SCI. MAR., 57(1): 51-63 SCIENTIA MARINA 1993

Fecal pellets collection as a method for assessing egesta of the marine cave-dwelling mysid *Hemimysis speluncola**

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SUMMARY: Egesta of a cave-dwelling mysid (*Hemimysis speluncola* Ledoyer, 1963) was studied in a submarine cave of Medes Islands, NW Mediterranean by *in situ* fecal pellet collecting. Fecal pellet production and gut fullness of mysids during incubation experiments are used to estimate mysid egestion rates. Intrinsic factors related with the natural history of this species such as population structure, density of mysids, daily rhythms and pellet decomposition rates are tested for their influence on the egestion rate. The effects of methodological artifacts, such as the stress induced by both incubation and preservation procedures, are also studied. An average mysid egests about 2.5 pellets per day into the cave. The time of day is the main factor affecting egestion. The highest deposition rate is between 2 to 4 hours after sunrise when about 38 % of the total daily pellet production becomes egested. Fecal pellet morphology changes with mysid demographic classes: immature mysids produce slender and thick pellets, whereas mature mysids produce only thick pellets. Immature classes show higher percentages of full guts than mature ones. Mysid density in the incubators does not affect the results on gut fullness, but it causes a decrease in the number of pellets collected after incubation. Coprorhexia seems to be the only plausible process to explain this paradox. The incubation procedure does not increase deposition rate significantly. Time of incubation is critical because the half-life of fecal pellets is about 2.5 hours. Fixation with liquid nitrogen decreases gut fullness and also deposition rates. Higher values are obtained with 70 % ethanol and 5 % formalin solutions which show very similar results for both gut fullness and pellet deposition rates. Nevertheless, ethanol is not suitable as fixative because it enhances the opacity of the body. Several suggestions are given in order to optimize the reliability of further *in situ* experiments for evaluation of egesta of *Hemimysis speluncola* in submarine caves.

Key words: Mysids, *Hemimysis speluncola*, egesta, fecal pellets, method, submarine caves.

RESUMEN: UN MÉTODO PARA LA EVALUACIÓN DE LA EGESTA DEL MISIDÁCEO CAVERNICOLA *Hemimysis speluncola* BASADO EN LA RECOLECCIÓN DE LOS PAQUETES FECALES. — La egesta de un misidáceo cavernícola ha sido estudiada en una cueva submarina de las Islas Medes, Mediterráneo occidental, mediante la recolección *in situ* de los paquetes fecales. La egesta de los misidáceos fue estimada a través del estudio de la producción de paquetes fecales y del contenido estomacal. Factores intrínsecos relacionados con la historia natural de la especie, tales como la estructura de la población, la densidad de misidáceos, el ritmo diario y la tasa de descomposición de los paquetes fecales, fueron analizados para determinar su influencia en la egesta. También se estudiaron los efectos de la manipulación metodológica, el estrés inducido por los procesos de incubación y fijación. Los misidáceos liberan un promedio de 2,5 paquetes fecales al día dentro de la cueva. El factor principal implicado en la egesta es la hora del día. La tasa de deposición más elevada se produce entre las 2-4 horas después de la salida del sol, período en el que se egesta el 38 % de la producción diaria de paquetes fecales. La morfología de los paquetes fecales depende de las clases demográficas de los misidáceos. Los misidáceos inmaduros producen paquetes fecales delgados y gruesos, mientras que las clases maduras sólo producen paquetes gruesos. Las clases inmaduras presentan porcentajes de contenidos estomacales mayores que las maduras. La densidad de misidáceos en los incubadores no afecta al contenido estomacal pero produce una disminución en el número de paquetes fecales recogidos después de la incubación. La "coprorexia" parece ser el único proceso capaz de dar explicación a esta paradoja. El proceso de incubación no incrementa significativamente la egesta. El tiempo de incubación es crítico debido a la corta vida media de los paquetes fecales (aproximadamente 2,5 horas). La fijación con nitrógeno líquido disminuye el contenido estomacal así como la tasa de deposición. Con etanol al 70 % y formol al 5 % se obtuvieron valores más elevados. Ambos fijadores ofrecieron resultados muy similares en cuanto a contenidos estomacales y egesta. No obstante, el etanol no es recomendado como fijador debido a la opacidad que produce en el cuerpo de los misidáceos. Se dan algunas sugerencias para la mejora de futuros experimentos *in situ* sobre la evaluación de la egesta de *Hemimysis speluncola* en cuevas submarinas.

Palabras clave: Misidáceos, *Hemimysis speluncola*, egesta, paquetes fecales, método, cuevas submarinas.

*Received February 9, 1993. Accepted April 2, 1993.
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INTRODUCTION

Fecal pellets produced by crustacean zooplankton have attracted considerable interest as a vehicle of supposedly rapid transport of material out of the euphotic zone to the deep trophic-poor zones (GAULD, 1957; HONJO and ROMAN, 1978; DUNBAR and BERGER, 1981; LAMPITT, 1985; LAMPITT *et al.*, 1990). Even if laboratory experiments support this view, field data present contradictory evidence (STEELE and BAIRD, 1972; PISKALN and HONJO, 1987; BATHMANN *et al.*, 1987). Sinking pellets are often collected by modified sediment traps which are moored in open waters in such a way that the effects of environmental parameters (such as temperature, water depth, ...), plankton structure and behavior, and handling of samples are difficult to test. In order to avoid some of these drawbacks, we test the adequacy of pellet collection (as a method to estimate egesta of crustaceans), by studying of a littoral epibenthic mysid which exhibits a very charateristic behaviour.

Hemimysis speluncola Ledoyer, 1963 (Crustacea, Mysidacea) is a gregarious species which forms dense swarms in submarine litoral caves on the NW Mediterranean coasts (MACQUART-MOULIN and PATRITI, 1966). Every day this species migrates horizontally in a predictable nycthemeral rhythm (RIERA *et al.*, 1991): during the daylight hours the mysids remain densely packed in the deepest holes of the cave, but at nightfall they go to the exterior of the cave where they search for food in the vicinity of the entrance, usually not farther than 100 m (PASSELAIGUE and BOURDILLON, 1985; MACQUART-MOULIN and PATRITI, 1966). Before sunrise the mysids go back to the darkest areas of the cave. With this behaviour, *H. speluncola* acts as a carrier of matter picked up as food outside the cave and released as fecal pellets inside (RIERA *et al.*, 1991). Although some aspects of the *H. speluncola* metabolism such as respiration, excretion (GAUDY *et al.*, 1980), and growth (GAUDY and GUERIN, 1979) have been evaluated in the laboratory, egesta has never been studied.

