Decay and Mineralization of Shrimps

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Experiments on decay and early diagenetic mineralization of shrimps in artificial sea water resulted, for the first time, in extensive mineralization in association with soft-tissues. The investigation focused on taxa with a cuticle which is not heavily mineralized in life: the shrimp Crangon, *and the prawn* Palaemon. *A series of experiments was monitored in terms of morphological change, weight loss, change in chemical composition, and the formation of minerals. The major controls investigated were the amount of oxygen present at the outset, and whether the system was open or closed to diffusion. Decay resulted in a continuum of five morphological stages: 1. swollen, due to osmotic uptake, 2. ruptured, as the exoskeleton split, 3. hollow, as the muscles shrunk, 4. disarticulated, as the elements of the exoskeleton began to separate, and finally 5. fragmented. There was no indication that rate of decay (measured by weight loss) was strongly influenced by the availability of oxygen.*

Two categories of mineralization occurred, the precipitation of crystal bundles of calcium carbonate, and the replacement of soft-tissue in calcium phosphate. Where the system was open to diffusion, mineralization was dominated by the precipitation of crystal bundles in the shape of discs, hemispheres, rods and dumbbells; soft-tissue was rarely extensively mineralized. Where the system was closed, precipitation of crystal bundles was not as widespread, but mineralization of soft-tissue (muscle, hepatopancreas, eggs) in poorly crystalline calcium phosphate commonly occurred. The major source of phosphate was the carcass itself. In some specimens mineralized soft-tissue was subsequently overgrown by calcium carbonate crystal bundles. Crystal bundles began to precipitate within a few days; mineralization of soft-tissue started within two weeks but increased by four weeks. The main control was pH which decreases most where the system is closed, inhibiting the precipitation of calcium carbonate in favor of calcium phosphate. Where phosphate was added to the sea water the rate of morphological decay increased; soft-tissue was mineralized in a similar proportion of experiments but it was more extensive sooner. Doubling the content of bicarbonate resulted in an increase in the extent of crystal bundle formation, but the proportion of individuals in which they precipitated remained the same. The excess bicarbonate inhibited the phosphatization of soft-tissue. Control experiments showed that both types of mineralization also occurred when the shrimps were buried in sediment.

Crystal bundles have not been reported from fossils (they would be very susceptible to diagenetic recrystallization). Phosphate minerals similar in texture and composition to those formed in the experiments preserve soft-tissues in exceptional preservations such as the Jurassic Cordillera de Domeyko of Chile and the Cretaceous Santana Formation of Brazil. The results of the experiments show that although the oxygen content of the sea water may not have a pronounced impact on rate of decay, the open or closed nature of the system influences pH and determines the type of early mineralization and hence the nature of preservation.

INTRODUCTION

Non-biomineralized animal tissues may be preserved in two main ways (see Allison and Briggs, 1991a, b). Structural organic tissues like cuticle may resist decay and, as a result of transformation into complex inert biopolymers, become fossilized as organic remains (Butterfield, 1990). More labile tissues like muscle, on the other hand, are normally rapidly metabolized by bacteria, and usually can be preserved only as a result of very early replication by authigenic minerals (Allison, 1988b; Briggs and Kear, 1993b). Minerals may also precipitate within a decaying carcass without replicating the soft-tissue morphology.

Research on the processes which control the preservation of soft-tissues has become focused on an increasingly finer level. Seilacher et al. (1985) emphasized broadly sedimentological or environmental criteria, recognizing three major influences, oxygen levels, rapid burial and the formation of microbial films. These factors do not, however, prevent decay; the first two inhibit scavengers, the third may be instrumental in stabilizing the sediment and carcass, and in generating geochemical gradients critical for diagenesis. Allison (1988b) recognized that early diagenetic mineralization was an important factor in preventing information loss through decay, and he modelled the conditions required to

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promote the formation of pyrite, carbonate and phosphate minerals in association with soft-tissues. Most discussions of the mineralization of soft-tissue have been based on data from fossils. Experiments with decaying organisms have led to the formation of calcium soaps (Berner, 1968; Allison, 1988a) but not to the extensive mineralization of soft-tissue. Here (and elsewhere, Briggs and Kear, 1993a, b, c) we describe experiments undertaken to investigate rates and controls on the decay and mineralization of different organs and tissues in specific taxa.

Only a small proportion of arthropods have a heavily biomineralized exoskeleton in life (e.g., some trilobites, crustaceans, millipedes). The extensive fossil record of the group (see Briggs and Clarkson, 1989; Shear and Kukalova-Peck, 1990) reflects the resistance of organic structural tissues to decay under certain conditions. A number of examples are known, however, in which more labile tissues such as muscles are also preserved, as a result of mineralization (e.g., Wilby and Martill, 1992). Here we describe experiments on decay and mineralization in two marine decapod crustaceans which lack a heavily biomineralized cuticle, the shrimp *Crangon* and the prawn *Palaemon*. The results have implications for the preservation of soft-tissues in general.

MATERIAL AND METHOD

In an experimental investigation of the factors that control decay and early diagenetic mineralization the number of variables must be reduced so that their effects can be monitored independently as far as possible. A series of experiments was carried out on the decapod crustaceans *Crangon crangon* (Decapoda, Caridea, Crangonidae) and *Palaemon elegans* (Decapoda, Caridea, Palaemonidae) (Fig. 1) to record the morphologic sequence of decay states, to monitor changes in pH and oxygen levels, to determine decay rates under different conditions, and to study the controls on the formation of early diagenetic minerals. The major variables explored in these experiments were the availability of oxygen at the onset of decay, and whether or not the system was open or closed to diffusion. These conditions reflect differences in natural systems in response, for example, to rates of sedimentation or the growth of microbial mats. In order to facilitate observation and recovery of the carcasses most of the experiments were carried out without sediment, but a number of controls were run with sediment to test whether it affected the outcome. It has been argued that elevated concentrations of phosphate are a prerequisite for the phosphatization of soft-tissue (e.g., Allison, 1988b, c; Martill, 1988). Hence the result of increasing the concentration of phosphate ions, or of the carbonate ions which precipitate under different conditions, was also explored.

Material

Live specimens of the common shrimp *Crangon crangon* (n = 360; size range 0.09 g to 1.77 g) and the common prawn *Palaemon elegans* (n = 142; size range 0.10 g to 1.95 g) were obtained by trawling (Plymouth Marine Laboratory's boats operating in the Plymouth Sound region) or hand-netting (Poole Harbour; Starcross Harbour) in southern England. Animals were held in aquaria (3–6° C, 35–37‰ artificial sea water, i.e., normal marine salinity) until required (for periods from 1 day to several months). Morbid or dead animals were not used in experiments. Fresh moults (*Crangon*, n = 70) were obtained from the stock tanks by removing discarded exuviae every 1 or 2 days. Animals were not manipulated or induced to moult in any way.

Method

Shrimps and prawns were killed by anoxia. They were placed in an empty beaker in the air-lock of an anaerobic cabinet. The air was pumped out of the chamber which was then flushed twice with oxygen-free nitrogen and filled with anaerobic gas mixture (CO_2, N_2 and H_2). They were left in the chamber for 40 to 60 minutes to ensure death but avoid excessive dehydration.

Carcasses were dried by blotting with tissue, weighed, and each transferred singly to a screw-top glass experimental vessel (ointment jar) with 50 ml of standard artificial sea water (ASW—see Table 1) where they sank to the bottom. The ASW had first been inoculated with water (50 ml/l) and sediment (ca 0.5 ml/l) from the Tay Estuary, Dundee, Scotland, as a source of bacteria, and yeast extract (0.1 g/l) was added to act as a bacterial substrate (see Briggs and Kear, 1993a). The inoculated ASW was allowed to incubate for at least 48 hours at room temperature before the carcasses were introduced. pH was adjusted to 8.00. The Tay Estuary was used as a source of inoculum as this site is characterized by high rates of organic matter degradation as a result of a range of processes, both aerobic, and anaerobic sulphate-reduction (Parkes and Buckingham, 1986; Parkes et al., 1989).

Our standard ASW recipe includes no phosphate (Table 1). Nonetheless impurities in the salts may contribute up to 15.6 mg phosphate per liter ASW (product information). This potential concentration (overlooked in our initial report of these experiments (Briggs and Kear, 1993b)) is orders of magnitude higher than that in normal sea water (which is variable: Lucas and Prévôt (1991), for example, list values from 0.02 to 0.27 mg PO_4 per liter). However, in Long Island Sound (FOAM site), where bottom water concentrations do not exceed 0.2 mg PO_4 per liter, pore-water concentrations approach 10 mg per liter just below the surface and 15 mg per liter at 1.5 m in sediment cores (Ruttenberg and Berner, 1993). Thus any phosphate introduced as impurities in our ASW would have resulted in maximum concentrations comparable to those in sediment pore-waters in non-upwelling, continental margin environments. The composition of the ASW in our experiments was varied (Table 1) in order to explore the effect of adding phosphate, and of increasing the concentration of bicarbonate ions. A quantity of phosphate (PO_4) was added equivalent to just over 25% of that in an average shrimp (Table 1) as a combination of $Na_2HPO_4 \cdot 12H_2O$

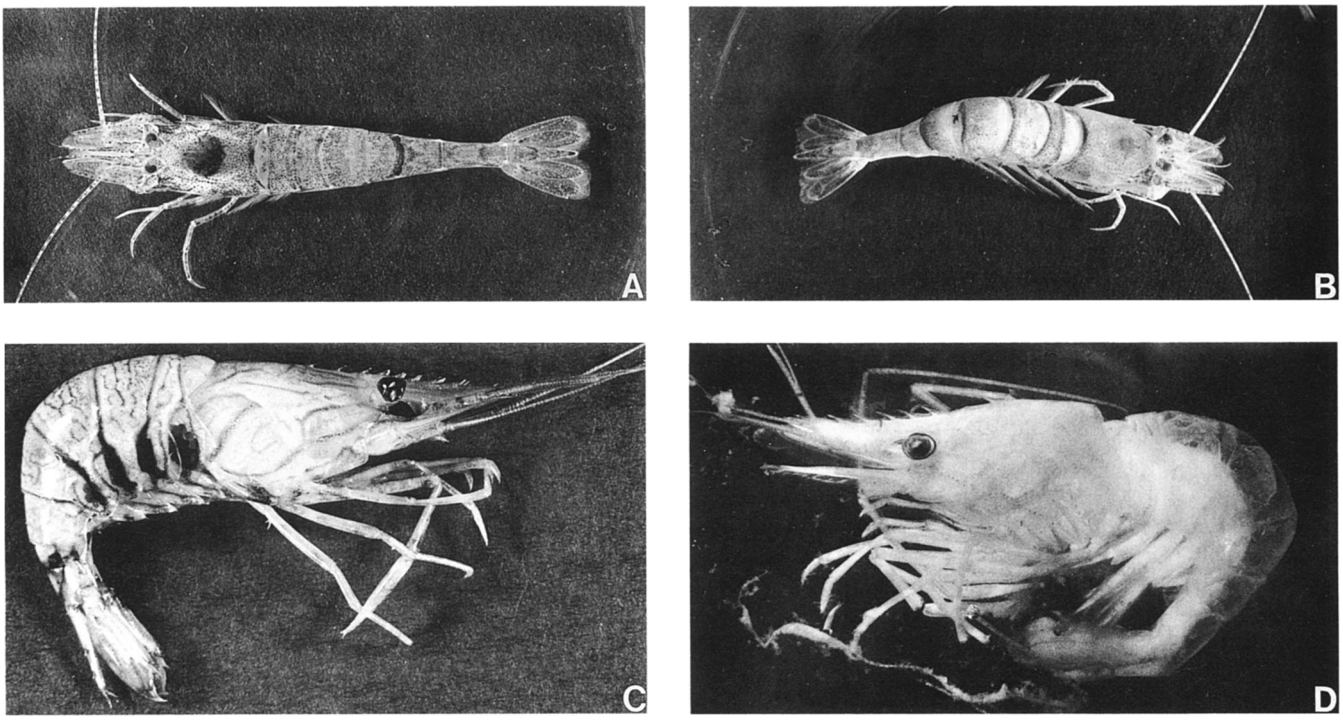


FIGURE 1—(A) Freshly killed *Crangon*. The dark hepatopancreas-stomach area is visible through the cuticle. (B) *Crangon* day 2, experiment 1a. The arthrodial membranes have stretched as the carcass swells due to osmosis (Stage 1). (C) Freshly killed *Palaemon*. (D) *Palaemon*, 25 weeks, experiment 1d. The cuticle of the cephalothorax has pulled away from the abdomen, and the muscles have shrunk in volume, leaving the abdomen partially "hollow" (Stage 3). All ×1.3.

and $NaH_2PO_4 \cdot 2H_2O$ (to prevent automatic precipitation at the initial pH of the experiments!) (this gives a *maximum* potential concentration, including impurities, of 32.3 mg (i.e., 16.7 + 15.6) per liter, compared to 145 mg per liter of HCO_3). Bicarbonate was increased twofold by doubling the amount of $NaHCO_3$ in the original ASW recipe. In both cases salinity was adjusted to the target 34.6‰ by reducing NaCl (Table 1). The vessels were incubated at 20 ± 0.5° C.

Where sediment itself was added to the experimental jars this too was obtained from the Tay Estuary. A layer about 2 mm deep was spread on the bottom of the jar. A piece of nylon cloth (100 μm mesh) was placed on the sediment to facilitate recovery of the remains of the shrimp. The carcass was positioned on top of this and covered by a further layer of sediment about 1 cm deep (total sediment volume was 23 to 35 cc).

The amount of oxygen in the ASW at the start of the experiments, and its subsequent availability, was varied in six ways (Fig. 2): 1a—starting oxygen 50% saturation, re-oxygenation by rapid diffusion; 1b—starting oxygen 50% saturation, re-oxygenation by slow diffusion; 1c—starting oxygen 50% saturation, no diffusion; 1d—completely anoxic; 1e—starting oxygen 50% saturation, diffusion from partially oxygenated air space; 1f—no oxygen in ASW, diffusion from partially oxygenated air space.

Where rapid diffusion of oxygen was required the vessel

TABLE 1—Recipe for standard artificial seawater (ASW) (pH adjusted to 8.00); adapted from Lyman and Fleming (1940). Recipes for additional phosphate and bicarbonate are also shown.

	Normal ASW (g/litre)	Extra phosphate (g/litre)	Extra bicarbonate (g/litre)
NaCl	23.5	23.44	23.3
$MgCl_2$	5.0	5.0	5.0
$CaCl_2$	1.1	1.1	1.1
KCl	0.66	0.66	0.66
$NaHCO_3$	0.2	0.2	0.4
KBr	0.096	0.096	0.096
H_3BO_3	0.024	0.024	0.024
$SrCl_2$	0.026	0.026	0.026
Na_2SO_4	4.0	4.0	4.0
$Na_2HPO_4 \cdot 12H_2O$	—	0.0603	—
$NaH_2PO_4 \cdot 2H_2O$	—	0.0012	—
Total	34.606	34.6075	34.606
Salinity	34.6	34.6	34.6
Yeast extract	0.1	0.1	0.1
Tay water, etc	50 ml	50 ml	50 ml

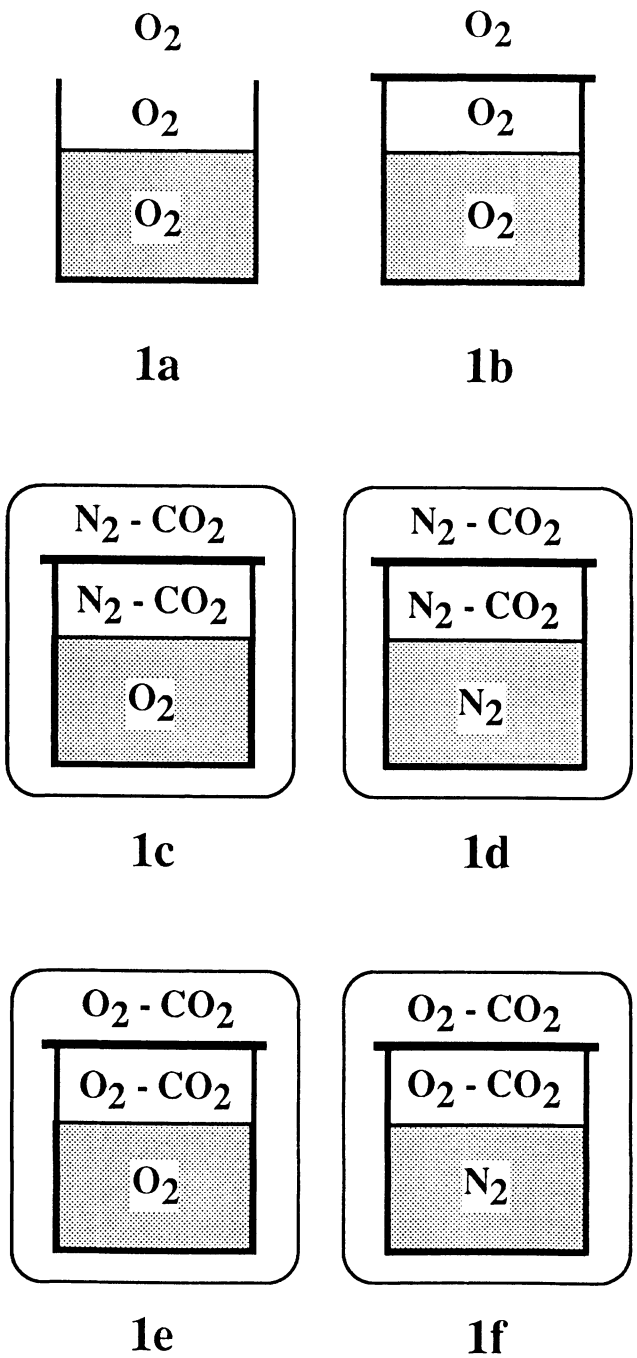


FIGURE 2—Diagrammatic representation of oxygen availability in the experimental runs. The vessels contained artificial sea water (ASW) and an airspace, and were either open to the atmosphere, closed, or closed and sealed in airtight aluminum bags. Gaseous content of the ASW, airspace, and surrounding atmosphere is indicated. 1a, starting oxygen 50% saturation, rapid diffusion; 1b, starting oxygen 50% saturation, slow diffusion; 1c, starting oxygen 50% saturation, no diffusion (Anaerocult A); 1d, completely anoxic (Anaerocult A); 1e, starting oxygen 50% saturation, diffusion from partially oxygenated air space (Anaerocult C); 1f, no oxygen in ASW, diffusion from partially oxygenated air space (Anaerocult C).

was covered only with sterile cotton wool or tissue (Experiment 1a). Oxygen exchange was limited to slow diffusion by closing the vessel with a screw cap (Experiment 1b). Oxygen exchange with the atmosphere was prevented by sealing the vessels in a plasticized aluminum bag (method of Cragg et al., 1992) with Merck Anaerocult A (Merck Ltd., Poole, Dorset, U.K.) which removes gaseous oxygen by reacting with it to form CO_2 (Experiment 1c). Oxygen was completely eliminated from the outset by degassing the ASW overnight with oxygen-free nitrogen (OFN) before sealing in an aluminum bag with Merck Anaerocult A (Experiment 1d). Anaerocult A results in a starting CO_2 level of approximately 18% of gas volume reducing O_2 to zero within one hour (Merck product information). Where atmospheric oxygen was to be reduced rather than eliminated from the outset the experiments were sealed in an aluminum bag with Merck Anaerocult C (Experiments 1e, 1f). This lowers gaseous oxygen levels to approximately 8% within 2 hours and to 5–6% in 24 hours by replacing it with CO_2 (~9% of starting gas volume) and H_2 (~0.5%) (Merck product information). O_2 levels produced by the anaerocults were verified using an oxygen probe. Where oxygen was present in the water at the outset (1e) it was rapidly eliminated by decay, but levels recovered to about 2% (in weeks 4 to 6) as a result of diffusion of oxygen remaining in the air space. Where oxygen was eliminated from the water at the outset by degassing with OFN (1f), decay consumed the airspace oxygen (0% at 8 weeks).

