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PRANIKEE

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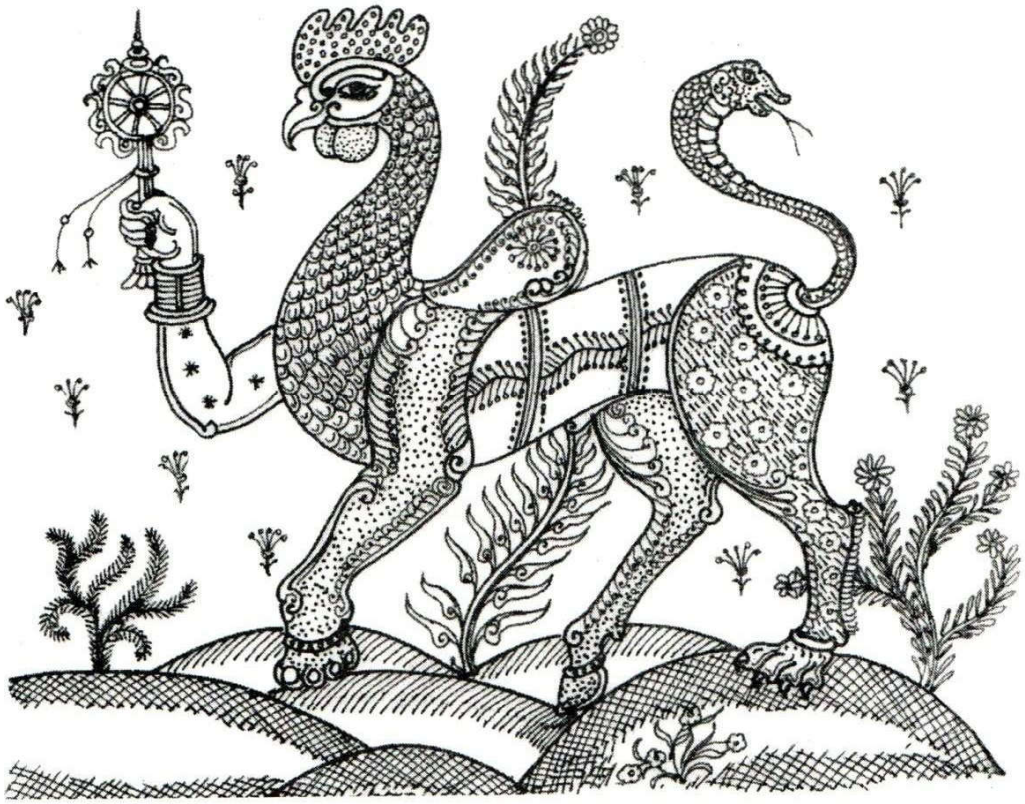

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PRANIKEE

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The emblem of Pranikee

The emblem "***NABAGUNJARA***" is a chimeric animal and a common motif of Odishan art and literature. It literally means "Nine form". This form has been described by poet Sarala Das in the Odia version of the epic Mahabharata. Apparently, Lord Krishna appeared in Nabagunjara form consisting of the body of an elephant, a leg each of a horse, a deer and a tiger respectively; throat of a peacock, tail in the form of a serpent, waist of a lion, hump of a bull and head of a cock, to fool his friend Arjuna. The Chimera was holding a lotus flower in a human hand. Arjuna had never seen such a creature in his life and guessed that this could not be a real animal but a form assumed by Lord Krishna and immediately bowed down at his feet. It is said that the human hand with the lotus provided the clue. In the paintings and sculptures however, the lotus is often replaced by a "Chakra" or the "stylized discus" of Lord Krishna. Chimeric forms are encountered in literature and art all over the world. However, a chimera of nine animals is uniquely Odishan. Therefore, it was considered to be an appropriate emblem for the Journal of Zoological Society of Odisha.

Padma Shri Prof. Priyambada Mohanty-Hejmadi

Former Editor

From the Editor's desk

The present edition of the Journal (Volume XXXIII) carries five research articles which cover different aspects of Zoology. The role of news coverage on protection of wildlife has been represented in the first article. The second article covers species diversity, abundance and conservation of cetacean in the state of Odisha. Besides, research papers entitled antifungal activity of phytochemicals from freshwater microalgae; melanophores during tail regeneration in the Indian tree frog, *Polypedates maculatus*; and aquatic habitats and nesting preference of anuran larvae in and around Bhubaneswar, Odisha, India have been described. I believe that this volume will contribute to the expanding knowledge in Zoology.

PK Mahapatra

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CONTENT

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NEWS COVERAGE ON WILDLIFE IN LOCAL NEWSPAPERS OF MEGHALAYA

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ABSTRACT

Newspaper plays a vital role in creating awareness. The news coverage on different aspects of wildlife and its conservation are studied by selecting an English daily and a Khasi daily, namely The Shillong Times and U Mawphor respectively. It is observed that the U Mawphor, the Khasi daily has given higher priority to local news and news on education while the national and international news are prioritized by The Shillong Times. The local print media needs to give more coverage to wildlife news to create sense of responsibility towards wildlife and its protection among the citizen of Meghalaya

Keywords: Newspaper, wildlife, awareness, Meghalaya

INTRODUCTION

The northeast India is known to the entire world as a rich treasure of natural resources from time immemorial. The British when ruled India followed one agendum: to collect and supply materials of Botany, as well as Zoology and Mineralogy from the territory of the East India Company (Grove, 1998; Bastin, 1981). This tradition of exploring and extraction of resources are still in practice. As a consequence, there occurs a large depletion of forest and other natural resources. IUCN recognizes the importance of sensitizing people in a way so as to minimize the stresses on biodiversity. The article 13, Convention on Biological Diversity also gives emphasis on "Promote and encourage understanding of the importance of, and the measures required for, the conservation of biological diversity, as well as its propagation through media, and the inclusion of these topics in educational programmes" (Secretariat of the Convention on Biological Diversity, 2005). In this regard, newspapers can play an important role in shaping a civilized society. Saikia (2017) opined that mass media functions in providing vocational and professional information and spreading awareness and civic responsibility. Loker et al. (1999) stated that the readers obtained information on species in New York State and the residents were more worried about nuisance, damage, and health/safety issues. The media serves as direct forum for public dialogue on sprawl and reporting on arguments occurring in all other forums (Bengston et al., 2005).

Out of 61 newspapers circulated in the state of Meghalaya, Khasi language (21 number) and English language (18 number) newspapers dominate the scenario (Sonowal, 2014). The literacy rate of the state (74.43%, as per 2011 census) is suggestive of reaching large section of people through print media.

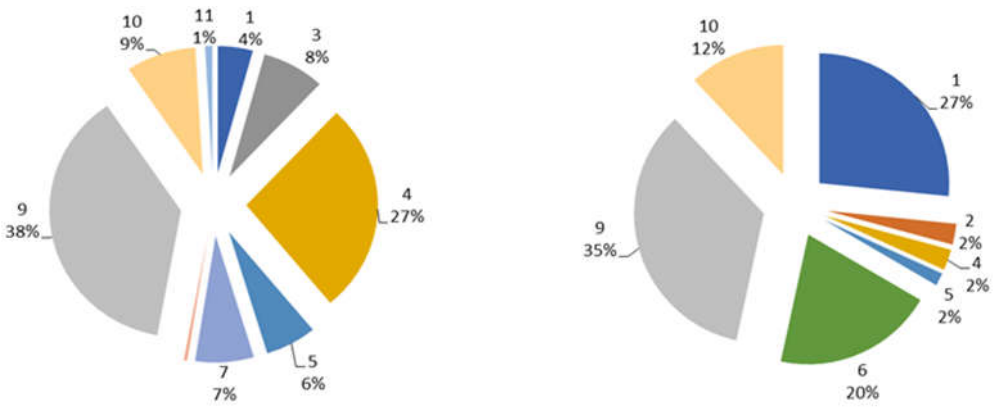
Newspaper, can act an active role in alerting people about environmental damages, corporate failure to meet its legal obligations, truthful analysis of new legislations, steps to protection and preservation of environment and in forming the positive attitudes of the people towards the environment and wildlife (Kushwaha, 2015). The present investigation describes the print media coverage of news related to wildlife conservation and management in Meghalaya.

MATERIALS & METHODS

Based on circulation two daily newspaper one English, The Shillong Times, and another Khasi, U Mawphor, were selected for the study. Following research questions were set for the study:
i. How much importance is given to different wildlife news?
ii. What are the different categories of news covered?
iii. Which category news is given more importance?
iv. How the newspapers try to generate awareness on conservation amongst the readers?
v. Do they publish international news on wildlife?
The data collected for content analysis are printed press articles published in the selected local daily newspapers over two years (2016 through 2018). News items on wildlife, appeared on Monday and Friday of a week during the study period have been covered. The frequency of the news and pagination were recorded. News on wildlife was categorized as: Conservation reliant, Trade, Death, Entertainment, Education, Conflict, Crime, Discovery, Showing affection and Achievement.

RESULTS

In both the newspapers highest number of news related to environment and wildlife are featured in page number 9. These are 37.6% and 34.6% of the total wildlife news in The Shillong Times and U Mawphor respectively. The said category of news was given much importance in the Khasi daily (U Mawphor) then the English daily, by placing them more in the front page to attract attention of the readers. It appeared that except placing important news in the front page, the environment and wildlife news were placed arbitrarily without any page preference (Fig 1). Figures 2-16 represent some news items published during the study period.



a. The Shillong Times b. U Mawphor

Fig. 1 Frequency of publication of news on wildlife in different pages of two local dailies of Meghalaya

The U Mawphor had given much stress on education both in term of frequency of appearance space covered. News on wildlife crime was covered by both language dailies. Conflict, death and discovery news were given importance in both the dailies (Table 1).

Table 1 Frequency of publication of different categories of wildlife related news in two local dailies of Meghalaya

Categories	The Shillong Times (in %)		U Mawphor (in %)	
	Coverage (%)	Covered (cm^2)	Coverage (%)	Covered (cm^2)
Conservation	13.7	49.33	4.0	124.6
Trade	4.4	101.8	9.3	179.63
Death	9.8	149.01	8.0	201.84
Entertainment	5.8	123.18	2.6	174
Education	7.8	214.36	29.3	225.1
Conflict	8.3	93	12.0	220.87
Crime	13.7	85.57	20	120.07
Discovery	6.8	129.08	5.3	17.17
Affection	2.4	82.8	0	0
Achievement	1.4	49.33	0	0
Rescue	2.4	130.4	0	0
Zoo	7.8	111.9	0	0
Miscellaneous	14.2	98.18	6.60	109.12

The Shillong Times paid less attention to the local news on wildlife and higher priority was given to national and international news. On the contrary, national and local environmental news were given privileged over the international news in U Mawphor. Further, the U Mawphor published more news supported by related photographs to spread awareness effectively among the readers. In Shillong times only news without photographs were published. Wild animals were addressed more frequently as compared to domesticated and cultured animals in both newspapers. Coverage on protected areas remained minimum in both newspapers (Table 2).

Table 2 Categories of coverage and types of news published in two local dailies of Meghalaya.

Category/Type	The Shillong Times (in %)	U Mawphor (in %)
Local news	13.23	35.66
National News	39.70	44
International News	47.07	20.34
Only photograph	20.23	2.60
News article with photograph	14.58	64.00
News article without photograph	65.19	33.40
News related to domesticated/ cultured animals	40.25	62.56
News related to wild animals	56.60	25.54
News related to Protected areas	3.15	1.9

DISCUSSION

The Indian print media mostly reported large, endangered, terrestrial mammals (Lyngdoh et al., 2017) and often ignored small and non-charismatic animals (Sharma, 2019). More than half of the wildlife reports by the dailies are on tigers, elephants, rhino and other fascinating and iconic species as compared to the domesticated and culturable animal species. Present observation is in conformity with earlier workers. In the study it is also noted that news on protected areas are very scanty. Narayan and Sekhsaria (2019) studied media reporting on the Protected areas in Maharashtra and found out that the news coverage was biased towards two protected areas, namely Sanjay Gandhi National Park and Tadoba- Andhari Tiger Reserve and a third of the protected areas were not reported at all.

KHYNDIAT HALOR KI JINGSHISHA

Ki Dodo ki dei ki jait sim ki bym lah her

Dodo (Raphus cucullatus) ki de kita ki jait sim ba khlem sner bad kiba im ha ka dewlynnong Mauritius jong ka duriaw bah Indian Ocean. Kine ki sim ki don ïa ka jingjrong ba kumba shi meter lane 3.3 phut bad khia kumba 20 kilo. Kine ki jait sim ki duh noh ïa ka bor ban her namar ka bam na ka bynta jong ki ka pahuh bha bad kita ki mrad ba thombor ïa ki kim don ha Mauritius. Kine ki sim ki don ïa ka jingïadei bad ki lyngdkhur bad paro bad kiba don jingïadei jan eh ki dei ki Rodrigues Solitaire, kiba long ruh kita ki jait sim ba la duh jait. Kine ki sim dodo ruh mynta ki la duh jait bad ia ka jinglong ka dur ka dar jong ki la tip tang na kita ki jingdro bad ki jingthoh ba don naduh ka spah snem ba 17. Tangba kine ki jingdro ruh ki ïapher kawei na kawei pat bad tang khyndiat eh ki dur la dro bad ai rong shaphang jong ki.

Ïa kine ki sim Dodo nyngkong eh la kdew da ki nongniah lieng Netherland ha ka snem 1598. Ha ka snem 1681, baroh ki dodo ki la shah pynïap ha kita ki nongñiah lieng ba tngan ban bam doh bad ha kita ki jingri jong ki. ïa kane ym shym la sngewthuh ha kito ki por, naba ym shym la ïohi shuh satia ïa ki hadien ba la duh jait bad wat hadien ba la duh jait ka jingngeit tang ha ki jingmutdur ba kine ki sim ki dang don haduh ka spah snem ba 19 haba la pynlong ïa ka jingwad bniah halor ki katto katne ki jait sim ba dang im ïa kiba la rah shapoh Europe ha ka spah snem ba 17.

