THE COMPOSITION OF PHOSPHATE GRANULES IN THE DIGESTIVE GLANDS OF MARINE PROSOBRANCH GASTROPODS: VARIATION IN RELATION TO TAXONOMY

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ABSTRACT

The composition of some 1150 phosphate granules in the digestive glands of over 40 species of marine prosobranch gastropods has been surveyed using a simple preparation technique and semi-quantitative SEM x-ray microanalysis. Spectral peaks for Mg, K, Ca, Mn, Fe and Zn were compared to that of P. Four major types of phosphate granule can be recognised, each generally characteristic of a taxonomic grouping: high Mg in archaeogastropods and littorinids, multiple metal in higher mesogastropods, and, in neogastropods, Mg-Ca in muricoideans and high Zn in buccinoideans. At least one *Conus* species (*C. ventricosus*) has high-Mg granules. Some causes of variation in granule composition are discussed: speculatively, it is suggested a palaeoenvironmental influence seems possible.

INTRODUCTION

Spherical, intracellular phosphate granules occur in a variety of invertebrates and can be a conspicuous feature of certain tissues. The cations are magnesium and calcium and the mineral form is amorphous (Taylor & Simkiss, 1984; Simkiss, 1994). The granules have a concentric structure and they are each enclosed in a membrane within specialized cells (for reviews see Mason & Nott, 1981; Brown, 1982; George, 1982; Simkiss & Mason, 1983; Mason & Jenkins, 1995). Amorphous minerals lack the regular repeating lattice structure (long range order) associated with crystalline forms and, as a result, they are less stable, more soluble and able to incorporate foreign ions, thus becoming sites of metal accumulation (Taylor, Simkiss & Greaves, 1986; Simkiss, 1994). Phosphate granules found in the digestive glands of marine gastropods have been

shown to exhibit wide variation in their composition. Some conform to the pattern of calcium phosphate with additional magnesium, as in *Hexaplex trunculus* (Nott & Nicolaidou, 1989a), some have magnesium in greater ionic concentration than calcium, as in *Monodonta articulata* (Nott & Nicolaidou, 1989a) and *Littorina littorea* (Simkiss & Mason, 1984; Nott & Langston, 1989), whilst others may be magnesium phosphate with no detectable calcium, as in species of the opisthobranch *Runcina* (Kress, Schmekel & Nott, 1994). The granules can contain an additional metal, such as zinc (Coombs & George, 1978; Ireland, 1979; Mason & Nott, 1981; Nott & Langston, 1993) or manganese (Mason & Simkiss, 1982; Simkiss, Jenkins, McLellan & Wheeler, 1982; Taylor, Simkiss, Greaves, & Harries, 1988) or a mélange of various metals (Mason & Nott, 1980; Mason, Simkiss & Ryan, 1984; Simkiss & Mason, 1984; Nott & Nicolaidou, 1990). Phosphate granules in species tolerant of highly polluted conditions, for example *Cerithium vulgatum*, can contain chromium, manganese, iron, cobalt, nickel, copper and zinc (Nott & Nicolaidou, 1989a–b, 1994). Whilst there is much information on the structure and composition on the phosphate granules of the gastropod digestive gland (hereafter referred to as 'P-granules'), for the most part, the data are limited to relatively few species selected for their potential as bioindicators of metal contamination. Such studies require detailed quantitative analyses; methods are time-consuming (e.g. microincineration of cryosections: Mason & Nott, 1980, 1981) and thus not appropriate for broad surveys. In the present study, a simple method of sample preparation has been employed to provide material suitable for semi-quantitative

evaluation by x-ray microanalysis of P-granule composition over a wide range of species.

Initial studies of the composition of P-granules showed several features; (i) the magnesium-calcium ratio tends to be remarkably uniform in any particular species, regardless of the animal's habitat (Nott, unpublished); (ii) the magnesium/calcium ratio can vary markedly from species to species, from predominantly magnesium to predominantly calcium (Nott & Nicolaidou, 1989a,b); and (iii) there are species in which zinc is consistently the dominant metal in P-granules even in animals taken from habitats without elevated levels of the metal (Nott, unpublished). These observations suggest that P-granule composition may provide a useful character of broad taxonomic groupings. As a test of this idea, the metallic composition of P-granules in over 40 species, collected from a wide range of localities and habitats, has been examined.

MATERIALS AND METHODS

Specimen preparation

Samples of marine gastropods were collected at various sites around southern England, north-west France, south Portugal and south-east Greece,

during the period 1994–96 (see Table 1). All specimens were collected intertidally, mostly between levels of low water neap and spring tides except for several species trawled offshore (marked [T] in Table 1). Identifications of the majority of species were confirmed from the descriptions given in Graham (1988), the remainder (Mediterranean forms) using those given in Parenzan (1970) and D'Angelo & Gargiullo (1978). Within a few hours of collection, small pieces of tissue were taken from the digestive glands of 1–10 individuals of each species from each site according to availability. Tissues were immersed briefly in distilled water to remove excess salt, damp-dried on filter paper, smeared thinly on a scanning electron microscope (SEM) graphite specimen stub and allowed to dry. Samples prepared by this simple 'pâté' technique were examined in a Jeol 35C SEM, operated in backscatter mode using a Robinson detector. The images differentiated the P-granules sharply from the background cytoplasm (Fig. 1A) and these were readily distinguishable from other granule types (e.g. carbonate and sulphide: see Nott & Nicolaidou, 1989a). In some specimens the digestive gland was highly mineralized and P-granules formed the dominant feature of the tissue. In all species, P-granules were perfectly spherical with a maximum diameter of about 5μm.

