Parameters:
1. Temperature and pH

Temperature is accessed with a help of thermometer and pH by pH paper or pH meter.

2. Turbidity

To access the water quality, turbidity or transparency is also an important factor that canbe analysed with the help of a Secchi disc. Secchi Disc is used by lowering it into the water with the help of a graduated dropper. It is a method for measuring of light penetration in water. Limitation of visibility and light penetration can be estimated. The lowering of disc into the water noting the depth when it disappear and then lifting the disc noting the depth at which it reappears and the average of these two readings are considered to be limit of visibility and calculated by the help of following formula:

Formula: A+B /2= turbidity of water measured in NTU
Where A = Depth of water where the disc just disappear.
B = Depth of water where the disc just reappear.

3. Chemical Oxygen Demand

50 ml of water sample was taken in a clean conical flask. 5ml of $KMnO_4$ was added and heated in a water bath (62^0 C) and cooled for 10 min. Later 5ml of KI solution was added, followed by 10 ml of 2M H_2SO_4. 35ml of this solution was taken and titrated against 0.1 M sodium thiosulphate until appearance of pale yellow colour. Few drops of starch solution were added and blue colour appeared. This was titrated until the disappearance of blue colour. The titrated reading was noted and COD was calculated with the help of following formula:

Formula: A+B /2= turbidity of water measured in NTU
Where A = Depth of water where the disc just disappear.
B = Depth of water where the disc just reappear.

4. Chemical Oxygen Demand

50 ml of water sample was taken in a clean conical flask. 5ml of $KMnO_4$ was added and heated in a water bath (62^0 C) and cooled for 10 min. Later 5ml of KI solution was added, followed by 10 ml of 2M H_2SO_4. 35ml of this solution was taken and titrated against 0.1 M sodium thiosulphate until appearance of pale yellow colour. Few drops of starch solution were added and blue colour appeared. This was titrated until the disappearance of blue colour. The titrated reading was noted and COD was calculated with the help of following formula:

COD = [8× C (A – B) × 0.23]/S
Where C = conc. of titrant
S = vol. of sample, A = titrant used for blank and B = titrant used for sample.

5. Dissolved Oxygen

The DO content in water sample was estimated by using the light and dark bottlemethod. A bottle of known volume was taken and it was filled water sample. 1ml of manganous sulphate and 1ml of alkaline iodide were added and content was mixed well. The bottle was kept in dark. After 15 minutes the ppt was formed. Then 1ml of conc. sulphuric acid was added to dissolve the precipitate. 25 ml of this water sample was taken in a conical flask and 1ml of starch solution was added. Blue color appeared. The blue coloured sample was titrated against sodium thiosulphate (0.025N) solution until the disappearance of blue color. The oxygen content was determined by using the following formula.

Oxygen=K*200*U*O.698/volume of sample)mg/L
K= vol. of bottle/vol. Of bottle-vol. of reagent used
V=volume of water sample taken, U= vol. Of sodium thiosulphate used for titration

6. Biological Oxygen Demand (BOD)

The water samples for BOD were collected in the field in BOD bottles (300ml capacity). Initial dissolved oxygen was determined in the first set of BOD bottles and the second set was kept for incubation in BOD incubator at 20^0 C for 5 days. After 5 days the bottle from the BOD incubator was removed and the dissolved oxygen from the incubated set of bottles were determine and the readings were noted.

7. Total Alkalinity

Total alkalinity was measured by phenolphthalein and methyl orange alkalinity.OH- and ½ CO3 were measured by the phenolphthalein alkalinity. 100ml of water sample is taken in a flask and 5 to 6 drops of phenolphthalein were added. Pink colour appeared and it was titrated with acid till pink colour disappears. The P was noted. The remaining CO3 and HCO3 were measured by methyl orange alkalinity.5-6 drops of methyl orange were added and titrated with acid till the colour change from yellow to orange (M).

Total titrate value T = P+M. Formula is as follows:
P alkalinity
P=A× N× 50000/ml of sample
Total alkalinity=B× N× 50000/ml of sample, Where A=ml of P; B=ml of T; N=0.02

3. Results

The tadpoles of *Polypedates maculatus*, stage 35-39 (Gosner, 1960) were collected from the temporal ponds of northern Odisha. The thorough study on tadpole morphology, microscopic study of the gut content, physico-chemical parameters of water showed both the expected results and some new results also. All the findings and data are hereafter presented as statistical as well as the tabulated data.

The morphological characteristics of *P. maculates* tadpoles like total length(cm), body length(cm) and body width(cm) and gut length(cm)of tadpoles in six studied temporal ponds showed no significant difference. The body length and the gut length of each tadpole were positively correlated (Table-2; Fig-1).

Table-2: Total length and gut length of *Polypedates maculatus,* tadpoles collected from different sites of Baripada.

Tadpole no.	GL	TL	BL	BW
1	19.5	4.65	1.40	0.76
2	21.06	5.01	1.51	0.79
3	17.9	3.39	1.20	0.54
4	13.4	3.45	1.19	0.51
5	9.2	2.32	0.95	0.32
6	19.7	3.34	1.30	0.61
7	20.5	4.95	1.76	0.79
8	17.3	3.01	1.01	0.34
9	9.6	2.85	0.91	0.29
10	15.7	3.08	1.03	0.32
11	21.5	4.95	1.69	0.81
12	19.4	3.32	1.23	0.44
13	21.5	3.37	1.19	0.53
14	11.3	3.24	1.12	0.49
15	19.7	3.09	1.05	0.37
MEAN ± S.D.	17.15 ± 4.30	3.60 ± 0.85	1.2 ± 0.25	0.25 ± 0.19
CORRELATION OF GL AND TL: 0.65				

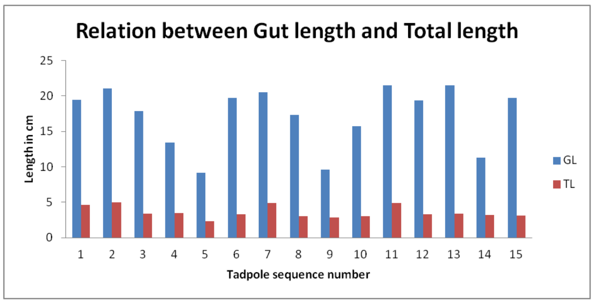


Fig- 1: Relation between Gut length and Total length

A. Tadpole morphology:

The tadpoles had an oval body, slightly rounded and depressed snout, and laterally placed eyes. Nostril was dorsal and nearer to snout than eyes. Spiracle was single, sinistral. Dorsal fin height was greater than the ventral fin. Both the fins gradually tapered towards the pointed tip. Black spot was present all over the body including tail. Ventral side of the body was not pigmented and transparent at the abdomen region. Hence, the intestinal spiracle was clearly visible through the transparent abdominal(Table-3; Plate-4).