Figure 2

Kmen ki nongshong shnong ba ïoh kem ïa ki shrieh kiba ju thombor kum ki lehnoh

Barpeta, K'lyngkot: Shi kynhun ki shrieh khlaw kiba la kylla long ki nongpynthut bad ju hiarthma kum ki lehnoh ïa ki nongshong shnong ha kylleng ki bynta ka noogbah Barpeta Town la ioh kem da ki nongshong shnong ha ka step ingi Saitjain ba lalah.

Ki nongshong shnong ka thaiñ ki la ong ba kane ka kynhun ki shnrieh ki ju pyathut, hiarthma bad pynher synglew ïa ki nongshong shnong ba kiba bun ki lang ym tang ïa karep ka riang, ki lingki sem bad kiwei ki jingdon jingem hynrei kaba buh jingma ruh ia ka jingim briew khunlam ki khynnah.

Kumta ka kynhun ki shrieh ha kane ka nongbah ïa suba jur ba ki la wan hiarthma hadien ba ki la lait phet na knao kna ka iing ba la longtrai nds mano ba la ri ïa kine ki shrieh. Naduh ba la wan pot kane ka kynhun ki shrieh, ka la long kum ka jingshah shah pyndik, pynjynjar kum ki lehnoh ia ki nongshong shnong

Ka kynhun ki shrieh ba ju thombor bad pyndik ïa ki nongshong shnong ha ka thaiñ ha Barpeta kiba la ïoh kem noh da ki nongshong shnong

ha kylleng ki bynta jong ka thaiñ ha nongbah Barpeta.

Kine ki shrieh hadien ba ki la lait phet na kano knao ka iing ba la ri bad longtrai ia ki, ki ju pynjynjar bha ïa ki nongshong shnong ban tuh ïa ki jingbam su ki ting briew bad pynjurlor ruh ïa ki jingdon jingem ki ting briew bad wat ban thombor pyamynsew kynthup ka jingshah dait ki briew hapoh shnong bad haba ki leit ki wan sha ki kam ki jam jongki.

Bun ki briew la kylleng ki bynta jong ka nongbah ki ju myntaw ha ki kam thombor da kine ki shrieh.

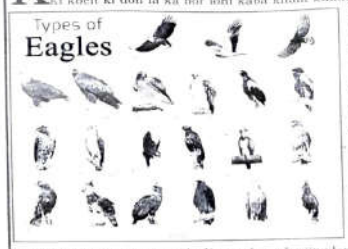
Ki briew ka tnat khlaw ki ju tai pyrtang ban ioh kem ïa ki hynrei baroh ki jingpyrshang jongki jam manla sngi na ka por sha ka por ym shym la seisoh satia. Ha kaba ka timna bat ioh kem ïa kine ki shrieh da ki briew ka tnat khlaw ki shah jop-ha kine ki shrieh

Figure 3

KHYNDIAT HALOR KI JINGSHISHA

Shaphang ki kbeit ki kynja sim ki ba don ïa ka bor ba kyrpang

Ki kbeit ki dei ki jait sim kiba heh bad ki dei na kajuh ka longiing heterogeneous jong ki sim. kynthup ta ki pukni. Ki kbeit ki don ïa ka bor iohi kaba khami khlain. tangba ki don pat ïa ki ajat kaba ksah bad ki sla kjat kiba khlain haduh katta katta. Ki don ïa ka shyntur kaba nep bha bad long hnoh kaba ki pyndonkam na ka bynta ban dait bad ban patar ïa ka doh. Namar kane ka jingdon ia ki tyrsim bad ka shyntur kaba nep. ki kbeit ki lah ruh ban kem in kiwei pat ki mrad kiba ki bam wat ha ka por ba ki her. Ka thliew khmut jong ki ka don haneng jong ka shyntur ha ka bynta ba long doh. Ki kbeit ki long kiba sted bha ban her bad ki lah ban kot ha ka jingsted kaba haduh palat ia ka 150 mer ha ka shi kynta ha ka por ba ki suh khlieh na suin sha tbian. Ki don haduh palat ia ka 270 jait ki kbeit bapher bapher ba kynthup ha kane ka longiing jong ki bad 25 jait na kine ki kbeit lah ban shem hapoh ri US. Kine ki jait kbeit ki ïapher ha ka jingheh bad ka rukom long bad ki don kiba rit bad kiba khia tang 4 ounce haduh kiba heh bad khia haduh 52 ounces.



Ki kbeit ki ju im bad her tang ha kajuh ka jaka ne u pud u sam man la u snem wat lada ki lah ban don katto katne ki jaka skum ba ki dem. Ki ju bujli ïa kane ka jaka sah jong ki na kawei ka skum ha kawei pat man la u snem. Ki kbeit ba don ia u tdong rong saw ne kita ki Red-Tailed Hawk barabor ki long kiba ju shah pyndik bha ha kiwei kiwei pat ki jait sim, kum ha ki dihoh, ki tyngab, simsong bad wat ha kiwei kiwei pat ki jait kbeit. Ki kbeit Rough-legged Hawk pat la tip ba ki long kiwei na ki jait kbeit ba heh duh. Kine ki jait kbeit ki don ïa ka jingjrong kaba haduh 22 inshi bad ka thapniang jong ki ka lah ban pyiar haduh palat ia ka 55 inshi.

Ha kiba bun ki khep, ki kbeit kynthei ki long kiba kham heh ban ïa ki kbeit shynrang. Ha kiwei kiwei ki jait kbeit, kum ki Sharp-shinned hawk, ki kbeit kynthei ki lah ban don ia ka jingkhia kaba haduh ar shah ban ïa u shynrang. Kumba long kiwei kiwei pat ki sim bam doh, ki kbeit ruh ki ju leit wad bam ne kthong ïa kiwei kiwei pat ki mrad ba ki bam ha ka por mynsngi. Ki kbeit ki don ïa kata ka bor iohi kaba khlain hha haduh katta katta bad ki lah ban iohi ïa kiei kiei ha ka jingjngai kaba phra shah na kaba u khumbynriew u lah ban iohi. Ym tang katta, ki kbeit ki long ruh kiba lah ban ithuh ïa ka rong.

Katba ki kbeit kiba heh ki lah ban kha pylleng tang kawei lane ar tylli ha ka shi snem, ki kbeit kiba kham rit par ki lah ban kha kumba lai haduh san tylli ki pylleng ha ka shi snem. Ka shim por kumba 3 haduh 6 taiew na ka bynta kine ki pylleng ba kin long khun. Ki kbeit ki lah ban im ha ka ryta kaba haduh 21 snem bad bunsien ki dei ki jait sim kiba bam doh da kaba bam ïa kiwei kiwei pat ki mrad bad ki ju bam ruh ïa ki metiap jong kiwei pat ki mrad. Bun na ki kbeit ki long kiba bam naphang kat kaba ki ioh ban kem. Tangba ka jingbam ba kongsan eh jong ki ka kynthup ia ki khniang, ki japih, jakeid, bseiñ, khnai, bsong, han, rabbit, bad kiwei kiwei pat ki sim.

Hadien ba la mih na ka sup pylleng, ki khun kbeit ki heh ki san kloi. Ki kbeit rit kum ki Kestrels bad ki Sharp-shinned ki lah ban heh pura tang hapoh ka shi bnai katba ki jait kbeit ba heh kum ki pukni ki shim por haduh 11 bnai ban heh pura. Ki kbeit ki kot ia ka jinglong sumla ne por shongicha ynda ki ia dap 2 snem. Ki im bad ki shong ki sah hapoh ki jaka khlaw, ki law bniap bad wat ha ki jaka ba long madan bad duna dieng. Ki kbeit ki shna ia ki skum jong ki da ki dieng bad ki synrum dieng. Ki kbeit bunsien ki ju slina ia ki skum jong ki ha kliar jong ki dieng ba jrong, lait noh tang ki jait kbeit kiba im ha ki law bniap kiba thaw skum ha madan.

Figure 4

Kem ki BSF ia u Nongkhaïi masi tuh, kurup 49 tylli ki masi

Shillong, Iaiong 03: Ki shipai Border Security Force (BSF) kila ïohkem ïa uwei u nongkhaïi masi tuh sha Bangladesh hynnin ka sngi na Lukaichar, South Garo Hils kaba la long ka jaka ba jur eh ka jingiakhaïi masi tuh mynta.

Une u Briew u kyrteng u Rubil Miya ba 35 snem ka rta uba dei na Mankachar District, Assam bad na une u briew ki shi pai kila ïoh kurup ïa 8 tylli ki masi.

Ha kawei pat ka jingjia ki BSF ba pahara ha Gopinathkilla, South West Garo Hills kila ïoh kurup ïa 41 tylli ki masi ba thmu ban khaïi tuh sha Bangladesh.

Ki shipai BSF kila pynjur bha ïa ka jingpahara hban khang lad ïa ka kam khaïi tuh na khappud khamtam ïa ka masi.

Figure 5

Four new frog species identified in Peru

Figure 6

Assam forest dept to destroy rhino horns, ivory stored in treasuries

From Our Special Correspondent

GUWAHATI, Aug 24: The Assam environment and forest department has decided to destroy the rhino horns, elephant tusks (ivory) and body parts of other protected animals stored in district treasuries.

The destruction of the horns and other animal articles, such as leopard and tiger skin, et al, would be in compliance with Section 39 of the Wildlife (Protection) Act, 1972.

However, those which are required as exhibits in court cases or for education, awareness and scientific purposes, would be preserved.

"Five percent horns, which are of unique character, would be preserved for education, awareness and scientific purposes," Assam chief wildlife warden, M.K. Yadava said in a statement.

A state-level committee has been constituted for the purpose while a public hearing is scheduled to be held on August 29, 2021 on the premises of Assam Forest School here in compliance with a Gauhati High Court order dated December 13, 2010.

The verification of the animal parts was done at treasuries in Morigaon, Barpeta, Mangaldai and Kamrup Metro between August 18 and August 23, 2021.

Of the 261 rhino horns verified so far, 241 have been marked for destruction and 18 for preservation.

Seven zonal committees and a technical panel constituted by the chief wildlife warden is conducting the exercise. The entire operation (destruction) would be screened live for public viewing and transparency.

The previous statewide counting and inspection of rhino horns was conducted in 2016. During the inspection, as many as 2,020 horns were found in 12 treasuries.

Figure 7

Pyllait ki nongtong dohkha ïa u Whale Shark ba sahkut ha ka jar

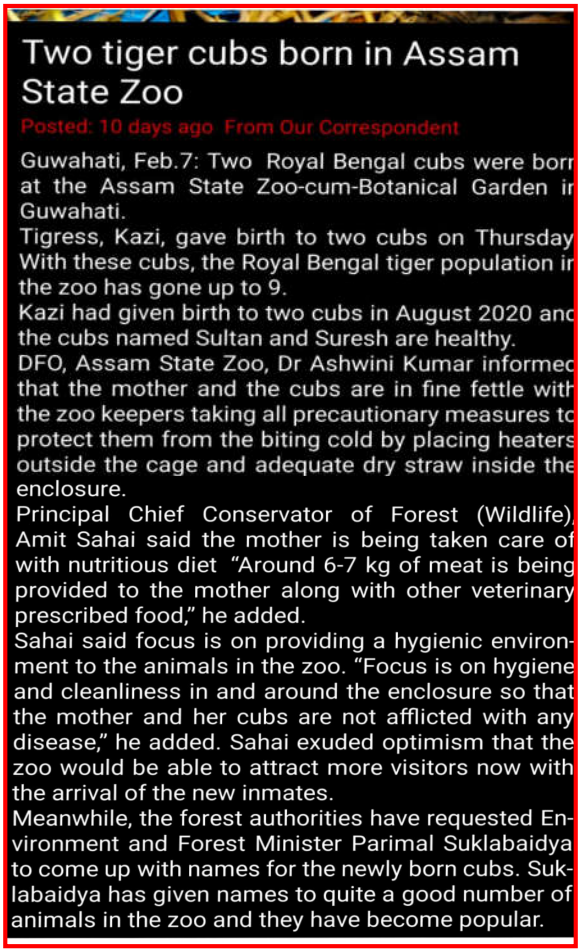
Ha ka jingjia kaba ym ju kham ïohi koit, kumba 60 ngut ki nongtong dohkha na Thiruvananthapuram ha Kerala ki la pyllait biang ïa u Whale Shark shapoh duriaw hadien ba u la ngat ha ka jar khwai jong ki. Hadien ba la mih ka jingmaham 'eriong, ki nongtong dohkha kim ïoh ban leit sha pdeng duriaw, bad ki la shu khwai noh tang najan. Ka dei ha kane ka por ba u Whale Shark u la ngat ha ka jar jong ki bad mar kumta hi ki la pyllait biang ïa u shapoh um.

"Ki don haduh kumba 60 ngut ki nongtong dohkha kiba la ïaleh shitom ban pyllait ïa une u Whale Shark. Ha kaba nyngkong ki pyrkhat bad une u Whale Shark un ym lah ban jngi, hynrei hadien arsien jong ki jingpyrshang une u Whale Shark u la lah ban jngi shapoh duriaw. Haba nga poi ha kane ka jaka, u Whale Shark u la sdang ban jngi. Ka long ka khyllipmat kaba kyrpang bha ïangi kiba ki la sakhi ïa kane," ong u Ajith Shanghmukham, uba la pyllait ïa kane ka video ha social media.

