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Reproductive Ecology of Narrow mouthed frog *Microhyla ornata*

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ABSTRACT
Reproductive ecology of Narrow mouthed frog *Microhyla ornata* was studied at selected breeding sites around Dharwad, Karnataka India during the wet periods between May 2017 and December 2018. The breeding activity varied depending upon the amount of rainfall. It was higher during monsoon when there was higher rainfall, whereas, when rainfall was scanty less activity was observed. Males begin the calling activity in the evening after the sunset at 1098min reaching its peak at around 1200min and extend till the early morning of the next day. Gravid females arrive at the breeding site one to one and half hour after the calling was initiated in the early pre-monsoon and monsoon period and amplexus took place soon after the arrival of females within one hour during peak calling days. Size assorted matting was observed in *M. ornata* and showed significant positive correlation. The female body size was positive correlation with clutch size. However, there was no significant correlation between female body size and egg size. Breeding activity of *M. ornata* spanning for seven months suggest that, this species is a prolonged breeder. The eggs and larvae of *M. ornata* were under constant threat by the predatory larva of the aquatic insects like Dragonfly and Water beetle. Few clutches during non rainy period faced the risk of desiccation. The breeding sites of many anurans are affected by urbanization and habitat fragmentation. There is a need to adopt the strategy to conserve the habitats to protect the species.

Key words: Breeding ecology, Breeding period**,** *Microhyla ornata,* Size assorted mating, Clutch size

INTRODUCTION

The natural history and reproductive biology of microhylid frogs are poorly known (Wassersug 1980, Donnelly et al. 1990, Lehr et al. 2007, Bowatte and Meegaskumbura 2011). Presently it consists of 58 genera and over 715 species (AMPPHIBIA WEB 2021). In India the family consist of 31 species with 9 genera (Dinesh et al. 2020).The reproductive ecology in this family is studied well in species *Kaloula verrucosa* (Fei et al.2009) and *Kaloula rugifera* (Chen et al. 2015)*Microhyla heymnosii* (Sheridan 2009, Shahriza 2017). The breeding behaviour studies on Indian anurans are limited to acoustic and breeding period of few species of non microhylids like *Polypedatus maculates* (Girish and Saidapur 1998) *Rhacophorous malabaricus* (Kadadevaru et al. 2000) *Philautus variabilis* (Kadadevaru and Kanamadi 2001).*Rhachophorous maximus* (Khongwir et al. 2016) from Rhacophoridae and a Dicroglossid *Minervariya* (*Limnonectus*) *syhadrensis* (Kadadevaru et al. 2002). Though microhylids constitute 30% of the Indian anuran, the studies on reproductive biology in this group are limited to *Uperodon* (*Ramanella*) *vareigata* (Kanamadi et al. 1993), *M. rubra* (Kanamadi et al. 1994) *Uperodon* (*Ramanella*) *montana* (Kadadevaru et al. 2000). The narrow mouthed frog, *Microhyla ornata* is widely distributed species in the entire south Asia, studies on this species are limited to spawning (Padye and Ghate 1989), Skeletochronology (Kumbar and Pancharatna 2001) morphometric study (Mithra and Abhik 2001) Normal development (Satoshi and Hidetoshi 2003) and ecology (Jwngma Narzary 2013). Hence, the present work is undertaken to study the reproductive ecology and elevate the size assorted mating in *Microhyla ornata* at selected ponds of Dharwad area, Karnataka, India.

MATERIALS AND METHODS

The study was conducted in 2017 and 2018, starting from Pre-monsoon (May), covering Monsoon (June-September) and lasted till Post-monsoon period (October-December). The survey duration in 2017 was from 17th May to 9th November, and in 2018 it was between 26th may and 1st November. The work was conducted at different breeding grounds at Dharwad and nearby places (15º26’ N, 74º58’ E). The study sites were visited for every two to three

days in a week every month. The calling males and amplexed pair were observed during night while the counting of the clutches was done on the next day. The counting of calling males and amplexed pair was done with analogue counter by single man power using LED LENSER MH6 head torch. Same method was followed in all the sites. Snout Vent Length (SVL) was measured in the field. Few amplexed pairs from the site were collected and shifted to laboratory to measure SVL of males and females using digital calliper, to check the size based mate choice and clutch size. The captured pairs were released back to the breeding site after measurement. Pearson's correlation was used to corroborate the relationship between the amplexed pair, female body size and reproductive outcomes (egg number and egg size). Data related to rainfall was obtained from Karnataka Government meteorological department Dharwad, India. Atmospheric temperature and water temperature at breeding grounds was measured using digital thermometer. Correlation was calculated using Past 3.24 software (Hammer et al. 2001), values are represented with range and mean ± SE.