There are two important components in the study of egesta: quantity and quality of defecated materials. Only the quantitative aspects of egesta have been considered in this paper. Egesta of mysids was approached by studying the response of individuals held in dark incubators as in a previously reported paper (MURTAUGH, 1984). The amount of egesta was mainly estimated by calculating fecal pellet production rates, although complementary estimates based on gut fullness and gut depletion time have been used in some cases.

Some factors affect both pellet production and gut fullness, and they can be grouped in two categories: 1) intrinsic factors related to the natural history and behaviour of the species, and 2) methodological factors induced by handling. These two categories may interact. The main intrinsic factors which are supposed to affect the egesta of *H. speluncola* are: a) the daily rhythms of activity which could introduce variation in both pellet deposition rates and gut fullness during the day, b) mysid density, which could produce stress and also accelerate the speed of the fragmentation or decomposition of pellets by enhancing coprophagia or coprorhexia (breaking down of the fecal pellets by the mysids themselves, while ingesting only a small proportion), c) pellet fragmentation rates which could affect the number of pellets counted depending on the time before fixation, d) demographic structure of populations, which could affect the number and size of pellets, and e) seasonal changes in metabolic rhythms and food availability. The *in situ* approach in this study (see methods) avoids some of the commonest problems associated with handling marine organisms in laboratory experiments.

Furthermore, possible alterations caused by unavoidable methodological artifacts, such as those related with incubation and preservation procedures, have also been studied.

MATERIAL AND METHODS

The site

The cave is located in the Meda Xica island (Medes Islands, NW Mediterranean, Spain) and described in previous papers (GILI *et al.*, 1986; ZABALA *et al.*, 1989). It is roughly cylindrical, 50 m long, 2-8 m wide and 2-4 m high, and rises from −12 m depth (at the entrance) to the surface (at the inner part). It ends in an air-filled chamber ($4 \times 4 \times 3$ m^3) where mysid manipulations can be carried out (Fig. 1 a-c). ZABALA *et al.* (1984) estimated the total volume of the cave to be about 756 m^3.

Description of mysid and pellet populations

Eleven different demographic classes have been distinguished in previous demographic studies of *H. speluncola* populations (GAUDY and GUERIN, 1979) but they are hardly distinguishable when only elementary biometric parameters — such as length or body area — are considered. Cephalothorax length

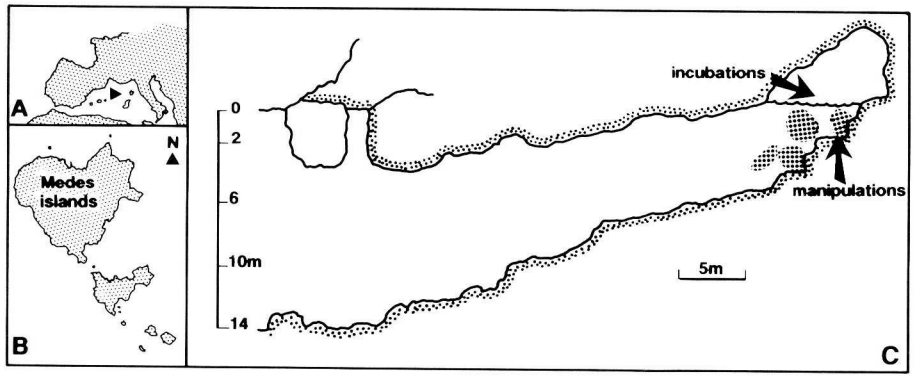

FIG. 1. — Sketch of study cave. A) General location in the western Mediterranean. B) Medes Islands site. C) Diagrammatic Section of the cave showing where the incubations and manipulations were performed. Shaded areas represent the position of mysid swarms during the daylight hours.

(LC) is the best body size estimator; it is better than total body length or body perimeter because these are influenced by body position during observation. Nevertheless, due to the great number of individuals to be analyzed, only area — measured by an automatic Image Analyzer System (IBAS)— was recorded. In the first step, mysids were sorted into visually distinguishable classes. Then, 1) all the juvenile classes were pooled in a single juvenile class; 2) the two mature female stages — those with eggs or embryos in the marsupium — were grouped in an ovigerous female class; and 3) the post-ovigerous mature females — those with an empty marsupium — and the mature pre-ovigerous females were grouped in the mature non-ovigerous female class. So, only six demographic classes — juveniles, immature males, immature females, adult males, mature non-ovigerous females and ovigerous females — were distinguished (Table 1). Several hundred individuals class were recorded to characterize the following parameters: 1) gut length, 2) gut diameter, 3) cephalothorax length, 4) body area, 5) perimeter and 6) dry weight.

In the second step, relationships between cephalothorax length and area (IBAS) of the six demographic classes were calculated. Because of the overlap among the length ranges of some classes, which made it difficult to differentiate between them, the six classes were further reduced to three : 1) juvenile, which includes the first class; 2) immature, which includes the second and the third classes; and 3) mature, which includes the last three classes. Furthermore, for some purposes juvenile and immature classes were grouped together in a single immature class which was opposed to the mature class.

Dry weight was obtained by placing individuals or pellets in a heater at 100 °C for 3 or 4 hours, followed by immediate weighing with a precision balance (accuracy 1 μg).

By using length and diameter as descriptors, pellets were classed into six discrete size-categories: large-thick, short-thick, thick-fragments, large-thin, short-thin and thin-fragments. Nevertheless, the last class was rejected due to its scarcity. Limits between classes are described in Table 2.

TABLE 1. — Biometrical parameters of six demographic classes of *Hemimysis speluncola*: cephalotorax lenght (LC, mm), dry weight (μg), gut lenght (mm) and gut diameter (mm) (N, number of individuals; n, number of weight replicates of hundred individuals).

		JUVENILE	IMMATURE		MATURE		
			males	females	males	Ov. females	Non ov. females
LC	min-max	0.6-1.6	1.25-1.6	1.4-1.95	1.45-2.4	1.95-2.4	1.9-2.45
	N	218	74	42	95	25	57
Weight	min-max	66-83	156-221	90-300	265-360	501-567	230-396
	n	5	6	6	2	3	2
Gut lenght	AVG	1.14	2.3			2.54	
	SDX	0.19	0.45			0.14	
	N	25	11			24	
Gut diameter	AVG	0.025	0.041			0.044	
	SDX	0.004	0.005			0.008	
	N	25	11			24	

TABLE 2. — Biometrical parameters of five classes of pellets of *Hemimysis speluncola*: lenght (μm), diameter (μm) and dry weight (μg).