Tang hapdeng khyndiat minit la ïoh ban pyllait ïa une u dohkha heh hadien ba la ïoh kem ïa u, baroh 60 ngut ki nongtong dohkha ki la ïatreilang ban tar ïa ka jar khwai jong ki. Katkum ka jingïathuh u Ajith, u la ong ba une u Whale Shark ba la sdang duhjait uwei napdeng ki dohkha baheh tam ha ka pyrthei u khia la kumba 1,000 kg. Kum kane ka jingjia ka la jia ruh ha ki por ba ladep, tang kumba 2 km na rud duriaw Shanghumukham ha Thiruvananthapuram.

"U Whale Shark u dei u jait dohkha ba la sdang ban duhjait. Ngi la ïakren bad ki nongtong dohkha bad ai khublei ïa ki na ka bynta kaei kaba ki la leh. Na ka liang jong ka tnad Forest ruh kan sa ai buskit ïa ki ban ïaroh bad ai mynsiem ïa ki ban pyllait im ïa ki mrad um kiba sahkut ha ki jar khwai jong ki," ong u Shaji Jose, Range Officer.

Figure 8



Two tiger cubs born in Assam State Zoo

Posted: 10 days ago From Our Correspondent

Guwahati, Feb.7: Two Royal Bengal cubs were born at the Assam State Zoo-cum-Botanical Garden in Guwahati.

Tigress, Kazi, gave birth to two cubs on Thursday. With these cubs, the Royal Bengal tiger population in the zoo has gone up to 9.

Kazi had given birth to two cubs in August 2020 and the cubs named Sultan and Suresh are healthy.

DFO, Assam State Zoo, Dr Ashwini Kumar informed that the mother and the cubs are in fine fettle with the zoo keepers taking all precautionary measures to protect them from the biting cold by placing heaters outside the cage and adequate dry straw inside the enclosure.

Principal Chief Conservator of Forest (Wildlife), Amit Sahai said the mother is being taken care of with nutritious diet "Around 6-7 kg of meat is being provided to the mother along with other veterinary prescribed food," he added.

Sahai said focus is on providing a hygienic environment to the animals in the zoo. "Focus is on hygiene and cleanliness in and around the enclosure so that the mother and her cubs are not afflicted with any disease," he added. Sahai exuded optimism that the zoo would be able to attract more visitors now with the arrival of the new inmates.

Meanwhile, the forest authorities have requested Environment and Forest Minister Parimal Suklabaidya to come up with names for the newly born cubs. Suklabaidya has given names to quite a good number of animals in the zoo and they have become popular.

Figure 9

Lap ki stad wadbniah ba dang don ki Huleng ha ki khlaw Sonapyrdi

Khliehrait,Rymphang, 24: Ki stad wadbniah ka Ashoka Trust Research in Ecology and Environment (ATREE) ha ryngkat ka jingiatreilang bad ka Society of Rural and Urban Empowerment

Figure 10

235 rare turtles recovered from train coach by RPF at Kamakhya Station

Posted: 12 days ago From Our Correspondent

Guwahati, Feb 5: Railway Protection Force (RPF) personnel recovered an unclaimed consignment of 10 bags containing 235 rare turtle species from a train at Kamakhya Railway Station during a routine search operation this afternoon. The turtles were then handed over to Guwahati Forest Range office for rehabilitation.

The turtles were found by RPF personnel; Bhanita Barman Talukdar (ASI) and Head Constable Narayan Das when they were conducting search inside the coach number WR-201255 (Sleeper) of Train No. 19305 Up (Indore-KYQ) on the Platform No-4 of Kamakhya Railway station. The turtles were concealed in 10 unclaimed bags

The RPF ASI then handed over the turtles to Guwahati Range under Kamrup East Division for further investigation, rescue and rehabilitation. Accordingly, a wildlife case has been registered and same have been seized and taken to Assam State Zoo for treatment.

Figure 11

Lap ïa ka dkar ba phylla ha ri lum Garo

Figure 12

Figure 13

Animal welfare programme

Meghalaya's first Animal Welfare Volunteer Programme of the Stray Animal Rescue Society will be launched on July 15. The programme will be followed by orientation at Meghalaya Bharat Scouts and Guides Hall at 2 pm.

Figure 15

3 cattle smugglers, 2 poachers held in Bengal, Assam

From Our Correspondent

GUWAHATI: Three cattle smugglers and two poachers were arrested by the BSF from the Indo-Bangladesh border under Cooch Behar in West Bengal and Kokrajhar in Assam on Wednesday. The BSF troops deployed at the border outpost of Balabhut and Talai

three cattle smugglers identified as Md Sayeb Ali Sardar, Md Yusuf Ali of Assam and Manowar Hussain of Charbhasani in Dhubri along the international border when they were trying to escape to Bangladesh with 45 animals.

In another joint operation carried out on Wednesday, the BSF together with the Bagaribari Police and the Wildlife Crime Control Bureau apprehended two persons, Sarrar Ali and Anowar Ali Sheikh from Assam while they were negotiating the price of a Tokay Gecko (endangered wildlife species), which is worth of Rs 5 lakh. The arrested persons along with the seized species were handed over to Divisional Forest Officer of Parbajhora, Assam for investigation.

Figure 14

Ki hepri bad ka dkhoh *Dur Sheemi T Malngiang*

Shillong, Rymphang 07 : Lai ki hepri hynne ka step ki byrta jong ka Nongrah Nongpdeng.

Figure 16

The Assam Tribune gives a major focus on the conservation management (Sharma, 2019) and so also is The Shillong Times but crime issues were not emphasized. The Khasi newspaper, U Mawphor, however gives more emphasis on education. Lyngdoh et al., (2017) noted that the top five subjects in relation to wildlife in the Indian print media were tiger, issue/policy, birds, leopard and elephant. Similar trend is also found during the study in both the newspaper but items on canines and cattle were also featured heavily. Marukatat (1991) did a comparative content analysis of environmental news coverage in Time and The Weekly Review and found that both paid more attention to the problem of wildlife or wilderness conservation than other categories. There are similar findings and patterns about the coverage of wildlife from the present study in Meghalaya and others from the rest of the country. Meghalaya being a part the Indo-Burma biodiversity hotspot houses a number of endemic species. Hence, it is important for the media to relay the information and the necessity to conserve these species by encouraging awareness. Different animals that are portrayed indicate importance of the species in the fabrics of ecosystem and human welfare. It is also apparent that wildlife has faced serious threats over the years. The Khasi newspaper, U Mawphor has given more importance to local news than The Shillong Times. It can therefore be suggested that equal importance should be given by both English and Khasi local newspaper on local news, in order to inculcate the spirit or sense of awareness related to local fauna, among the readers of all tiers of the society. From the study conducted, it is evident that media does play a role in wildlife conservation but they have to be more proactive in dissemination of information and role of citizen in conservation. There is an increasing need to spread the concern for species conservation and reduction of conflict. Conflict seems to be one of the main causes of death among the animals especially elephants and leopard. Crime in Meghalaya shows an upward trend (Mawrie, 2018) especially when it comes to cattle smuggling (Das, 2020). Seeing the prevalence of

these issues, it is highly recommended that the local issues need to be addressed widely and should be given more emphasis particularly by the English newspaper for better coverage.

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AN OVERVIEW OF SPECIES DIVERSITY, ABUNDANCE AND CONSERVATION OF CETACEAN IN ODISHA

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ABSTRACT

Whales, dolphins and porpoises are purely aquatic mammals; collectively known as cetaceans. Most cetaceans are marine species, few are facultative and freshwater species. Cetaceans occupy the apex of the ecological pyramid and play a significant role in aquatic ecosystems. Study on Irrawaddy dolphin started in Odisha from 2002 and was extended to other cetacean species to evaluate their population in near-shore coastal waters and monitor mortality of various species in Odisha coast. The territorial water of Odisha provides shelter to 18 species of cetaceans (i.e., ≈ 56.3% of India's cetacean diversity) which includes 3 species of baleen whales, 14 species of toothed whales and dolphins and a single species of porpoise. Surveys in coastal Odisha documents eight cetacean species living within 5 Km of coastal water. The Shannon Diversity Index for cetaceans in Odisha ranges from 1.8 to 2.0 based on survey conditions from good to best in terms of visibility and Beaufort (Sea State Condition). The conservation issues discussed in the present paper include over-fishing, by-catch, pollution at any part of Indian coastline, collision, harassment, noise pollution, habitat loss and degradation. Despite several initiatives been undertaken by state, national and international level with participatory approach of local stakeholder, much more efforts are essential to balance the ecosystem services and ecological resilience with wise-use of resources for welfare of cetaceans.

Key Word: cetacean, dolphin, porpoise, species diversity, ecosystem services.

INTRODUCTION

Cetaceans are charismatic species alluring special attention of public, media and political interest (Hoyt, 2011). They are also considered as an indicator for the health and integrity of an ecosystem (Bowen 1997; Sergio et al., 2008; IOC, 2001; Moore 2008; Godard-Codding et al., 2011). The word 'Cetacea' (Latin- *cetus*, means "whale" or "large sea animal") and study of these aquatic mammals is known as 'Cetology'. Mammalian Order: Cetacea or Cetartiodactyla includes about 91 species representing marine and freshwater species consisting of whales, dolphins and porpoises. It includes two Sub-orders: Mysticeti (baleen whales) and Odontoceti (toothed whales, dolphins and porpoises). They evolved in early Cenozoic era and fully adapted to aquatic mode of life by profound modifications from early Tertiary to middle Eocene. These mammals have evolved from terrestrial ancestors to occupy aquatic niches and fully aquatic life histories (Thewissen et al., 2007). Their body is torpedo-shaped, neck indistinct, skin devoid of fur, covered by smooth cuticle, underneath blubber for insulation, fore-limbs transformed to paddle-shaped flippers, hind-limbs reduced to vestiges. Presence of numerous vertebrae serve as flexible backbone. Long muscles and tendons attached to the vertebrae run backward to the tail. The development of neomorphic horizontal tail (fluke) provides galloping-like propulsion and locomotion in water. Fleshy dorsal fin acts as upright rudder preventing rolling over. External nostrils or blow-hole on dorsal side of the skull has musculature flap acting as valves and close completely underwater.

Physiological adaptation for increased efficiency of respiration is achieved by highly elastic lungs. Ear lacks external pinnae and externally ear tube is reduced to pinhole. They are sensitive to underwater vibrations and noise that is transmitted through highly dense bone formed by a fusion of the periotic and auditory bulla. Sophisticated sonar apparatus is used for communication using modulated phonation. Whales use infrasonic sound while dolphins and porpoise use ultrasonic sound for communication, echolocation, habitat surveillance and navigation. Although considered highly intelligent animal for having highly developed brain but lack olfactory lobe and olfactory sense. They are carnivorous and feeding preferences are diverse. Baleen whales or true whales strain plankton, krill and anchovies (filter feeder). Toothed whales, dolphins and porpoises hunt upon larger preys. Young ones are well developed for their aquatic mode and capable of swimming with mothers and suckles milk. Cetaceans typically are top predators in the aquatic ecosystems and are recognized as ecologically important species (Hoyt 2011).

In the state of Odisha, India, the Chilika Development Authority (CDA) initiated project Irrawaddy dolphin in Chilika lagoon since 2002. Annual population estimation survey was undertaken following concurrent survey teams taking records of dolphin encounters on Line Transect Survey Method since 2003. This method is internationally accepted and has been validated adopting underwater acoustics survey. Near-shore coastal cetacean population research in Odisha including Chilika lagoon, Gahirmatha (Marine Sanctuary and National Park) and major river confluences was initiated in 2015 by the Wildlife Wing of Odisha, Forest Department to evaluate the total cetacean diversity and population in Odisha (Khan et al., 2015).

Ecological Significance of Cetaceans

Cetaceans play a variety of important roles as apex species in global aquatic ecosystem.

a. Help in sustaining fish stock by feeding on weak, ill or sick individuals allowing healthy stock to flourish. They cut-down infection and acts as cleaners.

b. Strike balance between zooplanktons-phytoplanktons by preventing planktonic bloom detrimental to aquatic health.

c. The cetaceans feed at different depth of water column on diverse species and cannot poop in deep water due to water pressure. They poop near the surface of the water and play an important role in nutrient cycle of an aquatic ecosystem. They provide nutrients like - iron, nitrogen and phosphorous to phytoplanktons that sequester hundreds of thousands of tons of carbon and produce O_2 helping to combat climate change.

d. Many cetaceans are migratory across latitude and deep divers that signify their role in re-distribution of nutrients.

e. Cetaceans act as biological pump to increase the overall health of the aquatic ecosystem by counteract stratification or biomixing.

f. Occurrence of cetacean population and diverse species are best indicator of the biodiversity hotspots. Chilika lagoon, Gahirmatha Marine Sanctuary and Bhitarkanika Sanctuary and National Park in Odisha are best examples. Sick, injured or dead cetaceans indicate that

something is wrong in their environment like – pollution from agriculture, residential or industrial runoff which may affect the safety of humans too (Khan, 2013).

g. Carcasses of cetaceans act as carbon-sink and assist to develop mini-ecosystem supporting about 200 marine organisms in sea-bed as well as on sea-beach.

h. Warns of illegal fishing activity and harmful human-made underwater noise in aquatic ecosystem. Help US and Russian Navy to locate underwater mines and trained to diffuse. They help to locate people lost at sea.

i. They support growing economy relying on cetacean watching activities through tourism.

