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# Burrow morphology and mating behaviour of the thalassinidean shrimp *Upogebia noronhensis*

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Burrow morphology and mating behaviour of *Upogebia noronhensis* was studied using resin casting of burrows in the field and observation of laboratory maintained animals. Burrows of *U. noronhensis* showed a typical Y-shaped pattern in over 70% of the cases analysed. The remaining 30% comprised U-shaped burrows lacking the lower tunnel (shaft) and burrows with long additional branches projecting from the U portion. Results from animals left to construct burrows in the aquarium closely matched those found in nature. Field and laboratory burrows showed that different shapes are related to the collapse of the burrow walls, the burrowing activities of other individuals and species, and to the behaviour of the species itself. U-shaped burrows form as a result of the partial construction of the burrow (the U part is always built first) or owing to the collapse of the shaft. Burrows with additional branches always belonged to males and result from their search for a female with which to reproduce. This process also produced connected burrows. Mating occurs within the female burrow and this is the only time when two animals occupy the same burrow. After mating, the male returns to its burrow and immediately closes the connection. Larvae are planktonic and probably settle in adult areas, since the smallest juvenile burrows were always associated with adult burrows. This may contribute to the high population densities found in the field ($\sim 200\,\mathrm{ind\,m^{-2}}$), which in consequence facilitates fossorial encounters for reproduction. This is the first time fossorial encounters for reproduction are reported for an *Upogebia* species and probably for all Thalassinidea.

## INTRODUCTION

Thalassinidean decapods are common bioturbators living in almost permanent burrows in many marine and estuarine environments, especially in soft sediments of the intertidal and shallow subtidal. These animals may reach high population densities (Dworschak, 1983, 1987; Turnberg, 1986) and have an important bioturbation activity associated with their burrowing behaviour (Roberts et al., 1981; Nickell et al., 1995; Astall et al., 1997). Dworschak (1987) reported densities $<2420\,\mathrm{ind\,m^{-2}}$ of juvenile *Upogebia pusilla*, while adults may reach $>200\,\mathrm{ind\,m^{-2}}$. During the burrow construction they have both direct and indirect effects on bottom sediment characteristics, by means of digging and turning over the substrate. These activities promote the remobilization of sediment grains and nutrient cycling, changing the physical and chemical features of the local environment (Griffis & Suchanek, 1991).

Despite the ecological significance attributed to the group, little is known about some important aspects of the thalassinidean biology, including copulation. This has been observed only in *Callianassa turnerana*, which leaves the burrow for reproduction (Monod, 1927). However, the vast majority of the studied species live singly (e.g. *Upogebia deltaura*, Hall-Spencer & Atkinson, 1999) and probably leave their burrows only rarely. Fossorial encounters for copulation have been proposed by some authors (Pohl, 1946; Rodrigues, 1976), but they have never been observed.

The present paper describes the burrow morphology and the mechanism of encounter and mating behaviour of *Upogebia noronhensis* Fausto-Filho, 1969 based on the analysis of a wide sample of *in situ* resin casts and aquarium observations. The morphological characteristics of thalassinidean burrows, which reflect their general ecology, are well known and resin casting is the most accurate technique to studying burrow morphology, providing information such as the feeding behaviour of its inhabitants.

This is the second contribution to the knowledge of the biology of a subtidal thalassinidean in the Brazilian coast. The first study was carried out by Coelho et al. (2000) at São Sebastião Island, south-eastern Brazil. The lack of studies on subtidal thalassinideans is, in part, due to the difficulties of collection. Grabs and dredges used in shallow areas often fail to collect organisms which burrow deeper than the penetrability of the gear into the sediment. Hall-Spencer & Atkinson (1999) report that none of their 190 van Veen grab and 26 ring-dredge samples caught specimens of *Upogebia deltaura*.