		Large-Thick	Short-Thick	Thick-Fragments	Short-Thin	Large-Thin	Standard
Lenght	Min-max	580-780	300-425	170-270	180-400	450-1050	
	AVG(SDX)	692(70)	358(43)	230(60)	265(73)	640(220)	493
	N	120	201	350	27	18	
Diameter	Min-max	46-52	37-65	39-66	20-33	13-36	
	AVG(SDX)	49(2.5)	52(9.7)	50(9.2)	26(4.2)	21(2.6)	50
	N	120	201	350	18	27	
Weight	AVG(SDX)	16.85(2.5)	8.4(0.9)	—	—	—	12
	N	5	9				

Incubation. Basic procedures for pellet collection

Mysids were caught inside the cave with a plankton net (cone of 20 cm in diameter; 50 μm aperture mesh) handled by SCUBA-divers. Because of the high density and the location of swarms, mysids were caught and transferred to incubators in less than 1 minute. Several hundreds of individuals were carefully placed in dark plastic bottles (1 l volume, 85 mm diameter) previously filled with filtered sea water. Incubators were closed with a piece of plankton net which allows gas — but not pellet — exchanges. Less than 5 minutes later, incubators were replaced into the water. Mysids were incubated *in situ*, surrounded by free conspecifics, which live only 3 m below the surface of the air chamber. (Fig. 1c). After 2 hour-incubations (sufficient time to obtain pellets and minimize their fragmentation) samples were immediately fixed (the nature of fixatives depending upon each experiment). The water was filtered through a plankton net (1 mm mesh size) to separate mysids from fecal pellets.

Pellets

Pellet egestion rates were estimated as the number of pellets per mysid in an hour. To calculate this ratio, we assumed that 3 fragments are equivalent to 1 entire pellet. Long and medium pellets were counted as equal. Moreover, all mysids within the different classes were considered to produce the same amount of pellets.

Gut fullness

Three different parameters concerning gut fullness of mysids are studied: 1) number of full guts; 2) degree of fullness; and 3) gut content position. Mysids were examined individually by transparency, to discern between empty and full individuals. Moreover, gut contents were carefully investigated in full individuals. The existence of the six abdominal segments allowed the partition of the gut into the same number of divisions. This is useful when referring to degree of fullness — which can be estimated as number of full segments— and food position in the gut segments, which reflects the state of digestion.

Effect of the population structure

EXPERIMENT 1 (June 6th, 1991). To evaluate the relationship between mysid size and pellet class distributions, mysids from two different swarms of the cave — which were known to have a strongly biased population structure (Ribes, non published data) — were caught separately with a plankton net, and incubated from 2 to 4 hours after sunrise by the method described above and then fixed with 5 % formalin solution. One of the two incubators — hereafter referred to as immature — was filled with a population dominated by immatures (95 %); the second incubator —hereafter referred to as mature— was dominated by mature individuals (73 %). After fixation, both mysids and pellets of each incubator were separately sorted into different classes as described above, and counted.

Effects of mysid size on gut fullness

All the individuals of both the mature and immature incubators described above were examined to determine gut fullness; the number of full mysids and the number and position of full segments were also counted.

Time effect : daily rhythm

On pellet deposition

EXPERIMENT 2 (July 23th, 1988). A cycle of incubations was carried out by the basic incubation

procedure in summer. Incubations were repeated every 2 hours during the time mysids remained inside the cave (from sunrise to sunset). Four replicates of each incubation were made. At the laboratory, mysids and pellets were classified and counted as described above.

On pellet fragmentation

EXPERIMENT 3 (October 13th, 1988). Three sub-samples of pellets were obtained just after deposition as described above. Pellets were separated from mysids, re-incubated *in situ* for periods of 1, 2, 3, 4 hours, and fixed in 5 % formalin solution. Fragments and entire pellets were counted after re-incubation to calculate the pellet fragmentation rates. Decomposition of pellets can be described morphologically as the ratio of the number of fragmented pellets to the number of whole pellets.

Effect of mysid density

EXPERIMENT 4. (July 23th, 1989). Four incubators with increasing densities of mysids were used to estimate the influence of density on pellet egestion rate. No selection between different mysid demographic classes was done during capture, to ensure a similar population structure for all incubators. 5 % formalin solution was used as fixative. Two replicates were performed from 2.5 to 4.5 and from 4.5 to 6.5 hours after sunrise. Mysids and pellets were counted and pellet egestion rates were estimated as above.

EXPERIMENT 5 (June 6th, 1991). In this experiment, which was carried out simultaneously with the next one, two incubators were filled with a dominant immature population (95 %) identical in demographic structure but with different densities (590 versus 2069 individuals). After fixation, mysids and pellets were counted and pellet egestion rates were estimated.

Incubation and fixative effects

EXPERIMENT 6 (June 6th, 1991). Mysids were caught inside the cave by the described method 2 hours after sunrise. One sample was immediately fixed with a 5 % formalin solution to determine the fullness of guts before any manipulation. Several hundred individuals were carefully distributed into nine different incubators and were incubated by the basic procedure. After 2 hours, three samples were fixed with a 5 % formalin solution, three with liquid nitrogen and three with a 70 % ethanol solution. Simultaneously, in order to compare incubated and non-incubated mysids, several hundred free individuals were caught from the cave by a SCUBA-diver in the same plankton net as before, and were distributed into six incubators; three of them were immediately fixed with a 5 % formalin solution and the remaining three with liquid nitrogen.

All the mysids were examined for their gut fullness: the number of full individuals, the extent of fullness among full guts and food position in the abdominal segments were noted.

RESULTS

Population structure and pellet size distribution

Values of biometrical parameters characterizing the six demographic classes are presented on Table 1. In June, the juvenile class represented 71 % of the total mysid population, and the remaining percentage was equally distributed between immature (15 %) and mature (14 %) classes.

Relationship between cephalothorax length (LC) and area (IBAS) of the six demographic classes was calculated and the results are shown in Fig. 2. There was a great overlap between the area of the different demographic classes, which discouraged the use of areas as a criterion for class sorting.

