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Bioturbation by Common Port Phillip Bay Benthic Invertebrates.

Fiona L. Bird[1], Phillip Ford[2] and Simon Heislers[1]

[1] Department of Crustacea, Museum of Victoria, 71 Victoria Crescent, Abbotsford, Victoria, 3067, Australia

[2] Centre for Environmental Mechanics, CSIRO, GPO Box 821, Canberra, Australian Capital Territory, 2601, Australia

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EXECUTIVE SUMMARY

The burrowing and feeding ecology of three numerically important bioturbating species from Port Phillip Bay was studied. *Biffarius arenosus* and *Neocallichirus limosus* are callianassid ghost shrimps which inhabit permanent burrows. *Echinocardium cordatum* is a burrowing heart urchin which ploughs through sediments whilst maintaining a connection with the surface via a respiratory siphon. The very different burrowing habits of the callianassids and the heart urchin invited this comparative study.

Both species of callianassid built similar burrow shapes extending to a mean burrow depth of 26 cm. The burrow wall was not a discrete structure in either species' burrows, but was smooth and compacted, and there was no evidence of a 'leathery' mucous bound tube. Gut contents confirmed that both species were deposit-feeders, ingesting sediment and assimilating the bacteria and fungi adhered to the sediment grains. Sediment was processed during burrowing and feeding activity. Turnover rates were similar for both species, and a combined Baywide rate was estimated between 6.3×10^6 and 9.2×10^6 tonnes/day.

The urchin, *E. cordatum*, burrowed deeper in sandy than in muddy sediments, but speed of burrowing did not differ between sediments. The urchin mixed the surrounding sediments during movement, and a Baywide sediment mixing rate was calculated at 2.1×10^6 m^3/day. This corresponds to a burial rate (of surface sediments) of 32 days in sandy sediments and 52 days in muddy sediment. Sediment was ingested from the surface and sub-surface layers by *E. cordatum* at a rate of 4.9×10^4 tonnes/day. This deposit-feeder would also gain nutrition from the bacteria and fungi adhering to sediment particles.

Both the presence of callianassids and echinocardium (in double natural densities) in sandy sediment enhanced diffusive flux approximately 3.5 times that of sediment without animals. Callianassid burrows increased the water-sediment interface by at least 8 % which corresponded to an extra 8.6 km^2 of interface below the Bay's sediment surface. Both passive and convective flow of burrow waters would have increased diffusive flux of solutes across the water-sediment interface. During movement the urchins mix sediment, and pump water through the sediments. Both processes would alter pore waters and increase diffusive flux.

The bioturbating activity of these three species results in significant sediment turnover and mixing, and enhanced diffusive flux from sediments. Additionally, the burrows create a unique micro-environment for the survival of aerobic bacteria far into the sediment. The few millimetres of oxygenated sediment in the burrow wall provides a site for nitrifying bacteria, adjacent to sediments. In this way the nitrification/ denitrification processes are closely coupled so that the transformation of ammonia to nitrogen gas (via nitrification and denitrification) occurs in quick succession.

Abundance of callianassids appear to have decreased 18 fold between 1969-73 and 1994-95 Port Phillip Bay benthic studies. Urchin numbers have decreased by half over the same time. Quantification of ioturbation due to the two species, enabled prediction of the impact of population decline on the physical and chemical processes occurring in Port Phillip Bay sediments.

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1. GENERAL INTRODUCTION

1.1. The Effects of bioturbation

Most marine sediments underlying oxygenated bottom waters are inhabited by a variety of large, bottom dwelling organisms (Aller 1988). These burrowing organisms are known to alter physical properties of sediments (Rhoads and Boyer 1982, Suchanek *et al.* 1986, Meadows and Tait 1989, Davis 1993), redistribute organic matter (Kristensen 1988, Sloth *et al.* 1995), increase oxygen penetration into sediments (Hylleberg and Henriksen 1980, Fukuhara and Sakamoto 1987), increase microbial production (Aller 1982, Miller 1984) and promote nitrification and denitrification in sediments (Henriksen *et al.* 1983, Hüttel 1990).

A burrow structure or burrowing action creates a unique microenvironment in the sediments, the extent of which depends on taxon, life habit, mobility, life history stage and feeding type (Aller and Aller 1992, Steward *et al.* 1996). Oxygen availability in burrows also depends on the pattern of activity of the burrow inhabitant (Henriksen *et al.* 1983, Kristensen *et al.* 1985, Boudreau and Marinelli 1994, Forster and Graf 1995).

Both meiofauna and macrofauna are known to affect solute transport and reaction rates, each in a different way. Meiofauna affects processes near the sediment-water interface and so promotes aerobic decomposition reactions such as nitrification. Conversely, macrofauna burrow deeper, and influence pathways, such as denitrification, in the anaerobic sediments (Aller and Aller 1992). Muddy sediments, rich in organic matter, but with a poor oxygen supply would favour anaerobic metabolism such as denitrification. In comparison, sandy sediments would favour nitrification (Vanderborght and Billen 1975, Koike and Hattori 1978).

Excretion plays an important role in ammonia production in sediments. In the deposit feeding bivalve *Theora lata*, excretion liberates more inorganic nitrogen than bioturbation (Yamada and Kayama 1987). Macrofaunal excretion is known to contribute more than 80% of ammonia flux from sediments (Regnault *et al.* 1988). The excretion products are often flushed out of the burrow via irrigation (Henriksen *et al.* 1983), so that diffusion of these products, such as ammonia, increase in the presence of burrows (Henriksen *et al.* 1983, Waslenchuk *et al.* 1983).

Once oxidised, nitrogen will diffuse in one of two directions: into surrounding sediments to be denitrified, or into burrow water to be pumped into the overlying water (Sayama and Kurihara 1983). In low burrow oxygen concentrations, it appears that nitrifying bacteria can survive for prolonged periods. When oxygen is introduced to previously anaerobic sediments, nitrification starts quickly (Jensen *et al.* 1993). Available oxygen in the burrow walls is rapidly used up between irrigation bouts (Forster and Graf 1995).

Convection of water and solutes through interstitial spaces is induced by pressure gradients and pumping activities of burrowing organisms. These organisms promote diffusion by providing holes and by irrigating them (Ebenhöh *et al.* 1995). Bio-irrigation also introduces oxygen into usually anaerobic sediments. Nitrification potentials in burrow walls are significantly greater than in the surrounding sediment and in the surface sediment (Henriksen *et al.* 1980,1983, Kristensen et al 1985, Mayer *et al.* 1995, Tomaszek 1995).

Denitrification is enhanced in the presence of burrowing animals (Sayama and Kurihara 1983, Kristensen *et al.* 1991, Law *et al.* 1991). Denitrification in the sediments occurs in close proximity to the oxygenated sediment surface. Irrigated burrows can provide a site for nitrification and also bring the nitrate in close proximity with the denitrifying bacteria thereby coupling the nitrification/denitrification process (Revsbech *et al.* 1988, Kemp *et al.* 1990, Jensen *et al.* 1993, Rysgaard *et al.* 1993, Seitzinger 1993, Tomaszek 1995). The two processes can occur concurrently because of oxidised micro-zones in anaerobic layer and reducing micro-zones in the aerobic layer of sediments (Tomaszek 1995).

Denitrification accounts for all or nearly all of the nitrate uptake from the marine sediments (Binnerup *et al.* 1992). Denitrification accounts for 80-90% of the nitrogen removed from Port Phillip Bay sediments (Harris *et al.* 1996), and in doing so plays an important role in controlling the degree of eutrophication in the Bay. Understanding the burrowing ecology and feeding biology of some of the important bioturbating species is vital in the process of understanding the roles burrows and burrowing activity play in the nutrient cycling of Port Phillip Bay.

1.2. Aims of this study

The major aims of this study were to describe burrowing and feeding activities of three species of bioturbator from Port Phillip Bay, and to quantify effects of bioturbation such as sediment turnover rates and enhancement of diffusive flux.

2. CALLIANASSID GHOST SHRIMPS

2.1. Introduction

2.1.1. Review of Callianassid burrowing

Callianassid ghost shrimps are well documented bioturbators. The shrimps build burrows to differing depths in sediments, and a species' burrow shape is often unique (Suchanek *et al.* 1986). Burrow architecture has been characterised ecologically; filter/suspension feeders, detritus/deposit feeders, and seagrass/algae harvesters, each building distinctive burrows (Suchanek 1985; Griffis and Suchanek 1991). Recently this classification has been expanded to include additional characteristics (Nickell and Atkinson 1995). Filter-feeders have simple U-shaped burrows, deposit-feeders have a complex series of tunnels and chambers extending from the base of the U, and seagrass/algae harvesters have simple holes and no mound, and deep chambers for storage of plant material (Suchanek 1985). Understanding burrow shape of a species is important so that structure of a burrow can be related to function, and dimensions such as depth, burrow volume and surface area can be described.

Burrows in marine sediments are often lined presumably to give the burrow structure, and to protect the inhabitant. The wall of callianassid burrows has been observed to be different from the surrounding sediment. De Vaugelas *et al.* (1986) investigated *Callichirus armatus* burrows, and found that a thick lining was observed in burrow walls in sand but not mud. No description was made of this lining. Shinn (1968) describes burrow linings in *Callianassa* sp. B burrows as sticky, coherent, fine grained sediments. Dobbs and Gurket (1988) found the burrow wall of *Callianassa trilobata* also to be skewed towards fine particles. They suggested that the closeness of the matrix surrounding the wall indicates compaction by the shrimp. Some species have been observed to cement walls together with mucous (Frey *et al.* 1978). An anatomical description of the genus *Callichirus* (another genus in the family Callianassidae) describes structures on the third to fifth abdominal somites which may comprise the exoskeletal components of an abdominal apparatus, perhaps related to cementing burrow walls (Manning and Felder 1986). Cement producing glands have been described in other species, located in the hindgut of *Upogebia pugettensis*, and in glands at the bases of mouth parts in *Callianassa californiensis* (Thompson 1972, in Manning and Felder 1986). *Callianassa major* has been observed secreting a milky, gelatinous mucous from a gland in the thoracopod, with which it binds sand grains into a pellet. These pellets are pushed together to form the burrow wall (Frey *et al.* 1978). There is no evidence of these glands in either *N. limosus* or *B. arenosus* (Poore 1975), suggesting that mucous does not play a large role in burrow wall construction in these species.

A burrow lining is known to affect diffusive permeability of the water-sediment interface (Aller 1983), which is important in predicting the influence of a species' burrow on net diffusion from sediments. Permeability of sediments can be reduced by 10-20% (Aller 1988) and 60-90% (Aller 1983) depending on the taxa of marine invertebrate studied. Aller (1983) shows that linings can act as molecular sieves, so that some classes of solute will be affected more by the presence of irrigated burrows, than other classes. Some linings appear to have negatively charged regions which repel anions, but attract cations, increasing cation diffusion relative to anion diffusion.