*Upogebia noronhensis* is one of the eight species of the family Upogebiidae referred to the Brazilian coast. Its distribution was thought to be restricted to Fernando de Noronha Island (~3°50′S 32°25′W, Fausto-Filho, 1969), but sampling during the present study has extended this southwards to São Paulo State coast (23°33′S 45°05′W).

## MATERIALS AND METHODS

The present study was carried out in the subtidal zone of a small cove on the west coast of Anchieta Island, Ubatuba, south-eastern Brazil (23°33′S 45°05′W). The area is dominated throughout the year by high temperature (>20°C) and low salinity (<36 psu) water mass (Coastal Water; Castro Filho et al., 1987).

A 140 m long and 1 m wide transect was positioned on the seabed, perpendicular to the coastline, using a nylon rope. A 1-$m^2$ steel frame was used to estimate the density of burrows along the transect. The transect enclosed a clear sedimentary gradient between the subtidal limit of the rocky shore (6 m deep) and the 15-m isobath, composed of coarse sand, medium sand, fine sand, very fine sand and coarse silt. The *Upogebia noronhensis* population was distributed between the 55 and 133 m marks of the transect (at 8 and 12 m depth, respectively), reaching the highest average density of 200 individuals $m^{-2}$ in the fine sand strip limited between the 94 and 109 m marks of the transect (at ~10 m depth). Over 200 photographs were taken from the transect and analysed in the laboratory.

SCUBA diving was used to enable resin casting and 60 1-h dives were necessary to complete the work. Burrow casts were made in spring 1992 according to the Shinn method (Shinn, 1968), using a Ciba-Geigy epoxy resin (Ciba-Araldite® MY 757 and hardener HY 830; 100:60 parts by weight). The mixture was prepared on-board and carried by divers to the bottom, where it was poured into a 20×20×10 cm metal frame that surrounded some burrow apertures. Denser than seawater, the resin penetrated by gravity into the burrows and after three days casts were ready to be removed. Cast measurements were made according to Dworschak (1983). Casts frequently showed incomplete parts, which prevented the measurement of some dimensions. The resin transparency allowed an accurate sex determination of the animal trapped in the cast. The ratio propodus of the cheliped width/carapace length is larger in males than females. In females, the ratio is 1.2 SD ±0.1, and 2.3 SD ±0.5 in males. All casts were photographed and analysed in the laboratory.

In order to complement cast information, 60 *in situ* tests of burrow opening interconnection were carried out by injection of a jet of water into a hole using a syringe and the observation of its exit (or not) through another hole(s). A water dye was used in some tests, but it was replaced by plain seawater, which gave the best results. Caliper measurements of burrow wall thickness, opening diameter and distance between interconnected openings were also performed *in situ*.

A sample of 145 animals was collected near the transect by a combination of digging with a small spade and manual resuspension of sediment. It is a simple and efficient method for collecting these animals, live and in excellent condition, for observation in aquarium. The animals were collected during the austral winter and spring 1992, autumn and spring 1993, summer 1993/1994 and autumn 1994.

Two sets of observations were carried out in the laboratory, during which the burrowing and mating behaviours were checked. During the first set, ten animals were maintained in two narrow, sediment-filled aquaria (six and four animals on each, respectively) for 86 days. In addition, six glass artificial burrows were kept with animals for the first two days. In the second set of observations, four animals were maintained for 73 d in a single aquarium. All aquaria were maintained at 20–23°C water temperature and 10 h $d^{-1}$ artificial lighting. The animals were given four different food types: *Tetraselmis* sp., *Artemia salina* nauplii, shrimp powder and fish muscle. During both periods, day time (0800–1800 h) observations were made hourly during the first 20 d, and three times a day afterwards. Behavioural aspects were examined both directly and with the help of ~2 h of videotape and photograph prints.

## RESULTS

### *Burrow casts and* in situ *observations*

The burrow openings of *Upogebia noronhensis* are a very conspicuous feature of the sandy sediments at the study site. They appear as circular holes surrounded by a dark sediment wall or as funnel-shaped structures. The burrows are distributed very close to each other, forming a maze of tunnels beneath the sediment surface (Figure 1A).