Sampling

Replicate experiments (5 for each sampling interval under each set of conditions) were run and terminated with sampling. The time allowed to lapse between sample dates increased in order to monitor the important stages in decay. Due to its availability, *Crangon* was the main subject of investigation. Runs were sampled at 3 days and 1, 2, 4, 6 and 8 weeks. Experiments 1a and 1b were further sampled at 10, 15, 20 and 25 weeks. Subsequent experiments (1c, d, e, f and runs with additional phosphate and bicarbonate) were terminated after 8 weeks as all the major changes have occurred by that time. Experiments on *Palaemon* were run under conditions 1b (sampled at 1, 2, 3, 4, 6, 8, 10, 15, 20, 25 and 75 weeks), and 1d (at 4, 6, 8, 10, 15, 20 and 25 weeks).

The procedures adopted and instruments used in sampling are detailed in Briggs and Kear (1993a). The colour of the ASW was noted, and the presence or absence of bacterial films at the air interface and on the bottom of the jar was recorded by visual inspection. The pH and oxygen content of the ASW were measured using probes (pH: Jenway model 3030, accuracy to ±0.002; oxygen Jenway model 9010, accuracy to ± 2%, electronically compensated for salinity and atmospheric pressure). The state of decay was recorded, and the wet and dry weights of the remains were measured. If the remains could not be removed from the vessel without disintegration, then any surface bacterial film was removed, and the rest filtered through a GF/C grade glass microfiber filter before wet

and dry weights were taken. The wet weight was measured when the filter paper appeared completely dry, all the water having been removed from the filter paper by water-driven vacuum pump (and a standard value for the weight of the filter paper after filtering ASW only was subtracted from the total). The carcass (and filter paper where used) was placed on a piece of aluminum foil and dried in an oven at 105° C. Dry weight stabilizes after 2–6 hours depending on specimen size; all samples were left for at least 24 hours to ensure standardization before the dry weight was measured (a standard dry weight for the filter paper was obtained by oven-drying the blanks used for wet weight). A similar procedure was followed for moults, but measuring weight loss proved impracticable due to their small mass.

When required for microscopic examination of the state of decay, wet tissue was fixed in 1% glutaraldehyde in 0.1 M cacodylate buffer. Immersion in hexamethyldisilazane (HMDS) was substituted for critical point drying (Nation 1983). Both fixed and dried samples were coated in gold before being examined by SEM at 8.0 kV. Oven dried material was not otherwise treated prior to coating.

Experiments in mud were sampled by pipetting off the surface water and removing the carcass and overlying sediment by lifting out the nylon cloth. The carcass was freed from the mud by carefully washing away the sediment using a pipette. Experiments in mud were designed to monitor mineralization. Weight loss was not measured due to the difficulty of totally removing the sediment and of calculating background weights.

Chemical Analysis

Specimens for chemical analysis were oven dried on glass fiber filter paper or aluminum foil. Dried carcasses (removed from the filter paper or foil) were powdered and homogenized with mortar and pestle. Total organic carbon (TOC) and CHN ratios were analyzed using standard methods (Perkin Elmer 240C elemental analyzer; C in carbonate with a Coulamat 702 analyzer). The residue method of Hunt and Nixon (1981) was adapted to determine the amount of "structural tissue" resistant to alkali and acid hydrolysis (see Briggs and Kear 1993a, c).

Where mineralization occurred in association with carcasses, moults, or bacterial films, samples were examined under the scanning electron microscope (SEM), and analyzed using x-ray diffraction (XRD) and electron microprobe (EM). Specimens for analysis in the microprobe were placed in holes in a perspex plate, immersed in resin, and polished. Some of the minerals were difficult to polish because of their "soft" nature. Specimens were coated in carbon for analysis with the EM.

Statistical Comparison

The final wet weight was calculated as a percentage of the original wet weight. A wet : dry weight linear regression was calculated for freshly killed specimens (*Crangon,* $n = 15$, $r^2 = 0.946$; *Palaemon,* $n = 17$, $r^2 = 0.894$). This was used to predict initial "baseline" dry weight values from the initial measured wet weights. Final dry weights were expressed as a percentage of these "baseline" values. Data were processed using the Minitab Statistical Package (Minitab Version 8.1). A two-way analysis of variance (General Linear Model) was performed to determine whether the results of decay (weight loss, pH profile) under different treatments were statistically different. Means and standard deviations for weights and pH values are given in Appendix 1.

MORPHOLOGICAL STAGES IN DECAY

Disintegration of *Crangon crangon* and *Palaemon elegans* takes place in a sequence of morphological stages which, although they form a continuum, allow the state of decay to be defined qualitatively. The descriptions of the stages below include details of the usual sequence of changes, both external and internal; muscle fibers sometimes remained evident for longer, however, in parts of the carcass. This was particularly the case, of course, where the soft-tissues formed a locus for mineralization. The carcasses in our experiments rarely floated to the surface of the water, except where additional bicarbonate was added to the artificial sea water. The stages represent the effects of decay where the carcass is undisturbed by currents. They are as follows:

0. *Freshly killed.*—Killing the shrimp in the anaerobic cabinet makes no difference to its appearance (Fig. 1A, C). The cuticle remains rigid and the carcass essentially transparent. All pigmentation is in the epidermis, which lies below the cuticle, and in the retinal cells of the eyes. The hepatopancreas may be yellow-orange or dark brown (Fig. 1A).

1. *Swollen.*—Expansion of the internal tissues (due to osmotic uptake) leads to ventral flexure of the abdomen and stretching of the arthrodial membranes, which may begin to split (particularly in the abdomen) (Fig. 1B). The ventral curvature of the abdomen ensures that the shrimp comes to rest on its side. Rigor mortis often results in flexing of the antennae; the uppermost curves upwards and may become trapped in the surface film. The carcass remains intact but the cuticle begins to lose its rigidity. It easily separates from the underlying epidermis which bears the pigment. The muscles become opaque white, and are then stained by the release of granular pigment from the disintegrating epidermis. They retain much of their internal strength and cohesion. The boundaries between individual muscles in the abdomen, and the fibers within them, are clearly evident under the binocular microscope.

The water in the vessel becomes pale yellow and cloudy. A thin surface film develops.

2. *Ruptured.*—The exoskeleton usually begins to split at the junction between the cephalothorax and abdomen, and along the boundaries between the abdominal tergites starting at the junction between 3 and 4. The cuticle of the venter and appendages becomes flaccid, followed by the thinner cuticle along the margins of the cephalo-thorax and tergites. The slightest disturbance promotes the

separation of the tergites and the exoskeleton disarticulates when removed from the water. The pigment begins to disappear from the appendages, persisting longest where the cuticle remains in contact with the underlying epidermis. The muscles lose their internal strength and few fibers remain, although muscle boundaries may still be seen. The gut is represented only by a furrow in the abdominal muscles.

A thin organic precipitate, presumably bacterial in origin, covers the bottom of the vessel.

3. *Hollow.*—The muscles shrink and settle to the bottom of the body cavity (i.e., the lowest point, depending on the attitude of the shrimp in the vessel), leaving a hollow above (Fig. 1D). The cephalothorax separates from the abdomen. The entire cuticle is flaccid and, while remaining relatively intact, collapses and disarticulates on removal from the water. In many instances the muscles degenerate to an amorphous white semi-liquid state, with no evidence of fibers or boundaries remaining.

The water in the vessel has become clear; the surface film is noticeably thickened.

4. *Disarticulated.*—The tail fan separates from the abdomen and the uropods become detached as a result of the slightest movement. The entire exoskeleton begins to disarticulate, releasing any semi-liquid soft-tissue that remains, although some muscle is still evident. The legs separate from the trunk. Only the antennae remain fairly robust and attached to the cepahlon.

5. *Fragmented.*—Even the antennae and thickest parts of the tergites lose their rigidity. The exoskeleton is thin and flaccid, and some elements no longer retain the original outline. The eye pigment is still present and in place, and an organic residue may still remain in the abdomen even at 75 weeks under conditions of slow diffusion (1b).

The films on the surface and the bottom of the jar begin to disappear.

This sequence of decay is characteristic of both *Crangon* and *Palaemon* regardless of the experimental conditions. Traces of the stomach and hepatopancreas may persist for much longer, however, where oxygen diffusion is prevented.

The progress of decay through morphological stages provides a rough measure of rate of decomposition (Fig. 3). In the case of *Crangon,* Stage 1 (Swollen) was reached within 3 days and did not persist beyond 6 weeks (normally disappearing much earlier). Stage 2 (Ruptured) was reached within 3 days to 1 week, and rarely persisted beyond 8 weeks. Stage 3 (Hollow) was reached by 1 to 2 weeks but persisted in some cases to at least 25 weeks. Stage 4 (Disarticulated) was rarely reached before 6 weeks and Stage 5 (Fragmented) was not observed before 10 weeks except where bicarbonate or phosphate were added to the ASW (Fig. 3). Even under conditions of rapid diffusion (1a) the exoskeleton and traces of the muscles may remain for in excess of 25 weeks. Because decay rates are temperature sensitive (in general, a twofold increase in decay rate can be expected for a temperature increase of 10° C—Swift et al., 1979), a lower temperature than 20 ± 0.5° C should delay the onset of the morphological stages of decay (see experiments of Kidwell and Baumiller, 1990, on echinoid disarticulation).

The stages of morphological decay only allow its progress to be monitored by observation rather than measurement. Comparisons between decay under different conditions are complicated by the necessity for variations in handling (e.g., closed experiments must be removed from the aluminum bags for sampling) which may result in slightly differing degrees of agitation and consequent susceptibility to disarticulation. However, comparisons of the results of decay under different experimental conditions suggest faster rates under rapid (1a) as opposed to slow (1b) oxygen diffusion (based on the timing of the appearance of the later stages in *Crangon*) (Fig. 3). The onset of Stage 4 (Disarticulated) within 2 weeks in anoxic conditions (1d) (Fig. 3) is based on a single specimen; other examples did not reach this stage until week 8. The addition of bicarbonate and phosphate did, however, hasten morphological decay, particularly the onset of disarticulation. These differences in rates of morphological decay are not reflected in the weight loss profiles (except where phosphate was added in anoxic conditions—see below) but this is not surprising as the great bulk of the weight loss takes place in the first week. The observations also suggest slightly faster rates of morphological decay in *Crangon* than in *Palaemon* particularly in the later stages, disarticulation and fragmentation. This presumably reflects the smaller size range and less robust cuticle of *Crangon* (less than 0.1% Ca whereas *Palaemon* has ca 8% Ca), and the lower proportion of tissue resistant to acid and alkali hydrolysis relative to total weight (8.6% and 14.4% in *Crangon* and *Palaemon* respectively).

The later stages of morphological decay (disarticulation and fragmentation) are hastened if the carcass floats. Neither *Crangon* nor *Palaemon* showed a tendency to become buoyant in standard ASW (*Crangon* up to 8 weeks, conditions 1a–f, 1% floated, n = 180). With the addition of extra bicarbonate, however, 10% of *Crangon* specimens floated (1c, d, n = 60). Under these conditions specimens also showed a greater tendency to disarticulate (Fig. 3). In contrast to our results, Allison (1986) reported that carcasses of *Palaemon* allowed to decay in sediment and seawater floated to the surface of the experimental vessel within three days, buoyed up by decay gases. He argued that the generation of gas would likely prevent the carcasses from being buried in bottom sediment, and allow it to be transported by the gentlest of currents. Because Allison's decay experiments were run at lower temperatures than ours (5–8° C as opposed to 20° C), a reduced rate of decay and slower build-up of gas at the lower temperature might have increased gas retention in the tissues. On the other hand, there was no reduction of decay rate corresponding to the greater tendency for carcasses to become buoyant in our experiments with elevated concentrations of bicarbonate (Fig. 6C). The greater concentration of bicarbonate in the ASW may have hindered dissolution of decay-produced CO_2, and promoted its build-up in the carcass.

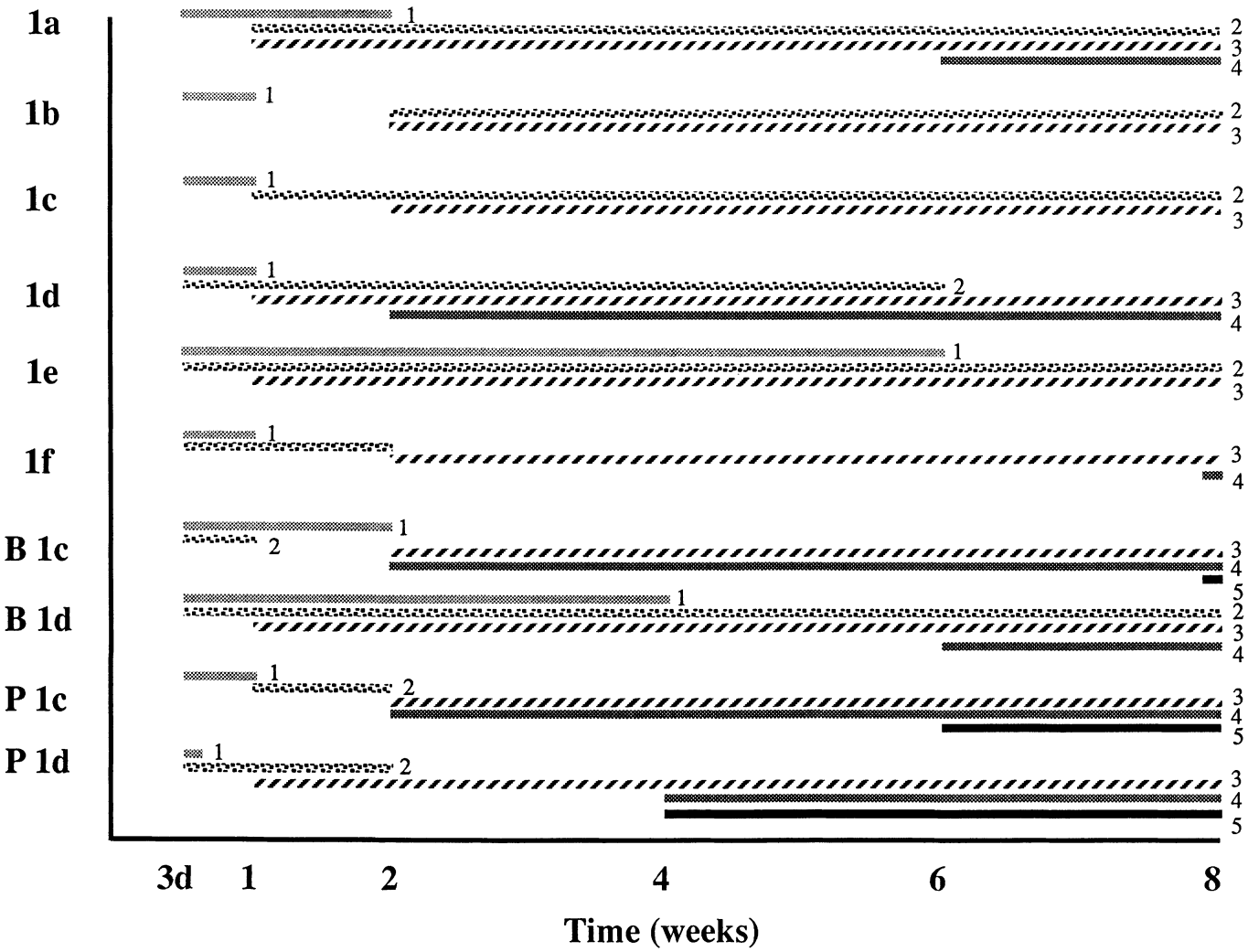


FIGURE 3—Sampling times when Stages of Decay were observed in different experimental runs on carcasses of *Crangon* (see Fig. 2). The ranges indicate the total period over which a particular stage persisted. Observations were made after 3 days, and 1, 2, 4, 6 and 8 weeks.

