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Cover: Green Sea Turtle *Chelonia mydas* watercolour by Elakshi Mahika Molur.

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COMMUNICATION

Observations on the courtship behaviour of Deocata Pipefish *Microphis deocata* (Hamilton, 1822) (Actinopterygii: Syngnathiformes: Syngnathidae) in an aquarium

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Abstract: The Deocata Pipefish *Microphis deocata,* is the sole freshwater syngnathid found in the Himalayan range. This species is categorised as 'Near Threatened' by the IUCN Red List and is also listed as a Schedule I species under the Wildlife Protection Act of 1972. This species is highly sought after in the ornamental fish trade due to its attractive colouration and uniqueness. The courtship behaviour of *M. deocata* consists of three distinct phases (initial courtship, spawning, and swaying) characterized by substantial behavioural alterations. *M. deocata* is a species where the action of females is more visible for commencing courting and strengthening at later phases. The females exhibit skin fold ornamentation to signal their readiness to spawn mature eggs. The first courtship phase is characterised by female quivering along with head pointing by both sexes and often tail entangling. As a sign of embracing the male often overlies upon the female. In the second phase, the female transfers her eggs onto the male incubating ventral surface with an upside-down body posture. During the final phase, the male forms his body into a contracting wave and lastly settles down in the bottom of the tank.

Keywords: Conservation, captive breeding, male pregnancy, sex-role reversal, spawning.

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Author contributions: The experiment was planned and critically examined by AS and DS; the methodologies were carried out by AS and JKN; the manuscript was prepared by AS and JKN; the final editing was done by DS. All authors reviewed and approved the final version of the manuscript.

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INTRODUCTION

Although the family Syngnathidae largely consists of a marine taxon that includes seahorses, pipefishes, and seadragons, there are a few pipefishes of the genus *Dorichthys* and *Microphis* that inhabit freshwater habitat (Dawson 1979, 1984). These species often exhibit a unique pre-copulation nuptial dance in the water column (Gudger 1905) and paternal care where male investment in offspring typically is in the form of embryo incubation, aeration, protection, and provisioning (Ridley 1978; Wilson et al. 2001; Stölting & Wilson 2007; Kvarnemo et al. 2011). In the Broadnosed Pipefish *Syngnathus typhle*, males possess a brood pouch that enables efficient nursing of offspring (Kvarnemo et al. 2011). In contrast, *Nerophis ophidion* lacks such a brood pouch (Berglund et al. 1986). Predominant competition for mates in females is more pronounced in species where the male potential reproductive rate is lower than that of females (Vincent et al. 1992). Moreover, among species the male shows a variation in the degree of structural and physiological specialization of parental care (Herald 1959; Vincent et al. 1992; Wilson et al. 2001). Within this family the operation of sexual selection (Trivers 1972; Thornhill & Gwynne 1986) can work on either of the sex (Shuker & Kvarnemo 2021), by the process of competition for mating or differential mate choice (Trivers 1972; Shuker & Kvarnemo 2021). If males compete among themselves for females, then the sexual selection pressure will be primarily on males, whereas predominant female competition results in sexually selected traits and ornaments in females (Vincent et al. 1992). However, in the conventional courtship pattern of the animal kingdom, where males are typically more competitive, females may invest a greater portion of their energy towards offspring than males (Trivers 1972; Masonjones 1996).

In this article, we present data on the courtship and spawning of *M. deocata.* Some brief notes on several aspects of syngnathid behaviour are also presented, to allow comparisons with *M. deocata*'s specific reproductive behaviour patterns. As this species is the only threatened freshwater syngnathid from Himalayan range therefore it should be our prime concern to save this species and their natural populations. Its conservation is much more crucial as by protecting this species, the habitat and rest of the fish communities will also get protected (Vincent et al. 2011a). In addition, this species has got a huge market demand in the ornamental fish business because of its uniqueness (Vincent et al. 2011b). Overexploitation and habitat destruction have resulted in a significant loss of wild stocks. Acquiring an understanding of courtship behaviour will improve our knowledge of their reproductive ecologies, and how to protect them in nature, but it can also enable captive propagation, thereby reducing pressure on wild stocks.