Callianassids continually process sediment during burrowing and feeding activity. Large size classes of sediment are stored in chambers and small particles are ejected from the burrow (Suchanek 1983, 1985). The sediment ejected forms distinctive 'volcanoes' which characterise callianassid burrows.

Sediment processing disrupts the vertical profile of sediments, and changes physical properties such as shear strength (Rhoads and Boyer 1982), permeability (Meadows and Tait 1989), turbidity of the water column (Colin *et al.* 1986), and grain size distributions (Suchanek *et al.* 1986). Spatial segregation of particle sizes will change chemical composition and reaction rates in the sediments because many chemical properties are related to grain size (Aller 1982).

2.1.2. Port Phillip Bay callianassid populations

Three species of callianassid ghost shrimp are found in Port Phillip Bay, *Neocallichirus* (formerly *Callianassa*) *limosus*, *Biffarius* (formerly *Callianassa*) *arenosus* and *Callianassa ceramica* (nomenclature changes were formalised by Poore 1994). Each species inhabits a specific sediment type and depth range. *N. limosus* occurs in deep silty clay sediment, *B. arenosus* inhabits shallower silty sand sediments, and *C. ceramica* is found in low numbers in shallow sandy sediments (Poore 1975).

Estimated abundances of both *N. limosus* and *B. arenosus* have changed since the survey of1969-73 (Wilson *et al.* 1996, Appendix A). *C. ceramica* habitats were not sampled by Wilson *et al.* (1996), so no change in numbers for this species could be detected. *B. arenosus* abundance has decreased by 25%, and *N. limosus* abundance has decreased by 99% since 1969-73 (Wilson *et al.* 1996). In the 1969-73 study, juvenile recruits of *N. limosus* were collected in huge numbers (~200 individuals/grab), but unfortunately a breakdown of immature/mature individual abundances was not recorded (Poore, pers. comm.). In the 1994-1995 samples, only adults were collected, so the two numbers are not directly comparable. Not all recruits would have survived to adulthood, so the absolute abundances recorded from 1969-73 are not directly comparable to the adult abundances recorded in 1994-95. A population decline since 1969-73 was expected, following the pattern of the total macrobenthic population decrease (Wilson *et al.* 1996). The decline in callianassid numbers in Port Phillip Bay would decrease the effect that burrowing and feeding activity of these bioturbators have on physical and chemical processes.

This project originally set out to study one species of callianassid, *Neocallichirus limosus*. A second species *Biffarius arenosus* was also collected and included in this study. *N. limosus* inhabits muddy sediments and *B. arenosus* inhabits sandy sediments, thus results gained from these experiments more accurately reflect the sediment strata found in Port Phillip Bay, and the biologically driven processes occurring there.

2.2. Methods

Animals were collected using Smith-MacIntyre grab samples collected by the Museum of Victoria in October 1995. Specimens of both callianassid species were housed in temporary aquaria in their preferred sediment types awaiting experimentation.

2.2.1. Burrow shape and dimensions

Seven *B. arenosus* and three *N. limosus* individuals were left to build burrows in sediment tanks for several weeks. Burrows were cast using an Araldite epoxy resin (CIBA-Geigy LC 3600 resin and LC249 hardener mixed in 3:1 proportions), which was poured into burrows in a continuous stream. The resin was left to cure for 48 hours and the casts were washed out of the sediment. Casts were described and cast length, volume and surface area were measured.

Due to the odd shape and depth of the casts, determining burrow volume via water displacement was impossible. Hence, burrow volume was estimated by weighing casts and dividing the weight by resin density. Surface area of the casts was estimated by wrapping the cast in a single layer of foil. One square centimetre of foil had a known weight and the total foil wrapped around each cast was weighed and converted to an area.

2.2.2. Burrow lining

The periodic acid-Schiff (PAS) reaction was used to investigate muco-polysaccharide content of burrow linings. This PAS reaction is based on the oxidative action of periodic acid (HIO_4) on glycol groups (present in glucose residue) which gives rise to aldehyde groups. The aldehyde groups react with Schiff's reagent producing a compound with a purple colour indicating presence of muco-polysaccharides (Junqueira *et al.* 1986).

Samples of burrow wall and surrounding sediment were attached to a microscope slide with gelatine (pure protein) and soaked in 1% aqueous periodic acid for 5 minutes. The excess acid was washed off the slides for 5 minutes under running water, and the sample was stained for 10 minutes in Schiff's reagent. Finally, the samples were washed for 10 minutes, and the colour change observed.

2.2.3. Feeding

Gut contents were analysed from preserved museum specimens. The entire alimentary canal was dissected out of individual specimens, opened up and the contents washed onto a slide.

2.2.4. Sediment turnover

Sediment turnover rates were investigated initially using *B. arenosus*. Three *B. arenosus* individuals were left to make burrows in tanks containing sandy sediment. Any sediment ejected was collected by syringe once a day for a week, and then once a week for three months. The sediment was dried and weighed. Sampling over three months was considered to be representative of sediment processing under natural conditions.

Daily collection of samples were shown to be unnecessary from the *B. arenosus* experiment, so samples were collected weekly for two *N. limosus* individuals. After four weeks (one tank) and seven weeks (the other tank), no more sediment was ejected from the burrows, suggesting that the inhabitants had died.

2.2.5. Baywide sediment turnover

Baywide estimates were calculated using abundance data from Wilson *et al.* (1996). For each species, abundances were extrapolated from number of individuals per grab sample (0.1 m^2 area) up to the area of sediment type in the Bay. Muddy sediments comprise approximately 55% of the Bay's sediments, so *N. limosus* abundance was estimated for 55% of the Bay area, and *B. arenosus* for 45% of the Bay area (sandy sediments). A range of rates was calculated for each species, using mean and maximum values. Current turnover rates were also compared to past rates using 1969-73 abundances (Poore *et al.* 1975).

2.2.6. Burial rate

If sediment is periodically dumped onto the surface by shrimp, it will assist with the burial of reactive organic matter. The number of days taken to bury sediment to a depth of 0.5 cm (approximately the lower limit of the oxic zone at the sediment surface) was calculated by multiplying the Bay area by the depth of burial, and dividing this by the sediment turnover rate by the abundance of shrimp.

2.3. Burrow shape and dimensions

All burrows in Figures 1, 2 and 3 are of the same scale. Diameter of a tunnel is directly related to size of an individual (Dworschak 1983, F. Bird pers.obs.), so the narrower burrows are probably built by smaller individuals, and thicker burrows by larger individuals. Major descriptive features of a cast are labelled in Figure 1.

Burrow casts should only be used as an indication of burrow shape, as casts were usually incomplete. When the casts were washed out of the tanks, additional tunnels and chambers are often observed continuing from where the cast ends.

2.3.1. Inter-specific similarities

Biffarius arenosus burrow shape can be seen in Figures 1 and 2. The six burrows described are all different, but based on a similar design. *Neocallichirus limosus* burrows were a similar shape to those of *B. arenosus* (Figure 3). Both species burrows included: burrow openings (mean number of 3); opening shafts originating from lower parts of the burrow; constriction at the base of some opening shafts; a spiral section of tunnel; turning chambers and a lack of storage chambers.

All burrows had a section of tunnel shaped as a spiral. In their discussion of burrow characteristics, Nickell and Atkinson (1995) describe a ‘tightly layered lattice’ indicative of full exploitation of sediments in a given area. This description gives no mention of a spiral shaped lattice as seen in this species. This spiral shape has been recorded in *Callianassa bouvieri* (Dworschak and Pervesler 1988) and *Callianassa* sp. 1 (Braithwaite and Talbot 1972, Griffis and Chavez 1988). Bromley (1990) suggested that many thalassinid burrows were based on a spiral plan, but this spiral may be disguised by branching tunnels and nodules. The spiral in Port Phillip Bay species is probably just a variation on the ‘tightly layered lattice’ discussed by Nickell and Atkinson (1995). Thorough exploitation for food of a given area of sediment is probably the main function of the spiral, but perhaps competition for space controls burrow shape as well.

Turning chambers were present at junctions, corners and at regular intervals along tunnels, in all the casts. These chambers form when the shrimp consistently changes direction in one place. The shrimp uses a somersaulting action which enlarges the turning place into a sphere. Turning chambers have been documented in burrows of many callianassid species (for example, *U. pusilla* in Dworschak 1983).

The apparent lack of deep chambers in these casts contrasts with casts made from burrows of *B. arenosus* from an intertidal site in Western Port (Bird, unpublished data). Casts of the Western Port population were made insitu, and large chambers coated with coarse sand were present at the lower reaches and periphery of the burrow.

Compacted sediment from tunnels walls does not adhere to the resin whereas the loosely-packed chamber contents do adhere. These chambers have been documented in other species (Suchanek 1983) and are thought to be storage areas for refuse, too large to eject from the burrows.

It is possible that burrows cast in the field in Port Phillip Bay would reveal chambers. In the aquaria, sediment was sieved to remove any animals and large pieces of shell and detritus such as wood, so that the sediment was relatively homogenous. In the natural environment the sediment probably contains coarse material that would need to be stored because it is too large to eject from the burrows.