RESULTS AND DISCUSSION

Breeding activity

Total of 2361 *Microhyla ornata* individuals were encountered in two breeding seasons in two years of survey period at 13 different breeding sites. The breeding sites were either temporary or permanent water bodies whose size varied from 0.0077 to 0.7785 ha. The sites were surrounded with grass and smaller shrubs of 22-60 cm in height. The permanent water bodies were larger and shallow with submerged aquatic vegetation and surrounded by medium sized vegetation and trees. The rainfall during the study period ranged between 0.4 and 197 mm with an average of 74.9±15.30 mm. The atmospheric temperature at the study site ranged from 22.6 to 25.1 ^{o}C with an average of 23.68±0.21^{o}C and the water temperature ranged from 22.1 to 24.6^{o}C with average of (23.24±0.28^{o}C). The breeding activity of *M. ornata* in Dharwad was initiated from premonsoon in the month of May, and extended up to post monsoon(November). The activity varied depending upon the amount of rainfall. It was higher during monsoon when there was higher rainfall. When rainfall was scanty less activity was observed. During peak breeding months calling male number was more which was coincided with temperature variation (Fig. 1). Rainfall and temperature related breeding activity were also observed in different families of Buffonidaes, Ranidaes, Rhacophoridaes, Pelobatidaes and Microhylidaes (Wells 1997, 2007, Fogarty and Vilella 2002, Zina and Haddad 2005, Daniel Saenz et al. 2006, Lai et al 2003, Cabanzo-Olarte et al. 2013, Maria camila Basto Riascos et al. 2017). *Rana boylii* (Wheeler and Welish 2008) *Nanorana parkeri* (Xin Lu et al. 2016). The microhabitats for calling in *M.ornata* were small mud crevices, under the grass blades or twigs of small herbs and some were observed calling from open land. Calling males (SVL=21.00 ± 0.19cm, n=90) were observed at a distance of 3-4 meters away from the spawning site. Calling activity begins in the evening after the sunset at about 18:30 hrs reaching its peak at around 20hrs and extends till the early morning of the next day. Gravid females (SVL=22.67 ± 0.13mm, n=90)arrive at the breeding site one to one and half hour after the calling was initiated by the males in the early pre-monsoon and monsoon days. Amplexus took place within one hour (45.47.2±8.3 min. n=35) after the arrival of females to the breeding ground during peak calling days. In post-monsoon days with the gradual decrease of rains the calling male number was reduced and females reached the pond after 2 to 5 hrs of call initiation and the time taken for forming the amplexus was more than 3hrs (3.35±0.13hrs. n=15). Maximum number of amplexed pairs was encountered at a distance of 2 to 4 mtrs away from the spawning site and only few pairs were observed at the periphery of the pond. The amplexus in case of *M. ornata* was axillary (Fig. 2).The Amplexus period varied from 3 to 4 hrs (4.16±0.14hrs n=10). Size assorted matting was observed in *M ornata* and showed significant positive correlation (r=0.564, r^2=0.318, t=5.763, P=0.001, N=73) (Fig. 3a). Tibetian frog *Nanorana parkeri* also showed positive correlation regarding size assorted matting in early breeding season (Xin Lu et al. 2016). Reproductive mode of *M. ornata* is mode 1 i.e., eggs are laid in lentic water as thin film (Duellmann and Trueb 1994). In the present observation, eggs were typically released in shallow, near-shore regions of water bodies with low emergent

