

Seasonal variability in the quantitative structure of the digestive tubules of *Littorina littorea*

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Abstract

Seasonal variability in the morphology of the digestive tubules was investigated by means of a morphometric procedure in a population of *Littorina littorea* (Mollusca, Gastropoda) collected from the Abra estuary (Bay of Biscay, Basque Country) at four different seasons (September, December, May and July). Some biometric characteristics of the winkles were recorded throughout the study period. Size parameters did not change significantly but high values of flesh condition (wet weight of flesh in mg per wet shell weight in g) were recorded in December and the lowest values in May. The reproductive state was determined using a planimetric procedure to calculate the relative tissue volume of digestive gland, gonad and storage tissue in the visceral mass. The relative volume of the digestive gland was significantly reduced in May. Gonad tissue volumes were maximal in May and minimal in September. Reserve connective tissue reached a maximum value in September. The quantitative structure of the digestive tubules was also investigated. The following five parameters were used to quantify digestive tubule morphology: mean epithelial thickness (MET), mean diverticular radius (MDR), mean luminal radius (MLR), MLR/MET ratio and MET/MDR ratio. The seasonal changes in the tissue composition of the visceral mass do not affect significantly any of these parameters. Thus, reserve storage/consumption and gamete development did not appear to affect the morphology of the digestive tubules in winkles. Reduced MET and MET/MDR values and augmented MLR/MET values were, however, observed at the breeding-spawning period. Such changes resemble a general stress response which might be related to the high metabolic cost of this phase of the reproductive cycle.

Keywords: *Littorina littorea*, morphometry, seasonal variability, digestive tubules.

Variations saisonnières de la structure quantitative des tubules digestifs de Littorina littorea.

Résumé

La variabilité saisonnière de la morphologie des tubules digestifs a été examinée au moyen d'une procédure morphométrique sur une population de *Littorina littorea* (Mollusque Gastéropode) récoltés dans l'estuaire de l'Abra (Pays Basque) à quatre saisons différentes (septembre, décembre, mai et juillet). Quelques caractéristiques biométriques des bigorneaux ont été enregistrées durant la période d'étude. Six paramètres n'ont pas changé de façon significative mais de fortes valeurs de condition de la chair (poids de chair humide en mg/poids de la coquille humide en g) ont été enregistrées en décembre et les valeurs les plus basses en mai. L'état reproducteur a été déterminé au moyen d'une procédure planimétrique pour calculer le volume relatif de tissu de la glande digestive, de la gonade, et des tissus de réserve de la masse viscérale. Le volume relatif de la glande digestive était significativement réduit en mai. Le volume de la gonade est maximal en mai et minimal en septembre. Le tissu conjonctif de réserve atteint une valeur maximum en septembre. La structure quantitative des tubules digestifs a été examinée. Les 5 paramètres suivants ont été utilisés pour quantifier la morphologie des tubules digestifs : l'épaisseur épithéliale moyenne (MET), le rayon diverticulaire moyen (MDR), le

rayon de la lumière moyen, le rapport MLR/MET et le rapport MET/MDR. Les changements saisonniers dans la composition du tissu de la masse viscérale n'affectent pas significativement ces paramètres. Ainsi, les réserves/consommation et le développement des gamètes ne semblaient pas affecter la morphologie des tubules digestifs chez les bigorneaux. Cependant, lors de la saison de reproduction, on observe une MET et un rapport MET/MDR réduits, et des valeurs du rapport MLR/MET en augmentation. De tels changements se rapprochent d'une réponse générale de stress qui pourrait être liée au coût important du métabolisme de cette phase du cycle reproducteur.

Mots-clés : *Littorina littorea*, morphométrie, variabilité saisonnière, tubules digestifs.

