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FEEDING, REPRODUCTION, AND DEVELOPMENT OF THE SUBTERRANEAN PERACARID SHRIMP *SPELAEOMYSIS BOTTAZZII* (LEPIDOMYSIDAE) FROM A BRACKISH WELL IN APULIA (SOUTHEASTERN ITALY)

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ABSTRACT

A population of the 'eyeless' hypogean shrimp *Spelaeomysis bottazzii* was studied over a three-year period in a shallow brackish-water well about 1 km from the Mediterranean coast. Mature males and immature females were numerous year round, whereas breeding females and juveniles were rare. The main stages of young in the brood pouch were embryos, nauplioids, and postnauplioids; all were unpigmented, unlike the postnauplioids in a congeneric species. In this well, the free-living stages fed mainly on autotrophic microorganisms. The accumulation of fat reserves was judged from the amount of subcuticular fat bodies and from body colour. Fat status improved with increasing body size in both sexes; seasonal variations were not significant. Only 'fat' specimens produced eggs. Females incubating eggs were fatter than those with larvae. Field and laboratory findings suggest that fat accumulation near the photic zone is necessary for egg formation, whereas larval incubation is very long and mostly occurs elsewhere, probably in deep groundwater under unfavourable nutritional conditions. The observed post-reproductive reduction of oöstegites may indicate a peculiar strategy to avoid a new breeding cycle before reconstitution of fat reserves. The findings on feeding and reproduction, particularly regarding fecundity and natality, are interpreted as a combination of typically hypogean features along with epigean environmental adaptations.

KEY WORDS: brackish water, fat status, fecundity, ground water, hypogean habitats, marsupial incubation, secondary sexual characteristics, *Spelaeomysis bottazzii*

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INTRODUCTION

In a continuation of studies (Ariani and Wittmann, 2002) on adaptations in a semi-hypogean species of Mysidae, we are now using a hypogean species of Lepidomysidae as a case study for investigating the extent to which traits associated with subterranean life are integrated with typical epigean habits. This approach may provide a better understanding of the conditions under which a subterranean trait could be sustained over millions of years, as suggested by a possible Tethyan origin of these crustaceans (Băcescu and Orghidan, 1971; Pesce and Iliffe, 2002). The stability of subterranean characters is mostly explained by both the stability of the environment (Coineau, 2000), and by strategies to escape from typical epigean competitors or predators. However, an equally important problem (Hüpopp, 2000) was scarcely considered for Lepidomysidae until now: what strategies are employed to counter food scarcity?

Due to the strongly limited or entirely lacking primary production, caves and other subterranean biotopes have scarce to very scarce availability of food. Survival strategies of animals in such environments involve behavioral, physiological, and morphological adaptations including low metabolism, long lifespan, and utilization of particular food sources such as feeding on organic matter imported by infiltration from photic environments (Ginet, 1955). The subterranean waters inhabited by crustaceans were depicted as a world of detritivores (Pesce, 1975) and scavengers (Ruffo, 1955) depending on surface environments. In line with this, the first records of aquatic crustaceans in caves of Apulia (eastern Mediterranean) densely populated by bats revealed a feeding strategy based on insect remnants in guano (Caroli, 1924a).

A further problem is that egg- or larvae-carrying females are very rarely found in Lepidomysidae, so far all referred to the genus *Spelaeomysis* Caroli, 1924. Ortiz et al. (2005) presented the only morphological data concerning larvae for *S. nuniezi* Băcescu and Orghidan, 1971, from Cuba.

Spelaeomysis bottazzii Caroli, 1924 is the only representative of its genus known from European subterranean waters. As in most congeneric species, the eyestalks lack ommatidia and the body is completely unpigmented. *Spelaeomysis bottazzii* is endemic to Apulia (SE Italy) and is recorded from brackish pools in a few caves and from a number of brackish (rarely freshwater) wells (Ruffo, 1955; Pesce et al., 1978; Ariani, 1982). The breeding habitat may be different from the shallow ground water, where mostly immature specimens are found.

After discovery (Ariani, 1980) of a small well inhabited by *S. bottazzii* with peculiar light and hydro-geological characteristics (see 'Study Site') near the Adriatic coast, we undertook a three-year study on the largely obscure biology of this species. In line with the questions raised above, our study focused on habitat utilization for feeding and reproduction. This regards the main sources of nutrition, relationships between nutrition and reproduction, numbers and characteristics of diverse life stages, and reproductive status.

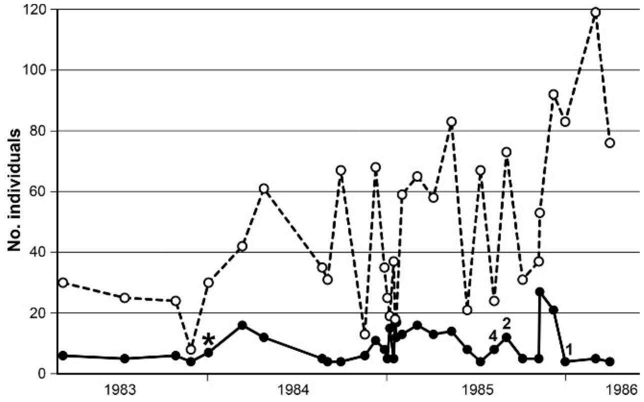

Fig. 1. Numbers of *Spelaeomysis bottazzii* counted (dashed line) or sampled (continuous line) in the well of Difesa di Malta between March 1983 and April 1986. Ciphers associated with data points indicate numbers of incubating females sampled; asterisk marks an egg-producing female which had deposited its eggs in laboratory.

MATERIALS AND METHODS

The Animals

Field observations, measurements, and sampling were performed during 33 inspections between March 1983 and April 1986 of the study site, indicated below (Fig. 1). We collected only 4-27 specimens per inspection (total 307 specimens), although we observed up to 119 individuals upon one single inspection. More intensive sampling was avoided in order not to impact the population. Specimens were collected during day time with small hand nets (mesh size 0.3 mm) and most were fixed in 80% ethanol. Some individuals were reared in the laboratory for up to 16 months in the dark at 20 ± 0.5°C, as reported by Ariani (1982). Water and scraps of rock in the rearing chambers were taken from the sampling site.

