

Nuno Monteiro · Maria da Natividade Vieira
Vitor C. Almada

The courtship behaviour of the pipefish *Nerophis lumbriciformis*: reflections of an adaptation to intertidal life

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Abstract The courtship behaviour of *Nerophis lumbriciformis* (Pisces: Syngnathidae) consists of three distinct phases (initial courtship, spawning, and embrace) marked by prominent behavioural changes. The first courtship phase is characterised by female quivering. Courtship activity increases from low to high levels of quivering, causing undulatory head movements in the female. In the second phase, the female transfers her eggs onto the male incubating ventral surface. During the final phase, the male wraps his body around the female (embrace). Females actively initiate courtship indicating that these pipefishes are courtship role reversed. The complete lack of swimming and vertical movements in the courtship behaviour of *N. lumbriciformis*, unlike in the behaviour of other syngnathid species, suggests an adaptation to intertidal conditions.

Keywords Behaviour · Courtship · Intertidal · *Nerophis lumbriciformis* · Spawning

Introduction

Fishes of the family Syngnathidae are exceptional models for the study of sexual selection (Darwin 1871; Barlow 1989; Johnstone 1995; Sherman and Reeve 1999), parental care (Trivers 1972; Ridley 1978; Blumer 1979; Smith and Wootton 1995), and the influence of the operational sex ratio (OSR) in the expression of sex role reversal (Vincent 1990; Clutton-Brock and Vincent 1991; Clutton-Brock and Parker 1992; Vincent 1994a, b). They show wide variation in the degree of male anatomical and

physiological specialisation for paternal care (Herald 1959; Vincent et al. 1992). Seahorses (genus *Hippocampus*) possess the greatest specialisation, with males incubating eggs within a highly vascularised brood pouch, where females deposit their eggs directly (Vincent 1990). In some pipefishes, such as *Nerophis ophidion*, females attach their eggs to the males' ventral surface (Berglund et al. 1989; Rosenqvist 1990). However, there are still very few descriptions of syngnathid courtship behaviour. Comparative studies would certainly provide excellent opportunities for understanding the evolution of this family's unique reproductive behaviour and morphological adaptations. In this respect, the genus *Nerophis* is of special interest since, as pointed out by Herald (1959), it has the least developed structure for carrying eggs. Furthermore, there is almost no information on the behaviour and ecology of *N. lumbriciformis* (Jenyns, 1835).

In this article we present data on the courtship and spawning of *N. lumbriciformis*. Some brief notes on several aspects of syngnathid behaviour are also presented, to allow comparisons with *N. lumbriciformis*' particular reproductive behaviour patterns.

Methods

Study organism

N. lumbriciformis occurs mainly in the intertidal zone, from Norway to Morocco (Dawson 1986), and is the most abundant intertidal syngnathid on the Portuguese rocky coast, although it is also present in estuaries (Almeida 1994). It is a small and slender green-brown pipefish, with a vermiform shape and an upwardly pointing snout. The sexes are dimorphic in size (females are larger), colour (females are more conspicuously coloured), and behaviour. During the breeding season, females develop ventral and dorsal skin folds, whereas males have a flattened ventral surface (N. Monteiro, personal observation).

Courtship behaviour trials

About 60 adult individuals were kept for 18 months in three tanks (approximately 85 l), with a sand, algae, and rock bottom. Fishes were caught in the rocky intertidal zone of Viana do Castelo

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N. Monteiro (✉) · M. da Natividade Vieira
Faculty of Sciences, University of Oporto,
Praça Gomes Teixeira, 4099-002 Porto, Portugal
e-mail: nmonteir@fc.up.pt
Tel.: +351-22-3401514, Fax: +351-22-3401511

V.C. Almada
ISPA, Rua Jardim do Tabaco, 34, 1149-041 Lisbon, Portugal

(41°41'45"N, 8051'09"W). The water temperature in the aquarium was constant (14–15°C) and the diet consisted of fresh *Artemia nauplii*. The tanks were illuminated with 18 W fluorescent light, with a photoperiod of 13L:11D. More than 200 h of ad libitum observations were made (*sensu* Martin and Bateson 1993), at random intervals, with about 60 h of videotape recordings. Observations were conducted after a 1 to 2-week period, allowing the fish to adapt to the observer's presence.

Results

Females willing to mate showed contrasted flanks. This ornament (stripes), presented throughout the entire courtship process, is a temporary amplification of the normal contrast and is very similar to the one described for *Syngnathus typhle* (Berglund et al. 1997). In addition, there was a rise in female activity level. The genital papilla was also clearly visible. The male's mating predisposition is more difficult to determine. Generally, they showed an extremely flattened ventral surface, sometimes forming a concavity, always without the presence of alveoli from a previous pregnancy.

