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In September of 1989, the site of an active seafloor methane vent was confirmed approximately 20 km off the coast of Oregon in shelf waters 135 m deep. The location of the vent had been described to Oregon State University oceanographers by a local commercial fisherman. Preliminary observations in 1989 suggested that biological activity near the vent was high. In analogy to other known venting phenomena, it was proposed that the methane served as an energy source fueling enhanced biological productivity. This thesis addresses this hypothesis as a portion of a larger project which investigates the biology, geology and chemistry of shallow-water methane vents on the Oregon shelf. The data presented within are the results of a second oceanographic cruise to the site in September, 1990.

Samples of benthic and pelagic organisms were collected near the vent and compared to collections from background sites in terms of quantitative abundance of organisms, qualitative community composition and ratios of the stable isotopes of C, N, and S. The stable isotope analyses indicate that a significant fraction of vent methane-derived carbon is present in most samples collected near the vent. Despite this, there does not appear to be a major impact on the abundance of any organisms sampled. Several hypotheses are put

forward which might explain these results. The findings at this site are compared to what is known about other types of seafloor vents and seeps.

The Biology of a Shallow-Water Methane Vent

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THE BIOLOGY OF A SHALLOW-WATER METHANE VENT

INTRODUCTION

The primary focus of this thesis is a preliminary assessment of the biological impacts of localized methane release from sediments on the continental shelf of Oregon. This phenomenon is well known in the shallow waters of other areas of the world's oceans (King and McLean, 1970; Hovland and Judd, 1988). Venting of fluids from unconsolidated sediments leads to the erosive formation of crater-like depressions in the seafloor which have been called pockmarks (King and McLean, 1970; Hovland and Judd, 1988). Pockmarks are generally associated with subsurface natural gas deposits and are very common in areas with known petroleum or oil deposits, such as the North Sea (Hovland and Judd 1988). Because of the association with fossil fuel reservoirs, it is understandable that pockmarks were well known to geologists. Despite the fact that methane is a potentially utilisable source of biological energy, they had not been investigated by biologists until recently. Hovland and Judd (1988) describe a number of anecdotal reports of high abundances of organisms associated with pockmarks. Because of these reports, it has been proposed that pockmark methane venting enhances fish production in the North Sea (Hovland *et al.*, 1985; Hovland and Thomsen, 1989).

The existence of a pockmark with an active gas vent, located on the Oregon shelf approximately 28 km southwest of Coos Bay, Oregon, was verified by Oregon State University oceanographers in September, 1989 (see map, figure 1). It had originally been discovered by a local commercial fisherman who had seen the images of rising bubbles on his acoustic fish finder and deduced their nature from repeated observations over a period of seven years. Catches of yellowtail rockfish (nicknamed "greenies") were high near this

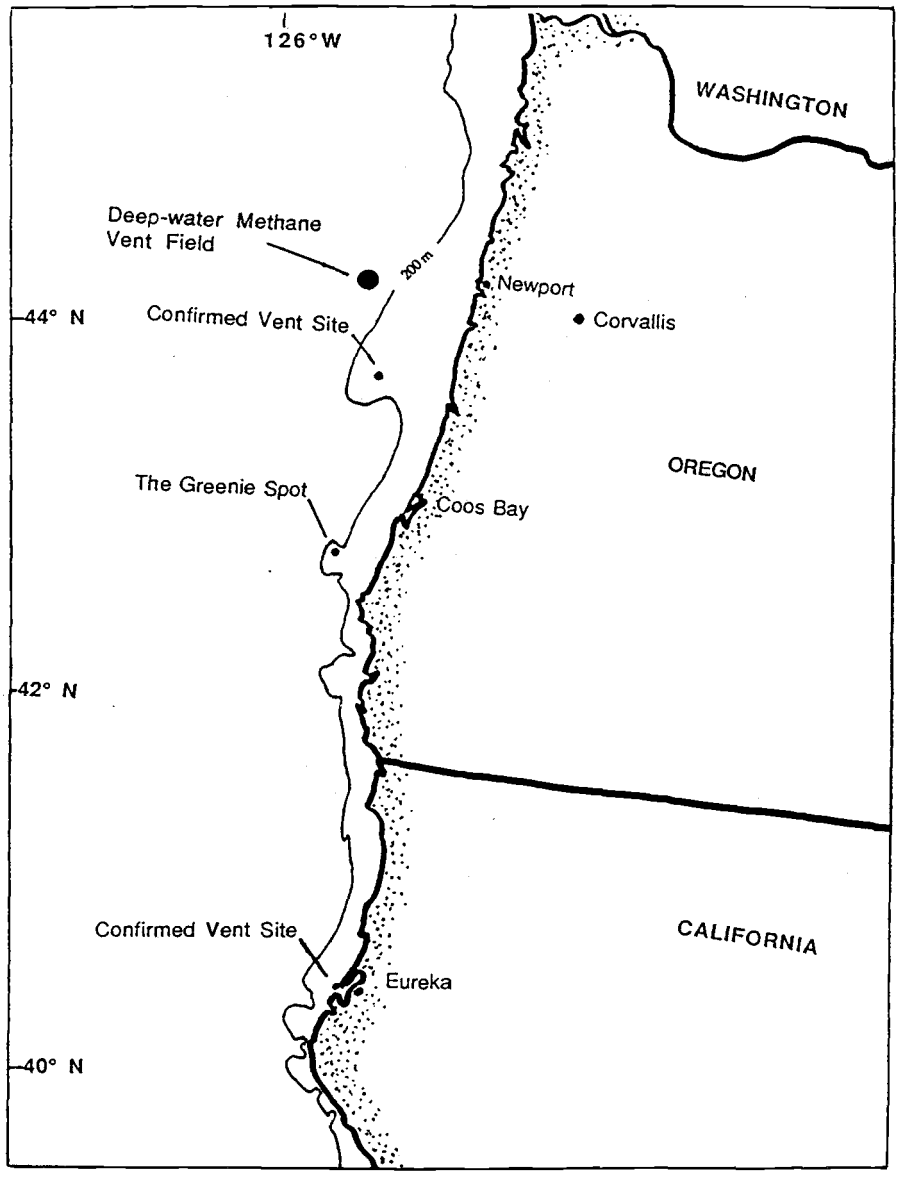


Figure 1 - Sites of known methane vents off the coast of the Pacific Northwest. Two other sites of shallow-water methane venting are known besides the Greenie Spot. The site near Eureka, CA consists of at least 3 and probably more individual vents. A large area directly west of Newport, OR is known to contain multiple deep-water methane vents.