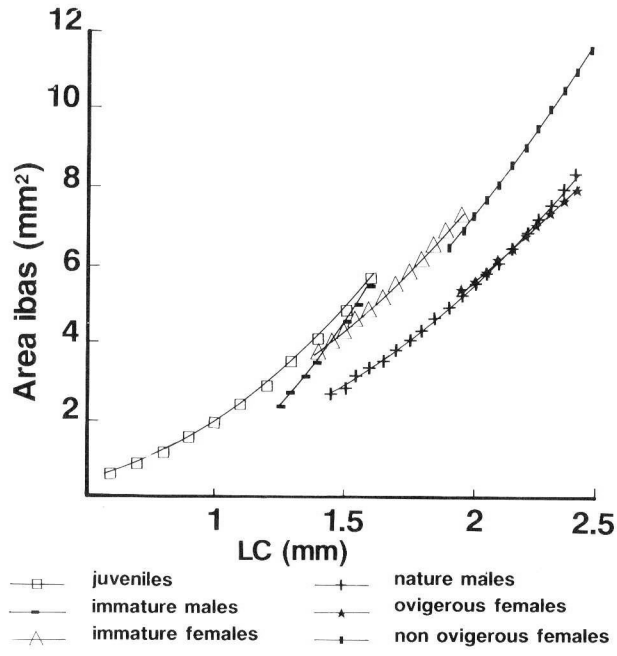

FIG. 2. — Relationships between cephalotorax length (LC) and Ibas area of the six demographic classes of *Hemimysis speluncola* which have been considerated in this study.

TABLE 3. — *Heteromysis speluncola*. Experiments 1 and 5. A) Population structure of mysids and pellets in three different experimental conditions: (column 1) immature dominance — high density; (column 2) immature dominance — low density; (column 3) mature dominance — low density. B) Comparison of four different ratios between the three experimental conditions mentioned above.

A) Mysids demographic class	Immature incubator Column 1	Column 2		Mature incubator Column 3	
Ovigerous female	15	5		84	
Non ovig. female	20	10	5 %	17	70 %
Mature male	68	25		93	
Immature female	72	103		26	
Immature male	60	4	95 %	43	30 %
Juvenile	1834	443		44	
Density (ind./liter)	2069	590		307	
Fecal pellets					
Short-thick	1405	343		458	
Large-thick	528	142	64 %	216	94 %
Fragmented thick	625	145		232	
Short-thin	563	254		32	
Large-thin	103	40	36 %	3	6 %
Fragmented thin	302	67		24	
Density (pellet/liter)	2908	991		965	
B)					
Pellets/mysid	1.4	1.7		2.6	
Thin/thick pellets	0.36	0.59		0.06	
Thin pellets/immature	0.39	0.57		0.38	
Fragmented/entire pellets	0.36	0.27		0.36	

Gut length ranged between 1.14 and 2.54 mm with an average value of 1.5 mm (SE = 0.07) due to the predominance of immature mysids (Table 1).

The dry weight of a mysid ranged from 66 to 567 μg DW/individual (Table 1).

Only three of the six initial classes of pellets were numerically important (Table 2). Fine short pellets are so scarce that morphological data on them are imprecise.

Thick fragments were always the most frequent class (57 %), followed by short-thick (24 %) and large-thick ones (10 %); large-fine pellets represented only 8 % of the total amount.

Length of pellets ranged between 0.26 and 0.69 mm with an average value of 0.49 mm (SD = 0.01).

Values of pellet diameter and length agreed with those of guts, although pellets had a slightly higher diameter than guts.

Considering these measurements, a full gut (or a length equivalent to the six abdominal segments) could carry three standard pellets; hence, the gut content of two full segments (0.25 mm average length/segment) corresponded to one entire pellet (0.49 mm average length).

Effect of mysid population structure

On pellets

No simple relationship between pellet length and mysid classes could be established.

By comparing columns 2 and 3 of Table 3, it is evident that thick and thin pellets were produced by mysids of different size. Although immature classes were represented in very different proportions (95 % in the immature incubator versus 27 % in the mature incubator), the thick pellets dominated both incubations (94 % versus 64 %, respectively). Obviously both immature and mature mysids were able to produce thick pellets. Furthermore, the ratio between thick pellets and mature mysids showed great differences between the two incubations (15.7 in the immature versus 3.8 in the mature), also suggesting that not all thick pellets came from mature mysids.

Thin pellets were very scarce when mature mysids became dominant (column 3, Table 3), suggesting that thin pellets were only produced by immature mysids. The same conclusion is suggested by the ratio between thin pellets and immature mysids, which remained roughly constant irrespective of the propor-

TABLE 4. — Raw values (%) of gut fullness in *Hemimysis speluncola* non-incubated individuals. Effect of population structure and fixatives on: full guts (column 2), full segments (column 3), and distribution of contents among the abdominal segments (columns 4 to 10). Percentages have been calculated for each segment.

		Mysids	% Full mysids	% Full segments	% Full segments						
					C	1	2	3	4	5	T
Formalin 1	Immature	165	25.4	4.3	0.0	2.4	6.1	4.2	4.2	3.0	10.3
	Mature	40	32.5	7.1	0.0	2.5	7.5	7.5	10.0	7.5	15.0
	Total	205	26.8	4.9	0.0	2.4	6.3	4.9	5.4	3.9	11.2
Formalin 2	Immature	101	46.5	7.5	0.0	3.0	5.9	7.9	14.9	18.8	2.0
	Mature	52	26.9	4.9	0.0	1.9	3.8	9.6	1.9	13.5	3.8
	Total	153	39.8	6.6	0.0	2.6	5.2	8.5	10.5	17.0	2.6
Formalin 3	Immature	131	33.5	6.2	0.0	0.8	6.9	13.0	7.6	9.9	5.3
	Mature	41	29.5	12.9	0.0	2.4	4.9	9.8	9.8	43.9	19.5
	Total	172	32.4	7.8	0.0	1.2	6.4	12.2	8.1	18.0	8.7
Formalin TOTAL	Immature	397	33.4	5.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mature	133	29.4	8.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Total	530	32.4	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrogen 1	Immature	146	29.4	2.9	1.4	5.5	8.2	4.8	0.7	0.0	0.0
	Mature	26	26.9	4.9	0.0	7.7	15.4	0.0	11.5	0.0	0.0
	Total	172	29.1	3.2	1.2	5.8	9.3	4.1	2.3	0.0	0.0
Nitrogen 2	Immature	30	20.0	2.9	0.0	16.7	3.3	0.0	0.0	0.0	0.0
	Mature	17	17.7	3.4	0.0	11.8	11.8	0.0	0.0	0.0	0.0
	Total	47	19.2	3.0	0.0	14.9	6.4	0.0	0.0	0.0	0.0
Nitrogen 3	Immature	80	11.3	2.1	0.0	6.3	3.8	3.8	1.3	0.0	0.0
	Mature	45	11.1	1.9	0.0	8.9	2.2	2.2	0.0	0.0	0.0
	Total	125	11.2	2.0	0.0	7.2	3.2	3.2	0.8	0.0	0.0
Nitrogen TOTAL	Immature	256	22.6	2.7	0.8	7.0	6.3	3.9	0.8	0.0	0.0
	Mature	88	17.0	3.1	0.0	9.1	8.0	1.1	3.4	0.0	0.0
	Total	344	21.2	2.8	0.6	7.6	6.7	3.2	1.5	0.0	0.0

tions on mature versus immature mysids placed in the incubators.

