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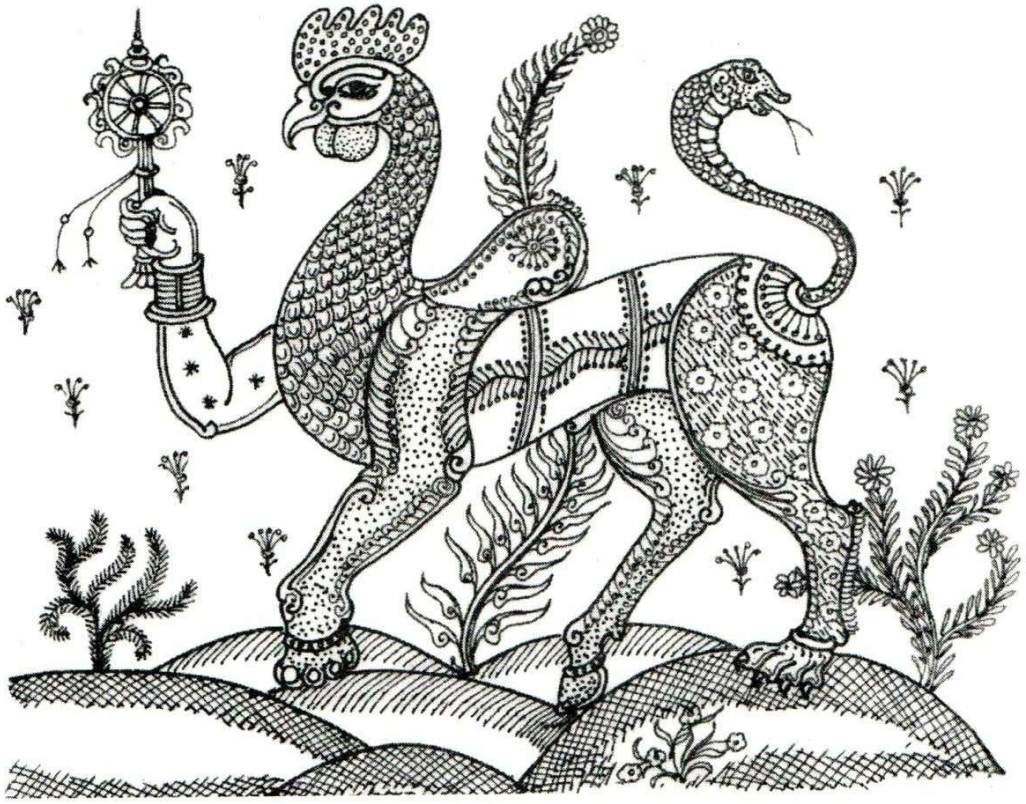

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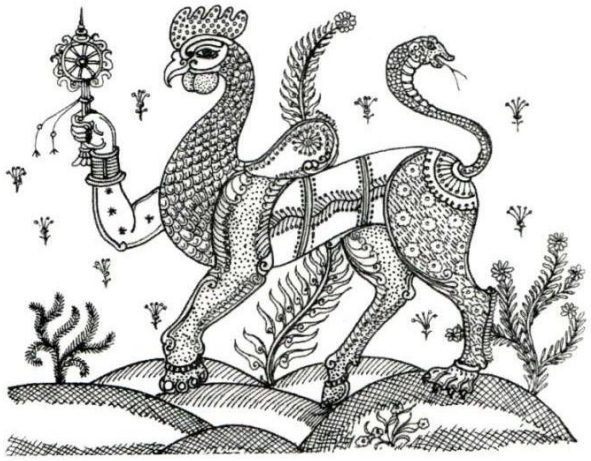
The emblem of Pranikee



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 XXXIV) carries five research articles covering different aspects of Zoology. The first article describes prevalence and severity of menopausal symptoms and anxiety in peri- and post-menopausal tribal women of northern Odisha**.** The second article covers the behavior of Asian elephant (*Elephas maximus indicus*) in free range Kuldiha wildlife sanctuary of the state of Odisha. Distribution of narrow headed soft-shell turtle, *Chitra indica* in the delta of river Mahanadi and its threats has been deliberated in the third article. Effects of urea fertilizer on some developmental stages of the common Asian toad, *Duttaphrynus melanostictus* inhabiting ephemeral pools of Mawsynram, Meghalaya during their breeding period has been presented in the fourth article. The concluding entitled describes retinoic acid and regeneration of skeletal muscles during tail regeneration in the tadpoles of the Indian tree frog, *Polypedates maculatus*. I hope the present volume will contribute to the growing knowledge in Zoology.

PK Mahapatra

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CONTENT

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PREVALENCE AND SEVERITY OF MENOPAUSAL SYMPTOMS AND ANXIETY IN PERI- AND POST-MENOPAUSAL TRIBAL WOMEN OF NORTHERN ODISHA

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ABSTRACT

Menopause is one of the inevitable phases of women's life. People talk less about the difficulties associated with menopause for various social and psychological reasons. However, due to increased life expectancy, women spend about one-third of their life in menopausal phase. Hence, people have started taking interest in the study on menopause plights and their in-time management. Thus, the present study aims at assessing the extent and severity of menopausal symptoms and mental health prevailing menopausal women. 118 peri- and 136 post – menopausal women gave their consent for the study. Data on sociodemographic aspects were collected using a pretested sociodemographic questionnaire. The incidence of menopausal symptoms among participants were evaluated using Menopausal Rating Scale (MRS). Anxiety was assessed with Hamilton Anxiety Rating Scale (HAM-A). Chi-square test, odd ratio, Mann -Whitney tests was carried out in SPSS version 20 for computing and comparing the data. Study results revealed that significantly higher post-menopausal women suffered from vasomotor and urinogenital problems whereas peri-menopausal women encountered severe psychological problems as assessed by MRS and HAM-A scale.

Keywords: Peri-Menopause, Post-Menopause, HAM-A, MRS, Anxiety

INTRODUCTION

Menopause is one of the predictable and unavoidable phases in women's life. It brings many undesirable physical and psychological changes in females. People talk less about problems of menopause due to various reasons and therefore, complications associated with menopause got ignored for quite a long time. The representational measurement of menopause, which marks the cessation of reproductive life further strengthens with the feeling of unwantedness from family and social front (Mendonsa and Appaya, 2010; Rajkumari et al., 2021). Women who have taken the utmost pride for their reproductive feature considers menopause as the termination of their career as women. They consider it as a deadly assault on their self-esteem (Hay et al., 1994; Rajkumari et al., 2021). It thus, leads to hypochondriasis, irritability, depression, sleeping disorders, mood swings etc.(Rajkumari et al., 2021).

Menopause, in terms of endocrinology can be defined as the termination of ovarian functionality to produce estrogen and continue reproductive cycle (Munro, 1969; Rajkumari et al., 2021). In women, menopause arrives in middle age and continues. Perimenopause is the time of transition into menopause and is defined as the years usually of a duration of 2-8 years preceding menopause and twelve months spent after the final menstrual period whereas the post - menopause is defined as permanent cessation of menstrual periods for more than twelve consecutive months in the absence of pregnancy and lactation (Harlow et al., 2012; WHO, 1996a, 1996b).

During peri-and post-menopause, changes in female sex hormones fetches psychosocial stress which initiates psychological complaints like anxiety, depression, mood swings, sleeping disorders and others. Till date there is a

great debate on establishment of relationship between psychological problems and menopause and thus has been considered to be one of the most argumentative issues in menopause research (Discigil et al., 2006; Rajkumari et al., 2021).

Tribal women have been considered to be one of the backward sections of our society with a low literacy rate and socio-economic status. They hardly seek any modern medical help for the management of menopausal problems due to unawareness, low educational and economic status, and various socio-cultural taboos. Hence, this study has been taken up to assess the incidence of menopausal symptoms and their severity and to estimate the level of anxiety in the tribal women of Mayurbhanj district, Odisha, India.

MATERIAL AND METHODS

Study Design

A total of eight villages of four subdivisions of Mayurbhanj district of Odisha, India were chosen for the study. The details of the research sites have been given in Table 1 and Figure 1. One hundred and eighteen peri-menopausal and 136 post-menopausal tribal women, belonging to Santal, Bathudi, Khadia and Mankidia tribes ageing between 40 -65 years participated in the study. Face-to-face interview were conducted after receiving a written consent from them. Sociodemographic attributes were collected employing a pretested sociodemographic questionnaire and incidence of menopausal symptoms were measured using the Menopause Rating Scale (MRS) questionnaire (Khatoon et al., 2018; Rahman et al., 2010). MRS is one of the popular and most tested instruments to measure incidence of menopausal symptoms among women and have been used for various menopause related studies all across the globe (Khatoon et al., 2018; Rahman et al., 2010). The MRS score ranges from 0 - 44. Asymptomatic women score <11 whereas women with mild to moderate menopausal symptoms score between 12-35 and severely affected women score > 36 on MRS scale. Hamilton Anxiety Rating Scale (HAM-A) was used to measure the level of anxiety (Chojnacki et al., 2015; Maier et al., 1988). The HAM-A questionnaire consisted of fourteen questions. Each question was scored on a 5-point Likert Scale ranging from 0 (symptom not present) to 4 (presence of very severe symptom). A total score per individual was calculated to estimate the severity of anxiety. The scores ranged between 0 – 56. Scores > 17 indicates mild anxiety, scores between 18-24 specifies mild to moderate level of anxiety and score between 25 -30 specify moderate to severe anxiety (Maier et al., 1988). Both MRS and HAM-A questionnaire were translated to Odia for better understanding by tribal women.

Table 1. Table Shows the Details of Sub-divisions, Blocks, Villages and Types of Tribes taken for the Study

Sub-Division	Block	Village	Tribe
Kaptipada	Khunta	Baniabasa	Mankidia and Kharia
	Kaptipada	Sagunabasa	Mankidia and Kharia
Baripada	Suliapada	Bhandarisole	Santali
	Suliapada	Patharnesa	Lodha
Bamanghati	Kusumi	Burudi	Santali
	Kusumi	Timtima	Santali
Panchpir	Jashipur	Tainsira	Mankidia and Kolha
	Jashipur	Ektali	Santali and Bathudi

Ethical Permission

The study was conducted between December 2018 to February 2020 with due approval of Ethical Committee of Maharaja Sriram Chandra Bhanja Deo University (Erstwhile North Orissa University), Odisha, India (IEC No. NOU/IEC/017, 7th March 2018).

Statistical Analysis

Statistical analysis was carried out using IBM SPSS statistics version 20. Quantitative data were computed as means and standard deviation. Kolmogorov Smirnov test was used to perceive the distribution pattern of the data. Descriptive statistics, Chi-square and odd ratio test were employed for computing mean and standard deviation of the sociodemographic and menopausal symptoms. Difference in the severity of menopausal symptoms and anxiety level of peri- and post-menopausal women were calculated using Mann-Whitney test.

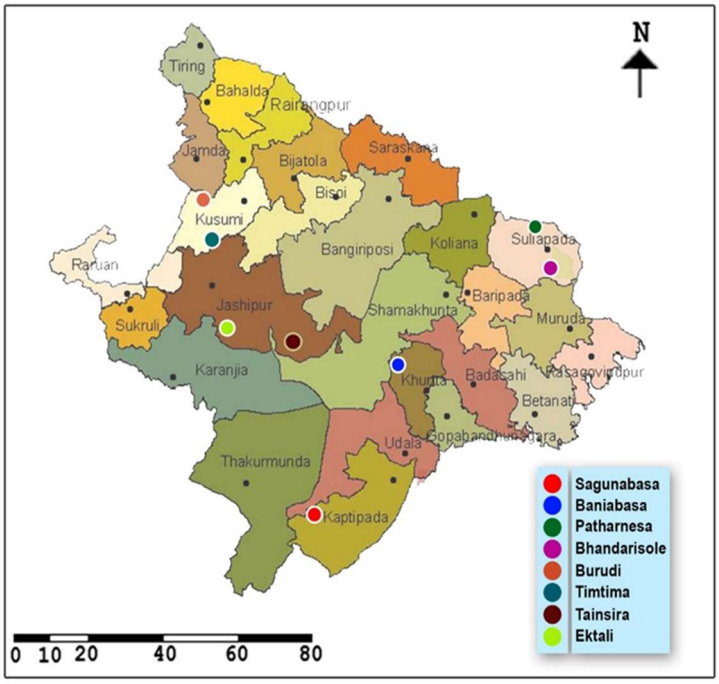

Figure 1: Map showing 26 Blocks and Villages of Sample Population drawn for the Study of Mayurbhanj District of Odisha, India.

RESULTS

Information regarding the average age of respondents in peri- and post-menopausal groups along with the average age of menarche, marriage and menopause; educational, marital, employability, socio-economic status and residence has been presented in table 2. Mean age of peri- and post-menopause women were found to be 39.33 and 54.26 years, respectively. Around 40% in peri-menopausal women and 63% post-menopausal women were reported to be uneducated. Likewise, ~12% peri-menopausal women and 2% post-menopausal women were having professional jobs. It was recorded that > 55%women of both groups lived under poverty line. Likewise, around >57 % women in both groups resided in urban areas (Table 2). Table 3 shows the extent of menopausal symptoms occurring in women of both the groups. Comparing the prevalence of somatic symptoms between peri- and post-menopausal women, occurrence of heart disorders, as well as muscular and joint pain was significantly higher in post-menopausal group whereas incidence of hot flushes was significantly more in peri-menopausal women. Likewise, frequency of irritability and anxiety was significantly higher in peri- menopausal women. However, in urinogenital domain, both bladder and sex-related problems in post-menopausal women was higher than their corresponding counterparts. The odd ratio revealed that the incidence of heart discomfort, muscle and joint pain, mental and physical exhaustion, and bladder to be 2-3 times higher in post-menopausal women. Sex-related problems in post-menopausal women was about 7 times higher than peri-menopausal (Table 3).