site, leading fishermen to call it "The Greenie Spot". This site provides the focus of this thesis.

Another active vent was discovered in late September, 1990, north of the Greenie Spot on Heceta Bank. A few weeks later, in October, at least three pockmarks with active vents were verified within 5 km offshore of Eureka, CA with the assistance of scientists and students from Humboldt State University. These finds, along with reports from fishermen of more vents, as well as evidence in the form of dredged-up carbonate chimneys (termed "Chinese anchors" by fishermen), indicate that pockmark venting is common off the coast of the Pacific Northwest.

The novelty of the system and its potential impact on coastal ecology and commercially exploited species would by themselves make this study interesting. Additionally, venting of methane from shallow-water sediments can be placed within the larger context of vent and seep phenomena in general. The most famous of these are hydrothermal vents, first discovered at the Galapagos Spreading Center in 1977 (Corliss *et al.*, 1979). New sites of hydrothermal activity and other types of related phenomena have been discovered since then in many places under the world's oceans. Most examples of vent and seep phenomena have been studied in the deep ocean, but there are increasing reports from shallow, coastal waters. Regardless of location and depth, all types of venting phenomena are related in the sense that they provide a very localized, essentially point source of energy to the surrounding environment. Often this energy is used by bacteria which then form the base of unusual food webs with unique animals.

The three categories of vent-type phenomena which have been studied to date are hydrothermal vents and seeps, hydrocarbon vents and seeps and brine seeps. All three

types can be found in deep as well as shallow water. Below I will summarize the main characteristics of these various phenomena before describing the Greenie Spot in more detail.

Deep-Water Vents and Seeps

Deep Hydrothermal Vents

One of the most important and exciting recent advances in oceanography was the 1977 discovery of hydrothermal vents located at the Galapagos Spreading Center beneath the equatorial Pacific Ocean (Corliss *et al.*, 1979). Prior to this discovery, the ruling paradigm considered the deep ocean to be the stablest of environments which, due to food limitation, high pressure and cold temperature, had little biological activity. The few organisms which were present were generally small and grew very slowly (Grassle, 1978; Turner, 1981). The first investigation of hydrothermal vents revised this view in a dramatic fashion. It was obvious that the ecology of the deep sea could be quite dynamic, that organisms could grow to be large and very active. It became clear that pressure and cold were not obstacles to organisms (Grassle, 1985), that the limiting factor was the small amount of euphotic zone energy reaching the deep sea floor (Hecker, 1985).

Hydrothermal venting can occur wherever geothermally heated rocks and water come into close proximity. In reality, hydrothermal systems are not unlike freshwater hotsprings such as are found in geothermally active areas on land. Hydrothermal vents are best known from the volcanic mid-ocean ridges (eg. Corliss *et al.*, 1979; RISE project group, 1980; Enright *et al.*, 1981; Rona *et al.*, 1986; Tunnicliffe, 1988) but they have also been found in island back-arc basins (Hessler *et al.*, 1988; Halbach *et al.*, 1989; Fouquet *et al.*, 1991),

near volcanos (Zhirmunsky and Tarasov, 1990) and along geologic faults (Lonsdale, 1979). Hydrothermal vents have been found at the bottom of Lake Baikal (Crane *et al.*, 1991) and are postulated to exist at the bottom of Crater Lake (Dymond *et al.*, 1989). Almost all hydrothermal vents have been found deeper than 1500m, the exceptions will be addressed in a separate section below.

At mid-ocean ridges the mechanism of vent formation is as follows. As cold seawater percolates down through fissures in the newly formed seafloor it comes into contact with hot, new basalts. The water becomes superheated and rises back to the seafloor carrying with it dissolved sulfides and other compounds extracted from the rocks (figure 2). At the seafloor, the water may erupt like a continuous undersea geyser at temperatures up to 400°C (Fouquet *et al.*, 1991), or it may gently seep out in a diffuse flow at the comparatively cold temperatures of 5 - 25°C (Jannash and Mottl, 1985). Large chimney structures may form around the mouths of high flow vents as precipitates from the hydrothermal fluids build up over time.

It may be premature to make generalizations about the biological communities at hydrothermal vents; only a fraction of the areas which might support hydrothermal activity have been investigated and the majority of these have been in the eastern Pacific. Even the sites that are known are undersampled and understudied. Most of the organisms found at hydrothermal vents belong to new taxa and many are as yet undescribed (Grassle, 1985, 1986; Tunnicliffe, 1988; Desbruyeres *et al.*, 1985). Additionally, every large area with venting activity consists of multiple smaller fields separated by many kilometers. There is variation in community structure between fields as well as from area to area. Nevertheless, the known sites of deep hydrothermal venting share the following similarities. At any individual field with active venting, one generally finds high biomass and density of macro-