QUANTITATIVE CHANGES DURING DECAY

Oxygen Concentrations

Experiments were run under six different oxygen conditions (Fig. 2), but in the longer term they converged to two which can be characterized as oxygen *available* and oxygen *eliminated*. Where initial oxygen saturations in the ASW were ca 50% (1a–c, e), this was reduced by bacterial activity to below 18% (the level at which organic matter decomposition begins to be oxygen-limited; Parkes and Buckingham, 1986) within 1 day. Indeed oxygen reached an effective zero saturation in 1 day even in conditions of rapid diffusion (1a) (Fig. 4). Thus decay within the carcass must have become oxygen-limited and probably anaerobic within hours. Where rapid rediffusion was permitted, oxygen levels recovered once the more metabolizable tissues were consumed, reattaining the initial 50% level within 10 days for 0.2 g shrimp (Fig. 4) and within 2 to 8 weeks for 0.4 and 0.6 g shrimps. In conditions of slow diffusion, oxygen levels approached starting levels within 8 weeks but did not always recover to 50%. Where oxygen was absent (1d) or consumed (1f) and diffusion was prevented, levels remained at zero.

pH

All ASW was prepared with an initial pH of 8.00 (Fig. 5). Data for experiments on *Crangon* were recorded over 8 weeks, except for conditions 1a and b where experiments were run for 25 weeks. Where rapid diffusion of oxygen was possible (1a) pH rose within three days and remained above 8.3 (only falling below 8.00 in week 20). Under other conditions pH fell to its lowest value within 3 days. Where only slow diffusion was permitted (1b) it dropped to 7.34

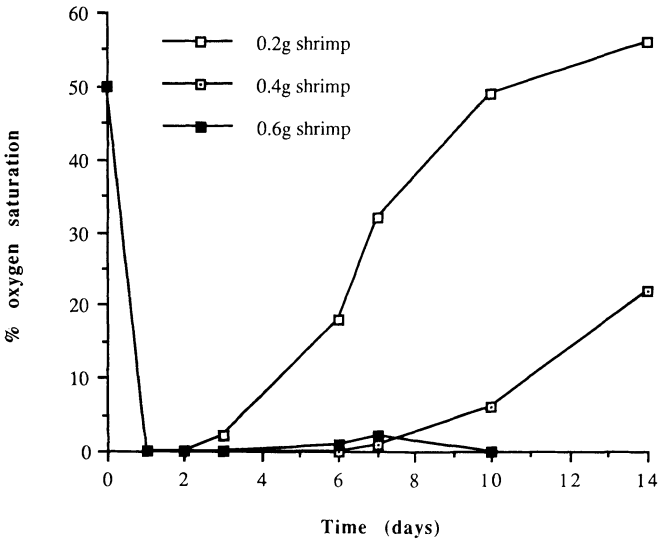


FIGURE 4—Trends in the percentage oxygen saturation of ASW over time for three specimens of *Crangon* of different sizes decaying under conditions of rapid diffusion (1a). The oxygen saturation in standard ASW is ca 50% at day 0.

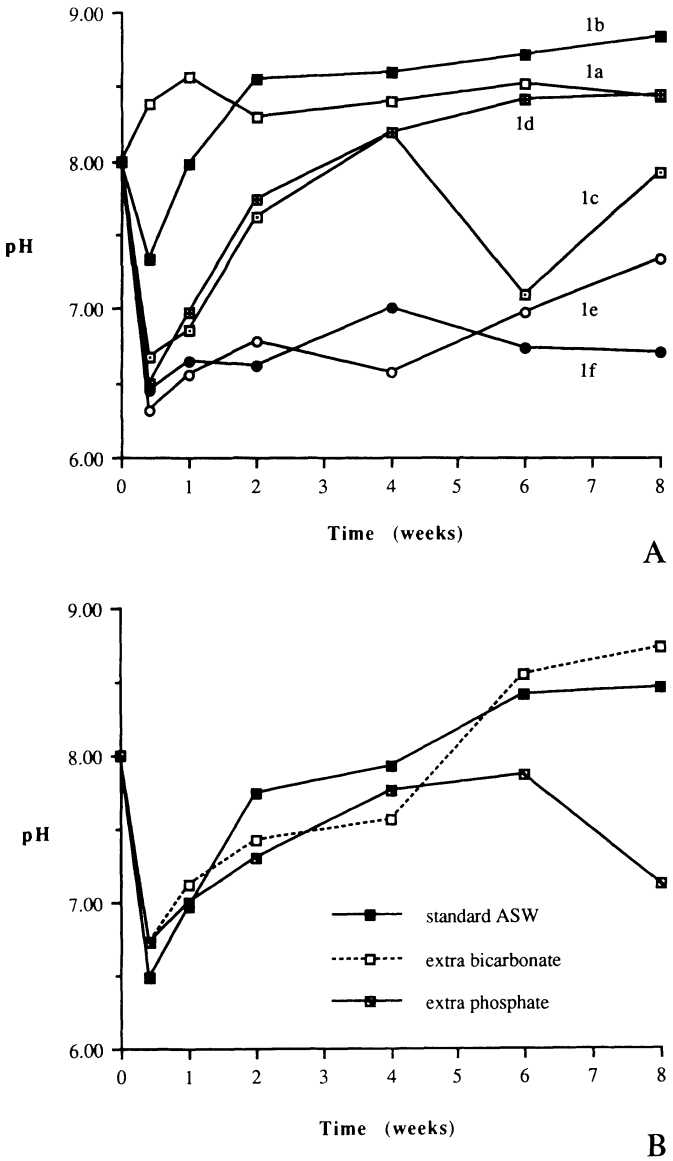


FIGURE 5—(A) The variation in pH over time where *Crangon* was decayed under different oxygen conditions: rapid diffusion (1a); slow diffusion (1b); no diffusion (1c); completely anoxic (1d); limited diffusion (1e); limited diffusion, anoxic water (1f). (B) pH under conditions 1d with additional phosphate and bicarbonate. The pH at day 0 is 8 in all cases.

but recovered within 1 to 2 weeks, only falling below 8.00 in week 25. In vessels which were sealed in aluminum bags with a N_2/CO_2 atmosphere (1c, 1d) the pH fell further (to 6.67 and 6.49 respectively). It did not recover to the original level until week 4 whether or not oxygen was present in the ASW at the outset. Where limited oxygen was available (1e, 1f) pH dropped to the lowest levels of all (6.32 and 6.41 respectively) and remained relatively constant, mainly below 7.00. All these pH profiles are significantly different (1e and 1f only at the 5% level) (Table 2).

Where the composition of the ASW was altered by adding bicarbonate or phosphate under closed conditions (1c, 1d) the pH induced by decay reached a minimum within 3 days and generally rose thereafter. The profile was significantly different from that in normal ASW; the only exception was where bicarbonate was added in total anoxia (1d) (Fig. 5B; Table 2). With excess bicarbonate, where oxygen was initially present in the ASW (bicarbonate 1c) pH fell to 6.83 and recovered to its former level by week 4. Where oxygen was completely eliminated (bicarbonate 1d) (Fig. 5B), pH fell further, to 6.73, and did not recover until week 6. Where the amount of phosphate was increased and some oxygen was present at the outset (phosphate 1c), pH fell to 6.49 but recovered within 4 weeks. In the absence of oxygen (phosphate 1d) (Fig. 5B), however, pH fell to 6.73 but did not recover its former level, reaching a maximum of 7.86 by 6 weeks.

To determine the effect of the anaerocults alone on pH, they were sealed in aluminum bags in vessels containing deoxygenated ASW but no carcass. The pH stabilized within one to two days at around 6.24 (n = 6, SD = 0.16) with Anaerocult A, and around 5.92 (n = 6, SD = 0.09) with Anaerocult C.

Weight Changes

The progress of decay was monitored by change in weight (Fig. 6) and in chemical composition, as well as by changes in decay stage. The size of the shrimp affects levels of oxygen in the early stages (Fig. 4) but there is no correlation between percentage weight loss (dry weight) and the initial weight of the carcass.

Whereas dry weights provide a reliable indication of differences in decay rate, wet weights are very variable.

TABLE 2—Results of statistical comparison of the pH data for *Crangon* using the General Linear Model on the Minitab 8.1 statistical package. Results are expressed as P values. NS = not significant.

	1a	1b	1c	1d	1e	1f	Phos 1c	Phos 1d	Bic 1c
1a									
1b	0.008								
1c	<0.001	<0.001							
1d	<0.001	<0.001	<0.001						
1e	<0.001	<0.001	<0.001	<0.001					
1f	<0.001	<0.001	<0.001	<0.001	0.015				
Phosphate 1c	—	—	<0.001	—	—	—			
Phosphate 1d	—	—	—	<0.001	—	—			
Bicarbonate 1c	—	—	<0.001	—	—	—			
Bicarbonate 1d	—	—	—	NS	—	—			

These presumably reflect other factors, particularly osmotic uptake (Briggs and Kear, 1993a) and a greater susceptibility to experimental error. Wet weight in *Crangon* (Fig. 6A) rose slightly over the first three days (except under completely anoxic conditions (1d)) but declined within a week to 50–72% of the starting weight. By two weeks wet weight rose again; where conditions were anoxic from the outset (1d) it reached a mean value of almost 120%. Weight then declined, but much more slowly where rediffusion of oxygen was permitted than where it was eliminated. It fell to below 30% by week 4. By week 8 this value had declined further except where rapid diffusion was permitted (Fig. 6A).

The oscillation in the wet weight profiles (Fig. 6A) over the first 4 weeks seems at odds with the morphological sequence, which suggests that swelling due to osmosis in the early stages of decay is halted by rupturing of the cuticle and loss of internal fluids. The wet weight profile, on the other hand, shows that osmotic uptake may take place not in one but in two phases, during the first 2 or 3 days as the body fluids equilibrate with sea water only to be lost as the arthrodial membranes tear, and subsequently during the second week as the muscle tissues decay and liquefy. This second phase of osmotic uptake is particularly pronounced in the complete absence of oxygen (1d) and may reflect a slightly more rapid transition through the earlier stages of morphological decay (Fig. 3).

Dry weight profiles (Fig. 6B) show that the major weight loss occurs within the first week when it falls to about 30% or lower. The decay profiles are not significantly different where oxygen levels were allowed to recover through rapid diffusion (1a) or where anoxia was maintained by sealing the experiments in aluminum bags with Anaerocult A either with (1c) or without (1d) oxygen in the ASW at the outset. (The standard deviations on data for dry weight loss under conditions of slow diffusion (1b) were too large to allow reliable inferences to be drawn.) Where limited gaseous oxygen was present (1e, f) weight did not exceed 12.5% from week 2 to 8 (Fig. 6B). These profiles show significantly faster decay than 1c and 1d respectively ($P < 0.001$) (Table 3), although they do not differ significantly one from the other. This is unlikely to be the result of differences in oxygenation (1f converges on 1c and 1d) but may reflect in some way the contrast in pH (Fig. 5A).

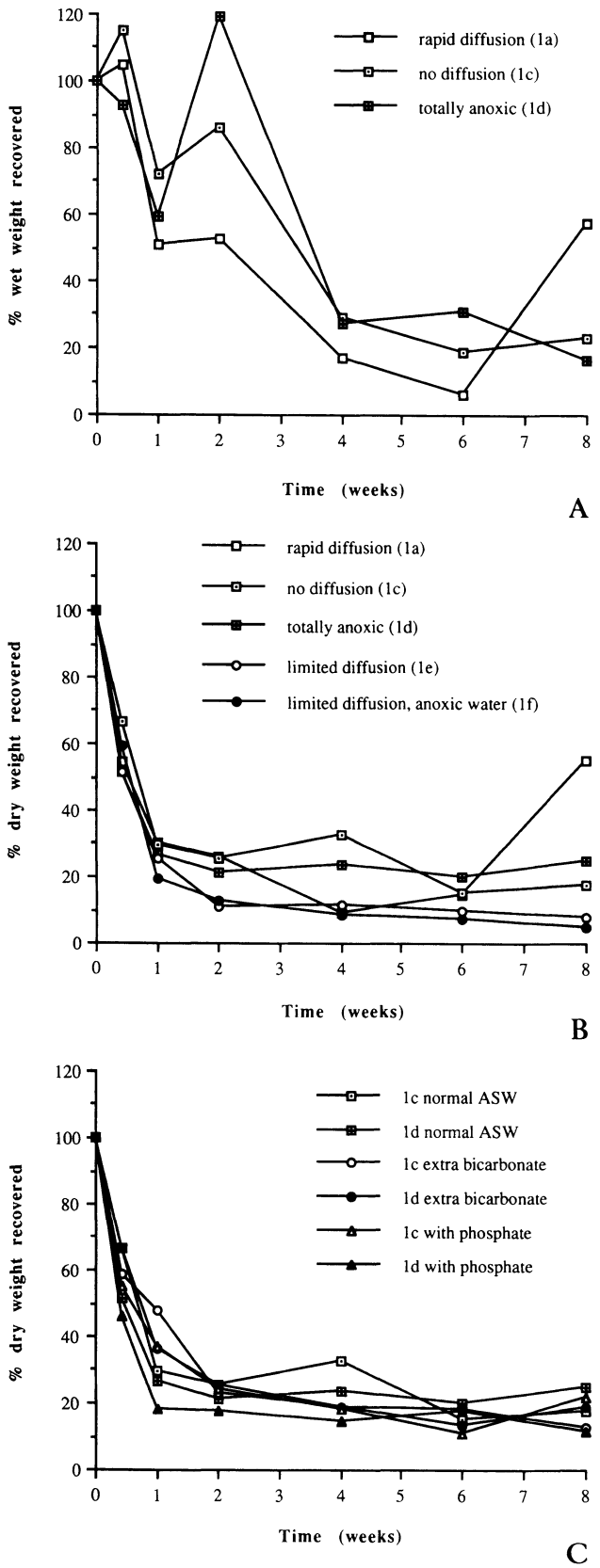
Where phosphate was added to the ASW decay was accelerated, but only in the complete absence of oxygen (1d; Table 3) (Fig. 6C). Where concentrations of bicarbonate were doubled, disarticulation was hastened but there was no significant difference in the dry weight profile (Table 3; Fig. 6C).

CHEMICAL DEGRADATION

Changes in the composition of *Crangon* at varying stages of decay can be expressed as a percentage of the starting dry weight, or as a percentage of the material remaining (Table 4). Over 50% of the starting composition of *Crangon* (expressed as a dry weight) consists of organic C, N, H, PO_4, C in carbonate, and S (total 52.96%: Table 4); the remainder is predominantly O, with 0.046% Ca, 0.002% Mg, and other minor elements. The starting CHN ratio is 1:1.93:0.22 (Fig. 7). Analyses were carried out on specimens decayed under conditions of no diffusion but where the ASW was oxygenated at the outset (1c). TOC dropped over 30% to less than 2% of starting dry weight within a week, declining only minimally to week 8 (Table 4; Fig. 7). H and N also declined dramatically. Predictably the decline in C in CO_3 was much less pronounced. The CHN ratio shifted from 1:1.88:0.19 (Week 1) through 1:1.78:0.12 (Week 4) to 1:1.94:0.14 (Week 8). The fall in the proportion of N reflects the decay of proteins. Only PO_4 showed an increase in percentage compared to the starting dry weight, rising from 3% to over 16% by week 8 (Table 4).

MINERAL PRECIPITATION

Decay of the shrimp carcasses under experimental conditions was accompanied by the precipitation of minerals (Briggs and Kear, 1993b). The styles of mineralization fall into two broad but distinct categories: (1) crystal bundles which form within carcasses and moults, particularly within envelopes formed by the cuticle, and (2) minerals



consisting of microspheres which replicate the soft-tissue itself, especially muscle. These different types of precipitation tend to occur in different parts of the animal (Fig. 8).

Crystal Bundles

Crystal bundles are aggregates of hundreds of micron-sized crystals (Buczynski and Chafetz, 1991). Those produced in our experiments took a variety of forms up to about 300 μm in dimension, including spheres (Fig. 9G), some with a rim (Fig. 9B), rods (Fig. 9E), and dumbbells (Fig. 9A, F). The axes of the individual crystals were oriented parallel to the long axis of the rods, whereas in the spheres they were arranged radially (Fig. 10A–C). The orientation of the individual crystals in dumbbells was a combination of both: parallel to the long axis in the narrow neck of the dumbbell, and radiating out in the swollen extremities (Fig. 9A, F). Where the crystal bundles grew on the cuticle of a moult or the surface of a bacterial film (Fig. 10E, F), or where the meniscus of the water made contact with the side of the glass vessel containing the experiment (Fig. 10A–C), they formed hemispheres rather than spheres. Those which grew on glass clearly show the internal structure: the individual crystals radiate from the nucleus, and irregular concentric bands indicate stages in the growth of the bundle (Fig. 10A–C). Occasionally the bundles were flat or disc-shaped, with a thickened center (Fig. 9B). The rounded surface of the crystal bundles was porous (Fig. 10D). The bundles formed most readily on the cuticle of moults, but they also occurred on the cuticle of carcasses and rarely within muscle and other soft-tissue. The crystal bundles do not reflect the morphology of bacteria: precipitation was bacterially induced, not controlled within bacterial cells.