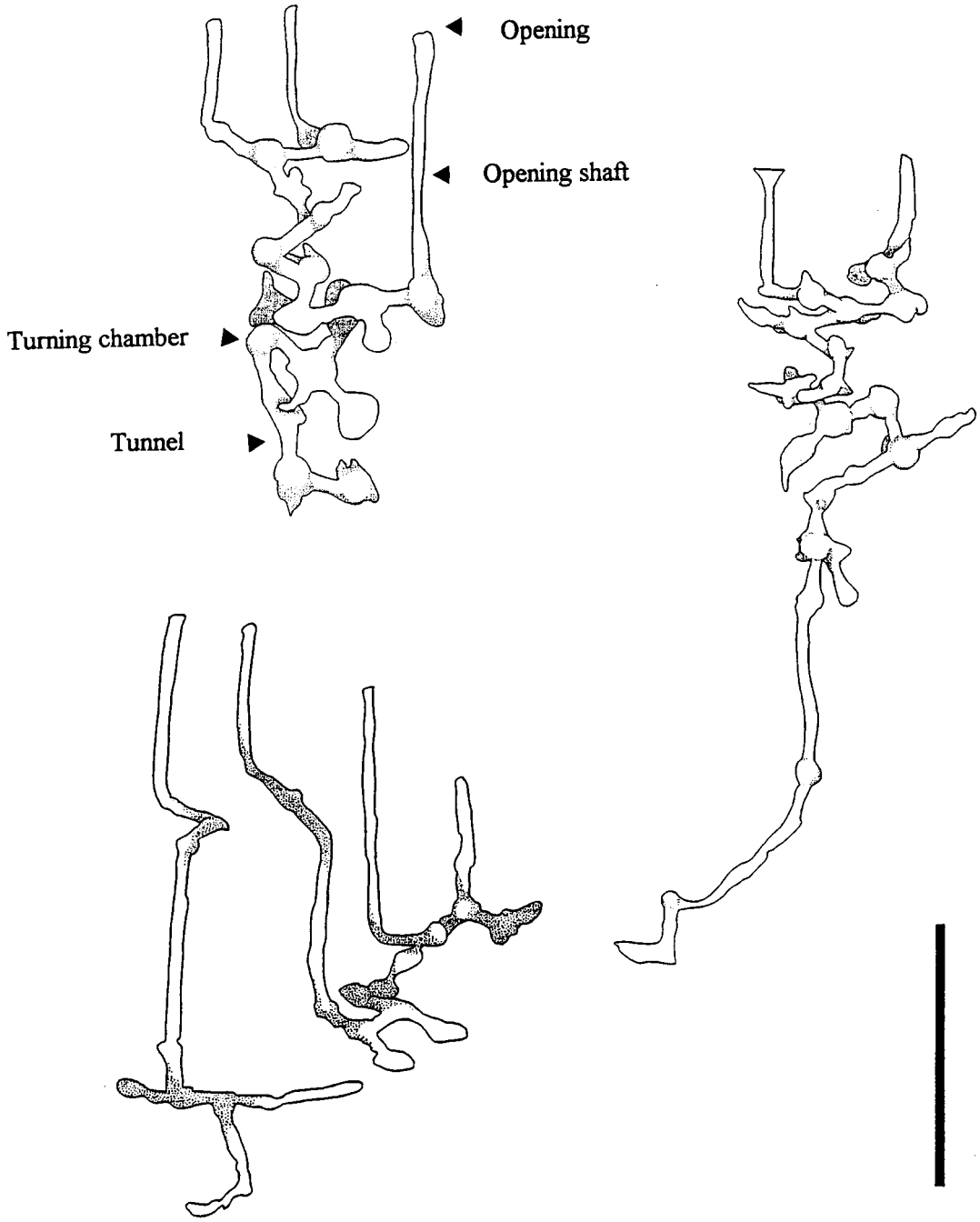

Figure 1: ***Biffarius arenosus*** **burrows, one cast showing general burrow features. Scale bar is 10 cm.**

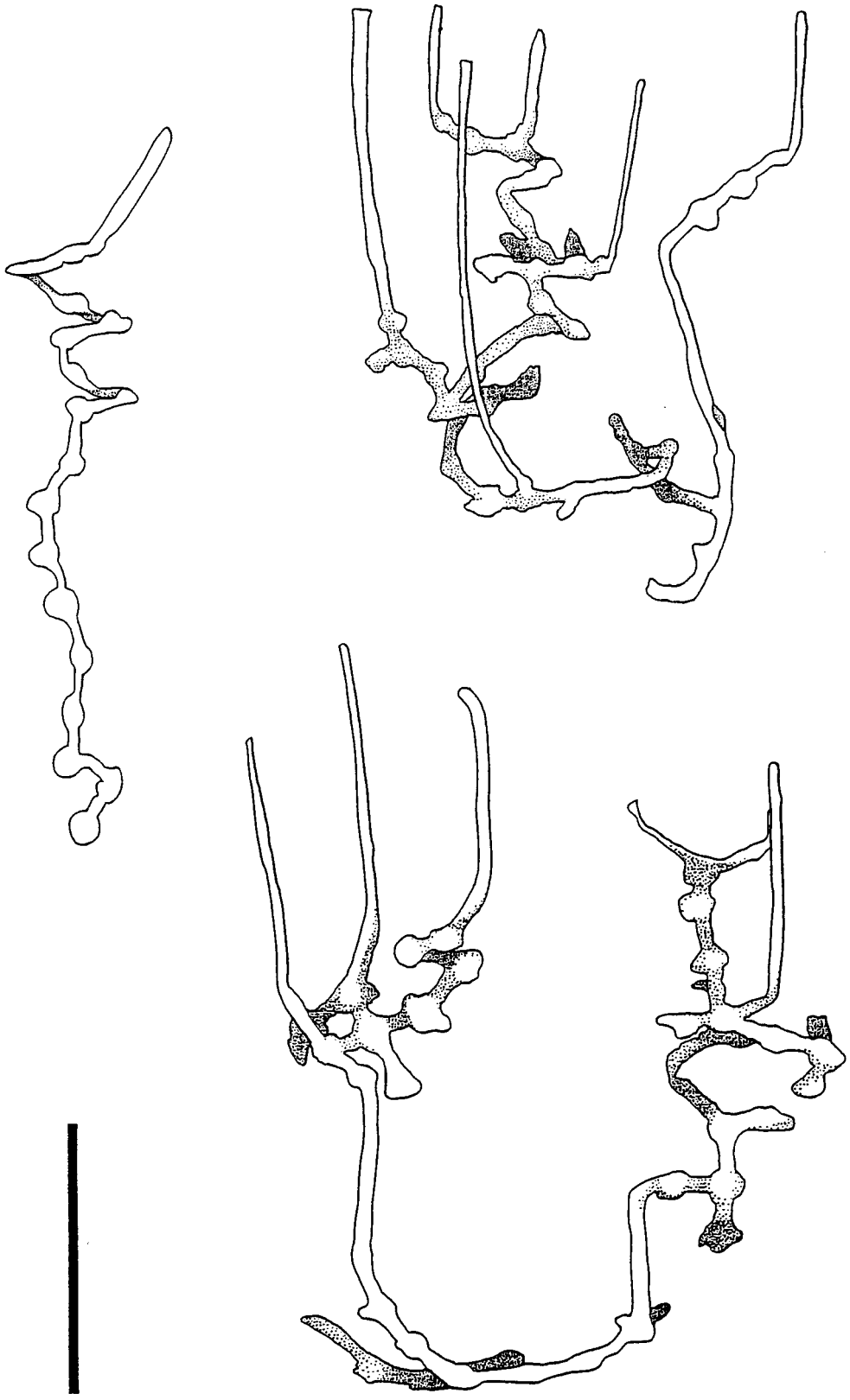

Figure 2: ***Biffarius arenosus*** **burrows. Scale bar is 10 cm.**

Figure 3: ***Neocallichirus limosus*** **burrows. Scale bar is 10 cm.**

2.3.2. Inter-specific differences

The main difference between the two species' burrows is overall shape. *Neocallichirus limosus* burrows are at least as wide as they are deep whereas *B. arenosus* burrows are always deeper than they are wide. This suggests a more horizontal burrowing habit in *N. limosus*. Burrow depth was similar for both species. Mean depth was shallower in *N. limosus* burrows (Table 1) but this difference was not significant (MS 60.2, df 1, P 0.46).

Table 1: Burrow dimensions of *B. arenosus* and *N. limosus.*

	Range	Mean	N	Standard deviation
B. arenosus				
Depth (cm)	18 - 40	28.0	5	8.0
Volume (ml)	37.7 - 60	44.7	5	10.4
Surface area (cm^2)	18.0 - 27	23.1	5	3.9
N. limosus				
Depth (cm)	15 - 37	22.3	3	12.7
Volume (ml)	45 - 93	64.5	3	25.0
Surface area (cm^2)	27.6 - 32.7	29.7	3	2.9

The different burrowing shapes may be related to the sediment type the species inhabit. Muddy sediments (which *N. limosus* inhabits) may not support long opening shafts, because they are less stable than sandy sediments. Burrows could extend deeper into sandy sediments because of the greater stability. Muddy sediments have a higher organic content than sandy sediments, which provides food for the burrower. If burrow shape is controlled by food availability, then a burrow in muddy sediments would not have to extend as deep to encounter the food resources.

Higher organic content of sediment would mean that muddy sediments were more reducing than sandy sediments. A shallow burrow would be easier to ventilate so that the burrow waters would contain enough oxygen for survival. The ideal burrow for living in highly anoxic sediments would be shallow with many openings. *Neocallichirus limosus* burrows were slightly but not significantly shallower and showed no significant increase in opening number compared to *B. arenosus*.

2.3.3. Burrow dimensions

Burrow volume and surface area were generally larger for *N. limosus* than *B. arenosus* (Table 3), but again these results were not significant (volume: MS 672.6, df 1, P 0.204 and surface area: MS 36.98, df 1, P 0.211).

2.3.4. Burrow openings and consequences for irrigation

Number of burrow openings ranged from 1 to 6 (Mean 3.8, SD 1.9), and opening shafts originated from different parts of the burrow. In most of the burrows, opening shafts formed the U-shape at the top of the burrow. In four out of the six *B. arenosus* casts, opening shafts originated from lower parts of the burrow. Creating a connection between lower tunnels and the sediment surface would enhance water circulation through the burrow.

Water movement is enhanced in the U-shape section of the burrow via the Bernoulli effect (Vogel 1981). The combination of a sunken and a raised opening (on top of the ejecta mound) creates differing water pressures in the opening shafts, which causes water to move through the U-shaped section of the burrow. Fresh water movement into the lower section of the burrow would occur due to movement by the inhabitant. If the lower section of the burrow was connected to the surface by an additional opening, then active irrigation may not be as necessary to control water quality. In the *B. arenosus* burrow casts the additional opening shafts originated from parts of the burrows which were separated from the U-shape by either a spiral section of tunnel, or a large length of tunnel, possibly providing an additional obstacle to water movement.

The opening shafts of all burrows have a reduced diameter compared to burrow tunnels. This supports the observation that once a shrimp buried itself, it was never seen leaving the burrows. One reason for reducing diameter of the shaft is to take advantage of the Venturi effect (Nickell and Atkinson 1995). The Venturi effect is the increased velocity fluid experiences when passing from a wider to a narrower tube. This would enhance the efficiency of sediment ejection from burrows. As the shrimp creates a current at the base of the shaft, the water and sediment suspension moves into the narrower shaft and increases velocity sufficiently to force the suspension from the burrow.

A constriction followed by an enlargement in the burrow diameter was often observed at the base of the opening shafts, an observation that has also been made in other species. Dworschak (1983) found that *Upogebia pusilla* constricted shafts specifically in response to increased salinity. Frey *et al.* (1978) suggested the constriction helped prevent material and predators from entering burrows.

2.4. Burrow lining

Trials with Schiff's reagent were unable to distinguish sediments with and without muco-polysaccharides, because all samples stained darkly. The staining technique was designed for staining tissue samples a few microns thick, so sediment embedded in gelatine was far too bulky, and continually overstained. The burrow wall of both species did not have a discrete structure, but was smooth, shiny and compacted due to animal movement and to the animal tending the burrow wall. Dworschak (1983) described the same for *Upogebia pusilla,* in which the inner burrow wall was smooth and outer parts of the turning chambers and the bottom of the U sometimes rougher due to particles sedimenting there.