B. Oral disc:

Mouth was antero-ventral. Teeth are blunt and these were not same in height. Lower jaw was 'v' shaped and jaw sheath was finely serrated. Upper jaw arch was shaped with a weak median convexity, both jaw sheaths were edged with black. Sub marginal papillae were present. LTRF is 4(2-4)/3(1). First row of the upper labium was continuous whereas the 2nd, 3rd, and 4th rows were interrupted. Innermost row of lower labium was slightly interrupted whereas the two other rows were continuous (Table-4; Plate-5).

Table 3: External Morphology

Eye position	Lateral
Location of nostril	Near snout, lateral
Shape of narial aperture	Elliptical
Location of the oral disc	Antero lateral
Spiral tube location	Sinisterl, single
Configuration of the spiral tube	Inner wall present at slight ridge
Number of spiracles	1
Vent aperture opening	Dextral
Snout shape	Cylindrical
Widest part of the body	Anterior, plane of eyes
Dorsal fin origin	Anterior to the body-tail junction
Ventral fin origin	Ventral axis
Shape of the tail tip	Pointed

Table 4: Oral disc Morphology

Oral disc	Emarginated
Marginal papillae	Present , near ventral
Rows marginal papillae	1
Sub marginal papillae	Absent
Jaw sheath	Both jaws present , long and wide
Jaw sheath serration	Serrated both jaws
Oral sucker	Absent
Number of denticle per row	$A_4(3)/P_3$

C. Food spectrum:

The food spectrum of tadpoles mostly included phytoplanktons and relatively very low amount of detritus, involving dead leaves, plant fragments, mud etc. The phytoplanktons were represented by four classes namely Chlorophyceae, Bacillariophyceae, Cyanophyceae and Euglenophyceae involving 37 genera. The individuals from chlorophyceae were *Actinastrum, Ankistrodesmus, Closterium, Cosmarium,Lagerheimia, Oocystis, Scenedesmus, Staurastrum,ulothrix, volvox etc.* The individuals from Bacillariophyceae were *Amphiplura, Cocconeis,Navicula, Cymbella, Pinnularia, Stauroneis, Fragillaria* etc.. Similarly the other two class Cyanophyceae and Euglenophyceae were represented by *Anabaena, Nostoc, Microcystis, Oscillatoria* and *Euglena, Phacus, Trachelomonas* respectively (Table-5; Plate-7a-c).

On the basis of numeric frequency and important index, most of the microalgae found from tadpole gut belonged to classes Bacillariophyceae followed by Chlorophyceae. Among Bacillariophyceae, *Closterium, Cosmarium, Ankitrodesmus, Chlamydomonas and Cyclotella, Fragillaria, Navicula, Cymbella*whereas Chlorophyceae including *Closterium, Cosmarium,Pinnularia*were the important food items consumed by the tadpoles in the study region. At the same time the genus *Microcystis*from class Cyanophyceae and *Phacus*from Euglenophyceae were also consumed at a remarkable rate (Table-8; Figs-3-6)

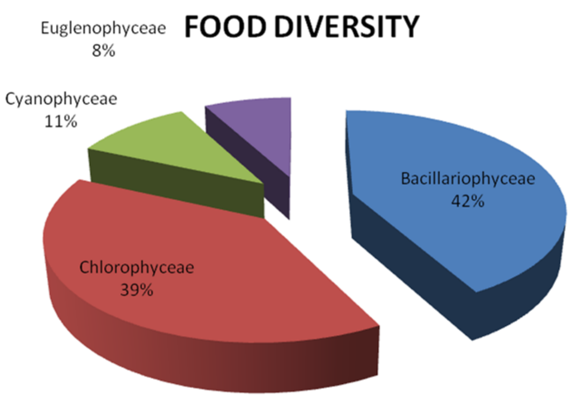
Table 5: Phytoplankton diversity in the intestine of *Polypedates maculates* tadpoles

Class	Food items identified
Bacillariophyceae	*Cyclotella, Fragillaria, Navicula, Nitzscia, Synedra, Cymbella, Pinnularia, Stauroneis, Amphipeura, Cocconeis, Craticula, Diadesmis,, Fragillariaforma, Frustulia, Gomphonema, Synedra*
Chlorophyceae	*Actinastrum, Ankistrodesmus, Cosmarium, Closterium, Mougeotia, Oedogonium,Spirogyra,Chlamydomonas,Ulothrix, Scenedesmus, Oocystis, Pediastrum, Zygnema, Volvox, Pandorina*
Cyanophyceae	*Microcystis, Oscillatoria, Nostoc, Anabaena*
Euglenophyceae	*Phacus, Trachelomonas, Euglena*

Table 6: Relative frequency of different class of algae in tadpole diet.

Class	Relative Frequency
Bacillariophyceae	0.41
Chlorophyceae	0.41
Cyanophyceae	0.10
Euglenophyceae	0.08

Fig 2: Food diversity of *Polypedates maculatus*



Based on Shannon-Weiner function and Simpson Index, the tadpoles of *Polypedates maculates* collected from different temporary ponds showed the value 3.2 and 0.95 respectively, which signifies the conspicuous food diversity considering the standard range of the two being 1.5 to 3.5 and 0 to 1.0, respectively. By applying Levin's measure, these particular tadpoles are found to have a broad niche breadth (Table-7). The FO% and NF% of each class of food item is highly correlated which indicates the species richness as well as abundance (Table -9).