Diversity of cetacean species in Odisha

Global cetacean diversity comprises of 91 species including 15 species of baleen whales, 26 species of toothed whales, 44 species of dolphins and 6 species of porpoises. India has 32 species of cetaceans (≈ 35.2% global cetacean diversity) which includes 6 species of baleen whale (≈ 40% global diversity of whales), 25 species of toothed whales and dolphins (≈ 35.7% of global diversity of toothed whales and dolphins) and a single species of porpoise (≈ 16.7% of global diversity of porpoise). The territorial water of Odisha provides shelter to 18 species of cetaceans (i.e., ≈ 56.3% of India's cetacean diversity) which includes 3 species of baleen whales, 14 species of toothed whales and dolphins and a single species of porpoise (Annandale, 1915; Khan et al., 2015)

Species Diversity, Abundance and Dominance

The diversity of cetaceans and its abundance in coastal and freshwater of Odisha; gives an empirical idea about the status of its freshwater and marine ecosystem, its species richness and support systems that sustain this diversified form of higher vertebrates. The Species Diversity or Shannon Diversity Index in real ecological data ranges from 1.5 to 3.5. The Shannon Diversity Index for cetaceans in Odisha ranges from 1.8 to 2.0 based on survey conditions from good to best in terms of visibility and Beaufort. The relative abundance or species evenness for different cetacean species is given in the Fig. 1.

Species Composition

The Irrawaddy dolphin prefers shelter bays to reside and does not share habitat with other near-shore coastal cetaceans, while the Finless porpoise prefers the river estuaries and very near-shore coastal waters. The Ganga River dolphin only resides in the freshwater of the river while often seen to venture into coastal waters when the salinity drops drastically to freshwater due to high flood in rivers.

The near-shore coastal population of Indo-Pacific Bottlenose dolphin or Indian Ocean Bottlenose dolphin, Indo-Pacific Humpback dolphin, Indian Ocean Humpback dolphin, Striped dolphin, Spinner dolphin and Spotted dolphin are sympatric population and are seen in large schools during group feeding or following a shoal of fishes (Figs. 3 to 9). Smaller groups of these species are resident while large group of 50 plus individuals are transient species and explore the near-shore coastal waters of Odisha and their abundance is variable (Table 1 and Fig. 2)

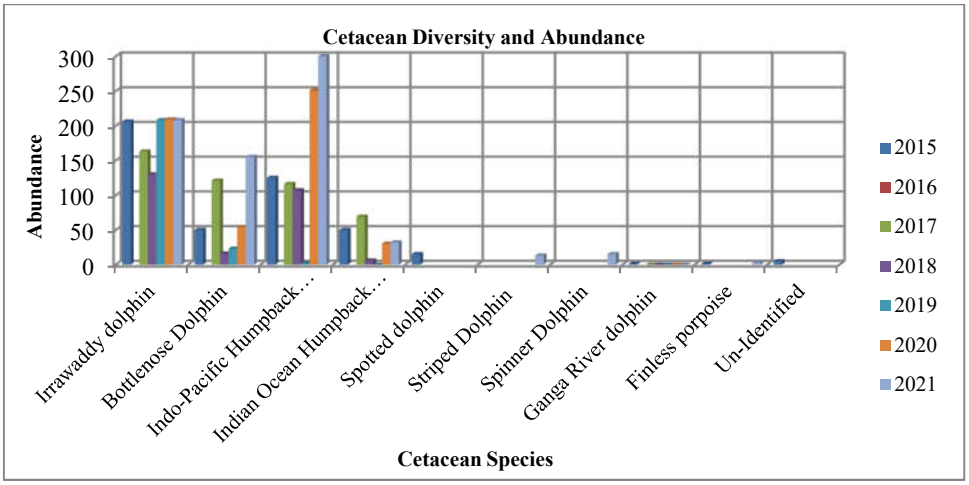

Fig. 1 Relative abundance of cetacean species

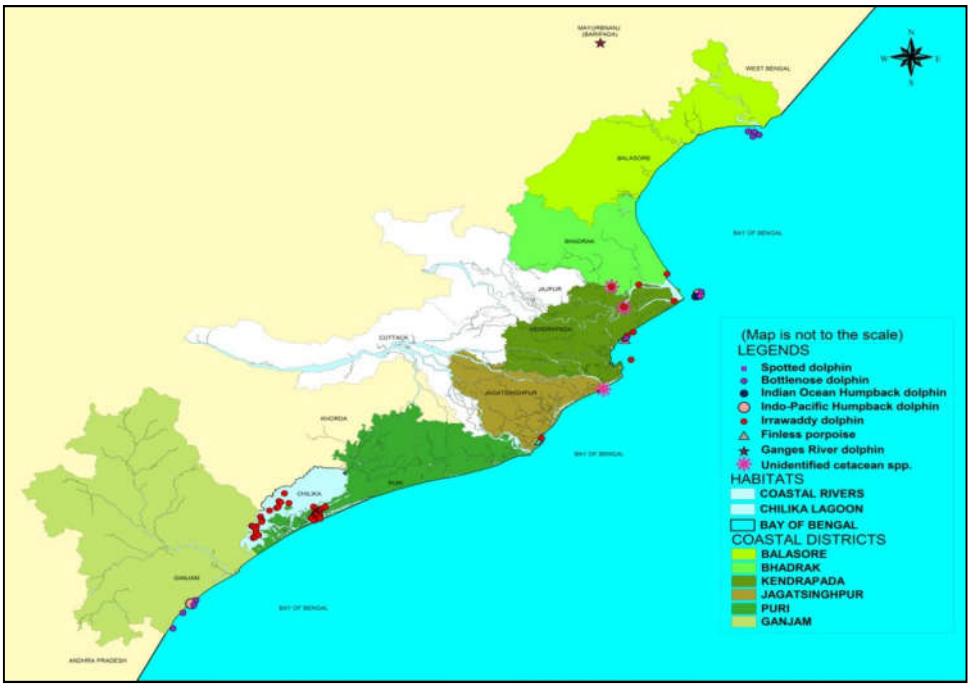

Fig. 2 Distribution of cetaceans in near-shore coastal waters of Odisha

Fig. 3 Bottlenose dolphin

Fig. 4 Indo-Pacific Humpback dolphin

Fig. 5 Indian Ocean Humpback dolphin

Fig. 6 Spotted dolphin

Fig. 7 Irrawaddy dolphin

Fig. 8 Ganga River dolphin

Fig. 9 Finless porpoise

Conservation Issues and Threats for Cetaceans

Many cetacean species are highly migratory and transient in nature found in diverse habitats from rivers and shallow coastal waters to abyssal depths. The threats are multiple due to their variable distributional range. Many of these cetacean populations have been listed as vulnerable or endangered and several species are in danger of extinction (IUCN SSC Cetacean Specialist Group / Status of the World's Cetaceans 2020). The following factors are known to be detrimental to cetacean population.

a. Over Fishing and By-catch

Higher demand for sea food and lucrative market has led to intensified fisheries over the years which impact the cetaceans in by-catch and loss of their prey species. This has been a universal phenomenon for many cetacean populations and other wildlife around the world. By-catch of cetaceans is increasing by entangled in many fishing gears which include gill nets, drift nets, purse-seine nets, long-lines, drift nets, trap lines and trawl nets. Globally, the proportion of fully fished stocks and overfished, depleted, or recovering fish stocks increased from above 50 percent of all assessed fish stocks in the mid-1970s to about 75 percent in 2005 (FAO, 2007) and to almost 90 percent in 2013 (FAO, 2014 and World Bank, 2017). The percentage of stocks fished at biologically unsustainable levels also increased, from 10 percent in 1974 to 34.2 percent in 2017 (FAO, 2020).

b. Marine pollution

Plastics and polyethene ingestion have been observed by the authors from the carcasses of cetaceans those readily prey upon cephalopods and jelly fishes in Odisha, industrial discharges, seepage from waste dumping sites, accidents of passenger and carriage ships and spills from oil tankers at sea, oil exploration, mining and agricultural run-off along with the persistent organic pollutants, pesticides, polychlorinated biphenyls (PCBs), heavy metals, dioxins and polycyclic aromatic hydrocarbons are major threat to cetacean population (Kannan et al., 2005; Capanni et al., 2020).

c. Noise pollution

Noise pollution by human (i.e., vessels, military, fisheries, pingers, wind farms, scientific research using acoustic devices and survey for oil/gas deposits) has increased in the marine environment and contributes significantly to natural biological and ambient level of sound. These noises cause physical damage to the cetaceans altering their behaviour, increasing stress and leading to displacement from its habitats. Moreover the cetacean watch program harass the cetaceans which may bring about a cumulative and long-term impact on the cetacean population (Aguilar et al., 2006; Malme et al., 1984; National Marine Fisheries Science (NMFS), 2006; Olesiuk et al., 2002; Rendell and Gordon 1999; Richardson et al., 1999; Simmonds and Lopez-Jurado 1991; Southall et al., 2006; Stone and Tasker, 2006; Thomsen et al., 2006; Todd et al., 1996; Van Parijs and Corkeron 2001; Weilgart, 2007; Würsig and Richardson 2002).

d. Habitat modification, loss and degradation

Various cetaceans use diverse kind of aquatic habitats. Freshwater river dolphins rely on the health and condition of the rivers for survival. Similarly, the near-shore or coastal cetaceans and off-shore cetaceans explore various water depths for survival. They show significant preference to various habitats based on various factors like salinity, temperature, nutrient concentrations and productivity. Any loss of habitat or degradation shall limit the critical areas those support the particular cetacean species with highly patchy environment and raise difficulties for securing their prey species. Habitat loss and degradation are more evident in freshwater habitats and near shore coastal waters due to higher dependency of human on these habitats (Sanganyado and Liu, 2021).

International and National Conservation Efforts and Status

Since the 1960s, the International Union for Conservation of Nature (IUCN, 2020) through Species Survival Commission (SSC) and Cetacean Specialist Group (CSG) has played a major role in identifying conservation problems for the world's whales, dolphins and porpoises. The IUCN-SSC-CSG promotes and facilitates the conservation of cetaceans worldwide. It functions as a catalyst, clearing house and facilitator for cetacean related research and conservation action. All the cetacean species are evaluated for the IUCN Red List of Threatened Species.

Convention on the Conservation of Migratory Species of Wild Animals (CMS) or Bonn Convention adopted in 1979 and came into force in 1983 is an environmental treaty of the United Nations that provides a global platform for the conservation and sustainable use of terrestrial, aquatic and avian migratory animals and their habitats. It provides two means of protection for migratory species. Migratory species threatened with

extinction are listed in Appendix I of the Convention. Migratory species that need or would significantly benefit from international co-operation are listed in Appendix II of the Convention. Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is a multilateral treaty of members of the International Union for Conservation of Nature (IUCN) and enforced on 1st July 1975 to protect endangered plants and animals.

Wildlife (Protection) Act, 1972 of India facilitates establishment of Wildlife Sanctuaries and National Parks. It prohibits hunting of endangered species and provides licenses for the sale, transfer and possession of the wildlife species. All cetaceans are protected under Schedule – I of the above Act. Table – 2, indicates international and national conservation efforts and status for all cetaceans documented in Odisha,

Initiatives and Community Conservation Efforts by the State Government

a. Continuous monitoring of cetacean population, distribution, habitat, documentation of all dead cetaceans and assessing threats by all Coastal Wildlife Divisions and Chilika Wildlife Division have addressed various knowledge gaps (Jayasankar et al., 2011; John et al., 2012; Khan et al., 2011; Khan, 2013; Khan et al., 2015; Panda et al., 2008) related to cetaceans of Odisha.

b. Annual population estimation survey is conducted by State Wildlife Organisation; pooling experienced team members from different Departments/Agencies of Government, NGOs, State Universities and Under Graduate Educational Institutes, Local Representatives, Boat Owners and Boat Operators. The method is rigorously carried out and estimates are consensus which is further subjected to rectification of double counts taking into account of GPS track route, location, time of sighting, distance and angle of sighting.

c. Dolphin Watching Protocol for visitors and boat operators were developed by Chilika Development Authority

d. Installation of propeller guard in tourist boats of outer channel of Chilika lagoon has significantly reduced mortality of dolphins from propeller injury.

e. Underwater acoustic studies had been undertaken in Chilika lagoon since 2006 (Akamatsu et al., 2013a and b; Bahl et al., 2006a and b; Inoue et al., 2007a and b; Ura et al., 2006; Yokiko et al., 2007)

f. Annual capacity-building training program are conducted for boat associations in Outer Channel by Chilika Development Authority and Chilika Wildlife Division.

g. Dolphin Protection Squad by Chilika Wildlife Division monitors the daily activity of tourist boats and movements of dolphins.

h. Enforcement of The Orissa Marine Fishing Regulation Act 1982 by Fisheries Department

i. Enforcement of Odisha Boat Rules 2004 by the Department of Commerce and Transport

j. Enforcement of Coastal Regulation Zone rule 2019 by Ministry of Environment and Forest

k. Established of Nalaban Bird's Sanctuary, Bhitarkanika Sanctuary, National Park and Gahirmatha Marine Sanctuary is directly protecting, conserving and serving as refuge for all aquatic life form; from minute to mega fauna.

l. State Pollution Control Board enforced ban on sell and use of polythene bags in Chilika, Sanctuary, National Park and Biosphere reserve

m. The tourist boat association, Chilika also deputed their staffs for monitoring the boats involved in chasing dolphin and penalised the boat operators by suspending their boats or its operator. The association has adopted Odisha Boat Rules 2004 and improvised the standard of boats and other safety protocols.

