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Health Status of Common Frog *Fejervarya limnocharis* (Anura: Ranidae) in Rice-Paddy Agroecosystems of Western Ghats, India

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Abstract: Frogs living in an agrochemical contaminated rice paddy fields are exposed to the chemical residues. These chemical residues produce negative effects on their population. Health statuses of such frogs are detected using Body Condition Factor (CF), Gonado-somatic Index (GSI) and Acetyl Cholinesterase (AChE) activity in gonads. In this study we have examined the health status of *Fejervarya limnocharis,* a common rice field frog living in agro-chemical contaminated and uncontaminated reference sites. CF of frogs living in contaminated sites showed negative correlation with those in reference sites. GSI of the male frogs collected from reference and contaminated sites showed subtle and insignificant difference ($F_{2, 183} = 2.559$, $p = 0.0802$). GSI of female frogs collected from reference and contaminated sites did not show differences between them ($F_{2, 83} = 0.449$, $p = 0.6395$). However, difference in AChE activity of gonads in reference and contaminated site were highly significant ($F_{2, 264} = 168.8$, $p<0.0001$) indicating the negative influence of agrochemical exposure on the health status of the frog.

Key words: Body Condition Factor · Gonado-somatic Index · *Fijervarya limnocharis* · Pesticide contamination · Agroecosystem

INTRODUCTION

Many species of frogs use shallow waters of croplands for breeding and early development. Soil and waters of the croplands are contaminated with residues of agrochemicals [1]. Frogs in crop lands showed variety of physiological, histological, developmental, morphological and biochemical alterations including reduced growth and development [2-6]. Reduced growth and development in the early and subsequent stages of frogs are considered as the less availability of energy for detoxification of xenobiotic compounds for which they were exposed [7, 8]. These reduced growth and malformations in frogs were observed frequently in crop lands where agrochemicals are used [9-11]. In many croplands of Western Ghats, the breeding of the frogs and application of pesticide and/or fertilizers coincides [1, 12] and it was considered as a major factor negatively influencing on their population [11, 13, 14]. It has been reported that status of frog population dwindling in croplands are linked with agrochemicals [4, 6].

Most of the ecotoxicological studies conducted on frogs have examined the direct effect of individual, or a few combinations of pesticides in laboratory or mesocosm [12, 15]. These results are important at individual level to those tested chemicals. While, these observations are essential to understand how an individual species respond to the selected chemical [16]. Field studies are also important to know the health status of species in the wild population. Therefore, in this study we have collected field informations and conducted experiments to assess the health status and reproductive conditions of frogs living in agrochemical contaminated and uncontaminated croplands of rice paddy cultivations. We used *Fejervarya limnocharis* (common paddy field frog, Anura: Ranidae) as a model species and tested the body condition factor (CF) as explained by Schulte-Hostdde *et al*. [17] and its relationship with Gonado-Somatic Index (GSI) and Acetyl Cholinesterase activity (AChE) as a tool and biomarkers to assess complex responses of the frog to field contaminations.

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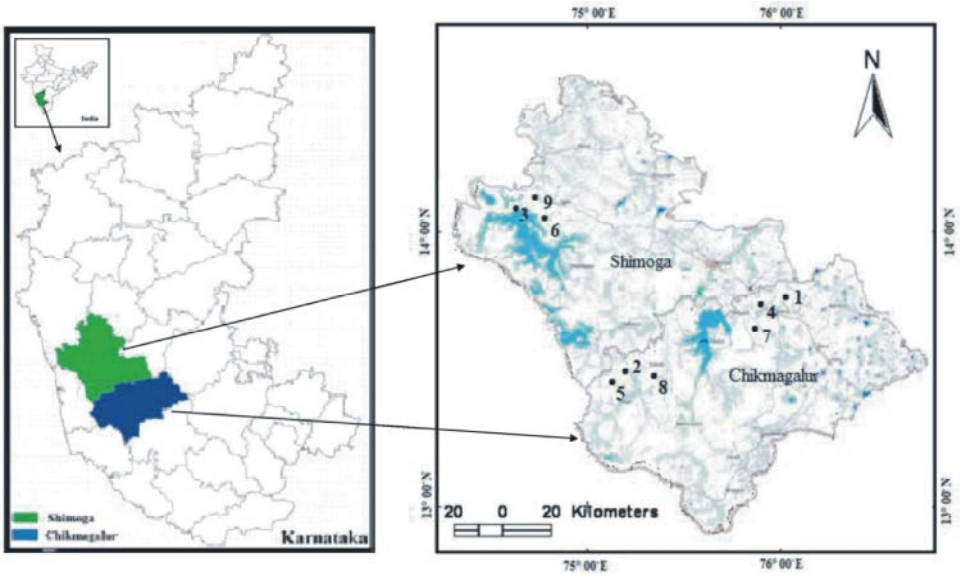

Fig. 1: Location of the study area. Number 1-9 represents the study sites.