Body size was measured as total length from the anterior margin of the carapace to the posterior margin of the telson, without spines. Size of the subspherical to slightly pyriform eggs was expressed as the geometric mean of apparent length and width in episcopic view. Developmental stages in the marsupium were classified according to Wittmann (1981) as embryonic (egg) stage, nauplioid larvae, and postnauplioid larvae (Fig. 2a-d). The sexes were distinguished by the presence of penes or oöstegites, respectively. Males were also distinguished from females by a three-segmented instead of a four-segmented exopod of the second pleopod. Juveniles were distinguished from immatures by the absence of secondary sexual characteristics. Based on detailed inspection and the results below, the following additional distinguishing features were used: 1) immature males with fewer and shorter setae on the exopod of the second pleopod than in mature (adult) males; 2) early immature females with small, loose oöstegites, not forming a brood chamber by overlapping themselves – the apex of the ultimate oöstegites not extending beyond the posterior margin of the sixth sternite; 3) advanced immature females with brood chamber present, but oöstegites shorter than in adults; 4) egg-producing females differing from advanced immatures by egg mass visible in the ovarian tubes; 5) adult females (all incubating eggs or larvae in the present material) with the four posterior oöstegites, when artificially stretched, extending beyond the merus of their corresponding thoracic endopod; and 6) post-reproductive females with small oöstegites (Fig. 3d), but bulging more than in advanced immatures and, therefore, not fitting together to form a well-developed common brood chamber.

The accumulation of fat reserves was judged from body colour in living animals or from the extent of subcuticular fat bodies in fixed specimens. Fat bodies were also visible upon microscopic inspection of living animals. Fat status visibly affected the entire body but could best be semiquantitatively estimated by microscopic inspection of the telson in dorsal view. The status was classified as 'low' if $< 15\%$ telson area was occupied by, if any, small fat bodies (Fig. 4a, b, d). When alive or freshly fixed, such animals showed a pale, whitish to light yellow body colour. The status was 'high' if $\geq 15\%$ telson area was occupied by fat bodies of various sizes (Fig. 4c). Such animals were distinctly yellow to orange.

The statistical contingency between columns in $2 \times m$ tables was tested with Fisher's Exact Test for $m = 2$ or with Pearson-Chi^2-Test for $m > 2$, respectively. Neighbouring intervals ($m > 2$) were matched if expected frequencies were < 5. Student's t test was used for slope of linear regression and for differences between means of metric variables. The two-tailed tests were performed at a significance level of $P < 0.05$. The calculations were made with SPSS 14.0 (SPSS Corp., U.S.A.).

Study Site

The study was performed from 1983-86 in a well at 40.804955°N, 17.515875°E, in a rural locality with the field name 'Difesa di Malta.' This well was excavated in the 1970s during a failed search for freshwater at the bottom of a 9 m deep, abandoned limestone pit (photograph in Ariani, 1980: Fig. 2), 920 m from the Adriatic coast of Apulia (north-eastern Mediterranean). The well extended from the bottom of the limestone pit 3 m down to the groundwater level, which was about 6 m above sea level. Several brackish springs were present along the adjacent seashore (Cotecchia et al., 1975; Ariani, 1982). The topographic situation (map in Ariani, 1980: Fig. 1) suggested that the well water emerged at 740 m distance in the brackish spring Fiume Morello (40.810113°N 17.521520°E), densely populated by the mysid *Diamysis mesohalobia* Ariani and Wittmann, 2000. At the bottom of the well, there were two small intercommunicating, brackish pools (1.2 and 2.2 m^2) with a depth of 0.3-0.5 m. Both pools ceased to exist during the decades after our study due to the intrusion of surface soil. In the 1980s, daylight penetrated down to the bottom for several hours per day, supporting some primary production by green algae, diatoms, and cyanobacteria (Fig. 5a, b; Ariani, 1982). The dim natural illumination was sufficient for regular visual inspection of the macrofauna in the well. Continuous automatic recordings in the well indicated tidal fluctuations of about ± 3 cm associated with temperature fluctuations of up to ± 4°C (Ariani, 1982). This indicates that the well had an open connection with the deep brackish groundwater floating above seawater. In fact, in the karstic underground of Apulia, seawater intrudes deeply into vast areas of the land mass (Cotecchia, 1977). Salinity in the well, expressed as a dimensionless equivalent of conductivity, varied in the range of S = 2.3-7.1 in 1983-86, mainly fluctuating with rainfall events; pH ranged from 7.1-7.9. Water temperature showed mainly seasonal variations in the range of 9.8-21.6°C. Further physico-chemical measurements together with faunistic data and (hydro)-geologic characteristics of the area are available in Ariani (1982).

RESULTS

Field Population

In the well of Difesa di Malta, *Spelaeomysis bottazzii* was found at all inspections during all seasons from 1983-1986. A total of 1526 individuals, including possible repetitive counts of same specimens, were counted by visual observation (Fig. 1). Between 8 and 119 specimens were encountered on each of the 33 inspections. The numbers of observed specimens fluctuated strongly (Fig. 1) but significantly increased during the investigation period ($t = 3.57$; $P = 0.001$).

The following data on frequency and size of the diverse free-living stages refer only to sampled specimens ($n = 307$): most *S. bottazzii* were immature females or mature males, whereas juveniles ($n = 5$) and incubating females ($n = 8$; including one which deposited eggs after sampling) were rare. Most of the latter appeared in summer 1985 (Fig. 1). No other field records of breeding females have ever been made in this species.

The body length of mature males was 8.44 ± 1.00 mm (± SD; 6.1-10.5 mm; $n = 94$). Egg-producing females measured 9.88 ± 0.78 mm (8.6-11.0 mm; $n = 23$), incubating females 10.08 ± 0.51 mm (9.1-10.6 mm; $n = 8$). The latter carried 8-14 eggs ($n = 5$) or 9-12 nauplioid larvae ($n = 3$; one female with additionally two eggs), respectively; pooled together, this yields a mean brood size