The burrow wall was also a lighter red-brown colour compared to the surrounding sediment. The lighter sediment is oxidised and the colour is due to ferric oxyhydroxides. The darker colour of the surrounding sediment is due to reduced compounds such as FeS (Matisoff *et al.* 1985). This colouration has been observed in burrows of many species (Henriksen *et al.* 1983, Matisoff *et al.* 1985, Hüttel 1990, Davey 1994), and is a clear indicator of an aerobic environment inside the burrow lumen.

2.5. Feeding

2.5.1. Gut content analysis

Gut contents confirm that both are deposit-feeders. Contents reveal a diet of sediment grains and a small amount of unidentifiable organic matter. Deposit feeders cannot gain sufficient energy from sediment grains (Cammen 1980), but the bacteria and fungi adhering to the grains are a much more important food source (Odum and Heald 1972, Berrie 1975, Cammen 1980).

2.5.2. Discussion

The structural parts of plants, such as cellulose and lignin, are not readily digested by organisms (Bernie 1975). However, the nutrient content of the plant material is often increased by decomposition and microbial action (Tenore 1975, Cundell *et al.* 1979, Fell and Master 1980, Rice 1982). Also, bacteria assimilate inorganic nutrients while decomposing plant matter, and the complex of detritus and microbial biomass becomes enriched for deposit-feeders (Fenchel and Harrison 1976).

Faeces formation by deposit feeders increase surface area for colonisation by micro-organisms (Hargrave 1976). Hargrave found that faecal material was rapidly colonised and activity (measured via oxygen uptake) reached a peak within 2-3 days. A feedback relationship is thought to exist between the deposit feeder and the microbe, whereby increased micro-organism number increases deposit feeder abundance (Driscoll 1975). Deposit-feeders enhance their own food source by stimulating decomposition of organic matter via faecal production (Hargrave 1976) and by grazing of micro-organisms which is thought to keep the latter in a highly productive growth phase (Aller 1982).

2.5.3. Burrowing behaviour

Individuals were not ever seen on the sediment surface; they fed on sediment within their burrow either from the burrow wall or from sites of excavation. The shrimp appeared to be continually moving within their burrows and were often observed carrying sediment in their appendages. Sediment (from either feeding or excavation) was occasionally ejected from the burrows. This sediment could be from anywhere in the burrows, so potentially sediment from the base of the burrow (up to 30 cm deep) may be brought to the surface.

2.6. Sediment turnover

2.6.1. Turnover rates

All *B. arenosus* tanks showed a decrease in ejecta rate after week 4 (Fig. 4). This trend continued in the following weeks signalling that the main burrow had been excavated, and further digging and ejecting sediment was related to burrow maintenance and feeding. Hence, sediment processing estimates were calculated from week 4 onwards, to avoid any 'start-up' effect. It is assumed that the shrimp inhabit a single burrow in their life-time, but if this is not the case, sediment processing rates listed here will be an underestimate.

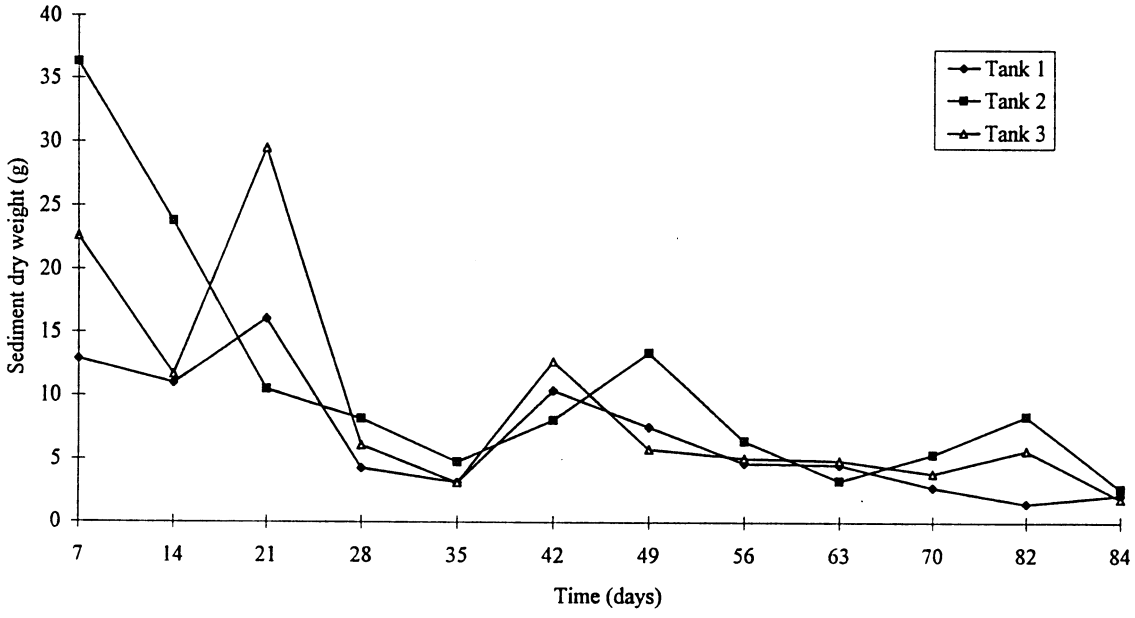

Figure 4: Sediment ejected from *Biffarius arenosus* burrows over time

Biffarius arenosus individuals burrowed in tanks containing sandy sediment, and ejected between 0.66 and 0.96 g sediment per day (Mean 0.79). Ejecta rates varied within and between tanks, suggesting that burrow building and feeding rates may not be consistent across individuals or over time. A single factor ANOVA comparing ejecta rates between tanks showed no significant difference (MS 0.43, df 2, P 0.247). However, a power analysis revealed that we had an 88% chance of not detecting a difference between replicates if there was one. Obviously more replicates would be ideal, but due to low capture and survival rates of individuals, these are the only data available.

Neocallichirus limosus tanks were run for only half as long as the *B. arenosus* tanks due to death of the animals (Figure 5). No significant decrease in ejecta rate was observed after week four in *N. limosus* tanks, and ejecta from both tanks was not significantly different (MS 5.4, df 1, P 0.237). Mean turnover rates were slightly lower than *B. arenosus* rates, ranging between 0.48 and 0.71 g sediment per day (Mean 0.56), but again the two species rates were not significantly different (Table 2).

Table 2: Nested ANOVA comparing sediment turnover rates of *B. arenosus* and *N. limosus*.

Source	Mean Square	DF	F-Ratio	P
Species	0.214	1	0.728	0.401
Individual (species)	0.287	3	0.976	0.418
Error	0.294	29		

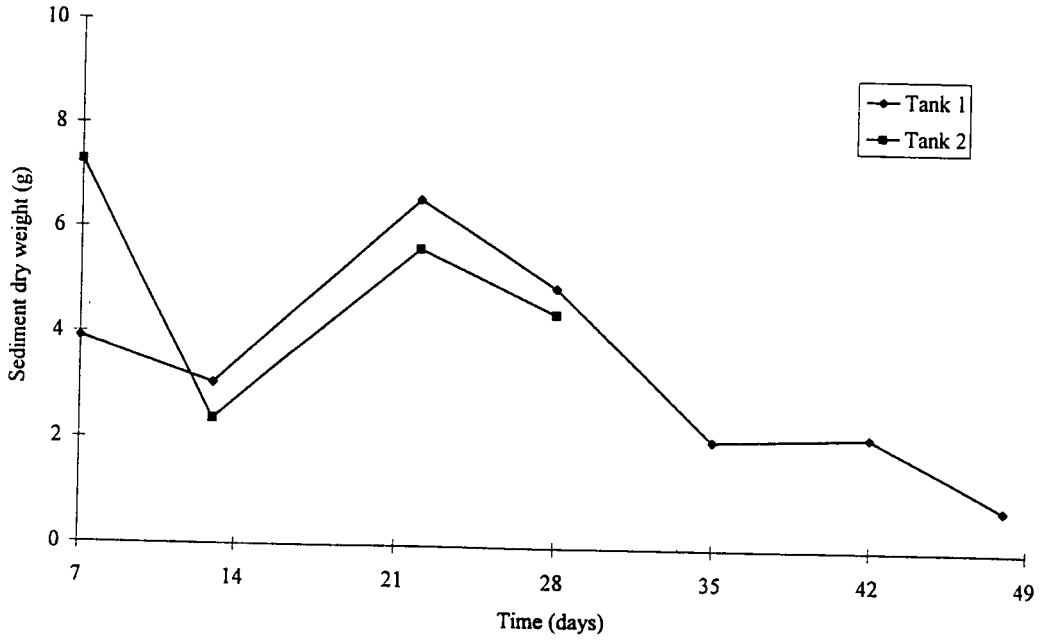

Figure 5: Sediment ejected from *Neocallichirus limosus* burrows over time

2.6.2. Comparison to other species

Sediment turnover rates for these two local species were similar to documented turnover rates. A deposit feeding species from the North Sea, *Callianassa subterranea*, turned over approximately 1.75 g (dry sediment)/day per burrow (Witbaard and Duineveld 1989). This species is similar in size to both *B. arenosus* and *N. limosus*, and is found in muddy sediments. It had been thought that sediment turnover rates might differ between different sediment types, but from the present study did not support the hypothesis. No published turnover rates from a similar sized temperate species residing in sandy sediments were available for comparison.

2.6.3. Baywide estimates

Baywide sediment turnover rates for both species are shown in Table 3. Because overall abundances of callianassids has decreased 20-fold in Port Phillip Bay since 1969-73, sediment turnover rates have also decreased. If sediment turnover is reduced, then the chemical and physical processes influenced by turnover will also be affected. In other studies, callianassids have been shown to redistribute sediments relative to their particle size, with burial of coarser material and ejection of finer material from burrows (Suchanek 1983). Fine particles have a large surface area to volume ratio, so will hold more organic matter and bacteria than coarser particles (Rashid 1985, Self and Jumars 1988). Fine grains containing more bacteria would be more reactive for processes such as nitrification (Aller 1982). Ejected sediments are aerated, either promoting reactions (if already aerobic) or changing reactions (if sediment was anaerobic).

Table 3: Baywide sediment turnover estimates of *B. arenosus* and *N. limosus*.

	1969-1973	1994-1995
B. arenosus		
Estimated abundance	2.5×10^{10}	1.8×10^{10}
Baywide sediment turnover ($\times 10^6$ tonnes/day)	6.0 - 8.8	4.3 - 6.2
N. limosus		
Estimated abundance	2.1×10^{12}	1.1×10^{10}
Baywide sediment turnover ($\times 10^6$ tonnes/day)	370 - 540	2.0 - 3.0
Both species combined		
Baywide sediment turnover ($\times 10^6$ tonnes/day)	380 - 550	6.3 - 9.2

Using turnover rates calculated for *C. subterranea* (Witbaard and Duineveld 1989) and abundances of *N. limosus* from the 1969-73 study (Poore *et al.* 1975), a Baywide turnover rate was estimated at 8×10^5 tonnes/day (Bird 1994). Using actual rates and current density data a rate of 4.6×10^3 - 6.8×10^3 tonnes/day was calculated. So the estimate from Bird (1994) was approximately 1000 tonnes/day too high for current *N. limosus* densities.