MATERIALS AND METHODS

Study Area: The present investigation was made in nine rice paddy fields located 13°25`46"— 14°02`48"N and 75°02`46"— 75°42`26"E alt: 601-647 m asl in Central Western Ghats (Fig. 1). Each sampling site was ≈ 3.6 ha area. Among these, sites 1, 2 and 3 are uncontaminated rice paddy fields, where no synthetic agrochemicals are used. We have collected the information from the farmers on agrochemical usage and managerial practices through an appropriate questionnaire and found that sites 4-9 are intensively managed and different pesticides and synthetic fertilizers are used for cultivation. The major insecticides used are malathion, methyl parathion, monocrotophos (organophosphate pesticides), butachlor (organochlorine), cypermethrin, chloropyriphos (pyrethroid). The total pesticide used per crop was found to be 528±59.7 g/ha. The major synthetic fertilizers being used are nitrophosphate and potash (209±74.2 kg/ha/crop).

Frog Sampling and Body Condition Factor: Frog sampling was made during premonsoon (March to May) of 2012. In each site frogs were collected by spending 9.4±1.25 man hr/collection. Samplings were made by searching all microhabitats of crop fields and frogs were handpicked. Once the survey of frogs is over then each frog was measured for its Snout-Vent-Length (SVL) using a digital calipers (make: Tresna, precision: 0.01mm) and Body Mass (BM) was recorded to nearest 0.1 g using a portable balance (make: Dura Scale, D2, precision: 0.1g). From every catch, ≈ 30% of the frogs were randomly picked up and were used for laboratory analysis. The remaining frogs were released back to their habitat.

The SVL of these frogs were ranged between 21 - 39 mm (males) and 25 - 36 mm (female). Later, according to the procedure of Brodeur *et al*. [6] body condition factor (CF) was estimated by considering the regression of BM on SVL. In this method we observed both negative and positive values, in which positive values (residue) of an individual represent healthy status, while negative value (residue) represent low energy status [17]. We subjected the SVL of frogs recorded at contaminated sites to regression equation (BM on SVL) of reference sites and BM was derived. Later, we subtracted the derived BM with actual BM recorded for those frogs to check any differences in SVL-BM ratio between contaminated and reference sites.

Estimation of GSI and AChE: we have analysed 88 frogs (male-60, Female-28) from site 1-3, 96 frogs (male-70, Female-26) from site 4-6 and 86 frogs (male-55, Female-31) from site 7-9 for GSI and AChE content in their gonads. Each frog was weighed in the laboratory using Ohaus electronic weighing scale (model: 214C) to a precision of 0.1mg, then dissected, gonads were removed carefully and weighed to a maximum precision. Then GSI (%) was calculated by -gonad weight x 100/ Body weight, as stated by Murphy *et al*. [18]. To compare the means in GSI and significance difference between the sites, male and female

frogs are tested separately. Then using the same gonad tissue, Acetylcholine Esterase (AChE) activity was analysed following the method of Ellman *et al*. [19].

Statistical Analysis: For all statistical inferences, we used mean values of each criterion tested. The data on SVL, BM, GSI and AChE concentrations of GSI did not fallow normality test as confirmed by Bartlett Statistics. Therefore data were transformed to logarithmic values. Then data on CF, GSI, AChE were checked for significant variation between the different sampling sites. We used ANOVA and ANCOVA with multiple comparisons to test the significance differences between sites. SPSS (ver 20.0) was used for all statistics.