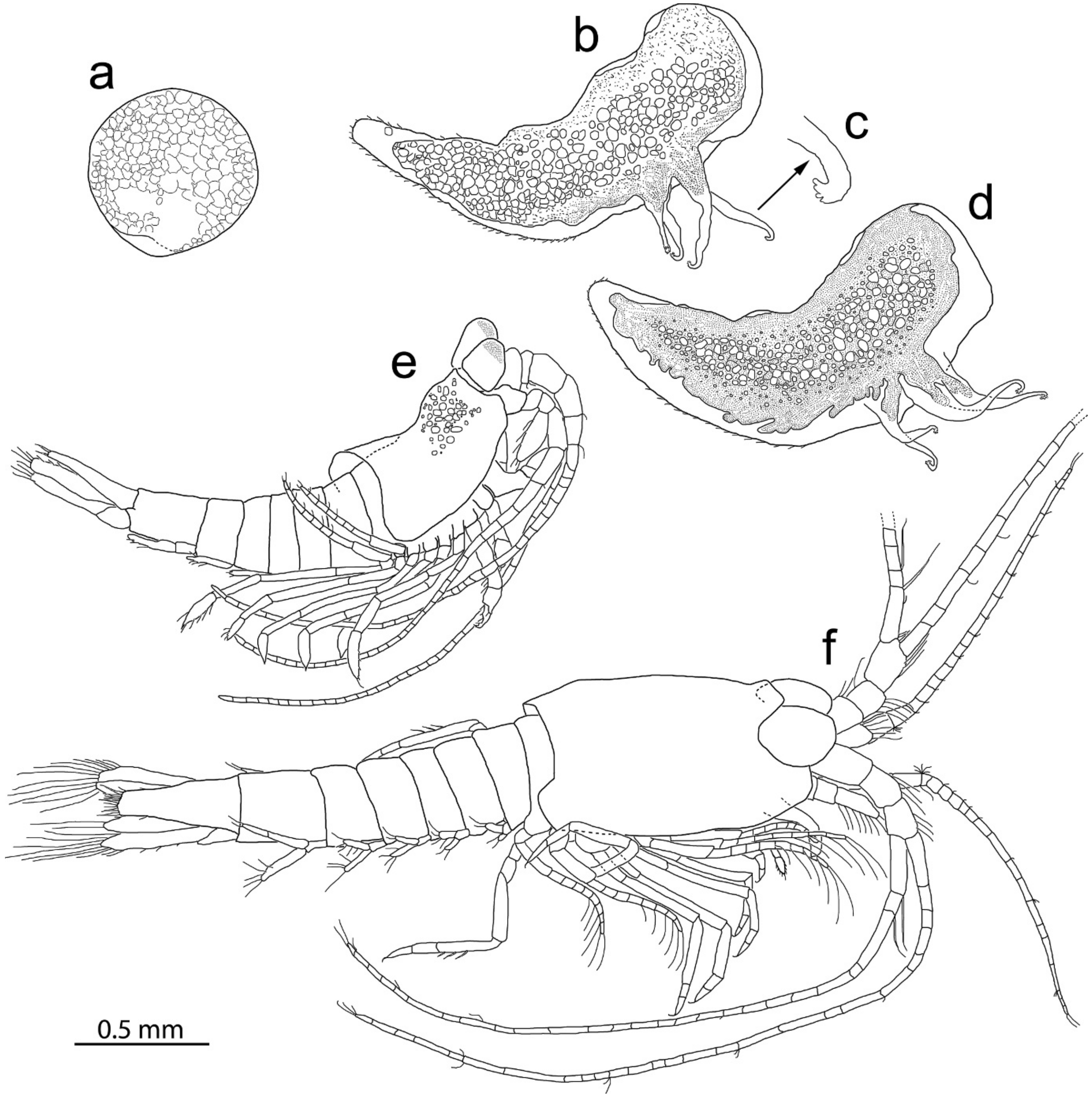

Fig. 2. Main stages of marsupial development and morphology of freshly released young ('neonates') in *Spelaeomysis bottazzii*. a, embryonic stage (egg); b, early-mid nauplioid larva, lateral; c, four-times enlarged detail of (b) showing tip of antennula; d, advanced nauplioid larva, lateral; e, postnauplioid larva, (dorso)-lateral, thoracic exopods 1-6 omitted; f, freshly released, free living juvenile, dorsolateral, thoracic exopods 4-6 omitted.

of 10.13 ± 2.10. Egg diameters were 0.68 ± 0.03 mm (0.61-0.71 mm; $n = 37$). Post-reproductive females measured 9.2-10.0 mm ($n = 2$).

Feeding and Nutritional State

When observed in the field that the animals rarely swam or crawled but usually were motionless on the surface of rock, much less frequently on the bottom sediment. Dead terrestrial molluscs and insects were often present on the bottom of the well. Such carcasses were occasionally covered by hydrobiid snails *Semisalsa aponensis* (von Martens, 1858), but rarely with *S. bottazzii*.

Stomach contents and feces (Fig. 5c, d) from animals freshly collected throughout the year in the well showed green algae, diatoms, and cyanobacteria; these were enclosed to a high degree by mineral particles. These contents corresponded well to the micro-organisms found on the surface of rock walls (Fig. 5a, b) and on the bottom sediment of the well (Ariani, 1982).

The number and size of fat bodies differed considerably between certain stages (Fig. 4). Egg-producing females and