Table 2: Table shows the Sociodemographic Characteristics of Respondents

Sl. No.	Sociodemographic Parameters of the Participants	Tribal (total n= 265)	
		Peri-Menopausal (Total n = 118) (Mean ± SD)	**Post- Menopausal (Total n=136) (Mean ± SD)**
1	**Age**	39.33 (7.10)	54.26 (6.97)
2	**Average Age of Menarche (in Years)**	13.47 ± 1.21	13.72 ± 1.28
3	**Average Age of Marriage (in Years)**	19.18 ± 2.04	18.05 ± 2.24
4	**Average Age of Menopause (in Years)**	Not yet attaine menopause	46.16 ± 1.27
5	**Educational Status**	**Peri-Menopausal Number (%)**	**Post- Menopausal Number (%)**
	No formal Education	42 (35.59)	86 (63.23)
	Primary Schooling	32 (27.11)	23 (16.91)
	Secondary	18 (15.25)	19 (13.97)
	Graduation and Higher	26 (22.03)	8 (5.88)
6	**Marital Status**		
	Married	97 (82.20)	102 (75)
	Widow/Divorcee	17 (14.40)	34 (25)
	Single	4 (3.38)	0 (0)
7	**Employment Status**		
	Professional Engagement	14 (11.86)	3 (2.20)
	Semi-Skilled / Skilled Worker	73 (61.86)	44 (32.35)
	Unemployed/Homemaker	31(26.27)	89 (65.44)
8	**Socio Economic Status**		
	Lower [Below poverty line (BPL)]	67 (56.77)	89 (65.44)
	Middle Income Group (between 5 lakhs to 25 lakhs per annum)	49 (41.52)	47 (34.55)
	Higher Income Group (above 25 lakhs per annum)	2 (1.69)	0 (0)
9	**Residence**		
	Urban	69 (58.47)	88 (63.23)
	Rural	49 (41.52)	48 (35.29)

Table 3. Comparison of menopausal symptoms in different domains of Menopausal Rating Scale between peri- and post-menopausal groups of women

MenopausalSymptoms (Subscale)	Peri - Menopause (118) Number (%)	Post-Menopause (136) Number (%)	Chi Square	OR with CI. (Lower-Upper)	p-value
Somatic (Vasomotor)					
Sweating/ Hot Flushes	76(64.40)	36(26.47)	36.886**	0.199(0.116-0.340)	<0.001
Heart Discomfort / Palpitation	31(26.27)	64(47.05)	11.661**	2.495(1.468-4.240)	<0.001
Sleeping Problems	61(51.69)	71(52.20)	0.007	1.02(0.623-1.672)	NS
Muscular and Joint Pain	98(83.05)	126(92.64)	5.586*	2.571(1.151-5.744)	<0.05
Psychological					
Depressive Mood	41(34.74)	53(38.97)	0.484	1.199(0.719-2.001)	
Irritability	53(44.91)	42(30.88)	5.314*	0.548 (0.328-0.916)	<0.05
Anxiety	58(49.15)	57(41.91)	1.337	0.746 (0.454-1.226)	
Physical and Mental Exhaustion	88(74.57)	117(86.02)	5.323*	2.099(1.109-3.972)	<0.05
Urinogenital/Sexual					
Bladder Problems	38(32.20)	74(54.41)	12.641**	2.513 (1.505-4.196)	<0.001
Sexual Problems	46(38.98)	111(81.61)	48.653**	6.950 (3.929-12.291)	<0.001
Vaginal Problems	86(72.88)	103(75.73)	0.27	1.161 (0.661-2.042)	NS

*** Significant difference in the occurrence of symptoms between peri- and post-menopausal women * p<0.05, **p<0.001, NS- Not significant**

About 79% peri-menopausal women and ~71% post-menopausal women had mild menopausal symptoms (Table 4). The occurrence of moderate level of menopausal symptoms among post-menopausal women were found to be high as compared to peri-menopausal women. In this study only one participant of post-menopausal group showed severe menopausal symptoms.

Table 4. Severity of Menopausal Symptoms according to Menopause Rating Scale

Factor (Severity level of MRS)	Peri- Menopause (n=118) Number (%)	Post - Menopause (n = 136) Number (%)	Chi-square	p - value
<11(Mild)	79(66.94)	71(52.20)	147.61	<0.001***
12-35 (Moderate)	39(33.06)	64(47.05)		
>36 (Severe)	0(0.00)	1(0.75)		

signify occurrence of significant difference in the occurrence of symptoms between severity factors among peri- and post-menopausal women ***p<0.001

Information about the level of anxiety that persisted in peri- and post-menopausal women is presented in Table 5. A significantly higher percentage of peri-menopausal women exhibited moderate to severe anxiety level in comparison to post-menopausal women. However, higher percentage of post-menopausal women showed mild to moderate anxiety level (Table 5).

Table 5. Level of anxiety in Peri – and Post-menopausal women

Anxiety Level [Scores of HAM(A)]	Peri-Menopause (118)	Post-Menopause (136)	Chi-square	p - value
	Number (%)	Number (%)		
Mild (<17)	12(10.16)	3(2.20)	78.424	<0.001***
Mild to Moderate (18-25)	40(33.89)	78(57.35)		
Moderate to Severe (>26)	66(55.93)	55(40.44)		

DISCUSSIONS

The demographic profile of peri- and post-menopausal women in this study is consistent with the sociodemographic profiling of other such studies conducted in different states of India and all across the globe (Khatoon et al., 2018; Rahman et al., 2010; Satpathy and Lenka, 2020). Menopause Rating Scale has been used as an important instrument in the assessment of menopausal symptoms and its severity in pre, peri- and post-menopausal women. In our study, we recorded the prevalence of muscle and joint pain as well as heart discomforts more in post-menopausal women as compared to peri-menopausal women (Table 5). Such results have been obtained due to the fact that in post-menopausal women, low serum oestrogen level exerts a restraining effect on bone turnover and contribute in loss of bone and calcium intake which in turn causes calcium depletion in the body leading to manifestation of arthritis, osteoporosis, osteopenia (Heshmati et al., 2002). Reason of heart discomforts might be due depletion of Vitamin D and calcium in menopausal women which arises because of an increase level of anxiety (Alkhatatbeh et al., 2019).

Very often it is found that in peri- and post-menopause hot flushes initiates trouble in sleep which leads to insomnia and further starts inducing anxiety, depression and irritability (Dar et al., 2020; Terauchi et al., 2012). Prevalence of such symptoms varies from 35% to 94% in peri- and post-menopause groups (Borker et al., 2013; Karmakar et al., 2017; Lenka, 2016; Sarkar et al., 2014). A study on tribal women of West Bengal, India reveals that around 40% of women suffer from hot flushes (Dasgupta et al., 2015; Kalarhoudi et al., 2011). Likewise, Chowta et al., (2008), Kaulagekar, (2011) and Poomalar and Arounassalame, (2013) reported that around 89%, 80%, and 70% of women encountered hot flushes in their menopausal phase. Correspondingly, Mahajan et al., (2012), Sarkar et al., (2014), Lenka, (2016), Karmakar et al., (2017) have reported that hot flushes are common in women undergoing menopause, and their incidence varies between 47% to 80%. It has been observed that ~ 60% of women experience hot flushes for less than 7 years from the attainment of menopause. However, in 15% it has been reported to persist up to 15 years or more (Kronenberg, 1990). Reports from several studies indicate that the quality of life of around 20% to 25% of female individuals becomes deprived because of somatic disorders (Schoebi and Randall, 2015; Utian, 2005; Whiteman et al., 2003).

Incidence and severity of urinogenital problems such as bladder problems and sexual problems were recorded to be significantly higher in the post-menopausal women as compared to their corresponding peri-manopausal counterparts. However the difference in vaginal problems in these two groups were not significant. In post-menopausal phase, it has been reported that synthesis and release of oestrogen and other female sex hormone deplete which brings atrophy of the oestrogen receptors present in the urinogenital tract of the females. This results in vaginal dryness, itching sensation and decrease interest for sexual activities (Iosif and Bekassy, 1984; Oriba and Maibach, 1989; Reid et al., 2014).

Findings of several related studies have enlightened that difference in menopause severity and type is depended upon racial differences, variances in genetic makeup, environmental factors, diet and other social aspects (Masjoudi et al., 2017). Chuni and Sreeramareddy (2011), Masjoudi et al., (2017) reported that women experience menopause symptoms caused by oestrogen deficiency in a different way and fluctuates from one country to other. Variation in the results of current study from the results of other studies done earlier might be due to diverse socio-cultural and social-underlying features of race, people's perception on menopause, difference in size of sample, design of the study and type of instruments used (Masjoudi et al., 2017; Norozi et al., 2013; Tehrani et al., 2002; Waidyasekera et al., 2009).

Employing the Hamilton Anxiety Scale (HAM-A) for assessment of frequency of anxiety and to compare between peri- and post-menopausal groups, it was found that incidence and severity of psychiatric disorders including depression and anxiety scores were more prevalent in peri-menopausal women as compared to post-menopausal conditions. Our results corroborate with that of Mohamed et al., (2015), Dennerstein et al., (2004) and Gyllstrom et al., (2007). Mohamed et al., (2015) attributed these problems to poor personal health, social stigma and taboo, daily hassles, and stressful circumstances both from family and societal front leading to psychological problems such as mood change, depression, and anxiety. Psychological changes in peri-menopausal women have been correlated to the hormonal changes causing disturbances in biological mechanisms involving dysregulation of the hypothalamic-pituitary-adrenal axis (Ghianda et al., 2014; Mohamed et al., 2015). In the present study, we recorded more hot flushes, vaginal dryness, anxiety, depression, irritability along with sleep disorders among peri-menopausal women. Intensification in the incidence of all such symptoms have been suggested to be related to sleep disturbances (Greene and Cooke, 1980; Schmidt et al., 2004; Slopien et al., 2020).

The peri-menopausal period of women's life is an important transitional stage and full of high-risks. Incidence of menopausal symptoms and their severity increases and becomes more challenging when stress components like personal illness, financial crisis, loss of close relatives, social burdens, and children leaving home are added up with the hormonal and physiological changes (Alkhatatbeh et al., 2019; Mohamed et al., 2015). Thus, proper assessment of physical and mental health status of peri-menopausal women should be conducted and medical intervention is required for a quality life during their transitional phase.

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BEHAVIOUR OF ASIAN ELEPHANT (*ELEPHAS MAXIMUS INDICUS, LINN.)* IN FREE RANGE KULDIHA WILDLIFE SANCTUARY, BALESHWAR, ODISHA, INDIA

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ABSTRACT

Behaviour of the free ranging Asian elephants were studied for a period of two years (20 March 2019 to April 2021) in the Kuldiha Wildlife Sanctuary (KWLS), Baleshwar, Odisha, India.The data were collected by both direst sighting and indirect observation. Besides, data from field staff and local community were collected for the investigation. During this study period major behaviours detected include movement, consumption of food and water, mating, bathing and swimming, resting and sleeping, defensive and aggrasive and parental care of herds. Findings of this research is suggested to contribute towards developing predictive models for a better human-elephant interactions in the future.

Keywords: Asian Elephant, KWLS, Movement, Defensive, Interaction

INTRODUCTION

A natural organism's home range is determined as magnitude of the region that it covers yearly inside its native environment (Alfred et al., 2012). Home range distribution is purely based on the availability of fodder plant species (Sukumar, 1989a,b; Alfred et al., 2012). As majority of fodder plants are classified as endangered category, there is growing worry that the elephant's home range degradation will have unintended and catastrophic repercussions for the long-term sustainability of ecological processes (Joshi, 2009). A variety of communication methods have been developed including acoustic, chemical, visual, tactile and seismic by the elephants for communication between their herds (Herler and Stoeger, 2012). An elephant activity budget is the sum of all the activities an elephant takes part in or is exposed to perform during the year (McKay,1973; Baskaran, 1998; Vanitha and Baskaran, 2010; Samarasinghe and Ahmad, 2016). Animal degree of functionality, along with the energy costs and performance behaviors, can be used to determine a population' energy consumption. It is dependent on the ecosystem as well as climatic conditions like precipitation and temperature (Samarasinghe and Ahmad, 2016). Understanding wild animal behaviour is an effective conservation technique that may improve the controlling and preservation of any vulnerable species from the verge of extinction. Female-led social groupings might be a technique for enhancing the safety of their young by allomothering activity (Gadgil and Nair, 1984, Vidya and Sukumar, 2005). African elephant populations have more adult females than Asian elephant herds, and African elephant females are thought to be more sociable than Asian elephant females (De Silva and Wittemyer, 2012; Petraccione et al., 2017). In contrary to the diverse groups of African elephants (Fernando et al., 2000; Shaffer et al., 2019) and their greater relationship indices of > 0.6, research of wild Asian elephants have shown that interactions is confined within immediate female lines and have linkage indices of 0.3 (De Silva et al., 2011; Petraccione et al., 2017).