Apart from its simplicity, use of the pâté technique has the added advantage of conserving the integrity of the P-granule and its constituents. This is in contrast to other methods employing acid extraction to eliminate the associated carbonate granules: this
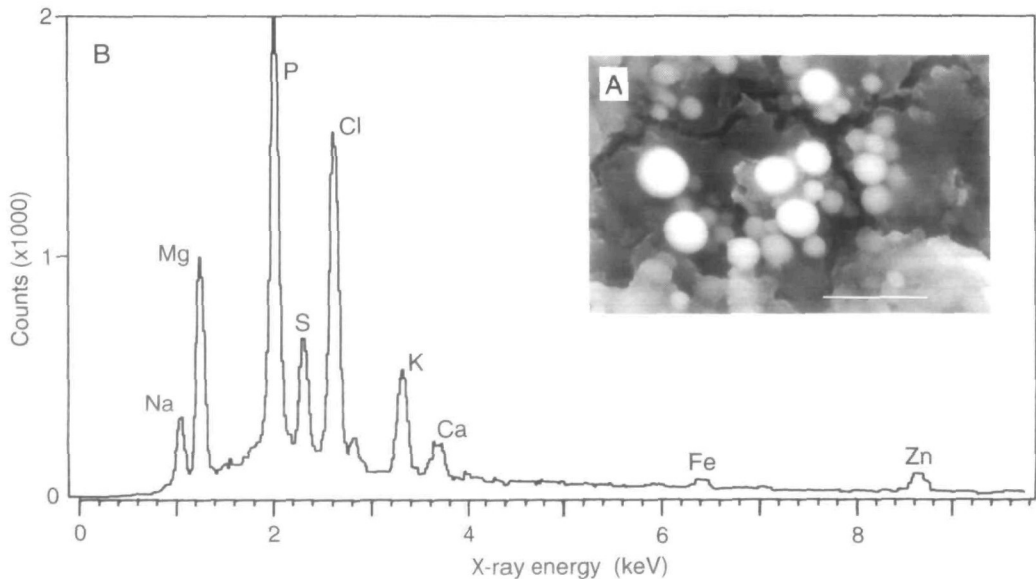

Figure 1. *Melarhaphe neritoides* (Tinside, Plymouth Sound). **A.** Scanning electron micrograph (backscattered image) of a group of P-granules in digestive gland tissue prepared by the 'pâté' method. Scale bar: 10 μm. **B.** X-ray microanalysis spectrum of a P-granule obtained with the beam operating as a stationary spot until the peak for P reached 2000 counts.

procedure affects P-granule composition, notably causing potassium to disappear and also reducing the magnesium and calcium contents.

X-ray microanalysis (XRMA)

The SEM was operated at 25kV and a magnification of x10K, producing images 5 cm in width from granules 5 μm in diameter. P-granules projecting clear of the cytoplasm were selected and were probed centrally with the beam operated as a stationary spot. X-ray spectra were produced with a Link 860 microanalyser. The system counted x-rays until the peak for phosphorus reached full vertical scale at 2000 counts (Fig 1B). For each tissue-specimen, four to ten P-granules were analysed; where possible, six or seven specimens of each species per sampling locality were examined. For some rarer species only a single individual was available. On each spectrum the K_α peak heights were measured and the non-specific background subtracted; mean values and standard deviations were then calculated for each element.

Semi-quantitative XRMA

The XRMA system generates elemental peaks with different degrees of efficiency. When the Jeol 35C SEM is operated at 25kV the efficiency is highest for potassium (3.31keV) and calcium (3.69keV) and it is progressively reduced at lower and higher energies. Elements with an atomic number less than sodium produce low-energy x-rays that cannot be detected. The relative peak efficiencies were calculated by analysing droplets of an isoatomic solution. This solution consisted of 0.768 g $Mg(NO_3)_2.6H_2O$, 0.408 g KH_2PO_4 (in 2 ml dilute HNO_3), 0.300 g $CaCO_3$ (in 2 ml dilute HNO_3), 0.834 g $FeSO_4.7H_2O$ and 0.408 g $ZnCl_2$, all dissolved in 25 ml of distilled water. This mixture is isoatomic for Mg, P, S, Cl, K, Ca, Fe and Zn. About 1 ml of the solution was pipetted into a glass microspray (Agar Scientific Ltd.) and sprayed onto formvar-coated, single-holed copper and titanium grids. The grids were mounted over holes drilled in graphite stubs to reduce the background component of the spectra.