INTRODUCTION

The cellular medium is quickly affected by changes in the external environment (Storch, 1987). Since the natural conditions are very variable, these variations might be reflected in the cell (Thompson *et al.*, 1974; Thomson *et al.*, 1978; Morton, 1983; Storch, 1987; Marigómez *et al.*, 1990; Marigómez *et al.*, 1991). In addition, it is known that the cell may respond to the presence of pollutants in the environment. The response of an organism to pollutants at this level of biological organization is prompt, sensitive and easily measurable (Sastry and Miller, 1981). Interestingly, some recent investigations on molluscs have outlined the relevance of cellular indices of stress when assessing the hazard of environmental pollutants (Lowe *et al.*, 1981; Bayne *et al.*, 1982; Tripp *et al.*, 1984; Marigómez *et al.*, 1986; Recio *et al.*, 1988).

The digestive cells of marine molluscs are target cells capable of indicating stress and environmental pollution (Lowe *et al.*, 1981; Vega *et al.*, 1989). Environmental factors relevant to the shore and in estuaries (salinity, nutrient levels, tide or photoperiod) may affect digestive cell structure (Marigómez *et al.*, 1990; Marigómez *et al.*, 1991). Variable life cycle factors such as age, reproductive stage, and health state might be also responsible for a high variability in the morphology of the cells in the digestive gland of marine molluscs. The present investigation deals with the seasonal changes in the quantitative structure of the digestive cells which comprise the digestive tubules. These changes might affect the reliability and the significance of some histological parameters in environmental stress studies.

The tubules of the digestive gland extend into the visceral haemocoel and are bathed by haemolymph. Only a layer of connective tissue separates the digestive cells from the haemolymph. A few muscle fibres are normally present around them. Cells containing glycogen may be found in the connective tissue. The amount of material which is stored here depends not only on such obvious factors as the degree of starvation of the animal but also on the season and the reproductive stage (Fretter and Graham, 1962). Linke (1934) showed that in *Littorina littorea* the bulk of the visceral hump consists of digestive gland and

gonad during the breeding season with a minimal amount of connective tissue containing reserve food separating the tubules of these two organs. During the intervening resting period, however, much of the reproductive system is broken down and the visceral hump is primarily made up of digestive gland and connective tissue. Martel *et al.*, (1986) also found variations in the digestive gland index of the prosobranch *Buccinum undatum* in relation to gametogenesis and sex.

The aim of this study is to elucidate if these changes in the tissue composition of the visceral mass and if the different functions of the digestive gland at different periods of the reproductive cycle are expressed in the histological structure of the digestive tubules.

MATERIAL AND METHODS

Individuals of *Littorina littorea* were collected at extreme low tides from the Abra estuary (Zierbena, Bizkaia) in September, December, May and July (12+12+12+12=48 individuals). All specimens belonged to the same population since they were collected from a restricted rocky area which emerges monthly when extreme low tides occur. Some size parameters (Moore, 1937) and the flesh condition index (Lobel and Wright, 1982) were measured. The calculation of the Flesh Condition (FC, Soft body wet weight in mg/shell dry weight in g) was performed on the basis of wet weight values because the digestive gland/gonad complex had to be further excised and processed for histological examination. After excision, the tissue was fixed in Bouin's liquid (Martoja and Martoja-Pierson, 1970), paraffin embedded, sectioned at 7 µm and stained with haematoxylin-eosin (Pearse, 1976). The reproductive stage at each sampling was calculated by a planimetric procedure on 7 µm-thick paraffin-sections of the digestive gland/gonad complex on the basis of the method of Morvan and Ansell (1988) and of the peculiarities of the visceral mass of *L. littorea* described by Linke (1934). The procedure is illustrated in figure 1.

