Small-scale spatial heterogeneity and seasonal variation in a population of a cave-dwelling Mediterranean mysid

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Abstract. Seasonal changes in small-scale spatial distribution patterns of abundance and composition of Hemimysis speluncola Ledoyer (1963) (Crustacea, Mysidacea) were studied in a population of cave-dwelling mysids off the Medes Islands (NW Mediterranean Sea). The distribution pattern was characterized all year round by marked spatial segregation of juveniles, which occupied areas closer to the cave entrance, while adults were concentrated mainly in the innermost part of the cave. The small-scale spatial heterogeneity observed appears to be regulated by biological factors, particularly social and reproductive behaviour. Nevertheless, a certain adaptation of the distribution of the swarms to hydrodynamic factors suggests that physical factors may also play a role. The heterogeneous aggregation pattern recorded would clearly appear to be an adaptive strategy by the population to enable it to thrive within its habitat. That adaptation is designed to reduce predation mortality, enhance mating efficiency and regulate the population generally. Marked seasonal fluctuations in the density and composition of the population were recorded, with high density levels in winter and throughout the spring. Two methods were employed to quantify the population: hauls with plankton nets towed by divers and collection of faecal pellets. The patterns observed using both these methods were similar, although the latter method yielded total density values that were an order of magnitude greater.

Introduction

Littoral zooplankton populations exhibit high temporal and spatial variability, reflected as patchiness. Although the view that biological processes affect the zooplankton distribution on a temporal scale while physical processes affect distribution on a spatial scale (e.g. Haury et al., 1978; Longhurst, 1981; Denman and Powell, 1984) is widely accepted, the effect is not so clear in the inshoremost littoral zone, where small-scale interrelationships between species and the habitat are intensified. On that scale, patchiness of the zooplankton is regulated more by social behaviour (feeding, predation, reproduction, etc.) than by fluctuations in physical factors, because of the basically low level of variation in environmental parameters in the region between 1 and 100 m (Mackas et al., 1985). Nevertheless, hydrodynamic mechanisms (such as turbulence) may be a source of small-scale heterogeneity in zooplankton populations (e.g. Haury et al., 1990), and such mechanisms have been accorded greater importance in recent studies. This means that the social behaviour of swarms or schools may display a certain adaptability to more or less vigorous physical conditions which may contribute to the formation and dispersal of aggregations.

Littoral mysid populations tend to exhibit a high degree of patchiness and are a good example for studying which physical or biological mechanisms are responsible for the spatiotemporal heterogeneity of epibenthic crustaceans. The swarming behaviour of littoral mysids is comparable to that of many other littoral or demersal zooplanktonic organisms that display high variability over short...
distances or short time spans (e.g. Williams, 1984; Jacoby and Greenwood, 1988; Lewis and Boers, 1991). To date, a number of studies have been published on the causes of small-scale variability in littoral mysids (e.g. Clutter, 1969; Mauchline, 1980; O'Brien, 1989; Modlin, 1990). On the whole, the distribution pattern of each species has been reported to be closely related both to the structure of the substrate with which they are associated and to specific social behaviour. In other words, the temporal changes observed in mysid populations may be attributable to growth, reproduction, death and migration of the population (Clutter, 1967; Ritz, 1994) or may simply reflect heterogeneous composition and density distribution (Mauchline, 1980). The difficulty in distinguishing between the causes is a result of the sampling system itself and the possible artifacts associated with it. In the case of tows using nets, the associated problems include the extent to which the different swarms are combined as a consequence of the loss of information on patchiness and individual net avoidance (Wiebe et al., 1982). Other sampling methods involving suction pumps (Rey et al., 1987), surface nets (Clutter, 1965) and plankton traps (Hobson and Chess, 1979) have gained currency, and have been supplemented by new bioacoustic (Roe and Griffiths, 1993) and video profiling techniques (Bauscunt et al., 1993). A new method based on the collection of faecal pellets has recently been proposed (Carola et al., 1993). This new sampling method avoids the artifacts caused by patchiness and individual net avoidance.