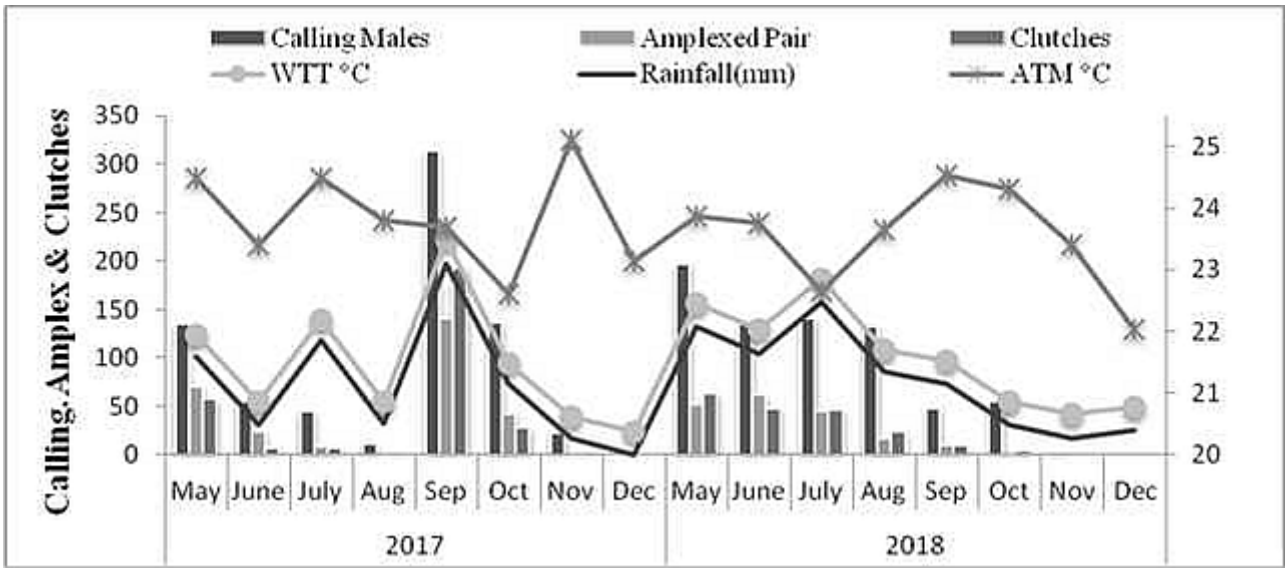

Figure 1. Breeding activities in relation to rainfall and temperature during study period.

Figure 2. Axillary amplex in *Microhyla ornata*

vegetation. The distance of oviposition site varied from 10 – 70 cm with an average of 39.96 ± 2.76 cm(n=35) from the shore and the depth at which the eggs were laid ranged between 7 cm and 32 cm with an average of 19.812 ± 2.844cm (n=15). The gravid female body size showed positive correlation with clutch size (r=0.089, r^2=0.008, t=0.761, p=0.44). However female body size and egg size were negetaviely correlated and non-significant (r= -0.022, r^2= 0.004, t= -0.186, p= 0.849) (Fig. 3b, c). In species like *Kaloula rugifera* (Chen et al. 2015), *Nanorana parkeri* (Xin Lu et al. 2016), *Espadarana prosoblepon* (Maria Camila et al. 2017) also show similar kind of observations. The egg mass was in the form of an irregular circular sheet the diameter of which ranged from 44.55 mm to 143. 79 mm with an average of 106.76 ± 5.2 (N = 23). A total of 486 clutches were recorded during study period. A total of 486 clutches were recorded during study period. Number of clutches varied depending upon the rains. The number of eggs ranged from 245-1207eggs with an average of 925.64 ± 24.10, eggs per clutch. Eggs were transparent covered with jelly coat the egg size ranged from 0.53 to 2.0 mm with an average of 1.05 ± 0.05mm(N= 73). Correlation between the egg number and egg size appeared to be negative and non-significant (r = -0.049, r^2 =0.0024, t = -0.413, p=0.688) (Fig. 3d). Breeding activity of *M. ornata* spanning for seven months suggest that, this species is a prolonged breeder (Wells 1977). *Kaloula rugifera* a microhylid from China has a breeding activity of four month (Chen et al. 2013). *Microhyla heymonsi* too showed breeding activity for about four months but differed in the months at Thailand and Singapore (Sheridan, 2009). In Indian micohylids the breeding period of *Microhyla rubra* extends from April to October (Kanamadi et al. 1994, in *Ramanella (Uperodon) Montana* it is between June and September (Kadadevaru et al. 1998), in *Kalaula (Uperodon) pulchara* it is between April and August (Kanamadi et al. 2002).

Risk factors

Breeding of aquatic insects like, dragonfly (*Diplocodes trivialis*) and water beetle (*Thermonectus marmoratus*) coincide with the *M. ornata* breeding season in the present study at few