Images of single droplets about 2 μm diameter were viewed with a variable-sized raster which was adjusted to include the entire droplet. Spectra were collected from different droplets and the mean values for K_α peak heights calculated. The peaks for K and Ca were equal highest (Fig. 2A) and the relative heights of the other peaks were used as factors to adjust the peaks in the granule spectra so as to approximate the atomic ratios. With K and Ca at unity, these factors were: Mg – ×3.17; P – ×1.26; Fe – ×1.34 and Zn – ×2.5. The factor for Mn (×1.2) was found by extrapolation. The XRMA peak data were adjusted to give the atomic ratios of Mg, P, K, Ca, Mn, Fe and Zn in P-granules from different species; for example, those obtained for *Melarhaphe neritoides* P-granules (Fig. 1) are shown adjusted in Fig. 2B according to the isoatomic efficiencies indicated in Fig. 2A. This method demonstrates that P-granule
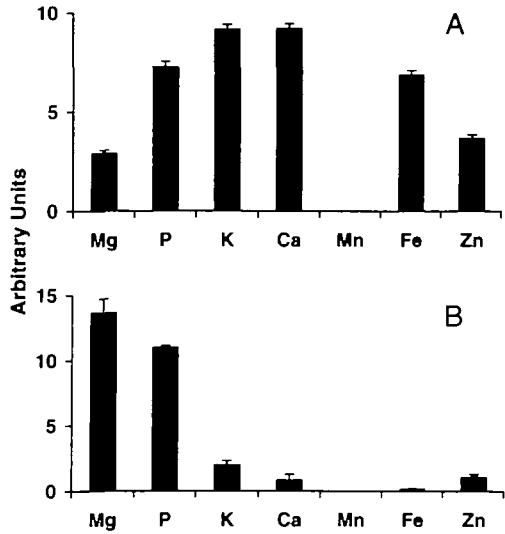

Figure 2. A. Mean relative peak efficiencies obtained using the XRMA system in analysing droplets ($n = 10$) of an isoatomic solution of Mg, P, K, Ca, Fe and Zn (no peak is shown for Mn as this was estimated by extrapolation). **B.** Mean relative proportions of elements in *M. neritoides* P-granules in spectra ($n = 12$), such as that shown in Fig. 1, after peak efficiency adjustment according to isoatomic ratios shown in Fig. 2A. Vertical bars represent standard deviations.

composition in individual species is remarkably constant and results are reproducible irrespective of specimen provenance. For those granules with few component elements, standard deviations are small; for example, in *Littorina littorea* (Fig. 3A) Mg and Ca both have SDs of <5%. For those granules containing a variety of metals, SDs exhibit a wider range; for example, in *Pirenella conica* (Fig. 3B) SDs vary between 1.4% (for P) and 52% (for Fe). For a general overview of P-granule composition in different species (Table 1) the adjusted peak heights for metals are expressed as percentages of adjusted peak height for P.

OBSERVATIONS

General trends

The results of analyses of some 1150 P-granules in over 40 species (230 specimens) representing 20 families are shown in Table 1. (As a framework for the presentation of data the largely traditional classification and nomenclature employed by Poppe & Goto (1991) has been used, but littorinid taxa follow Reid (1989) and superfamily endings have been changed). The

Table 1. Relative concentrations of Mg, K, Ca, Mn, Fe and Zn in phosphate granules occurring in the digestive glands of gastropods from various localities. See text for explanation.