A total of 91 resin casts of the burrows were made in spring 1992, representing a total bottom area of 4400 $cm^2$. From those, 64 (70.3%) present a U-shaped upper portion with a central shaft originating at its deeper point (hereafter referred as Y-shaped burrows) (see Figure 1B for definition of burrow terminology). Eighteen casts (19.8%) showed a U-shape without the shaft (Figure 1C). Among

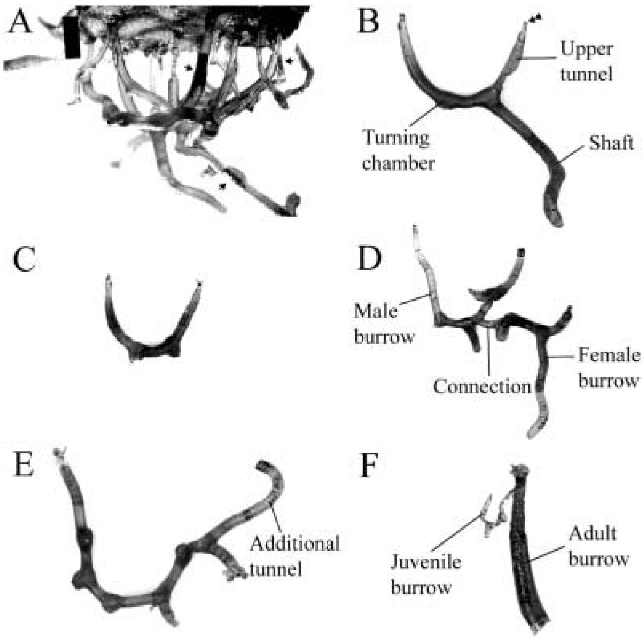

**Figure 1.** *Upogebia noronhensis* burrow casts: (A) large number of casts removed from the sediment showing the complex spatial structure of burrows within the sediment. Some animals can be seen through the resin (arrows); (B) Y-shaped burrow showing the constricted portion at the burrow opening (double arrow); (C) U-shaped burrow; (D) connected burrows; (E) male reproductive burrow. Note the irregular shape of the burrow and the large number of turning chambers; and (F) juvenile Y-shaped burrow attached to the upper tunnel of an adult burrow (only partly shown).

the 64 Y-shaped burrows found, three comprised female burrows connected to male reproductive burrows (Figure 1D). Reproductive burrows are considered burrows with additional tunnels extending from the U-portion of the burrow, which are thought to be made by the males to search and connect with burrows of females with which reproduction can take place. Another six reproductive male burrows occurred isolated (Figure 1E).

Eleven casts of juvenile burrows (>0.6 mm in diameter) were connected to the uppermost tunnels of adult burrows. Burrows with mean diameter <0.3 mm were only recorded connected to an adult burrow. Juvenile burrows with diameter between 0.3 and 0.6 mm occurred both connected and isolated. The morphology of these small burrows follows the Y-shape pattern described above (Figure 1F).

The inclination of the U-part of the burrow varied from completely vertical to 45° (x=14.8° SD ±10.7; N=34). The shaft can be straight or curved, with varying degrees of inclination, sometimes being parallel to the sediment surface.

At least one globular enlargement, used by the animal to change the direction of motion, is present in one of the upper tunnels of the burrow. One or two additional chambers can also occur in the opposite tunnel or in the shaft. The point of trifurcation of the upper tunnels and the shaft is also used as a turning chamber (Figure 1B).

