

Why is there no sperm competition in a pipefish with externally brooding males? Insights from sperm activation and morphology

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Nerophis ophidion sperm activation and morphology were investigated with the aim of explaining the apparent lack of sperm competition in this syngnathid with externally brooding males. *Nerophis ophidion* sperm were activated by a mixture of ovarian fluid and sea water, but not by sea water alone. This indicated that sperm were not shed into the water but needed to be released near the eggs, which probably restrained sperm competition.

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Sperm competition is widespread among animals and affect a variety of life-history traits (Smith, 1984; Birkhead & Møller, 1998). In fishes, external fertilization often exposes males to sperm competition from sneaker males that gain fertilizations by shedding their sperm nearby a mating couple (Petersen & Warner, 1998). In syngnathids (pipefishes and seahorses), eggs may be fertilized externally and brooded attached to the males ventral surface or fertilized and brooded in the male's brood pouch (Breder & Rosen, 1966). Although species with external fertilization are expected to be more exposed to sperm competition through sneaking, all paternity studies on syngnathids to date show that the male gains exclusive paternity to the young in his brood, with or without brood pouches (Jones & Avise, 2001; Avise *et al.*, 2002). Furthermore, all paternity studies on syngnathids up to date show that the male gains exclusive paternity to the young in his brood, with or without brood pouches (Avise *et al.* 2002). So why does there seem to be no sperm competition among these fishes if they indeed are external fertilizers? The aim of this study was to investigate the fertilization mode in one of the externally brooding syngnathids, the straight-nose pipefish *Nerophis ophidion* (L.). In this species, males carry the eggs

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attached to the bare skin and have been thought to fertilize them externally by distributing the sperm over the eggs after the egg transfer (Fiedler, 1954).

As sperm are sensitive to osmotic changes, the environmental conditions required to activate sperm give indications of the fertilization mode. In virtually all externally fertilizing fishes, sperm are activated by the change in osmolality when they are diluted in the aquatic environment (Morisawa & Morisawa, 1990). One exception is the Pacific herring *Clupea pallasii* Valenciennes in which sperm are activated by a particular egg protein in addition to a change in osmolality (Pillai *et al.*, 1993, 1994; Lahnsteiner & Patzner, 1998). This initiation process is equivalent to internally fertilizing teleost sperm being activated by some other factor in the isotonic and ionic environment in the female reproductive organ (Morisawa & Morisawa, 1990). Therefore, tests were conducted to investigate whether *N. ophidion* sperm were activated by sea water, ovarian fluid (which plays an important role in fertilization in many fishes, *e.g.* *Gasterosteus aculeatus* L., Elofsson *et al.*, 2003), as well as a mixture of both. The sperm morphology may also hint at the fertilization mode, as internal fertilization in percomorph fishes (to which Syngnathidae belongs) is generally associated with more elongated heads and tails compared to externally fertilizing species (Jamieson, 1991).

Fish were collected in May 2002 and 2003, in shallow eelgrass meadows in the Gullmar fjord at the Swedish west coast (58° 15' N; 11° 28' E), using a small beam trawl pulled by a boat. They were kept in a built-in tank measuring 3 × 1 × 1 m with a large glass window along one side at the Klubban Biological Station (Gothenburg Animal Research Ethical Committee permit Dnr 103-2002 and 142-2003). The lid was kept open during the day to allow natural light. The aquarium contained artificial eelgrass and was supplied with constantly running ambient sea water. Fish were fed daily and spawned readily under these conditions.

Sperm activity and morphology were investigated under a light microscope (×40 objective). Because these fish are not easily stripped, they were killed by decapitation and the gonads dissected from the body cavity and cut open to obtain sperm and ovarian fluid. To ascertain sexual maturity, only specimens showing courtship behaviours were used. Ovarian fluid was collected with a pipette from between the eggs of dissected ovaries. At dissection, each pair of testes were cut into small pieces and diluted in two of three treatment media, being sea water (salinity 30, 15° C), pure ovarian fluid, or a mixture (*c.* 1 : 1) of both. Sperm activity was examined instantly after dilution and within 1 min of dissection. In addition, the samples diluted in ovarian fluid were subsequently mixed with sea water after 5 min and *vice versa*.