Family	Species	No. of granules & animals	Elements as a percentage of phosphorus (means of atomic ratios)						Site	Area
			Mg	K	Ca	Mn	Fe	Zn		
Haliotidae	*Haliotis tuberculata* L.	15 (1)	74	15	28	0	0	0	Ile de Sieck, W. of Roscoff	F
Fissurellidae	*Diodora graeca* L.	15 (1)	95	14	25	0	1	2	Batten Bay, Plymouth Sound	E
Patellidae	*Patella vulgata* L.	10 (1)	97	22	10	0	1	2	Tinside, Plymouth Sound	E
	Patella ullysiponensis Gmelin	3 (1)	100	25	5	0	1	11	Tinside, Plymouth Sound	E
Trochidae	*Calliostoma zizyphinum* (L.)	– (3)	No phosphate granules found						Batten Bay, Plymouth Sound	E
	Gibbula adansoni Payraud.	10 (2)	86	29	24	0	0	9	Ag. Marina, Petalion Gulf	G
	Gibbula cineraria (L.)	12 (3)	118	19	5	0	0	0	Tinside, Plymouth Sound	E
	Gibbula cineraria	15 (3)	121	21	11	0	0	0	Widemouth Bay, N. Cornwall	E
	Gibbula magus (L.)	12 (3)	95	17	43	0	2	3	Ilha da Barreta, Ria Formosa	P
	Gibbula umbilicalis (da Costa)	30 (6)	120	24	7	0	0	0	Crackington Haven, N. Cornwall	E
	Gibbula umbilicalis	24 (6)	122	18	9	0	1	0	Tinside, Plymouth Sound	E
	Monodonta lineata (da Costa)	15 (3)	114	18	12	0	0	0	Renney Rocks, Plymouth Sound	E
	Monodonta mutabilis (Philippi)	12 (2)	121	22	18	0	0	0	Porto Rafti, Petalion Gulf	G
	Monodonta turbinata (Born)	12 (2)	116	25	12	0	0	0	Vromopoussi, Petalion Gulf	G
Littorinidae	*Melarhaphe neritoides* (L.)	12 (3)	124	18	8	0	1	10	Tinside, Plymouth Sound	E
	Melarhaphe neritoides	15 (3)	118	22	8	1	1	20	Vromopoussi, Petalion Gulf	G
	Littorina littorea (L.)	24 (4)	108	28	4	0	0	0	Jennycliff Bay, Plymouth Sound	E
	Littorina littorea	28 (6)	109	30	4	0	0	0	Widemouth Bay, N. Cornwall	E
	Littorina mariae Sacchi & Rast.	15 (3)	102	24	5	0	1	2	Tinside, Plymouth Sound	E
	Littorina obtusata (L.)	24 (6)	100	28	7	1	1	0	Stonehouse Pool, Plymth Sound	E
	Littorina saxatilis (Olivi)	24 (6)	104	28	8	0	0	0	Tinside, Plymouth Sound	E
	Littorina saxatilis	30 (6)	99	28	6	0	1	2	Widemouth Bay, N. Cornwall	E
Turritellidae	*Turritella communis* Risso [T]	28 (2)	51	19	27	4	3	63	Rame Mud off Plymouth Sound	E
Vermetidae	*Bivonia triquetra* (Bivona)	12 (3)	149	17	14	1	11	10	Glyfada, Saronikos Gulf	G
	Lemintina arenaria (L.)	10 (1)	123	36	7	2	3	22	Vromopoussi, Petalion Gulf	G
Potamididae	*Pirenella conica* (Blainville)	15 (3)	20	25	70	42	18	22	Porto Rafti, Petalion Gulf	G
Cerithiidae	*Bittium reticulatum* (da Costa)	12 (5)	79	13	27	10	3	36	Pte du Bindy, Rade de Brest	F
	Bittium reticulatum	27 (6)	86	23	21	16	5	30	Cacela Velha, E of Tavira	P
	Bittium reticulatum	15 (3)	112	28	25	9	5	19	Ilha Ramalhete, Ria Formosa	P
	Cerithium vulgatum (Bruguière)	11 (2)	40	19	69	16	15	15	Ilha da Culatra, Ria Formosa	P
	Cerithium vulgatum	24 (4)	28	23	41	33	17	41	Larymna, Vorios Evoikos G.	G
	Cerithium vulgatum	12 (3)	24	27	55	56	9	14	Vouliagmenis, Saronikos G.	G
	Cerithium vulgatum	10 (1)	27	23	65	52	7	7	Molos, Maliakos Gulf	G
Calyptraeidae	*Calyptraea chinensis* (L.) [T]	6 (3)	31	39	11	5	19	126	Ile de Sieck, W. of Roscoff	F
	Crepidula fornicata (L.)	– (6)	No phosphate granules found						Bembridge, Isle of Wight	E
Aporrhaidae	*Aporrhais pespelecani* (L.) [T]	24 (6)	72	23	6	0	1	90	Cawsand Bay, Plymouth	E
Ranellidae	*Cymatium parthenopeum* (v.Sal.)	12 (2)	60	26	44	0	2	36	Ilha da Culatra, Ria Formosa	P