Pellet egestion rates seem to be affected by population structure. Values for the immature incubator (1.71) were around 3/4 the value obtained for the mature incubator (2.58).

In contrast, the frequency of fragmented pellets, which was always around 33 %, is not affected by population structure.

On gut fullness

In non-incubated samples, immature classes showed a higher percentage of full individuals than mature classes. Conversely, the degree of fullness is higher in mature classes than in immature ones (Table 4). Nevertheless, differences in both the percentage of full mysids ($X^2 = 0.61 < 3.84$; $p \leq 0.05$) and the degree of fullness ($X^2 = 3.6 < 3.84$; $p \leq 0.05$) are not significant. On the other hand, immature and mature mysids had a similar pattern of distribution of full segments : fullness increasing from the first to the fifth segment and then decreasing in the last one (Fig. 3). In the first four segments, the degree of fullness was almost the same for both mature and immature mysids, the differences appearing in the last two segments where mature mysids have higher values of fullness than immature ones.

Time effect: Daily rhythms

On pellet deposition

The dependence of *H. speluncola* egesta on the time of day is clearly reflected in Fig. 4. This dependence suggests metabolic rhythms which could be controlled by a solar clock. After a short initial increase (0-2 to 2-4 h after sunrise) fecal pellet deposition rates decreased with time, first steeply (4-6 to 6-8 h after sunrise) and then very slowly, reaching values nearly zero. The maximum rate is found 2-4 h after sunrise, and, in this interval, about 38% of the pellets egested daily were produced. By integration of all rates obtained throughout the time that mysids re-

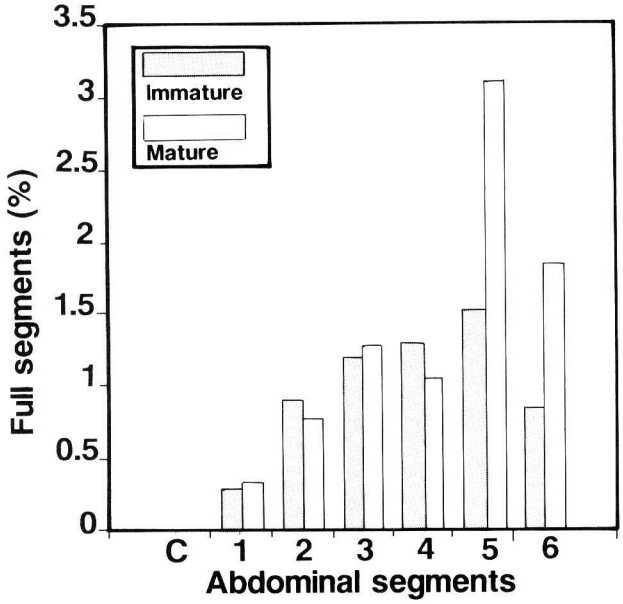

FIG. 3. — Effect of population structure on gut fullness of *Hemimysis speluncola*. Distribution of gut contents among the different abdominal segments in mature and immature non-incubated mysids.

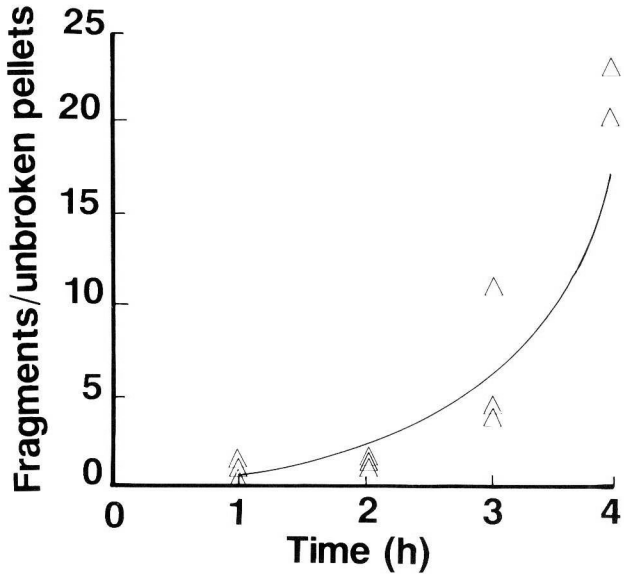

FIG. 5. — *Hemimysis speluncola*. Time course of the fragmented pellets to entire pellets ratio (13-10-88); regression: $Y = 0.21*e^{1.1*x}$, $r = 0.94$, $P < 0.001$. Temperature: 19° C.

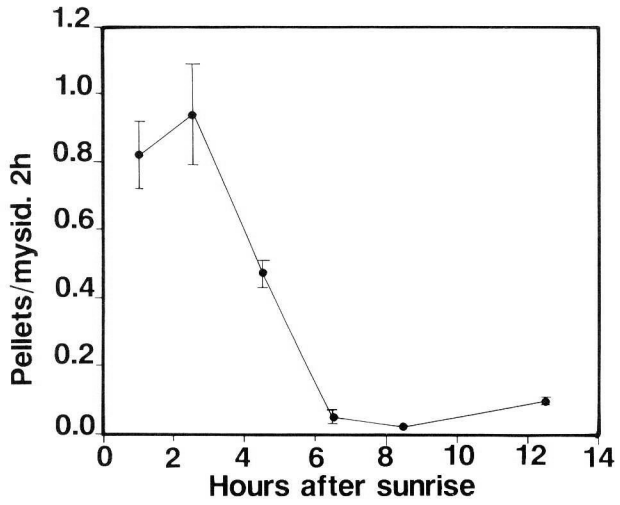

FIG. 4. — Egestion rates of *Hemimysis speluncola* as a function of time of day. Results of a series of 2 hours-incubations carried out every 2 h during the period mysids remain into the cave (7-7-88). Each point represents the mean value (± SD) obtained from five replicates with several hundred individuals each one.