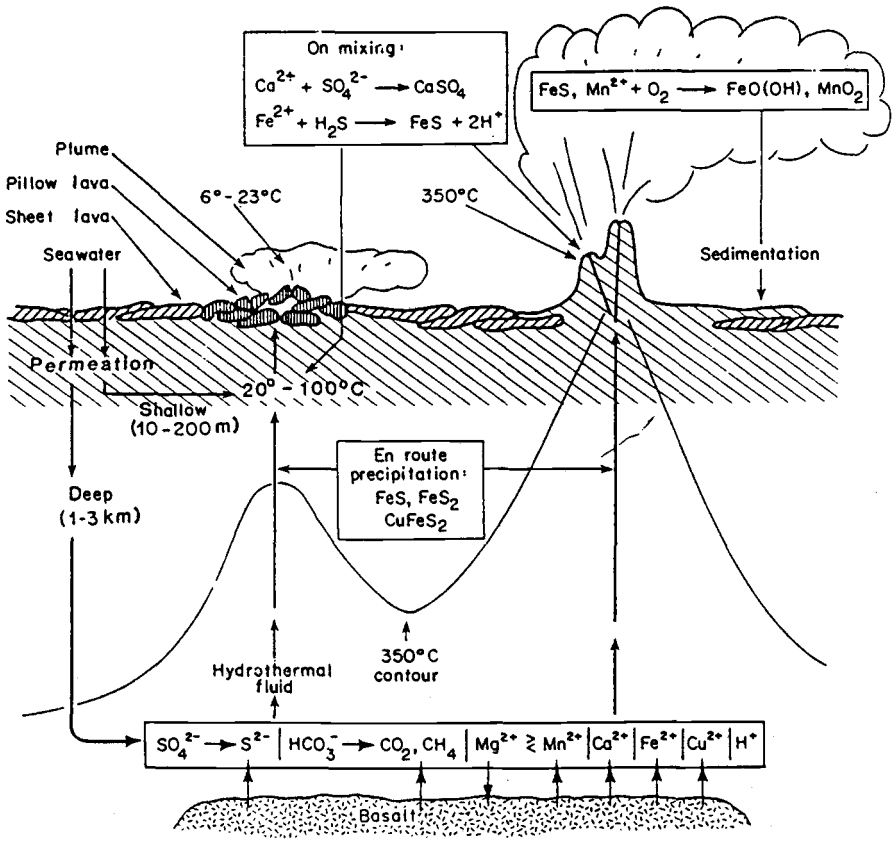


Figure 2 - Schematic diagram showing inorganic chemical processes occuring at warm- and hotwater hydrothermal vent sites. Deeply circulating seawater is heated to 350 - 400°C and reacts with crustal basalts, leaching various species into solution. The hot water rises, reaching the seafloor directly in some places and mixing first with cold, downwelling seawater in others. On mixing, iron-copper-zinc sulfide minerals and anhydrite precipitate. From Jannasch and Mottl (1985; modified from Jannasch and Taylor, 1984).

and megafauna. However, this biomass is dominated by a small number of species (Grassle, 1985; Tunnicliffe, 1988). Using most indices this would indicate low diversity although the rest of the deep sea environment is known to be one of this planet's most diverse habitats (Grassle, 1989). This may be due to the highly unstable nature of the vent habitat. Individual vent fields have lifetimes measured only in decades (Corliss *et al.*, 1979; Rise Project Group, 1980; Grassle, 1985, 1986). Presumably, this instability, combined with the rigorous physical and chemical regime, has maintained low diversity despite the long evolutionary history (see below) available to vent organisms (Sanders, 1969; Grassle, 1986).

Most deep hydrothermal vent species are not known from other environments. It appears that hydrothermal habitats were exploited early in metazoan evolution and that many modern vent organisms are the direct descendants of those early animals. Newman (1985, p. 239) finds that vents are "inhabited by an astonishing number of old taxa ... primarily Mesozoic, but apparently also some Paleozoic relics found no place else on Earth." Sulfide deposits which are interpreted as hydrothermal in origin have been dated to the Ordovician (Duke and Hutchinson, 1974). Fossil worm tubes have been found in similar sulfide deposits from the Cretaceous (Haymon and Koski, 1985) and the Carboniferous (Banks, 1985). Tunnicliffe (1988) concludes that vent habitats have likely been exploited since the first metazoan radiations in the Paleozoic. It has even been suggested that hydrothermal vents provide the most likely environment for the origin of life (Corliss *et al.*, 1981).

The communities of separated areas are often comprised of different but analogous species which are congeneric or confamilial (Tunnicliffe, 1988; Petrecca and Grassle, 1990). When populations of the same species are present at different areas, they may be genetically separated (J.P. Grassle, 1985). The species present at any single field are a

subset of the larger community of the area; temperature and effluent flow rate seem to be important factors in determining which subset of species is present.

Sulfide-oxidizing bacteria utilize the sulfur compounds in the vent fluids to fix carbon and thus form the base of the unusual animal communities associated with hydrothermal vents. These bacteria may be free-living or associated with megafaunal organisms in a symbiotic relationship. Methane and other reduced compounds in the vent fluids could also serve as energy sources for autotrophic bacteria (Jannasch and Mottl, 1985). The "classic" megafaunal community at hydrothermal sites contains large tube worms of the phylum Vestimentifera, vesicomyid clams, and mytilid mussels, all of which harbour endosymbiotic chemoautotrophic bacteria. Along with the free-living bacteria, these organisms are the primary producers of these environments, making this one of the few ecosystems on the planet which is independent of solar energy. The primary consumers are heterotrophic bacteria, suspension feeding polychaetes, molluscs and planktonic crustaceans as well as deposit feeding polychaetes and crustaceans. Predaceous polychaetes, brachyuran and galatheid crabs and zoarcid fishes are the top consumers of this simple food web (figure 3).

Deep hydrothermal vent communities can also be characterized by the absence of xenophyophores, sponges and bryozoa. In general, asexually reproducing colonial animals appear to be absent from hydrothermal vents. Echinoderms and coelenterates are also rare. These groups are among the more common taxa elsewhere in the deep sea (Grassle, 1985; 1986).