Sperm did not become activated in sea water ($n = 10$ males), nor by pure ovarian fluid ($n = 6$ males). Sperm, however, did activate in a mixture of ovarian fluid and sea water, showing activity for a few minutes, although the percentage of active sperm was low (*c.* 10%, $n = 3$). Sperm that first had been diluted in sea water could not be activated by subsequently added ovarian fluid ($n = 6$) nor *vice versa* ($n = 6$).

The results show that *N. ophidion* sperm are immobile in sea water. The hypothesis that sperm are released directly into the water therefore proves unlikely. Since a mixture of ovarian fluid and sea water did activate the sperm,

however, it seems more plausible that sperm are released in close proximity of the eggs. This fertilization mode could also explain why externally brooding syngnathids have smaller testes than expected if sperm were released into water, based on the expected effect of increased dilution and sperm competition on testis size (Kvarnemo & Simmons, 2004).

Sperm heads were elongated (4–5 μm) and slightly cone shaped, thinning towards the anterior end. Sperm tails measured 30–40 μm (Fig. 1). The oval heads and long tails of *N. ophidion* sperm are similar to sperm found in a pouch-brooding pipefish (seaweed pipefish *Syngnathus schlegeli* Kaup). In this species, fertilization is proposed to occur internally, in the brood pouch of the male (Watanabe *et al.*, 2000). Internal fertilization, which has evolved several times in the percomorph fishes, is associated with an elongation of both heads and tails (Jamieson, 1991). *Nerophis ophidion* sperm have longer heads compared to the spherical sperm nucleus of the externally fertilizing *G. aculeatus* (Hara & Okiyama, 1998), which is a close relative to syngnathids (Nelson, 1994). This sperm morphology thus indicates that the environment that *N. ophidion* sperm encounter is more similar to internally fertilizing fishes.

Since *N. ophidion* is a basal lineage in Syngnathidae (Wilson *et al.*, 2001) and males gain exclusive parentage in their broods in all syngnathids studied thus far (Avisé *et al.*, 2002), sperm competition was probably low in the ancestor to the syngnathids. In the sister group to syngnathids, Solenostomidae, the female broods the eggs within her fused pelvic fins and the eggs are fertilized internally (Blumer, 1982). Therefore sperm moving in ovarian fluid might be ancestral to the syngnathids and may be prevalent among other pouchless syngnathids as

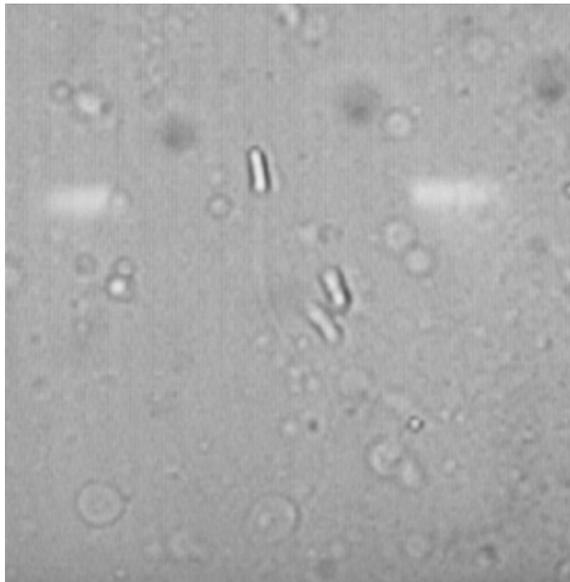


FIG. 1. Photograph of *Nerophis ophidion* sperm. The sperm heads were elongated and measured 4–5 μm , and the sperm tails measured 30–40 μm .

well, which would explain the lack of dilution effect on testis size and cuckoldry in these fishes.

Sperm activation and morphology both indicate that sperm are not shed into the water. The necessity of sperm being deposited in close proximity of the eggs (*i.e.* in the ovarian fluid) undoubtedly limits the possibility of sperm competition.

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