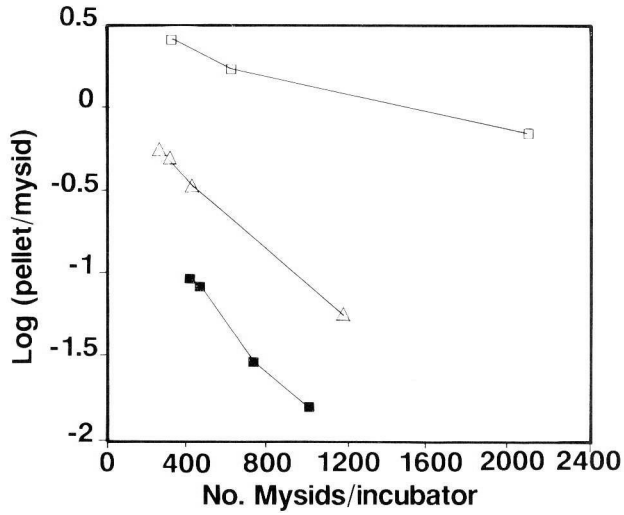

FIG. 6. — Effect of the density of mysids on the pellet egestion rates of *Hemimysis speluncola* at three different incubation times: 8-10 h during June 6th 1991 (open squares); 9-11 h during July 7th 1988 (filled triangles); and 11-13 h during July 7th 1988 (filled squares).

main in the cave, an average egesta of around 2.4 pellets per mysid a day has been estimated.

On fragmentation of pellets

The number of fragmented pellets in incubations from which mysids have been removed, increased exponentially with incubation time. So, if the fragmented/unbroken pellets ratio is plotted against time, a fairly precise exponential regression is obtained ($r \pm 0.94$, $p < 0.001$) (Fig. 5).

From the biometric results a conversion factor of 3 fragments to 1 entire pellet can be deduced (Table 2). Then a 1:1 ratio indicates that 75 % of fecal pellets still remained unbroken; a 2:1 ratio is equivalent to 60 % and a 3:1 ratio to 50 %. Then the time spent until the 3:1 ratio is reached corresponded to the half life of fecal pellets egested into the collector. As this process could be temperature-dependent, a minimal half-life of about 2.5 h (July, 22° C) could be retained as a reference value.

The effect of density

Fig. 6 summarizes the results of the first experi-

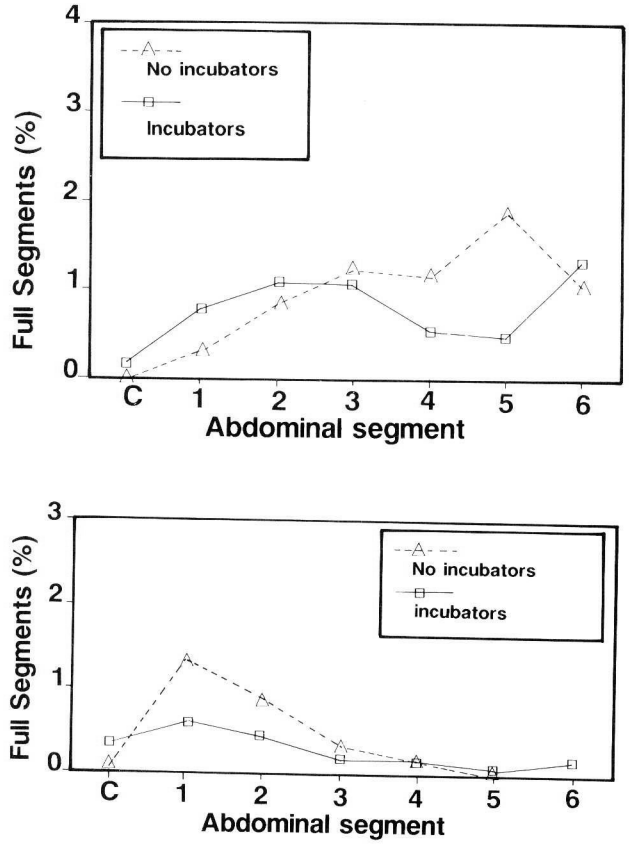

FIG. 7. — Effect of incubation on gut fullness of *Hemimysis speluncola*. Distribution of gut contents among the different abdominal segments in incubated and non-incubated mysids.(A) Formaline fixed samples. (B) Liquid nitrogen fixed samples. Note that the *y*-axis corresponds to an scale of only 0-5 %, as a evidence of the weak effect of the fixation treatment.

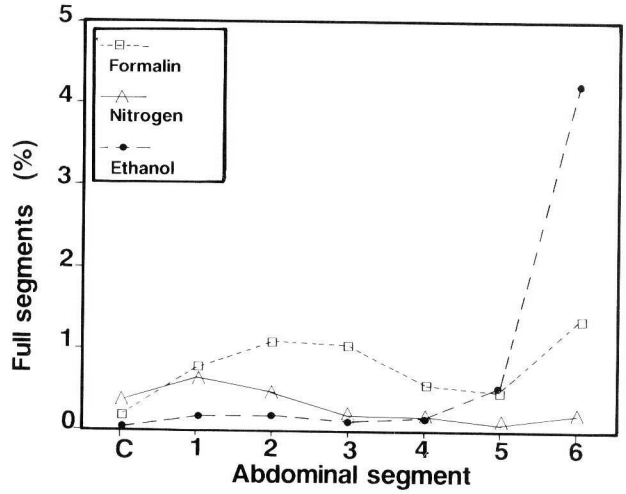

FIG. 8. — Effect of fixatives on gut fullness of *Hemimysis speluncola*. Distribution of gut contents among the different abdominal segments in mysids fixed with different fixators: formaline, ethanol and liquid nitrogen. Note that the y-axis corresponds to an scale of only 0-5 %, as a evidence of the weak effect of the fixation treatment.

ment on the effect of density. As suggested by these results, measured egesta seem to be dependent on mysid density, the egestion rates decreasing with the increasing number of mysids in incubators (note that only data from the same experiment can be considered together because of the effect of time of day on egestion rates; see above).

No clear trend arises from the second experiment: despite the strong differences in density between the two samples — which, on the other hand were very similar in their demographic structure— the egestion rates were only slightly different (Table 3; columns 1 and 2).