The above scheme has been described for the majority of the deep hydrothermal vents which have been studied but the exceptions are notable. While the vent communities in the Marianas back-arc basin are comprised of many of the genera found in the eastern Pacific,

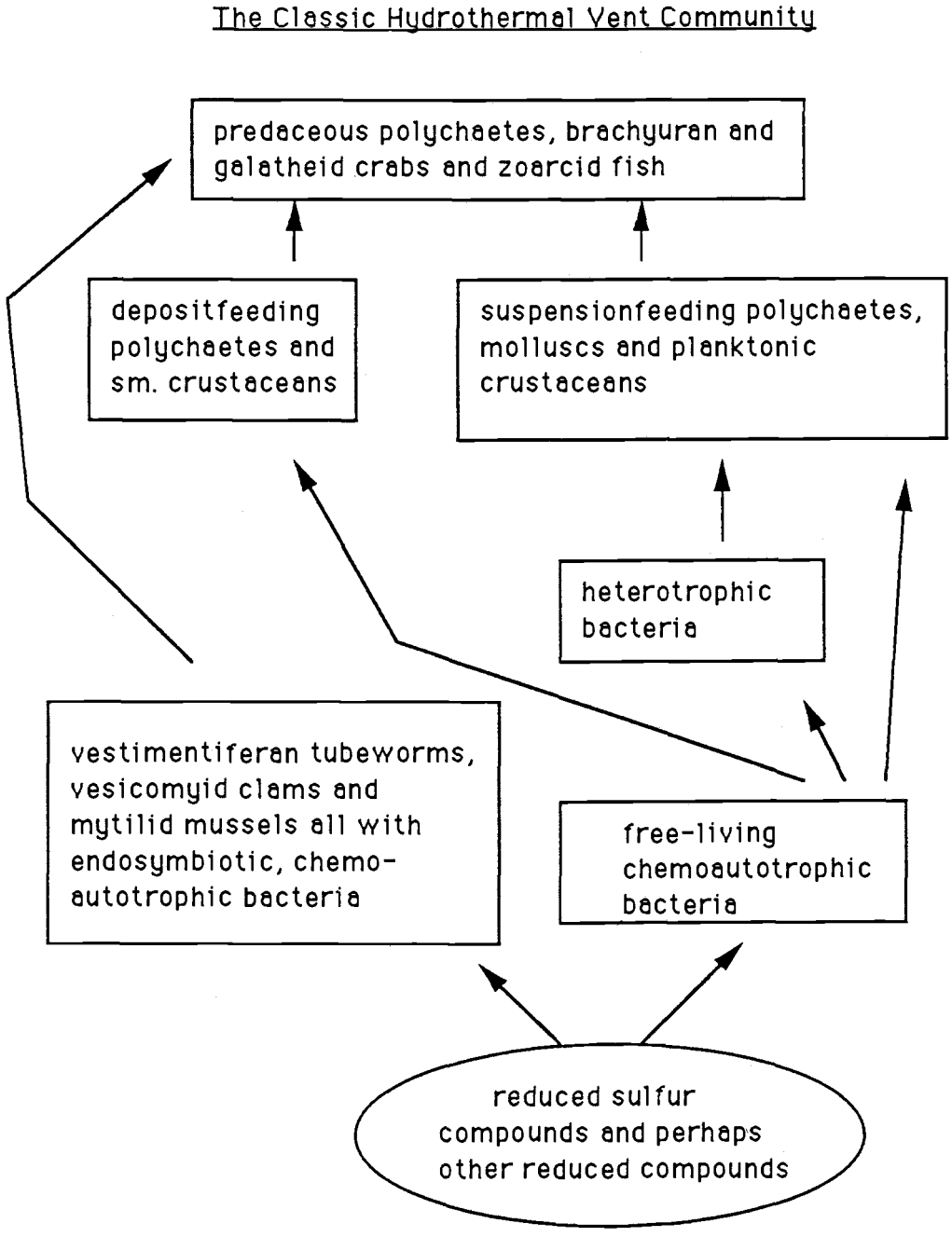


Figure 3 - Schematic diagram of the "classic" hydrothermal vent community food web.

vestimentiferan worms appear to be totally lacking (Hessler *et al.*, 1988). The major endosymbiont-containing megafaunal organism is a large snail, which has been found nowhere else (Hessler *et al.*, 1988). At sites along the Mid-Atlantic Ridge the dominant organisms are two species of shrimp in the new genus *Rimicaris*. These shrimp do not contain endosymbionts, they feed directly on free-living bacteria (Van Dover *et al.*, 1988). Interestingly, this genus of shrimp has recently been found at the Marianas vents although it has not been seen elsewhere in the Pacific (Hessler *et al.*, 1988). A final example is the Red Sea, which is known to support active hydrothermal vents but does not appear to have animal communities associated with them (Monin *et al.*, 1982).

Deep Hydrocarbon Vents and Seeps

Deep hydrocarbon vents and seeps have been found on the continental slopes off of Louisiana (Kennicutt *et al.*, 1985; Brooks *et al.*, 1987), Oregon (Kulm *et al.*, 1986), Japan (Juniper and Sibuet, 1987), Nova Scotia (Mayer *et al.*, 1988) and northern California (Kennicutt *et al.*, 1989). Where measured, temperatures at these sites have approximated ambient bottom waters. For this reason these sites have been called "cold seeps".

Cold hydrocarbon vents and seeps are analogous to hydrothermal vents in the sense that they provide a point source of reduced compounds where oxygen is also readily available (Cavannaugh, 1985). Bacteria can utilise the chemical energy at this oxic/anoxic interface. Once again this leads to high biomass communities in the deep sea. In fact, the organisms present are generally closely related, if not the same species, to those of hydrothermal vent communities.