Recommendations and Suggestions for conservation of Cetaceans

a. Establishment of "Centre for Dolphin Research and Management in Odisha".

b. A Committee of Subject Experts with field work and experience must be constituted. Annual Cetacean Population Estimation Survey preferably be undertaken in the month of February taking cues of favourable dates and time in accordance to Lunar Calendar, Tidal Cycle and abundance of prey-species (sardines).

c. Dedicated scientific study on bioacoustics need to be addressed by deploying adequate numbers of hydrophone arrays systems in strategic locations such as rivers, lagoons, river confluences, near-shore and offshore coastal waters.

d. Monitoring of the cetacean population and fixing of satellite transmitter in few numbers of cetaceans should be considered for off-board monitoring of cetaceans and their movements and migration on annual basis. Cetacean carcasses from Odisha coast must be considered as valuable information capsules to fill various knowledge gaps and develop reliable and scientifically validated conservation strategy. PhotoID method and MARK-RECAPTURE software integrated with GIS and GPS locations may be adapted.

e. Irrawaddy dolphin populations are resident and dominant cetacean species in Odisha, with regular sighting, generates state revenue and provides alternative livelihood to the local villagers. Declaration of Irrawaddy dolphin (*Orcaella brevirostris*) as the "State Aquatic Animal" will necessitate more focus for its conservation.

f. Recognition of "*Brand Ambassador for the cause of Cetacean*" conservation should be adopted for fruitful results.

g. More capacity building training programmes for the officers and field personnel of Govt. Department/Agencies be conducted for effective enforcement of law.

h. The Orissa Marine Fishing Regulation Act and Rules, 1982; Odisha Boat Rules 2004 and Wildlife (Protection) Act, 1972 in cetacean habitats need to be enforced throughout the year for resident population of cetaceans and temporarily for transient population of cetaceans as transient populations are more prone to entanglement and collision.

i. Carrying capacity of tourist, fishing boats and fishing activities in cetacean habitat is very important to balance the ecosystem services and ecological conservation measures. Cetacean friendly fishing gears should be identified and promoted.

j. Access to tourism activity in water must be limited so that the ecosystem and the species can recover from the stress. Speed of vessels and boats in cetacean habitats be regulated.

k. Involvement of the local community in conservation programs through participation and socio-economic benefits, awareness and capacity-building programs on alternative livelihood may be adopted.

l. All mechanized boats and vessels should deploy GPS telemetry system and registered mobile numbers to be accessed by Central and State Government Agencies - OSDMA, Fisheries Dept., Forest and Wildlife Dept., Tourism, Coast Guard, Port Authority and Marine Police for effective monitoring of traffic, safety of the boats/vessels/crew staffs, passengers and cetaceans. It will also help to identify non-state entry of any boats and vessels that do not comply to the laws of the sea and security aspect.

m. Collaborative scientific studies with neighboring states/countries need to be undertaken for understanding the status and migration patterns of cetaceans.

Table – 1 Cetaceans diversity and abundance within near-shore coastal waters of Odisha

Year → / Species ↓	2015	2016*	2017	2018**	2019**	2020	2021
Irrawaddy dolphin	206	---	163	130	208	209	208
Bottlenose Dolphin	50	---	121	16	23	54	155
Indo-Pacific Humpback dolphin	125	---	116	107	2	251	300
Indian Ocean Humpback dolphin	50	---	69	6	0	30	32
Spotted dolphin	15	---	---	---	---	---	---
Striped Dolphin	---	---	---	---	---	---	13
Spinner Dolphin	---	---	---	---	---	---	15
Ganga River dolphin	1	---	0	0	0	0	0
Finless porpoise	1	---	---	---	---	---	3
Un-Identified	5	---	---	---	---	---	---
TOTAL	453	---*	469	259	233	544	726

2006* The Survey was cancelled due to high Beaufort above 4 resulting in rolling conditions.
**In 2018 and 2019 the Beaufort was above 3 with foggy weather conditions.

Table – 2 International and national conservation efforts and status of cetaceans

Near-shore cetacean Species	IUCN Red List*	CMS**	CITES***	Wildlife (Protection) Act, 1972
Bottlenose dolphin	Near Threatened	Appendix – II	Appendix – II	Schedule – I
Indo-Pacific Humpback dolphin	Vulnerable	Appendix – II	Appendix – I	Schedule – I
Indian Ocean Humpback dolphin	Endangered	Appendix – I	Appendix – I	Schedule – I
Spotted dolphin	Least Concern	Appendix – II	Appendix – II	Schedule – I
Irrawaddy dolphin	Vulnerable	Appendix - I & II	Appendix – I	Schedule – I
Ganga River dolphin	Endangered	Appendix - I & II	Appendix – I	Schedule – I
Finless porpoise	Vulnerable	Appendix – II	Appendix – I	Schedule – I

*International Union for Conservation of Nature (IUCN)

**Conservation of Migratory Species of Wild Animals (CMS) or Bonn Convention

*** Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

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ANTIFUNGAL ACTIVITY OF PHYTOCHEMICALS FROM FRESHWATER MICROALGAE, A COMPARATIVE STUDY

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ABSTRACT

The aim of the study was to investigate antifungal activity of different extracts of three fresh water algae i.e, *Euglena viridis*, *Microcystis aeruginosa* and *Chlorella vulgaris*. Two different solvents such as ethanol and methanol were used to extract the bioactive compounds from the above selected algae to screen the antifungal activity against fungal pathogens, *Saprolegnia parasitica* and *Candida albicans* by disc diffusion method. The maximum antifungal activities were observed in ethanolic extract of *Euglena* and *Microcystis* (13.5±0.28 mm, 12.00±0.28mm) at 200 µg against *Saprolegina parasitica* and *Candida albicans*, respectively. The minimum inhibitory concentration (MIC) of the algal extracts against the selected fungal pathogens was between 250 µg to 500 µg.

Key Words: Antifungal, Alkaloid, *Candida albicans*, *Euglena viridis*, *Microcystis aeruginosa*

INTRODUCTION

The use of antimicrobial drugs against infectious diseases has certain limitations due to their side effects and resistance in pathogens. Among modern medicines, use of antibiotics against disease causing microbes is a great achievements. However, this kind of drugs are beginning to lose their usefulness due to the development of resistance on the part of microbes. There is demand for research on development of drugs with new antimicrobial agents (Al-Haj et al., 2009). Thus, the search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia (Latha and Kannabiran, 2006). In the past 25 years there is significantly increased incidence of fungal infection. Now *Candida* species are the most extensive and frightening fungal pathogens, and are accountable for various invasive and non-invasive fungal infections. *Candida albican* causes oral and genital infections in humans (Ryan and Ray, 2004, Hube, 2007). It has been reported that microbial infections are mostly responsible for inflammation (Du-shieng et al., 2005). The microalgae are natural source of bioactive compounds, and in culture they produce potentially bioactive molecules, which are tough to synthesize chemically (Borowitzka and Borowitzka, 1989). Several esearchers have intended to study the potential natural source of different functional compounds from algae and microalgae (Zheng et al., 2013, de Jesus Raposo et al., 2013, Maadane et al., 2017). It has been revealed that phycochemical and pharmacological studies on algae is available in the literature with special references to terpenoids and steroids (Parameswaran et al., 1944, Rodrı´guez-Meizoso et al., 2008). Thus, attention has been given to the microalgae for production of biologically active novel compounds such as phycobiline, phenols, terpenoids, steroids and polysaccharide (Patterson, 1968 and Li et al., 2007). The investigations on various aspects of antibiotic activities of marine algae have been done both

in India and abroad (Maadane et al., 2017). There are number of reports on antifungal activities related to marine algae against different pathogens (Kolanjinathan and Stella, 2009).
It has also been reported that extracts of green algae, diatoms and dinoflagellates have a broad range of *in vitro* antifungal activities. Whereas different strains of Cyanobacteria are known to produce intracellular and extracellular metabolites with varied biological activities such as antifungal, antiviral, antialgal and antibacterial activities (Rania et al., 2008, Duda-Chodak, 2013, Ramesh Babu et al., 2017). Some microalgae like *Chlorella vulgaris*, *C. protothecoides*, *C. minutissima*, and *Ulva lacuta* have fungicide potentials. To prevent the loss of stored apple fruit, some workers investigated the antifungal activities of above microalgae against soft-rotting fungi *Aspergillus niger*, *Alternaria alternata*, and *Penicillium expansum* fungi (Vehapi et al., 2020). The phytochemical analysis and antifungal activity of aqueous extract of various solvent extracts of *Spirulina platensis* from Rankala Lake, Kolhapur, Maharashtra, India were tested against *Candida albicans* and *Aspergillus fumigatus* (Chakraborty et al., 2019). The *Chlorella vulgaris* is also equally effective for opportunist fungal pathogens, particularly towards *Candida albicans* and *Candida tropicalis* and to some extent *Candida parapsilosis* (Sarkar et al., 2021).

Despite the potential, attention has been centered on marine algae, with very little on fresh water algae. In this study three freshwater microalgae, *Euglena viridis* (Ehren), *Chlorella vulgaris* and *Microcystis aeruginosa* (Kütz) are chosen for subject. Some of the previous studies on these freshwater microalgae were reported (Das and Pradhan, 2010; Pradhan et al., 2012a, 2b; Das et al., 2012). Therefore, the purpose of the present study was to evaluate the protective effects of various extracts of three freshwater microalgae, *E. viridis*, *C. vulgaris* and *M. aeruginosa* and their comparison with active principle antifungal activity and biomolecules of potential therapeutic interest. The prescreening results reported herein could serve as a basis to isolate and identify the antifungal compounds from these algal extracts as a source of natural antifungal agents for application in pharmaceutical industries.

MATERIALS AND METHODS

Collection and Culture of Microalgae

Samples of freshwater algae (*Euglena* and *Microcystis*) were collected from ponds of Central Institute of Freshwater Aquaculture and from Bindusagar, Bhubaneswar, India in the month of October and March. All samples were collected using plankton net and brought to the laboratory then washed properly with distilled water to separate contaminants. These two algae were identified as belonging to families Euglenophyceae and Chlorococcaceae respectively following Records of Botanical Survey of India (Biswas, 1949).

Axenic cultures of *C. vulgaris* (procured from Algal Culture Unit, CIFA, Bhubaneswar) were grown in freshly prepared culture medium. For growth, a temperature of 28 ± 2°C and illumination by cool white fluorescent lamps of intensity 25 Wm^{-2} was provided. The cells of 20 day old cultures were then harvested by centrifugation (5000×g) at 4°C.

Dry Weight Determination

To remove the suspended particle in collected algal samples, were washed repeatedly in Millipore water (Millipore Corporation, Bangalore India) and centrifuged at 1000×g at 4°C using macro rotor (Sorvall CE,

Buckinghamshire, UK). Biomass was transferred to a pre-weighed dry filter paper and placed in a dryer at 60°C for 24-72 h to reach constant weight.

Preparation of the Extracts

Harvested dry algal samples were ground using electric grinder and the resulting powder was dissolved in lipid soluble polar solvents (Hexane, Ethylacetate, Ethanol and Methanol) with constant stirring for 24 h. All samples were refluxed until saturation (24 h) and the respective extracts were dried in Rotavapor (Bu¨chii rotary evaporator-II, Flawil, Switzerland). Then the crude extracts were suspended in the respective solvents to a final concentration of 20 µg μl^{-1} (Das and Pradhan, 2010).

Test organisms

Antifungal activity was tested against two fungal pathogens, *Candida albicans* and *Saprolegnia parasitica*. These pathogens were maintained and the fungal isolates were sub cultured on a Potato dextrose agar (PDA) (Hi-Media) for 72 h at 25°C in the Fish Health Management Division (FHMD), CIFA, Bhubaneswar.

Preliminary Phytochemical Screening

The phytochemical constituents of *Euglena, Microcystis* and *Chlorella* were tested by following standard methods of Sofowora (1993), and Trease and Evans (2002) with minor modifications. One gram of the ethanol and methanol extract of algae was dissolved in 100 ml of its mother solvents to make a stock of concentration 1% (v/v).

Test for Alkaloids

a. Dragendroff's reagent test: The algal extract of 5 ml of was added to 2 ml of HCL. Then 1 ml of Dragendroff's reagent was added to this acidic medium. Production of an orange or red precipitate immediately indicates the presence of alkaloids.

b. Mayer's test: Two ml of the extract was added to 0.2 ml of dilute HCL and to this 0.1 ml of Mayer's reagent was added. The presence of alkaloids in the sample was indicated due to white precipitation.

c. Wagner's reagent test: To 0.2 ml of dilute HCL, 2 ml of the extract was added and then subsequently 0.1 ml Wagner's reagent was added to it. Reddish brown colour indicates presence of alkaloids.

Test for Flavonoids

A few drops of dilute sodium hydroxide were added to 1 ml of the algal extract. An intense yellow colour was produced in the extract, which become colourless by adding a few drops of dilute acid indicates the presence of Flavonoids.

Test for Tannin

Ferric chloride test: Five ml of the extract and 1 ml of 5% Ferric chloride solution were added. A blackish green colour was formed, indicates the presence of tannins.

Test for Steroids

Algal extract of 1 ml was dissolved in 1 ml of chloroform and sides of the test tube poured equal volume of concentrated sulphuric acid. The upper chloroform layer turns red and sulphuric acid layer showed green flouorescence, indicated the presence of steroids.

Test for Reducing Sugar

To 5 ml of solution of extract 5 ml of Fehling's A and B solution were added and allowed for boiling for 5 minutes at 60°C on water bath. Break red colour precipitate indicated the presence of reducing sugar.

Test for Saponins

The algal extract was diluted with 20 ml of distilled water and followed by shaking in a graduated cylinder for 15 minutes. The presence of saponins is confirmed by the formation of 1 cm layer of foam on it.