2.6.4. Fate of sediment

Recent experiments investigating sediment movement by *B. arenosus* using stratified rare earths, have suggested that major sediment redistribution is restricted to the top 7-8 cm (G. Batley unpublished data). Some sediments from lower levels were detected in the ejecta mound, but these amounts were insignificant relative to redistribution of the top few centimetres. Burrows of *B. arenosus* in Port Phillip Bay extend to a maximum depth of 40 cm (Table 1), and G. Batley (unpublished data) suggests that sediment at depths lower than the top 10 cm are redistributed at similar depths by filling in disused tunnels and chambers, rather than being ejected at the surface. Another study has found that burrow depth does not correlate with the depth of sediments returned to the surface. The echiuran worm *Maxmuelleria lankesteri* builds burrows to depths of 80 cm, but returns little deeply-buried material to the sediment surface (Hughes *et al.* 1996).

Few studies have shown how callianassids affect vertical transport of sediments. Suchanek *et al.* (1986) has shown that callianassids burrow to depths of over 2 metres in the Enewetak Atoll lagoon, and burrows intersect a layer of sediment with a high concentration of radionuclides. Suchanek *et al.* suggested that sediment in that area may be returned to the surface, but no evidence was provided.

2.6.5. Sediment burial rate

Using current abundances, burial time of surface sediments to a depth of 0.5 cm was estimated at 42 years. This value is much higher than that gained from 1969-73 abundances, which estimated a 5 year burial time due to callianassid activity. Once the reactive organic matter reaches the anoxic zone, its presence may enhance denitrification rates.

3. THE HEART URCHIN *ECHINOCARDIUM CORDATUM*

3.1. Introduction

Echinocardium cordatum is a cosmopolitan heart urchin (order Spatangoida), with a maximum test length of 50 mm (Buchanan 1966,1967), and is distributed throughout Port Phillip Bay, in both sandy and muddy sediments (Poore *et al.* 1975).

Echinocardium cordatum is an important bioturbator, but using a different method than the callianassids. The urchin does not live in permanent burrows, but burrows through sand with an action resembling a plough. The space surrounding a buried urchin is known as its burrow. Buried individuals maintain contact with the sediment surface via a respiratory siphon if the urchin is buried deep (Nichols 1959), or via a tuft of apical spines positioned on the urchin's dorsal surface (Chesher 1969) (Figure 6). De Ridder *et al*. (1987) suggests that the presence of a respiratory siphon linking a burrow to the sediment surface is a specialisation for successfully colonising fine sediments.

Echinocardium cordatum is a deposit feeder, and ingests sediments as it moves along. Particles are either gathered by the oral tube feet from the excavated area in front of the test (Buchanan 1966), or from the sediment surface (De Ridder 1982). Surface sediment falls down the respiratory siphon, accumulates between the apical spines, and is transported to the mouth by a conveyor belt arrangement of spines, tube-feet and mucous strings (Buchanan 1966, De Ridder *et al.* 1984). Once ingested, the sediment is lubricated by mucous and compacted and packed by actions of muscles and secretions in the gut (De Ridder and Jangoux 1993).

Rather than being sediment size selective, it appears that *E. cordatum* selects food particle type. Individuals digest and absorb only detrital material associated with the sediment, not organic matter adsorbed to the sediment grains (De Ridder *et al.* 1984).

Echinocardium cordatum movement through the sediment disrupts the horizontal layers to the depth and width of the urchin (Chesher 1969). Whether this disruption of sediments results in vertical mixing or burial of surface sediments, is unknown. Chesher (1969) found that burrowing action shifts large particles to the surface sediments, and that sediment is moved posterior in the burrow by successive waves' of spines. If sediment is ingested from the surface, it will be buried deeper in sediments after digestion. Once food passes through the guts, it is excreted into the sanitary tube at the posterior end of the urchin (Nichols 1959).

In clean sand, *E. cordatum* moves water for respiration through the sediments (Chesher 1969, Foster-Smith 1979). Because of the decreased porosity in silty sediments, water is obtained by drawing surface water through the apical spine opening or the respiratory siphon (Chesher 1969). Once water is drawn into the burrow, it flows outward from the apex, past the respiratory tube feet and is forced out of the burrow through the interstitial spaces of the surrounding sediment. Drainage tunnels or sanitary tubes, which assist burrow drainage, are constructed behind the urchin (Chesher 1969, De Ridder and Lawrence 1982). Chesher (1969) found that water rarely returned to the surface once it was drawn into the burrow.

Increased water flow into sediments changes pore water content (Foster-Smith 1979), and increases concentration of oxygen in sediments, which enhances aerobic reactions such as nitrification. *E. cordatum* has been shown to stimulate aerobic microbial respiration and re-oxidation of reduced inorganic compounds such as sulphide (Osinga *et al.* 1995). This effect was more obvious under increasing eutrophic conditions.

A comparison of current abundances of *E. cordatum* to those from the 1969-73 reveals a 50% decrease (Wilson *et al.* 1996) (Appendix A). This population decline will decrease the urchins' effects on the physical and chemical sediment processes.

3.2. Methods

Animals were collected from Smith-MacIntyre grab samples taken by the Museum of Victoria in October 1995. Specimens were housed in temporary aquaria awaiting experimentation. *E. cordatum* was sampled from both muddy and sandy sediments, and the populations were housed in separate aquaria in their appropriate sediments.

3.2.1. Depth and speed

Depth was measured as the distance from the dorsal surface of an individual to the sediment surface. One observation (n) is one individual's depth measured once on a day. Movement speed of individuals was estimated by measuring distance of an individual from a reference point over time.

3.2.2. Sediment mixing

During movement *E. cordatum* disrupts the sediment surrounding it, to the width of its test and to the depth of its lower edge. The amount of sediment mixed by individuals was estimated by multiplying mean width of test, depth to lower edge of test and rate of movement (Chesher 1969). Bay wide estimate were calculated using 1969-73 and current abundance data.

3.2.3. Burial rate

Reactive organic matter drawn into the urchin's burrow during movement will either be ingested or buried. The number of days taken to bury sediment to a depth of 2.0 cm (in mud) and 2.5 cm (in sand) was calculated by multiplying the Bay area by the depth of burial, and dividing this by the mean sediment mixing rate times the abundance of urchins.

3.2.4. Ingestion rate

Sediment ingestion rate was studied by allowing urchins to feed in finely crushed pink quartz. Ingested quartz was an obvious marker band which provided a point of reference at time zero. Urchins were then placed in normal sediment for a known time. The amount of sediment in the guts between the marker band and the mouth was dried and weighted, and related to the time spent feeding.

3.3. Burrowing behaviour

Burrowing behaviour of individuals varied over time. When added to tanks, some individuals would almost immediately begin to bury themselves, while others did not move for a considerable period of time.

Kanazawa (1991) categorised spatangoid echinoids by their test profile and related this to sediment type inhabited. Kanazawa found that *E. cordatum* had a 'globular' test shape which was suited to deep burrowing in fine-medium sandy sediments. A 'wedge' shaped test was more suited to burrowing in muddy sediments. Thus *E. cordatum* may not be well adapted to living in muddy sediment, possibly accounting for the observation that a population living in clean sand was more successful than one living in silty sand in the North Sea (Buchanan 1966). Buchanan found that the urchins burrowed shallowly (< 2 cm) and moved slowly through muddy sediments. The population inhabiting the mud also had a much slower growth rate.

3.3.1. Depth

Individuals burrowed deeper in sandy sediments than in muddy sediments (Table 4), and a significant difference in burrowing depths was found between sediment types (MS 2.07, df 1, P 0.016). Depth range was also much larger in urchins living in sandy sediments (Table 4), suggesting that a wider range of depths could be successfully utilised. Urchins were often located in both sediments by the apical spines sticking out of the surface, especially during movement.

Table 4: Burrowing speed (cm/hr) and depth to the dorsal edge (cm) of *E. cordatum* individuals.

Substrate	Sand		Mud	
	Depth	Speed	Depth	Speed
Mean	0.71	0.68	0.46	0.59
Standard deviation	0.67	0.62	0.42	0.60
Range	2.40	2.50	1.70	3.07
Count	82	60	54	39

Buchanan (1966) suggests that inefficient working of the feeding mechanisms is the reason why *E. cordatum* burrows deeper in sand than mud. Fine silt is thought to congest the spine tract which needs to be cleared with copious amounts of mucous (Buchanan 1966). Ability to maintain an open respiratory siphon in muddy sediment would have an important impact on burrowing depth. Buchanan (1966) and Foster-Smith (1978) found that in sand mixed with silt and clay, the respiratory siphon was absent, but the apical tuft (collection of long spines on the dorsal surface) keeps a funnel to the surface open in these experiments (Figure 6).

Burrowing depths of both the mud and sand populations of Port Phillip Bay were similar to those found in a New Zealand population inhabiting medium-fine sands (Higgins 1974), and to depths reported in mud in an intertidal population in the North Sea (Buchanan 1966). However, much larger depths have been reported for *E. cordatum* species in both sand (15-20 cm) (Nichols 1959, Buchanan 1966) and sand with clay (10-15 cm) (Nichols 1959).

3.3.2. Speed

Individuals generally burrowed faster through sandy sediments than muddy sediments (Table 4), but due to high levels of variability, no significant difference was found (MS 0.203, df 1, P 0.462). Burrowing speeds were much slower than previously quoted values of 6-8 cm/hr in clean sand and 2 cm/hr in silty sand (Buchanan 1966). There is an obvious reduction in speed relative to increasing silt/mud content of sediments (Buchanan 1966), perhaps indicating that Port Phillip Bay sediments are finer and higher in organic content than those investigated by Buchanan.

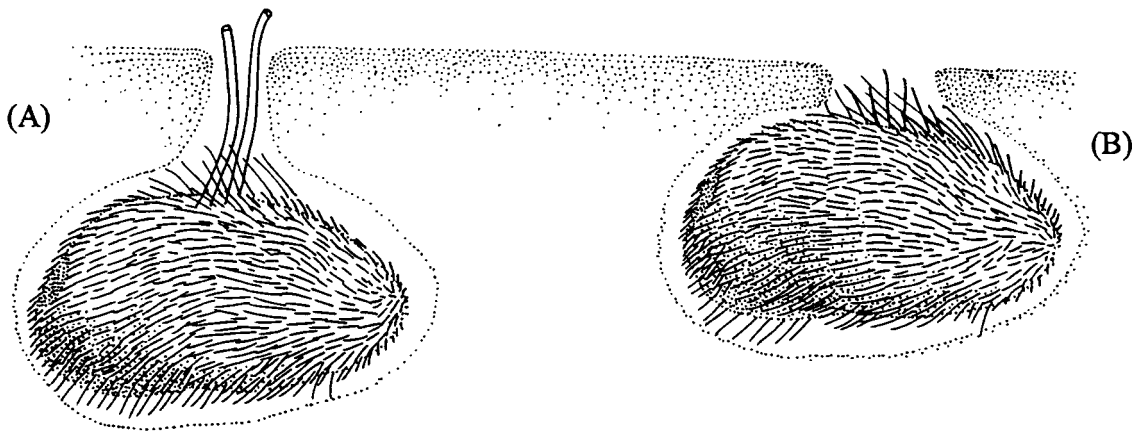

Figure 6: Burrowing behaviour of *E. cordatum*, with connection to overlying water via (A) respiratory siphon and (B) apical spines.