On the Louisana slope in the Gulf of Mexico (approx. 500 - 900 m depth) and off the

coast of northern California (approx. 450 - 600 m depth) hydrocarbons, predominantly methane, but also gasoline range (C5+) and higher order hydrocarbons (C15+) (Anderson *et al.*, 1983; Kennicutt *et al.*, 1985; Kennicutt *et al.*, 1989) are released from subsurface oil and natural gas deposits. Sediment cores from the Louisiana system contained up to 12% oil (Kennicutt *et al.*, 1985). In both cases the high organic loading depletes oxygen in the sediments, promoting sulfate reduction and thereby producing high concentrations of sulfides in the sediments.

The Louisiana community includes vestimentiferan tube worms, pogonophoran tube worms, vesicomyid clams and mytilid mussels, remarkably similar to hydrothermal vent communities in the eastern Pacific (Kennicutt *et al.*, 1985; Brooks *et al.*, 1987). The Vestimentifera and clams utilise endosymbiotic sulfide-oxidizing bacteria, while the mytilid mussels analogously utilise methane-oxidizing endosymbionts (Childress *et al.*, 1986).

The only clearly vent/seep related organisms at the California site are vesicomyid clams (Kennicutt *et al.*, 1989). The California clams utilise sulfide oxidation (Kennicutt *et al.*, 1989).

Venting on the slopes of Oregon (several sites between approx. 700 - 2000 m) and Japan (many sites between 3800 - 6000 m) is driven by large scale plate tectonic processes. Both sites are associated with active subduction zones where Pacific Ocean plates are being overridden by continental plates (Kulm *et al.*, 1986, Juniper and Sibuet, 1987). As the oceanic plate is subducted under the continental plate its overlying oceanic sediments are scraped off and accreted onto the continental margin. As furthur material is accreted onto the margin, previously deposited material is "squeezed". The methane-rich porewaters of these sediments are forced out, particularily at faults or other weak spots (Kulm *et al.*, 1986; Ritger *et al.*, 1987). This leads to the slow seepage of methane-rich

water or actual venting of methane as bubbles (R. Collier, pers. comm.). The methane is biogenically formed by the bacterial degradation of organic matter deep within the sediments (Kulm *et al.*, 1986; Ritger *et al.*, 1987).

The fauna associated with the Oregon sites includes vestimentiferan tube worms (same species as is found at the Louisiana slope community), vesicomyid and solemyid clams (also known to harbour symbiotic bacteria) (Kulm *et al.*, 1986). Preliminary analyses of the organisms at the Oregon site suggested that the clams and worms depended upon methane-oxidizing symbionts (Kulm *et al.*, 1986). However subsequent investigations have failed to confirm this conjecture, while good evidence for sulfide-oxidizing symbionts has been found (G. Taghon, pers. comm.). However, it is likely that the symbiont-containing organisms are still indirectly linked to methane-oxidizing bacteria in the sediments. Methane oxidation could occur anaerobically using sulfate as the reductant instead of oxygen:

$$CH_4 + SO_4^{-2} \text{---> } CO_2 + 2H_2O + S^{-2}.$$

There is some chemical evidence which indicates that this is indeed occuring at the Oregon sites (Ritger *et al.*, 1987; Suess and Whiticar, 1989); this reaction would provide a large source of sulfide for the megafauna. Certainly, massive methane oxidation (either using oxygen or sulfate) is mediated in the sediments by free-living bacteria. The CO_2 produced in the reaction precipitates out of the porewaters, forming large slabs and stacks of carbonates (Kulm *et al.*, 1986; Ritger *et al.*, 1987; Suess and Whiticar, 1987).

The Japan communities are dominated by vesicomyid clams, although high abundances of "normal" fauna are found on the periphery of the clam colonies (Juniper and Sibuet, 1987). It has also been speculated that the vesicomyid clams from the Japan slope sites rely on methane oxidation, but it is more likely that they utilize sulfide oxidation as at the

Oregon sites. They may be supplementing any chemosynthetic strategy with filter feeding (Juniper and Sibuet, 1987).

The communities found off the coast of Nova Scotia (approx. 3800 m) were discovered by accident while geologists were using the *Alvin* to study the deposits of the 1929 Grand Banks earthquake. Very large communities of vesicomyid, solemyid and thyasirid clams (all of which are known to contain endosymbiotic bacteria) as well as pogonophorans, gastropods and galatheid crabs were seen through the ports of the submarine (Meyer *et al.*, 1988, Petrecca and Grassle, 1990). Sulfide oxidation is the probable source of energy for this community but the site has yet to be investigated by biologists.

Deep Brine Seeps

At the juncture of the West Florida Escarpment and the abyssal plain sediments (approx. 3300 m), hypersaline water slowly seeps into the sediments carrying sulfides and methane. Temperature anomalies are not associated with the seepage areas (Hecker, 1985).

The fauna are dominated by dense assemblages of vestimentiferan tube worms and beds of mytilid mussels. Vesicomyid clams, galatheid crabs, polychaetes and various other molluscs are also found. The taxonomy of the organisms and the high biomass are highly reminiscent of eastern Pacific hydrothermal vent fauna (Hecker, 1985). Like the Louisiana slope community, separate trophic resources are exploited by different organisms. The vestimentifera and vesicomyid clams seem to utilise sulfide-oxidizing symbionts while the symbionts of the mytilid mussels use methane oxidation (Cary *et al.*, 1989).

Summary and Comments

Deep-water venting and seeping phenomena are found throughout the worlds oceans. Although there are several different types of phenomena driven by separate geological processes they are all linked chemically by the release of reduced compounds utilizable by bacteria. They are additionally linked by biological communities which are similar by virtue of their high biomass, by the unusual animals which are rare elsewhere and by the general absence of typical deep-sea fauna.