Family	Species	n							Location	Area
Muricidae	Bolinus brandaris (L.)	10(1)	71	25	23	0	0	1	Cacela Velha, E of Tavira	P
	Bolinus brandaris	10(2)	54	27	29	0	0	0	Ilha da Culatra, Ria Formosa	P
	Bolinus brandaris	18(3)	69	20	24	0	0	2	Glyfada, Saronikos Gulf	G
	Bolinus brandaris	12(3)	65	24	24	0	0	0	Molos, Maliakos Gulf	G
	Hexaplex trunculus (L.)	12(3)	56	23	38	0	0	4	Molos, Maliakos Gulf	G
	Hexaplex trunculus	10(2)	71	32	19	0	0	2	Ilha Ramalhete, Ria Formosa	P
	Hexaplex trunculus	12(3)	61	25	27	0	0	0	Cacela Velha, E of Tavira	P
	Hexaplex trunculus	6(1)	66	22	28	0	4	4	Larymna, Vorios Evoikos G.	G
	Hexaplex trunculus	10(1)	58	26	30	1	0	0	Ilha da Culatra, Ria Formosa	P
	Hexaplex trunculus	12(3)	68	20	26	0	0	3	Glyfada, Saronikos Gulf	G
	Hexaplex trunculus	10(2)	65	28	33	0	0	0	Vouliagmenis, Saronikos G.	G
	Ocenebra erinacea (L.)	15(3)	66	25	34	0	1	10	Batten Bay, Plymouth Sound	E
	Ocenebra erinacea	11(1)	62	27	28	0	0	6	Ilha da Culatra, Ria Formosa	P
	Ocenebra erinacea	10(1)	59	25	37	1	0	13	Cacela Velha, E of Tavira	P
Thaididae	Nucella lapillus (L.) [1]	16(4)	78	20	19	0	1	2	West Looe, south Cornwall	E
	Nucella lapillus [2]	9(4)	61	23	20	0	1	11	West Looe, south Cornwall	E
	Ocinebrina aciculata (Lam.)	9(3)	74	31	19	0	1	6	Ilha da Culatra, Ria Formosa	P
	Ocinebrina aciculata	14(2)	76	27	22	1	0	7	Cacela Velha, E of Tavira	P
	Ocinebrina aciculata	8(5)	65	32	19	0	1	7	Ile de Sieck, W. of Roscoff	F
	Ocinebrina edwardsi (Payraud.)	15(3)	81	31	19	0	0	4	Vromopoussi, Petalion Gulf	G
	Thais haemastoma (L.)	10(1)	82	26	12	0	0	0	Breakwater, Vila Real S.Anton.	P
Buccinidae	Buccinum undatum L.	24(4)	21	24	14	0	3	94	West Muds, R.Tamar	E
	Buccinum undatum [T]	30(5)	17	16	16	3	1	96	5m S of Plymouth Breakwater	E
	Colus gracilis (da Costa) [T]	30(2)	26	10	1	1	1	91	Irish Sea, 10m E. of Douglas	IoM
	Pisania striata (Gmelin)	10(1)	39	39	11	0	5	97	Vromopoussi, Petalion Gulf	G
	Cantharus dorbignyi (Payraud.)	12(3)	62	27	35	1	1	1	Vouliagmenis, Saronikos G.	G
Columbellidae	Columbella rustica (L.)	16(3)	95	25	5	0	1	2	Vromopoussi, Petalion Gulf	G
	Columbella rustica [female]	12(3)	97	37	5	0	0	5	Cais Comercial, Ria Formosa	P
	Columbella rustica [male]	20(4)	105	37	6	4	7	5	Cais Comercial, Ria Formosa	P
Nassariidae	Nassarius incrassatus (Ström)	20(5)	14	30	4	7	0	177	Tinside, Plymouth Sound	E
	Nassarius reticulatus (L.)	10(3)	25	38	7	0	8	135	Batten Bay, Plymouth Sound	E
	Nassarius reticulatus	29(7)	21	41	4	0	7	125	Ilha da Culatra, Ria Formosa	P
	Nassarius reticulatus	16(4)	29	43	7	0	3	116	West Looe, Cornwall	E
Turridae	Raphitoma purpurea (Montagu)	11(1)	80	33	5	3	1	52	Ile de Sieck, W. of Roscoff	F
Conidae	Conus ventricosus Gmelin	11(1)	106	11	2	1	3	2	Cacela Velha, E of Tavira	P
	Conus ventricosus	11(1)	113	6	2	0	5	4	Ilha Ramalhete, Ria Formosa	P
	Conus ventricosus [female]	12(3)	114	7	2	5	0	6	Praia Cavacos, Ria Formosa	P
	Conus ventricosus [male]	12(3)	117	9	2	0	0	2	Praia Cavacos, Ria Formosa	P
	Conus ventricosus [female]	12(3)	114	9	2	0	0	4	Cacela Velha, E of Tavira	P
	Conus ventricosus [male]	12(3)	114	9	1	1	1	2	Cacela Velha, E of Tavira	P
	Conus ventricosus	16(5)	104	11	2	0	0	2	Vouliagmenis, Saronikos G.	G
Epitoniidae	Epitonium clathrus (L.)	10(1)	26	28	15	5	38	12	Cacela Velha, E of Tavira	P

Abbreviations: [T] – trawled; [1] feeding on *Gibbula*; [2] feeding on barnacles.
Areas: E – England (south-west); F – France (north-west); G – Greece (eastern Sterea Elada); IoM – Isle of Man; P – Portugal (Algarve).

composition spectra of all P-granules are consistent in that P invariably produces the highest peak and K usually occurs at between 10–30% of the P peak, *Conus ventricosus* being a notable exception. Other elements show marked variation in both presence and quantity but overall certain trends are apparent. Archaeogastropods, including species of

Patella, Gibbula and *Monodonta*, plus the mesogastropod *Littorina*, all have spectra with high percentages of Mg. Other mesogastropods, chiefly cerithioids, have multiple metals. Amongst the neogastropods, the Buccinoidea are prominent in having high percentages of Zn. In the cases of those genera and species which have been sampled from different geographical areas, the uniformity of P-granule composition is impressive. Various P-granule types can be recognised:

Type 1: high magnesium (Fig. 3A)

All the species in this group have a peak for Mg which is comparable in height to that of P. The peak for Ca is minor by comparison. Some show traces of Fe and/or Zn. This type of spectrum is typical for the genera *Patella* (2 species analysed), *Gibbula* (4 spp.), *Monodonta* (3 spp.), *Melarhaphe* and *Littorina* (4 spp.). Species show consistent P-granule composition regardless of location; for example, P-granules in *Melarhaphe neritoides* from SW England and SE Greece are virtually identical and both include a Zn component.