RESULTS

Relationship between SVL, BM and CF: The SVL of frogs in reference and contaminated sites did not show a considerable differences (male: site 1-3; 26.8±1.61 mm, site 4-6; 26.4±1.97 mm and site 7-9; 26.6±2.61 mm, $F_{2,182} = 0.79$, $p = 0.455$, Female: site 1-3; 23.5±1.73 mm, site 4-6; 29.9±2.12 mm and site 7-9; 29.41±1.90 mm, $F_{2,182} = 0.56$, $p = 0.576$). The BM of frogs ranged between 0.9 and 5.0 grams for males and 1.4 and 4.4 grams for females. Similar to SVL, the BM for males and females between sites did not show significant variations (male: site 1-3; 1.8±0.37 grams, site 4-6; 1.7±0.40 grams and site 7-9; 1.8±0.61 grams, $F_{2,183} = 1.32$, $p = 0.270$; female: site 1-3; 2.8±0.60 grams, site 4-6; 2.9±0.71 grams and site 7-9; 2.7±0.62 grams, $F_{2,83} = 1.054$, $p = 0.353$). However, compared to female the SVL and BM of males are smaller (SVL: male: 26.6±2.06; female: 29.6±1.91, $F_{1,269} = 131.90$, $p < 0.0001$; BM: male: 1.8±0.47; female: 2.8±0.65 $F_{1,269} = 212.67$, $p<0.0001$). Fig. 2 presents SVL-BM relationship of males and females in reference sites while, Fig. 3 details the deviation in CF from length weight relationship of reference. In site 4-6, a total of 42 males (67.74% of the total) and in sites 7-9 a total of 55 males (63.95% of the total) and showed negative CF compared to reference. Differences in CF between all sites are highly significant ($F_{2,183} = 405.9$, $p < 0.0001$) (Wilks' Lambda = 0.085, $F_{2,83} = 291.35$, $p < 0.0001$, Partial $n^2 = 0.915$).

GSI: GSI did not show significant variation for male and female frogs between sites (male: site 1-3, 0.33±0.080% ; site 4-6, 0.34±0.102%; site 7-9, 0.37±0.091%; female: site 1-3, 9.57±4.466%; site 4-6, 9.24±5.443%; site 7-9, 10.43±5.087%). GSI of male frogs between sites showed a subtle and insignificant difference ($F_{2,183} = 2.559$, $p = 0.0802$). Female frogs collected from different (reference v/s contaminated) sites did not show significant differences between them ($F_{2,83} = 0.449$, $p = 0.6395$).

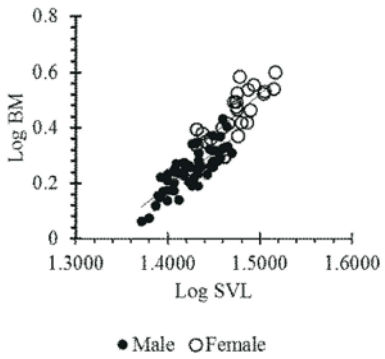

Fig. 2: SVL-BM relationship of males and females in reference sites.

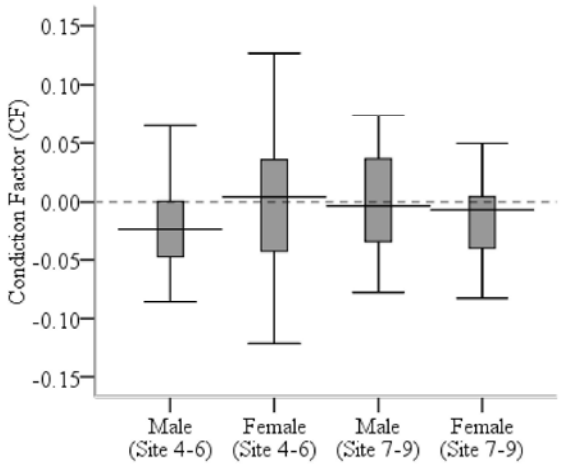

Fig. 3: Box-plot represents the condition factor (CF) recorded in males and female frogs from contaminated sites.

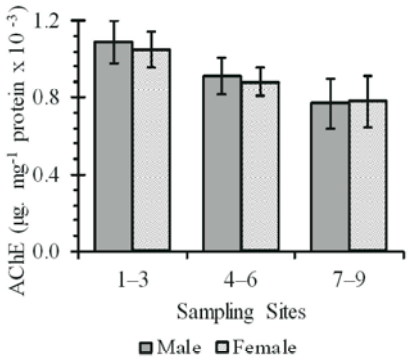

Fig. 4: Mean differences in AChE activity (μg.mg^{-1} protein) of male and female frogs recorded from reference and agro-chemical contaminated sites.