Initial courtship took place throughout the entire day, especially after dawn and during the afternoon. Fish activity decreased significantly during the night (N. Monteiro et al., unpublished data). The first sign of the onset of courtship was a considerable rise in female activity. The courtship ritual may occur underneath stones, on the sand, or in algae, but always in close contact with the substratum. A survey of the complete courtship and spawning sequences is summarised as follows:

- Initial courtship

The female approaches a male, assuming a side-by-side position, and starts to quiver (these tremors result from vigorous vibrations of the dorsal fin and affect all of the body but not the head). This phase is characterised by repeated occurrences of quivering (only the female quivers, in continuously decreasing time intervals). Then, the female slowly moves forward and waits for the male to follow. If the male follows the female, the quivering continues, in a crescendo. Otherwise, the female returns to the original position and repeats the quivering or abandons that specific male and immediately looks for another available mate. After a short period (5–10 min), if the male follows, the female starts to quiver in physical contact with the male. As the quivering intensity rises, the female's head starts to vibrate, in a vertical movement (Fig. 1, a). Sometimes, the male's body shakes as a result of the contact with the quivering female. This period can be relatively long as a result of a low level of male's mating predisposition or by disruptions caused by other females. Indeed, the mating pairs were frequently disturbed by other females (four at a time in one observation) that quivered to the male and attempted to place their bodies between those of the pair. Nevertheless, this initial courtship period is considerably shorter (30 min) than the one described for seahorses (Vincent 1990, 1994b, 1995; Vincent and Sadler 1995).

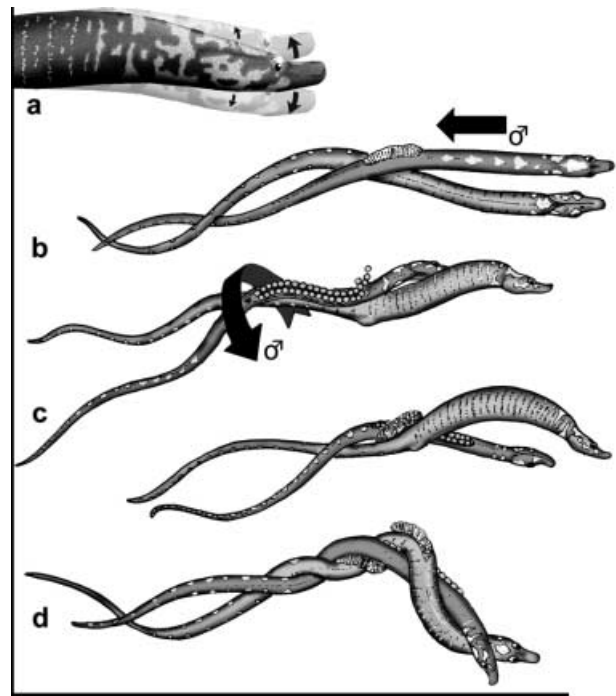


Fig. 1 Schematic representation of *Nerophis lumbriciformis* main courtship and spawning sequences (a-female quivering; b-spawning; c-male rotation; d-embrace)

- Spawning

After the initial phase, the female starts to tilt its body along its longitudinal axis, turning its genital papilla, extremely distended, to the male. Generally only after a few female attempts, the male adopts a similar position, turning its ventral surface to the female (Fig. 1, b). The male starts to vibrate its dorsal fin vigorously (about 6 s) as soon as their genital regions are in contact. After this contact, the female immediately starts egg deposition and the male slowly moves backward, always with its incubating surface turned to the female's genital papilla. Observations of unfertilised eggs under a stereomicroscope showed that they are not released individually. Instead, they form long strings that may have a length greater than 20–25 eggs, arranged in two to four parallel rows (average n°. of eggs, 49; $n=114$; range, 18–84; $SD=10.84$), with a mucilaginous layer in the portion that is in contact with the male's incubating ventral surface. Once the incubating surface is completely occupied, the male ends its backwards movement and starts to rotate along its longitudinal axis (usually three full turns), thus promoting the removal of the surplus eggs (not directly attached to the incubating surface) (Fig. 1, c).

- Embrace

Immediately after spawning, the male wraps himself around the female (Fig. 1, d) in a close and prolonged embrace (10–15 min). During this period, both individuals wander, apparently erratically, always in close contact with the substratum. After a while, the female

slowly tries to disengage, but it is the male that apparently determines the end of this period.

Discussion

We argue that like *N. ophidion* and *S. typhle* (Berglund et al. 1989, 1997), *N. lumbriciformis* is a sex role reversed species. Females are the courting sex and show more intense changes in colour patterns during reproduction than males. They are the “more ornamented sex”. In addition, the frequent disruptions of mating by intruding females that we observed are likely to represent female–female competition, a process that was not observed in males. Our aquarium observations also suggest that the potential reproductive rate of females may exceed that of males (one female re-mated during a male pregnancy period).

The courtship behaviour of several seahorse (Vincent 1990, 1995; Vincent and Sadler 1995; Masonjones and Lewis 1996) and pipefish species (Vincent et al. 1995) has already been described. The general patterns of behaviour are extremely similar. Nevertheless, there is a striking difference in *N. lumbriciformis* courtship. Unlike that of other syngnathid species, which rise up before, during, and/or after egg transference (*S. acus*, *S. typhle*, *N. ophidion*, and *Hippocampus* spp.), the entire courtship ritual takes place in close contact with the substratum. We suggest that, at the behavioural level, the reduction of vertical and swimming elements may constitute an adaptation to typical intertidal conditions, since *N. lumbriciformis* inhabits areas exposed to strong wave action, an interpretation similar to that proposed by Almada and Santos (1995) to explain the reduction of water column displays in intertidal blennies.