Antifungal Activity

The antifungal sensitivity test was done by disc diffusion technique (Darah et al., 2006). The sterile potato dextrose agar plates for fungus were prepared and over it the fungal test organisms were spread. Subsequently sterile discs of 5 mm diameter were embedded with 10 µl of the algal solvent extracts. After solvent evaporation, the discs were placed on the organism-inoculated plates with equal distance along with control discs. All the fungal plates were incubated at 24°C for 72 h and the measurement of the diameter of the zone of inhibition was taken in mm (Izzo et al., 1995). Triplicate test were performed and the antifungal activities of algal extracts were compared with a commercial antifungal, Fluconazole (Hi Media, India).

Determination of Minimum Inhibitory Concentration (MIC)

The values of minimum inhibitory concentration (MIC) were determined following tube dilution assay (Khan et al., 2007). Where 24 h broth cultures of fungal pathogens were taken and the minimum dilution of algal extract that inhibits the growth of the organism was considered as MIC.

Statistical Analysis

The statistical analysis of the results were done by using one way analysis of variance (ANOVA) and significant difference of various extract of algae among different concentration were compared using Duncan's multiple range test (DMRT) (Duncan, 1955).

Results and discussion

The preliminary phytochemical analysis of ethanolic and methanolic extract of *E. viridis* (E:EtOH, E:MeOH), *M. aeruginosa* (M:EtOH, M:MeOH) and *C. vulgaris* (C:EtOH, C:MeOH) were cited in Table. 1, which revealed the presence of alkaloids, flavonoid, steroid, saponin and reducing sugar. Similar findings have been reported in ethanolic extract of plants (Egwaikhide and Gimba, 2007; Ayandele and Adebiyi, 2007).

Table 1. Preliminary phytochemical analysis of various extracts of freshwater algae

Extracts	Phytochemical tests							
	Alkaloid test			Tanin	Flavonoid	Steroid	Saponin	Reducing sugar
	Dragendroff's Reagent	Wagner's Reagent	Mayer's Reagent					
E:EtOH	+	+	+	-	+	-	-	+
E:MeOH	-	-	-	-	-	-	+	-
M:EtOH	-	-	-	-	-	+	-	+
M:MeOH								
C:EtOH	-	-	-		+	-	+	+
C:MeOH	-	-	-	+	+	-	+	+

+: present, -: absent

Note: E: EtOH; E:MeOH- Ethanolic and methanolic extract of *Euglena*, M:EtOH; M:MeOH- Ethanolic and methanolic extracts of *Microcystis*, C:EtOH;CMeOH- Ethanolic & methanolic extracts of *Chlorella*

Whereas Steroids and alkaloids were absent in both ethanolic (C:EtOH) and methanolic (C:MeOH) extract of *C. vulgaris*. Usually organic solvents were more efficiently extract antimicrobial compounds (Tuney et al., 2006). Cox et al. (2010) revealed that the extraction of antimicrobials from the different species of seaweeds was solvent dependent; acetone was better solvent for red and green species whereas methanol was for brown seaweed. There is wide range of natural antimicrobial compounds from algae have been represented under chemical classes such as phenols, fatty acids, indoles, terpenes, volatile halogenated hydrocarbons, and acetogenins (Zheng et al., 2013, de Jesus Raposo et al., 2013).

The antifungal activity of various extracts of freshwater algae exhibited concentration dependent effect against *S. parasitica* and *C. albicans*. Present study revealed that all the extracts showed concentration dependent inhibition (50, 100, 150 and 200 µg/ 10µl) against *S. parasitica* and *C. albicans*, which was well compared with standard drug Fluconazole (500 µg) (Table. 2 and Fig. 1).

Table 2. Antifungal activity of various extracts of freshwater algae against *Saprolegnia parasitica* (48 h culture)

Extracts	***Saprolegnia***			
	Disc potency in µg/10µl			
	50	100	150	200
	Zone of inhibition in mm			
E:EtOH	9.00±0.28^{c}	9.00±0.50^{c}	11.0±0.28^{b}	13.5±0.28^{a}
E:MeOH	8.00±0.28^{c}	7.83±0.16^{c}	10.0±0.28^{b}	11.5±0.28^{a}
M:EtOH	7.83±0.33^{b}	8.16±0.33^{b}	9.83±0.16^{a}	10.0±0.28^{a}
M:MeOH	7.83±0.16^{c}	9.00±0.50^{bc}	10.0±0.50^{ab}	11.0±0.30^{a}
C:EtOH	-	8.00±0.28^{b}	10.0±0.16^{a}	10.6±0.28^{a}
C:MeOH	-	7.00±0.28^{b}	11.0±0.33^{a}	11.2±0.30^{a}
	Zone of inhibition in mm (Standard antifungal)			
Fluconazole (500 µg)	15			

Values represent mean±S.D.,

Values bearing common superscript are not significantly different ($p<0.05$)

The ethanolic and methanolic extracts at higher concentrations showed significantly higher antifungal activity against *S. parasitica*, where as both ethanolic, C:EtOH and methanolic, C:MeOH extract of *Chlorella* was devoid of activity at lower concentrations. Similarly ethanol and methanol extracts of *Euglena*, *Microcystis* and *Chlorella* inhibited *C. albicans* on all the selected concentrations except *Chlorella* extract at 50 and 100 µg/10µl concentration, which showed deficient inhibition (Fig. 1).

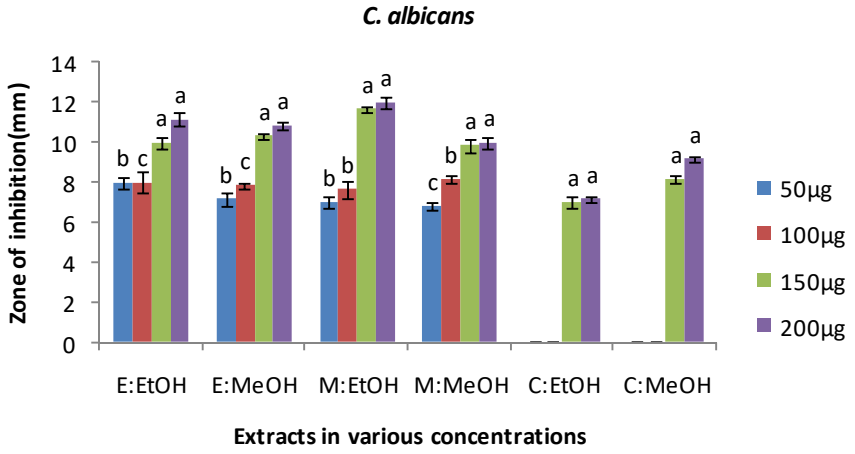

Figure 1. Antifungal activity of various extracts of freshwater algae against *Candida albicans* (48 h culture)

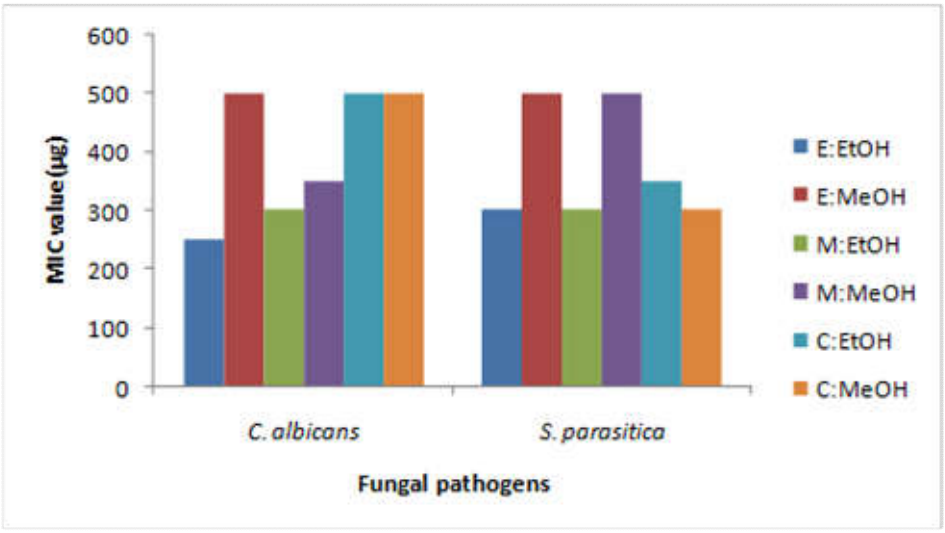

Figure 2. MIC values of various extracts of *Euglena viridis*, *Microcystis aeruginosa* and *Chlorella vulgaris* against *Candida albicans* and *Saprolegina parasitica*

Significantly different ($p<0.05$) zone of inhibition was observed in 150 µg and 200 µg concentration of both ethanolic, E:EtOH and methanolic, E:MeOH extract of *Euglena* against *S. parasitica*. Whereas, no significant difference ($p<0.05$) was found in above concentrations of extracts of all the algal extracts treated against *C. albicans.* It was noticed that with the increase in concentration (50µg to 200µg) of the extracts there was increase in zone of inhibition. A similar type of observation was observed by Kanwal et al. (2010). Eight steroid saponins from *Tribulus terrestris* L., were tested against fluconazole-resistant yeasts

encountered clinically, especially *C. albicans* (Zhang et al., 2006). Hebsibah and Dhana Rajan (2010) reported that partially purified ethanolic fractions of seaweed extracts are more active against fungi such as *C. albicans*, *C. tropicalis, Aspergillus fumigatus, A. flavus*, and *A. niger*. Similarly, in fungi the ethanol extract showed maximum activity at 400 µg/well against *C. albicans* (Mathad and Mety, 2010). The Minimum Inhibitory concentration (MIC) of all the extracts showed that *S. parasitica* and *C. albicans* showed an MIC ranges from 250µg-500 µg. The lowest MIC was observed by ethanolic extract of *Euglena* against *C. albicans* (Fig: 2). In comparison to other solvent extracts, the aqueous extract of *Spirulina platensis* composed of dominant bioactive compounds showed antifungal and antioxidant activity (Chakraborty et al., 2019).

The present investigation concluded that the freshwater microalgae constitute a new source of antifungal formulation with stable bioactive compounds. Future studies are essential to identify the chemical structure of the isolated compounds and examine their protective nature against fungal pathogens.

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MELANOPHORES DURING TAIL REGENERATION IN *Polypedates maculatus*

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ABSTRACT

Melanophores are pigment cells present in amphibians. They are seen to be regenerated along with the regenerating tail in the tadpoles of the Indian tree frog, *Polypedates maculatus*. In course of retinoic acid mediated tail regeneration, the tail tissue is affected drastically resulting in the impairment of regeneration of its tissue components. However, an increase in the number and appearance of melanophores, the neural crest derivatives has been witnessed during the retinoic acid mediated tail regeneration in this species.

Keywords: Melanophores, melanocytes, pigment cells, retinoic acid, Indian tree frog.

INTRODUCTION

Melanophores are the main pigment cells in amphibians (Lin et al., 2007; Pshennikova and Voronina, 2018). These cells are highly differentiated neural crest derivatives (Olsson and Löfberg, 1992; Edmondson et al., 1999; Mull et al., 2015; Wang et al., 2016; Rodríguez-Rodríguez et al., 2020). They essentially function to produce melanin that is brown and black (Rodríguez-Rodríguez et al., 2020). In vertebrates the melanocytes are situated in the basal layer of the epidermis (Edmondson et al., 1999). In lower vertebrates the melanocytes are called as melanophores (Lin et al., 2007; Pshennikova and Voronina, 2018). However, reports do not indicate major difference in these cell types (Lin et al., 2007). Pshennikova and Voronina (2018) have suggested the melanophores of poikilotherms differ from melanocytes in terms of the movement and rearrangement of pigment granules. In context of the development and regeneration of the melanophores in lower vertebrates, the zebrafish has served as an important vertebrate model that has provided vital insights on the mechanism. It is also documented that the amphibian melanophores are similar to that present in fish (Lin et al., 2007). However, not much is known about them during retinoic acid mediated tail regeneration. The anuran tadpole tail thus can provide valuable information on the regeneration of melanophores during retinoic acid mediated tail regeneration. The Indian tree frog, *Polypedates maculatus* regenerates its tail while as a tadpole (Mahapatra et al., 2004) and hence, has been serving as a preferable laboratory model for regeneration studies. The tadpoles of this species competently restore the lost caudal appendage following injury (Mahapatra et al., 2004) and the new tail strikingly resembles the previous one morphologically within a fortnight (Hota et al., 2018). Interestingly, retinoic acid (RA) interferes during tail regeneration in this species and causes severe morphological and functional impairment (Mahapatra and Mohanty-Hejmadi, 1994; Pati et al., 2003; Mahapatra et al., 2004; Mahapatra et al., 2018). The most striking of it is homeotic transformation where limbs emerge from the tail (Mohanty_Hejmadi et al., 1992;

Mahapatra and Mohanty-Hejmadi, 1994; Pati et al., 2003; Mahapatra et al., 2004; Mahapatra et al., 2018). We have a wealth of knowledge on appendage regeneration however, our understanding of homeotic transformation of tail to limbs is very limited. Moreover, not much is known on the melanophores during RA mediated tail regeneration, hence it is investigated in the present study.

MATERIALS AND METHODS

Tail transection and RA treatment

The egg nests of the Indian tree frog were collected from the campus of Utkal University, Odisha, India (20°18'14.26" N 85°50'22.73" E). The eggs were allowed to hatch and reared following the standardised protocol (Mohanty-Hejmadi, 1977). The tadpoles were constantly monitored and were selected while in their hindlimb bud stage I and II (Taylor and Kollros, 1946). The tail transection was simple and carried out after the tadpoles were exposed to MS222 (Himedia) at the dose of 1:3000 to ensure minimal stress to the animals. The tadpoles were subsequently exposed to RA (Sigma Aldrich) at the standardised dose of 250 ng/ml for 24 h duration in low light condition. The control sets were also generated which lacked RA treatment for comparison and analysis.