The fauna of deep vents and seeps are members of a relatively small, coevolved community of extreme specialists which contrast sharply to the typical deep-sea organisms. The habitat they exploit is similar to islands, lakes and desert oases in terms of biogeographical and evolutionary questions. The similarity (and differences) of the communities at different sites despite huge geographical separation has led to considerable speculation about speciation and dispersal mechanisms, however too little is currently known for convincing patterns to be seen. In particular, examination of the fossil record of sulfide deposits attributed to paleo-vent activity could be revealing.

Numerous biochemical and physiological studies have been and will be conducted on deep vent and seep organisms. These organisms are fascinating and informative because they can function under the nocuous conditions of high pressure, extremes of temperature and high concentrations of normally toxic chemicals such as sulfur compounds, oil, petroleum, other hydrocarbons and heavy metals.

Shallow-Water Vents and Seeps

Although the chemical and geological nature of shallow-water vents and seeps is similar to those of the deep ocean, the biology is distinctly different.

Shallow Hydrothermal Systems

Hydrothermal vents have been described in shallow (0 - 110 m) waters near Los Angeles, CA (Kleinschmidt and Tschauder, 1985), in the Kurile Islands (Zhirmunsky and Tarasov, 1990) and near Iceland (Fricke *et al.*, 1989). The most obvious biological feature shared by the sites are extensive bacterial mats which appear to be dominated by *Beggiatoa*-type bacteria (a sulfide-oxidizing group of bacteria). Like deep sea vents, meio- and macrofauna are very abundant near the vents. Several new species were found at the Kurile Islands site, but no endosymbiont-containing organisms were found at any of the sites. Generally, in contrast to deep-sea systems, the communities present merely represent a subsample of the community typical for the surrounding area. Although diversity is comparatively low at vent sites, a large number of shallow-water fauna can apparently tolerate the harsh chemical and temperature regimes of hydrothermal systems.

Shallow Hydrocarbon Seeps

The biology of a natural petroleum seep located at 16 m depth about 0.5 km offshore from Santa Barbara, CA has been intensively studied since 1975 (Spies and Davis, 1979; Davis and Spies, 1980; Bauer *et al.*, 1990). The site was compared to a reference site of similar depth and sediment composition. High levels of hydrocarbons and sulfides were found at the seep site. *Beggiatoa*-type bacterial mats were found at the most active

seepage areas (Spies and Davis, 1979). The macrofauna of the two sites was very similar with 72% of the species, which constituted 90% of the biomass, being found at both sites. Diversity at the two sites was similar, although more variable at the seep site (Davis and Spies, 1980). Biomass of macrofauna at the seep site was consistently higher than at the reference site, particularly of deposit-feeding polychaetes (Spies and Davis, 1979, Davis and Spies, 1980). Although the mechanism has not been delineated, it appears that a significant portion of the macrofaunal carbon is derived from seep hydrocarbons. In contrast, the meiofauna of the seep site seem to be exploiting a primarily photosynthetically-derived carbon reservoir (Bauer *et al.*, 1990).

Hydrocarbon seepage has also been described on the continental shelf of Baffin Island. Seepage areas are overlain with white *Beggiatoa* mats (Levy and Lee, 1988).

Shallow Brine Seeps

A brine pond which overflows into the northwestern Gulf of Mexico has been studied by Powell *et al.* (1983; 1986). The brine contains very high concentrations of sulfides, which promotes the growth of bacterial mats (*Beggiatoa*, other bacteria and algae). The fauna near the seep is characterised by sulfide-dependant and sulfide-tolerant shallow-water organisms (thiobios, Powell *et al.*, 1983). As the brine is diluted by seawater, the community is taken over by non-sulfide dependent meio- and macrofauna typical for the region. The biomass of these organisms is enhanced by the detritus produced near the brine seep.

Pockmark Venting

Pockmark venting is sufficently different from oil and petroleum seeps to merit a

separate category. To date, the only published biological research on pockmark venting is a study by Dando *et al.* (1991) on an active North Sea pockmark. They found high water column methane concentration and high sediment sulfide concentration. The pockmark community was of low diversity but in contrast to other vent and seep environments it was also of low biomass relative to the surrounding area. This may have been due to the coarser sediment composition within the crater; as gas bubbles out, fine-grained sediments are selectively entrained by the plume. Dando *et al.* found two species of thyasirid clams and a nematode which very likely depend to some extent on sulfide-oxidizing endosymbionts, however the rest of the community did not demonstrate any direct or indirect reliance on methane-derived carbon.

The Greenie Spot

Oceanographers from Oregon State University briefly investigated The Greenie Spot during September 10 - 11, 1989 from the *R.V. McGaw* and the research submersible *Delta*. The following is paraphrased from a description of the submarine dives to the pockmark.

> *The sea was glassy calm on those two days allowing gas bubbles up to 20 cm in diameter to be be seen bursting at the sea surface. The pockmark is located in waters 132 m deep. It is a 4-6 m deep depression, 7-9 m wide and 15-18 m long, with 45 - 50° sloping walls on one side. Its floor is flat and is covered with a fine, white, powder-like material. Visibility inside and near the pockmark is generally poor due to suspended particulate matter. Just outside of the crater lie carbonate slabs and several, apparently extinct, donut-shaped carbonate chimneys interspersed with areas of broken clam shell. The surrounding area consists of anoxic sediments smelling of hydrogen sulfide covered by a thin layer of oxidized sediment.*
>
> *Extensive bacterial mats surround the pockmark. Thick, rugose, salmon-colored mats extend right up to the lip of the crater; father away, the mats are white and thinner. The densities of hagfish and flatfishes (including four commercially exploited varieties) are very high. Pink shrimp are also abundant. Larval and juvenile fishes are seen just above the bottom. Euphausiids and other zooplankton are extremely abundant within the the first several meters above the pockmark. Schools of hake, herring and smelt pass by irregularly. A large aggregation of yellowtail rockfish is consistently present near the site.*

Based on these observations and in analogy to what was known about other vent and seep phenomena, a comprehensive, interdisciplinary study was planned for September of 1990. The central hypothesis of the biological segment of the project was that methane from the vent provided an energy source for the local food web. We predicted that elevated abundances of organisms would be found near the vent, if this were the case. Additionally, the vent area might also provide a habitat for unusual animals.