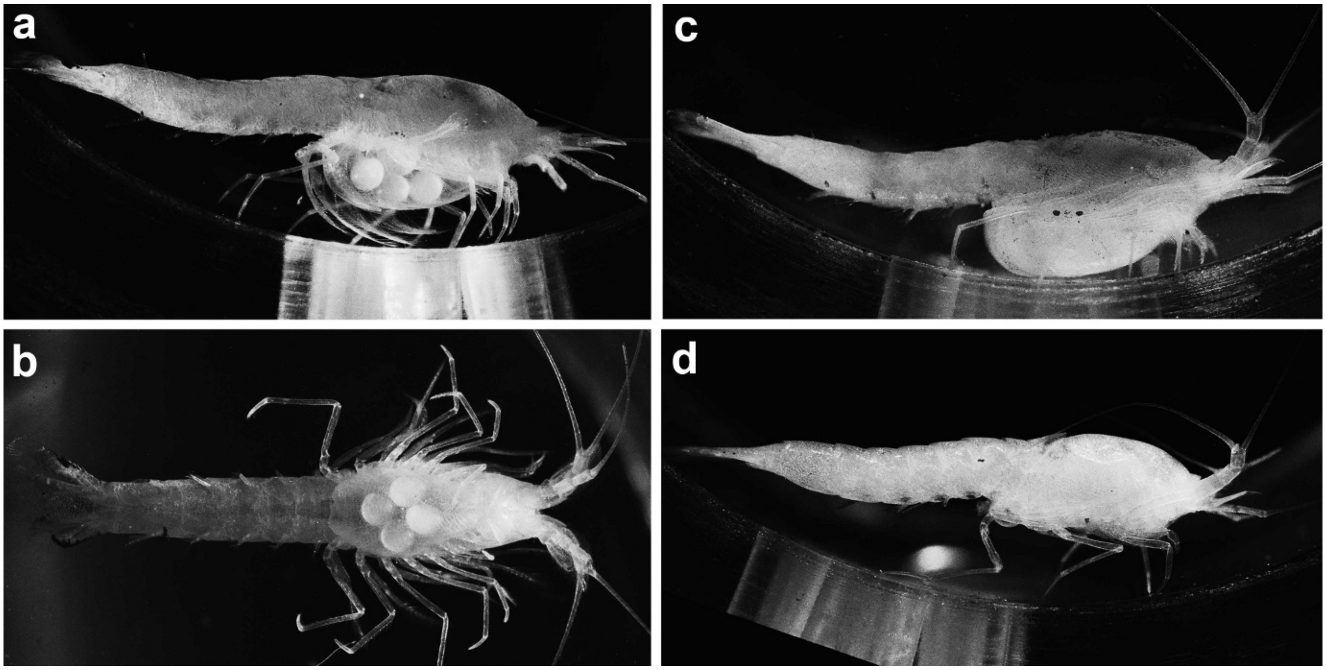

Fig. 3. Incubating (a-c) versus post-reproductive (d) females in *Spelaeomysis bottazzii*. a, b, female with body size 10.1 mm, incubating eggs (four of an initial eight eggs lost in captivity), in lateral (a) and ventral (b) view; c, female with nauplioid larvae, 10.6 mm; d, female four days after the moult subsequent to release of young, 10.0 mm. Photomicrographs of living specimens in the laboratory.

those carrying eggs (Fig. 4c) in the brood pouch had the telson area 20-80% filled by fat bodies; in contrast, this value was only 0-10% in females carrying larvae (Fig. 4d). The frequency of 'fat' females (fat bodies extending over $\geq$ 15% telson area; yellow/orange body colour) showed significant differences related to their reproductive status (Fig. 6; non-breeding females: $n = 190$; $\chi^2 = 20.84$; 2 *d.f.*; $P < 0.001$). The corresponding difference between immature and mature males was only marginally significant (Fig. 6; $n = 8 + 94$; Exact Test; $P = 0.061$). The frequency of high fat status increased with increasing body size in both sexes (Fig. 7; $n = 298$; $\chi^2 = 41.37$; 2 *d.f.*; $P < 0.001$). The females ($n = 200$) were on the average fatter (Exact Test; $P < 0.001$) but also larger ($t = 8.53$; $P < 0.001$) than the males ($n = 102$). The difference between sexes regarding fat content became non-significant when matching the data with body size ($n = 102$ pairs; Exact Test; $P = 0.172$). Nutritional state derived from fat content and corresponding body colours showed no significant differences between seasons ($n = 302$; $\chi^2 = 1.03$; 3 *d.f.*; $P = 0.795$).

Development

In the laboratory, the duration of embryonic development between capture of a 10.0 mm female with 14 already fertilized eggs and hatching from the egg membrane was 16-22 days. In this period, embryos and nauplioid larvae were simultaneously present in the brood pouch. This female released six fully developed young ('neonates') asynchronously between day 100 and 108 after capture. A further female with 10.6 mm body length, which had carried eight nauplioids upon capture, released only one 'neonate' 78 days after capture. Three other females lost their brood and one died during culture.

The sequence of main developmental stages (Fig. 2) in the marsupium was observed on field individuals held in culture in combination with material fixed shortly after sampling. After hatching from the egg membrane, the nauplioid larvae (Fig. 2b) were very similar to nauplioids of certain mysid shrimps, e.g., *Mesopodopsis aegyptia* Wittmann, 1992 (Fig. 2B loc. cit.). There were two pairs of naupliar appendages: the antennulae and the antennae. Both showed strongly recurved terminal portions with spine- to finger-like (Fig. 2c) processes near the tip. Advanced nauplioid larvae showed a number of additional cephalic and thoracic appendages developing below the old cuticle (2d). After hatching from this cuticle, the emerged postnauplioid larvae showed large, near-globular eyestalks with cornea-like structures situated terminally, yet without any trace of visual pigment (Fig. 2e). Yolk mass was still visible dorsally in the cephalic region. All thoracic and pleonal appendages were present, although many still showed a reduced number of segments. Apart from the absence of pigment, this stage also showed no essential difference from the situation in Mysidae (Wittmann, 1981). The postnauplioids moulted to the first free-living juvenile stage upon release from the brood pouch. The detailed timing was not observed, i.e., whether moulting occurred shortly before or shortly after release. In *S. bottazzii*, postnauplioids and 'neonates' are currently known only from our laboratory culture. The eyestalks of the freshly hatched juveniles were dorsoventrally slightly flattened, still large, but without cornea and without any pigment (Fig. 2f). Notably, the eyestalks of immatures and adults were relatively smaller and dorsoventrally more flattened than in juveniles.