Histological analysis

Histology of the tissue section is crucial to assess the changes manifested in the tissue anatomy following RA treatment. Hence, standardised histological procedure was adapted and tissue sections of 5 µm thickness were obtained and subsequent differentially stained by Mallory's triple stain technique. Histological sections were obtained for both control sets that lacked RA treatment and experimental sets with RA exposure.

RESULTS

The regenerating tadpoles tail of *P. maculatus* tadpoles revealed the melanophores were present in close proximity to the overlying epidermis (Fig. 1 A). Few melanophores were also evident in the mesenchyme next to the regenerating muscles (Fig. 1 B). Their shapes were found to be mostly globular while few of them were slightly dendritic in appearance (Fig. 1A-D). The melanophores were identified based on their morphology and the dark brown to black colouration in tissue sections (Fig.1 A-D). Interestingly, these melanophores were seen as large clusters along with the cells of the mesenchyme in RA treated sections unlikely to their control counter parts (Fig. 2 A-D). In fact, melanophores of the RA treated sections had profusely dendritic appearance (Fig. 2 A-D). Moreover, their proximity near the multi-layered thick epidermis and a dense collagenized matrix was evident (Fig. 2 A-D). Another distinctive feature was observed in terms of absence of melanophores near the regenerating caudal cord of the control tail tissue (Fig. 3 A). However, they were seen to be present near the spinal cord of the tail in the completely regenerated control tail tissues (Fig. 3 B). On the contrary, the melanophores with dendritic appearance were very much noticeable near the regenerating caudal cord throughout in the RA treated tissues (Fig. 3 C, D).

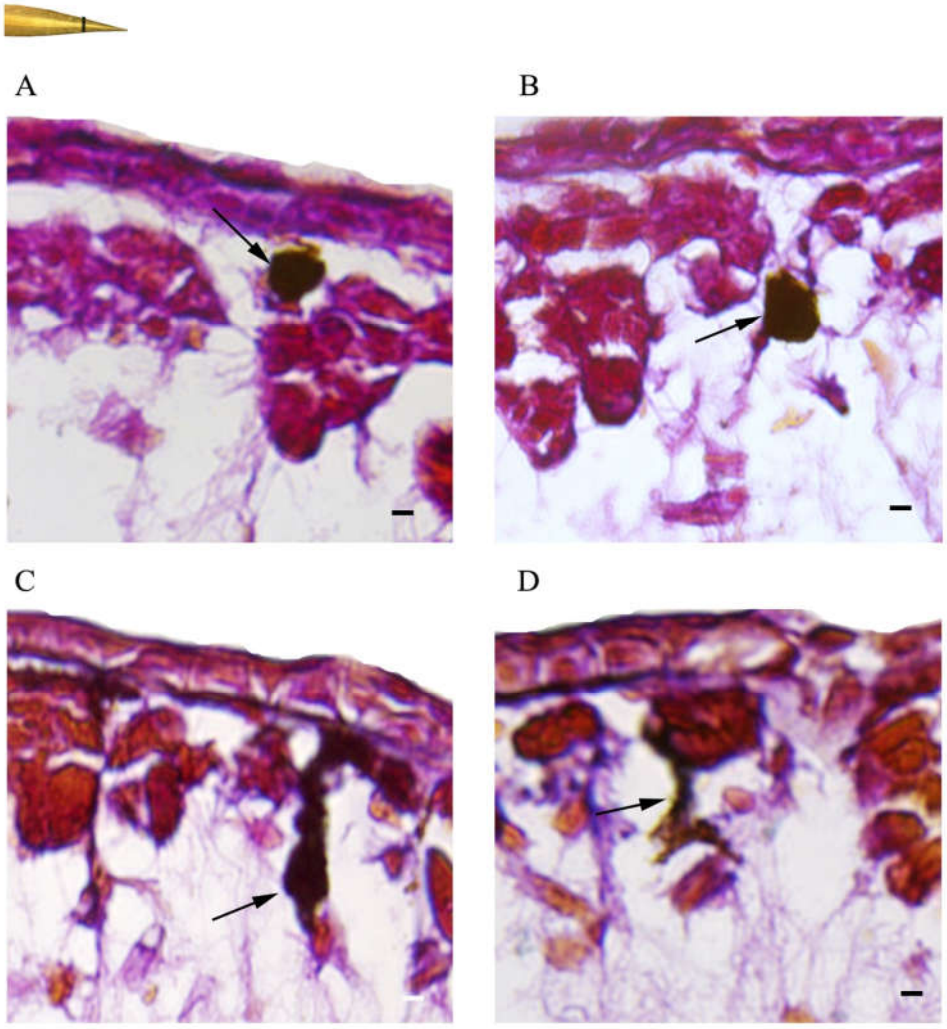

Figure 1 Histology of regenerating tadpole tail of *Polypedates maculatus*: (A-B) T.S. of regenerating tail of tadpoles of *P. maculates* showing melanophore below the epidermis having globular appearance. **(C-D)** T.S. of regenerating tail of tadpoles of *P. maculates* showing melanophore below the epidermis having slightly dendritic appearance. (Scale bar in panels A-D= 25 µm).

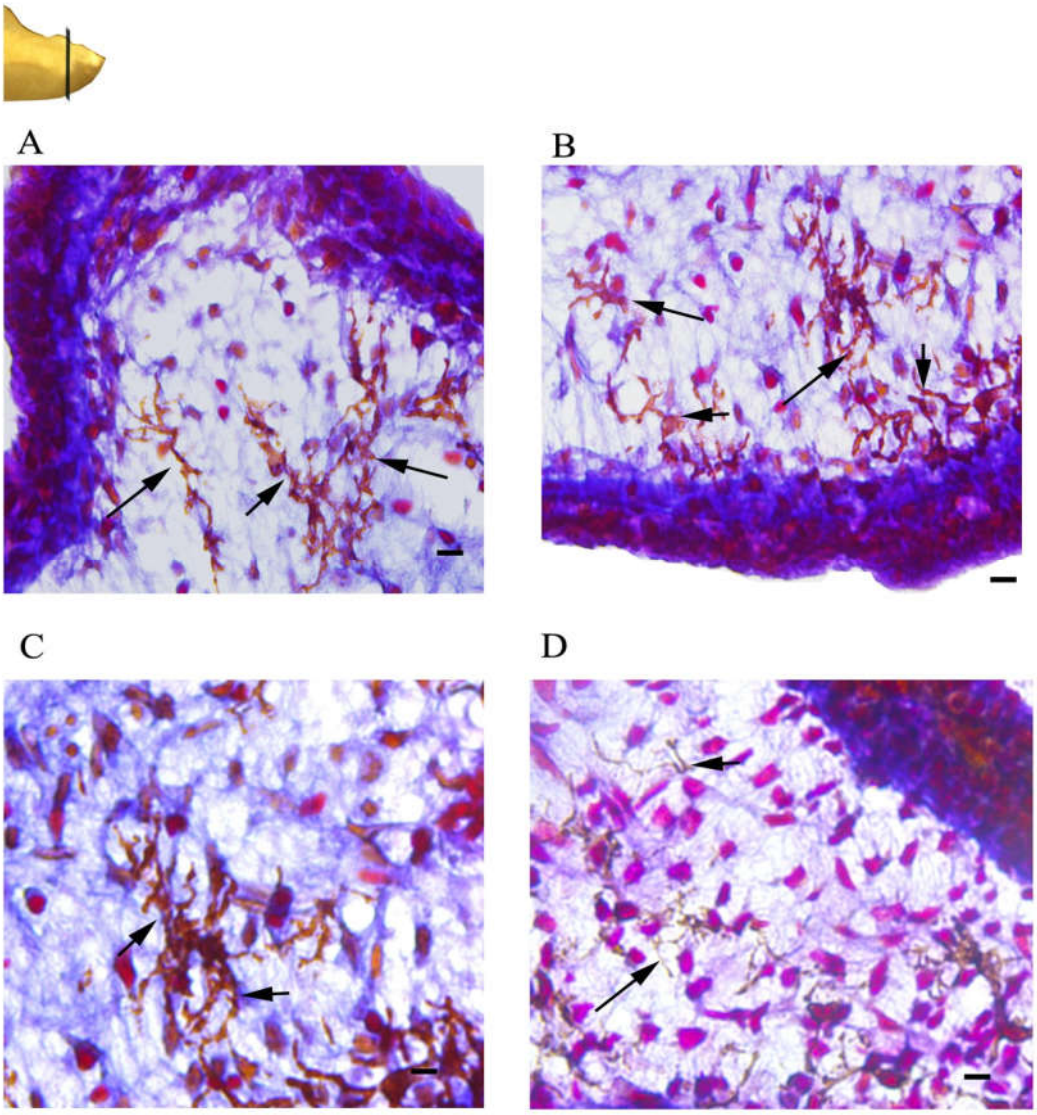

Figure 2: Histology of retinoic acid treated regenerating tails of the tadpoles of *Polypedates maculatus*: (A-B) T.S. of regenerating tail of tadpoles of *P. maculates* showing melanophore below the thick epidermis having highly dendritic appearance. **(C-D)** T.S. of RA treated regenerating tail of tadpoles of *P. maculates* showing highly dendritic melanophore in the mesenchyme and below the epidermis (Scale bar in panels A-D= 25 μm).

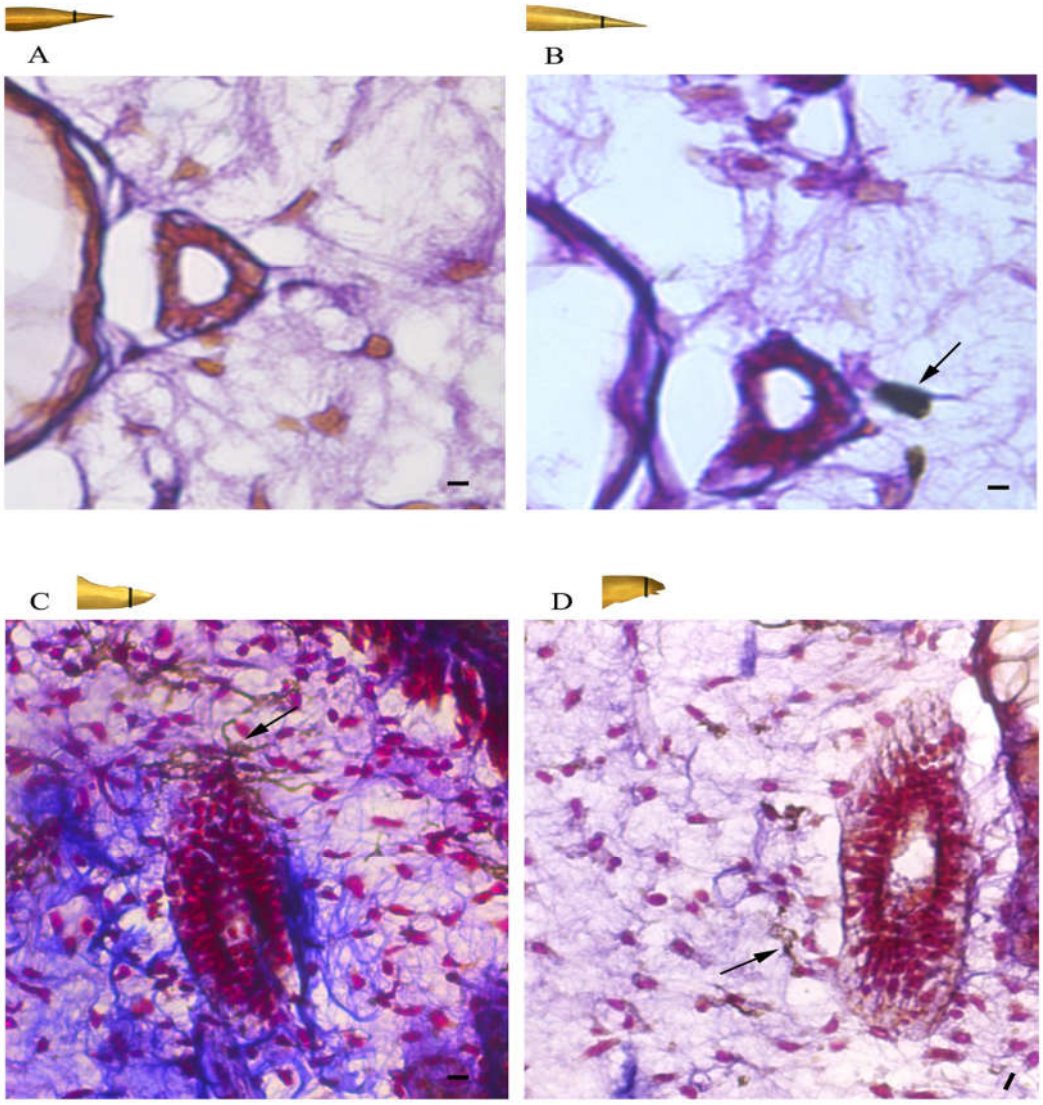

Figure 3: Histology of regenerating and retinoic acid mediated regenerating tails of the tadpoles of *Polypedates maculatus*: (A) T.S. of regenerating spinal cord in the tail of tadpoles of *P. maculates* having no melanophores in its proximity. (**B)** T.S. of regenerated spinal cord in the tail of tadpoles of *P. maculates* showing presence of melanophores in its close proximity. **(C-D)** T.S. of RA treated regenerating tail of tadpoles of *P. maculates* showing highly dendritic melanophore in close proximity of the regenerating spinal cord (Scale bar in panels A-D= 25 µm).