METHODS

The present study was approved by the Institutional Animal Ethical Committee of Gauhati University, Assam (Reference ID: IAEC/PER/2019/PP-IAEC/2018-034). *Microphis deocata* occurs mainly in rivers, streams and lakes (Menon 1999) and was found in rivers of northern Bengal and Bihar. Afterwards, reported from the foothills of eastern Himalaya, below Darjeeling (Hora 1921; Menon 1974), Brahmaputra drainage in Assam (Sehgal 1956; Sen & Choudhury 1977; Sen 1980) and Arunachal Pradesh (Sen 2000). The species has been categorized as 'Near Threatened' in the IUCN Red list of Threatened Species due to habitat destruction and overexploitation. The body is elongated and sub-cylindrical with a protruding snout. The sexes are dimorphic. Males are mainly brownish, with a dark lateral stripe on the snout and above the operculum (Dawson 1984). Adult females show distinctive Y-shaped markings on the lower half side of the trunk. During the breeding season, females extend their abdomen from their body axis with vibrant colour pattern whereas males show a continuous distinct white dotted line in its dorsal surface (Image 1) (A. Saikia, pers. obs. 29 August 2020).

About 105 adult individuals (Average total length±S.D: Male—113±3 mm; Female—141±3 mm) were kept for 12 months in three tanks approximately 90 l, depth—365.76 mm with sand and rock bottom, planted with *Vallisneria* sp. and *Hydrilla* sp. sponge and under-gravel filters were provided for the recirculating water. Fishes were kept in the sex ratio of 1:2 (Male: Female). The male produces 7–8 broods over a span of a year, with a brooding period of 25±2 days. The fish were collected as by-catch from local fishermen using scoop nets (mesh size: 1.0–2.0 mm) in January 2020 from the Manas River (Brahmaputra drainage), Assam, India. Thermostats (RS 300 W, 220–240 V) were fitted and maintained at 26°C and the diet consisted of *Artemia nauplii* or copepods/rotifers. The aquaria were illuminated with T5 tubes (24W) maintaining a 14L/10D cycle. More than 200 h of ad libitum observations were made at random intervals, with about 1–2 h of videotape

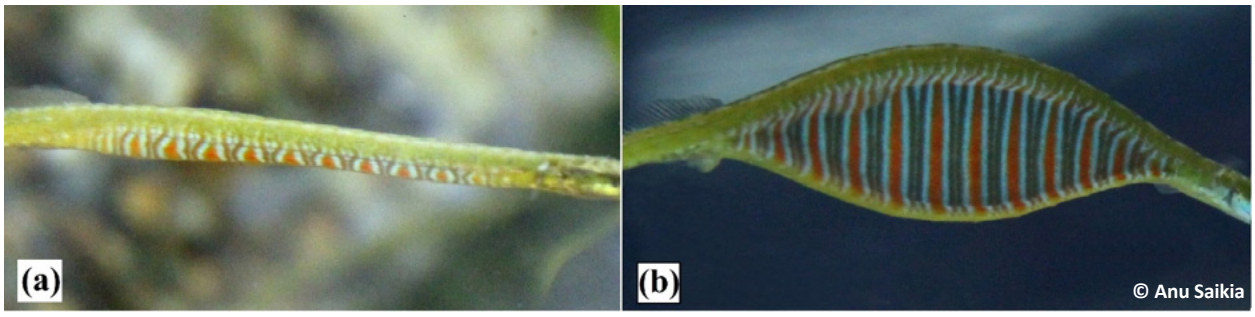

Image 1 . Female amplifiable ornament: a—in isolation | b—interacting with another female (Increasing contrast in the striped pattern).

recordings and the main stages of the courtship rituals were measured and described. In total, we observed eight pairs of courting fish. To give the fish time to get used to the observers, observations were made after a week or two.

RESULTS

Gravid females began to show pre-spawning displays, especially in the morning hours (0430–0600 h), by constantly inflating the ventral skin folds. It consists of a Y-shaped alternate banding pattern (Image 1). The ornament (contrasted bands) is presented throughout the entire courtship process that lasts for 1–2 h. Before the onset of courtship, there is an increase in basal activity of females accompanied by restless parallel movements with other females horizontally. A clearly distinct 1 mm extended ovipositor and contrasted colouration in the trunk region was visible (Image 1). Whereas, in males, the dorsal portion is singly lined with a discontinuous dotted pattern extending from anterior to posterior region, and has a swollen pouch fold. The spawning events of *M. deocata* consisted of three distinct phases marked by prominent behavioural changes and can be summarized as follows:

In the first phase, continuous quivering by females is observed. However, males were seen to dilate the opening of their pouch, inflating the pouch to balloon-like proportions. Both male and female swam side by side and the male often touched the female's belly with his head; the male and female exhibited head-pointing and their tails were often tangled together. The male often overlies upon the female as a sign of embracing the female. After approximately 2 h, the male and female began to swim towards the water's surface. This phase lasts for a long time as males have a low degree of mating propensity or due to interruptions brought about by other females. The interfering females attempted to place their bodies between those of the mating pair (four at a time in one observation). Males that were ready to receive the eggs moved their folds apart and approached the female, manifesting their readiness. (Figure 1a–c)

The mating pair steadily rose in the water column (up to 38 cm) before the actual spawning event. On approaching a male, the female starts to rotate her prehensile tail (4 rotations). Further, the female grasps the tail of the male, swirls it up, and immediately in an upside- down body posture relaxes its abdomen and lay the eggs facing the anterior region of the pouch within 1–2 s of the time period. During egg transfer, the male and female were usually suspended horizontally in the water column. The attempt for the egg-transfer process was usually repeated thrice at each swim towards the surface and it lasted one to three days during which the male was successful to receive the egg (Figure 1d).

The male continued to swim in the water column while bending his body into a contracting wave, while the female started swimming in an orderly directed way right after the egg transfer. The male maintains this posture for 6–8 s then swims back and repeats the movement. Perhaps, this behaviour was responsible for the packing of the eggs in the posterior end of the marsupium. Despite the fact that sperm ejaculation was not clearly visible, fertilization possibly takes place inside the marsupium right after egg transfer (Kvarnemo & Simmons 2004). After this stage, the male progressively sank to the substratum and remained there immobile, occasionally adopting an S-shaped stance, like other pipefish species (Fiedler 1954) (Figure 1e)

DISCUSSION

Before mating, the increase in activity of the female is more pronounced similar to as observed in *Syngnathus*

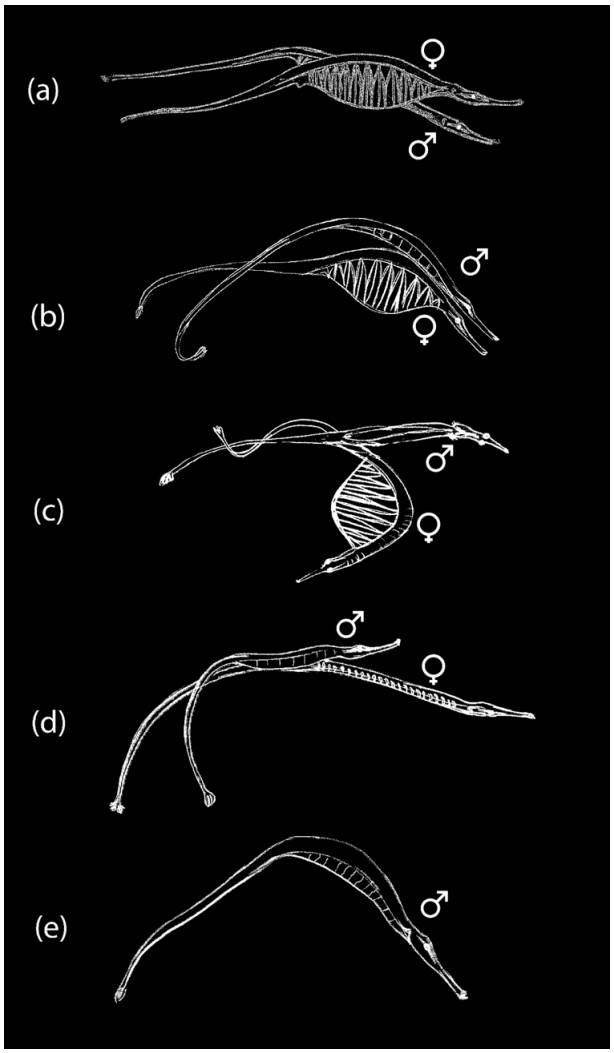

Figure 1. Schematic representation of *Microphis deocata* courtship and spawning sequences: a, b, c—Initial courtship | d—Spawning | e—Swaying. © Jayanta Kumar Nath & Anu Saikia.