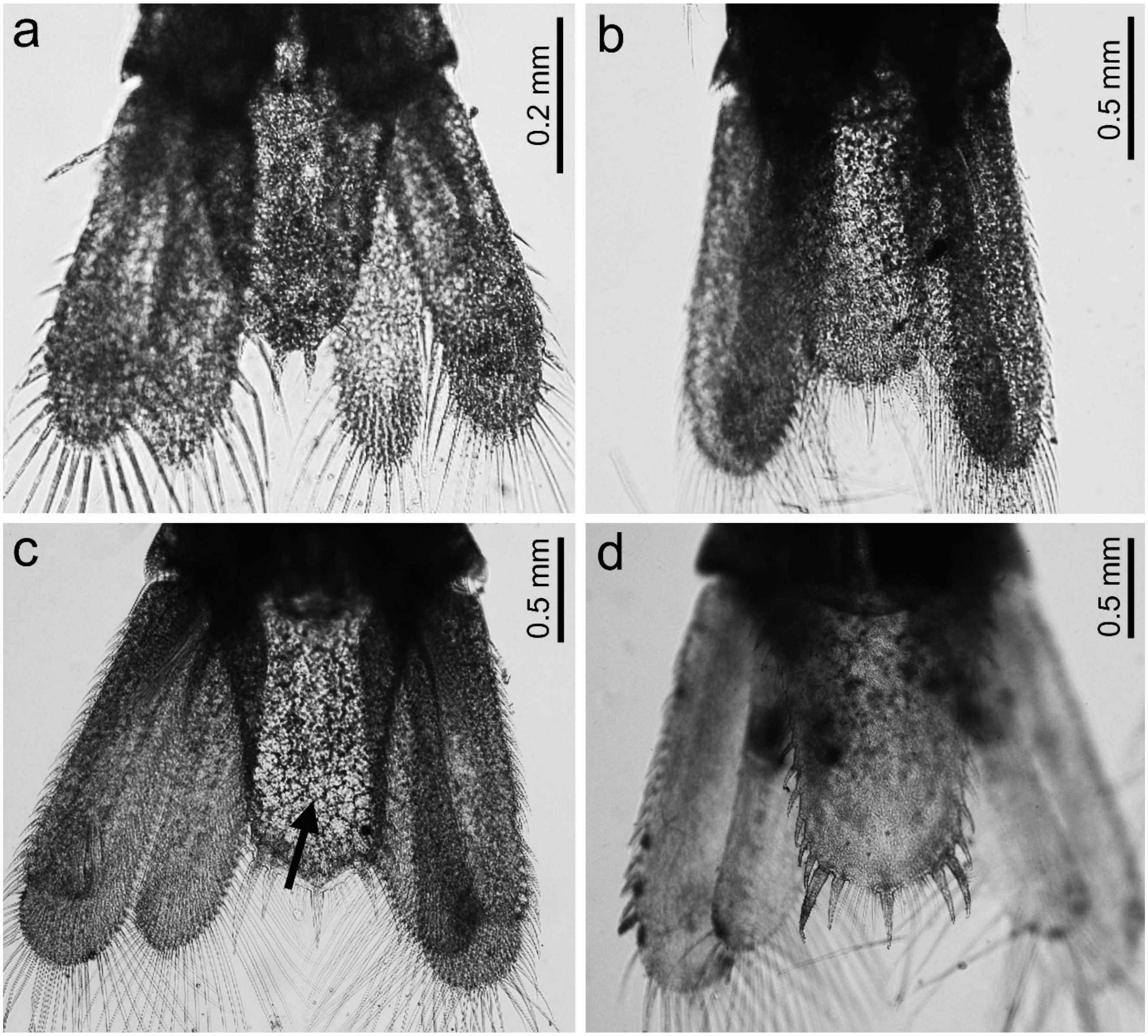

Fig. 4. Morphology of the tail fan during the life cycle of *Spelaeomysis bottazzii*. a, freshly released juvenile with body length 2.5 mm; b, immature female, 6.2 mm; c, female incubating eggs, 9.2 mm, arrow points to large fat bodies in the telson; d, female incubating postnauplioid larvae, 9.8 mm. Specimens from laboratory culture (a, d) and field samples (b, c).

Two breeding females from laboratory culture were examined after the moult subsequent to incubation. These post-moult females, plus two additional specimens sampled in Nov. 1985 in the well, showed a post-reproductive habit with empty ovarian tubes and with small, strongly bulged oöstegites (Fig. 3d) which did not match together to form a brood chamber. The bulge of these oöstegites was similar (even stronger) to that in adults (Fig. 3a-c), but their size (Fig. 3d) was as small as that typical for immatures.

DISCUSSION

Population Establishment

Based on reduced eyes and the absence of pigment, *S. bottazzii* is described in the literature as a classical inhabitant of subterranean waters (Caroli, 1924b; Riedl, 1966; Mauchline, 1980). However, our findings point rather to a combination of typically hypogean with epigean characteristics. So far, the well at Difesa di Malta is the only water body with records of incubating females of *S. bottazzii*. This particular site has also yielded more than half the total number of specimens ever sampled for this species, which was the first discovered and most cited taxon in Lepidomysidae; moreover, this site showed increasing densities during the 1983-1986 investigation period (Fig. 1). This and the findings concerning feeding and reproduction, discussed below, suggest that dwelling in near-surface environments is an essential component in the ecology of *S. bottazzii*.

A potential alternative hypothesis for the comparatively high densities at Difesa di Malta could be trapping or stranding of the animals. We judge this unlikely due to tidal