MATERIALS AND METHODS

Study Area

In the Indian state of Odisha, the Kuldiha Wildlife Sanctuary (KWLS) (21.4096° N, 86.7335° E) is located in the Balasore district. This sanctuary is within the Chota Nagpur Plateau area and covers 272.75 Sq. km^2. The Sukhupada and Nato hill ranges associate it to Similipal Biosphere Reserve. The whole area is known as Mayurbhanj Elephant Reserve, which encompasses the game sanctuaries of Similipal, Kuldiha, and Hadgarh. Tenda Elephant Reserve is the elephant reserve's traditional term in Kuldiha. The Sal (*Shorea robusta*) tree is the dominant species in this mixed deciduous woodland, other than *Aegle marmelos* (Bel), *Careya arbore* (Kumbhi), *Bauhinia racemose* (Jangalee Kanchan), *Kydia calycina* (Banakapasia), *Bauhinia vahli* (Siali), *Helicteres isora* (Orola), *Madhuca indica* (Mahulo), *Zizyphus mauritiona* (Bara Koli), *Artocarpus heterophyllus* (Panasa), *Limonia acidissima* (Kendu) (Panda and Behera, 2021). Faunal diversity of this sanctuary includes Leopard, Elephant, Wild Boar, Gaur, Sambar, Giant Squirrel, Hill Myna, Peafowl, Parakeet, Malabar Trogon, etc. Several migratory birds and reptiles are among the species that live in the protected area. The annual precipitation ranges from 1565 MM/ year. The annual temperature ranges between 24° to 26° Celcius, but during summer temperature ranges from 34°-42° Celcius.

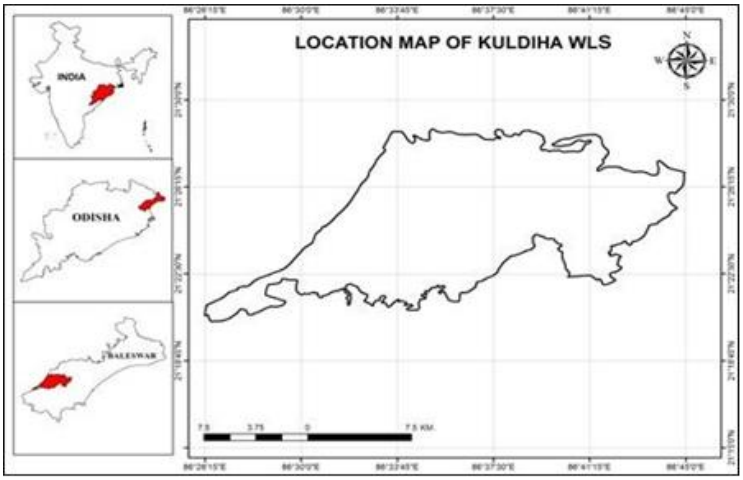

Figure 1 Study Area Map

Methodology

All of the observational data took place between October 2018 to December 2020. Due to the deep vegetation and misty environment, it was difficult to see elephants in densely wooded settings, particularly during the monsoon and winter seasons. Due to thick tropical foliage and the existence of hilly slopes with shrubs greater than the elephants, it was impossible to locatedirectly. Further to avoid any casualties both direct and indirect approaches were adapted.

Indirect Method

In a few cases, the indirect count approach (Dawson and Dekker, 1992; Ramakrishanan et al., 1991; Santiapillai and Suprahman, 1986; Joshi, 2009) was used to determine the number of elephants in the research region. This entails counting the number of elephant signs on the trail and the regularity with which they appear. All data collection was conducted from a vehicle using the road-strip count method (Santiapillai et al., 2003; Joshi, 2009) to track elephant population structure inside the forest.

Direct Method

The elephants could not be seen during the rainy (monsoon) since the regions were overgrown with thick grass species and seasonal water bodies. Thus, the summer and winter season were the ideal time to study them, specifically close to water bodies. In the present research the study area was visited on a weekly basis with elephant

sightings performed along forest trail. A few other related rocky paths that connect the grassland ecosystem with a motorable road were also surveyed. While some creatures were seen up to a distance of 100 meters, the majority of the sightings were made within 50 meters. In addition, all prospective habitats (water dominated regions, cool shaded areas, fodder enriched areas, and rough forest pathways) were examined on foot in the early morning, mid-day, and evening hours. Cool-shaded trees and other favorite forage species were studied mostly during the midday during the month of March-June, when elephants are most active. During the overnight hours, all water sources (perennial/annual) were explored in alternate days. Field binoculars were utilized to observe their range of motion from a respectable distance without displeasing the animal.

RESULT AND DISCUSSION

Inter Range Movement and Migration Behaviour

One of the most significant ecological characteristics of elephant's physiology is movement and migration from one place to another in search of food, water and breeding ground. To fulfill various bodily process seasonal movements is performed by KWLS resident elephants. Elephant of KWLS roam in whole area, but leave the parent ground to nearby villages in search of fodder and water. However, solitary bull may feed on same ground or move to nearby village in search of feeding. When the winter season begins in last week of October and there is a scarcity of fodder species inside forest land, elephants either a solitary male or ten to fifteen herds migrate to the nearby villages of Nilagiri Forest range and Soro forest range in search of paddy and other fruits and vegetable farmed by the local tribal community or other resident family. Incidence of free ranging elephants migrating during dry season (March-May) has also been evident. The Nilagiri Forest Range is situated in North East direction and the Soro Forest range situated in South East direction of the Kuldiha Wildlife Sanctuary. Many villages like (Tenda, Sajnagad, Gopalpur, Shyamsundarpur, Sindhua, Benagadia, Darkholi, Balimundali, Kamarapala etc.) are closer to the sanctuary. So, more cases of Human- Elephant conflict in form of crop field depredation, property damage, livestock and human death has been reported from the data of forest department (Annual Balasore Wildlife Division Report, 2021).. Another factor which attracts elephants towards human society is country liquor and rice brew which were stored by the tribal community. State Highway (SH-19) crosses through Kuldiha WLS to Tinkosia Reserve forest. In KWLS there is an inter district corridor which connects with Hadgarh Wildlife Sanctuary, Keonjhar. This corridor enables the passage of resident Kuldiha elephant and Hadgarh population. Few years back i.e. from 2010-2018 there was an illegal stone quarrying which was a major obstacle for their movement. It has been observed that all the incident of Human elephant conflict is due to the anthropogenic activity. Other prominent factor for the movement of elephant during summer is the forest fire which may be due to natural or anthropogenic reason. Forest fire damaged preferred fodder species which force the elephant to migrate from woods. But in rainy season, the migration towards human dominated landscape was reduced due to the availability of consumable foliage species in the forest.

Movement Behaviour

Elephants walk slowly by nature, yet they are capable of going quickly. Movements were very reliant on the activity they were engaged in. When an animal is feeding, it takes a sluggish walk, but when it is going towards waterbodies and passing a mountain road, it takes a quick walk. The trunk and tail positions are very varied in both the slow and rapid walk. Calves and juveniles move quickly owing to their playful temperament, chase one other and attempting

to capture their mothers while altering the track and raising their trunk and tail for a brief period. Adult animals only moved quickly when they were fleeing or being attacked.

During the survey period it was observed that mostly charging behaviour was exhibited by tuskers and this behaviour was more profound where numbers of calves were more in the herd. The tail was typically curved upward the trunk and coiled in between the forelegs. During the charge, the male elephant trumpeted. When elephants moved near human habitation zones, they walked faster, owing to anthropogenic disruptions and threats. When compared to groups and herds, herd migration was usually sluggish, but bull movement was enormous. Bulls created a wide seasonal area. This movement was dependent on the musth phenomenon in males, during which time their movements were more expansive as they sought to join groups and find potential females for reproductive purposes. The bull elephant ran at a speed of about 25-30 Km/Hr while the female elephant ran about 15-20 Km/Hr.

Consumption of Food and Water Behaviour

Elephants usually ate in the early morning and, more importantly, in the evenings, shortly before nightfall. They were spotted feeding in the middle of the day in the winter, but resting in the middle of the day in the summer seasons. Elephants have been seen eating constantly across the night time both during winter and summer seasons. In the summer, they slept in open woodland regions and moved into the deeper woodland as the day progressed. The elephants widely fed on the vegetal species like *Aegle marmelos* (Bel), *Careya arbore (Kumbhi), Bauhinia racemose* (Kanchan), *Kydia calycina* (Banakapasia), *Bauhinia vahli* (Siali), *Helicteres isora* (Orola), *Madhuca indica* (Mahulo), *Zizyphus mauritiona* (Bara Koli), *Artocarpus heterophyllus* (Jackfruit), *Shorea robust* (Sal), *Limonia acidissima (*Kendu*),* Bamboo species (*Bambusa vulgaris, Bambusa multiplex), Smilax zeylanica* (Muturi) and Diosporea species. They were fond of fruits of *Mangifera indica* (Mango) and *Artocarpus heterophyllus* (Jackfruit) in summer, bamboos (*Bambusa vulgaris, Bambusa multiplex*) during rainy and *Madhuca indica* (Mahula) during winter (Panda and Behera, 2021). Not all parts of fodder species were consumed by elephants, but some of the leftovers were consumed by other herbivores, indicating the associational behaviour of elephants with other organisms. This survey elucidated a total of 146 species of forest plants belonging to 55 families and 19 species of non-forest plants belonging to 8 families as the fodder species of elephants. Usually the twigs, bark, fruits and leaves of fodder plants were consumed by elephants.

It has been observed that elephants of KWLS drink water between early morning i.e., 4.00 AM to 6.00 AM and afternoon 2.00PM to 6.00PM. The drinking frequency behaviour is purely dependent over the presence of calf in their herd, temperature, humidity and other environmental factor. Numerous water holes are present inside Kuldiha for water consumption during dry season. Asian elephants drink and bath mostly in the mornings and evenings (Joshi and Singh, 2008), while elephants in the Wankie National Park (Africa) peak their drinking behavior at night (Shaffer et al., 2019).

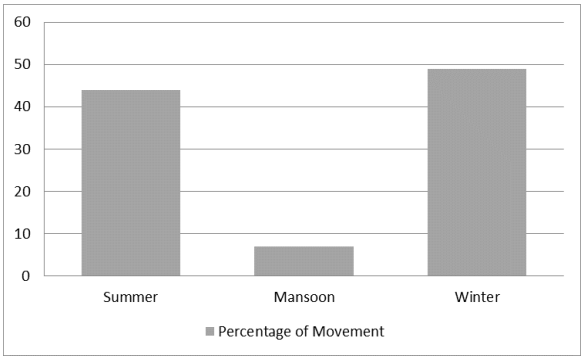

Fig. 2 Frequency of Migration in Different Season

Mating Behaviour

Elephants are social animals as we have observed that they always make their herd with 5 to 20 individuals depending upon the season (Laws *et al.,* 1975). The herd is led by the oldest female while males are solitary and spend their time alone in the forest. During the breeding season the bull returns back for mating with the female

partners (Sukumar, 2006: Joshi, 2009). The elephant performs its mating by the action of pheromones, which are the sex attractant basically act as magic bullet directly approaches the opposite partner for coietus, at a particular period of time that ranges from May to November, where various kinds of reproductive behaviour have been observed. The observed characters are like bull join with the group of herd where adult cow was present, after that various pre coitus sign are played between couple like, sniffing the dung, urine, genital and touching the genital or further coupulatory action. The whole copulation period is environment dependent, like during evening and nearby the water bodies, which is completed within 10-15 days. They copulate for 4-6 minute in a single mating action and in a day they copulate for 4-5 times. The entire process may be rapid and accomplished in a short period of time in younger bulls that are scared of grown-up leaders (Daniel, 1995). Musth is an important phenomenon in adult bulls during their reproductive periods, where the secretion from temporal gland has been observed which make them restless for 6 to 7 days. The copulation in general occurred where there were plenty availability of foliage species and water bodies. The key pre-contact boosting behaviour patterns such as trunk inspection, embracing, stroking the temporal area of bulls, and inhaling the feces have been described earlier (Nair, 1989; Joshi, 2009).

Fig. 3 Social Behaviour

Fig. 4 Solitary Bull Elephant

Fig. 5 Maternal Care

Bathing and Swimming

During our study period it has been observed that elephants enter into the water body during summer from 1400Hrs to 1900Hrs and generally spray water. We have observed that elephants submerged themselves in the water body with only their head and trunk visible above the water. Elephants also like to bath in dust and mud. During the summer season they like to crawl in muddy place and they frequently apply the dust over their body. They aslo cover their claves with dust. It has been observed that this peculiar activity is performed during the afternoon session when the temperature is comparatively higher ranging from 36°-44° celcius. The dust bathing has been described to protect them from heat and tiny flies (Joshi, 2009). Elephants are also capable of swimming in the high current of water (Joshi, 2009).

Resting and Sleeping Behaviour

Elephants frequently relax for many hours in the shade in close proximity to their foraging sites, particularly in hot seasons. No fixed resting place was observed in KWLS as the herd migrated from one part of the forest to the other. However, it was interesting to note that they took rest in the same preferred place wheneve they came back.

Defensive and Aggrasive Behaviour

Protective response amongst elephants was seen in the current study solely as a result of interference in any of their activities, particularly during their travels. Elephant herds are typically unafraid of danger, and if they sense or anticipate manmade disturbance, they first adjusted their course by gathering their calves. The leading female usually flaps her ears and trumpets loudly, notifying the other cows, who respond by forming a clump. Other adults and sub-adults follow her lead and create a closed or semi-circular defence configuration. It has been reported earlier that elephants are usually never violent inside the wild, but the fracturing of animal habitats has pushed them to alter their behavior from sociable to antagonistic. The mature males are naturally quite violent than females (Joshi, 2009). Elephants also exhibit the phenomena of "redirected aggressiveness" which is generally displayed by an item that develops anxiety at the same time (Oberoi, 1980; Joshi, 2009). Elephants' tactic is limited to the situation where the calves are present in the herd. Solitary bulls occasionally display defensive behaviour.

Parental Care

Elephants are extremely sociable creatures who exhibit parenting concern. Newborn infants are cared for 10 to 15 years by their elders, while the mother feeds and suckles the calf for another 2 to 3 years. Calf began to eat on smooth grass underneath the supervision of their elders at the same time. When a male elephant reaches the age of 16, he prefers a solitary existence and leaves the herd. Although all members of a community have equal responsibilities for the care of newly born offspring, mature cow elephants are much more cautious of calves than bull elephants. When the herds moves from one location to other their, calves were piloted by their mothers. The new born calves were guided by other family members along with their mothers. The calves prefer to move under the belly of their mothers. Whenever the calves wanted to move around and exhibit certain activities such as playing, swimming or crawling, they used to attract the atentation of their mothers or other members of the family.