One of the drawbacks to this method is that the full spatial extent inhabited by a population and the migrations that may be made by that population may be unknown. To avert that problem, a species (*Hemimysis speluncola*) dwelling in littoral submarine caves, a quite different habitat from those studied to date, was chosen for the present study. This species is common in the Western Mediterranean Sea (Ledoyer, 1963; Macquart-Moulin and Patriti, 1966; Macquart-Moulin and Passelaigue, 1982; Riera et al., 1991), where it follows a nychthemeral migration pattern involving horizontal migrations outside the caves at night and a return to the caves again when daylight comes (Riera et al., 1991).

The main objective of this study was to observe spatiotemporal variability in this cave-dwelling species using the conventional method of net tows and the new faecal pellet collection method, in an effort to describe the effects this species' particular swarming behaviour (in the context of the special features of its habitat) may have on density estimates and population dynamics.

**Method**

The study population of *H.speluncola* Ledoyer, 1963 was located off Meda Xica Island (Medes Islands, Northwestern Mediterranean, Spain). The cave occupied by that population is roughly cylindrical in shape, measuring some 50 m in length by 4-7 m in diameter [for a more detailed description, see Gili et al. (1986)]. The *H.speluncola* population is concentrated in the final 15 m of the innermost section of the cave and forms a series of readily locatable swarms (Figure 1). For the purpose of describing the distribution of the *H.speluncola* population, the cave was divided into four subzones based on the findings of preparatory work for this study (Riera et al., 1991; Carola et al., 1993): subzone 1 was the side to the left of the entrance (south wall); subzone 2 was the innermost part of the cave against the rear
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wall; subzone 3 was the side located to the right of the entrance (north wall); subzone 4 was the area closest to the entrance (Figure 1). Sampling was carried out monthly from March 1990 to April 1991 using the two methods described below.

**Plankton nets**

A series of four 1–2 min tows was carried out on each day of sampling using a plankton net, with a mouth opening 20 cm in diameter and a mesh size of 250 μm, towed by a diver. Tows were carried out successively in each of the four subzones (total time elapsed from the first to the last tow: 15 min). Samples were fixed in 5% formalin and stored in a refrigerator until examination at the laboratory. The individuals in each plankton tow were assigned to one of six separate population classes: juveniles (JUV), immature males (IM), mature males (MM), immature females (IF), females in berry (FIB) and post-berry females (PBF), based on the morphological classification described elsewhere (San Vicente and Sorbe, 1988) and counted. In samples comprising >4000 individuals, subsamples of between 25 and 50% of the sample were examined. In all, 61500 individuals were counted.

**Faecal pellets**

Containers termed 'collectors' were set out on the floor of the cave in all four subzones. They were deployed and opened with special care to minimize disturbance...
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and retrieved 2 h later. All samples were taken between 1 and 3 h after sunrise. The factor for converting pellet mass to number of mysids was estimated every sampling date (13 times) with four replicates each according to the method described by Carola et al. (1993). Mysids were caught inside the cave simultaneously with the plankton net towings and with a plankton net of the characteristics previously described. The sampling for the incubation experiment covered the four subzones of the cave. A total of 300–600 individuals were carefully placed in dark plastic containers (1 l volume, 85 mm diameter) previously filled with filtered seawater. Incubators were closed with a piece of plankton net which allows gas, but not pellet, exchanges. Less than 5 min later, incubators were replaced into the water. Mysids were incubated in situ, surrounded by free conspecifics. After 2 h, incubations were immediately fixed in 5% formalin.

To separate the faecal matter from other extraneous matter that was deposited in the collectors, the collector contents were added to a water column consisting of three layers (with different concentrations of LUDOX) with differing densities (Holme and McIntyre, 1984) and separated into three levels concentrating the material by centrifugation. Around 90% of the faecal pellets were concentrated in the two upper levels. These two levels were drawn off into a Petri dish using a Pasteur pipette, and all matter other than faecal pellets was then removed under a dissecting microscope. The contents of the Petri dish were then filtered through Whatman GF/C filters previously incinerated at 450°C for 4 h. The pellets were dried at 70°C for 48 h and then weighed. The pellets were incinerated at 450°C for 4 h. The faecal pellets in the incubators underwent the same procedure. At the same time, the number of mysids was counted to establish the relationship between the number of individuals and the faecal pellet weight obtained. That value was used to convert the data from the collectors set out and retrieved each month into the number of mysids.