So far, it has been impossible to describe accurately the fertilisation in this species, but it seems very likely that the sperm may be spread underneath the egg layer by the backward movement of the male. Initially concentrated in his genital area, the sperm is probably continuously pushed forward by the eggs that are being placed whilst the male is moving backwards. Thus, the fertilisation might occur during the embrace period, which may have evolved as a means of avoiding sneak fertilisations, a phenomenon widespread in fishes (see Taborsky 1994, for a review). If the sperm is indeed moved along the ventral surface of the male by the extending egg layer, one interesting consequence would be the inability of the male to fertilise spawnings of additional females since the genital area would become obstructed by the previous eggs, forcing monogamy upon the males.

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References

- Almada VC, Santos RS (1995) Parental care in the rocky intertidal: a case study of adaptation and exaptation in Mediterranean and Atlantic blennies. *Rev Fish Biol Fish* 5:23–37
- Almeida AJ (1994) Macrofauna acompanhante de zosteráceas. Importância na conservação do meio marinho. In: Professor Germano da Fonseca Sacarrão (1914–1992). Museu Nacional de História Natural, Museu Bocage, Lisbon, pp 125–144
- Barlow GW (1989) Choosy fishes: mating and related issues. In: Blanchard RJ, Brain PF, Blanchard DC, Parmigiani S (eds) *Ethoexperimental approaches to the study of behavior*. Kluwer, Boston, pp 267–287
- Berglund A, Rosenqvist G, Svensson I (1989) Reproductive success of females limited by males in two pipefish species. *Am Nat* 133:506–516
- Berglund A, Rosenqvist G, Bernet P (1997) Ornamentation predicts reproductive success in female pipefish. *Behav Ecol Sociobiol* 40:145–150
- Blumer LS (1979) Male parental care in the bony fishes. *Q Rev Biol* 54:149–161
- Clutton-Brock TH, Parker GA (1992) Potential reproductive rates and the operation of sexual selection. *Q Rev Biol* 67:437–456
- Clutton-Brock TH, Vincent A (1991) Sexual selection and the potential reproductive rates of males and females. *Nature* 351:58–60
- Darwin C (1871) *The descent of man and selection in relation to sex*. Murray, London
- Dawson CE (1986) Syngnathidae. In: Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J, Tortonese E (eds) *Fishes of the north-eastern Atlantic and the Mediterranean*. UNESCO, Paris, pp 628–639
- Herald ES (1959) From pipefish to seahorse – a study of phylogenetic relationships. *Proc Calif Acad Sci* 29:465–473
- Johnstone RA (1995) Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. *Biol Rev* 70:1–65
- Martin P, Bateson P (1993) *Measuring behaviour: an introductory guide*, 20th edn. Cambridge University Press, Cambridge
- Masonjones HD, Lewis SM (1996) Courtship behaviour in the dwarf seahorse, *Hippocampus zosterae*. *Copeia* 3:634–640
- Ridley M (1978) Parental care. *Anim Behav* 26:904–932
- Rosenqvist G (1990) Male mate choice and female–female competition for mates in the pipefish *Nerophis ophidion*. *Anim Behav* 39:1110–1115
- Sherman PW, Reeve HK (1999) Sexual selection and sensory exploitation. *Science* 283:1083
- Smith C, Wootton RJ (1995) The costs of parental care in teleost fishes. *Rev Fish Biol Fish* 5:7–22
- Taborsky M (1994) Sneakers, satellites and helpers: parasitic and cooperative behaviour in fish reproduction. *Adv Stud Behav* 23:1–100
- Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) *Sexual selection and the descent of man*. Aldine-Atherton, Chicago, pp 136–179
- Vincent A, Ahnesjö I, Berglund A, Rosenqvist G (1992) Pipefishes and seahorses: are they all sex role reversed? *Trends Ecol Evol* 7:237–241
- Vincent A, Berglund A, Ahnesjö I (1995) Reproductive ecology of five pipefish species in one eelgrass meadow. *Environ Biol Fish* 44:347–361
- Vincent ACJ (1990) Reproductive ecology of seahorses. PhD thesis, Cambridge University
- Vincent ACJ (1994a) Seahorses exhibit conventional sex roles in mating competition, despite male pregnancy. *Behaviour* 128:135–151
- Vincent ACJ (1994b) Operational sex ratios in seahorses. *Behaviour* 128:154–167
- Vincent ACJ (1995) A role for daily greetings in maintaining seahorse pair bonds. *Anim Behav* 49:258–260
- Vincent ACJ, Sadler LM (1995) Faithful pair bonds in wild seahorses, *Hippocampus whitei*. *Anim Behav* 50:1557–1569