Conflict of Intrest

Authors Declare no conflicts of intrest.

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DISTRIBUTION OF NARROW HEADED SOFT SHELL TURTLE *CHITRA INDICA* (GRAY, 1831) (TESTUDINES, TRYONICHIDAE) IN MAHANADI DELTA, ODISHA, AND ITS THREATS

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ABSTRACT

The endangered Indian Narrow Headed Soft Shell Turtle *Chitra indica*, native to Indian subcontinent, is found in major rivers and their tributaries in Odisha. Over the past few decades, the population of *Chitra indica* has declined due to exploitation for the consumption of its meat and eggs, fishing activities, habitat destruction resulting from illegal sand mining, urbanization, and water pollution. In the present study, an attempt has been made to study the distribution of the *Chitra indica* in the delta region of the Mahanadi River, specifically in three rivers, Chitrotpala, Paika, and Devi, which are the distributaries of the Mahanadi River system of Odisha. Existing threats to this species have also been studied through a questionnaire survey among local people, the fisherman community along with regular visits to the study locations. The findings indicated the illegal capture of this turtle by local communities across the Mahanadi Delta. Awareness of its ecological importance among the local population with the support of the environmental and eco-groups is the need of the hour along with the strict enforcement of legal provisions on the trade and marketing of this rare species of freshwater turtle.

Keywords: Paika, Chitrotpala, Devi River, Turtle trade, Balichhatri

INTRODUCTION

The Indian Narrow Headed Soft Shell Turtle *Chitra indica* (Gray, 1831) (Testudines: Trionychidae) is by far the largest among Indian freshwater turtles. It is also known as the small-headed softshell turtle or the Indo-Gangetic softshell turtle. It is an endangered species (IUCN, 2022) of softshell turtle native to waterways and rivers of the Indian subcontinent. Mostly aquatic, this species is widely distributed in the Indian subcontinent from Pakistan through India to Bangladesh and Nepal (Das and Singh, 2009). In India, this species is found in major river systems (the Indus, Ganga, Godavari, Mahanadi, Satluj, and Indus Rivers (Das and Singh, 2009). Its distribution in Peninsular India is localized and patchy but extensive, which is possibly the result of its highly specialized requirement for habitat (Das and Singh, 2009). In a review article on distribution in Indian Peninsula, Webb (1981) found the records from Dhond, Pune, Maharashtra. A skeleton of *Chitra indica* from Coleroon River, Tamil Nadu has been preserved in Madras Govt. Museum. This species has also been reported from the Chambal River in Rajasthan (Das and Singh, 2009) and rivers and tributaries of Jammu and Kashmir (Uetz and Hosek, 2021).

Chitra indica has been reported from the Brahmaputra plains of Assam and adjoining areas in lower eastern Himalayas, parts of Meghalaya and Mizoram in northeastern India (Das, 1990, 1996; Bhupathy et al., 1992; Frazier and Das, 1994; Choudhury, 1995; Datta, 1998; Sengupta et al., 1998; Pawar and Choudhury, 2000; Praschag and Gemel, 2002). This species was also reported from three Barak Valley districts of Cachar, Hailakandi and

Karimganj in Assam, north-eastern India, based on both live specimens and carapace records (Das and Gupta, 2011).

Indian narrow-headed softshell turtle (*Chitra indica*) has been recorded from the River Indus, Pakistan, and its tributaries (Akbar et al., 2006; Khan, 2006; Kanwal and Khan, 2018). In Bangladesh, this species occurs in all the major rivers, the Padma, Jamuna, Meghna, Bhiarab, Bramhaputra and Dholeswari (Khan, 1987; Rashid, 1991; Rashid and Swingland, 1997). In Nepal, populations of this species have been recorded from the Grewa River (Shrestha, 1996 a,b) and also from the central and eastern Terai of Nepal (Schleich and Kastle, 2002). The range of distribution also extends up to Mynamar and Western Malaysia (IUCN, 2022). In Odisha, India *Chitra indica* was recorded from the Mahanadi River at Satkosia Gorge, Kantilo, Kandarpur, Munduli area having a water depth of more than 40 feet (Mohapatra et al., 2009).

The body of *Chitra indica* is distinguished by the long narrow head with eyes situated close to the comparatively short proboscis. Carapace and head are olive grey in colour. Grey lines with black border radiates behind neck looking wavy reticulation pattern over the carapace. Plastron is creamish or white. The shell is oval, flattened, and soft with the presence of four plastral callosities. Between the first pair of costals, a single neural is present. Digits are completely webbed on the legs (Das and Singh, 2009). Adult males possess comparatively longer tails with thicker bases than females (Das and Singh, 2009). In females, the length of the tail remains within the carapace as compared to a male. They are oviparous, eggs are usually laid when the water level is low, and the shape of the egg is spherical. Females require sandy or sandy loam beaches to dig nests and lay eggs (Das and Singh, 2009). A purely freshwater species living in deep waters can achieve large size of up to 70 kg. (Behera et al., 2019). Females tend to achieve a greater overall size than males, reaching an overall mass up to 250 kg (Das and Singh, 2009; Uetz, 2015).

These are highly aquatic freshwater turtles living in deep river systems. The ideal habitat for *Chitra indica* is rivers of moderate to large size, preferably with sandy bottoms and low turbidity. *Chitra indica* buries itself in the sandy sediment of the rivers and spends most of the day submerged. Females require sandy or sandy loam beaches to dig nests and lay eggs (Das and Singh, 2009). They are carnivorous, and eat molluscs, fish, frogs, and macroinvertebrates. *Chitra indica* are ferocious ambush predators, it buries itself under the sand, and only the eye and proboscis remain above the surface of the sand. When a prey item passes by, usually a fish or other aquatic invertebrate, it shoots out its head and plunges at the prey (Das and Singh, 2009; Aryal et al., 2010; Ernst et al., 2015).

Within the past few decades, the population of *Chitra indica* has declined due to exploitation for the consumption of its meat and eggs, fishing activities, habitat destruction resulting from illegal sand mining, urbanization, and water pollution (Das, 2008; Mohapatra et al., 2009). In the present study, an attempt has been made to study the distribution of the *Chitra indica* in the delta region of the Mahanadi River, specifically in three rivers, Chitrotpala, Paika, and Devi, which are the distributaries of the Mahanadi River system of Odisha and threats to this species of turtle.

MATERIALS AND METHODS

Study Area

The river Mahanadi, 851 km long, arises at an altitude of about 450 m in the Kankar basin of Raipur District, Chhattisgarh, and flows into the Bay of Bengal. The drainage area of the Mahanadi extends over an area of 142000 km^2, spreading outside Odisha State into Chhattisgarh, Jharkhand, and Maharashtra. The river Mahanadi descends the hilly catchment to the west of Cuttack and starts branching to form the broad and flat deltaic plain (Mahalik et

al., 1996). The Mahanadi deltaic plain is divisible into two major regions: the upper, fluvial sector and the lower, marine-marginal sector. The fluvial sector occupies more than the western half of the delta plain and is primarily composed of sediments deposited by the river systems within the delta. The marine-marginal sector is a geomorphic belt running parallel and adjacent to the present-day shoreline (Mahalik et al., 1996). Below the delta head, the main Mahanadi channel divides into the Mahanadi on the north and the Kathjodi on the south (Fig. 1). These two are further divided own stream into many branches, which make up four active distributary systems. They are, from north to south: the Birupa system, the Mahanadi system, the Kathjodi-Debi system, and the Kuakhai system (Fig. 1). The present study on distribution and threats has been mostly from the Mahanadi system, the Kathjodi-Debi system, and the Kuakhai system (Mahalik et al., 1996).

Mahanadi system:

The main Mahanadi channel pursues an easterly course and forms a loop in the centre due to branching and reunion of branches. The major branches are the Chitrotpala, Nuna, and Paika (Fig. 1). After reunion of the branches, the Mahanadi flows as a single channel, deflects 90° to a north-easterly course parallel to the coast, and empties into the Bay of Bengal between Paradip and Hukitola (Mahalik et al., 1996).

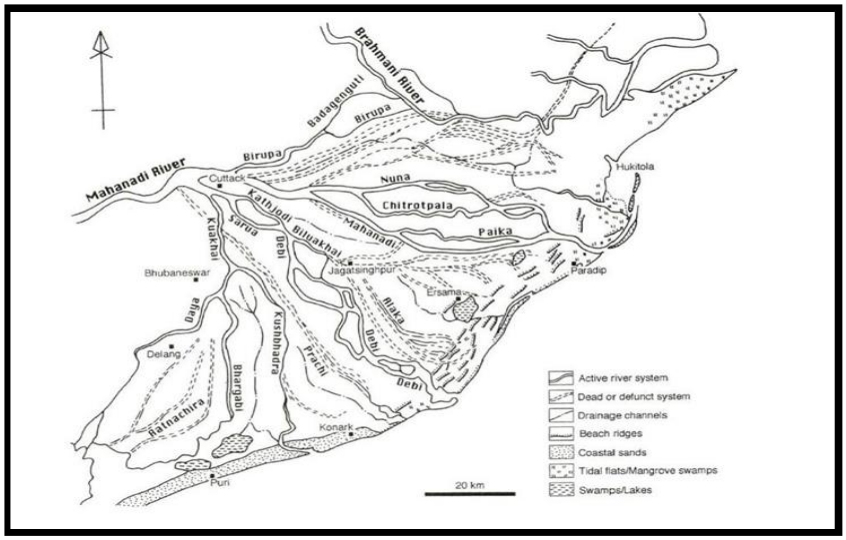

Fig. 1 Mahanadi Delta in Odisha, India (After Mahalik et al., 1996)

Kathjodi-Debi system:

This is presently the most active distributary system m the Mahanadi delta. The river Kathjodi, after separation from the Mahanadi and the Kuakhai, flows south-eastwards and splits into many anabranches which unite at many points downstream making many loops and enclosing channel islands. The major anabranches are the Sarua, Debi, Biluakhai, and Kandal, of which the Debi is the main channel of this system (Fig. 1). These channels ultimately join the Debi before draining into the Bay of Bengal (Mahalik et al., 1996).

Kuakhai system:

The river Kuakhai separates from the Kathjodi a few kilometres downstream of the delta apex, and branches into three distributaries: the Kushbhadra, Bhargabi, and Daya (Fig. 1). They flow in single channels without any anabranches. The Bhargabi and Daya drain into Chilika Lake, and the Kushbhadra into the Bay of Bengal. The Bhargabi River, after a southerly course, turns at right angles to the west, parallel to the coastal sandy belt, and flows along distance before debouching into Chilika Lake along with the Daya River. On the other hand, the Kushbhadra deflects about 900 m to the east from its southerly course before draining into the sea (Mahalik et al., 1996).

Methods

The study on Narrow Headed Soft Shell Turtle (*Chitra indica*) was carried out from October 2019 to December 2022 in Mahanadi Delta in three rivers i.e. Chitrotpala, Paika and Devi, which are the distributaries of the Mahanadi River system of Odisha.

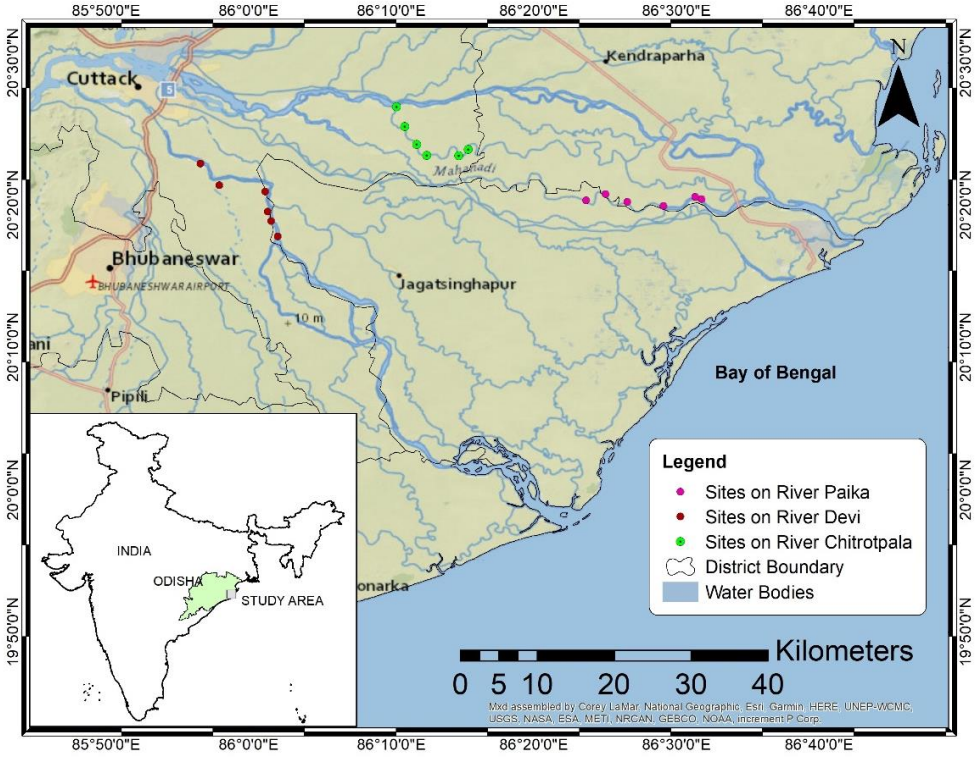

Fig. 2 Map of Mahanadi Delta, Odisha with *Chitra indica* sighting locations

Potential sites in these three rivers were visited and transect walks were carried out to observe turtles. Field surveys were carried out at selected six deepwater points each in the river Chitrotpala, Paika and Devi. The exact location and altitude of the area were recorded using GPS (geographical positioning system (Table 1). Depth of the river and width of the river were measured, from where Chitra indica were observed. River banks were surveyed during basking time from October to December. Turtles at a distance were observed through binoculars and whenever possible individuals were caught, photographed, and measured for future reference and released back (Ahmed and Das, 2010). The fishermen and local communities were interviewed by showing photographs of *Chitra indica*. A local network was developed in the study area and using a built network of fishermen, key local informants were identified through semi-structured interviews with residents living close to the river, helping to learn more about the species of turtles present there, and report any accidental hook-and-net catches along with crafts and gears if any used for catching freshwater turtles. Then photographs, measurement of the length and breadth of the carapace, and identification of sex were recorded through their help. Species identification was followed after Das (2002).