The descriptors selected were individual density and population class composition. Density values were expressed as number of individuals per square metre, since swarm volume was always greater than 1 m. Kruskal–Wallis non-parametric analysis of variance was applied to test the statistical significance of the differences in density values between subzones (Sokal and Rohlf, 1969). The composition was represented as the percentage contribution of each population class to the total number of individuals in a tow.

To test the significance of spatial differences in population composition, monthly and biweekly data for each of the subzones were combined on the basis of periods shorter than 1 year. The combination procedure was to average the values for successive months with low levels of variation. The periods used were: March–July (period 1), August–January (period 2) and February–April (period 3). A homogeneity test (Sokal and Rohlf, 1969) was applied to each of these periods. Four different cases were considered with a view to determining which aspect of populational composition was responsible for spatial segregation: (i) indeterminate sex (juveniles)-sex identifiable (adults); (ii) male-female; (iii) immature male-mature male; (iv) immature female-mature female (in berry and post-berry).
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The only descriptor furnished by the collector method is individual density. The defaecation rate (dry weight of pellets/mysid) was calculated for each day of sampling based on the incubator data. The total number of individuals in the water column above each collector was calculated by multiplying the defaecation rate by the weight of pellets (grams dry weight) in the collector. That value was then divided by the depth of the water column to yield the density. The characteristic density value for each subzone in the cave was calculated by averaging the density values for each of the collectors set out in each of the four subzones. Kruskal–Wallis non-parametric analysis of variance was applied to test the differences between the subzones.

Results

Individual density distribution

The density distributions presented consistently marked spatial heterogeneity, even though the subzones were only a few metres apart. Subzone 3 (the north wall of the cave) exhibited the highest individual abundance values at all times (Kruskal–Wallis test values; plankton net method: \( P = 0.0461 \); collector method: \( P = 0.0351 \)). The opposite (south) wall and the innermost area displayed lower individual density values (Table I). This spatial pattern persisted over the entire annual sampling period, despite quite intense seasonal fluctuations, with maximum density values recorded in period 1. The lowest individual density values were recorded in summer–autumn (period 2; Figure 2).

Spatial heterogeneity of population structure

Figure 3 represents the distribution of the population in each zone, showing the composition for each period and the annual mean. The results were also indicative of pronounced spatial heterogeneity. Mature adults aggregated in the innermost part of the cave (subzone 2), whereas immature individuals were concentrated mainly in the area nearest the cave entrance (subzone 4). Each of the four subzones presented differing composition breakdowns for each populational component considered (homogeneity test; Table II). A comparison of the two values (since all cases had the same degrees of freedom) showed that individual maturity stage was the most important determining factor, particularly in the early stages in which sex was not yet differentiable. In individuals in which sex was identifiable, the spatial distribution of this species was determined by whether or not individuals were actively reproductive (much more pronounced in the females). Sex was the factor with the least effect on the spatial distribution. From a season standpoint, the study population exhibited a preponderance of juveniles all year round. That preponderance was particularly marked during the summer (Figure 4), when hardly any adults were present. Spatial segregation of the population was at its peak in winter (period 3).
Table I. Mean individual density (10^6 ind. m⁻³) of *H. speluncola* for each zone in each period considered and over the entire annual sampling period, estimated by plankton net towings (A) and the faecal pellet method (B).

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SD, standard deviation.

Comparison of the two sampling methods

The results obtained using the two sampling methods followed the same seasonal pattern (Figure 2), although with a difference of an order of magnitude in absolute terms. The highest recorded density collected using plankton net tows exceeded 10^6 individuals m⁻³. The maximum density obtained using faecal pellet collectors was >10^5 individuals m⁻³.