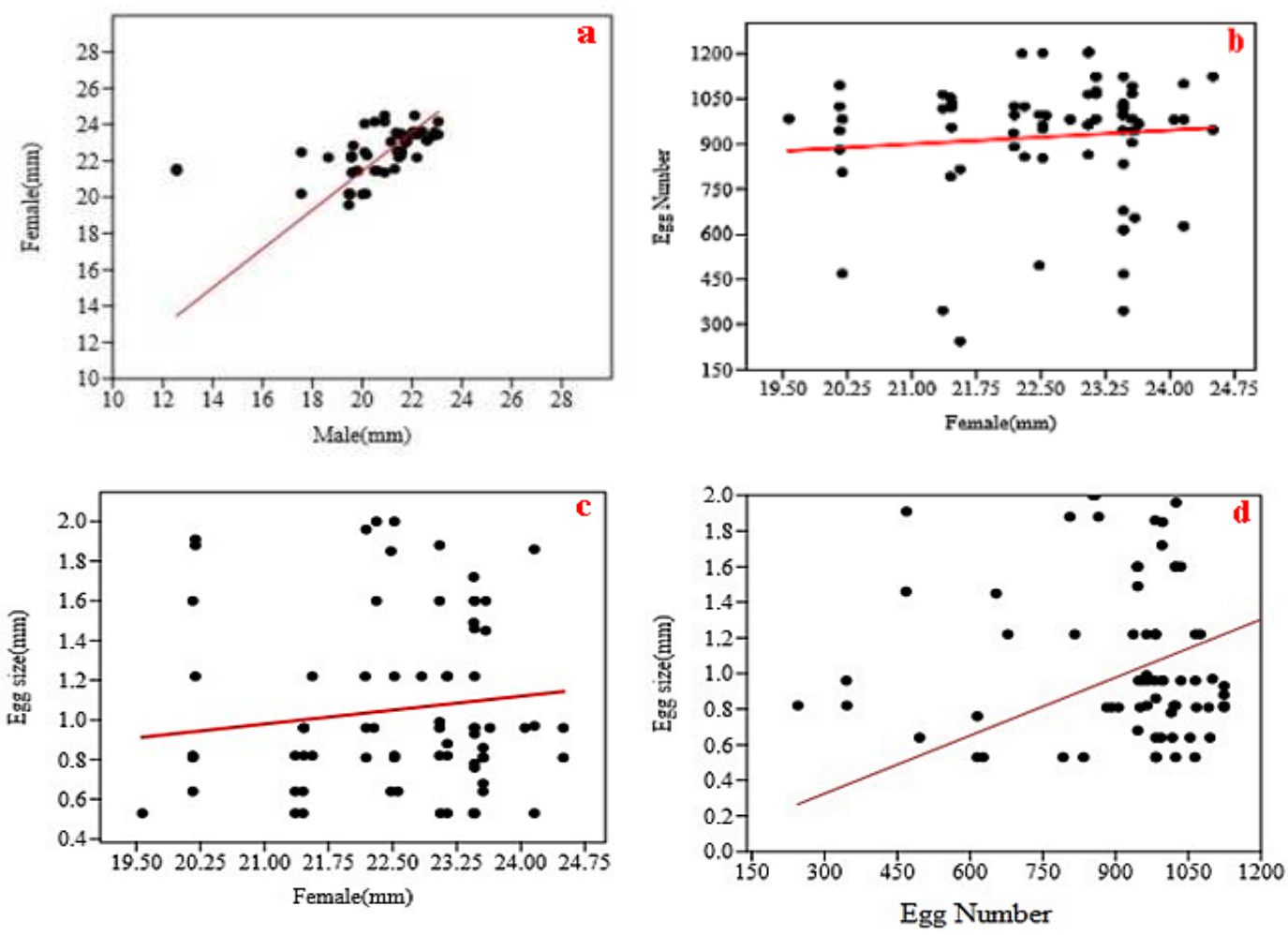

Figure 3. (a) Correlation of size assorted mating in Male and female *M. ornata*, (b) Egg number correlated with female body size, (c) Egg size correlated with female body size, (d) Egg size correlated with egg number

locations. As a result, the eggs and larvae of *M. ornata* were under constant threat by the predatory larva of these aquatic insects (n=32). Predators can present one of the most significant challenges to successful reproduction by anurans (Morin 1983, Wilbur 1987, Warner and Mc Peek 1994). Depositing eggs at a site with predators can result in complete failure of reproduction which may be problematic for short lived anurans with few opportunities to breed. In addition, when the rainfall was uneven there was random selection of spawning site, in such cases many egg clutches were observed at the periphery of the water body and such clutches faced the risk of desiccation as the water at the edges dries up due to evaporation leading to desiccation (n=17) (Fig.4).

CONCLUSIONS

The narrow mouthed frog *Microhyla ornata i*s widely distributed Microhylid. The breeding activity depends upon the rainfall. Its breeding period extends to seven months. Size assorted mating behaviour is observed in the present study. There is a significant positive correlation between female body size and clutch size. However there is no correlation between body size and egg size. In few sites the developing larva faced the risk of predators and also desiccation. Due to urbanization and habitat fragmentation the breeding grounds for many anurans are under threat. There is a need to adopt the strategy to conserve the habitats to protect the species.

Figure 4. Eggs exposed to desiccation

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Authors' contribution: Both the authors contributed equally.

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