The effect of incubators

The effect of incubators on gut fullness is not significant, even early in the morning, when the highest changes in fullness occur. Between 8.00 and 10.00, when the number of full mysids drops from 77.2 % to 33.1 %, differences between incubated and non-incubated mysids were just 7 %. Further, differences in the degree of gut fullness were not significant ($X^2 = 2.77 > 3.84$; $p \leq 0.05$) (Tables 4,5). Nevertheless a slight shift (around 1 % of total egesta) in the pattern of distribution of gut contents arises when incubated and non-incubated mysids were compared. There is an interaction between the incubators and the fixative effects (see below): while in formaline-fixed samples differences are mainly at the fourth and fifth abdominal segments (Fig. 7a), in liquid nitrogen-fixed samples differences were higher in the first and second segments (Fig. 7b).

The effect of fixatives

The samples which were fixed with liquid nitrogen always presented the lowest values in percentage of full mysids (7.5 %), gut fullness (2 %) and pellet egestion rates (Tables 4,5). There is a paradox in these results, because if incubators are closed volumes it is not possible to obtain the lowest values on gut fullness at the same time as the lowest values on pellet deposition rates. Nevertheless, the effect of liquid nitrogen on the distribution of contents throughout the gut should be cautiously interpreted due to the extremely low values registered in liquid nitrogen-fixed mysids (Fig. 7b). The same pattern of distribution of contents was obtained for both non-incubated and incubated mysids (Fig. 7b).

Gut fullness of samples fixed with ethanol and formalin were similar ($X^2 = 0.02 < 3.84$; $p \leq 0.05$).

TABLE 5. — Raw values (%) of gut fullness in *Hemimysis speluncola* incubated individuals. Effect of population structure and fixatives on: full guts (column 2), full segments (column 3), and distribution of contents among the abdominal segments (columns 4 to 10). Percentages have been calculated for each segment.

		Mysids	% Full mysids	% Full segments	% Full segments						
					C	1	2	3	4	5	T
Formalin 1	Immature	544	17.8	3.4	0.0	3.7	4.2	5.7	2.8	1.1	6.4
	Mature	35	31.4	5.3	0.0	2.9	14.3	5.7	0.0	0.0	14.3
	Total	579	18.6	3.5	0.0	3.6	4.8	5.7	2.6	1.0	6.9
Formalin 2	Immature	291	24.4	5.3	0.0	3.1	8.9	8.2	2.7	4.1	10.0
	Mature	25	40.0	9.7	0.0	12.0	4.0	8.0	12.0	16.0	16.0
	Total	316	25.6	5.7	0.0	3.8	8.5	8.2	3.5	5.1	10.4
Formalin 3	Immature	751	32.1	6.9	4.1	9.3	10.3	7.3	4.1	3.2	10.0
	Mature	107	34.6	7.9	0.9	1.9	2.8	12.1	14.0	8.4	15.0
	Total	858	32.4	7.0	3.7	8.4	9.3	7.9	5.4	3.8	10.6
Formalin TOTAL	Immature	1586	25.8	5.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mature	167	34.7	7.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Total	1753	26.6	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrogen 1	Immature	443	11.3	2.6	5.4	6.3	3.4	0.9	0.5	0.5	1.1
	Mature	65	21.5	4.2	7.7	6.2	3.1	1.5	0.0	1.5	9.2
	Total	508	12.6	2.8	5.7	6.3	3.3	1.0	0.4	0.6	2.2
Nitrogen 2	Immature	283	3.5	1.7	1.4	4.2	2.8	0.7	1.1	0.4	1.1
	Mature	40	7.5	2.1	2.5	7.5	5.0	0.0	0.0	0.0	0.0
	Total	323	4.0	1.7	1.5	4.6	3.1	0.6	0.9	0.3	0.9
Nitrogen 3	Immature	489	4.7	1.5	0.0	2.0	2.4	2.2	2.7	0.2	0.6
	Mature	71	5.6	1.2	0.0	4.2	2.8	1.4	0.0	0.0	0.0
	Total	560	4.8	1.4	0.0	2.3	2.5	2.1	2.3	0.2	0.5
Nitrogen TOTAL	Immature	1215	6.8	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mature	176	11.9	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Total	1391	7.5	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ethanol 1	Immature	521	38.2	6.1	0.0	1.0	1.5	0.8	0.4	3.3	35.7
	Mature	69	39.1	6.0	0.0	2.9	0.0	0.0	0.0	8.7	30.4
	Total	590	38.3	6.1	0.0	1.2	1.4	0.7	0.3	3.9	35.1
Ethanol 2	Immature	488	27.5	4.6	0.4	1.2	1.2	0.4	1.2	3.1	24.8
	Mature	111	25.2	5.1	0.9	0.9	0.9	1.8	3.6	7.2	20.7
	Total	599	27.0	4.7	0.5	1.2	1.2	0.7	1.7	3.8	24.0
Ethanol TOTAL	Immature	1009	33.0	5.4	0.2	1.1	1.4	0.6	0.8	3.2	30.4
	Mature	180	30.5	5.5	0.6	1.7	0.6	1.1	2.2	7.8	24.4
	Total	1189	32.6	5.4	0.3	1.2	1.3	0.7	1.0	3.9	29.5

Due to the high variability between replicates, the significance of the differences in the percentage of full mysids could not be evaluated. Some differences were observed in the distribution of the gut contents: ethanol seems to cause a shift in gut contents to the last abdominal segment (increase in a 4 % of total egesta)(Fig. 8). Nevertheless, this result could be an artifact caused by the enhanced opacity of mysid carapaces after fixation with ethanol. Formalin produced a nearly uniform distribution of contents inside the gut.

DISCUSSION

Length and diameter of pellets egested by *H.speluncola* are extremely variable parameters which preclude easy visual sorting into simple classes. This makes counting of pellets a time-consuming procedure, which resists automation by Image Analyzers because there is detritus of similar size and shape. Nevertheless, theoretical considerations based on biometric comparisons of both mysid gut and pellets suggest a good agreement between results coming from the two approaches followed in this work: pellet deposition rates and gut fullness.

Summarizing, on a broad average, no more than 3 standard pellets can be carried by a standard mysid in a daily migratory movement; probably, 0.5 are released before mysids reach the inner parts of the cave where only 2.5 pellets would be deposited.

When all samples were mixed, results on both pellet deposition rates and gut fullness showed a high variability which suggests the existence of powerful factors affecting egesta of *Hemimysis speluncola*. The effects of these factors must be properly controlled if an accurate estimation of egesta is the goal.

Rhythms

Quantitatively, the first factor of variability is the metabolic rhythm which introduces a strong asymmetry in the distribution of both pellet deposition rates and gut fullness along the day. Pellet deposition rate varies with time of day by up to two orders of magnitude (0.02 to 2 pellets per mysid in two hours). A strongly fixed daily rhythm of fecal pellet deposition is the only predictable pattern found in the extremely variable egestion process of *H. speluncola*.