The two estimates were fit by linear regression, which yielded a highly significant correlation coefficient ($r = 0.90; P < 0.001$):

$$D_{pel} = 2597 + 6.4 \cdot D_{tow}$$

where $D_{pel}$ was the density calculated based on the faecal pellet collector data and $D_{tow}$ was the density based on the plankton net data.

Discussion

Both methods showed the same pattern of spatiotemporal variation of the population. However, the densities obtained using the faecal pellet collector method were an order of magnitude higher and thus appear to bear out the view that tows with nets underestimate the total number of individuals in a population.
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Fig. 2. Changes in individual density of *H. speluncola* (*10^3* ind. m⁻³) over the sampling period: plankton net tows (top) and faecal pellet collection (below).

reaction of individuals in a population to the collection method using plankton nets towed by divers is comparable to the escape behaviour of swarms attacked by a predator (O'Brien, 1988; O'Brien and Ritz, 1988; Ritz, 1994). The effect of the diver, coupled with the ability of many zooplankton species to avoid nets which are not towed at sufficient speed or do not have sufficiently large mouth areas (Orr, 1981; Wiebe *et al.*, 1982), makes net-based estimates less reliable than the faecal pellet collector method. This latter method does not involve any disturbance of the population and may be a quite useful method for sampling cave-dwelling populations like the one studied here. The development of more specific methods for sampling highly patchy demersal zooplankton populations has been regarded as a very important factor to bear in mind before undertaking the study of a given population (Hamner and Carleton, 1979; Omori and Hamner, 1981). The faecal pellet collector method may entail handling problems that result in overestimation of mysid density. Sediment around the collectors may be resuspended during retrieval, thereby increasing the number of faecal pellets collected beyond the actual number produced in the water column above the collectors. On the other hand, the short decomposition time of <2 h (Carola *et al.*, 1993) and the absence of
pellet build-up on the bottom suggest that the risk of retrieval-induced contamination is negligible.

The high level of heterogeneity recorded, with the formation of swarms with differing compositions within a section of the cave only 15 m long, was consistent with other reports of spatial variability in mysids. For example, in several species individuals in each age class form distinct swarms in close proximity to each other within a single population (Clutter, 1969; Wittmann, 1978; O'Brien, 1989). Different species of mysids present differing depth distributions for juveniles and for adults. The extent of such depth differences varies according to the species: Clutter (1969) reported depth differences of <10 m in the distribution of juveniles and adults of Metamysidopsis elongata, but no special differences in the distribution of the sexes. Studies of Mysis relicta indicate that juveniles are spawned in deep water (50 m) in the lake, after which they migrate to shallower zones (20 m), where they remain until they reach reproductive size, when they migrate back to the deeper zone (Morgan and Threlkeld, 1982). There was a distinct segregation pattern for the H.speluncola population within a much smaller area (Macquart-Moulin and Patriti, 1966). Such segregation can normally be explained in social terms, since high levels of cannibalism, which this species appears to avoid, have been reported in mixed swarms (Pulliam and Caraco, 1984).

Light may be one of the factors responsible for the spatial distribution of H.speluncola. Studies of phototropism in this species conducted in the laboratory have shown these organisms to be very sensitive to low levels of illumination (0.0012

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Fig. 3. Mean composition of H.speluncola in each zone, presenting the data for each of the three periods considered and combined for the entire sampling period (sectors represent percentage composition). MF, mature females; IMF, immature females; MM, mature males; IMM, immature males; JUV, juveniles.
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Fig. 4. Changes in populational composition (in per cent) of *H. speluncola* over the study period. PBF, post-berry females; FIB, females in berry; IF, immature females; MM, mature males; IM, immature males; JUV, juveniles.

Seasonal variations in both total individual abundance and the composition of the study population followed the general pattern known for littoral mysid species. Such annual variation is a response to a reproductive cycle that maximizes population growth when sufficient food resources are available (Modlin, 1990; Ritz, 1994). Increased abundance of mature males and females in winter (period 3) precedes the recruitment period for juveniles in spring.