In order to quantify the morphology of the digestive tubules a planimetric procedure based on the

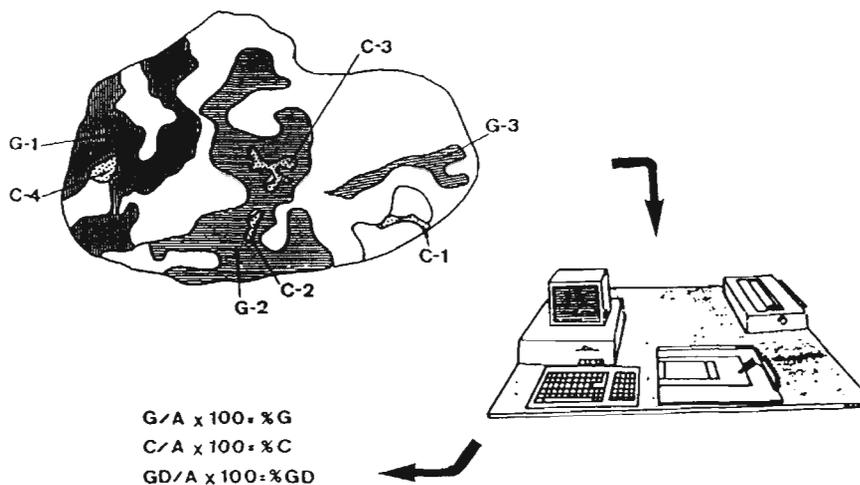


Figure 1. — Diagrammatical schemes of the two sections (approx. 500 μ m distant) measured in each specimen were drawn with the aid of a slide-projector. The total area covered by the section (A), the area covered by gonad (G) and the area covered by the connective tissue (C) were calculated, after digitizing their profiles, by an Olivetti M240 personal computer. The area covered by the digestive gland was calculated as follows: $GD = A - C - G$. Thus, the relative percentages of each tissue occupying the visceral mass were calculated as $GD \times 100/A$, $G \times 100/A$ and $C \times 100/A$.

geometrical transformation of a tubule section into a regular geometrical figure (an hypothetical trapezium) was applied (Vega *et al.*, 1989; Soto *et al.*, 1990). Six tubule sections were randomly selected in each of 5 sections of the digestive gland/gonad complex (60 μ m distant one from each other). Thus, 30 tubule sections were drawn per winkle, with the aid of a drawing-tube attachment on a Nikon Optiphot microscope. Section profiles were recorded by means of a Watanabe DT1000 digitizer and the planimetric measures were calculated by an Olivetti M240 personal computer. Five parameters were obtained: Mean Epithelial Thickness (MET), Mean Diverticular Radius (MDR), Mean Luminal Radius (MLR), MET/MDR ratio and MLR/MET ratio (Vega *et al.*, 1989; Soto *et al.*, 1990). The planimetric parameters were tested using a one-way ANOVA.

RESULTS

The biometric characteristics of the population of winkles collected at Zierbena are summarized in table 1. There was a low variability in the average wet weights throughout the year in this population, the columellar height being constant. However, the Flesh Condition (FC) increased from May to December (table 1). Accordingly, the relative tissue volume calculated by means of planimetry indicates that gametogenesis began in autumn, when the Flesh Condition is higher, and that breeding/spawning period occurred predominantly between May and July (fig. 2).

The four sampling periods represent four clearly distinct stages of the annual cycle of winkles. In September, gonad tissue was almost absent, storage tissue was abundant and the winkles exhibited an intermediate FC. In December, gonad tissue had increased and the specimens showed a good condition. In May, gonad tissue comprised 25% of the visceral mass and a minimum FC value was recorded. In July, the gonad tissue occupied less than 5% of the visceral mass and the FC values appeared slightly recovered. Despite these differences between seasons, the morphology of the digestive tubules exhibited no significant variations. MDR and MLR values were shown not to be significantly different ($p > 0.05$) in relation to the annual reproductive cycle (table 2). In contrast, there was significant reduction in the MET during the breeding season (May-July) as evidenced by analysis of variance ($p < 0.05$, table 2). Such reduction caused a concomitant increase in the MLR/MET ratio and decrease in the MET/MDR ratio ($p < 0.05$, table 2).