DISCUSSION

The melanophores are interesting pigment cells whose association with skeletal muscle during tail regression metamorphosis have been documented (Divya et al., 2010). However, their involvement during anuran tail regeneration is scarce and needs extensive investigations. In the present study, the melanophores were evident in the regenerated tails in tissue sections of *P. maculatus*. Similar findings have also been reported in *Xenopus* during tail regeneration (Lin et al., 2007). Grafting and labelling experiments in *Xenopus* have also revealed the proliferating and migrating pre-existing unpigmented melanophore precursor cells found near the amputation surface are crucial for the regeneration of these neural crest derivatives (Lin et al., 2007). In Zebrafish fin stripes, the same mechanism of melanophore regeneration is suggested to be operated as in *Xenopus* (Lin et al., 2007; Rawls and Johnson, 2000; Yang and Johnson, 2006; Iyengar et al., 2015). In the present investigation, the regenerated melanophores were found in more numbers in the RA treated tissue sections in *P. maculatus* where they had a distinctly dendritic appearance in comparison with their control counter parts. Earlier studies have suggested, in response to certain signals melanocytes can acquire dendritic architecture for their interactions with keratinocytes (Domingues et al., 2020; Knapp and Iden, 2020). Also, FGF2 is considered as an important keratinocyte derived factor in melanocytes (Dong et al., 2012). Furthermore, it is documented that in human, the survival and proliferation of melanocytes is largely dependent on bFGF or FGF2 (Halaban et al., 1988; Edmondson et al., 1999; Czyz, 2019). The melanophores in the present study were seen to be present in more numbers as clusters in the RA treated tissue sections associated with the epidermis and in dense collagenized mesenchyme. Further investigations in this direction are essential as our previous studies on Fibroblast growth factors (FGF) reported a significantly higher FGF2 immunofluorescence localization in the epidermis and in the mesenchyme during RA mediated tail regeneration (Hota and Mahapatra, 2022). Melanophores have been observed to be absent near regenerating spinal cord in the tail in the present study. Similar results were also suggested in our earlier investigation on the caudal cord regeneration where the melanophores reappeared only after the complete regeneration (Hota et al., 2018). In contrast to this the present study suggests the presence of these cells near the regenerating spinal cord of the tail throughout in the RA treated tissue regenerates. Further investigations in this direction are essential to establish the association of melanophores during spinal cord regeneration.

CONCLUSION

Our study provides information on the melanophores based on histological analysis of the RA mediated tail regeneration in *P. maculatus* tadpoles. Futuristic work in this direction is crucial to validate the involvement of melanophores in this context.

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AQUATIC HABITATS AND NESTING PREFERENCE OF ANURAN LARVAE IN AND AROUND BHUBANESWAR, ODISHA, INDIA

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ABSTRACT

Abiotic and biotic environmental factors limit the distribution of amphibian larvae. Some of these variables are still unknown in and around Bhubaneswar, Odisha, India, where *Duttaphrynus melanostictus*, *Microhyla ornata* and *Euphlyctis cyanophlyctis* are abundant. We sampled 52 sites in Bhubaneswar and measured biotic and abiotic water variables at the breeding site. The water variables: pH, water depth, water temperature, relativeair humidity and submerged vegetation were analyzed using multivariate statistical methods. The presence of anuran larvae is generally, but not always, associated with pH, water depth and submerged vegetation. *Microhyla ornata* was unaffected by any of the variables studied, whereas *D. melanostictus* was influenced by water depth and *E. cyanophlyctis* was influenced by pH, water depth, and submerged vegetation. Our findings point to a potential risk for these species as a result of changes in aquatic variables caused by climate change.

Key Words: Amphibians, anuran larvae, breeding sites, environmental parameters, species occurrence.

INTRODUCTION

Fresh water aquatic habitats are the primary requisites for the development of tadpoles of all the amphibians. Following the south-west monsoon rain, most Indian anuran species breed in ephemeral ponds, damp grounds, temporary puddles, permanent ponds, and rivers (Saidapur, 1989). The physical environment of such habitats influences the growth rates, larval duration, and survival until metamorphosis, and size at metamorphosis of the tadpoles (Alford, 1999). Aquatic breeding habitats differ in terms of their structure (e.g., pond area, and water depth), limnological characteristics (e.g., dissolved oxygen concentration, temperature, lentic or lotic,current), and hydroperiod (e.g., ephemeral, temporary, or permanent), all of which influence or determine the composition of their tadpole assemblages (Peltzer and Lajmanovich, 2004; Oliveira and Eterovick, 2009). Ultsch et al., (1999) found that abiotic parameters like pH, water temperature, dissolved oxygen content, and desiccation risk affect the breeding success of amphibians. Anuran species are restricted in number and dispersion by the presence/absence of the breeding sites (Rodrigues et al., 2010). Understanding the population dynamics of a species through information on habitat associations can help to improve conservation and management strategies (Morris, 2003). Studies by Gillespie et al., (2004); Afonso and Eterovick (2007); and others offered important details on the characteristics of anuran breeding habitat and its possible conservation uses.

Duttaphrynus melanostictus (Schneider, 1799), *Euphlyctis cyanophlyctis* (Schneider, 1799), and *Microhyla ornata* (Dumeril and Bibron, 1841) are widely distributed in Southeast Asia (Dutta et al., 2009; Bahuguna et al., 2016; Frost and The American Museum of Natural History, 2018; AmphibiaWeb, 2022). They choose to breed both in lentic (e.g., ephemeral pools, temporary ponds, permanent ponds, gutters, puddles, and cement cisterns in parks) and lotic (e.g., rivers, creeks, and streams) habitats. In this study, we establish a correlation between the existence of anuran tadpoles and the aquatic environment in order to provide a baseline for identifying the ecological needs of the target species and for subsequent research on the effects changes both at local and global level.

MATERIALS AND METHODS

Field survey

Fifty two water bodies were randomly surveyed over a period of four consecutive breeding seasons (May–September, 2017–2021) in and around Bhubaneswar, Odisha. The tadpoles were located by visually inspecting aquatic environments in the study area during early morning hours (6:00–10:00 AM). Water temperature, pH, water depth, relative air humidity and submerged vegetation were measured twice as close to the tadpoles as possible during each breeding season. The water temperatures, as well as the percentage of relative humidity were measured using a mercury bulb thermometer and a thermo-hygrometer. The pH value was determined using a digital pH metre (pH Tester 10, ecotester, eutech Instruments). A ruler was used to measure the depth of the water. Submerged vegetation was classified based on visual observation and rated as a presence (1) or absence (0) in the study locations.

Statistical analyses

We used a two-step approach to examine the effects of environmental variables on the presence of anuran larvae of all the species in the study area. The number of species found per site was treated as the response variable to identify a general pattern referring to the occurrence of all the studied species. We used a Generalised Linear Model with the presence of larvae as the dependent variable (binary encoded), weighted by the number of species found at the site (ranging from 1 to 3) and environmental variables as covariates. We then ran three independent multinomial logistic regressions with species presence/absence as the dependent variable and environmental variables as covariates to see how the variables affected the distribution of each species individually (only main effects). All statistical evaluations were carried out using IBM SPSS 20.

RESULTS

Out of the 52 water bodies surveyed, *D. melanostictus* tadpoles were present at 12 sites, *M. ornata* tadpoles at 18 sites and *E. cyanophlyctis* tadpoles at nine sites. The presence of tadpoles was related to a marginally slightly acidic pH (6.70 ± 0.63), shallow water depth (1.02 ± 0.36), and moderate submerged vegetation (0.66 ± 0.47) (Table 1).

Table 1 Descriptive statistics of the water parameters of 39 aquatic habitats in and around Bhubaneswar, Odisha, India.

Water parameters	Range (n = 39)	Mean ± SD
pH	5.7 – 7.8	6.70 ± 0.63
Water depth (Cm)	0.3 – 1.6	1.02 ± 0.36
Water temperature (°C)	19 – 29	26.22 ± 2.62
Relative humidity (%)	61 – 97	77.61 ± 9.62
Submerged vegetation	0 –1	0.66 ± 0.47

The Generalised Linear Model used to determine the impact of the variables on the presence of tadpoles of all the species together was significant ($\chi2 = 159.53$, df = 4, $p < 0.001$). However, the results of the model showed that only pH (Wald $\chi2 = 51.13$, B = 0.14, SE = 0.02, df = 1, $p = 0.005$), depth (Wald $\chi2 = 5.89$, B = 0.02, SE = 0.02, df = 1, $p = 0.030$) and submerged vegetation (Wald $\chi2 = 3.68$, B = 0.01, SE = 0.02, df = 1, $p = 0.018$) had a significant impact on the presence of the anuran tadpoles. Water temperature (Wald$\chi2 = 1.70$, B = 0.396, SE = 0.01, df = 1, $p = 0.514$) and relative humidity (Wald $\chi2 = 0.34$, B = 0.498, SE = 0.01, df = 1, $p = 0.490$) were not related to their presence (Table 2).

Table 2 Generalized linear model investigating environmental parameters affecting abundance of anuran larvae in and around Bhubaneswar, Odisha, India ($N = 52$, Wald $\chi2 = 159.53$, $df = 4$, $P < 0.001$). The model used a complementary log-log link function. S.E. – Standard error of the mean.

Water parameters	Wald $\chi2$	df	*p*	B	S.E.
pH	51.13	1	0.005	0.14	0.02
Water depth	5.89	1	0.030	0.02	0.02
Water temperature	1.70	1	0.514	0.396	0.01
Relative humidity	0.34	1	0.490	0.498	0.01
Submerged vegetation	3.68	1	0.018	0.01	0.02

The three independent multinomial logistic regressions showed that for *M. ornata*, the overall model was significant ($\chi2 = 32.70$, df = 4, $p < 0.001$) and explained 48% of the variation, although none of the variables was significantly explaining the presence of the species in relation to five environmental variables (Table 3).

In the case of *D. melanostictus*, the model was significant ($\chi2 = 15.42$, df = 4, $p = 0.002$) and explained 26% of the variation, and only water depth significantly explained the presence of the species (Table 3). A lower water depth was associated with the presence of the species (Table 3). Finally, for *E. cyanophlyctis,* the model was significant ($\chi2 = 97.23$, df = 4, $p < 0.001$) and explained 88% of the variation. The variables pH, water depth, and vegetation significantly explained the presence of the species, albeit marginally for vegetation (Table 3). A higher pH, but lower water depth and higher vegetation were associated with the presenceof the species (Table 3).

Table 3 Results of the multinomial regression analyses on the presence of three anuran larvae in relation to environmental variables in and around Bhubaneswar. pH and water depth were significant explanatory variables. Data were collected during the breeding season in between 2017-2021.

Species	Δ log likelihood	χ2	df	p	B	SE
M. ornata						
pH	105.17	0.51	1	0.343	0.12	0.16
WD	104.62	0.01	1	0.821	-0.01	0.06
WT	106.20	1.53	1	0.197	0.00	0.00
RH	105.54	0.88	1	0.224	-0.01	0.01
SV	104.28	0.05	1	0.426	0.14	0.07
D. melanostictus						
pH	103.36	2.37	1	0.051	0.29	0.16
WD	103.05	2.97	1	0.064	-0.10	0.05
WT	106.72	4.73	1	0.015	0.00	0.00
RH	101.96	0.97	1	0.200	-0.01	0.01
SV	103.01	2.30	1	0.052	-0.07	0.04
E. cyanophlyctis						
pH	24.21	8.68	1	0.003	1.01	0.30
WD	20.52	5.10	1	0.012	-0.18	0.12
WT	14.59	0.07	1	0.674	0.00	0.00
RH	18.21	2.70	1	0.040	0.13	0.11
SV	21.25	4.12	1	0.034	1.01	0.13

DISCUSSION

Our findings demonstrated the importance of water depth and pH in determining the abundance of anuran larvae in the study sites. These findings are consistent with the previous reports on anuran tadpoles, and the species found in this area appear to exhibit similar broad patterns (Rout et al., 2019). An important ecological factor that affects the survival and distribution of amphibian populations in nature is the pH level of the water (Gosner and Black, 1957; Saber and Dunson, 1978; Freda and Dunson, 1986). According to Pierce (1985), species that reproduce in alkaline waters are extremely susceptible to habitat acidification. The current findings demonstrated that the pH of the breeding sites of the tadpoleswas (5.7-7.8), supporting the claims made by Pierce (1985) and Freda and Dunson (1986) that amphibians as a group experience increasing mortality at pH 5 and below. Additionally, higher variations in pH values are anticipated in the near future as concerns from global changes, which locally result in comparatively rapid desertification and consequently related environmental factors, are becoming more and more significant (Zolotokrylin et al., 2016).

Amphibians opt their oviposition sites based on the water depth or the water holding capacity (Rudolf and Rödel, 2005). Embryos and tadpoles grow and develop more quickly in shallow environments since water depth and temperature are often inversely related (Skelly et al., 2002). The selection of anuran breeding sites has been shown to be significantly influenced by the thermal characteristics of the spawning water (Skelly et al., 2002; Ficetola and Debernardi, 2004). Tadpoles made their breeding site decision mostly based on the water temperature at the time of oviposition. Due to the larger concentrations of dissolved oxygen that are generally present in cooler water, adults may choose to oviposit in such habitats (Dougherty et al., 2005). Since tadpoles prefer to attach their egg strings between submerged objects and hide in the foliage to protect themselves from predators, submerged vegetation was another important predictor of spawning sites.

The sensitivity of *D. melanostictus* and *M. ornata* tadpoles to pH is probably related to regional fluctuations in the environment. The combined effects of environmental factors can also lessen the effects of local adaptations to the environment. There may be a connection between *E. cyanophlyctis*'s substantial response to pH, water depth, and submerged vegetation. Future research may correlate these patterns to additional factors, particularly heterogeneity between groups in terms of genetic connectedness and landscape connectivity. Intra-clade plasticity might be associated with environmental adaptability and, thus, with the capacity to adjust to climate change.

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