The primary goal of this thesis was to establish whether or not a trophic link existed between the biological community and the vent methane. Secondary goals included the posing of hypotheses concerning the importance of pockmark venting to the general ecology of the Oregon Coast, the relevance of methane venting to the production of commercially exploited species and the relationship of pockmark venting to other vent and seep phenomena.

MATERIALS AND METHODS

The biology, chemistry and geology of the Greenie Spot was investigated from the *RV McGaw* during September 1-14, 1990. The cruise was split into two legs with the bulk of the biological samples being taken between September 9-14.

The general methodology followed was to collect biological samples from the immediate area of the pockmark (these will be termed vent samples) as well as from areas which were far enough away as to be considered outside of the vent's influence (background samples). These two sets of samples were then compared along 3 lines. We compared qualitative community composition, in particular looking for any unusual organisms which might be associated with the vent. We made quantitative comparisons of the abundances of all groups of organisms which were collected. Finally, we collected samples to compare the stable isotope ratios of biological materials from both sites.

Water Column Bacteria and Microzooplankton Counts

Since the vent is well beneath the euphotic zone and since methane would presumably have its greatest effect on bacteria and their predators, only heterotrophic bacteria and microzooplankton (eukaryotic microheterotrophs larger than bacteria) were enumerated. Water samples for bacteria and microzooplankton counts were collected using standard bottle casts from the ship. Ten ℓ OSU hydrobottles were lowered on a new, steel hydrowire to depths of 10, 30, 60, 90, and 115 meters. A total of five water column profiles were completed. The first cast (Station 1) was carried out at the vent site between 1000 - 1100 on Sept. 11. The next two casts (Stations 2 and 3) were also conducted at the vent site between 2100 - 2300 that evening. The final two profiles (Stations 4 and 5) were taken

at a background site between 1000 - 1130 of the next morning (Sept. 12). While it would have been more appropriate to collect the vent and background samples closer in time to one another, this was simply not possible because of the constraints of running multiple sampling programs from a single ship.

As the hydrobottles were brought on board, they were gently inverted once or twice and then subsampled. Subsamples were collected in 125 ml plastic bottles which contained 61.5 ml of 4% ice-cold glutaraldehyde. Each plastic bottle was filled up to a mark such that the preservative/seawater ratio was 50:50, with a final preservative concentration of 2% glutaraldehyde (Sherr and Sherr, 1989). These bottles were stored in the dark at <4°C until their contents could be filtered out and mounted on microscope slides. This was done onshore after the cruise. Some of the samples were stored for several weeks before they could be processed.

The standard epifluorescence direct count technique was used to enumerate bacteria and microzooplankton. Preserved organisms were stained with fluorescent dye, filtered onto polycarbonate membrane filters and then counted using an epifluorescence microscope (Hobbie *et al.*, 1977; Sherr and Sherr, 1989). Separate slides were made for counting bacteria and larger organisms. Bacteria were stained with 4'6-diamidino-2-phenylindole (DAPI; Porter and Feig, 1980) only, while larger organisms were stained with both DAPI and primulin (Caron, 1983; Martinussen and Thingstad, 1989). DAPI forms a complex with DNA which fluoresces blue-white under UV excitation. Primulin stains protein and fluoresces yellow-orange. Thus the external cell morphology as well as the nuclear morphology can be clearly seen using this combination of stains. Additionally, since chlorophyll will fluoresce red under blue light excitation, phototrophic organisms can be distinguished from heterotrophs simply by observing the cells under blue light. It is usually not difficult to

distinguish chloroplast containing autotrophs from heterotrophs which have ingested algae in food vacuoles. It is not necessary to use multiple stains for bacteria; since their DNA is not localized in a nucleus, the whole cell is illuminated by the DAPI fluorescence. The smallest cells appear simply as blue points while the larger cells can be distinguished as bacilli, cocci, etc.

Bacteria and microzooplankton slides were made from the same 125 ml subsample. All solutions used were administered thru a 0.2 µm syringe filter to prevent contamination of the samples by bacteria which may have gotten into the solutions. 13 ml of a DAPI solution (10 µg DAPI/ml) was added to the sample bottle. After allowing 5 minutes for staining, a pipet with a sterilized tip was used to remove subsamples. Three replicate bacterial slides were made from each bottle using 4 ml of sample (actual vol. of seawater = 1.8 ml) which were sucked through a 0.2 µm prestained (2 g/ℓ Irgalan black in 2% acetic acid) Nuclepore polycarbonate membrane filter at 5 in. Hg vacuum pressure. The filters were then rinsed by vacuuming through 2 ml of sterile seawater. The filters were removed from the filter stand and mounted on a slide by sandwiching them between drops of immersion oil (Cargille type A), and then covered with a coverslip.

Three replicate microzooplankton slides were made, one with a 60 ml aliquot (actual vol. of seawater = 27.1 ml) and two from 25 ml aliquots (actual vol. of seawater = 11.3 ml). Prestained 1.0 µm Nuclepore polycarbonate membrane filters were used to allow the rapid filtration of these larger volumes. The maximum vacuum pressure used was <3 in. Hg. After vacuuming down the sample, the filters were rinsed twice with 1-2 ml of 0.1 M Trizma-HCl (pH 4.0). One to 2 ml of a primulin solution (250 µg/ml) was added and then immediately sucked through. The filters were then washed two more times with 1-2 ml of the Trizma-HCl solution (Martinussen and Thingstad, 1989). The filters were mounted on slides as above.