Table 7: Estimation of Simpson, Shannon Diversity Index, Evenness and standardised Levin's Measure of *P.maculatus.*

Estimation of Shannon(H'), Simpson Diversity Index (1-D), Evenness(e^H/S) and Standardised Levin's Measure	
Number of taxa found	39
Individual	27607
Dominance	0.04774
Simpson	0.9523
Shannon	3.241
Evenness	0.655
Berger Parkar	0.11
Levin's Measure	0.52

Table 8: Numeric Frequency (NF%), Frequency of Occurrence(FO%) and Important index of different food items.

CLASS	GENUS	NF%	FO%	IMP
Chlorophyceae				
	Closterium	11.00	100	61.00
	Cosmarium	7.17	100	57.17
	Mougeotia	3.54	100	53.54
	Oedogonium	0.42	80	40.42
	Spirogyra	0.06	46.66	23.39
	Ankitrodesmus	4.36	100	54.36
	Chlamydomonas	5.03	100	55.03
	Coelastrum	2.8	93.33	49.46
	Oocystis	1.46	80	41.46
	Pediastrum	2.52	93.33	49.18
	Scenedesmus	3.24	100	53.24
	Ulothrix	1.14	86.66	44.47
	Tetrastrum	0.56	80	40.56
	Zygnema	1.02	80	41.02
	Volvox	0.17	80	40.17
	Pandorina	0.03	33.33	16.69
Bacillariophyceae				
	Cyclotella	5.20	100	55.2
	Fragillaria	5.66	100	55.66
	Navicula	6.25	100	56.25
	Nitzchia	5.10	93.33	51.17
	Synedra	1.28	86.66	44.61
	Cymbella	4.19	100	54.19
	Pinnularia	3.81	100	53.81
	Stauroneis	3.00	86.66	46.33
	Amphipleura	2.55	93.33	49.21
	Cocconeis	0.78	86.66	44.11
	Craticula	2.24	93.33	48.90
	Diadesmis	2.79	100	52.79
	Fragilariaforma	4.11	93.33	50.77
	Frustulia	1.93	100	51.93
	Gomphonema	1.26	86.66	44.59
	Eunotia	0.66	73.33	37.32
Cyanophyceae				
	Anabaena	0.09	60	30.09
	Nostoc	0.08	73.33	36.74
	Microcystis	1.23	86.66	44.56
	Oscillatoria	0.13	73.33	36.79
Eugnenophyceae				
	Euglena	0.07	53.33	26.73
	Phacus	2.56	100	52.56
	Trachelomonas	0.39	80	40.39

Table 9: Correlation between NF% & FO% of different Phytoplankton classes in*Polypedates maculates* tadpoles diet.

Class	Correlation between NF% & FO%
Bacillariophyceae	0.69
Chlorophyceae	0.63
Cyanophyceae	0.82
Euglenophyceae	0.88