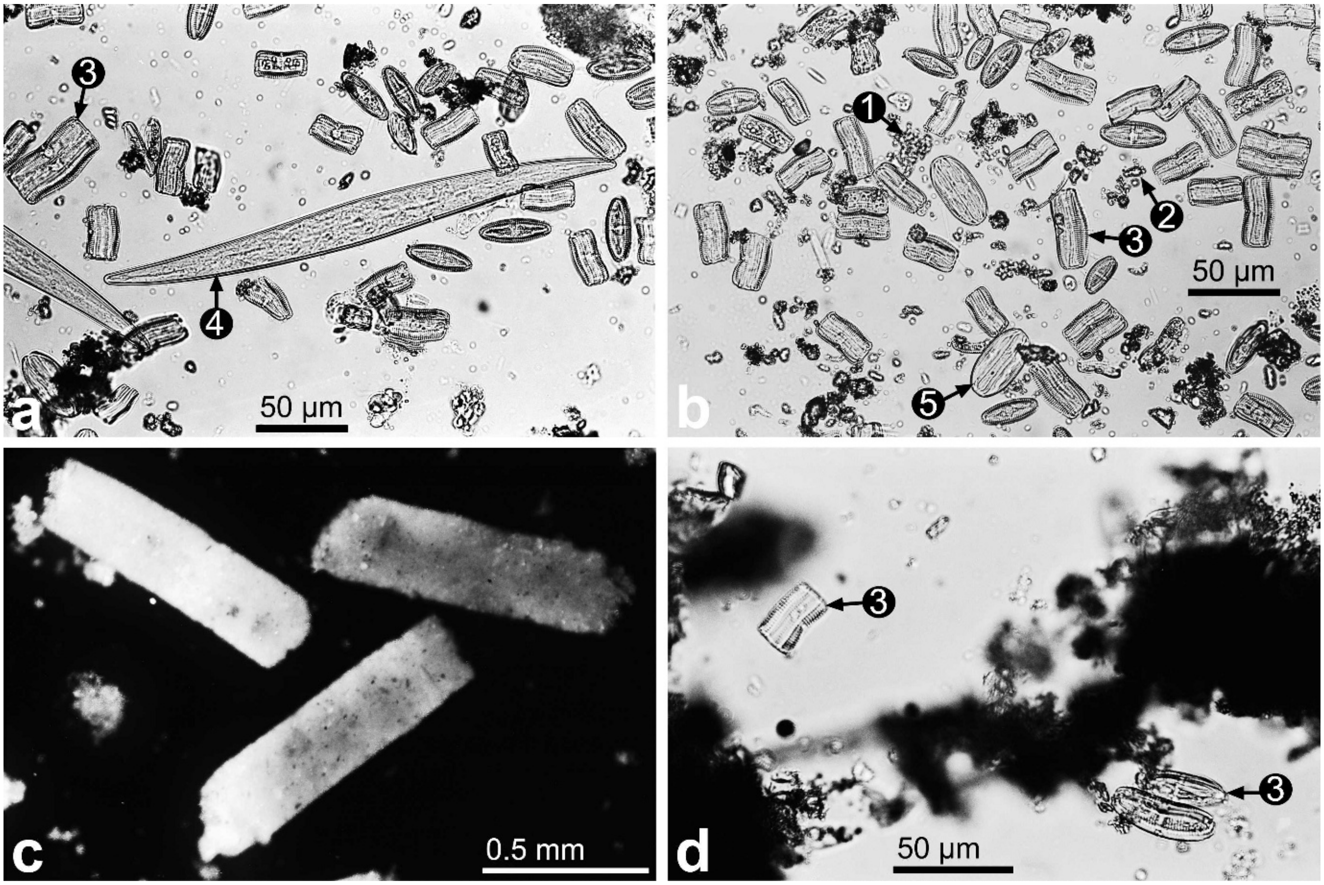

Fig. 5. Microalgae (a, b) scratched from rock walls in the well at Difesa di Malta versus faecal pellets (c) and pellet contents (d) in *Spelaeomysis bottazzii*. Note cyanobacteria (1), chlorophyceans (2), and the diatoms *Achnanthes brevipes* Agardh (3), *Pleurosigma longum* Cleave (4), and *Amphora* sp. (5).

movements indicating open connection of this well with the deep ground water extending under vast areas of Apulia (Cotecchia, 1977). The presence of more than ten brackish springs along the shore within 1-5 km of the well and of a great number of submarine springs along the coast suggest that the karstic underground here may be highly permeable for aquatic animals of that size of body and ones even larger as shown by Ariani (1982) for the decapod *Typhlocaris salentina* Caroli, 1923, in this well.

Feeding

Up to the 1970s, *S. bottazzii* was thought to depend only on allochthonous resources of epigean origin, e.g., by feeding on carcasses (Ruffo, 1955) or plant detritus (“organogenic bottom deposit” Pesce, 1975) in caves or wells. This picture changed in the 1980-90s by records from brackish waters at the margin of the photic zone in wells and dolinas; there, this species co-occurs not only with stygiophilic but also with typical epigean organisms (Ariani, 1982; Ariani and Wittmann, 2002). As shown above, *S. bottazzii* feeds mainly on autotrophic micro-organisms in the well of Difesa di Malta. This agrees with the strategy of another subterranean peracarid, *Thermosbaena mirabilis* Monod, 1924, which feeds on cyanobacteria in thermal springs of Tunisia (Delamare-Deboutteville, 1960).

Some immature specimens (11 out of 18) survived over 16 months in the laboratory, where they were kept in the dark and fed only with the coating of autotrophic micro-organisms off of limestone (Ariani, 1982). The strong mandibular apparatus (Ariani, 1982: fig. 28) is well suited for epilithic scraping and breaking up of diatom frustules, and even for attacking the surface of polished pieces of relatively soft limestone under appropriate experimental conditions. Based on indirect evidence, Ariani (1982) proposed that the scraping of microbial coats on limestone and possibly also fossil remnants in poorly coherent Quartenary rock (calcarenite) of Apulia yielded potential food sources for *S. bottazzii* in deep ground water.

The comparatively high densities at Difesa di Malta together with the above data on stomach contents and feces suggest that living off the resources from autochthonous primary production is an essential feature within a generally opportunistic habit in the feeding ecology of *S. bottazzii*. A mainly micro-herbivorous feeding habit was also found in the semi-hypogean mysid *Diamysis camassai* Ariani and Wittmann, 2002, marginally co-occurring with *S. bottazzii* along the southwestern coast of Apulia.

The observed frequency distribution of the fat status in immature and in egg-producing females of *S. bottazzii* suggests that a certain degree of fat accumulation is a prerequisite for egg formation in the ovarian tubes. The low

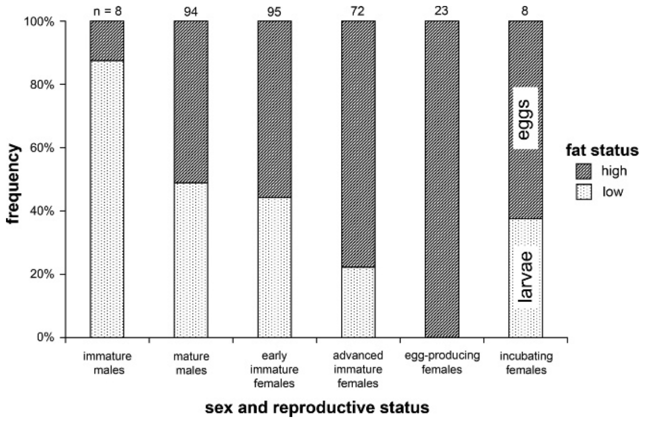

Fig. 6. Fat status versus sex and reproductive status of *Spelaeomysis bottazzii* in the well at Difesa di Malta.

fat status of females with nauplioid larvae may indicate that food intake is reduced during the long incubation of young.

Marsupial Development

The numbers and morphology of marsupial stages given above (Fig. 2) for *S. bottazzii* are essentially the same as in species of Mysidae (Wittmann, 1981). The embryonic stage, nauplioid larvae, and postnauplioid larvae, classified here according to Wittmann (1981), correspond to the stage I, II, and III larvae of Ortiz et al. (2005). Our findings provide the first evidence that all marsupial and free-living stages of a species of Lepidomysidae can totally lack pigment. In particular, the postnauplioid larvae show no trace of pigment, unlike the situation in *S. nuniezi* from Cuba (Ortiz et al., 2005), where the postnauplioids have well-pigmented, stalked eyes.

The rarity of incubating females near the water surface suggests that incubation may take place mainly elsewhere. Some indirect evidence for such locations in nature was gained from preference experiments in the laboratory; according to Ariani et al. (1984), the females tested (one with eggs) of *S. bottazzii* consistently preferred temperatures above the acclimatization temperature (= temperature at sampling). Cesaro et al. (1984) obtained analogous results for salinity. Applying these laboratory results to the prevailing hydrological situation in Apulia (Cotecchia, 1977) suggests a vertical descent of the animals to the warmer and more saline deep ground water.