Crystal bundles precipitated in four more-or-less sequential stages. Initially they formed at the extremities, within the cuticle envelopes created (1) by the scaphocerites (Fig. 11) and (2) by the telson and uropods, and the chelae of the pereiopods. They then precipitated (3) around the margins of the abdominal tergites, and finally (4) in the cephalothorax. The bundles are composed of calcium carbonate (Table 5). X-ray diffraction indicates that the dominant mineral is aragonite.

Formation in Carcasses

The precipitation of crystal bundles was similar in form and extent in carcasses of both *Crangon* and *Palaemon*.

←

FIGURE 6—The percentage weight recovery over time of *Crangon* decayed under different oxygen conditions: rapid diffusion (1a) versus no diffusion (1c), complete anoxia (1d), limited diffusion (1e), and limited diffusion, anoxic water (1f). n = 5 for all points. (A) Wet weight. The curve for 1a is significantly different from the other two ($P < 0.001$). (B) Dry weight. None of the curves is significantly different from the others. (The high values obtained for week 8 (1a) has a high standard deviation and is regarded as unreliable.) (C) Dry weight. Comparison of 1c and 1d where ASW is normal, and where it has added bicarbonate, and added phosphate. Only the 1d curve for added phosphate differs from that with normal ASW.

TABLE 3—Results of statistical comparison of the dry weight data for *Crangon* using the General Linear Model on the Minitab 8.1 statistical package. Results are expressed as P values. NS = not significant.

	1a	1b	1c	1d	1e	1f	Phos 1c	Phos 1d	Bic 1c
1a									
1b	—								
1c	NS	—							
1d	NS	—	NS						
1e	0.001	—	<0.001						
1f	0.002	—	—	<0.001	NS				
Phosphate 1c	—	—	NS	—	—	—			
Phosphate 1d	—	—	—	0.001	—	—	<0.001		
Bicarbonate 1c	—	—	NS	—	—	—	—	—	
Bicarbonate 1d	—	—	—	NS	—	—	—	—	NS

TABLE 4—Composition of shrimps as weight % of starting dry weight. TOC is total organic carbon; C-CO_3 is carbon present as carbonate.

	TOC		C-CO_3		H		N		PO_4		S	
	% start	% now	% start	% now	% start	% now	% start	% now	% start	% now	% start	% now
Crangon fresh	32.63		1.90		5.56		8.96		3.00		0.91	
Crangon week 1	1.88	27.71	1.02	1.20	0.31	4.52	0.44	6.31	1.82	5.10	0.94	1.07
Crangon week 4	1.74	24.03	1.07	1.11	0.27	3.74	0.26	3.59	2.59	10.43	0.38	0.39
Crangon week 8	1.26	19.56	0.72	1.29	0.22	3.19	0.21	3.12	2.45	16.09	0.43	0.93

Bundles formed in the large flat envelopes formed by the scaphocerites, and the uropods and telson. Otherwise they were concentrated around the margins of the dorsal exoskeleton, specifically in the rostrum, along the ventro-lateral margin of the cephalothorax, and in the pockets formed by the doublure fringing the abdominal tergites (Fig. 8).

Crystal bundles normally formed on the inner surface of the cuticle rather than within the soft-tissue. They were commonly flat (disc-shaped) and sometimes formed rosettes, but only occasionally precipitated in sufficient density to coalesce into sheets (Fig. 9D).

Isolated crystal bundles began to precipitate within 1 week. Crystal bundles precipitated in a higher proportion of carcasses in open than in closed systems (percentages of *Crangon* samples with bundles up to 6 weeks: 1a 68% (n = 25); 1b 60% (n = 15); 1a and 1b combined 65% (n = 40); 1c 32% (n = 25); 1d 40% (n = 25); 1c and 1d combined 36% (n = 50); 1e 40% (n = 20); 1f 20% (n = 15)). There is no evidence that the proportion of carcasses in which bundles formed increased with time, although the extent of mineralization in individual carcasses may increase (Fig. 12). Crystal bundles never formed in the tergites and cephalothorax in closed systems (1c, d, e, f, n = 105) but they formed in these areas in open conditions in 53% of specimens where crystal bundles precipitated (1a, b, n = 40).

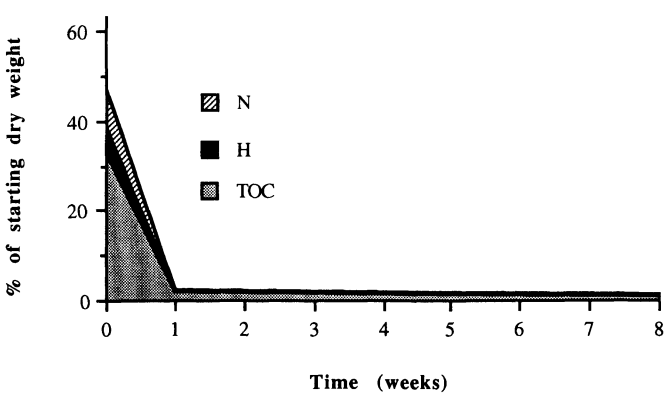


FIGURE 7—Variation in nitrogen, hydrogen, and organic carbon (expressed as a percentage of starting dry weight) in *Crangon* as it decays (1c).

Radiating bundles of widely spaced acicular crystals (Fig. 9C) (?aragonite) were observed in a small number of specimens of *Palaemon* growing within and displacing unmineralized soft-tissue in the earliest stages of decay (1a). Laths of magnesium phosphate 2–3 mm long were observed on a *Palaemon* carcass which had decayed for 75 weeks in conditions of slow diffusion (1b) (Table 5).

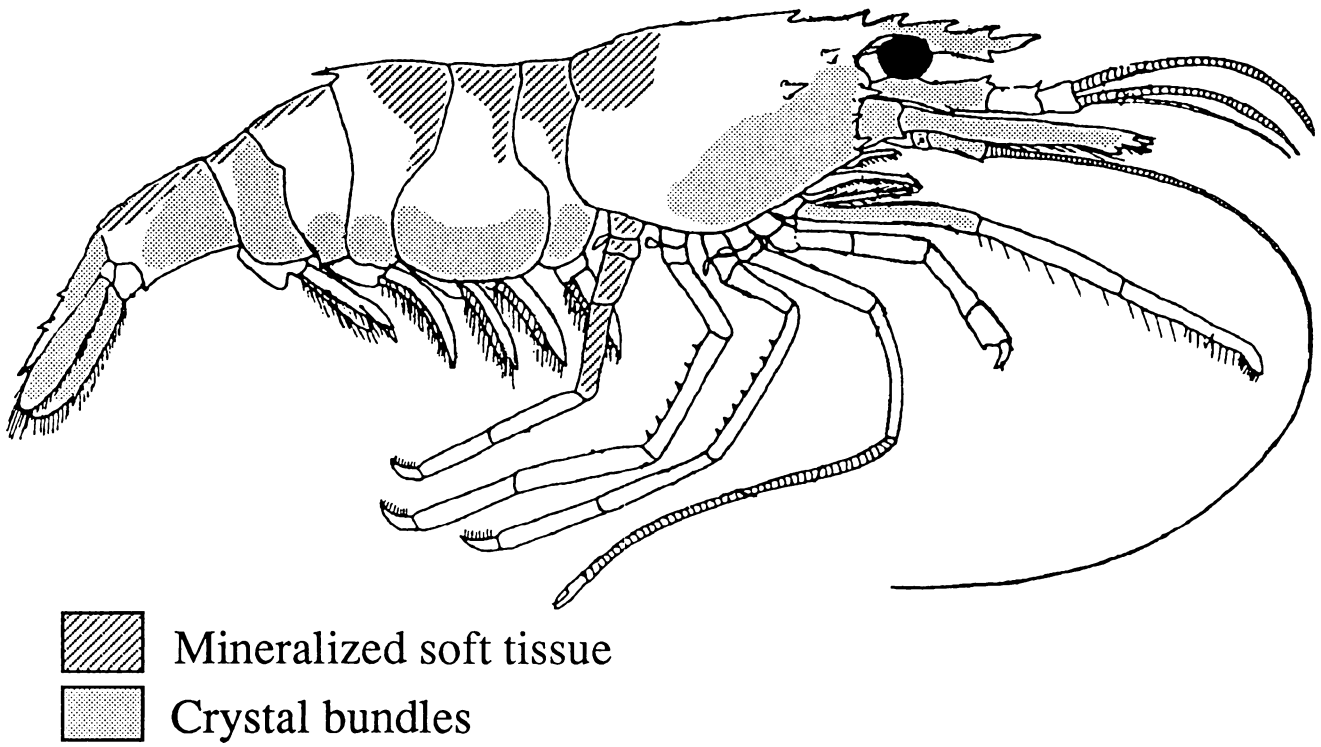


FIGURE 8—Idealized shrimp showing where the two different types of mineralization, crystal bundles and mineralized tissue, commonly occur.

Formation in Moults

Crystal bundles formed much more extensively in moults than in carcasses. As decay proceeded the cuticle of the moult became thinner and more flexible, and the arthrodial membranes weakened and ruptured. The small crystal bundles which formed initially—in the scaphocerites (Fig. 11A), rostrum, telson and uropods, and the limbs—were usually spherical or hemispherical. They were rarely flat, in contrast to those which formed in carcasses. They increased in size with time, and sometimes formed the nucleus of a "rosette" of similar bundles. Where large numbers of crystal bundles precipitated in close proximity they coalesced to form a sheet (Fig. 11B), with the individual bundles within remaining relatively small. Finally, as precipitation continued, sheets of bundles formed in all parts of the moult (but only very rarely in the eyes and antennae). The sheets were essentially two-dimensional, forming in association with one cuticle surface. They became dense and white in appearance and, particularly if the specimen was disturbed, they sometimes peeled away under their own weight or attached to an inner layer of cuticle. Crystals very rarely formed on parts of the exterior as well as the interior of the cuticle.

Crystal bundles precipitated in all experiments on moults. They formed in all parts of the exoskeleton including the tergites and usually the cephalothorax of all those moults decayed in open conditions (*Crangon,* 1a, b, periods of 1 to 8 weeks: n = 19) and to the same extent in 77% of those decayed in closed conditions (*Crangon,* 1c, d, periods of 2 to 6 weeks: n = 13; they formed in the scaphocerites of the remainder). A small number of single experiments demonstrated that crystal bundles also formed in most parts of the exoskeleton where additional bicarbonate, phosphate, or sediment was added to experiments in open conditions (1b).

The Effect of Increasing Bicarbonate Concentration

The effect of increasing the concentration of bicarbonate ions by a factor of two was explored by decaying *Crangon* in closed conditions (1c, 1d). The proportion of carcasses in which crystal bundles formed was not increased (1c 32% (n = 25); 1d 32% (n = 25)), but the crystal bundles formed extensive sheets in some specimens. In some instances areas of cuticle were completely covered on the inner surface by these sheets, and they even infilled the cuticle envelope (e.g., in scaphocerites and uropods) to form a crystalline "mould" (Fig. 13A–D). In places groups of crystal bundles deformed or even ruptured the cuticle (Fig. 13B). The dumbbells sometimes varied dramatically in size even within the same part of the carcass (?reflecting rates of diffusion) (Fig. 13C, D). The crystal bundles sometimes formed a sheet of coalescing spheres (Fig. 13E). Rhombs sometimes formed in association with dumbbells (Fig. 13F), presumably as a result of high levels of concentration. Specimens covered in crystal bundles in this manner would

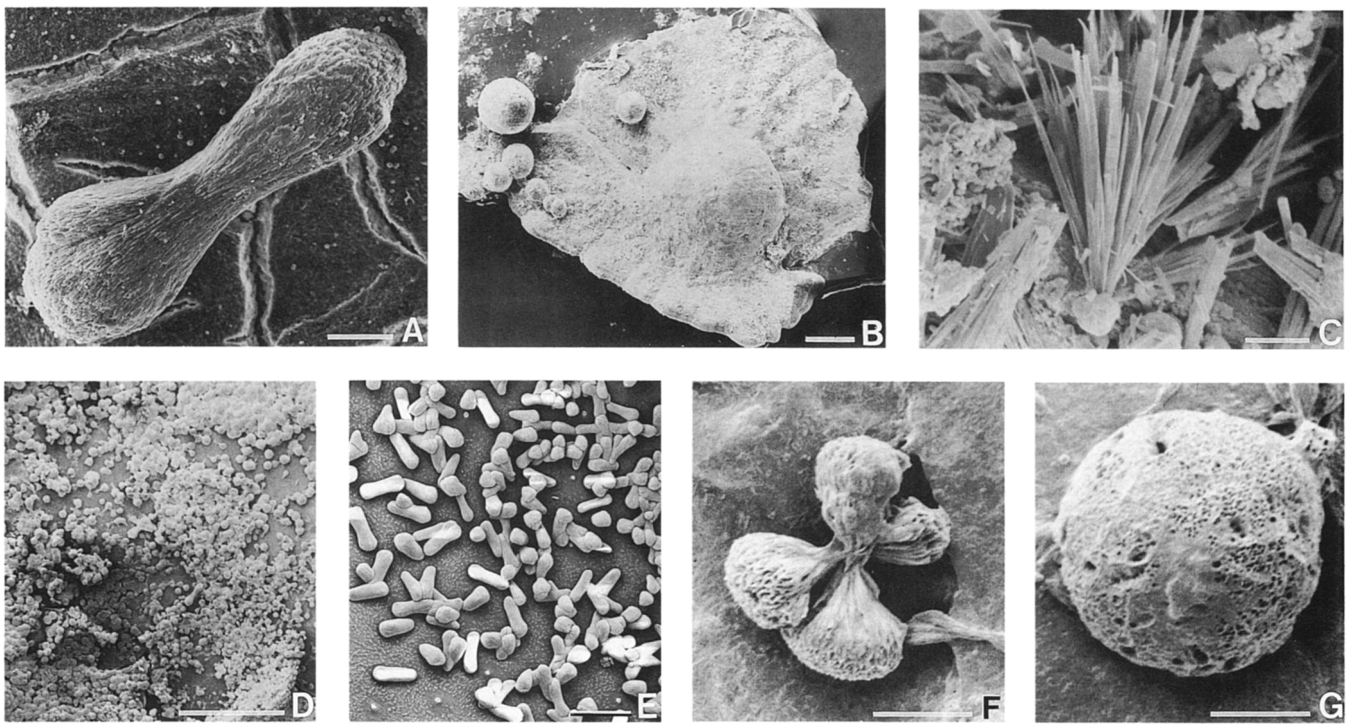


FIGURE 9—SEM illustrations of $CaCO_3$ crystal bundles: different morphologies. (A) Dumbbell in *Palaemon* carcass (10 weeks, 1b). Scale bar is 20 μm. (B) Sphere with rim and smaller isolated spheres in *Palaemon* carcass (5 weeks, 1b). Scale bar is 100 μm. (C) Needles in *Palaemon* carcass (2 days, 1a). Scale bar is 4 μm. (D) Crystal pavement on tergite of *Palaemon* carcass (10 weeks, 1b). Scale bar is 400 μm. (E) Rods in *Crangon* moult left in aquarium (2 days). Scale bar is 40 μm. (F) Dumbbells from *Crangon* moult (2 weeks, 1c). Scale bar is 10 μm. (G) Sphere from same specimen as (F). Scale bar is 10 μm.

obviously resist collapse and compaction, and they may be an important agent in fossilization. The increased concentration of bicarbonate presumably promoted the formation of sheets.

The effect of an increased concentration of bicarbonate on the precipitation of crystal bundles in moults was not systematically investigated, but a small number of single experiments on *Crangon* demonstrated that sheets of bundles formed in most parts of the exoskeleton in those instances where additional bicarbonate was added to moults decaying in open conditions (1b).

Discussion

A causal relationship between organic decomposition and calcium carbonate precipitation has long been recognized (see Chafetz and Buczynski, 1992). Spheres, rods and dumbbells similar to those which formed in our experiments have been produced experimentally before (Boquet et al., 1973; Krumbein, 1979; Buczynski and Chafetz, 1991; Chafetz and Buczynski, 1992), although not in association with decaying invertebrates. Naturally occurring examples have been reported from travertine deposits in Oklahoma, from gelatinous material secreted by bacteria and algae in Storr's Lake, San Salvador Island, Bahamas (Buczynski and Chafetz, 1991), and from modern microbial mats from the coast of Texas and the Bahamas (Chafetz and Buczynski, 1992).