Fig 3: Estimation of NF%, FO% and important index of class Chlorophyceae

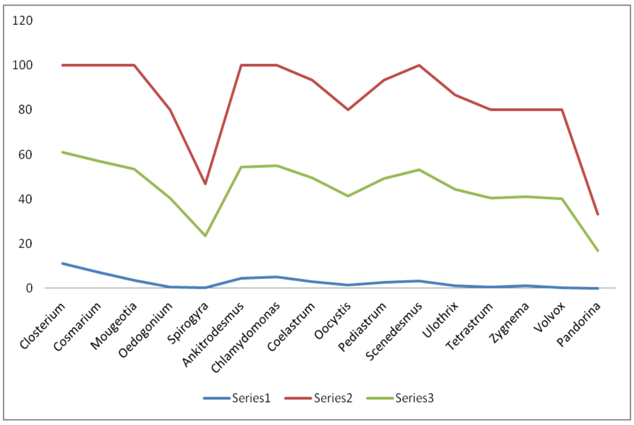


Fig 4: Estimation of NF%, FO% and important index of class Bacillariophyceae

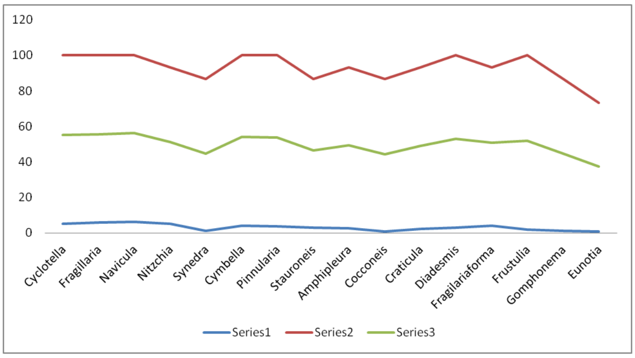


Fig 5: Estimation of NF%, FO% and important index of class Cyanophyceae

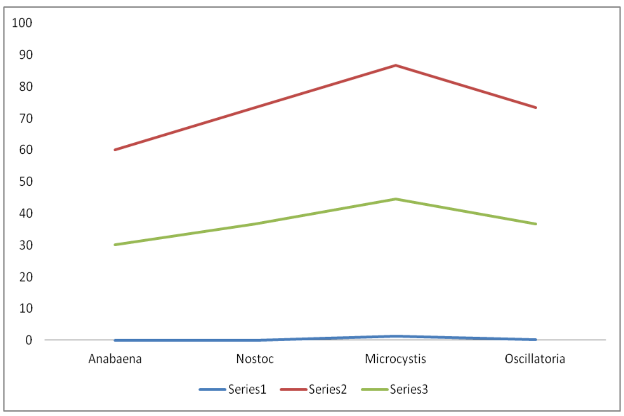
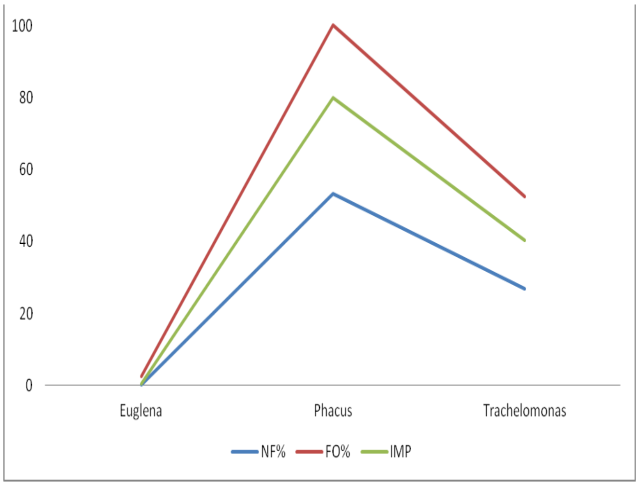


Fig 6: Estimation of NF%, FO% and important index of class Euglenophyceae



D. Physico-Chemical parameters of water:

Water is a precious resource, which has two dimensions; that are closely linked i.e. quality and quantity. Water quality is commonly defined by its physical, chemical and biological characteristics. A healthy environment is one in which the water quality supports a rich and varied community of organisms. As the study was conducted in temporary ponds, the water quality was closely linked to the surrounding environment and land use. Before studying the growth and food diversity of tadpoles of *P. maculatus*, the quality of water should be analyzed, because tadpoles, the specialized creature having the biphasic life cycle, are very sensitive towards water quality. At the same time, within the stipulated period, the tadpoles had to overcome larval life and finally become metamorphosed. During this alarming period, if they don't get proper environment to spawn, the population will head towards decay and devastation. So water analysis is very important aspect of analyzing the health of aquatic organisms.

Physical parameters:

TEMPERATURE

As the atmospheric temperature increases, the temperature of water was found to be little more i.e. 31^0C to 32.4^0C, which enhanced the productivity of water body, indicating the rich growth of phyto-planktons (Table-10; Fig-7).

TURBIDITY

Turbidity refers to the decreased ability of water to transmit light caused by suspended particulate matter and phytoplankton. In the present study, the water sampled from Kostha was more turbid i.e. 27.08, 26.05, supporting the fast growth of tadpoles (Table-10; Fig-7).

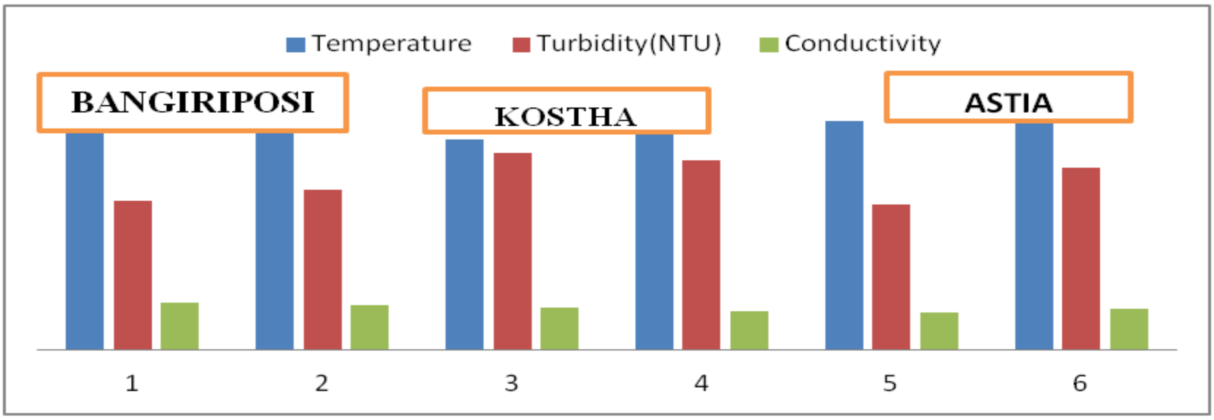
CONDUCTIVITY

This parameter is used for indicating the total concentration of ionized constituents of water (Huq and Alam, 2005). The conductivity was less in all studied habitats due to heavy influx of water during monsoon (Table-10; Fig-7).

Table- 10: Physical parameters of water bodies in the study area.

	Bangiriposi		Kostha		Astia	
	Pond 1	Pond 2	Pond 1	Pond 2	Pond 1	Pond 2
Temperature	31	31.42	29.02	30.2	31.47	32.4
Turbidity(NTU)	20.5	22	27.08	26.05	20.01	25.09
Conductivity	6.5	6.1	5.9	5.4	5.2	5.6

Fig. 7: Physical parameters of water bodies in the study area



Chemical parameters:

COD

The chemical oxygen demand of water represent the amount of oxygen required to oxidise all organic matters, bio-degradable and non-biodegradable by a strong chemical oxidant. This is an indicator of pollution. The water of the showed very less amount of COD i.e., 1.56 - 2.61mg/Lit, which ensures a pollution free habitat, supporting the varied aquatic organisms (Table-11; Fig-7).

BOD

The biodegradation of organic material exerts oxygen tension in the water and increases the BOD(Abida and Harikrishna, 2008). More the oxidisable organic matter present in water, more the BOD(Gupta, 2001). The BOD of ponds of three studied sites were estimated to be very low (1.34 to 2.01mg/Lit) which indicates the pollution free aquatic habitat (Table-11; Fig-7).

DO

Dissolved oxygen is a vitally important parameter of water that is required for aquatic organisms. In natural and waste water, DO levels depend on the physical, chemical and biological activities in water body (Huq and Alam, 2005). The DO in ponds of Astia with human interference was more followed by ponds of site-1 and site-2 near cultivated area and forest area. In the study DO ranged from 4 to 6 mg/l, which is little less due to heavy influx of water during monsoon. Despite this it supported the aquatic organism due to heavy run-off from catchment area (Table-11; Fig-7).