RESULTS AND DISCUSSION

In the Mahanadi Delta area, the Narrow Headed Soft Shell Turtles are locally called as Balichhatri. During the study period, a total of 18 sightings with six sightings each was observed in three tributaries, Paika River, Chitrotpala River, and Devi River of Mahanadi Delta from October 2019 to November 2022 (Fig. 2). Among the sightings in Paika River, the altitude varied from 3m to 8m (Mean ± SD : 5.67± 2.52m); with lowest in Naladiapalanda and highest in four sites, i.e. Gobardhanpur, Batria, Tikhiri and Mahanangala. The depth of the river varied from 4.572m to 7.62m (Mean ± SD : 5.945± 1.03m); with lowest in Gobardhanpur and highest in Naladiapalanda. The width of the river near turtle sighting varied from 400m to 1200 m (Mean ± SD : 700±282.84m), with lowest in Batria and highest in Naladiapalanda (Table 1).

Fig. 3 *Chitra indica* from Paika River at Tikhiri in Mahandai Delta, Odisha. A-B Dorsal Surface, C- Dorsal surface visible from the river water, D- Ventral surface

Fig. 4 *Chitra indica* rescued from Daya River Bed near Dhauli (close to Bhubaneswar city). A-B Dorsal Surface of a male specimen

In Chitrotpala River, among the six sightings, the altitudes varied from 8m to 17m (Mean ± SD : 12.33± 4.13m); the lowest in two sites Nageshpur and Narendrapur and highest in Babujanga. The depth of the river varied from 4.572m to 7.62m (Mean ± SD : 5.59±1.24 m), with lowest in three sites, Nageshpur, Manijanga and Narendrapur and highest in Babujanga. The width of the river near turtle sightings varied from 500m to 1000 m (Mean ± SD : 683.33± 248.33m), with lowest in three sites, Nemala, Nageshpur and Manijanga and highest in two sites, Barapada and Narendrapur (Table 1).

Table 1 Sightings of Narrow Headed Soft Shell Turtle *Chitra indica* in three tributaries (Paika, Chitrotpala and Devi) of Mahanadi Delta, Odisha, India.

Name of the Place	Date of sighting	Latitude	Longitude	Altitude (m)	Depth (m)	Width (m)
PAIKA RIVER						
Kathakota	10.10.2019	20.34587976º N	86.41719247º E	6.0	4.572	700
Gobardhanpur	18.10.2019	20.33854388º N	86.39454523° E	8.0	5.182	600
Batria	23.10.2019	20.33673951º N	86.44275245º E	8 .0	6.1	400
Tikhiri	03.11.2019	20.33226961º N	86.48469812° E	8 .0	6.1	500

Mahanangala	16.11.2019	20.34003474º N	86.52929842º E	8 .0	6.1	800
Naladiapalanda	27.11.2019	20.34258104º N	86.52209993º E	3.0	7.62	1200
Mean ± SD				5.67± 2.52	5.945± 1.03	700± 282.84
CHITROTPALA RIVER						
Barapada	09.10.2020	20.44861174º N	86.17301603º E	15.0	6.1	1000
Nemala	18.10.2020	20.42535421º N	86.18228724° E	17.0	6.1	500
Nageshpur	23.10.2020	20.40424308º N	86.19642809º E	8.0	4.572	500
Babujanga	07.11.2020	20.39148905º N	86.20796897º E	16.0	7.62	600
Manijanga	12.11.2020	20.39132106º N	86.24565837º E	10.0	4.572	500
Narendrapur	25.11.2020	20.39851407º N	86.25655779º E	8.0	4.572	1000
Mean ± SD				12.33± 4.13	5.59± 1.24	683.33± 248.33
DEVI RIVER						
Sankhartras	05.10.2022	20.3820632º	85.94366544º	15.83	7.62	300
Khaladra	10.10.2022	20.3564347º	85.9658141°	19.07	9.144	250
Daleighai	15.10.2022	20.3490224º	86.0195127º	25.7	27.432	1000
Govindpur	30.10.2022	20.3253948º	86.0222041º	27.5	9.144	1000
Satabhauni Ganda	09.11 2022	20.3144658º	86.0264045º	19.4	12.192	1500
Jharapada	12.11.2022	20.2964444º	86.0342311º	9.9	18.288	500
Mean ± SD				19.57± 6.45	13.97± 7.61	758.33± 490.32

In Devi River, among the six sightings, the altitudes varied from 9.9m to 27.5m (Mean ± SD : 19.57± 6.45m); the lowest in Jharapada and highest in Govindpur. The depth of the river varied from 7.62m to 27.432m (Mean ± SD : 13.97±7.61m) with the lowest in Sankhartras and highest in Jharapada. The width of the river near turtle sighting varied from 250m to 1500 m (Mean ± SD : 758.33± 490.32m), with the lowest in Khaladra and the highest in Satabhauni Ganda (Table 1). The weight of three specimens of *Chitra indica*, including their length and breadth of the Carapace was recorded, one from Tikhiri near Paika River (Fig. 3), the second one from Gobindpur near Daya River and the third one from Daya River from the Bindha-Ragunathpur village, close to the Buddhist site of Dhauli near capital city of Bhubaneswar (Table 2). While the weight of the specimen recorded from Paika River was 7.2 kg, followed by 7.0 kg from Devi River and 60 kg from Daya River respectively. The specimen from Daya River (Fig. 4) was rescued on 5th July 2021 from Daya River bed with the help of the villagers, and its carapace length was 127 cm and breadth was 60 cm (Table 2).

Table 2 Weight and carapace measurement of recorded Narrow Headed Soft Shell Turtle *Chitra indica* specimens from Mahanadi Delta.

Sl. No.	Name of Place	River Name	Latitude and Longitude	Weight (Kg)	Length (L)/ Breadth (B) (cm)
1.	Tikhiri	PAIKA	20.3322696° N 86.4846398° E	7.2	L-52 B-40
2.	Govindpur	DEVI	20.3253948° N 86.0222041° E	7.0	L- 50 B-40
3.	Bindha- Raghunathpur (Near Dhauli, Bhubaneswar)	DAYA	20.192434° N 85.839665° E	60.0	L- 27 B- 60

Threats

Chitra indica has been hunted throughout its distribution. The meat can be found at local markets in India, Bangladesh, and Nepal, where it is known as a delicacy. More important, is the extensive hunting of *Chitra indica* for the harvest and sale of its outer cartilaginous rim or "calipee". The dried calipee is exported for use in traditional medicine or as the stock of a luxury soup. (Das and Singh, 2009; Lowe et al., 2009). *Chitra indica* is listed as endangered on the IUCN Red List of Threatened Species (IUCN, 2022) globally and it has been placed in Schedule II of the Indian Wildlife Protection Act of 1972. Domestic trade of the species is a major concern, causing the population decline of the species (Asian Turtle Trade Working Group, 2000). The population decline of this species over the last few decades is attributed mainly to exploitation for consumption of its meat and eggs, fishing activities, habitat destruction resulting from illegal sand mining, urbanization, and water pollution (Das, 2008; Mohapatra et al., 2009).

Trading of *Chitra indica* has been rampant in recent times in various parts of India, including the Mahanadi Delta area of Odisha. Forest officials rescued 12 narrow-headed softshell turtles (*Chitra indica*) from an islet of Kathjodi River at Anlatalanga village near Bayalish Mouza on the outskirt of Cuttack city, Odisha. These turtles were about to be transported to West Bengal and each turtle weighed 30 to 35 kg (Anonymous, 2015; Rajvanshi, 2015). Forest officials of Dhenkanal, Odisha rescued a narrow-headed freshwater turtle (Chitra indica) in October, 2020 from Shadangi Forest Range, Dhenkanal close to River Barhmani (Anonymous, 2020). In May, 2021, sixty-seven endangered turtles including six of *Chitra indica* species, each weighing 50 kg were seized from poachers in Etawah, Uttar Pradesh, India (Siddique, 2021).

Fig. 5 *Chitra indica* fishing in Devi River, Mahanadi Delta with the help of a fishing rod attached to a plastic string with bait and iron hooks. A-B Multiple iron hooks and bait tied at the end of a long plastic string, C- fisherman with fishing rod (bamboo pole) attached to a plastic string with bait and multiple iron hooks.

The questionnaire survey among the local population and visits to study areas indicated that the Devi river system has a large population of softshell turtles including *Chitra indica* particularly in the Gobindpur area. Post monsoon, hundreds of people and fishermen do fishing for big fish and softshell turtles due to their abundance. The fishermen use fishing rods (usually bamboo poles) tied to a long plastic string. Towards the end of the plastic string, baits and multiple iron hooks are attached for capturing turtles from the river. Different baits for capturing turtles are prepared using flour, corn, breadcrumbs, and a binding agent (Fig. 5). They also use rats to attract softshell turtles.

Due to the strong demand for turtle meat from the neighbouring state of West Bengal and among the Bangladeshi community, the traders are now sourcing turtles from Odisha, particularly from major rivers like Mahanadi and Baitarani. Turtles are mostly caught at Dhabaleswar, Mundali, Padmavati, and Kantilo, on the Mahanadi River upstream of Cuttack. In these places, most of the poaching takes place in summer when water levels are low (Swain and Palita, 2011). However, the findings of the present study indicate that in the tributaries of Mahanadi Delta downstream of Cuttack poaching and trading of these turtles take place during the post-monsoon period.

Conservation measures

The globally endangered Indian narrow-headed softshell turtle *Chitra indica* population continues to decline. In spite of the turtle being worshipped as an incarnation of Lord Vishnu, and in spite of legal protection under the Wild Life (Protection) Act of 1972, its population decline continues unabated. Both the animals themselves, as well as their nesting grounds, require protection from exploitation. Greater monitoring and control of the illegal trade of this species is necessary to further protect *Chitra indica*. Upgrading the species to Schedule I of the Indian wildlife act as well would help the conservation efforts of *Chitra indica*. Due to the difficulty of maintaining this species in captivity, conservation efforts should focus on hatch-and-release programs and the reduction of mortality in mature individuals (Das and Singh, 2009; Rhodin et al., 2011)

In the areas of deep pools with good concentrations of freshwater turtles in the tributaries of the Mahanadi Delta, strict vigilance by Government bodies is necessary for the protection of this species. Awareness of its ecological importance among the local population with the support of the environment and eco-groups is the need of the hour along with the strict enforcement of legal provisions on the trade and marketing of this rare species of freshwater turtle.

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EFFECTS OF UREA FERTILIZER ON SOME DEVELOPMENTAL STAGES OF THE COMMON ASIAN TOAD, *DUTTAPHRYNUS MELANOSTICTUS*

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ABSTRACT

Most anurans often breed near agricultural sites and are thereby affected by the agricultural contaminants through surface run off. Larval amphibians inhabiting aquatic ecosystem are known to be excellent bio indicators in ecotoxicological studies because of their highly permeable skin. In the present study, we assessed the effect of short-term exposure to urea on some developmental stages (embryos, hatchlings and tadpoles) of the anuran species, *Duttaphrynus melanostictus*. Egg masses of the selected species were collected from Mawsynram, Meghalaya. Gosner stages 9 (embryo), 18 (hatchling) and 25 (tadpole) were subjected to sub lethal (1, 5, 10, 12) in g/L and lethal concentrations (14, 16, 18, 20, 21-28, 30) in g/L. The LC_{50} for all stages were carried out at exposure period of 48 and 96 hours. The findings of the study highlighted that the effect of urea on the amphibian larvae is dosage dependent and sensitivity occurs only at extremely high concentrations where hatchlings are more sensitive than embryos and tadpoles. Morphological changes include compressed and curved body, reduced and bent tail, mucous secretion in the oral sucker among the hatchlings, while swollen head, depigmentation and tail curvature was observed in tadpoles. The behavioural changes such as sluggishness, irregular movement and reduced swimming were observed in the treated larvae exposed to lethal concentrations. The analysis for calculation of LC_{50} values was done using Finney's Probit analysis method (SPSS computer software).

Keywords: Amphibians, larval stages, mortality, urea, nitrogenous fertilizers.

INTRODUCTION

Amphibians are group of vertebrates exhibiting their biphasic life cycle in two alternating habitats i.e., terrestrial and aquatic. They exhibit significant role in environment by serving requisite trophic links in the food chain whereby acting both as prey and predators (Junges et al., 2012). The breeding grounds of anurans ranges in a variety of water bodies such as ephemeral pools situated near agricultural fields, permanent ponds, streams and rivers. For decades, there has been an increasing awareness among biologists throughout the world on the widespread decline of amphibian populations at an alarming rate (Blaustein and Wake, 1990; Alford and Richards, 1999; Krishnamurthy et al., 2008; Sayim, 2008; Alton and Franklin, 2017). Multiple factors such as habitat loss, diseases, climate change, UV irradiation, exploitation and introduced species are major contributors to decline in amphibian population (Beebee and Griffiths, 2005; Blaustein et al., 2003). Further, another possible contributing factor includes the use of agrochemicals which favours this decline as amphibians are sensitive to environmental pollution (Blaustein et al., 1994; Blaustein and Wake, 1995).