Reproduction

At a given body size (10 mm) of the breeding females of *S. bottazzii*, the mean numbers (10) of young per brood are smaller (66%) and egg diameters (0.68 mm) are larger (145%) than expected from the data on epipelagic or coastal species of Mysidae and Lophogastridae (Wittmann, 1984). These crustacean families are considered to be comparable groups in the present context because they share essentially the same marsupial stages with *Spelaeomysis* (Mauchline, 1980; Wittmann, 1981; Ariani and Wittmann, 1997; Ortiz et al., 2005; Fig. 2). The values for *S. bottazzii*, however, do not exceed the range and 95% confidence intervals for these two families. As this species fits within the above confidence intervals, it only weakly meets the expectations of fewer but comparatively large eggs, as established for hypogean animals in general (Delamare Deboutteville, 1960; Vandel, 1964; Hüpopp, 2000). Fage and Monod (1936) found only two very large larvae in the brood pouch of a single female of the mysid *Heteromysoides cotti* (Calman, 1932) with 7 mm body size inhabiting an anchialine cave and wells of Lanzarote (Canary Islands). About eight eggs, at 8.5 mm parental size, were indicated by Villalobos (1951) for one female in connection with the description of *S. quinterensis* from cave waters in Mexico. A maximum of nine eggs were reported by Pillai and Mariamma (1964) for ten females (average body size 4.8 mm) in the description of *S. longipes* from a freshwater well in India. Coincidently with this, nine larvae were found by Ortiz et al. (2005) in the marsupium of one 4.8 mm female of *S. nuniezi* from Cuba. The incubation time of *S. bottazzii* was > 100 days at 20°C in the laboratory, i.e., > 6 times longer than in epigean Mysidae (exceeding the 95% confidence intervals given by Wittmann, 1984; no data are available for other species of Lepidomysidae). In summary, the duration of incubation and the resulting natality, i.e., number of offspring released per individual and unit time, in *S. bottazzii* are typically hypogean, whereas this is not clearly evident for egg size and fecundity, i.e., number of offspring per brood. So far, there is no evidence that any species of the genus *Spelaeomysis* produces more than one brood per lifetime. However, the available data do not suffice for conclusions on semelparity versus iteroparity.

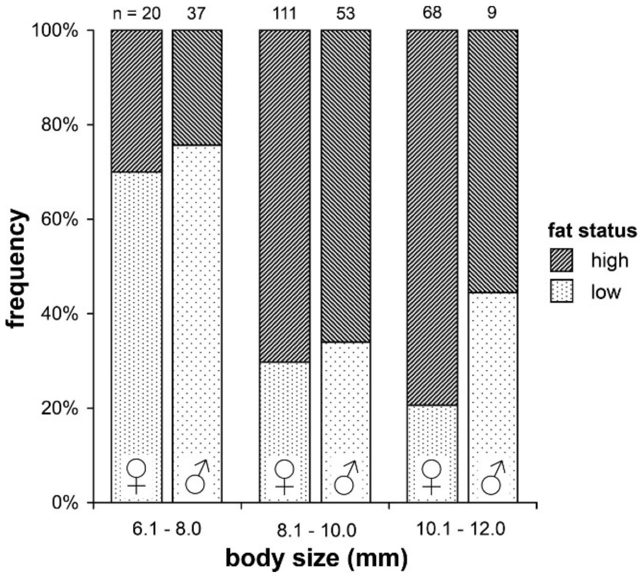

Fig. 7. Fat status versus body size in females and males of *Spelaeomysis bottazzii* in the well at Difesa di Malta.

Reduction in the size of the oöstegites with the moult that occurs after the release of young points to a potential reproductive strategy that involves prevention of an unsuccessful attempt at breeding while in a poor nutritional state. It remains unknown whether the appearance of anomalous small oöstegites heralds the formation of a normal brood pouch (iteroparity) or if it marks the end of the reproductive lifespan in females (semelparity).

The limitations of the present study arose from the rarity in the well of advanced female stages and of juveniles, and from the lack of (difficult) sampling in deep ground water. Given these limitations, we propose the following hypothetical scheme for the reproductive cycle and its dependence on nutrition in the Difesa di Malta population. A consistent scenario must integrate the following findings in the shallow water layer reached by the well: 1) *S. bottazzii* feeds mainly on autotrophic micro-organisms; 2) only well-nourished females form eggs in the ovarian tubes; and 3) breeding females and juveniles are rare in the near-surface environment.

Near the margin of the photic zone, the animals use the higher primary production to invest energy into egg formation. When the eggs are ready for deposition into the brood pouch the mature females descend to deep ground water for copulation and subsequent breeding. Here, the mating partners and their offspring are protected from predation by epigean organisms. The young *S. bottazzii* grow and mature slowly under the poor nutritional resources deep underground. Such slow growth fits conceptually with the low birth rate resulting from the long incubatory period of more than 100 days in this species. Having arrived at or near a mature state, the animals migrate upwards to the margin of the photic zone, where they can accumulate energy for egg formation. This cycle is essentially similar to that proposed by Ariani and Wittmann (2002) for *D. camassai*, a semi-hypogean mysid species, which marginally co-occurs with *S. bottazzii* in brackish-water dolinas of Apulia. That species breeds for the most part in the darker parts of its habitat. As a major difference from *S. bottazzii*, *D. camassai* has well-developed eyes, although these are smaller than in congeneric species.

If our conclusions hold true, from a more generalized view, *Spelaeomysis* profits from its stay in or near the photic zone during egg formation by a comparatively (for a hypogean animal) high degree of fecundity, and pays the price for the security that results when breeding in deep ground water with a low natality.

REFERENCES

Ariani, A. P. 1980. *Spelaeomysis bottazzii* Caroli (Crustacea, Mysidacea) nella falda freatica del litorale Brindisino. Annuario dell'Istituto e Museo di Zoologia dell'Università di Napoli 23: 157-166.

———. 1982. Osservazioni e ricerche su *Typhlocaris salentina* (Crustacea, Decapoda) e *Spelaeomysis bottazzii* (Crustacea, Mysidacea). Approccio idrogeologico e biologico sperimentale allo studio del popolamento acquatico ipogeo della Puglia. Annuario dell'Istituto e Museo di Zoologia dell'Università di Napoli 25: 201-326.

———, and K. J. Wittmann. 1997. Alcuni aspetti della biologia della riproduzione in *Spelaeomysis bottazzii* Caroli (Mysidacea, Lepidomysidae). In, Il carsismo dell'area Mediterranea. Thalassia Salentina 23 (suppl.): 193-200.

———, and ———. 2000. Interbreeding versus morphological and ecological differentiation in Mediterranean *Diamysis* (Crustacea, Mysidacea), with description of four new taxa. Hydrobiologia 441: 185-236.