ALKALINITY

Generally alkalinity of water is caused by ions like bicarbonate, carbonate and hydroxyl ions. The appropriate range of alkalinity supports the growth of aquatic organism properly. In my study the water of ponds in Astia showed higher alkalinity i.e.70.76 and 69.65, followed by other ponds due to anthropogenic activity (Table-11; Fig-8).

HARDNESS

Hardness of water depends on the dissolved solids and pH. Hardness gives a measure of the total concentration of the divalent metallic cations like calcium, magnesium etc. The hardness in the ponds of site-3 was found to be little more than other two areas. But it has no negative impact on growth and metamorphosis of tadpoles (Table-11; Fig-8).

pH

It is an important parameter of water, which plays an important role in the growth of aquatic organisms. The sites from where the water was sampled were not urbanized or industrialized. So the pH was maintained in the range 6.5 to 7.3 (Table-11; Fig-8).

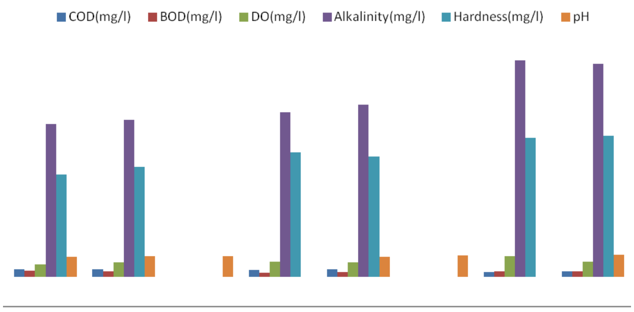
DISCUSSION:

Being a seasonal breeder, *P. maculatus* breeds in temporary ponds and other water bodies, which are filled by rain water during monsoon. Usually the larval life of this species is completed in 40-42 days. Relatively short period of development is characteristics of tropical species, which have to take advantage of transitional aquatic habitats during the monsoon (Heyer 1973). This stipulated period of time allows the tadpoles to metamorphose quickly and escape desiccation as the temporary ponds dry up. Mouth plays the most significant role in feeding behaviour of the tadpoles. The diversity of oral parts utilises diverse food items by different tadpoles. So the knowledge of the oral parts of the tadpoles is very important to access the diverse feeding ecology. The

Table- 11: Chemical parameters of water in the study region.

Chemical parameters	Bangiriposi		Kostha		Astia	
	Pond 1	Pond 2	Pond 1	Pond 2	Pond 1	Pond 2
COD(mg/l)	2.57	2.61	2.34	2.45	1.56	2.44
BOD(mg/l)	2.01	1.95	1.34	1.72	1.89	1.74
DO(mg/l)	4.05	4.89	5.01	4.78	6.90	5.08
Alkalinity(mg/l)	50	51.39	53.89	56.42	70.76	69.65
Hardness(mg/l)	33.53	36.05	40.65	39.45	45.46	46.23
pH	6.5	6.9	6.8	6.7	7.1	7.3

Fig 8: Chemical parameters of water in the study region.



present study on oral structure of *P.maculatus* showed labial teeth row formula as 4(2-3)/3(1). Altig (2007) reported that a labial tooth row formula (LTRF) 2/3 is the most common configuration, but the number varies from 0/0 –17/21 in many combinations throughout Anura. Variation in oral morphology of anuran tadpoles shows that the structure of the mouth and buccal cavities of anuran larvae are highly adaptive and correlated with the feeding ecology.

In the present study, the 15 number of *P.maculatus* tadpoles were taken for gut content analysis. Before analysis, the morphometric parameters like TL, BL, BW were observed. The ecology of tadpoles is related to their morphology and to the process of metamorphosis (Wassersug, 1975: Wilbur, 1980). Tadpole size can also influence community organization (Alford and Crump, 1982). The parameters were important factors for accessing the growth of these tadpoles in particular habitats. There were no significant changes in growth from three sampling sites. This showed the equitability and uniformity of ecology of habitats.

The NF% and FO% values of each genus of four individual classes are positively correlated. This shows the species abundance and species richness in the studied habitats. The diet preference and choice of algae as food indicates that the conservation of habitat in terms of algal diversity is essential for the survival and completion of life cycle of the tadpoles and for successful survival of anurans. Qualitative analyses of food spectrum of five species of anuran tadpoles (*Bufo malanostictus, Rhacophorus maximus, Amolops afghanus, Rana danieli* and *Euphlyctis cyanophlyctis*) from Arunachal Pradesh, India by Sinha *et al*., (2001) recorded the presence of Diatoms and Chlorophyta in all the five species which was also seen in the case of *Pmaculatus*used in the present study. Bacillariophyta are the principal food found in the gut of *Polypedates maculatus*. Most diatoms belong to the periphyton community indicating that the tadpoles of the first cluster mainly feed diatoms as a consequence of occupying the same microhabitat. However, microhabitat partitioning alone does not fully explain the two clusters, because *Phrynohyas venulosa* tadpoles, although occurs in midwater, presented a different diet compared to the remaining tadpoles that occupied the same microhabitat. *P. venulosa* tadpoles ingested *Trachelomonas*, a planktonic alga, whereas *Scinax fuscovarius* and *S. similis* tadpoles scrape surfaces for food, a fact which explains the large amounts of *Oedogonium* – a species that grows on leaves of aquatic macrophytes and other plants (Prescott, 1984) in its diet. *Leptodactylus fuscus* tadpoles, despite being typical bottom dwellers (Altig and Johnston 1986), consumed a diet that differed from those of all other tadpoles. These data demonstrate that food partitioning is related not only to the occupation of different microhabitats, but also to the feeding behaviour of the tadpoles. During the early stages of feeding, tadpoles feed mostly on detritus and plant materials and during the later stages of feeding they consumed both phytoplankton and zooplankton.