Many biologists considered amphibians as ideal bio indicators of environmental health and resilience (Blaustein, 1994; Blaustein and Wake, 1995) for studies on environmental pollution since they inhabit both aquatic and terrestrial habitats (Watt and Jarvis, 1997). It is suggested that these lower vertebrates are responsive to agricultural and environmental pollutants during their early aquatic life stages (Venturino et al., 2003). Freshly hatched larvae which are often restricted to the aquatic habitat can be greatly affected on exposure to pollutants introduced in the water

bodies (Cooke, 1981; Berger, 1989). Amphibian possesses glandular skin which lacks epidermal structures such as scales, feathers, hair (Duellman and Trueb, 1986) and acquiring moist, permeable dermal skin may likely be at risk of exposure to contamination since they use their skin for respiration and water uptake and readily absorb potentially harmful substances (Fryday and Thompson, 2012). Prolonged exposure of these chemical contaminants first to the aquatic and then to the terrestrial environment which may make amphibians sensitive to potential environmental stressors in particular (Berril et al., 1993). The most probable route of exposing amphibians to contamination in their breeding sites is agricultural surface run off (Storrs and Kiesecker, 2004).

The major source of nitrogen input in the environment is the anthropogenic usage of chemical fertilizers (Vitousek et al., 1997). As a consequence, extensive use of such fertilizer may have an impact on the freshwater ecosystem and affect non target aquatic biota due to the changes in the environment (Sangeetha et al., 2011; Maitra and Nath, 2014). Nitrogenous fertilizers applied in croplands and other agricultural areas have been reported to have a negative impact on the growth, development, survival and the behaviour of amphibians (Baker and Waights, 1993; Hecnar, 1995; Marco and Blaustein, 1999; Marco et al., 1999). Usually, nitrogenous based fertilizers applied to crops at the time of application become impenetrable below the surface and may flow over into nearby water bodies through ground water basins (Crews and Peoples, 2004).

Among aquatic organisms, amphibians are considered to be key group that may be susceptible to nitrogenous compounds (Griffis-Kyle and Ritchie, 2007) since they rely on water to complete their life cycles (Stebbins and Cohen, 1995). Embryos exposed to nitrogenous compounds can augment mortality later in development (Griffis-Kyle, 2005). Burgett et al. (2007) and Bibi et al. (2016) reported increasing levels of ammonium nitrate could reduce survival larval amphibians. Ecotoxicological work conducted in laboratory helps us understand the precedent sensitivity of amphibians to contaminants where the effect of other environmental stressors can be controlled (Srivastav et al., 2017).

Effects of nitrogenous fertilizers on amphibians are evident from the accounts made by several researchers (Marco et al.,1999; Hatch et al., 2001; Ortiz et al., 2004; Bibi et al., 2016 and Zhao et al., 2018). Our study location situated in Mawsynram, East Khasi Hills District, Meghalaya, Northeast India, receives exceptionally high rainfall and humidity and is home to a variety of fauna and flora including *Duttaphrynus melanostictus*. Urea (NH_2-CO-NH_2), a commonly used nitrogenous fertilizer is selected for the study and its solubility property make it easy to flow into nearby water bodies through irrigation water and surface runoff especially during rainy season (Gupta, 2016). Literature survey revealed that little is known on studies of acute effect of urea on embryonic and larval amphibians. Therefore, the present investigation aims to describe the acute toxic effect of this fertilizer on the three selected developmental stages of the anuran exposed to urea and to observe the morphological and behavioural changes.

MATERIALS AND METHODS

Egg masses of *Duttaphrynus melanostictus* were collected during the breeding period (April) from the breeding habitat (ephemeral pool) located at Mawsynram (25°17'52.6"N, 91°35'07.69"E; 1424 m asl; recorded using Garmin etrex 30 GPS). The eggs were kept in plastic trays filled with dechlorinated tap water in the laboratory and allowed to hatch. The tadpoles were reared under laboratory conditions and the experimental setup was maintained at room temperature of 20.67 ºC ± 1.53 (Mean ± SD; N=10). Three Gosner stages of development (Gosner, 1960) were selected for treatment with different urea concentrations i.e., embryos (stage 9), hatchlings (stage 18) and tadpoles (stage 25). Urea (IFFCO Urea 46% N) which was available commercially was procured from local agrochemical dealers.

Determination of LC_{50} values: Different urea solutions at varying concentrations were prepared in g/L with 1, 5, 8, 10 (low concentrations), 12, 14, 16, 18, 20, 21, 22 and 23 (high concentrations) and 24-28 and 30 (extremely high

concentrations). Five replicates each containing ten embryos and larvae were exposed to different urea concentrations kept in borosil bowls for 48 and 96 hours. A control group of five replicates for each developmental stage (embryos, hatchlings and tadpoles) were also maintained. Tadpole stages were not fed throughout the experimental period. Mortality of the embryos, hatchlings and tadpoles at 48 and 96 hours was subjected to Probit analysis method (SPSS computer software) (Finney, 1971) to calculate the LC_{50} values at 95% confidence intervals.

Morphological and behavioural studies: At the beginning of the experiment, the control embryos (stage 9), hatchlings (stage 18) and tadpoles (stage 25) were divided into seven groups. The mean size of the embryo and body lengths of the control larvae as well as those exposed to higher concentrations (18, 21, 23, 25, 27, 30) g/L of urea were measured at a duration of 0, 48 and 96 hours and measurements (mm) were taken with the help of a dial calliper (Mitutoyo series No. 505-671). The morphological and behavioural abnormalities seen in the treated groups (hatchlings and tadpoles) at 18, 21, 23, 25 and 27 g/L during the test at 96 hours were observed and photographed using the dissecting binocular microscope attached with photographic facilities (Motic SMZ-143 series).

RESULTS

The result of the present investigation showed no mortality among the embryos, hatchlings and tadpoles in the control group kept for 48 and 96 hours. Mortality was not recorded in the treated groups (embryos, hatchlings and tadpoles) at lower concentrations (1, 5, 8, 10 and 12) g/L exposed to the four-day test. On the fourth day, increased percentage of mortality were recorded in treated groups of the embryos, hatchlings and tadpoles from 14 g/L to 30 g/L as compared to the control groups (Table 1). The embryos exhibited 7% mortality at 14 g/L treatment. The percent mortality of embryos was found to increase with the increase in urea concentration and attained 100% mortality at 30 g/L. Similarly, hatchlings and tadpoles showed progressive increase of mortality from 14 g/L onwards. Hatchlings showed 9% death at 14 g/L and attained 100% death at 30 g/L. Tadpoles showed 5% and 100% mortality at 14 g/L and 30 g/L, respectively (Table 1).

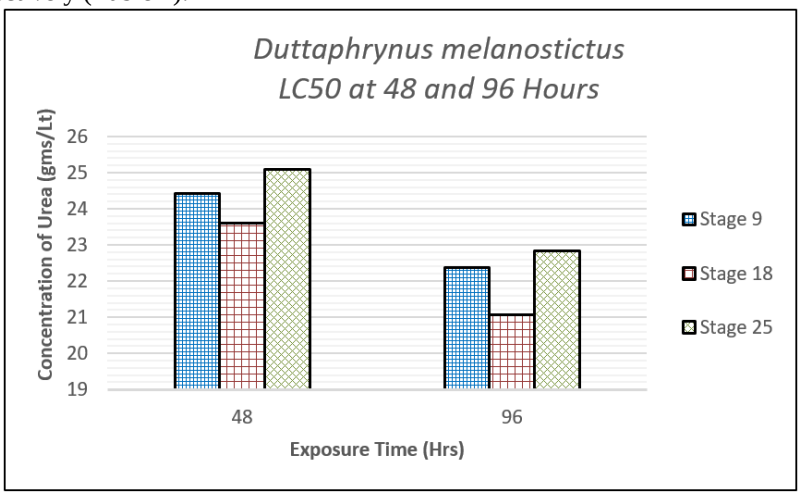

Fig. 1: Graph showing 50% mortality in *Duttaphrynus melanostictus* (Gosner stage 9, 18 and 25) after 48 and 96 h exposure to different concentrations of urea

The LC_{50} values of urea at 96 hours for embryos (stage 9), hatchlings (stage 18) and tadpoles (stage 25) were found to be 22.38 g/L, 21.06 g/L and 22.83 g/L, respectively. Simultaneously, the LC_{50} values of the treated groups exposed to urea for 48 hours for embryos (stage 9) was recorded to be 24.42 g/L. The hatchlings had an LC_{50} values of 23.61 g/L whereas the LC_{50} values of tadpoles subjected to the 48-hour test was observed to be 25.08 g/L (Table 2). The

two-day test conducted displayed similar finding with that of the four-day test (96 hours) where hatchlings were among the most susceptible group when exposed to urea treatment. It was noted that LC_{50} values decreased with increase in the time of exposure to urea in all three developmental stages (embryos, hatchlings and tadpoles) of *D. melanostictus* (Table 2).

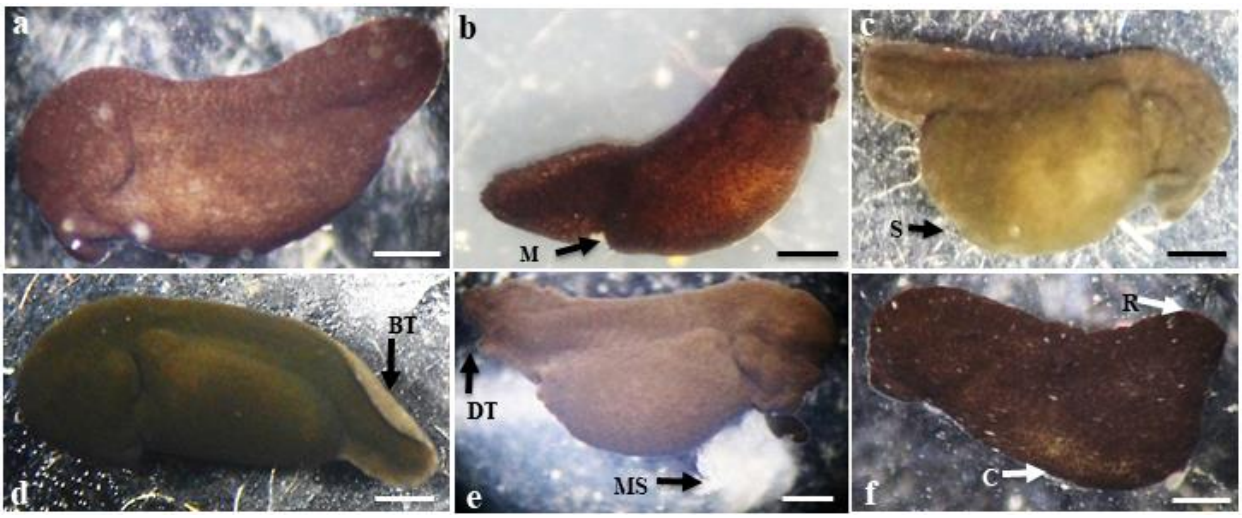

Fig. 2. Morphological abnormalities observed in the hatchlings of *Duttaphrynus melanostictus* exposed to urea (a) Control larva (Gosner stage 18); (b) Larva treated at 18 g/L showing mechanical injury (M); (c) Larva treated at 21 g/L showing swollen body (S); (d) Larva treated at 23 g/L showing bent tail (BT); (e) Larva treated at 25 g/L showing deformed tail (DT) and mucous secretion from oral sucker (MS); (f) Larva treated at 27 g/L showing compressed body (C) and reduced tail (R). Scale bar=0.5 mm

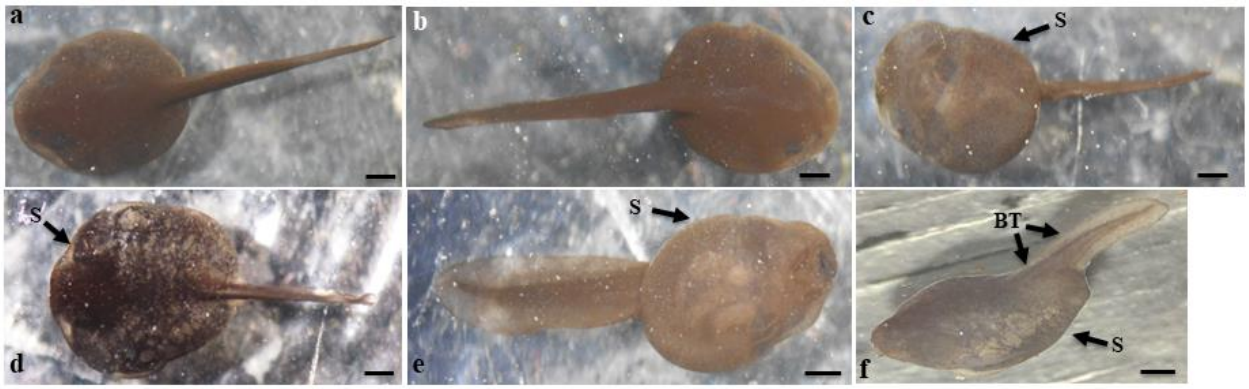

Fig. 3. Morphological abnormalities observed in the tadpoles of *Duttaphrynus melanostictus* exposed to urea (a) Control tadpole (Gosner stage 25); (b) Tadpole treated at 18 g/L (c) Tadpole treated at 21 g/L showing swollen body (S); (d) Tadpole treated at 23 g/L showing depigmentation and swollen body (S); (e) Tadpole treated at 25 g/L showing swollen body (S); (f) Tadpole treated at 27 g/L showing depigmentation, swollen body (S) and bent tail (BT). Scale bar= 2 mm