Our experimental results indicate that crystal bundles formed more consistently and extensively in open than in closed conditions. Whether the experiment is open or closed affected not only oxygen but also pH, which fell to a greater extent where diffusion was prevented. The precipitation of crystal bundles was enhanced where pH values remained high in open conditions (Fig. 5). Moults lack the highly labile tissues which generate much of the fall in pH values and thus favored more extensive mineral precipitation. There was a much less pronounced contrast in the extent of crystal bundle formation in open and closed conditions in moults compared to that in carcasses. pH values must have varied in different parts of the decaying carcass, remaining depressed for a longer interval where there was a greater mass of soft-tissue to undergo decay. This presumably explains the formation of crystal bundles mainly in the peripheral areas of a carcass, where the thin muscle tissues decay fastest. Slight evaporation in experiments where oxygen diffusion was permitted may also have contributed somewhat to the more extensive formation of crystal bundles. Buczynski and Chafetz (1991, p. 227) recorded that precipitation of crystal bundles occurred more rapidly in experiments which were aerated. However, the control may not have been oxygen, but some other effect

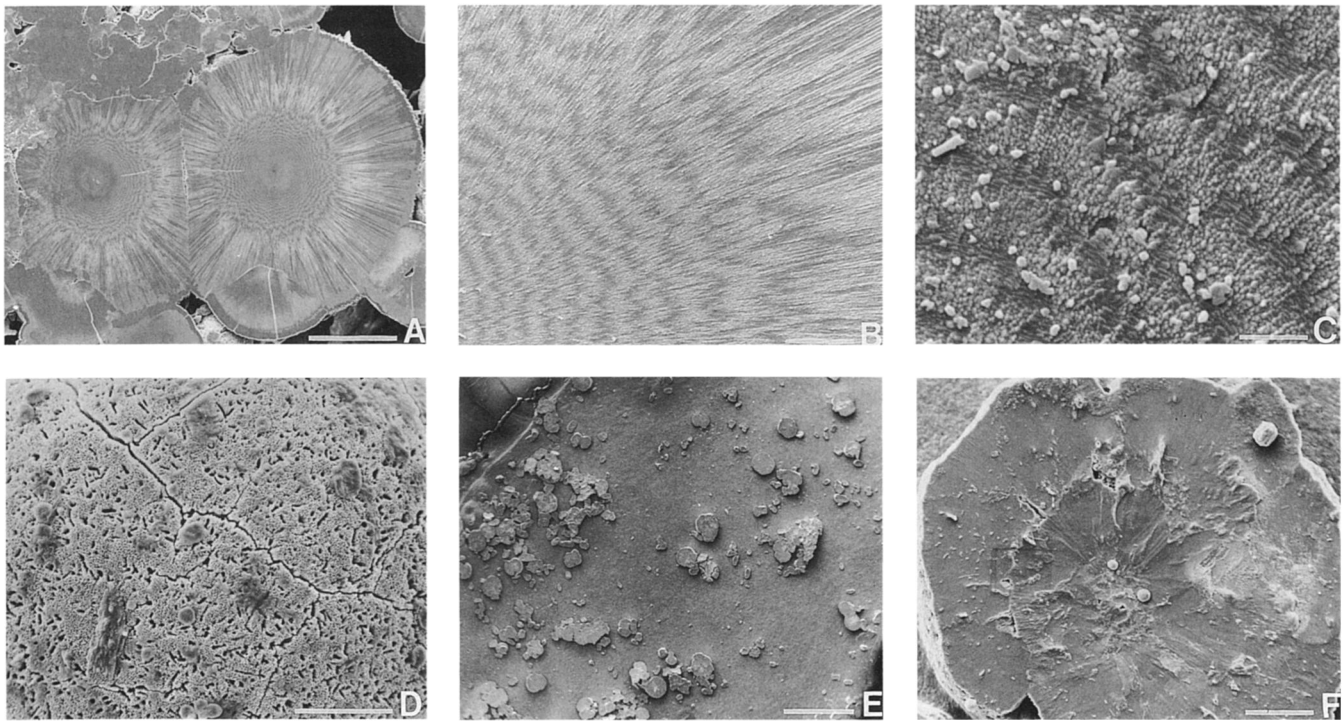


FIGURE 10—SEM illustrations of $CaCO_3$ crystal bundles from bacterial films associated with *Palaemon* carcasses: different textures. (A–D) Crystal bundles peeled away from the jar at meniscus of bacterial film (10 weeks, 1b). (A) Flat surface of crystal bundle which grew on the glass jar, showing the radial growth structure. Scale bar is 100 μm. (B, C) Details of (A). Scale bars 10 and 2 μm respectively. (D) Rounded surface of crystal bundle showing porous texture. Very few pores contain bacteria—compare with Fig. 19B. Scale bar is 10 μm. (E) Bacterial film from air-water interface (3 weeks, 1b) with crystal bundles. Scale bar is 400 μm. (F) Detail of (E) showing traces of radial structure of crystal bundle. Scale bar is 20 μm.

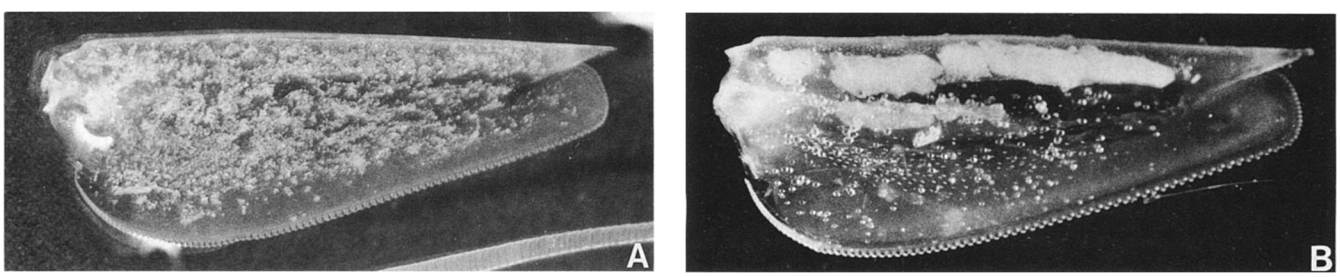


FIGURE 11—Scaphocerites from *Crangon* moults (1 week, 1b), showing differing crystal bundle morphologies (×12.5). (A) Small, scattered spheres and dumbbells. (B) Larger isolated spheres, and crystal pavement where spheres have coalesced.

of aeration such as enhanced evaporation or the prevention of a reduction in pH.

Rounded dumbbells are thought to be unique to bacterially induced precipitates (Buczynski and Chafetz, 1991). Were they to occur in fossil examples this might therefore be diagnostic of bacterially induced precipitation. In practice, however, crystal bundles are likely to be subject to recrystallization and have not been reported in fossil examples (Buczynski and Chafetz, 1991, p. 232). Buczynski and Chafetz (1991) noted that the composition of the minerals formed in their experiments reflected the viscosity of the medium in which they grew: aragonite in liquid, where the ion diffusion rate is high, and calcite in gelatinous media. This appears to be paralleled in the predominance of aragonite rather than calcite in our results.

Mineralized Soft-tissue

Various soft-tissues, including those making up muscles, hepatopancreas, and eggs (Briggs and Kear, 1993b), became mineralized. In contrast to the crystal bundles which precipitated inside the cuticle of carcasses, and more ex-

TABLE 5—Average composition of crystals and mineralized tissues in *Crangon* and *Palaemon* carcasses (decay times in weeks) and *Crangon* moults (decay times in days). Mineralized muscle tissue of a shrimp from the Solnhofen Limestone (Museum of Comparative Zoology MCZ 100836) is included for comparison (it contains 4.1% FO in addition). Oxide weights based on electron microprobe analyses (total given as weight percent of sample mineralized). Ratio of calcium phosphate to $CaCO_3$ based on the assumption that all P_2O_5 is incorporated into ideal OH-apatite [$Ca_5(PO_4)_3OH$]. The $CaO:P_2O_5$ ratio is 1:1.32 (based on molecular weights, ignoring H and excess O). The remaining CaO is assumed to form $CaCO_3$.

Material	Shrimp	Weight percent of sample mineralized	Na_2O	MgO	SiO_2	P_2O_5	SO_3	CaO	CaO in amorphous phosphate	Ratio of phosphate to $CaCO_3$ (as percent)
Crystal bundle in moult 2 d (1b)	*Crangon*	51.02	0.8	0.3	0.11	0.2	1.1	50.3	0.3	0.6:99.4
Crystal bundle in muscle 8 w (1d)	*Crangon*	51.54	0.8	1.6	0.01	1.2	0.6	47.3	1.6	3.4:96.6
Crystal pavement (bicarb) 8 w (1c)	*Crangon*	52.25	0.3	3.6	0.06	1.4	0.4	46.5	1.8	3.9:96.1
Mineralized muscle 8 w (1d)	*Crangon*	40.18	1.0	1.2	0.03	14.9	0.6	22.5	19.7	87.6:12.4
Mineralized muscle 15 w (1d)	*Palaemon*	33.72	0.3	1.4	0.18	12.2	0.6	19.0	16.1	84.7:15.3
Fresh cuticle	*Crangon*	44.22	1.3	0.8	0.13	2.1	0.4	39.3	2.8	7.1:92.9
Cuticle 6–8 w (1d)	*Crangon*	64.06	0.5	1.6	0.13	23.1	1.1	38.1	30.5	80.1:19.9
Egg 25 w (1d)	*Palaemon*	16.85	0	0.3	0.11	3.5	0.4	11.8	4.6	39.0:61.0
Crystal laths 75 w (1b)	*Palaemon*	64.04	0.4	21.5	0.19	41.5	0.2	0.02	0.02	—
Muscle from Solnhofen shrimp	*Antrimpos*	90.66	1.1	0.2	0.01	33.3	1.2	50.7	44.0	86.8:13.2

tensively in moults, these minerals consisted of microspheres and appeared essentially amorphous, and they replicated the structure of the tissue itself. Fresh muscle tissue shows the fibers with banding on the individual fibrils (Fig. 14A). Where the fibers are torn away the sarcolemma that encloses them is evident (Fig. 14B). As the muscle began to break-down it acquired a paste-like texture and collapsed if it was disturbed before mineralization took place (Fig. 14D). If this paste was fixed before it completely decayed (or became mineralized) it revealed no structure (Fig. 14C) except, in some cases, bacteria covering the surface (Fig. 14E, F). Even in such specimens, however, the muscles were sometimes mineralized where they insert on the cuticle. The attachment of the fibers sometimes survived (Fig. 14G, H) (giving the appearance of a dusting of icing sugar under the binocular microscope) or the sarcolemma which surrounded them (Fig. 14I).

Muscles were mineralized in many experiments, as traces of sarcolemma and/or fibers (Fig. 15A, B). The sarcolemma could be preserved by a coating of mineral even where the fibers enclosed by it had decayed (Figs. 15C, D). Where fibers were mineralized (Fig. 15E), structural details were rarely preserved (Fig. 15F), although traces of the banding on the muscle fibrils (probably corresponding to the M-lines where the fibrils rupture) were evident in a few examples (Fig. 15G, H). The microspheres of mineral replicating the muscles were normally less than 1 μm across (Fig. 15D, F, H). The hepatopancreas was frequently mineralized, although the resultant granular texture revealed no structural detail (Fig. 16A). Eggs, which are ca 0.5 mm in diameter, were normally covered with an irregular, relatively smooth, membrane. Inside this, scattered irregular spheres up to 5 μm in diameter occurred within a disorganized open meshwork (Fig. 16B). Although a thick carpet of bacteria coated the carcasses in the earliest stages of degradation (fixed preparations), they did not become mineralized *en masse*. However, isolated bacteria (spheres and rods) 1–4 μm in dimension were occasionally associated with mineralized material (Briggs and Kear, 1993b).

Analysis with electron microprobe revealed that the mineralized muscle tissue is predominantly calcium phosphate (>80%) (Table 5). The lack of definite peaks in the x-ray diffraction pattern indicates that this material is very finely crystalline or amorphous; some peaks may indicate that calcium soaps are also present. The amount of phosphate in the cuticle also increased with decay although the proportion of calcium remained similar. The eggs were less completely mineralized than the other tissues with a much lower proportion of phosphate to carbonate (Table 5).

Mineralization of soft tissue, like the precipitation of crystal bundles, took place in stages, and the amount of mineralization increased with time. Some decay is clearly required to promote precipitation; if the muscle was disturbed at an early stage it behaved like a mud flow, collapsing and flowing over the cuticle. The next stage involved the mineralization of small scraps of tissue (<1 mm, usually 0.1–0.5 mm in dimension) including muscle, scraps of the hepatopancreas, and even complete eggs. Finally blocks of tissue 1 to 3 mm in size became mineralized; muscle was common, and the entire hepatopancreas was mineralized in many specimens. Some of these larger blocks were in situ. Mineralization was concentrated where

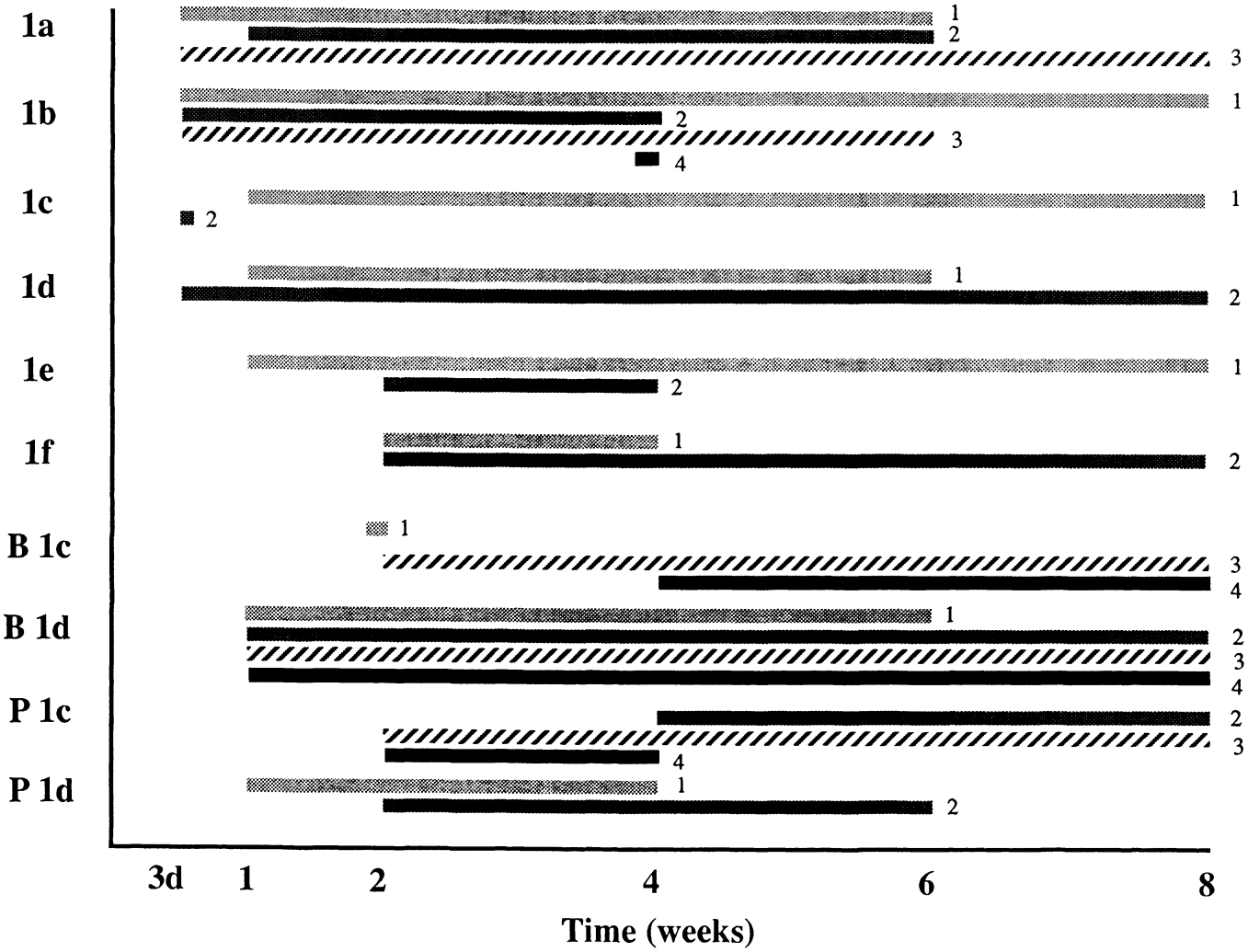


FIGURE 12—Sampling times when stages of precipitation of crystal bundles were observed in different experimental runs on carcasses of *Crangon* (see Fig. 2). The ranges indicate the total period over which a particular stage persisted. Observations were made after 3 days, and 1, 2, 4, 6 and 8 weeks.

the muscle was in contact with the surface of the tergites (Fig. 17). As the unmineralized muscle decayed it shrank away from the cuticle and collapsed into the lower part of the abdomen. Finally the tergites themselves collapsed, and this would lead ultimately to compression and compaction of mineralized tissue in a fossil (Fig. 17).

As in the case of the precipitation of crystal bundles, the extent of soft-tissue mineralization was influenced by whether the system was open or closed. Mineralization can be roughly quantified in three ways: in terms of the proportion of experiments in which it occurred, the degree of mineralization (scraps, blocks etc.), and the length of time required. Where oxygen was available, through either rapid or slow diffusion, preservation of muscle tissue occurred less frequently and consisted only of scraps (in weeks 4 to 8, 20%, n = 15, recorded in *Crangon* in conditions 1a, none in *Palaemon* or *Crangon* in conditions 1b; 13%, n = 80, in *all Palaemon* run under 1b). In closed conditions mineralization of recognizable muscle tissue in *Crangon* occurred in 53% of the experiments where the water was oxygenated at the outset (1c), and 60% where the experiment was completely anoxic from start to finish (1d) (4 to 8 weeks, n = 15, in both cases). Where limited diffusion was possible (Anaerocult C), mineralized tissue occurred in 67% of experiments where the water was oxygenated at the outset (1e) but only 27% where it was not (1f) (4 to 8 weeks, n = 15, in both cases). Mineralization occurred in 67% of *Palaemon* under the conditions in 1d (4 to 8 weeks, n = 15). A chi-squared test provided confirmation that there is a significant difference in the degree of mineralization under open and closed conditions. A consideration of the proportions of experiments where mineralization occurred under conditions 1a, b, c and d shows that there is a significant deviation from the average in

each case ($P < 0.001$). Conversely the proportions where mineralization occurred under conditions 1c, d, e and f show no significant deviation from the average. The low percentage of mineralization in experiments under conditions 1f is anomalous.