The present study of gut analysis showed 4 classes of microalgae represented by 39 genera and considerable amount of detritus. The detritus packed along the length of intestine is indicative of its habitat as a benthic detritus feeder which was also observed by Das (1996) and Khan (1999). Although the class bcillariophyceae and chlorophyceae were represented by 16 genera each, bacillariophyceae had more NF% and FO% followed by chlorophyceae. But euglenophyceae and cyanophyceae showed little less diversity. On the whole, the gut content of *P. maculatus* tadpoles showed the remarkable and noticeable food diversity.

There was no algal bloom in the tadpole habitats during the study period, as none of the specific algal variety is dominating the others. Hence, the habitat of tadpoles was good for larval development. The relative frequency is a very important factor. The relative frequency was as high as 0.41 in case of chlorophyceae and lowest for euglenophyceae (0.08). The following table (Table-6) made the statement more clear.

Water is one of the major requirements of amphibian's biphasic life cycle, because the amphibian reproduction and tadpole development is completed in water. Hence, physic-chemical parameters of tadpole habitats should be optimum for growth of food items. Phytoplanktons are main feeding constituent of tadpoles and these also depend upon certain consistent water quality for their survival. Factors supporting the phytoplankton growth are very complex and interaction between chemical and physical factors such as dissolved oxygen, temperature, visibility and the availability of nutrients, nitrogen and phosphorus (Goldman and Horne, 19830). Temperature increase should lead to greater rates and biomass accumulation under adequate resource supply (Padilla-Gamino and Carpenter, 2007). The present study on water parameter showed no such distinct abnormality indicating a polluted habitat that may affect the tadpoles.

CONCLUSION:

Metamorphosis is one of the most entertaining, peculiar events in the nature's creation and proceedings. Although the basic process is known, still many minute and sensitive changes and factors are to be analysed and discovered. These minuscule factors when analysed sincerely, vividly with the scientific approach the results are really interesting and hence provides a handful of data that can be announced as the new findings on that field of research. Rainy season is the most suitable time of egg-laying and metamorphosis since there is better chances of survival and successful metamorphosis. Different types of water bodies like permanent and temporary ponds are the main hub of the metamorphosis of larvae and their development. Rehydrated and reenergized phytoplankton, bacteria and fungi also find their way to multiply and spread in the water bodies after such a long adverse condition of summer that restricted them to grow and reproduce. As these microbes high up their numbers, they serve themselves as the regular and sufficient food source for the developing tadpole larvae. focus of the study was the analysis of qualitative and quantitative analysis food preference and feeding habit of *Polypedates maculatus* tadpoles which is also supported by the data on physico-chemical parameters of water.

The present study revealed that the food diversity of tadpoles including various microalgae were mainly from four classes i.e. Bacillariophyceae, Chlorophyceae, Cyanophyceae and Euglenophyceae. The huge diversity of algal fauna indicated the high level of nutrients consisting of dead leaves, plant fragments etc. The diversity in terms of species richness and abundance of food indicates broader niche breadth for these tadpoles.
Though amphibians are leading a biphasic life, water is the basic need for their early larval development. Within the short period of time the tadpoles have to be metamorphosed by utilizing the ample source of nutrients in water and escaping from desiccation. The physical parameters like turbidity, temperature, pH, DO etc. and chemical parameters like BOD, COD, etc. were supported the algal growth suitable for tadpoles.

It can be concluded that tadpoles are selective in their food preference when there is abundance of food variety i.e.*closterium, cosmarium, ankitrodesmus, chlamydomonas and cyclotella, fragillaria, navicula, cymbellaclosterium, cosmarium* etc.. The rainy season brings the buffet for them to eat on varieties of algae and plant residues. This ensures all the nutrient availability for the growth and development of tadpoles which leads to the successful attendance of mature stage. End of metamorphosis is only the beginning of a new tough job and target for breeding. Though the feeding habit completely changes in next phase of life, but still the nutritious feed during the larval development help them strengthen themselves overcome the hurdles during larval stage. And this particular habit and experience is transferred to the next progeny by the gene for the survival of race. Once again, the life of anurans follows the rule "Breed to free", "free to feed" and "feed more to be free".

ACKNOWLEDGEMENT:

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Pond-1

Pond-2

Plate.1: Sampling site near Bangiriposi area, Baripada (21º58'.353"N-086º46'.978"E)

Pond-1

Pond-2

Plate.2: Sampling site near Kostha area, Baripada (21º56'.759"N-086º47'.517"E)

Pond-1

Pond-2

Plate.3: Sampling site near Astia area, Baripada (21º58'.949"N-086º46'.978"E)