Table 1: Percent mortality of the embryo, hatchling and tadpole stages of *Duttaphrynus melanostictus* after 96 hours exposure to urea (g/L)

	Duttaphrynus melanostictus		
Concentration of urea (g/L)	**Gosner stage 9**	**Gosner stage 18**	**Gosner stage 25**
0	0	0	0
1	0	0	0
5	0	0	0
8	0	0	0
10	0	0	0
12	0	0	0
14	7	9	5
16	11	17	10
18	15	30	14
20	25	41	23
21	34	49	31
22	50	56	39
23	53	59	48
24	60	64	57
25	65	70	64
26	71	76	70
27	77	81	75
28	85	87	81
30	100	100	100

Table 2: LC_{50} values of *Duttaphrynus melanostictus* in g/L of urea using Probit analysis

Species	**Gosner Stage**	**Exposure time in hours**	
		48	**96**
Duttaphrynus melanostictus	Stage 9	24.42	22.38
	Stage 18	23.61	21.06
	Stage 25	25.08	22.83

Table 3: Body measurements (mm) of *Duttaphrynus melanostictus* for control and treated groups taken at an interval of two days

Concentrations (g/L)	Exposure time in hours								
	Stage 9 (Embryo)			Stage 18 (Hatchling)			Stage 25 (Tadpole)		
	0	48	96	0	48	96	0	48	96
0 (Control)	2.93 ± 0.02	3.56 ± 0.05	3.79 ± 0.12	4.02 ± 0.03	4.46± 0.14	5.28± 0.07	13.44 ± 0.41	13.72 ± 0.32	14.01 ± 0.24
18	2.95± 0.07	2.99 ± 0.03	3.24 ± 0.07	4.03 ± 0.05	4.38± 0.18	4.76± 0.12	13.42 ± 0.34	13.45 ± 0.39	13.56 ± 0.28
21	2.91± 0.12	2.94 ± 0.05	2.96 ± 0.11	4.01 ± 0.02	4.38± 0.09	4.61± 0.15	13.43 ± 0.42	13.45 ± 0.41	13.53 ± 0.27
23	2.92± 0.05	2.95 ± 0.04	2.96 ± 0.09	4.01 ± 0.06	4.37± 0.11	4.44± 0.11	13.41 ± 0.37	13.42 ± 0.29	13.5 ± 0.21
25	2.91± 0.11	2.93 ± 0.04	2.94 ± 0.12	4.02 ± 0.02	4.37± 0.12	4.39± 0.08	13.46 ± 0.39	14.43 ± 0.35	13.48 ± 0.31
27	2.93± 0.07	2.94 ± 0.03	2.95 ± 0.12	4.04 ± 0.03	4.33± 0.21	4.34± 0.12	13.47 ± 0.43	14.34 ± 0.33	13.38 ± 0.29
30	2.94± 0.05	2.94 ± 0.04	-	4.02 ± 0.07	4.13± 0.15	-	13.43 ± 0.36	14.26 ± 0.29	-

The current investigation showed differences in sensitivity to urea among the developmental stages for which hatchling stage was found to be more responsive to urea at 48 and 96 hours when compared with embryos and tadpoles. Further, the study revealed that embryo and tadpole stages were tolerant to urea and can withstand toxicity when compared with hatchling stage (Figure 1).

The initial measurements of the size of the embryos at 0 hour in the seven control groups were recorded to be 2.93 mm ± 0.02, 2.95 ± 0.07, 2.91 ± 0.12, 2.92 ± 0.05, 2.91 ± 0.11, 2.93 ± 0.07 and 2.94 ± 0.05. For hatchling and tadpole stages, the initial measurements were found to be 4.02 mm ± 0.03, 4.03 ± 0.05, 4.01 ± 0.02, 4.01 ± 0.06, 4.02 ± 0.02, 4.04 ± 0.03, 4.02 ± 0.07 and 13.44 mm ± 0.41, 13.42 mm ± 0.34, 13.43 mm ± 0.42, 13.41 mm ± 0.37, 13.46 mm ± 0.39, 13.47 mm ± 0.43, 13.43 mm ± 0.36, respectively. At 48 and 96-hour duration, there was a decrease in the size of the embryo, hatchling and tadpole of the treated groups (18, 21, 23, 25, 27 and 30 g/L) as compared to the control groups (Table 3). Moreover, no measurements of the treated groups (Gosner stage 9, 18 and 25) were recorded at 30 g/L as the mortality rate reached 100%.

Control groups did not show any behavioural and morphological changes throughout the test. However, hatchlings and tadpoles exposed to higher concentrations (18, 21, 23, 25 and 27 g/L) at 96 hours revealed some morphological abnormalities. The hatchling group showed compressed body, curvature of the body, reduced and bent tail and mucous secretion in the oral sucker (Fig. 2). The tadpole group exposed to urea have undergone morphological alterations and revealed changes in the body such as swollen head, depigmentation and tail curvature (Fig. 3). Behavioural changes such as sluggishness, irregular movement and reduced swimming were observed at concentrations of 25 and 27 g/L in the tadpole stage.

DISCUSSION

The toxicity of urea in the anuran increases with increased concentration and duration of exposure. Similar findings were also reported in fishes when exposed to fertilizers (Sangeetha et al., 2011) and in amphibians when exposed to ammonium nitrate (Bibi et al., 2016) and chlorpyrifos (Srivastav et al., 2017). In the present study, the decrease in LC_{50} values of all the three developmental stages (embryos, hatchlings and tadpoles) of *D. melanostictus* suggested that with a short duration time of exposure, mortality can occur only at a higher dosage of urea. It was suggested that some chemical compounds present at relatively low concentration in a clean environment can become harmful to various organisms with alternatively increasing concentration (Ortiz Santaliestra et al., 2011). The present investigation showed a stronger negative impact on survival of the hatchlings (Gosner stage 18) compared to embryonic (Gosner stage 9) and tadpole stages (Gosner stage 25). A possible explanation to this could be that embryos are protected by the perivitelline membrane which act as a protective barrier to the embryo from the external environment (Duellman and Trueb, 1986). Moreover, studies carried out by Meredith and Whiteman (2008) and Ortiz Santaliestra et al. (2006) revealed similar findings that embryos are least sensitive to contaminants than hatchlings as they have a protective mechanism comprising of the gelatinous coat covering around them that prevent them against the exposure to such contaminants (Edginton et al., 2007; Marquis et al., 2006).

The chances of freshly hatched larval stages to remain in contact with certain exogenous chemicals is relatively high in the first few weeks and months of their development (Duellman and Trueb, 1986; Laposata and Dunson, 1998; Kiesecker et al., 2001). The presence of external gills in newly hatched larvae could make them more vulnerable to nitrogenous compounds compared to advanced larval stages when gills have become internalized (Ortiz-Santaliestra et al., 2006; Wijesinghe et al., 2011). Similarly, the survival of larval newt is proportional to ammonium nitrate concentration, where higher concentration of the substance corresponds to greater mortality (Watt and Jarvis, 1997). In addition, sensitivity of pollutants in the young larval stages could be due to incomplete tissue and organ differentiation (Herkovits and Ferna´ndez, 1978).

It was suggested that the high ability of the tadpoles to detoxify chemical contaminants makes them more tolerant than early stages (Bucciarelli et al., 1999). In addition, Marco and Blaustein (1999) reported high tolerance of tadpoles to nitrite than those of early life stages. Naamane et al. (2020) also found increased tolerance of tadpoles to ammonium sulphate fertilizer. Studies reported that tolerance of tadpoles to toxic substances differs during their development or growth (Harris et al., 2000; Smith, 2001). Moreover, tadpoles having progressively thicker skin could provide more protection against osmoregulatory alteration and hence are not easily susceptible to contaminants (McDiarmid and Altig, 1999). It is also evident from the findings of Watt and Jarvis (1997) that tadpoles having a comparatively larger initial size than early smaller larval stages experiences low mortality.

The study of effects of urea on the mortality of non-target aquatic organisms such as amphibians and fishes have been reported by several workers (Schuytema and Nebeker 1999a; Ofojekwu et al., 2008; Adakole and Lawan, 2011; Sangeetha et al., 2011 and Asuquo et al., 2016).

It has been reported that amphibians with deformities are found near agricultural fields where applications of chemical fertilizers and pesticides takes place (Ouellet et al., 1997; Hayes et al., 2002). In the present study, certain morphological changes occurred in both hatchling and tadpole stages of *D. melanostictus* exposed to high dosage of urea (18 g/L, 21 g/L, 23 g/L, 25 g/L and 27 g/L). Similarly, some morphological abnormalities like bent tail and edema have been reported in ammonium nitrate-treated tadpoles of *Bufo calamita, Bufo spinosus and Discoglossus galganoi* (Ortiz et al., 2004). Effect of nitrate causing alteration in the morphology of *Nyctibatrachus major* (Gosner stage 25) including swollen heads and bodies, protruding mouths and darkening of skin was reported earlier (Krishnamurthy et al., 2006). Hatchlings of *D. melanostictus* exposed to a pesticide, chlorpyrifos showed reduced tail length with increased concentration (Kharkongor et al., 2018). Present investigation revealed mucous secretion in the oral suckers of the hatchling in the treated group which may be linked to the first defensive mechanism (Bernabo et al., 2011). Amphibians with deformities or malformations have a greater chance of getting infection due to weak immune and endocrine systems and are more susceptible to predation and low reproductive success (Bridges, 1997; 1999a; 1999b; 2000; Ouellet et al., 1997; Boone and Semlitsch, 2002).

The present study showed behavioural changes only at extremely high concentrations (25 g/L and 27 g/L). Ammonium nitrate has shown significant direct toxicological effects on behavior of tadpoles on the wood frog *Rana sylvatica* (Burgett et al., 2007). Abnormal activities such as sluggishness and paralysis were suggested to be due to the effect of the contaminants on the central nervous system (Hecnar, 1995).

It has been reported earlier that pure form of urea applied to croplands could affect larval amphibians only at extremely high concentrations (Schuytema and Nebeker, 1999a). In natural conditions, there is a potential risk of fertilizers in the shallow water, as concentration may be high and therefore careful measures needs to be taken while handling them near closed water bodies (Capkin et al., 2010). Poor handling of such fertilizers increases the nitrogen levels in amphibian habitats (Schuytema and Nebeker, 1999b). It has been suggested that the interactions between nitrogenous pollutants and amphibian survival may be critical to the long-term management and recovery of amphibian populations (Griffis-Kyle and Ritchie, 2007). From the study conducted we also observed that mortality occured only at a relatively high dose of urea (14 g/L, 16 g/L, 18 g/L, 20-28 g/L and 30 g/L) on *Duttaphrynus melanostictus* embryonic and larval stages. Such mortality seen among the treated groups is due to the adverse effect of urea at high concentrations where such concentrations are rare in the environment. The average rate of urea treatment, particularly in paddy cultivations, is between 200 and 300 kg/ha every cropping season (Divya and Belagali, 2012). The presence of urea residues in lake water was found to vary from 0.6 to 3.4 parts per million (ppm), in channel water from 0.1 to 0.12 ppm, and in ground water from 0.3 to 4.2 ppm. Therefore, application of urea fertilizers only at higher dose in agricultural practices may affect the survival of amphibians and hence needs to be monitored.

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RETINOIC ACID AND REGENERATION OF SKELETAL MUSCLES DURING TAIL REGENERATION IN THE TADPOLES OF THE INDIAN TREE FROG, *POLYPEDATES MACULATUS*

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ABSTRACT

The skeletal muscle is an important tissue component of the tadpole tail. Anuran amphibians competently regenerate their skeletal muscle of the tail. However, vitamin A and its metabolites such as retinoic acid interfere with the regeneration of the skeletal muscle during tail regeneration in the tadpoles of the Indian tree frog, *Polypedates maculatus*. During this time an increase in the FGF2 immunofluorescence is also observed.

Keywords: Asian Elephant, KWLS, Movement, Defensive, Interaction

INTRODUCTION

The tadpoles of *Polypedates maculatus* are regeneration competent organisms especially attractive to study the effect of retinoic acid (RA) and other vitamin A metabolites during tail regeneration. In this species, the tail regeneration is unconventional under the effect of exogenous RA treatment (Hota and Mahapatra, 2022). Development of multiple ectopic hindlimbs, formation of large bulbular tissue mass, incomplete and stunted regeneration are many of the manifestations of RA treatment (Hota and Mahapatra, 2022). The ectopic hindlimbs that emerge from the regenerating tail are shed off eventually as the tadpole begin to metamorphose (Tazawa and Yaoita, 2017). On the contrary, in the absence of RA treatment, the tail regenerates completely within a fortnight (Hota et al., 2018). Morphologically, it is the exact replica of the lost caudal appendage with functional restoration (Hota et al., 2018). In fact, the tissue components such as the notochord, nerve cord, skeletal muscles and blood vessels are regenerated during tail regeneration (Mahapatra et al. 2004). In this context, it is extremely important to scrutinize the regeneration regime of the individual components of the tail and validate their regenerative paths and procedures to derive vital insights into the mechanism as a whole. The tadpole larva of this species can be a promising anuran model for investigators to study and elucidate the intricate mechanism behind regeneration as well as homeotic transformation of tail to limbs.

Interestingly, upon RA influence during regeneration, a tissue turmoil is observed affecting all the components of the tail (Hota and Mahapatra, 2022). The majority of the tissue mass seen in the tadpole tail reportedly consists of the skeletal muscle composed of long striated muscle fibres formed by myofibrils (Divya, et al. 2010). In fact, the skeletal muscle helps in voluntary movement and aids in locomotion and can be studied to understand regenerative myogenesis (Baghdadi and Tajbakhsh, 2018). Hence, the regeneration of skeletal muscles of the tadpole tail can serve as a model to be explore and understand the mechanism of muscle regeneration in vertebrates.