Blocks of tissue were mineralized earlier in closed than in open systems (Fig. 18), forming within 4 weeks (in 1c, d, e) compared to 6 (in 1a—no mineralized tissue was observed within 8 weeks in 1b).

Where additional bicarbonate was added to *Crangon* decaying in closed conditions the proportion of specimens in which mineralization of recognizable muscle tissue occurred fell from 53% to 27% where the water was oxygenated at the outset (1c), and from 60% to less than 1% where the experiment was completely anoxic (1d) (4–8 weeks, n = 15 in all cases).

The Effect of Adding Phosphate

The addition of phosphate (Table 1) resulted in more rapid morphological decay in closed conditions. Specimens disarticulated within 2 weeks and fragmented within 6 where oxygen was present in the ASW at the outset (1c), whereas neither of these stages was reached within 8 weeks without added phosphate (Fig. 3). Specimens fragmented within 4 weeks where oxygen was absent (1d), a stage which was not reached within 8 weeks without added phosphate. These differences in the rate of morphological decay were only reflected in the weight loss profile where oxygen was absent (1d); decay was significantly faster with additional phosphate than without (Table 3; Fig. 6C) presumably reflecting increased bacterial activity. The proportion of specimens with some mineralized tissue after 4 to 8 weeks increases with added phosphate only where oxygen was present (1c: 67–73%, n = 15); it falls where oxygen was absent from the outset (1d: 40%, n = 15) (if all the data for closed systems sampled from 4 to 8 weeks is pooled the addition of phosphate has no impact). In both cases, however, blocks of muscle become mineralized *in situ* within 2 weeks compared to 4 (Fig. 18).

In a typical experiment (where a 0.4 g *Crangon* is added to 50 ml ASW) the shrimp contributed about 80% of the phosphate present even if the highest level of impurity is assumed (Table 6). Even where phosphate was added, the shrimp contributed about 65% (even assuming maximum impurity). The additional phosphate reduced the tendency of crystal bundles of calcium carbonate to precipitate (from 32% to 28% under condition 1c, and from 40% to 20% under condition 1d (in both cases n = 25, experiments terminating up to 6 weeks)—the reduction for the combined data is from 36% to 24% (n = 50)).

Crystal Bundles and Mineralized Soft-tissue in Association

Crystal bundles and mineralized soft-tissue do not often occur in association. Only 15% of experiments in closed conditions sampled from 4 to 6 weeks (1c–f, n = 60) were found to contain both, representing 31% of those specimens in which any soft-tissue mineralization occurred. In each case precipitation of crystal bundles was very limited, and confined to the scaphocerites.

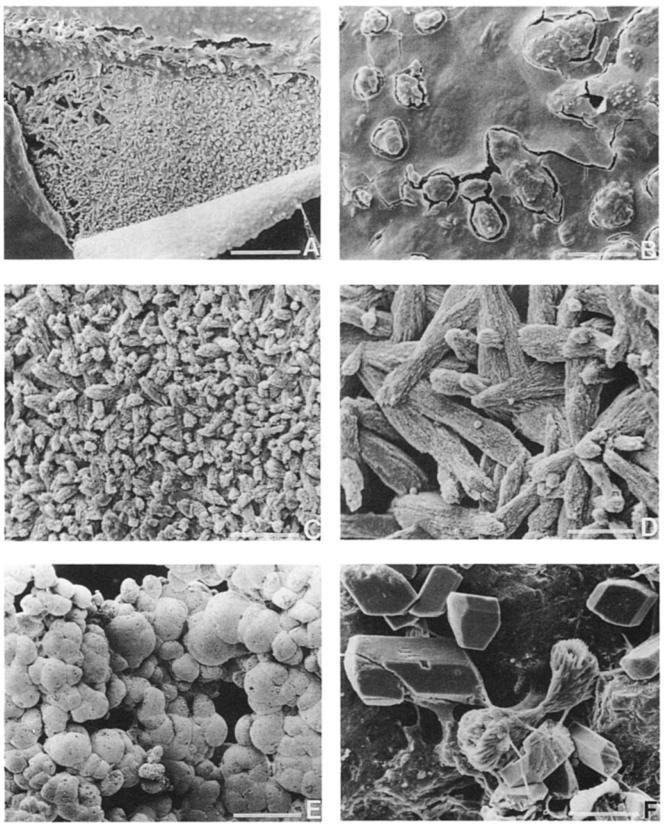


FIGURE 13—SEM illustrations of $CaCO_3$ crystal bundles in *Crangon* moults and carcasses decayed with added bicarbonate (see text for details). (A–D) "Internal mould" preservation in the uropod of a carcass (8 weeks, 1c). (A) Outer layer of cuticle peeled back to reveal inner mould made of dumbbells. Scale bar is 40 μm. (B) Dumbbells pushing up through the surface of cuticle. Scale bar is 20 μm. (C, D) Detail of dumbbells to illustrate the range of sizes preserved in the same uropod. Scale bars 10 μm. (E, F) Crystal form in moults (4 weeks, 1b). (E) Crystal pavement of coalesced spheres. Scale bar is 100 μm. (F) Dumbbells and rhombs. Scale bar is 10 μm.

On sampling some experiments in closed conditions (1c), spherical and dumbbell-shaped crystal bundles of calcium carbonate 100–200 μm long were discovered in association with mineralized muscle tissue. It is clear that the two represent separate phases of precipitation, because the crystal bundles overgrew the mineralized soft-tissue (Figs. 15, 19A, B). Crystal bundles may form in the scaphocerites and tail fan of *Crangon* within a week in such conditions (Fig. 12), and scraps of mineralized soft-tissue may be evident within 2 weeks, blocks forming within 4 weeks (Fig. 18). Overgrowth of crystal bundles on mineralized tissue, however, was not observed in specimens which had decayed for less than 8 weeks.

Electron microprobe analyses of specimens with both phases in association show that while the mineralized soft-

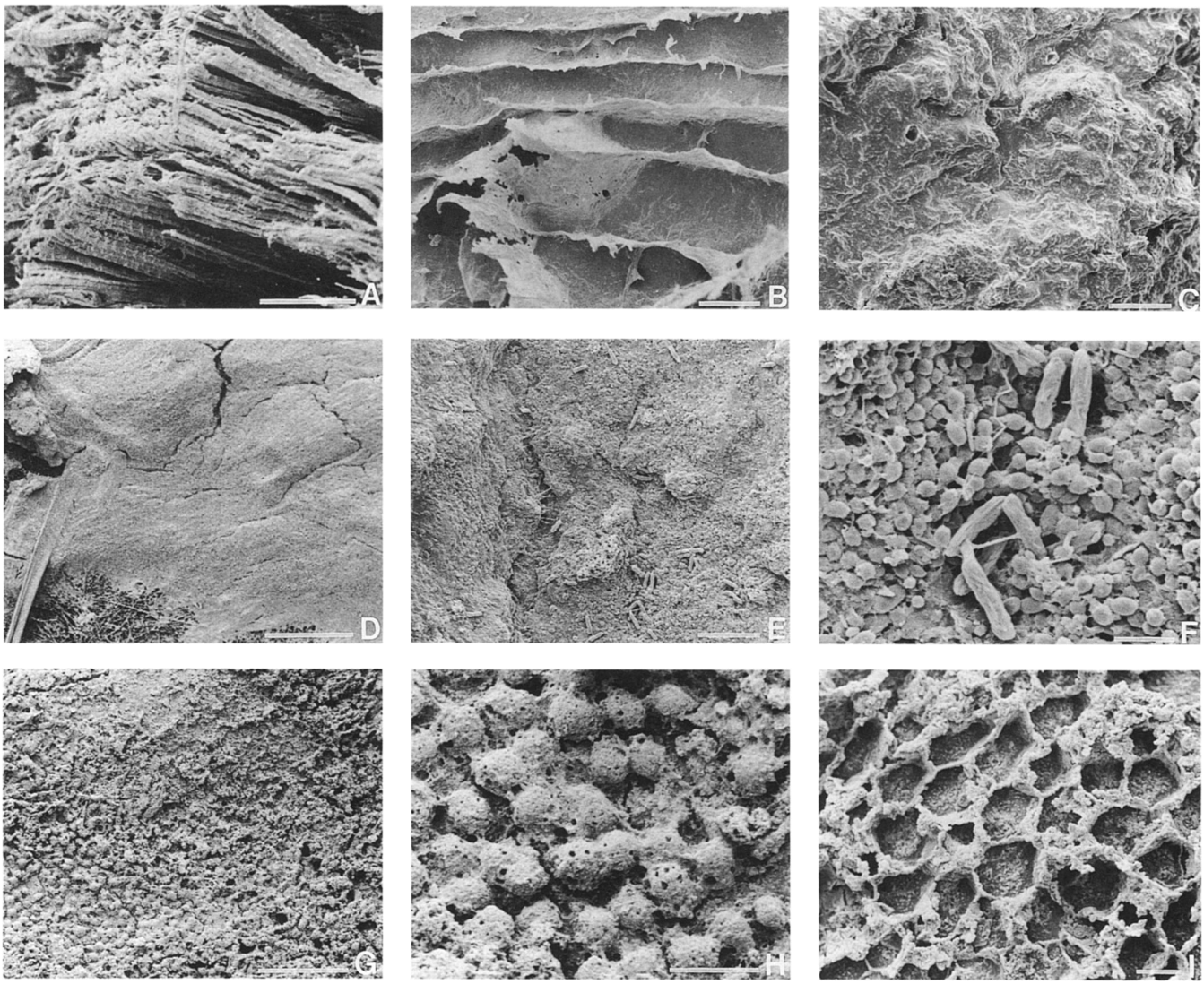


FIGURE 14—SEM illustrations. (A) Fixed fresh muscle fiber from *Palaemon elegans*. Z bands are evident on the individual fibrils. Scale bar is 20 μm. (B) Sarcolemma in a torn *Crangon crangon* muscle decayed for 1 day (1a) and fixed. Scale bar is 20 μm. (C) Dried muscle tissue from *Crangon crangon* decayed for 6 weeks (1c). The material is organic and reveals no recognisable structure. Scale bar is 20 μm. (D) Slumped "paste" of muscle tissue from *Crangon* decayed for 2 weeks (1d) and dried on a glass fiber filter paper. No fibers or structure are evident. Scale bar is 200 μm. (E) Slumped "paste" of muscle tissue from *Crangon* decayed for 4 weeks (1d) and fixed. No fibers or structure are evident, but bacteria are visible covering the surface. Scale bar is 20 μm. (F) Detail of rods and cocci from (E). Scale bar is 4 μm. (G) Thin coating of mineralised material on a *Crangon* tergite decayed for 2 weeks (1d). This is the texture reminiscent of a "dusting of icing sugar" which often incorporates original pigment patterns—see text. Scale bar is 100 μm. (H) Detail of (G). Each of the round blobs represents the remains of a muscle fiber mineralized where it inserts on the cuticle below. The sarcolemma is absent. Scale bar is 20 μm. (I) Polygonal "icing sugar" pattern in *Crangon* decayed for 26 weeks (1d). Here, in contrast to (G) and (H), the fibers have decayed but the sarcolemma is mineralized leaving a polygonal pattern where it inserts on the cuticle. Scale bar is 10 μm.

tissue is composed of calcium phosphate, the crystal bundles are calcium carbonate (Table 5).

Sediment

Sediment was omitted from the main series of experiments as it makes sampling (morphological observations, weight loss, mineralization) very difficult if not impossible to carry out with the accuracy required. A small number of experiments were run in closed conditions (1c) with *Crangon* buried in sediment in order to demonstrate that the occurrence of mineralization was not contingent upon its absence. Thin blocks of *in situ* mineralized muscle formed in all of these specimens and crystal bundles precipitated in the peripheral parts of the exoskeleton of 80% of them (sampled at 4 and 8 weeks, n = 10).

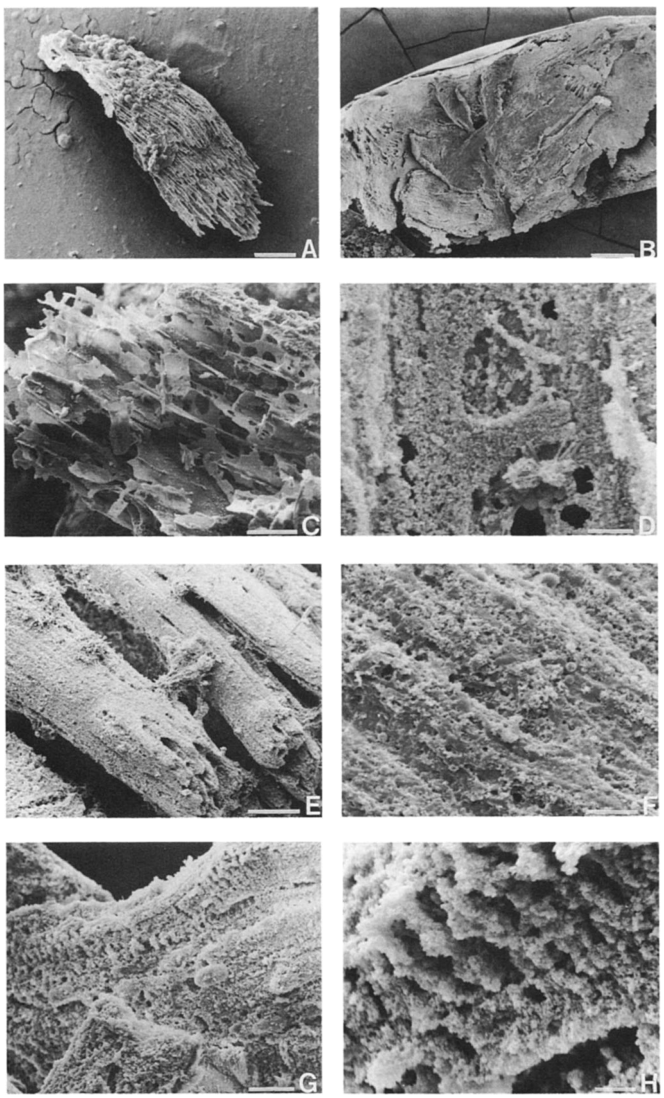


FIGURE 15—SEM illustrations of mineralized soft tissue. (A) Isolated block of mineralised muscle tissue from *Palaemon* decayed for 15 weeks (1c). The sarcolemma is clearly visible at the lower end, but it has been overgrown by $CaCO_3$ spheres and dumbbells at the top. Scale bar is 400 μm. (B) A tergite from *Palaemon* decayed for 10 weeks (1d) unfolded to show the thick coating of mineralized muscle on the inner surface. The sarcolemma is visible in places. Scale bar is 400 μm. (C) Mineralized sarcolemma in a fragment from *Palaemon* decayed for 15 weeks (1d); the fibers have decayed away. Scale bar is 40 μm. (D) Detail of mineralized sarcolemma in (C) showing microspherulitic texture. Some bacteria are present. Scale bar is 4 μm. (E) Mineralized muscle fibers from *Crangon* decayed for 6 weeks (1c). Scale bar is 20 μm. (F) Detail of (E) to show surface texture of fibers. No clear ultrastructure is preserved. Scale bar is 4 μm. (G) Banding (probably M-lines) on mineralized muscle fibrils from the sternite of *Palaemon* decayed for 15 weeks (1c). Scale bar is 20 μm. (H) Detail of (G) showing banding and microspherulitic texture in mineralized muscle. Scale bar is 4 μm.

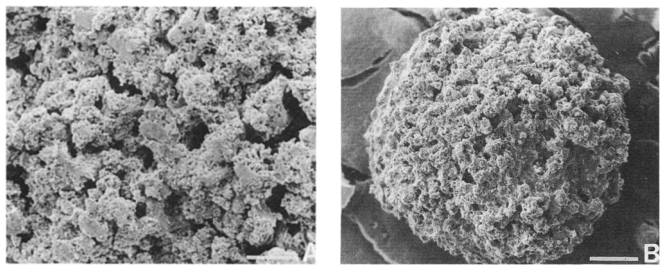


FIGURE 16—(A) Granular surface texture of mineralized hepatopancreas from *Crangon* decayed for 6 weeks (1c). Scale bar is 20 μm. (B) Partially mineralized egg of *Palaemon* after 20 weeks with the outer membrane decayed away (1d). Scale bar is 100 μm.

Discussion

Our experiments show that mineralization of soft-tissue, in contrast to the formation of crystal bundles, occurs more frequently and to a greater extent in closed than in open conditions in laboratory experiments. Decay rate is not significantly different under conditions 1a, 1c and 1d (Fig. 6B, Table 3) even though the amount of tissue mineralization contrasts markedly (Fig. 18). The likely controls revealed by the experiments are oxygen and pH. Where oxygen is eliminated the pH falls rapidly with decay (Fig. 5A) due to the production of CO_2 and volatile fatty acids. This presumably inhibits the precipitation of calcium carbonate and allows calcium phosphate to form (Nathan and Sass, 1981). This correlates with the very rare mineralization of soft-tissue in open conditions (Fig. 18) where pH remains high (Fig. 5). It is also reflected in the location of mineralized tissue away from the extremities of the carcass (Fig. 8), emphasizing that pH must vary in different parts, dropping further where larger masses of soft-tissue are present. The recorded examples of calcium carbonate overgrowing mineralized soft-tissue (Fig. 19A) are presumably the result of the rise in pH values after the initial decay of the most labile tissues (Fig. 5). Concentrations of bicarbonate are also critical. Where they were doubled mineralization of muscle tissue occurred in less than 1% of experiments with no oxygen (1d) compared to 60% in normal ASW, even though the pH profiles were not significantly different (Table 2).