DISCUSSION

The annual reproductive cycle of *Littorina littorea* from Zierbena (Abra estuary, Bizkaia) is similar to those reported in various populations from the British shore (Moore, 1937; Fretter and Graham, 1962). Thus, gonadal tissue is almost absent in September and reaches maximum volumes in spring when the increase in sea water temperature over a threshold

Table 1. — Biometric parameters (mean value \pm standard deviation) of *Littorina littorea* collected at Zierbena (Bizkaia) in September, December, May and July. P.S.W.: maximum shell width perpendicular to the columellar axis; M.S.W.: maximum shell width ($n=12$).

	September	December	May	July
Columellar height (mm)	16.00 \pm 1.19	15.41 \pm 1.06	16.46 \pm 4.03	15.92 \pm 0.73
P.S.W. (mm)	11.25 \pm 1.14	10.12 \pm 0.61	11.54 \pm 2.21	11.33 \pm 0.49
M.S.W. (mm)	14.25 \pm 1.32	11.83 \pm 0.72	13.67 \pm 2.84	14.08 \pm 0.06
Flesh wet weight (mg)	587.42 \pm 369.56	450.08 \pm 92.22	483.04 \pm 280.89	402.67 \pm 51.26
Shell wet weight (g)	0.94 \pm 0.28	0.80 \pm 0.19	1.16 \pm 0.69	0.85 \pm 0.11
Flesh condition	495.45 \pm 88.68	560.25 \pm 79.81	432.63 \pm 117.04	472.88 \pm 42.05

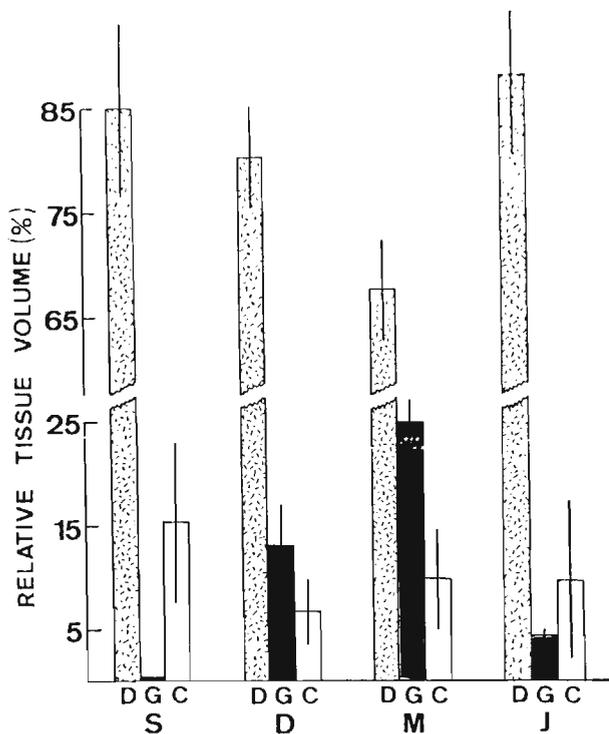


Figure 2. Different relative tissue volumes (D, digestive gland; G, gonad; C, connective tissue) calculated by a planimetric procedure for the winkles collected at Zierbena in four sampling months (S, September; D, December; M, May; J, July). Fine bars illustrate the standard deviations ($n=12$).

value might provoke breeding and spawning (Martel *et al.*, 1986). The requirement to initiate gametogenesis might be a high critical value of FC which occurs between September and December. The relative volume occupied by the connective tissue that substitutes the degenerative gonadal follicles increases during the summer, the highest value being recorded in September. In mussels, stereological analysis of the mantle tissues from individuals in early gametogenesis showed that, at this time, the volumes of nutrient storage cells were high, as would be anticipated of mussels entering a period of gamete production (Lowe *et al.*, 1982, Lowe and Pipe, 1987). In mussels, whose gametogenesis occurs in summer, the storage cell

Table 2. — One-way ANOVA tables for the five parameters (MET, MDR, MLR, MLR/MET and MET/MDR) used to quantify the morphology of the digestive tubules in *Littorina littorea* collected at Zierbena (Bizkaia) at different seasons. MET, Mean Epithelial Thickness; MDR, Mean Diverticular Radius; MLR, Mean Luminal Radius.