The spectra of P-granules in *Conus ventricosus* samples from Portugal and Greece correspond to type 1 in having high Mg peaks but are readily distinguished by their very low K and Ca peaks. Thus their composition is virtually pure magnesium phosphate.

Type 2: multiple metal (Fig. 3B)

Spectra for this P-granule type always include peaks for Mn, Fe and Zn. Typical examples of genera containing such granules (one species of each having been analysed) include the sediment burrowers *Turritella, Pirenella, Bittium* and *Cerithium* and also sessile/sedentary forms such as vermetids (*Bivonia* and *Lemintina*) and *Calyptraea*.

Both *Pirenella* and *Cerithium* are exceptional amongst the species analysed in that the Ca ratio exceeds that of Mg. The reason(s) for this is unclear but in the case of *Pirenella* this may reflect its estuarine habitat. The sample of *C. vulgatum* from Larymna comes from a metal-polluted site close to a ferro-nickel-smelting plant (see Nicolaidou & Nott, 1989) and spectra had further peaks for Cr, Co and Ni.

Type 3: magnesium-calcium (Fig. 3C)

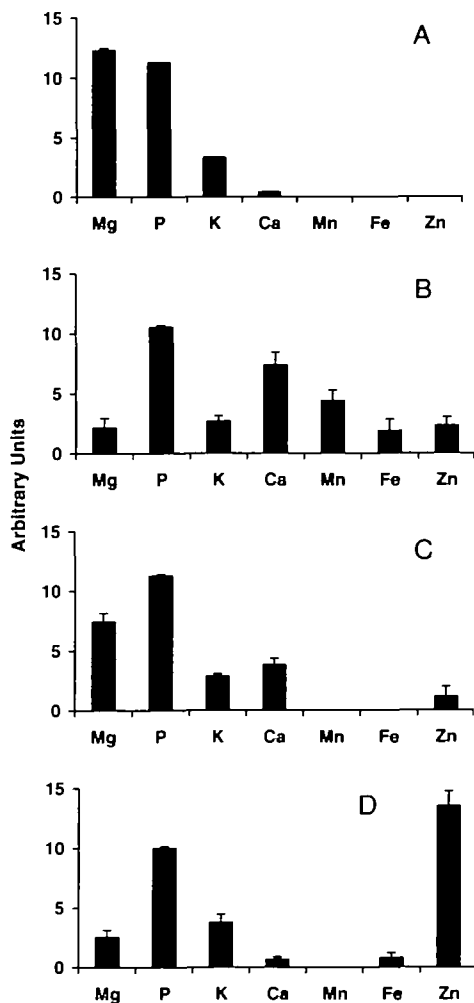Spectra produced by type 3 P-granules are typified by a Mg peak significantly lower and a Ca

Figure 3. Examples of the four types of P-granule composition recognised by differences in mean relative peak heights for elements after XRMA efficiency adjustment shown in Fig. 2A. **A.** Type 1: *Littorina littorea* from Widemouth, Cornwall—28 granules from 6 animals. **B.** Type 2: *Pirenella conica* from Porto Rafti, Greece—15 from 3. **C.** Type 3: *Ocenebra erinacea* from Batten Bay, Plymouth Sound—15 from 3. **D.** Type 4: *Nassarius reticulatus* from Batten Bay, Plymouth Sound—10 from 3. Vertical bars represent standard deviations.

peak higher than those found in P-granule type 1 but lacking the other metal peaks of type 2, although traces of Fe and/or Zn can be present. This type is characteristic of muricoid neogastropods including species of *Bolinus, Hexaplex, Ocenebra, Nucella, Ocinebrina* and *Thais*. Again, there is a specific consistency between samples from widely separated localities, e.g. *H. trunculus* from Portugal and Greece and *O. aciculata* from France and Portugal.

Type 4: high zinc (Fig. 3D)

P-granules in buccinoids, notably in *Buccinum, Colus, Pisania* and *Nassarius*, all show a pronounced peak for Zn that usually exceeds the peaks for both Mg and Ca and, in some cases, that of P. *N. incrassatus* from Plymouth Tinside contain remarkably high Zn. A trace of Fe is detectable.