The presence of more mature females and males in the innermost zone of the cave may be attributable both to heightened probability of mating success and to predator avoidance. The more compact swarms deeper inside the cave would facilitate mating encounters (Nicol, 1984; O'Brien *et al.*, 1986). The larger number of females observed would also be consistent with an increased likelihood of mating. Furthermore, situating the population's spawners furthest from the cave entrance, where most predators are located, would help provide protection from predation. Analysis of the stomach contents of various specimens of the fish species *Anthias anthias* collected inside the cave has shown it to be the main predator on this mysid. As a sight-based predator, *A. anthias* stays permanently near the cave entrance, but individuals accompanied the divers and their lights to the end of the cave and took advantage of the light to feed actively on the mysids (personal observation).

Subdividing the population into swarms is an evolutionary response by demersal organisms with a highly patchy distribution to ensure preferential protection from predation for certain individuals. This behaviour is usually accompanied by a
tendency to aggregate in more compact swarms during the daytime, also for the purpose of predator avoidance (Clutter, 1969; Hamner and Carleton, 1979).

The location of juveniles closest to the cave entrance is also related to protection of the population from predation. The presence of juveniles in the area closest to the entrance and hence most accessible to predators may be interpreted as a means of increasing the protection accorded the population's spawning potential, whereby the activity of a predator which chanced to enter the cave would be confined to juvenile individuals. The segregation of juveniles and adults in *H. speluncola* might also be related to 'breeding aggregation', a phenomenon reported for many mysid species (Mauchline, 1971), whereby mature males and females aggregate to spawn at certain times of year.

Aggregations of littoral mysids are closely related to the characteristics of the substratum. Swarm size, structure and distribution are adapted to habitat topography (Clutter, 1967; Fager and Clutter, 1968), and are reminiscent of the types of associations displayed by other demersal zooplanktonic groups, such as tropical copepods (Hamner and Carleton, 1979; Omori and Hamner, 1981). In the cave in the Medes Islands considered, the segregation pattern and swarm structure observed were related to the cave architecture. The swarms with the largest numbers of individuals were located in the largest open spaces (i.e. along the north wall and in the area closest to the cave entrance). Occupation of the largest area by juveniles may enable swarms to disperse over larger areas in the presence of predators (Modlin, 1990). In contrast, the concentration of individuals along the north wall may be related to the hydrodynamic features of the cave. In a preliminary
study of the flow of water masses inside the cave, Zabala et al. (1989) observed differing water flows which appeared to be more intense next to the roof of the cave and along the north wall. Thus, the location of the largest numbers of individuals in the cave would appear to follow a pattern of adaptation to hydrodynamic factors, with the swarms perhaps orienting themselves so that they faced the most intense flow (O'Brien, 1988).

The spatiotemporal distribution of the Medes Island population is also related to the pattern of horizontal migration recorded for this species (Riera et al., 1991). The extent of horizontal migrations is much larger than that for vertical migrations in this species, and indeed vertical migration is negligible, unlike the commonly accepted pattern for the zooplankton (Steel, 1978). Juveniles apparently spread out more widely than adults and females less than males, perhaps to avoid the risk of decreasing spawning potential (Zelickman, 1974; Wittmann, 1978; Fenton, 1992). The location of juveniles closest to the cave entrance and of mature females in the innermost area would contribute to an organized migration process, while at the same time allowing the swarms in the population to regroup spatially on their return.

On the whole, the spatial heterogeneity observed in *H. speluncola* in the Medes Islands appears to be regulated by biological factors, particularly social and reproductive behaviour. Although biological factors clearly seem to be preponderant on a small scale, the distribution of the swarms also exhibits a certain level of adaptation to the hydrodynamics of the cave and hence increased scope for the role of physical factors, although further experimental evidence will be required to substantiate this. The heterogeneous pattern of aggregation observed would appear to reflect an adaptive strategy by the mysids to maximize exploitation of their niche in the habitat. The population's position would be strengthened by a distribution pattern that tended to decrease predation mortality, enhance mating success and regulate population structure during migrations.

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