Source	d.f.	Sums of squares	Mean squares	F ratio
(MET)				
Between seasons	3	614.635	204.878	5.971*
Error	42	1 441.143	34.313	
Total	45	2 055.778		
(MDR)				
Between seasons	3	299.090	99.697	1.784
Error	42	2 346.669	55.873	
Total	45	2 645.759		
(MLR)				
Between seasons	3	66.851	22.284	2.045
Error	42	457.572	10.895	
Total	45	524.423		
(MLR/MET)				
Between seasons	3	917.043	305.681	7.611*
Error	42	1 686.882	40.164	
Total	45	2 603.925		
(MET/MDR)				
Between seasons	3	286.207	95.402	9.867*
Error	42	406.099	9.699	
Total	45	692.306		

(*) Significantly different at $p < 0.05$.

volume decreases during this season (Lowe and Pipe, 1987).

The distinct height of digestive cells may be indicative of digestive epithelium activity (Lowe *et al.*, 1981, Morton, 1983, Robinson, 1983) and of the pathological damage caused by environmental irritants (Lowe *et al.*, 1981; Couch, 1984; Tripp *et al.*, 1984; Marigómez *et al.*, 1986; Recio *et al.*, 1988; Vega *et al.*, 1989; Plana and Le Pennec, 1991; Cajaraville *et al.*, 1992). The MDR and the MLR are absolute estimators of tubule and lumen sizes, respectively. Tubule size may vary within individuals of the same population but

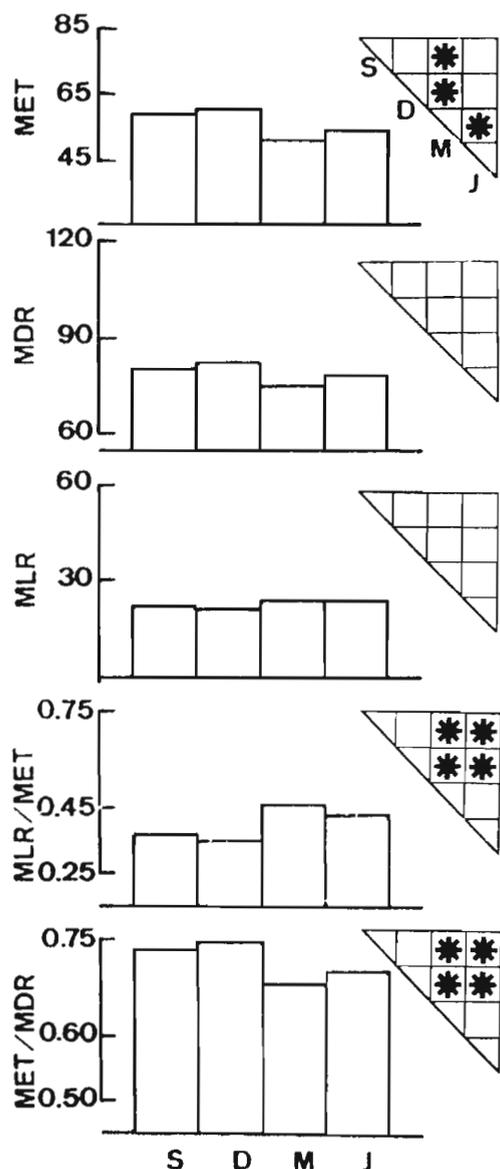


Figure 3. — Results of the planimetric analysis of the digestive tubule morphology for the four groups of winkles studied. Mean epithelial thickness (MET), mean diverticular radius (MDR) and mean luminal radius (MLR) are given in μm . MLR/MET and MET/MDR are relative values without units. Asterisks in the upper triangles indicate pairs of dissimilar groups ($p < 0.05$) according to the Student-Newmann-Keuls test for multiple comparison of mean values (S, September; D, December; M, May; J, July).