DISCUSSION

The phylogeny of gastropods is a subject of much discussion (for reviews see Taylor, 1996; Ponder & Lindberg, 1997) and consequently many systems of classification remain in current use. As explained above, for convenience Table 1 follows that employed by Poppe & Goto (1991). Table 2 categorizes the types of P-granule found in the species surveyed in three prosobranch orders. In general, the high-Mg type 1 of P-granule dominates amongst the archaeogastropods and lower mesogastropods, the multiple-metal type 2 of P-granule is typical of the higher mesogastropods and the Mg-Ca and high-Zn types are characteristic respectively of neogastropod muricoid and buccinoid species. Thus P-granule composition is not consistent throughout the Gastropoda but does appear to be fairly predictable for major groupings. Several species do not fit into their predicted grouping: *H. tuberculata* has a lower Mg than expected for an archaeogastropod; likewise *A. pespelecani* and *C. parthenopeum* lack the high Mn of other higher mesogastropods. Amongst the neogastropods, *C. rustica* lacks the high Zn found in other buccinoids; conoids are poorly represented with just one turrid individual (*R. purpurea* P-granules appear to be somewhat intermediate between types 1 and 4 with high Mg and Zn) and one conid species—*C. ventricosus*—which, perhaps surprisingly, has a variant of the type 1 granule.

Although direct evidence is lacking (see below), phosphate granule formation may contribute to the regulation of metals, because at the end of the cycle of digestion, the digestive epithelium, which includes the granule-containing basophil cells, disintegrates into the lumen of the digestive gland and is passed through the gut to be voided in the faeces. P-granules in faecal pellets remain complete with associated metals and their overall composition reflects the reactivities of different metals since only those that can bind electrostatically can bind with the phosphate radical. In this group are the class A, or hard acid, metals Na, Mg, K and Ca, together with transition metals such as Cr, Mn, Fe, Co, Ni, Cu(II) and Zn (Nieboer & Richardson, 1980). (The class B, or soft acid, metals Cu(I), Ag, Cd and Hg are not associated with the mineral component of P-granules.) It is suggested that the granules are basically calcium phosphate and that Mg ions can be trapped in the lattice since there is a large energy barrier to the substitution of Ca^{2+} into a $Mg_2P_2O_7$ lattice whilst the converse, i.e. adding Mg^{2+} to $Ca_2P_2O_7$ lattice, is energetically favourable (Simkiss, 1994). In marine species, substitution of magnesium—by Fe and Mn in type 2 and by Zn in type 4—appears to be significant.

In molluscs there is a good correlation between feeding categories and taxonomy: members of major groups feed in the same manner and occupy similar positions in the marine food webs (Hickman, 1984). Thus it is perhaps not surprising that the different P-granule types approximate phylogenetic groupings with different diets. The genera possessing type 1 granules are predominantly grazers of algae (and includes the neogastropod *Columbella rustica* which has been considered a herbivore: Taylor, 1987). Type 2 are typical of detrital feeders, type 3 of carnivores and type 4 of carnivore/scavengers. Uptake of metals by carnivores from prey is not always predictable; for example at Larymna (Table 1) *Hexaplex trunculus* feed on *Cerithium vulgatum* but the P-granules of the predator do not accumulate the array of metals constituting the P-granules of the prey (see Nott & Nicolaidou, 1989a, 1993). On the other hand, *Nucella lapillus* feeding on barnacles (which are known to contain zinc phosphate granules: Walker, Rainbow, Foster & Holland, 1975; Thomas & Ritz, 1986) have higher Zn (and lower Mg) than *N. lapillus* in an adjacent area of the same shore which were feeding on *Gibbula* spp. (Table 1). Overall, diet appears to cause only minor modification of P-granule composition.

The potential of certain gastropod species as

Table 2. Summary of gastropod species in relation to types of phosphate granules in digestive glands. Feeding categories (mostly based on data in Graham, 1988; Fretter & Graham, 1994): c, carnivore; d, detrital (including ciliary) feeder; h, herbivore; s, scavenger.

Type 1 High Magnesium	Type 2 Multiple metal	Type 3 Magnesium-calcium	Type 4 High Zinc
		Archaeogastropoda *Haliotis tuberculata* (h)	
Diodora graeca (c) *Patella vulgata* (h) *P. ullysiponensis* (h) *G. adansoni* (h) *Gibbula cineraria* (h) *G. magus* (h) *G. umbilicalis* (h) *Monodonta lineata* (h) *M. mutabilis* (h) *M. turbinata* (h)			
		Mesogastropoda	
Melarhaphe *neritoides* (h) *Littorina littorea* *L. mariae* (h) *L. obtusata* (h) *L. saxatilis* (h)			
	Turritella communis (d) *Bivona triquetra* (d) *Lemintina arenaria* (d) *Pirenella conica* (d) *Bittium reticulatum* (d) *Cerithium vulgatum* (d) *Calyptraea chinensis* (d)		
			Aporrhais pes-pelecani (d) *Cymatium* *parthenopeum* (c)

		Neogastropoda
		   Bolinus brandaris (c)
		   Hexaplex trunculus (c)
		   Ocenebra erinacea (c)
		   Nucella lapillus (c)
		   Ocinebrina aciculata (c)
		   O. edwardsi (c)
		   Thais haemastoma (c)	

			Buccinum undatum (c) *Colus gracilis* (s) *Pisania striata* (c)
		Cantharus dorbignyi (c)	
Columbella rustica (h)			
			Nassarius reticulatus (s) *N. incrassatus* (s) *Raphitoma purpurea* (c)
Conus ventricosus (c)			
	Epitonium clathrus (c)		