The internal diameter of the burrow is strongly dependent on the animal size ($r^2$=0.89; N=45; $P$=0.0086; Figure 2A), with most of the burrow being as wide as the animal carapace. The uppermost part of the upper tunnels of the burrow is constricted and varies in size. The openings comprise the narrowest parts of the burrow, presenting reductions ranging from 31 to 90% of the total burrow arm diameter. The constricted upper part of the burrow may open at the sediment surface as a small hole or as a funnel-shaped structure (17.4%; N=138) with a variable diameter. The width of the turning chambers is 1.1–2.7 times (x=1.65 SD ±0.33; N=62) larger than the portion of the burrow adjacent to it.

Carapace length shows a weak positive correlation with the distance between openings ($r^2$=0.48; $P$=0.2019; N=34; Figure 2B), the depth of the U ($r^2$=0.32; $P$=0.0151; N=37; Figure 2C) and the total depth into the sediment ($r^2$=0.06; $P$=0.0267; N=32; Figure 2D). The deepest burrows cast, penetrated <18 cm into the sediment.

The burrow wall is composed by a layer of dark, compacted sediment, but there is no granulometric difference between the wall and the circumjacent sediment (unpublished data). Observations made after a sediment resuspension caused by storm waves showed that the hard walls become exposed 1–3 cm over the sediment. The walls of neighbouring burrows frequently touched each other owing to the high population density present in the area.

Burrows were inhabited by only one animal and all but nine reproductive burrow casts typically showed two openings. Nevertheless, many casts presented one of the upper tunnels shorter than the other, not reaching the surface of the sediment. It is not clear, however, whether the shortness of the tunnels was a feature of the original burrow or it was the result of the incomplete penetration of the resin.

The 60 *in situ* interconnection tests showed that 18% of the burrows presented only one opening. In the field, some of the burrows had one of the openings blocked by the action of large epifaunal organisms, such as sand dollars and flatfishes. The relationship between the number of burrow openings and individual animals for density estimation derived through the count of apertures is 1.8/1.

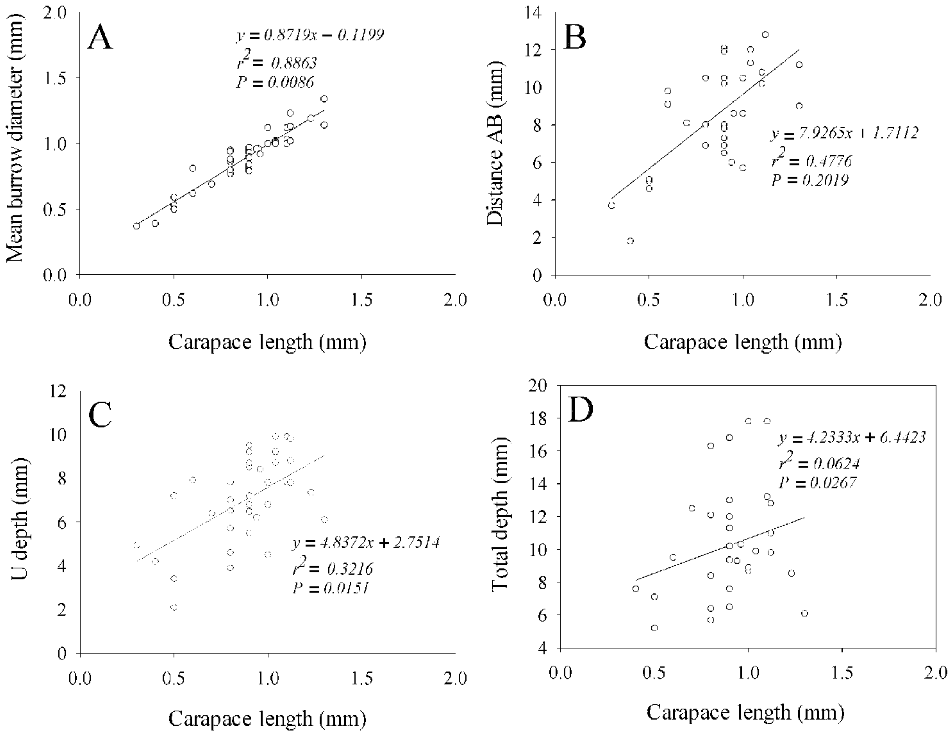

**Figure 2.** Correlation between *Upogebia noronhensis* carapace size and (A) internal diameter of the burrow; (B) distance between burrow openings; (C) depth of the 'U' part of the tube; and (D) depth of the burrow in the sediment.