———, and ———. 2002. The transition from an epigean to a hypogean mode of life: morphological and bionomical characteristics of *Diamysis camassai* sp. nov. (Mysidacea, Mysidae) from brackish-water dolinas in Apulia, SE-Italy. Crustaceana 74: 1241-1265.

———, G. Cesaro, and A. Balboni. 1984. Ricerche sulle preferenze termiche di *Spelaeomysis bottazzii* (Mysidacea, Lepidomysidae). Bollettino di Zoologia 51 (suppl.): 4.

Băcescu, M., and T. Orghidan. 1971. *Antromysis cubanica* n.sp. et *Spelaeomysis nuniezi* n.sp., Mysis cavernicoles nouvelles de Cuba. Revue Roumaine de Biologie – Zoologie 16: 225-231.

Calman, W. T. 1932. A cave dwelling crustacean of the family Mysidae from the island of Lanzarote. Annals and Magazine of Natural History (10)10: 127-131.

Caroli, E. 1923. Di una specie italiana di *Typhlocaris* (*T. salentina* n. sp.) con osservazioni morfologiche e biologiche sul genere. Bollettino della Società dei Naturalisti in Napoli 35: 265-267.

———. 1924a. Sulla presenza della *Typhlocaris* (*T. salentina* n. sp.) in Terra d'Otranto. Contributo alla conoscenza del genere. Annuario dell'Istituto e Museo di Zoologia dell'Università di Napoli 5, n. 9: 1-20.

———. 1924b. Su di un misidaceo cavernicolo (*Spelaeomysis bottazzii* n.g., n.sp.) di Terra d'Otranto. Rendiconti dell'Accademia Nazionale dei Lincei, Classe di Scienze fisiche, matematiche e naturali 33: 512-513.

Cesaro, G., A. P. Ariani, A. Balboni, and V. Saggiomo. 1984. Preferenze di salinità in *Spelaeomysis bottazzii* (Mysidacea, Lepidomysidae). Bollettino di Zoologia 51 (suppl.): 27.

Coineau, N. 2000. Adaptations to interstitial groundwater life, pp. 189-210. In, H. Wilkens, D. C. Culver, and W. F. Humphreys (eds.), Subterranean ecosystems, vol. 30; In, D. W. Goodall (ed.), Ecosystems of the World. Elsevier, Amsterdam.

Cotecchia, V. 1977. Studi e ricerche sulle acque sotterranee e sull'intrusione marina in Puglia (Peninsola Salentina). Quaderni dell'Istituto di Ricerca sulle Acque 20: 1-462.

———, G. S. Tazioli, and F. Tittozzi. 1975. Geochimica delle acque della Penisola Salentina in relazione ai rapporti tra le acque di falda, le acque marine sotterranee e il mare. Geologia Applicata e Idrogeologia 10: 205-224.

Delamare Deboutteville, C. 1960. Biologie des eaux souterraines littorales et continentales. In, Actualités scientifiques et industrielles 1280. Vie et Milieu (suppl.) 9: 1-740, pl. I.

Fage, L., and T. Monod. 1936. La faune marine du Jameo de Agua, lac souterraine de l'Ile de Lanzarote (Canaries). In, Biospeologica LXIII. Archives de Zoologie Expérimentale et Générale 78: 97-113.

Ginet, R. 1955. Études sur la biologie d'Amphipodes du genre *Niphargus*. I. – Le creusement de terriers; relations avec le limon argileux. Bulletin de la Société Zoologique de France 80: 332-349.

Hüppop, K. 2000. How do cave animals cope with the food scarcity in caves? pp.159-188. In, H. Wilkens, D. C. Culver, and W. F. Humphreys (eds.), Subterranean ecosystems, vol. 30; In, D. W. Goodall (ed.), Ecosystems of the World. Elsevier, Amsterdam.

Mauchline, J. 1980. The biology of mysids and euphausiids. In, J. H. S. Blaxter, F. S. Russell, and M. Young (eds.), Advances in Marine Biology 18: 1-681, Academic Press, London.

Monod, T. 1924. Sur un type nouveaux de Malacostracé : *Thermosbaena mirabilis* nov. gen., nov. sp. Bulletin de la Société Zoologique de France 49: 58-68.

Ortiz, M., A. García-Debrás, A. Pérez, and R. Lalana. 2005. Estudio del marsupio y las larvas de *Spelaeomysis nuniezi* Bacescu y Orghidan, 1971 (Crustacea, Mysidacea). Revista Biología 19: 83-84.

Pesce, G. L. 1975. A new locality for *Spelaeomysis bottazzii* with redescription of the species (Crustacea, Mysidacea). Bollettino del Museo Civico di Storia Naturale, Verona 2: 345-354.

———, and T. M. Iliffe. 2002. New records of cave-dwelling mysids from the Bahamas and Mexico with description of *Palaumysis bahamensis* n. sp. (Crustacea: Mysidacea). Journal of Natural History 36: 265-278.

———, G. Fusacchia, D. Maggi, and P. Tetè. 1978. Ricerche faunistiche in acque freatiche del Salento. Thalassia Salentina 8: 1-51.

Pillai, N. K., and T. Mariamma. 1964. On a new Lepidomysid from India. Crustaceana 7: 113-124.

Riedl, R. 1966. Biologie der Meereshöhlen. Topographie, Faunistik und Ökologie eines unterseeischen Lebensraumes. Eine Monographie. Paul Parey, Hamburg.

Ruffo, S. 1955. Le attuali conoscenze sulla fauna cavernicola della regione Pugliese. Memorie di Biogeografia Adriatica 3: 1-143.

Vandel, A. 1964. Biospéologie. La Biologie des Animaux Cavernicoles. Gauthier Villars, Paris.

Villalobos, A. 1951. Un nuevo Misidaceo de las Grutas de Quintero en el Estado de Tamaulipas. Anales del Instituto de Biología Universidad Nacional Autónoma de México 22: 190-218.

von Martens, C. E. 1858. Über einige Brackwasserbewohner aus den Umgebungen Venedigs. Archiv für Naturgeschichte 24: 152-208.

Wittmann, K. J. 1981. Comparative biology and morphology of marsupial development in *Leptomysis* and other Mediterranean Mysidacea (Crustacea). Journal of Experimental Marine Biology and Ecology 52: 243-270.

———. 1984. Ecophysiology of marsupial development and reproduction in Mysidacea (Crustacea). Oceanography and Marine Biology, An Annual Review 22: 393-428.

———. 1992. Morphogeographic variations in the genus *Mesopodopsis* Czerniavsky with descriptions of three new species (Crustacea, Mysidacea). Hydrobiologia 241: 71-89.

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