Plate.4-Morphology of *P. maculatus*

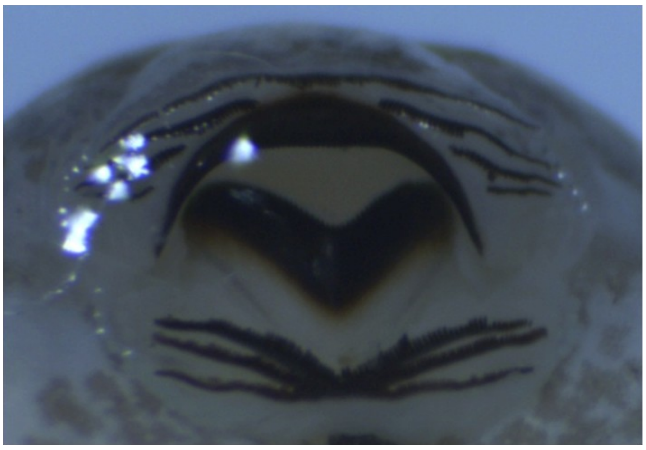


Plate.5- Oral structure of *P. maculatus*

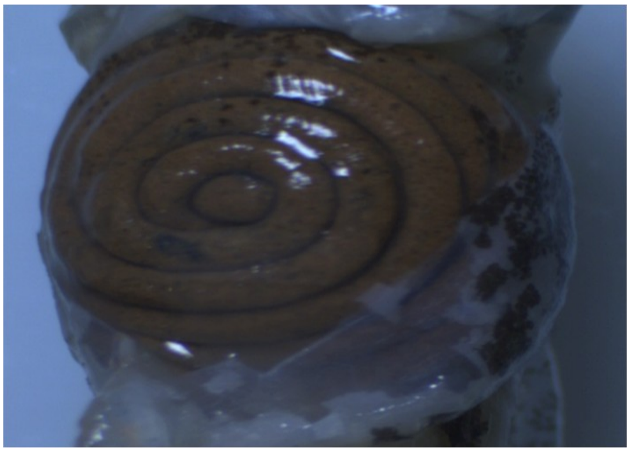


Plate.6: Coiled gut of *P.maculatus*

6a. Measurement of length of dissected gut of *P.maculatus*

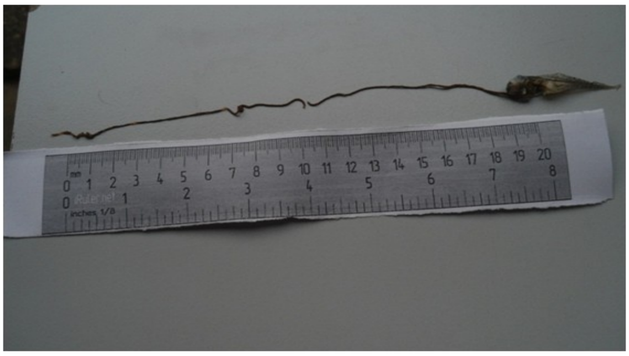
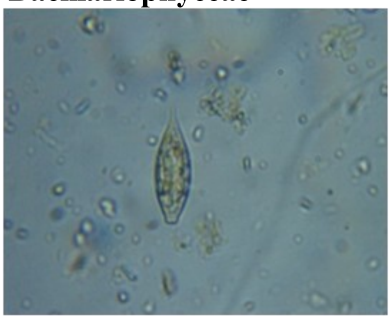


Plate 7: Image of algal species, observed under microscope from gut food remaining

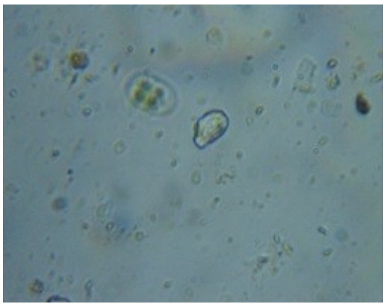
Bacillariophyceae

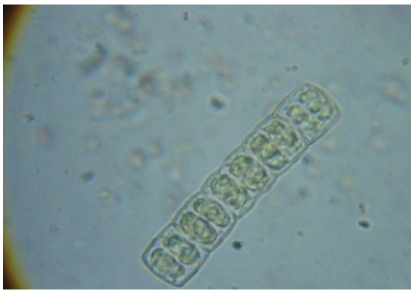


Amphipleura

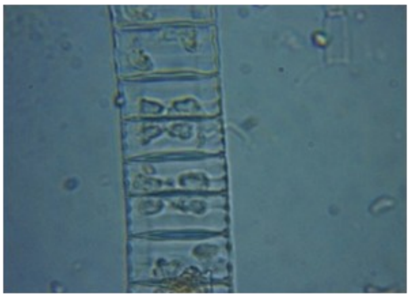
Navicula

Asterionella

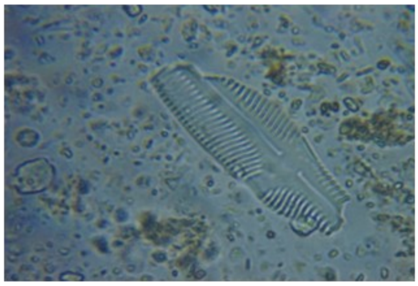

Cocconeis

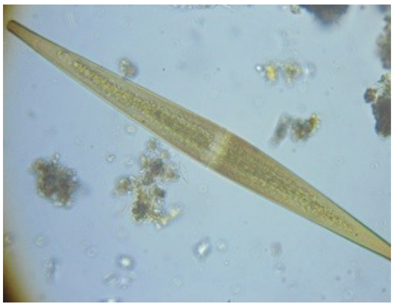

Aulacoseira


Cymbella

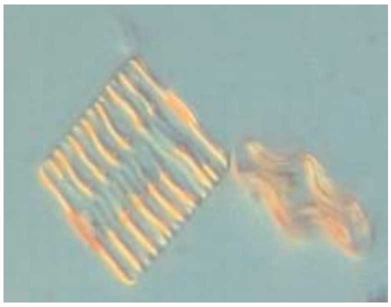

Craticula

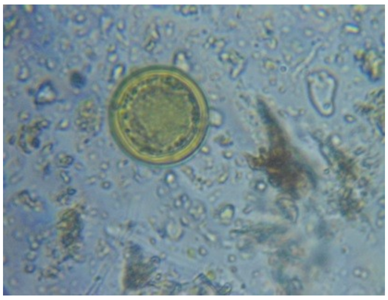

Diadesmis

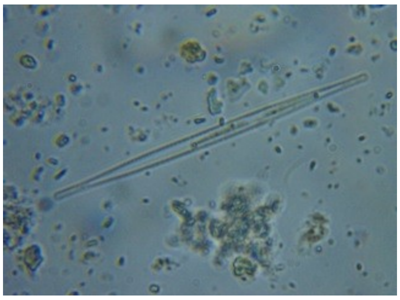

Gomphonema


Gyrosigma


Pinnularia


Tabellaria

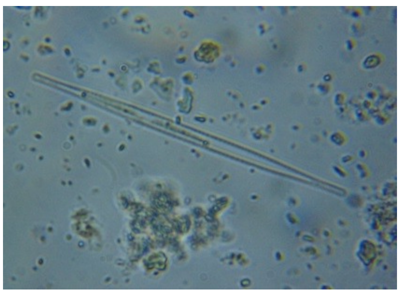

Cyclotella


Nitzschia


Diatoma

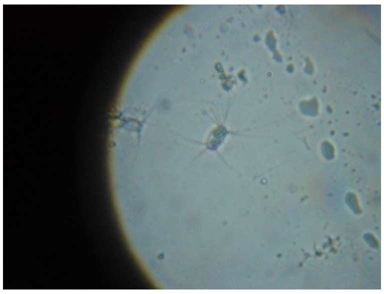
Chlorophyceae:


Actinastrum

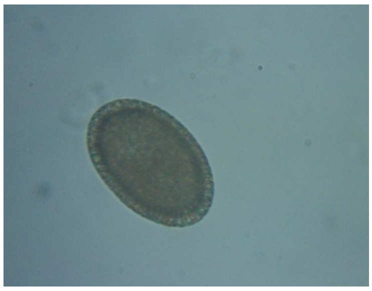

Ankistrodesmus


Closterium

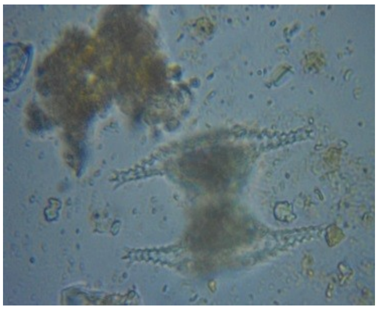
Cosmarium



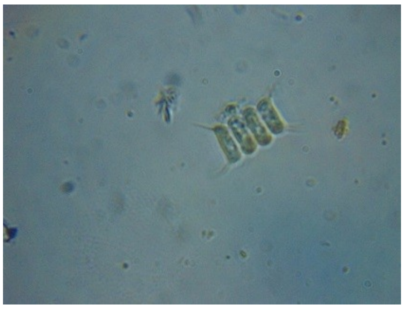
Lagerheimia



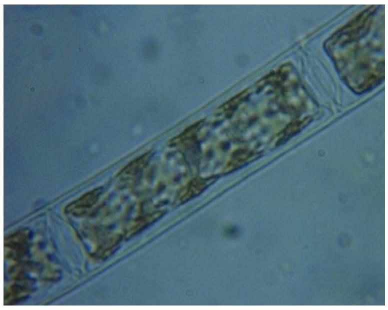
Oocystis



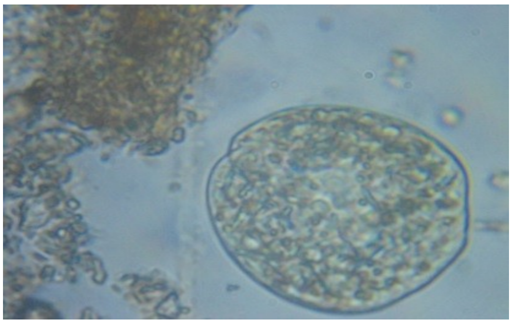
Staurastrum



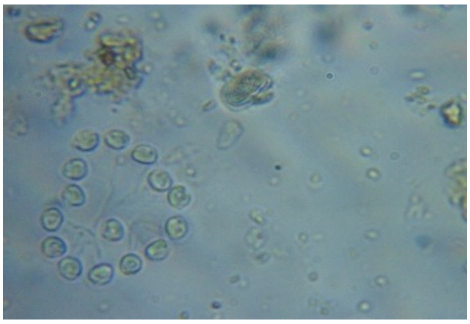
Scenedesmus



Spirogyra

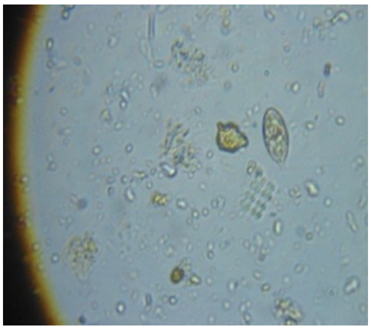


Micrasterias

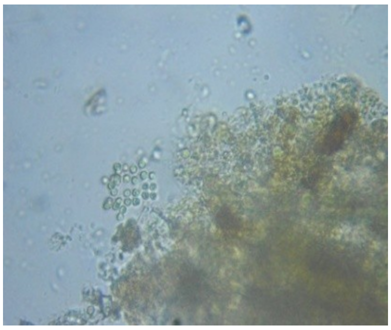


Micractinium

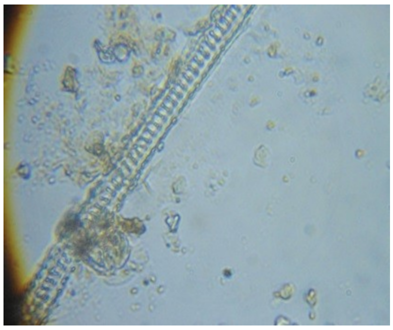
Cyanophyceae:



Merismopedia

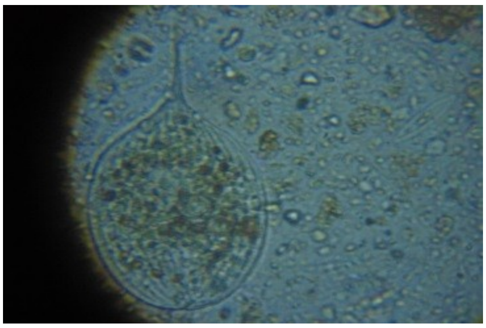


Microcystis

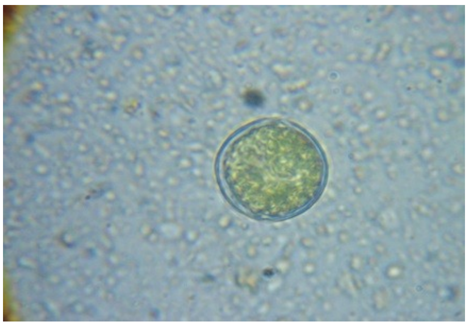


Oscillatoria

Euglenophyceae:



Phacus



Trachelomonas

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