not between individuals (Robinson, 1983), or even through the digestive cycle (Morton, 1970). Luminal size may also vary as a result of the digestive stage of the tubules (Langton, 1975; Robinson *et al.*, 1981) or due to the presence of environmental stressors (Lowe *et al.*, 1981; Couch, 1984; Tripp *et al.*, 1984). This complex variability may be checked by a combined index, as proposed by Robinson (1983). The currently used MET/MDR ratio is analogous to the

MPTW (Mean Proportion of Tubule Width) recommended by Robinson (1983) to assess digestion activity in bivalve molluscs.

The variability recorded in the morphology of the digestive tubules does not seem to be related to seasonal changes in the function of the digestive gland depending on the seasonal nutrient source or on spatial relationships with adjoining tissues. The only significant difference is the reduction in MET (May). An extra energy demand has been related to the late reproductive stages in mussels (Lowe and Pipe, 1986). Such demand might lead to the temporary weakening of the population. Thus, a reduced MET might indicate the occurrence of a general stress response associated to increased reproductive costs inherent to breeding and spawning activities. A reduction in digestive epithelium thickness has been described in various instances of environmental stress due to pollutants and interpreted as a non-specific response (Lowe *et al.*, 1981; Couch, 1984; Tripp *et al.*, 1984; Marigómez *et al.*, 1986; Vega *et al.*, 1989; Cajarville *et al.*, 1992). As a result of the reduction in MET, the MLR/MET and MET/MDR ratios show also significant changes at the late breeding season (May and July) in winkles. The changes in both ratios resemble the effects of sublethal concentrations of cadmium described in the same species (Vega *et al.*, 1989) indicating, in agreement with the MET values, the occurrence of a stress response between May and July. These ratios seem to be more sensitive than MET alone, because the stress response observed in July is evident when using MLR/MET and MET/MDR whilst MET only is indicative of the response which occurs in May.

Another aspect related to the annual variability in the digestive tubules must be commented upon. Seasonal trends in the prevalence of atrophic tubules (Couch, 1984) and necrotic tubules (Agirregoikoa, 1988) and for neoplastic diseases (Farley *et al.*, 1986) have been found in various bivalve molluscs. These seasonal trends appear related to the end of the reproductive cycle: in late-winter in *Mya arenaria* (Brousseau, 1987), in September in *Mytilus edulis* (Agirregoikoa, 1988), and in late-winter and late-spring periods in the oyster *Crassostrea virginica* from different estuaries (Couch 1984). Although the reasons still remain unclear (Farley *et al.*, 1986; Brousseau 1987), it is possible that a weakening of the organisms caused by the reproductive effort increases the susceptibility of individuals to environmental pathogens (Agirregoikoa, 1988). The present observations indicate that, although a stress response is evident in *L. littorea* collected from Zierbena during the breeding season, major degenerative changes such as atrophy or necrosis of the digestive tubules or such as neoplastic disorders do not appear to take place subsequently.

In summary, only minor changes occur in the morphology of the digestive tubules of winkles over the year. Nevertheless, the study of the reproductive cycle of sentinel populations is highly recommended in marine biomonitoring programmes based on reliable

environmental stress indicators. Reproduction might affect organism and digestive cell conditions at some critical moments (*i.e.*, breeding/spawning period) which should be clearly stated for each sentinel population.