abaster (Silva et al. 2006) that usually initiated displays, mainly consisting of vertical swimming movements indicating shallow intertidal habitats also reported in *Corythoichthys intestinalis* (Gronell 1984), *Nerophis ophidion* (L.) (Fiedler 1954). However, it contradicts what is reported for *N. lumbriciformes* where there is a reduction in vertical and swimming movements as they thrive in intertidal zones to avoid strong wave action (Monteiro et al. 2002). In contrast, *M. deocata* shows horizontal parallel movement signifying shallow river and stream habitats, similar as observed in *M. aculeatus* (Christie 2022). Courtship display shown by female of *M. deocata* occurs early in the morning and lasts for about 4.30–6 h. This is in contrast to *S. abster* (Silva et al. 2006) where courtship occurs throughout the day.

Many pipefishes are known to intertwine their bodies like twisted rope during courtship in *C. intestinalis* (Gronell, 1984) and *N. lumbriciformes* (Monteiro, 2002), but the distinct "S" shape curves exhibited here by *M. deocata* are similar to as observed in *M. acul*eatus and other species such as *S. floridae* and *S. abaster* (Breder & Rosen 1966; Gudger 1905; Silva et al. 2006). Male *M. deocata* exhibits a preference for larger-bodied females with greater ornamentation, resembling the characteristics observed in *N. ophidion* (Rosenqvist 1990) and *S. typhle* (Berglund et al. 1997; Berglund & Rosenqvist 2003, 2009). After completion of courtship, the body of the male will be in a bending structure, which indicates that the male has just received the egg. This kind of behavioural adaptation of males strongly signifies that it takes one batch of eggs from a single female at a particular time. During our study, one of the pair pouches was empty before mating but became full after mating. Further investigation is needed on brooding males receiving eggs from one or multiple females.

Afterwards, when the pouch is full, that male will be unavailable for mating with other females in the group. Females are the courting sex and show more intense changes in colour patterns during reproduction than males, as also reported in *N. lumbriciformes*, *N. ophidion* and *S. typhle* (Monteiro 2002; Berglund & Rosenqvist 2003). As in *N. ophidion* (Rosenqvist 1990), *S. typhle* (Berglund & Rosenqvist 2009), *S. abaster* (Silva et al. 2006), and *M. deocata* an overtly interactions among females suggests the female-female competition for mates resulting dominance one over another which occurs mainly through sexual signalling, having a more contrasted colouration in the trunk with more inflation tendency. They can be considered sex role reversed (Vincent et al. 1992) as predominantly females are much more active than males and female-female competition is seen for matings, similar as observed in *M. deocata.*

The incidence of disturbances during the initial stage of the courting ritual appears to be another common occurrence. Females were seen approaching the courting couple and starting to aggressively flicker or just following the pair in a parallel motion, a behaviour that might be seen as a sort of competitiveness (Matsumoto & Yanagisawa 2001). Strangely, the invading female frequently had trouble mating with the courting male. The prolonged courting display, however, appeared to come to a halt as a result of these disruptive females' far greater effectiveness in diverting the other female's attention. Similar observations have been described in *Corythoichthys haematopterus* (Bleeker) (Matsumoto &

Yanagisawa 2001), *Syngnathus schlegeli* Kaup (Watanabe et al. 2000) *N. lumbriciformis* (Monteiro et al. 2002) and *M. deocata*, indicating that the struggle for mates, which differs widely throughout the syngnathid taxa, is highly influenced by female-female interactions (Berglund & Rosenqvist 2003). However, an experiment carried out by Rosenqvist (1990) on *Nerophis ophidion* showed a female-dominance effect in order of the size of the skin fold of females. Similarly, the exhibition of female-dominance cannot be denied in *M. deocata* because the successful deposition of eggs was recorded in all males of the present study despite the maintenance of 1 male: 2 female sex ratio. As females of *M. deocata* exhibit an ornament, i.e., the colourful belly, and distension of the belly amplifies this ornament, there is always a greater tendency of males' choice to select a female having the largest skin fold during courtship and subsequent mating.

In conclusion, the present study provides insights of the breeding behaviour of *M. deocata* in captivity, i.e., in aquarium. Being the only threatened freshwater syngnathid of northeastern India, information shared here will aid in the formulation of effective captive breeding and rearing protocols along with proper identification of broodstock and their basic requirements in captivity. The information shared here aims to assist freshwater pipefish breeders worldwide and restore the population of this threatened species in the wild.

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