Density

Density of mysids in incubators seems to be the second main factor affecting pellet deposition rates. This is fairly clear when pellet deposition rates coming from incubations which have been carried out at the same season and time of day, and filled with a population of similar structure but different density, are compared. As shown in Fig. 6, pellet deposition rates drop by one or more orders of magnitude when the number of mysids is increased by an equivalent factor. So, incubations conducted in high mysid density conditions ($10^2 - 10^3$ ind.l^{-1}) can result in severe underestimations of pellet deposition rates (note that mysid swarms in natural conditions are at same or higher densities). Gut fullness seems to be a less dependent variable than pellet production on density of mysid (in incubators), this disagreement suggesting that pellet loss with increasing density occurs after egestion, and that mysids actively contribute to eliminate egested pellets.

Coprophagy or coprorhexia

Could coprophagy explain this decrease on the mysid egestion rates with increasing mysid density?

Bearing in mind that both stomachs and segments placed just after the stomachs of incubated mysids were generally empty, it can be concluded that coprophagy is not a trait of the *H.speluncola* trophic behaviour.

Moreover, gut fullness from experiment 1 was examined to answer this question (Table 3). If the pellet egestion rate deduced from the incubators with the lowest mysid density (2.6 pellet per mysid × 2h) is applied to the 590 mysids incubated at intermediate density, then not less than 1494 pellets should be found into the incubator; but only 991 were counted and hence, 503 were missing. The examination of guts from these 590 mysids revealed that only 145 segments were full, that is (by converting by the ratio: 2 segments = 1.2 pellet; see Tables 1,2 for measurements) not more than about 87 pellets could be justified in this way. Even if we assume all these contents to be the result of coprophagy, this behaviour would explain only 17 % of the missing pellets. Hence, the remaining 83 % must be explained by other causes.

The second hypothesis tests the posibility that pellets were broken in fragments by feeding activity of mysids. This action would lead to an increase of the fragmented pellet ratio with the density of mysids. Pellets from experiment 5 were examined to test this hypothesis (Table 3) and a nearly constant ratio, around 0.33, was obtained irrespective of the density of mysids.

If pellets were neither ingested nor fragmented, the loss of pellets could be explained by coprorhexy, an alternative process which has been recently described from copepods (LAMPITT *et al*, 1990). Coprorhexy consists in breaking down the fecal pellets while ingesting only a small proportion, mainly the perithrophic membrane which ensures the compactness of the pellet. Before the complete breakdown of the pellet, there is an intermediate step where pellets increase in diameter without changing length. This expansion could explain the differences obtained between biometrical parameters of pellets and mysid guts. Furthermore, this change in pellet morphology caused by mysid activity furnishes a satisfactory explanation for the ability of immature mysids to produce thick pellets, which are conspicuously higher in diameter than the guts. On the other hand, it is a congruent possibility that coprorhexy was able to decompose thin pellets faster than thick ones. This would give a satisfactory explanation for the lower pellet egestion rates obtained for immature mysids (see below).

Despite the absence of direct data, these results suggest the presence of coprorhexia in *H. speluncola*.

Effect of population structure

There is an apparent paradox when results on both pellet deposition rates and gut fullness of immature mysids are compared with those of mature ones (Tables 4,5). Immature mysids have significantly lower gut contents than mature mysids but they also have lower pellet deposition rates. Bearing in mind both that at the beginning of the experiments the gut fullness of both classes are similar and that incubators are closed volumes from which pellets cannot be missed, the only plausible explanation are faster decomposition rates of thin pellets (which are only produced by immature mysids) compared to thick ones.

If this is the case, the strong effect of demographic structure on egesta would be, in part, an artifact. This would lead to underestimation of numbers of pellets produced. Nevertheless, differences in size and weight of pellets produced by the two classes will enhance the effect on the total amount of egesta, which will be higher on the weight than on the number of pellets.

In any case, the effect of population structure on egesta should be considered moderate in comparison with the effects caused by the time of day and density: the pellet deposition rates of a population dominated by mature mysids only double those of immature ones (Table 3).

Incubators

In contrast with the importance of the effects described above, the effect of incubators on the measured egesta is neglegible. Differences between incubated and non-incubated mysids oscillate around 10 % in gut fullness and 20 % in the number of full mysids (note that pellet deposition rates cannot be estimated for non-incubated mysids).

Fixatives

Results from samples fixed with liquid nitrogen are quite different from samples fixed with both formalin or ethanol solutions. The lowest number of full mysids and gut fullness are found with this fixative. This is an unsolved paradox because these are also the samples in which lower pellet deposition rates are found. At present we are unable to explain the destination of the missing pellets.

There are not significant differences for two of the three variables related to gut contents —percentage of full mysids and gut fullness— when formalin and ethanol are used as fixatives. Nevertheless, there is a slightly different pattern of distribution of the gut content along the abdominal segments, these differences suggesting a different physiological reaction of mysids to each fixative. The opacity of mysid carapaces, which makes difficult both the evaluation of fullness and distribution of contents, is highly influenced by ethanol.

Formalin solution is chosen as the best fixative because it produces the best transparency level on fixed mysids.

The method of pellet collection has been succesfully tested in a closed site (a cave) inhabited by a dense, mono-specific population. Although this is a simpler system compared to open water, it may be safely assumed that pellet collection can be proposed as a useful method to obtain estimates of the egesta of gregarious, densely-packed crustaceans. We are not judging the applicability of the method to open waters, where mixed populations and migrational patterns can severely limit its usefulness, but there is a strong presumption that it can be applied, at least, to other epibenthic littoral crustaceans which may be playing an unexpectedly important role in the carbon cycling of benthic populations.

ACKNOWLEDGEMENTS

We would like to thank our colleagues at the Department of Ecology, University of Barcelona, for their help during this study, and particularly A. García, C. San Vicente and P.Maluquer; to authorities from the "Direcció General de Pesca" of the "Generalitat de Catalunya" for giving us permission and facilities to work in the Medas Islands Marine Reserve; to Mr. R. Algarra, the guard of the reserve, and Mr. J.M. Llenas for their help in our field work. P. Peiró and his colleagues at the "Servei de Microscopia Electrònica" for offering us all their expertise in Image Analyser System (IBAS) surveys. We are also grateful to Joan-Domènec Ros and two anonymous reviewers for their help in improving this manuscript. This work has been supported by a grant from "La Caixa de Barcelona".

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