No phosphate granules detected: *Calliostoma zizyphinum* (c/d); *Crepidula fornicata* (d).

bioindicators of metal levels in environments, both natural and polluted, has been fully investigated. *Littorina littorea* is probably the best-known (see for example Bryan, Langston, Hummerstone, Burt & Ho, 1983). Metal body burdens must be influenced by the P-granules type; indiscriminate use of species without regard to their P-granule type could confound their use as 'biomarkers'. Examples from the present survey are instructive in pointing to the

stability of the P-granule types. Table 3 summarises data for species collected at two different localities—Ile de Sieck, an exposed rocky site in NW Brittany, and Cacela Velha, a sedimented lagoon on the Algarve coast. Ambient metal levels can assumed to be the same for each collection at either site but nevertheless there is wide interspecific variation in P-granule composition at each site. (Importantly, the P-granule spectrum of *O. aciculata* is similar at both.) Other examples of wide variation between species at the same site are to be found in the survey data (Table 1). For example, compare the P-granule spectra for *C. vulgatum* with both that of *H. trunculus* at Larymna and with that of *C. dorbignyi* at Vouliagmenis; likewise at Tinside the highest ratio recorded, that for Zn in *N. incrassatus* (177%), is not replicated in other co-habiting species.

Apart from diet and environment, other biological factors that may affect P-granule composition include size and age, season and perhaps gender. Data on these aspects are limited but two size-age groups of *Buccinum undatum* were examined: in Table 1 young animals (1–2 years), taken at low water within the Tamar Estuary, are compared with large, old individuals dredged offshore and the spectra are similar. With respect to gender, separate analyses of granules from mature (ripe) males and females of *C. rustica* and *C. ventricosus* (Table 1) show no difference.

Whilst P-granules appear in the digestive gland of the great majority of gastropods, curiously they were not detected in two species—*C. zizyphinum* and *C. fornicata*. Possibly, the specimens examined were in a resting state but this seems unlikely. Based on their taxonomic affiliations, *a priori*, they would be predicted to have P-granules of type 1 and type 2 respectively.

The function of phosphate granules is poorly understood (Taylor & Simkiss, 1994). The deposition of metals, such as Ca, Fe, K, Mg, Mn and Zn, into discrete granules has been used as evidence for these structures being involved in metal metabolism, the general view being that they constitute either stores of calcium and/or phosphate or a detoxification mechanism for cations. Nevertheless, as Mason & Jenkins (1995) point out, it needs to be stressed that direct proof of the latter function is lacking. An alternative viewpoint in the case of P-granules is that detoxification by elimination may be purely incidental. The similarity of P-granule composition in related species (Table 1), the constancy of P-granule composition for any species regardless of location (Table 1), and also the differences in P-granule composition between species in the same location (Table 2), suggest composition may have a phylogenetic origin rather than be a reflection of contemporary ambient conditions. A question thus arises:– Does the composition of the phosphate granule in the digestive glands of different gastropods reflect, or have been influenced by, the geochemical composition of the seas of

Table 3. Comparison of digestive gland phospate granule composition in gastropods examined from two contrasting habitats – Ile de Sieck (Brittany) and Cacela Velha (Algarve, Portugal).

Locality/species	Elements as a percentage of phosphorus (means of atomic ratios)					
	Mg	K	Ca	Mn	Fe	Zn
Ile de Sieck						
Haliotis tuberculata	74	15	28	0	0	0
Calyptraea chinensis	31	39	11	5	19	126
Ocinebrina aciculata	65	32	19	0	1	1
Raphitoma purpurea	80	33	5	0	1	52
Cacela Velha						
Bittium reticulatum	86	23	21	16	5	30
Bolinus brandaris	71	25	23	0	0	1
Hexaplex trunculus	61	25	27	0	0	0
Ocenebra erinacea	59	25	37	0	0	13
Ocinebrina aciculata	76	27	22	1	1	7
Conus ventricosus	106	11	2	1	3	2
C. ventricosus [female]	114	9	2	0	1	4
C. ventricosus [male]	114	9	1	0	4	2
Epitonium clathrus	26	28	15	5	38	12

previous geological eras? P-granules feature in the digestive glands of all but two of the 41 species (representing 32 genera and 20 families) examined. This universality suggests these structures are part of a system that evolved early in the history of gastropods and one that has been retained as a fundamental, plesiomorphic character. Given the long, well-documented history of gastropods, it would be surprising if the composition of the phosphate granule produced by the digestive gland cell remained constant and unresponsive to variations in seawater chemistry.

The present survey is very fragmentary and includes only temperate, shallow-water species. Extension of the list to tropical and deep-water species would certainly uncover further aspects of the relationship between P-granule chemistry and taxonomy. For example, *C. ventricosus* is an inshore sand dweller whereas most *Conus* spp are coral reef inhabitants: whether or not P-granule composition in *C. ventricosus* is typical of this large genus (~500 species: Kohn & Perron, 1994) needs to be examined.

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