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REFERENCES

- Agirregoikoa M. G., 1988. *Mytilus edulis* L. bibalbioaren liseri-epitelioaren batezbesteko lodieraren aldakuntz espazio-tenporala Bizkaiko kostaldean. Licenciature thesis, Euskal Herriko Unibertsitatea, Bilbo (Basque Country), 76 p.
- Bayne B. L., J. Widdows, M. N. Moore, P. Salked, C. M. Worral, P. Donkin, 1982. Some ecological consequences of the physiological and biochemical effects of petroleum compounds on marine molluscs. *Phil. Trans. R. Soc.*, **297 B**, 219-239.
- Brousseau D. J., 1987. Seasonal aspects of sarcomatous neoplasia in *Mya arenaria* (soft-shell clam) from Long Island Sound. *J. Invert. Pathol.*, **50**, 269-276.
- Cajaraville M. P., J. A. Marigómez, G. Díez, E. Angulo, 1992. Comparative effects of the water accommodated fraction of three oils on mussels. 2-Quantitative alterations in the structure of the digestive tubules. *Comp. Biochem. Physiol. Ser. C*, **102 C**, 113-123.
- Couch J. A., 1984. Atrophy of diverticular epithelium as an indicator of environmental irritants in the oyster, *Crassostrea virginica*. *Mar. Environ. Res.*, **14**, 525-526.
- Farley C. A., S. V. Otto, C. L. Reinisch, 1986. New occurrence of epizootic sarcoma in Chesapeake Bay soft-shell clams (*Mya arenaria*). *Fish Bull.*, **84**, 851-857.
- Fretter V., A. Graham, 1962. British prosobranch molluscs. Ray Society, London, 755 p.
- Langton R. W., 1975. Synchrony of the digestive diverticula of *Mytilus edulis* L. *J. Mar. Biol. Assoc. U.K.*, **55**, 221-230.
- Linke O., 1934. Beiträge zur Sexualbiologie der Littorinen. *Z. Morphol. Ökol. Tiere*, **28**, 170-177.
- Lobel P. B., P. A. Wright, 1982. Relationship between body zinc concentration and allometric growth measurements in the mussel *Mytilus edulis*. *Mar. Biol.*, **66**, 145-150.
- Lowe D. M., M. N. Moore, B. L. Bayne, 1982. Aspects of gametogenesis in the marine mussel *Mytilus edulis* (L.). *J. Mar. Biol. Assoc. U.K.*, **62**, 133-145.
- Lowe D. M., M. N. Moore, K. R. Clarke, 1981. Effects of oil in the digestive cells in mussels: quantitative alterations in cellular and lysosomal structure. *Aquat. Toxicol.*, **1**, 213-226.
- Lowe D. M., R. K. Pipe, 1986. Hydrocarbon exposure in mussels: a quantitative study of the responses in the reproductive and nutrient storage cell systems. *Aquat. Toxicol.*, **8**, 265-272.
- Lowe D. M., R. K. Pipe, 1987. Mortality and quantitative aspects of storage cell utilization in mussels, *Mytilus edulis*, following exposure to diesel oil hydrocarbons. *Mar. Environ. Res.*, **22**, 243-251.
- Marigómez J. A., E. Angulo, J. Moya, 1986. Copper treatment of the digestive gland of the slug *Arion ater* (L.) 2. Morphometrics and histophysiology. *Bull. Environ. Contam. Toxicol.*, **36**, 608-615.
- Marigómez J. A., V. Saez, M. P. Cajaraville, E. Angulo, 1990. A planimetric study of the Mean Epithelial Thickness (MET) of the digestive gland of molluscs during the tidal cycle. *Helgol. Meeresunters.*, **44**, 81-94.
- Marigómez J. A., M. Soto, E. Angulo, 1991. Responses of winkles digestive cells and their lysosomal system to environmental salinity changes. *Cel. Mol. Biol.*, **37**, 29-39.
- Martel A., D. H. Larrive, K. R. Klein, J. H. Himmelman, 1986. Reproductive cycle and seasonal feeding activity of the neogastropod *Buccinum undatum*. *Mar. Biol.*, **92**, 211-221.
- Martoja M., R. M. Martoja-Pierson, 1970. Técnicas de histología animal., Toray-Masson, Barcelona, 350 p.
- Moore H. B., 1937. The biology of *Littorina littorea*. Part I. Growth of the shell and tissues, spawning, length of life and mortality. *J. Mar. Biol. Assoc. U.K.*, **21**, 721-742.
- Morton B. S., 1970. The tidal rhythm and rhythm of feeding and digestion in *Cardium edule*. *J. Mar. Biol. Assoc. U.K.*, **50**, 499-512.
- Morton B. S., 1983. Feeding and digestion in bivalves. In: The Mollusca, E. Wilburg Ed., Vol. 5, Physiology, Part 2, A.S.M. Salcuddin, A.S.M. ed., Academic Press, New York, 563-586.
- Morvan C., A. D. Ansell, 1988. Stereological methods applied to reproductive cycle of *Tapes rhomboides*. *Mar. Biol.*, **97**, 355-364.
- Plana S., M. Le Pennec, 1991. Altérations de la glande digestive et conséquences nutritionnelles chez la palourde *Ruditapes philippinarum* contaminée par une bactérie du genre *Vibrio*. *Aquat. Living Resour.*, **4**, 255-264.
- Pearse A. G. E., 1976. Histochemistry, theoretical and applied. Churchill Livingstone, Edinburgh, 759 p.