AChE Activity of Gonad Tissues: Compared to reference site, in site 4-6 and site 7-9 the AChE activity was reduced at an order ranged between 16.28% and 27.95%. Fig. 4 depicts the mean differences in AchE activity of male and female frogs respectively. Differences in concentration of AchE in male and female frogs and between sites were significant (male: $F_{2, 183} = 118.9$, $p < 0.0001$; female: $F_{2, 83} = 54.8$, $p < 0.0001$; overall between sites $F_{2, 268} = 168.8$, $p < 0.0001$). This indicates that frog lives in contaminated sites have low AChE activity in their gonadal tissue.

DISCUSSION

In contaminated agro-ecosystems, the different developmental stages of amphibians were continuously exposed to the chemical residues. Such chemical exposure produce stress, detrimental to the survival of many amphibian larvae by affecting their biological processes like metabolism, tissue repair, nervous and immune system, reproductive function [20, 21, 22]. These altered biological process finally leads to the negative impact on growth, deviation in development, retarded growth, morphological abnormalities in many species of frogs [11, 13, 21, 23, 24] and has even been linked to amphibian declines [4].

Female frogs are generally larger than male frogs due to difference in fat contents and gonadal development. In case of atrazine contamination, the mean BM and SVL of males and females were higher in reference sites compared to contaminated sites and SVL is highly correlates with BM [25]. In the present study, there are no differences in SVL or BM of the frogs of reference and contaminated sites, but females are larger and heavier than male frogs. Bencila *et al*. [26] recommend that the condition factor is to be considered as an indicator of overall health status. It has been reported that the frogs in contaminated ecosystem generally showed low condition factor [6]. In this study, the CF is low in contaminated sites and found to be negatively correlated with the data of reference site. The differences in CF between references and contaminated sites are statistically significant ($F_{2, 183} = 405.9$, $p<0.0001$). Since the difference in CF of the frogs inhabiting dissimilar habitat are considered as an indicator of overall health status [7, 17], the low CF observed in present study among the frogs living in contaminated site could indicate lesser health fitness of frogs.

It has been reported that, frogs collected during breeding season showed high body weight and GSI than the frogs collected during non-breeding season [27, 28]. According Ko *et al*. [29] during breeding season, active spermatogenesis increases testis weight and seminiferous tubule size, leads to increased GSI and are declined in the late stages of spermatogenesis. These variations are influenced by local climatic conditions. McCoy *et al*. [4] found that gonadal form and function in the anuran amphibian *B. marinus* are altered by agricultural practices as dose dependent fashion. McDaniel *et al*. [3] observed significant low GSI in female green frogs living in agricultural sites, while, males in the same area were less likely to be mature. Earlier studies showed little variations among GSI of frogs from agricultural against non-agricultural sites [18, 30]. In this study we found that there is no such significance differences between GSI of frogs (male: $F_{2, 183} = 2.559$, $p = 0.0802$; female: $F_{2, 83} = 0.449$, $p = 0.6395$) inhabiting in reference site and those in contaminated sites.

Amphibians are known to be vulnerable to pesticides [22, 31] particularly organochlorine and organophosphate pesticides, as they are cholinesterase inhibitors [32, 33]. In the studied areas, farmers use considerable amount of these pesticides. These pesticides are known to influence negatively on enzymatic actions at neurosynaptic junctions [34, 35]. Organophosphate and carbamate pesticides can reduce the AChE activity thus resulting in muscular twitching, extreme weakness and paralysis and damage to nerve cells [36, 37]. The effect of pesticides on AChE activity in agricultural field could be measured by comparing AChE activity of frogs from uncontaminated area. Lajmanovich *et al*. [38] found that the toads living in intensive agricultural sites were showed plasma ChE activity less than the reference site. In this study we found low AChE activity in gonad tissues of frogs living in contaminated rice fields and differences in AChE concentration with uncontaminated sites were significant ($F_{2, 268} = 168.8$, $p<0.0001$). The low concentration of AChE in gonadal tissue probably indicates long term or delayed consequences on the biology of the frogs.

CONCLUSIONS

The CF, GSI and AChE activity are the major markers depicting the fitness and health status of the frogs living in agroecosystem. GSI and AChE activity in gonad tissues are major key factor to control gonadal function.

The deviation in these factors among the frogs living in contaminated rice paddy fields represents the negative influence of agrochemicals on their health status.

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