Berried females were found throughout the year, suggesting reproduction is continuous. However, higher numbers were found during spring and summer months, when reproduction may be more intense. During the austral winter 1992, 28.6% (N=7; only females) of females carried eggs. In spring 1992 and spring 1993, the number of berried females was similar (60.0%; N=20 and 62.5%; N=8, respectively). During autumn 1993, no berried females were found (N=2), but in autumn 1994, 35.3% (N=34) of females carried eggs. Finally, during the austral summer 1993/1994, 80.0% (N=5) of females were berried.

### *Aquarium observations*

During both sets of observations, animals held in laboratory were very active and started to dig burrows in the sediment as soon as they were introduced. One of the animals took four hours to build a complete U-shaped burrow to a depth of 7 cm, including two surface openings and a turning chamber. The lower tunnel (shaft), however, was only built by the animals after a much longer time (nearly two months). The maximum depth of excavation attained by an animal in laboratory was 9 cm.

Animals systematically refused solid food (fish muscle), throwing it outside the burrow through either one of the openings. Nauplii of *Artemia salina* were also refused. Once nauplii were introduced to their burrows, *Upogebia noronhensis* started vigorously beating the pleopods to move them away from the burrow. Animals accepted *Tetraselmis* sp. and shrimp powder as food items, confirming the filter feeding habits of these animals.

During the second set of observations, after eight days the animals were introduced (two males and two females), a process possibly related to mating was observed. Male and female burrows were ~7 cm apart and both presented an exuvia close to the burrow opening on the surface of the sediment. The process started with the male digging a straight and almost horizontal 10 cm long connection from the U part of its burrow to the U part of the burrow of the female. As soon as the connection was completed, the male and female immediately paired their ventral parts within the U-part of the burrow, both lying with the carapaces turned to opposite sides. The animals remained almost immobile for nearly 30 min, only gently moving the pleopods. After separation, the male moved back to its burrow and promptly started to close the connection.

When put together in a same glass artificial burrow or a natural burrow, *U. noronhensis* individuals always showed marked antagonistic behaviour, either when both animals were of the same or opposite sex. Even within sampling buckets animals were very aggressive toward each other, and fights frequently caused the loss of chelipeds. Animals trying to move into the burrow of other animals were repelled immediately both in aquarium and in the field.

## DISCUSSION

### *Burrow characteristics*

The large sample of resin casts undertaken in the present work allowed us to examine differences in burrow structure, yielding important information about their occupants. Burrow morphology is a relatively simple Y-shaped pattern, with two surface openings and only one occupant per burrow. This pattern is similar to burrows of other extant members of the family Upogebiidae (Griffis & Suchanek, 1991).

There seems to be a strong correlation between burrow morphology and feeding mode, with the burrows of *Upogebia noronhensis* fitting into the category of filter feeders (Griffis & Suchanek, 1991; Nickell & Atkinson, 1995). In aquaria, *U. noronhensis* accepted only food of very small size (*Tetraselmis* sp. and shrimp powder), while rejecting larger sized food (fish muscle and *Artemia salina* nauplii), suggesting that this species is a filter feeder.