3.4. Sediment mixing

3.4.1. Fate of sediment

Overall, *E. cordatum* burrows deeper in sandy sediment but moves at the same rate as in muddy sediment (Table 4). Increased burrowing depth would increase the amount of sediment disturbed by a burrowing individual. Nearly all individuals observed moving were connected to the surface with their apical spines. This supports Chesher's (1969) observation that individuals with a high respiratory demand are found close to the sediment surface.

As previously mentioned, whether the urchins vertically mix sediments during movement and feeding is inconclusive. However, if urchins in this study only moved when connected to the surface by dorsal spines, and sediment was ingested from the surface anteriorly and excreted posteriorly, and sediment was continually moved posteriorly around the outside of the test by spines, then the authors suggest that sediment to the depth and width of the urchin is mixed, both vertically (from the surface) and horizontally (within the strata of movement).

3.4.2. Mixing rates

If an average urchin has a height of 1.54 cm, then urchins will mix sediment to a depth of 2.25 cm in sand and 2.0 cm in mud. Sediment mixing rates in sand were approximately double that in mud because the urchins burrowed about twice as deep in sand than mud (Table 5).

Table 5: Individual sediment turnover rates (cm^3/day) using both mean and maximum burrowing depths of *E. cordatum* in different substrates.

Substrate	Sand	Mud
Calculation using mean depths	90.0	55.2
Calculation using maximum depths	157.5	88.8

3.4.3. Baywide estimates

Baywide sediment mixing rates have halved (Table 6) since 1969-73 because of the decrease in urchin abundances. This would mean less water is introduced into the sediments during movement, which would decrease the effect *E. cordatum* has on aerobic microbially-mediated reactions and chemical processes such as oxidation of reduced compounds (Osinga *et al.* 1995).

Table 6: Baywide sediment turnover estimates of *E. cordatum* using means and maximums calculated from individual turnover rates.

	1968-1972	1994-1995
Estimated abundance	6.8×10^{10}	3.4×10^{10}
Sediment turnover (m^3/day)		
Sand		
Mean	2.8×10^{6}	1.4×10^{6}
Maximum	4.9×10^{6}	2.4×10^{6}
Mud		
Mean	2.1×10^{6}	1.0×10^{6}
Maximum	3.3×10^{6}	1.7×10^{6}
Total		
Mean	4.9×10^{6}	2.4×10^{6}
Maximum	8.2×10^{6}	4.1×10^{6}

3.4.4. Burial rate

Using current abundances, burial rate of surface sediment in sandy sediments, was estimated at 32 days. In muddy sediments, burial rate was slightly slower at 52 days. This means surface sediments are buried to a depth of 2.5 cm eleven times a year in sand, and to a depth of 2 cm seven times a year in mud. Organic material is brought in close contact with the anoxic zone by burial, and denitrification will be enhanced.

3.5. Feeding

3.5.1. Food source

Previous work revealed that *E. cordatum* also collects sediment from the surface by sending out search tubefeet up the respiratory siphon (De Ridder *et al.* 1984). These authors suggested that the main food source for *E. cordatum* comes from the detrital sediment containing high organic matter from the surface. Deep-seated sediment, anterior to the urchin, is ingested to bulk up the gut so that food is efficiently shifted along the alimentary canal. Sediment ingested anteriorly may have a purely mechanical function. Most urchins observed in this study were moving through the surface sediments, or just below them, keeping the respiratory connection open to the surface with the apical tuft of spines (Figure 6). These individuals were ingesting surface and just sub-surface sediment as they moved through.

3.5.2. Ingestion rate

Urchins ingest the sediments which they move through. Individual ingestion rates are listed in Table 7. There are no published reports of ingestion rates of species of a similar size for comparison.

Table 7: Ingestion rate of *E. cordatum*, including Baywide estimates.

	Range	Mean	Standard deviation
Ingestion rate (g/day)	0.03 - 0.15	0.06	0.05
Bay wide estimate (tonnes/day)			
1969-1973		9.8×10^4	
1994-1995		4.9×10^4	

3.5.3. Baywide estimates

Baywide ingestion rates are listed in Table 7, and again a decrease over time is obvious due to a drop in individual numbers in the Bay. The data suggests that 2.1×10^6 tonnes of the Bay's sediments are ingested and stripped of bacteria and organic matter daily by this species. This reduction in *E. cordatum* feeding due to population decline will cause a decrease in microbial processes and reaction rates in sediments because of diminished microbial grazing and faecal production. Assimilation of food from sediments would provide new surfaces for fungal and bacterial growth, and *E. cordatum* stimulates micro-benthic activity therefore maintaining the food source (Chesher 1969). Micro-organisms in sediments are tied to decomposition of organic matter and several important chemical pathways such as nitrification/denitrification. If microbial activity is enhanced so will be these processes. Microbial mediated decomposition reactions are stimulated by increased surface area of organic detritus (by feeding and by producing faeces) and increasing electron acceptor supply by aerating sediments during reworking and animal movement (Aller 1982).

4. DIRECT MEASUREMENT OF DIFFUSION RATES AND THE EFFECTS OF BIOTA

4.1. Introduction

Nicholson *et al.* (1996) state that pore-water profiles in Port Phillip Bay sediments are characteristic of benthic faunal activity. The profiles show an increase in solute concentration to a sub-surface maximum (at 5 cm) and then a decrease in concentration with depth rather than a gradual increase to an asymptotic value. This type of profile has been found in other sediments inhabited by bioturbating species (Grundmanis and Murray 1977, Emerson *et al.* 1984).

Henriksen *et al.* (1983) showed that nitrate/nitrite concentrations were highest in the profile at the depth a species was burrowing. For example, the species *Corophium volutator* burrows to a depth of 4 cm, resulting in a significant increase in nitrate concentrations in the upper 4 cm of sediment (Henriksen *et al.* 1983). The burrowing activity of *C. volutator* introduces oxygen into anoxic sediments, which enhances nitrification and therefore nitrate/nitrite production in the burrowed zone (Henriksen *et al.* 1983). The lugworm, *Arenicola marina*, also increases nitrate and decreases ammonia concentrations at the depth of its burrow by promoting nitrification (Hüttel 1990). Hüttel (1990) found a maximum concentration of ammonia and silicate at a depth of 3.5 - 5 cm (which was also the zone of redox potential discontinuity). Below this layer the redox potential decreased as did the concentrations of the two solutes. Matisoff *et al.* (1985) also found that pore water concentrations of chemical species usually found in high concentrations in reduced sediments were low within the irrigated burrow zone.

Nicholson *et al.* (1996) calculated a flux enhancement factor for Port Phillip Bay sediments, which compared diffusive flux (molecular movement of solutes passively over the water sediment interface) to the chamber flux (the actual flux measured under the chamber which was enhanced by bio-irrigation). This flux enhancement factor ranged from 0.27 (NO_x in cold temperatures) to 7.96 (SiO_4 in warm temperatures). These values suggest that burrowing activity of macrofauna, either by irrigation of permanent burrows or disruption of sediments during movement, may increase diffusive flux from sediments into the overlying water by 0.27 to 7.96 times. An oxygen flux enhancement factor was calculated in the same way by Clavero *et al.* (1992) for dissolved phosphate. Clavero *et al.* found that flux was enhanced by 1.7 to 5.8 times depending on density of the polychaete *Nereis* sp. These values compare well to those calculated by Nicholson *et al.* (1996).

Bioturbating fauna are known to increase net diffusion of solutes from and into sediments (Waslenchuk *et al.* 1983, Hopkinson 1987, Aller and Aller 1992). Permanent burrow structures, like those inhabited by the ghost shrimp of Port Phillip Bay, increase the surface area for diffusion along gradients, by extending the water-sediment interface deep into the sediments. In other callianassid species, surface area is increased between 0.25 and 16 times (Ott *et al.* 1976, Dworschak 1981, Koike and Mukai 1983, Dworschak and Pervesler 1988). An estimated increase to water-sediment interface by both Port Phillip Bay species was calculated at 8%. This means an additional 800cm^2 of interface exists due to callianassid burrows below every 1m^2 of sediment.

Burrows change the one-dimensional model of diffusion, whereby solutes can diffuse horizontally into the burrow lumen, as well as vertically from sediments into the overlying water (Aller 1982, 1983). Active irrigation and animal movement will increase diffusive flux by convective flow, where a water current literally drags solutes out of the surrounding sediment.

A burrow inhabitant needs to flush the burrow water regularly to remove toxic metabolites (Henriksen *et al.* 1983) and replenish oxygen supply. Regular replacement of burrow water will renew the solute concentration gradients and further enhance diffusive flux.

Bioturbators which do not create permanent burrows, like *E. cordatum*, will probably have a different effect on diffusive flux rate. These animals mix the sediments around them during movement, and if they remain within a specific horizontal layer, that section of sediment will be well aerated and mixed. Pore water is altered by *E. cordatum* forcing water through the sediments (Foster-Smith 1978). Net diffusion from the sediments is enhanced by sediment disturbance (Regnault *et al.* 1988). Pore water solute migrate more rapidly in a mixed layer disturbed by a bioturbator than can be accounted for by molecular diffusion (Matisoff 1982).

This investigation was prompted by preliminary results from the Nicholson *et al.* (1996) chamber study. Results indicated that burrowing activity may have been creating anomalies in benthic flux data. The aim of this study was to quantify the affect callianassid ghost shrimp and *E. cordatum* urchins (two numerically important bioturbators in Port Phillip Bay) had on diffusive flux from sediments into the overlying water, in a closed system.

4.2. Methods

Animals and sediments were collected from Smith-MacIntyre grab samples taken by the Museum of Victoria in October 1995. Mud and sand sediments were kept separate to house the different populations. Sediments were mixed to ensue homogeneity within treatments. Buckets were used as tanks, and were filled with sediment to 30cm depth. Seawater was added to the top and the water aerated continually throughout the experiment.

4.2.1. Experimental design

The experimental design included 4 treatments and 2 controls as listed below. Each treatment and control had 3 replicates.