PREVIOUS EXPERIMENTS

Shrimp Decay

Plotnick (1986) investigated the decay of shrimps both in the field and in open and sealed jars in the laboratory, with and without sediment. The results were recorded at intervals of 1, 3, 7, 21, and 28 days, by which time the shrimps were reduced to numerous tiny cuticle fragments. The shrimps were killed by freezing. Observations were qualitative and based on monitoring changes in individual specimens throughout the duration of the experiments; weight loss was not measured. Plotnick (1986, p. 290) concluded that decay "seemed to be somewhat faster in the

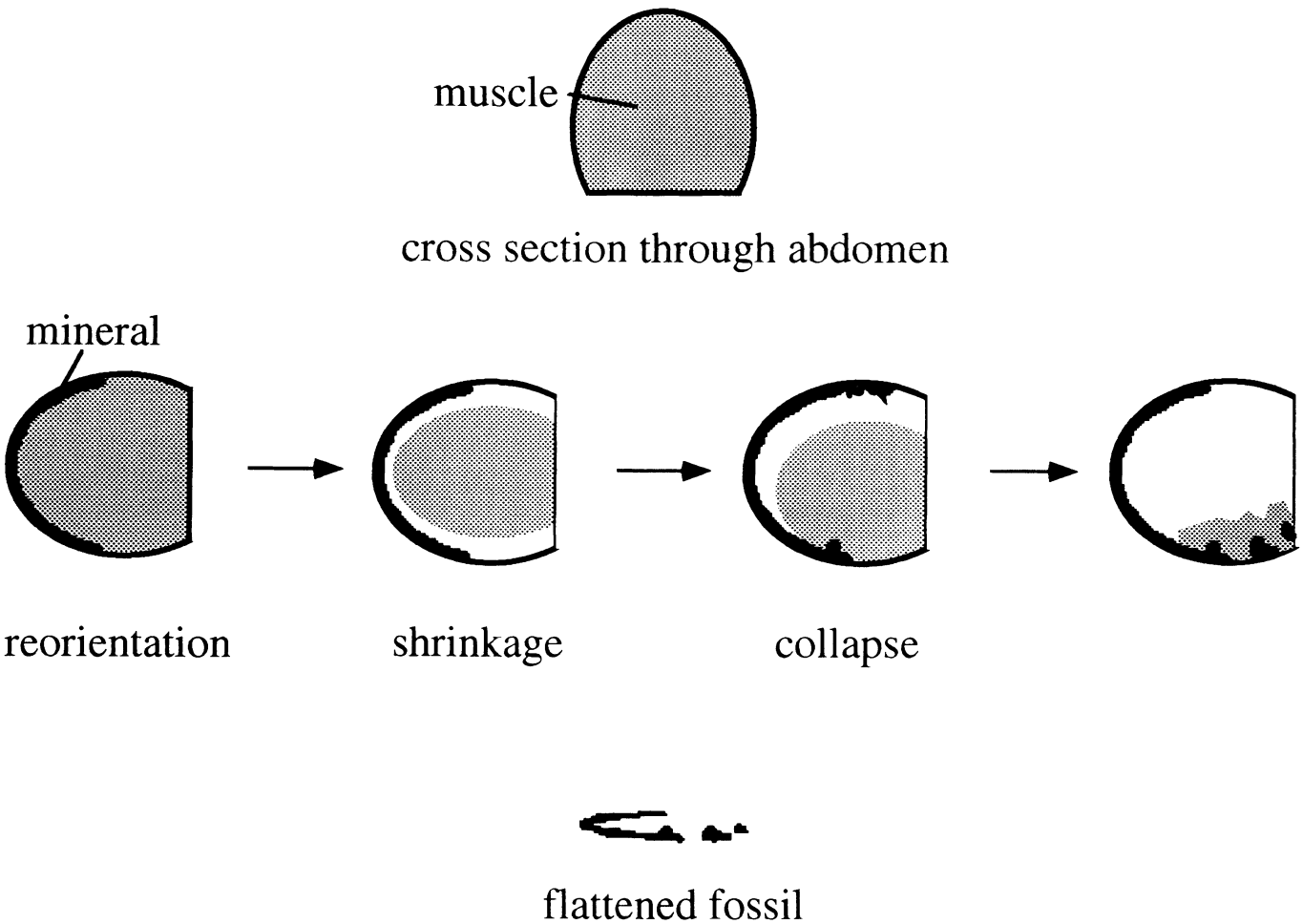


FIGURE 17—Diagrammatic representation of sequence of mineralization of muscle tissue in a carcass, as seen in cross-section. With the onset of decay the abdomen flexes and falls onto its side. Muscles are mineralized on the inner surface of the cuticle. The muscle tissue shrinks and collapses, leaving a hollow trunk. Finally the cuticle itself also collapses.

open jars" but that no difference was evident after 2 weeks. He did not monitor pH or oxygen levels, however, and although sea-water was passed through the tank in which the jars were placed, it is probable that oxygen concentrations converged at low levels in the open and sealed jars in just a few days. Plotnick did run experiments in sealed jars with no sediment (roughly equivalent to our 1b conditions) but he did not record the formation of crystals. His experiments were carried out at a lower temperature (12° C) than ours. He observed (p. 290) that "decomposition appeared to be only slightly faster when sediment was present." Our observations on shrimps showed no obvious difference in rates of morphological decay whether sediment was present or not. Similarly, simple experiments on decay in regular echinoids revealed no difference in the presence or absence of coral reef sediments (Greenstein, 1991). Experiments on the polychaete *Nereis,* however, showed more rapid weight loss in the presence of sediment in the first 6 days. Thereafter the difference was less striking, the percentage weight remaining after 30 days being almost identical in the presence or absence of sediment (Briggs and Kear, 1993a).

Allison (1988a) investigated decay in shrimps, including *Palaemon.* He placed the carcasses in jars containing sediment and water from marine, brackish, or lacustrine environments. The jars were sampled, and the experiments terminated, at intervals of 2, 6, 12, 18, and 25 weeks, i.e., a similar time scale to ours, but much longer than that used by Plotnick (1986). Weight loss was not measured. Allison concluded that anaerobic decay was rapid, virtually destroying shrimp carcasses in 25 weeks. Neither pH nor oxygen levels were monitored, however, but although oxygen may have diffused into the jar during the later stages of the experiments, it is unlikely to have penetrated the sediment to any significant depth. Allison (1988a) demonstrated a marked increase in calcium concentrations in the decaying tissue of *Nephrops,* and in the surrounding sediment through the period of the experiment. He did not record the presence of mineralized tissue nor of crystal bundles. A whitish material sampled from within the car-

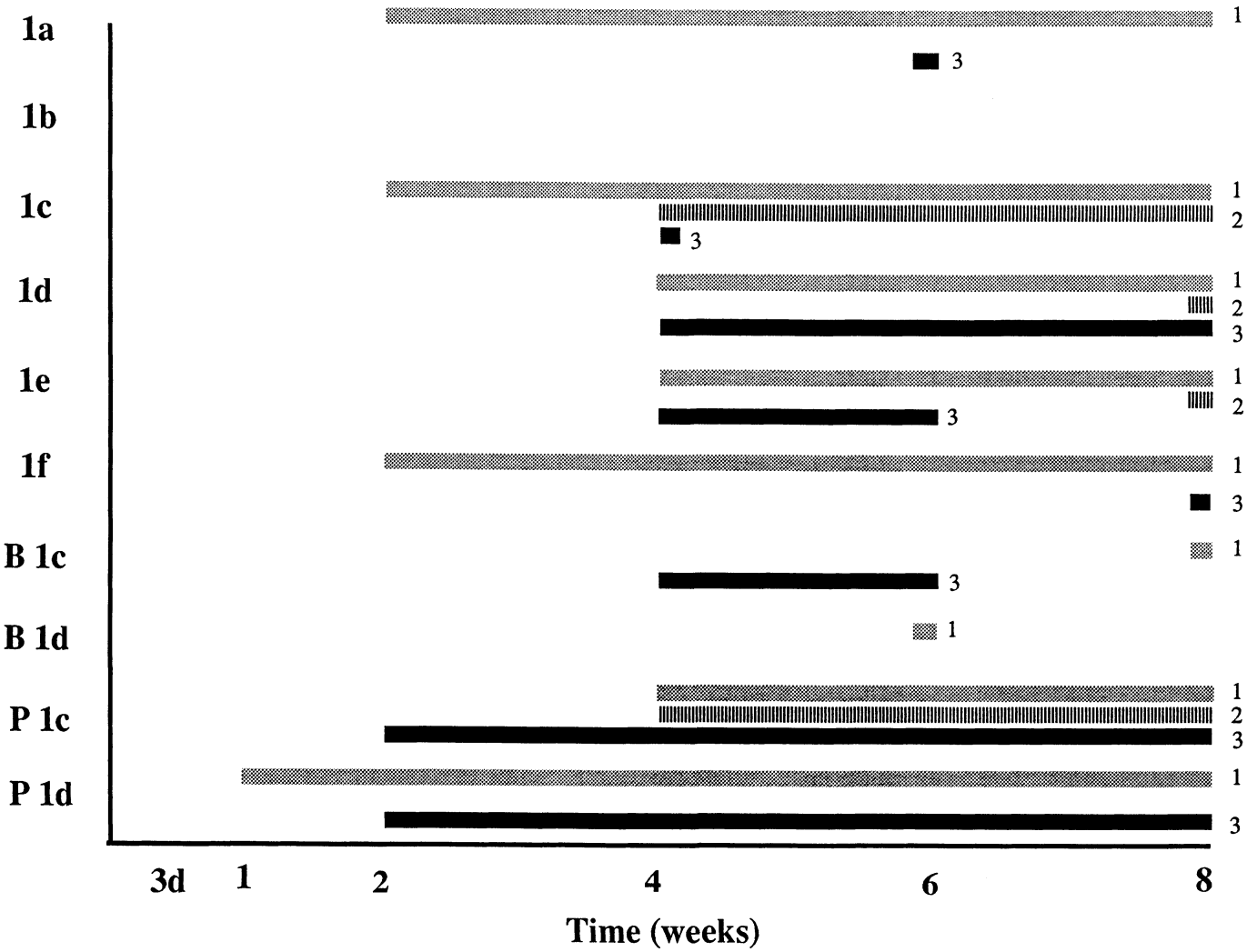


FIGURE 18—Sampling times when stages of formation of mineralized tissue were observed in different experimental runs on carcasses of *Crangon* (see Fig. 2). The ranges indicate the total period over which a particular stage persisted. Observations were made after 3 days, and 1, 2, 4, 6 and 8 weeks.

cass after eight weeks gave x-ray diffraction peaks corresponding to calcium stearate. Allison (1988a) interpreted the excess calcium as partly the result of the formation of this calcium soap, but also the development of calcium-enriched pore waters around the carcass, or the production of a calcium-organic complex. However, amorphous calcium phosphate may also have been present; it does not give definitive diffraction peaks. X-ray diffraction patterns for some of our samples of mineralized muscle indicate that calcium soaps may have been present in addition to calcium phosphate.

Mineralization of Soft Tissues

Allison (1987) sealed six strips of fish muscle, each weighing 30 g, in individual packets of dialysis tubing and placed them in two 1 litre kilner jars containing marine sediment and water to which 100 ml of 10% calcium phosphate solution had been added. After 8 days of fragments of fibrous material (muscle or connective tissue collagen) had become mineralized in an unknown phosphate, but these did not exceed 100 μm in dimension. The experiments were not repeated. This preliminary work is, as far as we know, the only previous example of the replication of morphological details of a labile tissue like muscle by mineralization in the laboratory. Other mineralization experiments have focused on the processes involved in the formation of concretions in association with decaying organic matter (Berner, 1968; Zangerl, 1971). More recent experiments have investigated soft-bodied fossils by attempting to reproduce the decay state of the organism and thereby deduce the conditions under which it became fossilized (e.g., Martill and Harper, 1990; Henwood, 1992; Briggs and Kear, 1993a, c).

TABLE 6—Amount of phosphate (PO_4) in experiments (including the maximum possible level of impurity in the ASW). An additional 0.84 mg was included in experiments on *Crangon* with "added" phosphate.

	Weight of typical specimen (g)		% PO_4 in dry tissue	Weight PO_4 in carcass (mg)	Volume of ASW (ml)	Max PO_4 impurity in ASW (mg)	Max total PO_4 (mg)
	Wet	Dry					
Nereis	5.0	0.76	1.48	11.2	150	2.34	13.54
Branchiostoma	0.25	0.05	5.30	2.7	50	0.78	3.48
Crangon	0.4	0.10	3.00	3.0	50	0.78	3.78
Palaemon	0.7	0.14	2.05	2.9	50	0.78	3.68

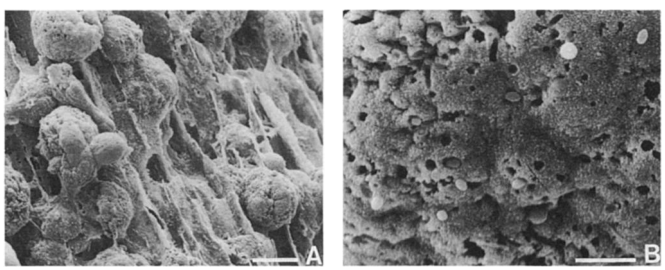


FIGURE 19—(A) Dumbbell shaped crystal bundles overgrowing mineralized sarcolemma in *Palaemon* decayed for 15 weeks (1c) (detail of Fig. 15A). Scale bar is 40 μm. (B) Porous surface texture of dumbbell. Bacteria sit within many of the pores. Scale bar is 4 μm.

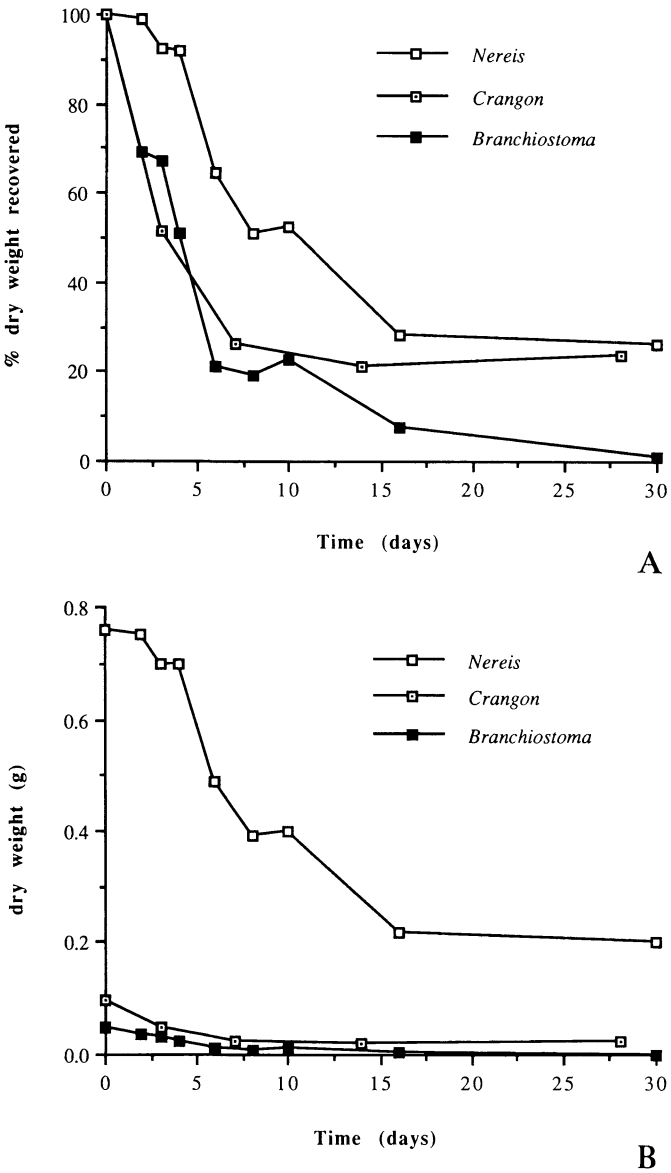


FIGURE 20—Comparison between dry weight loss in *Crangon*, the cephalochordate *Branchiostoma lanceolatum*, and the polychaete *Nereis virens* decayed in the complete absence of oxygen (1d). (A) As a percentage of starting dry weight. (B) As an average weight in grams.

Comparison with *Nereis* and *Branchiostoma*

Little is known about decay and associated mineralization in different organisms under controlled conditions. Similar decay experiments on the polychaete (*Nereis virens* (Briggs and Kear, 1993a) and the cephalochordate *Branchiostoma lanceolatum* (Briggs and Kear, 1993c) allow a comparison of the relative preservation potential of *Crangon* with that of these animals. Decay experiments on *Nereis* and *Branchiostoma* under the same conditions resulted neither in the mineralization of soft-tissue, nor in the formation of crystal bundles.

Shifts in pH resulting from decay are clearly an important control on early diagenetic mineralization (Briggs and Kear, 1993b) and even, perhaps, on decay rate itself. Crystal bundles formed most readily in open conditions and usually within one week. In experiments on *Crangon* in open conditions pH rose from the starting value of 8.00 to above 8.3 within 3 days where rapid diffusion of oxygen was possible (1a); where slow diffusion was permitted (1b) it fell to pH 7.34 but recovered within 1 to 2 weeks. In *Nereis* (Briggs and Kear, 1993a) pH fell initially in both cases, to pH 6.83 (1a, 2 days) and pH 5.99 (1b, 3 days) and did not recover to the same degree. Decay experiments on *Branchiostoma* in open conditions were not carried out, although observations on morphological decay were made (Briggs and Kear, 1993c). However, the drop in pH associated with *Branchiostoma* is unlikely to have been much greater than that in the shrimp experiments (based on a comparison of values under closed conditions), due to the comparable size of the carcasses (see Briggs and Kear, 1993c). In addition crystal bundles did form in *Crangon* in closed conditions (albeit to a lesser extent) at pH values lower than those recorded in *Nereis* decaying in open conditions (where no crystal bundles formed). Thus while the

formation of crystal bundles was presumably influenced by fluctuations in pH, the process is more complex. Precipitation was localized in specific parts of the shrimp carcass (Fig. 8) and controls must have operated at that level; values for the experiment as a whole only provide an indication of relevant conditions. The high proportion of calcium in the shrimp cuticle (Table 5) within which crystal bundles commonly formed may have had an influence.