Aquarium observations showed that the burrow can be divided into two functional parts: the upper U, which is used mainly for filtration of suspended food particles; and the shaft, which is probably used for protection against epifaunal predators. However, this protection is not complete, since *U. noronhensis* has been detected in the stomach of stingrays (L.C.C., personal observation), flat fishes (S.B. Lucato, personal communication), and other demersal fishes. The mulid fish *Pseudupeneus maculatus* and the serranid *Diplectrum radiale* rapidly eat *U. noronhensis* dislodged from their burrows. Burrows of *U. noronhensis* are relatively shallow (maximum length ~18 cm) in comparison to other species, such as *U. deltaura* (maximum length ~68 cm, Hall-Spencer & Atkinson, 1999), and predators may take advantage. Demersal fish may also predate upon the species when the animal is tending the entrance of the burrow. It is possible that the shaft may serve as a refuge and may reduce predation by the epifauna and demersal fish.

Deviations from the basic Y-shaped pattern were detected in some burrows. The most common alterations are the collapse of a burrow opening, resulting in a funnel-shaped structure, the complete lack of one of the openings and the absence of a shaft. Observations made during sampling suggest that the different shapes of the openings might be related to sediment disturbance caused mainly by storm waves and epifaunal organisms, such as stingrays, asteroids and sand dollars. It is not clear, however, whether the normal condition is a circular hole or a funnel-shaped opening, since both cases are present. In burrows of *U. noronhensis*, the funnel-shaped condition is probably abnormal, since this part of the burrow presents a different texture in relation to the remaining parts. In some species, however, a funnel-shaped opening may be a functional part of the burrow. Nickell & Atkinson (1995) suggest that a funnel-shaped opening may be useful for particle collection by filter-feeding animals. The absence of the shaft is, however, more difficult to explain and, in many cases, could be an artefact of the resin casting technique. The variation found in the field shows the importance of a wide sample of casts to accurately describe the morphology of thalassinidean burrows.

Juvenile burrows $<0.3$ cm diameter were always connected to adult burrows, suggesting that the presence of adults may serve as a cue for the settlement of larvae. This may contribute to the high population density found in the study area ($\sim 200$ ind m$^{-2}$) and elsewhere

(Dworschak, 1987). *Upogebia* species are known to infest shellfish cultures, attaining high densities and are considered pests (Dumbauld et al., 1996; Smith & Langdon, 1998). High population densities are probably important for successful subterranean encounters for reproduction.

### *Mating behaviour*

Field results showing unusual male burrows interconnected with Y-shaped female burrows, in addition to observations in aquaria, suggest that the interconnection between burrows of *U. noronhensis* are not permanent features, but transient stages related to the reproduction of the species. *Upogebia noronhensis* was never observed outside its burrow during the present study (60 h of *in situ* observations by divers) and, when artificially exposed, individuals were always eaten by fish, especially *Pseudupeneus maculatus* and *Diplectrum radiale*. Copulation probably results when males dig lateral burrows searching for the burrows of females, following some reproductive cue, which may include moulting by the female. In the Anchieta Island, exuviae of *U. noronhensis* were common mainly during autumn and summer months. These molts were normally eaten by the vagile epibenthos, such as hermit crabs (personal observations).

Copulation has been observed only once in the Thalassinidea (*Callianassa turnerana*, Monod, 1927). In this species, individuals leave the burrow to reproduce. It is possible that other species have some mechanism of encounter within the sediment. Interconnection between burrows has been observed before in *Upogebia* spp. Swinbanks & Murray (1981) found constricted passages between Y-shaped burrows of *Upogebia pusilla*. Coelho et al. (2000) reported interconnected burrows from a large number of casts of *Upogebia omissa* burrows. These data suggest that the same mechanism of encounter and copulation may be used by different species within the genus *Upogebia*. Despite the fact that reproduction has not been confirmed after the possible mating encounter observed in aquaria, several lines of evidence suggest that the animals were actually mating: (1) all casts of reproductive burrows with additional tunnels belonged to males; (2) interconnected burrows belonged to animals of opposite sexes; (3) the antagonistic behaviour of the animals when put artificially together in glass-made burrows or buckets; and (4) the burrow types resulting from the probable mating observed in aquarium closely matched those found in the field.

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