- Recio A., J. A. Marigómez, E. Angulo, J. Moya, 1988. Zinc treatment of the digestive gland of the slug *Arion ater* L. 2. Sublethal effects at the histological level. *Bull. Environ. Contam. Toxicol.*, **41**, 865-871.
- Robinson W. E., 1983. Assessment of bivalve intracellular digestion based on direct measurements. *J. Moll. Stud.*, **49**, 1-8.
- Robinson W. E., M. R. Pennington, R. W. Langton, 1981. Variability of tubule types within the digestive gland of *Mercenaria mercenaria* (L.), *Ostrea edulis* L. and *Mytilus edulis* L. *J. Mar. Biol. Assoc. U.K.*, **54**, 265-276.
- Sastry A. N., D. C. Miller, 1981. Application of biochemical and physiological responses to water quality monitoring. In: Biological monitoring of marine pollutants, F. J. Vernberg, A. Calabrese, F. P. Thurberg, W. B. Vernberg eds., Academic Press, New York, 265-294.
- Soto M., M. G. Agirregoikoa, M. A. Pérez, J. A. Marigómez, 1990. A planimetric study of morphological variability in the digestive diverticula of *Littorina littorea* (Linnaeus) and *Mytilus edulis* Linnacus. *J. Moll. Stud.*, **55**, 339-344.
- Storch V., 1987. Cell and environment: a link between morphology and ecology. In: Biología ambiental (II Congreso Mundial Vasco)-Tomo I. SEUPV-GV, Admon. C. A. Euskadi-Secr. Presid. Gobierno Vasco ed., Vitoria/Gasteiz (Basque Country), 179-190.
- Thompson R. J., C. J. Bayne, M. N. Moore, T. J. Carefoot, 1978. Haemolymph volume, changes in the biochemical composition of the blood, and cytological responses of the digestive cells in *Mytilus californianus* Conrad induced by nutritional, thermal and exposure stress. *J. Comp. Physiol.*, **127**, 287-298.
- Thompson R. J., N. A. Ratcliffe, B. L. Bayne, 1974. Effects of starvation on structure and function in the digestive gland of the mussel (*Mytilus edulis* L.). *J. Mar. Biol. Assoc. U.K.*, **54**, 699-712.
- Tripp M. R., C. R. Fries, M. A. Craven, C. E. Gricr, 1984. Histopathology of *Mercenaria mercenaria* as an indicator of pollutant stress. *Mar. Environ. Res.*, **14**, 521-524.
- Vega M. M., J. A. Marigómez, Angulo E., 1989. Quantitative alterations in the structure of the digestive cell of *Littorina littorea* on exposure to cadmium. *Mar. Biol.*, **103**, 5437-553.