Experimental procedures

Retinoic acid treatment and tail amputation

Regeneration of the tadpole tail begins post amputation and therefore selected for regeneration studies. The *P. maculatus* egg nests were locally collected from natural breeding grounds, procured to laboratory for rearing following standardised procedures (Mohanty-Hejmadi, 1977). Taylor and Kollros stage I and II of tadpole staging were selected for tail regeneration experiments (Taylor and Kollros, 1946). Prior to tail amputation the tadpoles were subjected to MS222 (Hi Media) at the dose of 1:3000. The selected tadpoles were categorized into three categories such as original tail (homeostatic tail), control (lacking retinoic acid treatment) and treated (RA treated). The tails were amputated from the middle and RA (Sigma Aldrich) at the dose of 250ng/ml for 24 hours under low light condition was administered. The RA treatment was based on standardisation in the laboratory in this species. The original sets were used to study the tissue architecture of the tadpole tail. The control sets lacked RA treatment and hence were analysed for changes manifested in the skeletal muscles of the tail during regeneration. All experiments were approved by the Animal Ethical Committee, Utkal University, Bhubaneswar, Odisha, India.

Histological analysis and Immunohistochemical Localization

Histological sections were analysed for histopathological changes following RA treatment. The tail regenerates of the three groups i.e., original, control and RA treated were subjected to formalin fixation for 24 hours and subsequent paraffin sections of 5 μm were obtained. Mallory's triple differential staining was carried out and serial sections were selected for immunofluorescence localization of FGF2. Immunofluorescence labelling was done as per the instructor's manual. Tissue sections were incubated in normal goat serum 10% (Santa Cruz Biotechnology, U.S.A) for 30 minutes. Phosphate buffer saline (PBS) was used for dilution purposes. Rabbit Polyclonal IgG specific for FGF2 (Santa Cruz Biotechnology, U.S.A.) at the dilution 1:50 in 1.5% antibody diluent (Normal goat serum- PBS) was applied on the tissue sections and incubated overnight at 4°C. Next day tissue samples were incubated under dark conditions with anti-Rabbit Goat-FITC conjugated secondary antibody (Santa Cruz Biotechnology, U.S.A.) diluted 1:200 with 2% antibody diluent (Normal goat serum-PBS) for 40 minutes. Ultracruz mounting medium (Santa Cruz Biotechnology, U.S.A.) was used as mounting medium. The tissue sections were observed under Leica DM 3000 LED microscope and photos were captures by Leica DFC450 C Camera fitted to the microscope. Adobe Photoshop software was used for preparing the photo plates.

RESULTS

The transverse section (T.S.) of the original tail consisted of muscle bundles below the basement membrane (Fig. 1 A). The chevron pattern was evident in the histological section of the (homeostatic) tail tissue. In the control tail tissue of the 5 days post amputated tail, the skeletal muscle fibres were mostly seen to be still in the stage of regeneration and appeared fibrillar in structure (Fig. 1 B). Several cells of the mesenchyme were seen to be accumulated in the vicinity of the regenerating muscle below the basement membrane (1B). In the control tissue sections of day 10 and 15 post amputation (pa), the muscle regeneration was seen to be complete (Fig.1 C, D). The regenerated skeletal muscles resembled the muscles of the original tail; however, they were seen to be present as patches in the control tissue sections (Fig. 1 C, D). RA effect on the skeletal muscle of the regenerating tail was clearly evident causing stunted muscle regeneration in the treated tissue sections of day 5, 10 and 15 pa (Fig.2 A-C). The regenerated muscle was seen as continuous patches, clumps and completely void of the typical chevron arrangement (Fig. 2 A-C). In fact, the mesenchyme in such tissue sections were collagenised, appeared dense and condensed (Fig A-C).

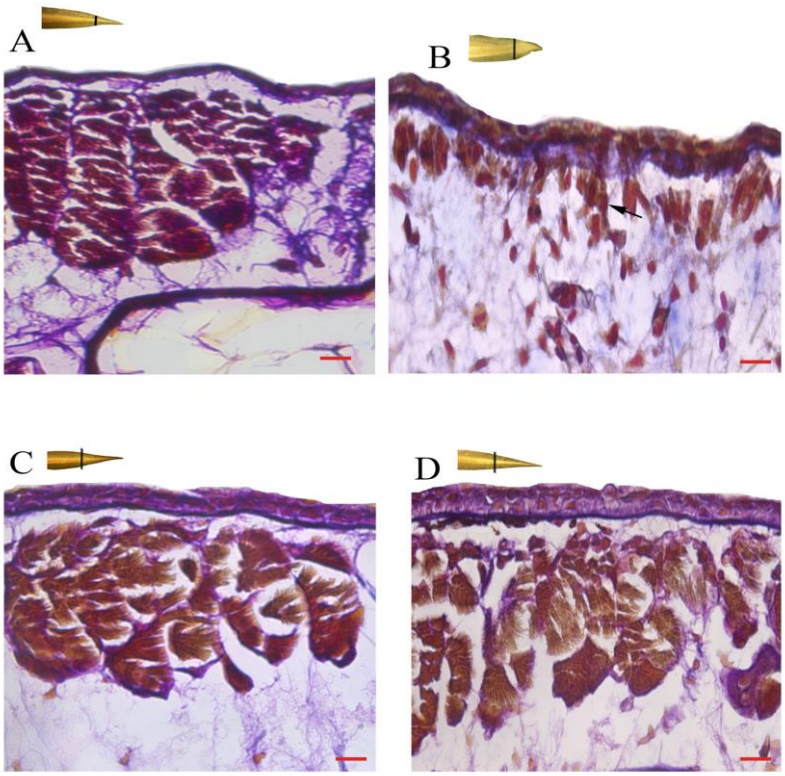

Fig. 1 Histology of original and regenerating tadpole tail (control) of *Polypedates maculatus showing* skeletal muscles: (A) T.S. of the original tail of tadpoles of *P. maculates* showing muscle bundles in typical chevron pattern (B-D) T.S. of regenerating tail of tadpoles of *P. maculates* of day 5, 10, 15 post amputation respectively showing regeneration of skeletal muscle below the epidermis. (Scale bar in panels A-D= 25 μm).

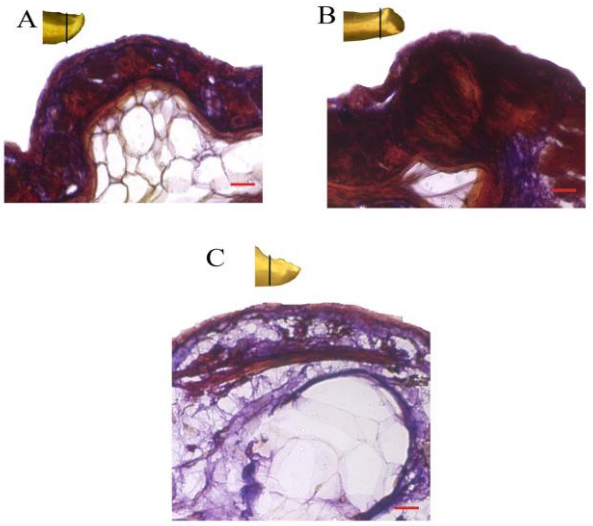

Fig. 2 Histology of retinoic acid treated regenerating tails of the tadpoles of Polypedates maculatus showing skeletal muscles: (A-C) T.S. of RA treated regenerating tail of tadpoles of P. maculates showing regeneration of skeletal muscle below the epidermis. (Scale bar in panels A-D=25 μm).

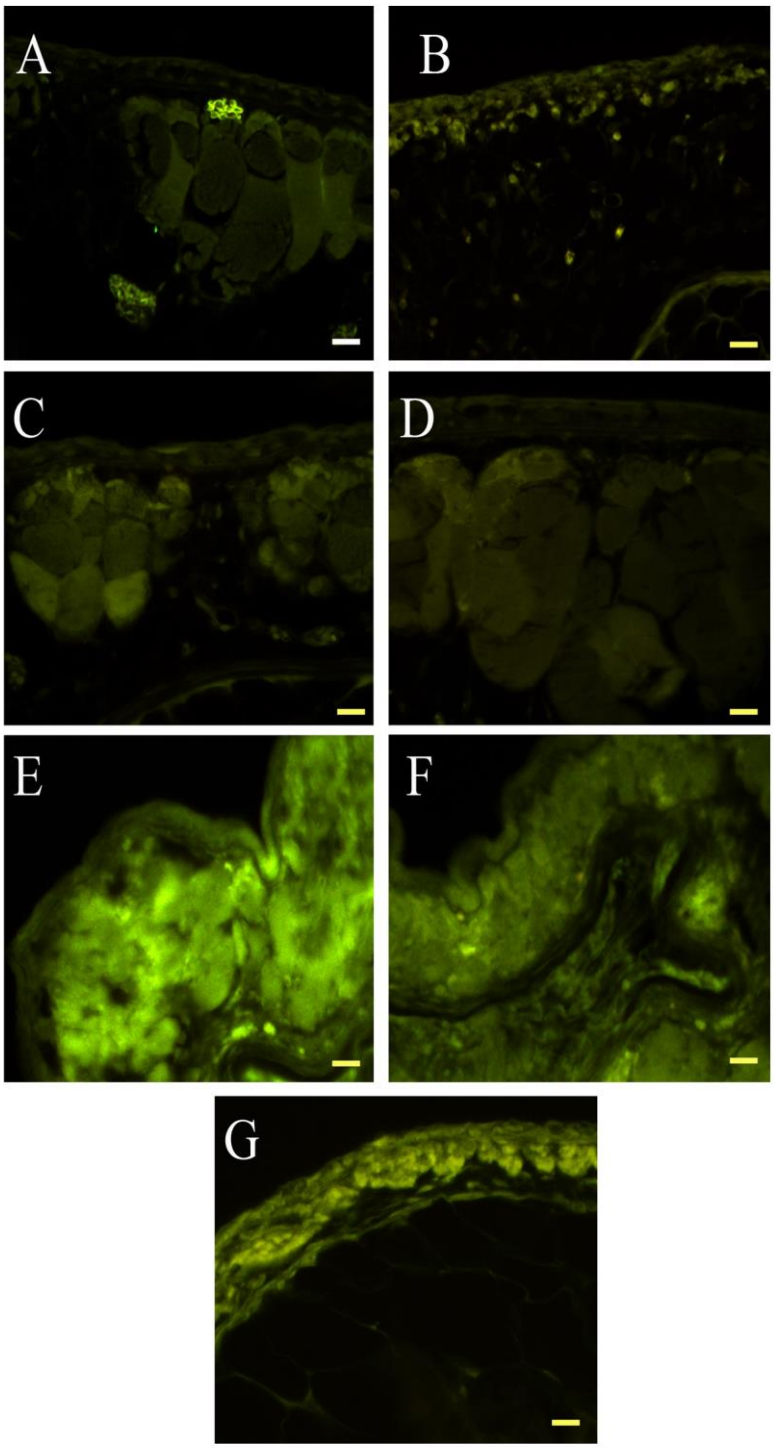

Fig. 3 FGF2 immunofluorescence localization in the skeletal muscle of the original, control and RA treated tail tissues of the tadpoles of *Polypedates maculatus*: (A) FGF2 immunofluorescence localization in the skeletal muscle of the original tail (B-D) FGF2 immunofluorescence localization in the skeletal muscle of the control tail of day 5, 10, 15 post amputation respectively (E-G) FGF2 immunofluorescence localization in the skeletal muscle of the RA treated tail of day 5, 10, 15 post amputation respectively (Scale bar in panels A-G = 25 μm).

Few melanocytes were also seen in the vicinity of the regenerating muscle (Fig. 2 A-C). FGF2 immunofluorescence was detected in the original, control (day 5, 10 and 15pa) and RA treated (day 5, 10 and 15pa) tissue sections (Fig.3 A- G). However, the FGF2 immunofluorescence was higher in the skeletal muscle regenerates under RA treatment.

DISCUSSION

Skeletal muscle is one amongst the most studied tissue that has major contribution during development (Baghdadi and Tajbakhsh, 2018). Owing to its remarkable regenerating capabilities during trauma and injury, regeneration of skeletal muscle has been extensively studied (Baghdadi and Tajbakhsh, 2018). In the present study, the regeneration of the skeletal muscle was seen to be stunted upon RA treatment. Similar findings were reported during vitamin A palmitate mediated tail regeneration in *Bufo melanostictus* where the regenerated muscles were seen to be clumped, unorganized and failed to appear as bundles (Das and Mohanty-Hejmadi, 1999). Likewise, bilateral muscle bundle formation was also reportedly altered and the muscle cells were seen to be scattered in the vitamin A palmitate treated tail tissue sections in the tadpoles of the Indian tree frog, *Polypedates maculatus* (Mahapatra et al. 2004). It is reported that regeneration of muscles is the most adversely affected out of the three axial tissues due to the effect of vitamin A (Niazi and Saxena, 1979). Furthermore, FGF2 immunofluorescence was detected in the original, control and treated tissue sections in the present study. It is reported that FGF2 is know to favour the skeletal muscle regeneration (Kashpur et al., 2013). Moreover, an increase in the FGF2 expression during muscle regeneration has been documented (Pawlikowski et al. 2017). Gargioli and Slack (2004) have revealed that the myofibres of the muscle during tail regeneration in *Xenopus* arise from mononucleate satellite cells instead of the pre-existing myofibres. Hence, marker related studies in futuristic work will provide vital insights into skeletal muscle regeneration during tail regeneration as well as retinoic acid mediated tail regeneration in the tadpoles of *Polypedates maculatus*.

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