- muddy sediments with 2 *N. limosus* individuals in each
- sandy sediments with 2 *B. arenosus* individuals in each
- muddy sediments with 4 *E. cordatum* individuals in each
- sandy sediments with 4 *E. cordatum* individuals in each
- control tanks with muddy sediments with no animals
- control tanks with sandy sediments with no animals

The animals were added to the tanks two months prior to the experiment date to allow the ghost shrimp to build burrows and the urchins to thoroughly plough through the sediments. The control treatments were set up two weeks prior to the experiment, using the same sediment, because tanks were not available immediately. The timing of treatments and controls may have created an anomaly in the results (as discussed in the results/discussion section).

The numbers of individuals added to the tanks were approximately double the natural densities in Port Phillip Bay, to ensure that an effect, if there was one, would be obvious. The experiment was run at a constant 15 ^{o}C.

Bubblers were positioned in the centre of the tank, and set at a constant rate in each tank using a measuring column. Air was bubbled into the column filled with water, and the volume measured over a given time. It was important that the water in the tanks be well mixed so that the tracer in the tank water was well mixed in a given time. This means the first sample could be taken when the tracer was at its maximum concentration in the water. Fluorescein solution was added above the bubbler and water samples were taken from the centre and four corners at 5, 10 and 15 minutes. These samples were analysed using a fluorimeter, with the sample excited at 490 nm and the emission read at 514.5 nm. After 15 minutes it was found that the dye was well mixed through the tank water.

Five ml of D_2O (99.8% ANSTO) were added to each tank at Time=0. Water samples were then removed from the centre of the tank at 15, 30 mins and 1, 2, 4, 8, 18, 24, 30, 42 and 48 hours. The sample was transferred to a 0.7 ml amber CHROMACOL vial and crimp sealed immediately. Standards were created by diluting 5ml D_2O to 1 litre with milli-Q water in a standard flask. Samples were taken from the standard flasks at the start and end of the experiment.

Samples were analysed in a VG Optima dual inlet isotope ratio mass spectrometer after reduction of the water to hydrogen gas over zinc at 500 ^{o}C. The results are reported as δ values relative to Vienna standard marine ocean water where

$$\delta = (R_{sample} / R_{standard} - 1) \times 1000$$

and R is the measured D/H ratio.

All results are collected together in Appendix B. Note that the concentration of D_2O continued to increase in the first few samples taken. This indicated that the overlying water of the tanks were not well mixed by the bubblers, and this lack of mixing was not consistent over the different tanks. For example, sand control tank 1 was well mixed after 15 minutes, compared to 120 minutes in sand control tank 3. Since the samples were not analysed until after the experiment was completed, we were unable to address the problem.

Water volumes above the sediment surface were calculated again using fluorescein. Five ml 1mM fluorescein was added to each tank, and after 15 minutes a water sample was taken from the middle of the tank and analysed using a fluorimeter (parameters described above). The concentration of fluorescein in the tank water was compared to the concentration of fluorescein in a standard of known volume. The water volumes were also calculated from the isotopic concentration data (Appendix C).

4.2.2. Analysis of data

The removal of the unreactive D_2O tracer added to each tank was treated as happening by a purely diffusive processes with the change in the concentration of the added tracer given by (Ford, Philip and Knight 1996) :

$$c(0,\tau) = e^{\tau}\, erfc\left(\tau^{1/2}\right),$$

where

$$\tau = \frac{\rho^2 Dt}{L^2}$$

and ρ is the porosity, D is the Diffusion coefficient (cm^2sec^{-1}), t is the time (sec), and L is the height (cm) of the chamber.

4.3. Results and discussion

The reduced concentration (actual δ value divided by the δ value extrapolated to t = 0) was plotted against time. On the same axes we plotted the reduced concentration, calculated from the above expression using the measured porosity and water column heights and a range of values of D. This provided an initial estimate of D which was subsequently refined by successive adjustments to achieve greatest concordance (visually estimated) with the experimental data. Figure 7 shows a representative example.

The measured diffusion coefficients (listed in Appendix D) had to be corrected for the tortuosity (^{2}) of the sediment to provide an estimate of the effective diffusion coefficient (Do) of D_2O in the pore water i.e. without the presence of the solid sediment matrix. The tortuosity was calculated from the relationship (Boudreau 1996)

$$\theta^2 = 1 - \ln(\rho^2)$$

and

$$D_o = \theta^2 D$$

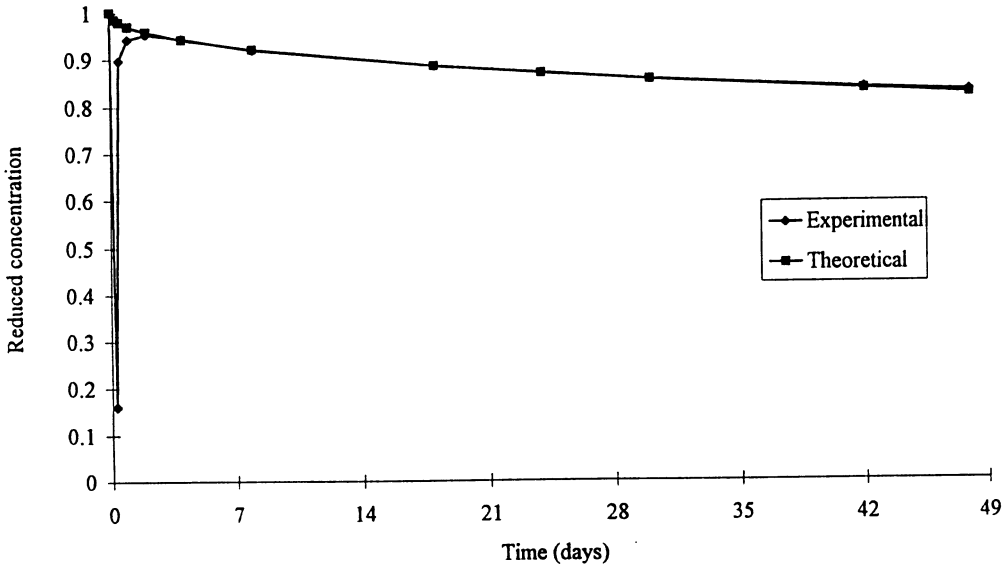

Figure 7: Theoretical reduced concentration of D2O compared to the measured reduced concentration in overlying water of Sand Control Tank 3.

Table 8 shows the calculated tortuosities of the various tanks together with the effective diffusion coefficients, and enhancement factors . The enhancement factor is the ratio of the measured effective diffusion coefficient to the actual diffusion coefficient for D_2O in water (1.97 +/- 0.02 x 10^{-5} cm^2 sec^{-1}) (Eisenberg and Kauzmann 1969) at the tank temperature (15 ^oC). It is a direct measure of the extent that flux of materials to the sediment is increased relative to purely diffusive transfer.

Table 8: Summary of porosity, tortuosity, measured Diffusion Coefficients and enhancement factors for all tanks and species (including controls).

Medium	Porosity	Tortuosity	Diffusion Coefficient (std) x 10^5	Enhancement factor
SAND				
Control	0.34	3.16	2.41 (0.27)	1.22
Callianassids	0.25	3.77	7.86 (2.98)	3.26
Urchins	0.22	4.02	8.49 (2.50)	3.52
MUD				
Control	0.31	3.34	7.92 (2.06)	4.02
Callianassids	0.58	2.09	3.45 (1.66)	1.75
Urchins	0.51	2.20	2.11 (1.16)	1.07

4.3.1. Enhancement of net diffusion by biota

For the sand tanks we see that the effective diffusion coefficient in the 'control' tanks is only marginally larger than the pure diffusive value measured under ideal laboratory conditions (enhancement factor 1.2). In marked contrast the diffusion coefficients for both the callianassid and urchin tanks are significantly greater than the controls (MS 0.958, df 2, P 0.007). There is no significant difference between the two species. We conclude that the presence of either callianassids or urchins at population densities comparable to those in Port Phillip Bay leads to an enhancement of the rate of transfer of dissolved substances between the sediments and the water by a factor of 3.3 for callianassids and by a factor of 3.5 for the urchins. We note that the variation between tanks was not large, giving power to the results.

4.3.2. Comparison with literature values

This measured increase compared well with the enhancement factors due to benthic activity calculated by Nicholson *et al.* (1996) and by Clavero *et al.* (1992). Previous studies have also reported an enhancement of diffusive fluxes by benthic activity of the same orders of magnitude. Using a Cl- conservative tracer, Aller and Yingst (1985) found that macrofauna increased diffusive flux by 2 - 5 times in the upper 8 - 12 cm of marine sediment. The polychaete *Nereis virens*, which inhabits a permanent burrow, enhanced diffusive flux of inorganic nitrogen by 2 - 4 times (Kristensen *et al.* 1991). Aller and Aller (1992) found that meiofauna increased diffusive flux rates of the tracers Cl- and Br- by 1.7 - 2.3 times. Matisoff *et al.* (1985) found that silica fluxes were enhanced by 3.1 times over the controls by chironomid larvae in freshwater sediments. Additionally, flux of ammonium in lake sediment was enhanced 7 - 18 times by different species of freshwater bioturbators (Fukuhara and Sakamoto 1987).

4.3.3. Explaining the muddy sediment results

The results from the tanks containing the muddy sediments are much less clear-cut. Firstly the control tanks show a significant enhancement over purely diffusive transfer (enhancement factor = 4.0). Similarly the callianassids have a relatively large transfer rate (enhancement factor = 1.8) relative to the theoretical value for pure diffusion. In marked contrast the diffusion coefficients for the urchin tanks are only marginally larger than the theoretical value.

There are several possible confounding factors in play. Due to logistic difficulties the mud control tanks were set up much later than the experimental tanks (2 weeks before the experiment as opposed to 8 weeks prior). Sandy sediment particles are larger and settle more quickly than muddy sediment particles, so once settled the sandy sediments should be relatively homogeneous. In comparison, muddy sediments have a high concentration of silt particles, which often remain suspended in the water for some time after addition of sediment to the tanks. During settlement, the mud falls in distinct layers with the finer particles settling on top. This stratification of muddy sediments would have occurred in both the control and experimental tanks, but the animals would have altered this distribution during the six weeks of burrowing and feeding activity prior to the experimental date.

Despite this the measured porosity of the control tanks (0.31) indicates that considerably greater compaction of the sediments had occurred relative to the previously prepared tanks containing the biota (porosities 0.58 and 0.55).

This raises the question whether the mud used in the control tanks may have different mechanical properties vis-a-vis the other two tank series, even though it was collected from the same nominal locality.

Alternatively, the activity of the biota may cause greater sediment mixing/bioturbation leading to resuspension which is reflected in the higher porosity. If this process is slow relative to the time scale of the experiments (2 days) it will not lead to a great increase in the measured diffusion coefficient nor will it be reflected in fast rates of nutrient transfer in benthic chambers. This hypothesis may be tested by examining the porosities of mud of similar particle size and mineralogical characteristics collected from regions of widely differing macrofaunal activity.