Reduction in pH values in the early stages of decay appears to have been critical in initiating the formation of calcium phosphate as opposed to calcium carbonate in *Crangon* and *Palaemon*. Comparisons with *Nereis* and *Branchiostoma*, where no soft-tissue mineralization occurred, can be made for completely anoxic conditions (1d). Values of pH fell further in both *Nereis* and *Branchiostoma* than in *Crangon* (5.32 and 6.23 compared to 6.49); thus differences in pH are unlikely to explain the non-occurrence of mineralization in these animals. Neither is mineralization of the soft-tissue controlled by the proportion of phosphate in the carcasses (Table 6). While the percentage of phosphate in *Nereis* is lower than that in the crustaceans, that in *Branchiostoma* is higher. In addition, the total amount of PO_4 available in the *Nereis* carcass is more than three times that in the crustaceans. Similar levels of impurities were present in the ASW in all the experiments. Nor does the proportion of calcium appear to be critical. It is greater in the worms (0.25% of dry weight) than in *Crangon* (0.046%), although *Palaemon* contains 7.90%. The *distribution* of these elements may, however, have been critical. Phosphate liberated from the decaying soft-tissues in *Crangon* appears to have combined with calcium in the cuticle (Table 5 shows that Ca levels in cuticle remain similar, while those of P_2O_5 increase in proportion by more than a factor of 10), which provided a locus for the mineralization of the adjacent muscle tissue. Eggs, which were independent of the cuticle, are not so heavily phosphatized.

The dry weight profiles for the three taxa (1d) reveal some striking differences (Fig. 20). The initial rate of decline is much more rapid in *Crangon* and *Branchiostoma* than in *Nereis*. The percentage weight remaining after 30 days, however, is similar in *Crangon* and *Nereis* (20–25%) whereas that in *Branchiostoma* is less than 1%. The percentage TOC remaining after 30 days is less than 2% in *Crangon*, ca 3% in *Branchiostoma*, but ca 11% in *Nereis* (although the absolute weight in the latter is much higher). These differences in residual weight are reflected in the proportion of tissue resistant to acid and alkali hydrolysis. The tissue measured by our residue method is not all equally resistant to degradation. The proportion present in fresh carcasses of *Nereis* (46%), however, is much larger than that in *Crangon* (8.6%) and in *Branchiostoma* (1.42%). Thus phosphatization may be an important process in preserving not just muscle tissue, but even the delicate cuticles of shrimps like *Crangon*.

The only experimental conditions which resulted in a significant difference in the dry weight profiles in *Crangon* were those where limited gaseous oxygen was present at the outset (1e, f; Table 3). The major difference was a greater weight loss in samples at 2 weeks and later (Fig. 6B). The oxygen levels in these experiments, however, converged on those in others (1f on 1c and 1d) but there was a greater drop in pH (Fig. 5A). In experiments on *Nereis* (Briggs and Kear, 1993a) only the profile of decay in the complete absence of oxygen (1d) was significantly different (experiments 1e and 1f were not run). Here, however, decay rate was slower, and mainly in the first 15 days. This reduction in decay rate in the absence of oxygen was not evident in *Crangon*. This is probably a reflection of differences in composition between *Crangon* and *Nereis* (rather than differences in sample intervals): *Nereis* has a higher content of TOC (46.19% vs 32.63%), and of tissue resistant to acid and alkali hydrolysis (46% vs 8.6%) (Briggs and Kear, 1993a). Differences in rates of decay in the presence or absence of oxygen may be much greater for some categories of organic compound than for others (e.g., Briggs et al., 1992).

PHOSPHATIZED SOFT-TISSUE IN FOSSILS

Phosphatized muscle and other soft-tissues have been reported in fossil arthropods, for example from the Jurassic Solnhofen Limestone (G. Viohl, pers. comm.; pers. observation) and from fish stomachs from the Lower Cretaceous Santana Formation of Brazil (Wilby and Martill, 1992). The most completely documented occurrences of phosphatized soft-tissue in fossils, however, are those reported in fishes from the Santana Formation (Martill, 1988, 1989, 1990; Martill, et al., 1992; Wilby and Martill, 1992) and from the Upper Jurassic of Chile (Schultze, 1989). The different mineral morphologies which formed in association with soft-tissue in our experiments are similar to those reported from these fossil examples (Briggs, 1993). The finest details preserved in Santana specimens are comprised of crystallites up to 0.3 μm in length, aggregated into microspheres ca 1 μm in diameter (Wilby and Martill, 1992). In the Santana fossils these microspheres may replace or coat arthropod cuticle and preserve internal tissues in fish and arthropods. This texture sometimes preserves even cell nuclei (Martill, 1990) and other cell organelles (Martill, pers. comm.), although we do not have unequivocal examples of this in our experiments (perhaps because the nuclei in shrimp muscle are surrounded by fibrils). Fish muscle from Chile is preserved in microspheres 0.5–1 μm in diameter (Schultze, 1989, Pl. 3, fig. 6), and cephalopod muscle from the Jurassic of Wiltshire, England, in microspheres 1–2 μm in diameter (Allison, 1988c). Spheres 2 to 5 μm in diameter, which represent autolithified bacteria, occur in Santana specimens (Wilby and Martill, 1992). Spheres of this size were produced in experiments to demonstrate the involvement of bacteria in apatite genesis (Hirschler et al., 1990a) using aragonite from crushed cuttlebone as a source. The outline of the soft-tissues in most of the Santana fossils is not replicated

in a bacterial film, however, a mode of preservation characteristic of some soft-bodied fossils (e.g., body outlines of Eocene Messel fossils preserved in siderite: Wuttke, 1983; Franzen, 1985, Fig. 5). This type of "photographic" preservation did not occur in our experiments. The preservation of soft-tissue in calcium phosphate in our experiments and the majority of fossil examples is bacterially induced, not bacterially controlled (*sensu* Hirschler et al., 1990b).

Martill (1988, p. 6) reported that the muscles of the Santana fish are preserved in phosphate, specifically a cryptocrystalline francolite. Schultze (1989, p. 197), however, argued that the muscle in the Santana fishes is preserved in calcite, based on x-ray diffraction of muscle tissue. Maisey (1991, p. 80) offered an explanation for these apparently incompatible views noting that "much of the fossilized muscle is dissolved readily in formic or acetic acid" and that "where phosphatized soft-tissues occur, their component parts are held together by calcium carbonate." Thus interstitial calcium carbonate in Schultze's mechanical preparations may have swamped any phosphate peaks on the XRD (Wilby and Martill, pers. comm.). The preservation of muscle tissue in calcite in the Santana fish is not supported by petrography or EDAX elemental mapping (Wilby and Martill, pers. comm.). Electron microprobe traces of arthropod soft-tissue preserved in the stomach of *Notelops* (Wilby and Martill, 1992, Fig. 5) indicate the presence of both calcium hydroxylapatite and calcium fluorapatite (reflecting the substitution of F^- for OH^- ions). Electron microprobe analysis of mineralized muscle from a specimen of the shrimp *Antrimpos* from the Solnhofen Limestone (Table 5) reveals an essentially identical ratio of phosphate to carbonate to that in the muscle mineralized in our experiments. Our experimental results confirm that while calcium carbonate precipitated in carcasses at an early enough stage, only calcium phosphate replaced soft-tissues. The calcium carbonate always precipitated as crystal bundles. Both minerals sometimes formed in the same carcass just as they did in the Santana fishes, although additional calcite was precipitated in the fossils at a later stage (Martill, 1988; Maisey, 1991).

Schultze (1989) argued that the soft-tissues of the Chile fishes were phosphatized in life by a process of calcinosis. Martill and Harper (1990) deduced that mineralization of the Santana fishes occurred within 5 hours on the basis of comparisons between the details preserved in the fossil tissues and the state of fish decayed in laboratory experiments. Our experiments indicate that, in favorable circumstances, the process may have taken longer to initiate. Mineralization of the shrimps was not evident prior to 2 weeks and the degree of mineralization increased up to 4 weeks and longer (Fig. 18). Lower temperatures would have further slowed decay (e.g., Kidwell and Baumiller, 1990). It is not known to what extent replacement of the muscle in the fossil fish might have required higher concentrations of phosphate than those available in the carcass itself (as suggested by Martill, 1988, 1989). A comparison of the likely original chemistry of the fishes, and the amount of calcium phosphate formed, has not been made.

CONCLUSIONS

The experiments show that under the same set of starting conditions (carcass, bacterial population, and ASW) either calcium carbonate or calcium phosphate mineralization may be more important, depending on whether the system is open to diffusion or closed. As neither type of mineralization occurred in similar experiments on *Nereis* and *Branchiostoma*, the composition of the shrimps and the distribution of elements (particularly Ca) within them must be fundamental. A number of variables determine the diagenetic process, including the concentration of bicarbonate and phosphate, pH, and oxygen availability. The precipitation of crystal bundles ($CaCO_3$) occurred more readily than the mineralization of muscle tissue. They formed under the range of experimental conditions, although their precipitation was enhanced in open systems, and where the concentration of bicarbonate ions was increased. The mineralization of soft-tissue (by calcium phosphate) was more sensitive to experimental variables. It formed more readily where the system was closed and was significantly inhibited by increased concentrations of bicarbonate. The amount of mineralization (both precipitation of $CaCO_3$ crystal bundles and mineralization of soft-tissue by phosphate) was very variable even under the same experimental conditions, reflecting differences in decay between and within carcasses. The process is clearly determined by dynamic changes within the carcass and a detailed understanding of the controls involved will only emerge when variables are monitored continuously in different parts of a decaying organism.

This investigation clearly demonstrates that phosphatization of soft-tissue may occur without the build-up of unusual concentrations of phosphate external to the carcass (although this may play a role in the case of some fossil deposits). It shows that, at least in certain conditions, the process may take weeks rather than hours or days. The absence of oxygen may be less important than the prevention of diffusion. This delays or minimizes the recovery of pH values which fall as a result of the build-up of fatty acids and CO_2 during preliminary decay. Closed conditions might be the result of a number of influences in natural systems, for example: deep burial, the formation of microbial films on the surface of a carcass, or simply the size of a large carcass which might promote relatively closed conditions internally. Although precise conditions are required for the phosphatization of soft-tissue, this kind of preservation is more widespread in the fossil record than is usually realized (Allison and Briggs, 1991b; Briggs and Kear, 1993b). Only a few examples have been subjected to SEM study and many others would likely reveal similar details to those described here.

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APPENDIX

Means and standard deviations for data from experimental runs on *Crangon* and *Palaemon*: wet weights, dry weights and pH. Note that the standard deviations for weights beyond 8 weeks become increasingly high due to the difficulty of recovering and weighing very small quantities of residue. (* indicates n = 4; ** is n = 3; n = 5 in all other cases).

Crangon crangon - wet weights

	1a		1b		1c		1d		1e		1f		Bic 1c		Bic 1d		Pho 1c		Pho 1d	
Time	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD
3d	104.7	9.5	106.3	16.1	114.9	10.0	93.0	16.5	85.1	6.6	89.1	14.4	106.3	8.0	125.1	14.5	103.9	17.8	83.3	15.0
1w	51.2	21.2	66.4	37.4	71.9	20.1	59.6	23.6	60.9	18.9	22.5	27.6	104.6	19.3	79.5	26.4	76.6	15.1	40.8	8.3
2w	52.7	23.0	74.2	35.3	86.2	15.1	119.2	67.0	13.8	19.0	25.8	18.5	38.6	6.0	78.4	37.1	36.0	9.6	24.4	24.1
4w	16.9	29.2	67.5	15.4	29.1	11.1	27.1	15.2	21.8	12.2	0.0	0.0	6.7	11.0	51.2	63.1	34.2	27.4	35.3	9.4
6w	6.2	8.0	49.3	43.7	18.9	13.4	31.1	11.7	4.4	9.3	0.0	0.0	6.4	7.5	33.0	23.6	29.2	4.8	38.9	6.0
8w	57.7	22.1	133.2	50.8	23.1	10.6	16.5	10.6	0.8	1.9	6.9	9.5	28.6	16.6	53.2	14.4	39.8	22.5	23.1	12.2
10w	25.6	15.2	42.3	15.9																
15w	66.3	17.9	79.5	32.8																
20w	51.0	21.0	45.2	24.5																
25w	58.8	20.6	65.1	35.4																

Crangon crangon - dry weights

	1a		1b		1c		1d		1e		1f		Bic 1c		Bic 1d		Pho 1c		Pho 1d	
Time	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD
3d	54.6	10.7	60.7	5.8	66.5	7.5	51.3	15.4	51.6	7.5	59.6	10.0	58.9	10.5	66.5	3.1	55.1	9.8	46.1	7.9
1w	30.2	12.5	61.2	26.7	29.6	6.3	26.5	6.3	25.7	16.9	19.6	10.8	47.8	5.0	36.2	9.8	36.7	3.7	18.0	8.3
2w	25.9	4.5	41.6	27.2	25.3	7.8	21.4	9.7	10.7	9.1	12.5	9.1	23.3	7.2	25.2	11.9	24.3	4.3	17.3	5.7
4w	8.9	7.1	29.2	8.6	32.5	9.2	23.5	5.2	11.7	5.8	8.4	1.0	18.8	4.1	18.9	2.9	18.2	4.0	14.7	4.4
6w	14.6	7.3	65.5	23.6	15.2	9.2	20.2	6.6	9.7	5.9	7.2	6.0	18.1	1.4	13.2	7.1	11.2	5.1	17.4	5.3
8w	55.2	33.2	69.1	15.3	17.7	4.6	24.8	7.6	7.6	4.2	5.1	4.6	12.9	5.5	18.5	13.5	21.9	1.9	11.5	6.9
10w	83.5	39.9	62.9	33.5																
15w	122.0	52.8	73.9	35.6																
20w	39.1	11.0	23.0	16.6																
25w	38.9	22.0	25.2	14.3																

Crangon crangon - pH

	1a		1b		1c		1d		1e		1f		Bic 1c		Bic 1d		Pho 1c		Pho 1d	
Time	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD	x	SD
3d	8.38	0.06	7.34	0.18	6.67	0.04	6.49	0.13	6.32	0.05	6.45	0.07	6.83	0.06	6.73	0.04	6.49	0.10	6.73	0.06
1w	8.57	0.08	7.98	0.11	6.80	0.13	6.97	0.06	6.55	0.09	6.65	0.09	6.88	0.06	7.12	0.11	6.91	0.07	7.00	0.03
2w	8.30	0.05	8.36*	0.41	7.62	0.22	7.74	0.22	6.78	0.06	6.62	0.06	7.63	0.06	7.43	0.03	7.76	0.09	7.31	0.15
4w	8.40	0.16	8.59	0.09	8.19	0.06	8.19	0.11	6.57	0.10	7.01	0.07	8.01	0.03	7.56	0.10	8.33	0.12	7.75	0.49
6w	8.52	0.38	8.71	0.05	7.10	0.16	8.41	0.12	6.98	0.07	6.74	0.10	8.21	0.69	8.54	0.04	8.24	0.14	7.86	0.18
8w	8.43	0.16	8.83	0.05	7.92	0.21	8.45	0.14	7.34	0.08	6.71	0.19	8.65	0.10	8.72	0.15	8.54	0.10	7.12	0.10
10w	8.48	0.22	8.95	0.05																
15w	8.48	0.17	8.86	0.08																
20w	7.90	0.40	8.45*	0.78																
25w	7.11	0.65	7.80	0.66																

Palaemon elegans - wet weights

	1b		1d	
Time	x	SD	x	SD
1w	107.7	8.4		
2w	70.2	20.7		
3w	56.8	13.0		
4w	73.5	13.1	53.2	15.7
6w	89.1	24.4	75.9	11.1
8w	96.6	35.1	31.5	12.7
10w	59.5	38.1	52.8	19.7
15w	42.1	23.2	128.0	73.2
20w	65.4	49.4	104.4	78.1
25w	36.6	20.3	71.6	34.0
75w	15.3	13.4		

Palaemon elegans - dry weights

	1b		1d	
Time	x	SD	x	SD
1w	62.0	19.8		
2w	35.6	6.6		
3w	18.6	5.9		
4w	27.0	7.2	29.2	11.4
6w	70.5	26.3	40.2	7.1
8w	78.3	29.5	31.7	9.1
10w	34.7	16.7	39.7	10.7
15w	34.0	14.6	43.8	29.1
20w	35.0	15.9	44.1	43.3
25w	22.0	25.1	36.4	16.0
75w	26.7	14.0		

Palaemon elegans - pH

	1b		1d	
Time	x	SD	x	SD
1w	7.84**	0.17		
2w	8.25	0.27		
3w	8.54*	0.16		
4w	8.45	0.28	7.41	0.19
6w	8.88*	0.02	7.93	0.20
8w	8.61	0.94	7.82	0.14
10w	8.29*	1.06	8.45	0.36
15w	8.50	0.77	8.39	0.16
20w	7.04	0.23	7.15	0.35
25w	7.81	1.16	8.38*	0.70
75w	7.97	1.37