The transfer of dissolved tracer clearly takes place by an increase in the advection induced by the activities of the biota. All organisms do not use the same mechanical processes in producing these fluid motions. The pumping by the callianassids due to the rapid action of their pleopods and their piston-like motion up and down the burrows is quite different in character to the bulldozer like action of the echinoids as they push subterraneanly through the sediments. These two mechanisms may be affected differently depending on the particle size.

Advection depends on the permeability which is strongly dependent on both porosity and particle size. In a mud it is possible that the mud acts as fluid which flows smoothly around the advancing echinoids generating very little advective motion while the suppression of advection is less pronounced for the more dynamic callianassids. This offers an explanation why there are differences between the two species in mud but not in a sand, but it does not explain the similarity between the control and the callianassids in mud, nor the enhanced transfer seen in the controls.

5. CONCLUSION

The decline in callianassids and *E. cordatum* numbers since 1969-73, have lead to decreased bioturbation rates for Port Phillip Bay. A lower density of animals would also reduce the effect burrowing activity has on diffusive flux. A smaller Baywide burrow surface area would decrease the available microenvironment for nitrifying bacteria to exist in close association with the denitrification process, possibly diminishing the amount of denitrification occurring in the Bay's sediments.

Wilson *et al.* (1996) found that the composition of the benthic community has changed since 1969-73. A shift from a dominance of deposit-feeders to filter-feeders has occurred, as well as an overall decrease in macrofaunal numbers. Decline of callianassid and *E. cordatum* numbers are thus part of a wider phenomenon. The most interesting result, with the most relevance to this study, is the shift towards higher numbers of filter-feeders. Filter-feeders collect food from the water column, and although some species bury themselves in the sediment, they rarely establish extensive burrows. A community change from deposit-feeder to filter-feeder would decrease burrow surface area in sediments, microbial production that is enhanced in burrowed sediments, and the rate of sediment turnover and mixing of the Bay's sediments. These processes are known to be linked to diffusive flux, and the coupling of nitrification/denitrification, so that any further shift towards filter-feeders may alter the chemical dynamics of the Bay's sediments detrimentally.

This report shows the importance of sampling benthic macrofaunal populations. Bioturbation effects and rates can be investigated via experiments, but if population densities are not available for a system, then Baywide extrapolations can not be made. Repeat monitoring is also important because it shows how a population changes over time, and using calculated rates, how these changes influence bioturbating effects of the region.

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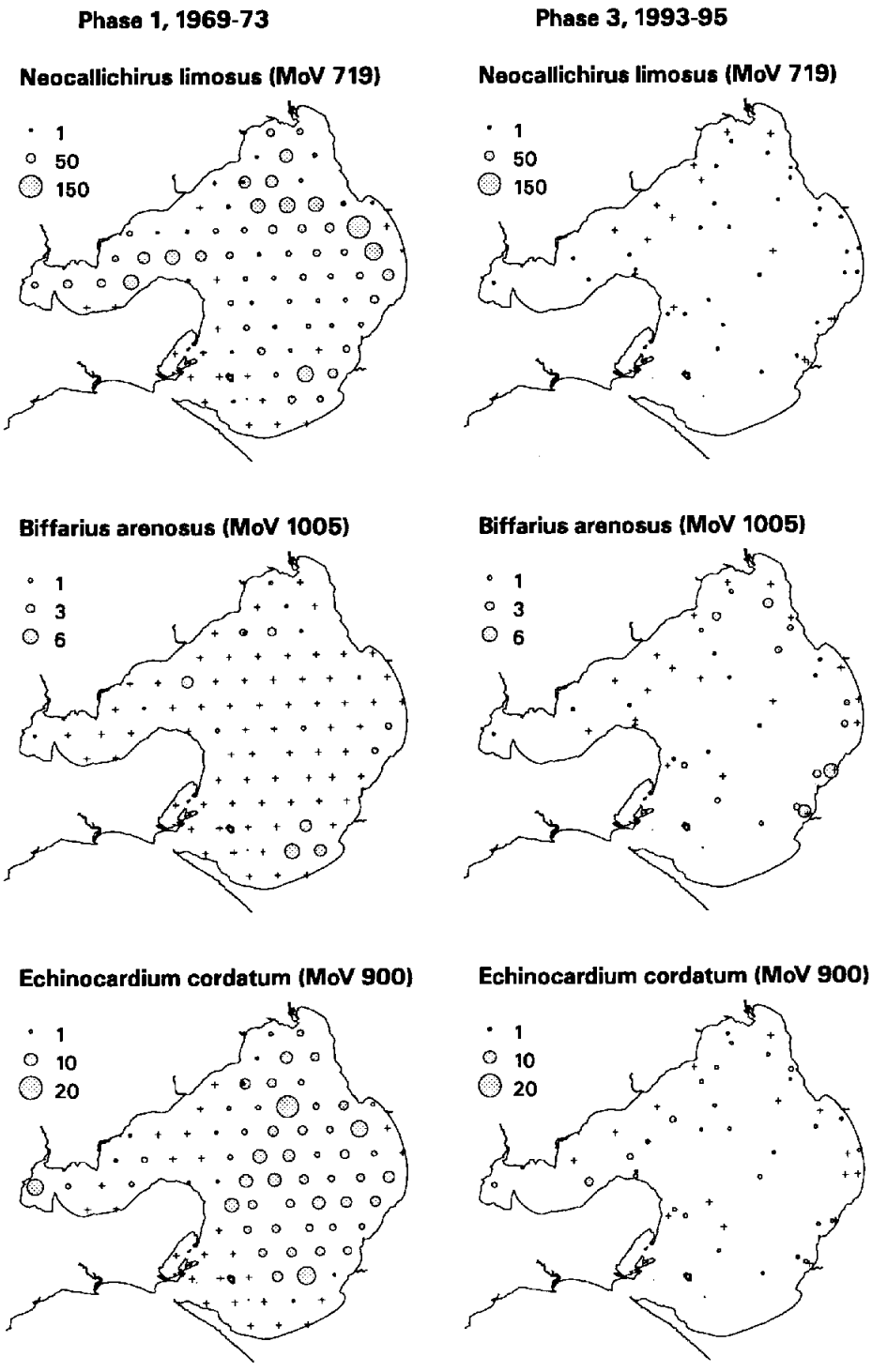
8. APPENDICES

Appendix A. Distribution and abundances of *Biffarius arenosus*, *Neocallichirus limosus* and *Echinocardium cordatum* in Port Phillip Bay sediments in 1969-1973 and 1993-1995.



Appendix B. Delta values for the diffusion experiment.

Tank	Time of sample (hours)	SAND Delta value Tank 1	SAND Delta value Tank 2	SAND Delta value Tank 3	MUD Delta value Tank 1	MUD Delta value Tank 2	MUD Delta value Tank 3
Control	0.25	25635.221	29583.657	4099.176	8104.892	18978.909	19559.188
	0.5	25232.841	29200.483	22863.488	14014.511	19393.242	19696.008
	1	24674.296	28757.801	24029.398	17046.232	19734.841	19686.681
	2	24079.320	27890.073	24314.065	17455.796	19793.343	19512.987
	4	23250.627	26664.956	24053.839	17096.048	19310.429	18897.103
	8	22122.746	25408.748	23426.202	16843.421	18574.753	18262.375
	18	20780.450	23381.747	22540.783	16046.489	17427.141	17090.300
	24	20374.974	22137.901	22122.165	15734.599	17025.621	16646.543
	30	20062.410	22451.390	21767.680	15506.257	16687.322	16489.628
	42	19390.216	21718.196	21258.234	15131.164	16102.767	15876.882
	48	19255.681	21379.089	21063.216	14988.006	15954.788	15673.052
Callianassids	0.25	15824.738	13342.936	14355.727	11478.580	12220.835	13529.066
	0.5	16800.793	13523.135	15824.577	12169.333	12685.397	14481.332
	1	16960.340	13442.140	15992.037	12540.122	12747.608	15485.952
	2	16768.168	13240.355	15732.100	12361.468	12560.248	15738.512
	4	16392.706	12917.110	15354.438	11919.485	12227.383	15363.324
	8	15604.399	12417.171	14445.936	11090.306	11525.225	14774.261
	18	14613.009	11737.125	13233.903	10186.339	10917.144	13377.913
	24	14106.039	11464.081	12658.055	9653.934	10569.831	12862.849
	30	13728.625	11205.141	12259.223	9197.524	10441.672	11791.316
	42	12770.935	10752.419	11499.426	8687.892	10010.723	11639.501
	48	12641.409	10539.280	11055.274	8427.943	97772.570	11394.238
Urchins	0.25	13230.798	16141.006	19107.062	9277.261	9675.733	10402.811
	0.5	13845.009	16966.445	17972.870	11189.980	11129.733	11345.426
	1	14318.689	17288.810	18075.132	12064.692	11948.295	12315.547
	2	14257.730	16870.342	17693.377	12464.665	11886.972	12601.732
	4	13797.688	16234.835	16940.379	13230.047	11710.393	12383.005
	8	13181.792	15390.023	15983.857	12951.033	11245.026	11847.088
	18	12311.815	14383.092	14799.592	12188.675	10668.935	11018.571
	24	11904.560	13945.995	14412.355	11901.110	10497.083	10692.847
	30	11707.401	13717.320	14111.007	11712.063	10310.945	10423.412
	42	11205.248	13320.348	13636.955	11265.909	9998.717	9958.070
	48	11079.416	13098.051	13372.391	11113.306	9875.475	9818.080

Appendix C. Volume of overlying water in tanks of the diffusion experiment.

Sediment	Tank	Replicate	Volume (litres)
Sand	Control	1	0.922
		2	0.749
		3	0.974
	Callianassids	1	1.480
		2	1.923
		3	1.621
	Urchins	1	1.967
		2	1.466
		3	1.478
Mud	Control	1	1.410
		2	1.226
		3	1.356
	Callianassids	1	1.855
		2	1.974
		3	1.877
	Urchins	1	1.980
		2	2.118
		3	2.423

Appendix D. Diffusion coefficients (cm^2 sec^{-1}) of mud and sand tanks containing no animals (control), callianassids or urchins.

Sediment	Tank	Replicate	Diffusion coefficient
Sand	Control	1	6.71×10^{-6}
		2	8.44×10^{-6}
		3	7.74×10^{-6}
	Callianassids	1	1.83×10^{-5}
		2	1.45×10^{-5}
		3	2.97×10^{-5}
	Urchins	1	2.81×10^{-5}
		2	1.68×10^{-5}
		3	1.83×10^{-5}
Mud	Control	1	1.76×10^{-5}
		2	2.36×10^{-5}
		3	2.99×10^{-5}
	Callianassids	1	2.41×10^{-6}
		2	8.25×10^{-6}
		3	1.72×10^{-5}
	Urchins	1	6.13×10^{-6}
		2	7.01×10^{-6}
		3	1.57×10^{-5}