Blanks were made to check if there had been any contamination of the slides from the solutions. DAPI was added to the 4% glutaraldehyde solution to achieve the same final concentration as in the samples. After 5 minutes, this solution was filtered through a 0.2 μm membrane filter at 5 in. Hg vacuum pressure. The filter was rinsed first with sterile seawater and then twice with 1-2 ml of the Trizma-HCl solution. One to 2 ml of the primulin solution was added and then the filter was rinsed twice more with the Trizma-HCl solution. The filter was mounted on a slide as above.

All the slides were stored in the dark in a freezer. Slides stored in this manner will keep for months without deteriorating (Porter and Feig, 1980; Bloem and Baer Gilissen, 1988). A Zeiss epifluorescence configuration (blue excitation: Zeiss filter set 48 77 09, exciter filter BP 450-490, chromatic beam splitter FT 510, barrier filter LP 520; UV excitation: Zeiss filter set 48 77 01, exciter filter BP 365/12, chromatic beam splitter FT 395, barrier filter LP 397) was used to enumerate the organisms on the slides.

Bacterial Cell Size

In addition to abundance, the relative cell sizes of bacteria from the two sites was determined since if average cell size was different, a large difference in biomass could exist even with similar numerical abundance (B. Sherr and E. Sherr, pers. comm.).

Photomicrographs of bacterial slides were made using Kodak T-max 400 black and white film in an Olympus photomicrographic system (model PM-10AK) mounted on the same microscope used for the counts. Four to 7 fields were photographed per microscope slide.

After developing, the film negatives were cut into single frames and mounted as if they

were slide transparencies. They were projected onto sheets of blank newsprint. Transects across each image were selected and each bacterial cell along the transect was outlined with a pen. After about 40 bacteria per microscope slide had been outlined, the newsprint sheet was taken down and the longest diameter of each cell outline was measured using a ruler. These measurements could be calibrated against projected photographs taken of an optical micrometer.

Stable Isotope Analysis

General Information

Stable isotope analysis has been used in a wide range of ecological studies (see review in Petersen and Fry, 1987) and has been extensively used in studies of vent and seep phenomena (eg. Kulm *et al.*, 1986; Powell *et al.*, 1986; Van Dover *et al.*, 1988; Van Dover and Fry, 1989; Dando *et al.*, 1991). Using a mass spectrometer the ratios of the various isotopes of common elements can be easily measured. Different information can be extracted from different elements. For biological questions the ratios of $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ measured in natural materials, including organisms themselves, are most valuable.

There are two types of information contained in stable isotope ratios, source and process information. Information about the source of a particular element within some material can be extracted from stable isotope ratios if there exist characteristic differences in the ratio of the stable isotopes of that element in the possible sources of that element. For example, the ultimate source of biological carbon (the end-member) on land is the atmospheric carbon reservoir, which has a clearly distinguishable ratio of ^{13}C to ^{12}C when compared to dissolved marine carbonate, the ultimate source of biological carbon in the

marine environment. Thus marine and terrestrial organisms can usually be distinguished by their $^{13}C/^{12}C$ value since their carbon comes from different sources.

Process information can be extracted from stable isotope ratios if the chemical reactions of the process discriminate against one or the other of the isotopes of the element in question. Usually the heavier isotope is less reactive because of its higher mass, this results in fractionation, whereby the end product of the process is enriched in the lighter isotope while the initial reservoir becomes enriched with the heavier isotope. Using carbon again as a example, photosynthesis discriminates against ^{13}C with the result that photosynthetically fixed carbon is about 20‰ lighter (relative to a standard) than the immediate source of carbon.

Del Notation

The values of stable isotope ratios are generally reported as parts per thousand difference relative to an established standard. They are known as del values (δ values).

$$\delta X = [(R \text{ sample}/R \text{ standard}) - 1] \times 1000,$$

where X is ^{13}C, ^{15}N, ^{34}S or the isotope in question and R is the corresponding ratio with the lighter isotope. The standard for carbon is PeeDee Belemnite (a limestone deposit), the nitrogen standard is the atmospheric reservoir and the usual standard for sulfur is sulfur from the Canyon Diablo meteorite. Typical measurement precision is usually 0.1-0.2‰.

$^{13}C/^{12}C$

After carbon is incorporated into biological material the ratio of the stable isotopes changes only slightly as that carbon cycles through an ecosystem. The change is usually

less than 1‰ per trophic step. Because this change is small compared to the variation in potential carbon sources, carbon isotopes are generally used to define the carbon sources to a system.

$^{15}N/^{14}N$

The ratio of the stable isotopes of nitrogen are known to change predictably as nitrogen cycles through an ecosystem. Typically, the value of $^{15}N/^{14}N$ measured in organisms will increase by about 4‰ (relative to the standard) with each increase in trophic level. This is due to the selective excretion of the lighter isotope in nitrogenous wastes. Thus nitrogen isotopic ratios can be used to gain information about trophic relationships. Additionally, the intensity of bacterial nitrification and denitrification can be determined across systems using N isotope ratios since a large increase in $^{15}N/^{14}N$ accompanies these processes.

$^{34}S/^{32}S$

This ratio changes even less after incorporation into organic matter during biological cycling than does $\delta^{13}C$. The exception to this rule is the large $\delta^{34}S$ decrease which accompanies anaerobic sulfate reduction. Therefore $^{34}S/^{32}S$ can be used to determine whether this process is important to an ecosystem. Sulfur isotopes have frequently been used to examine organic matter cycling in systems with both terrestrial and marine inputs. Organisms utilizing marine-derived organic matter should have higher $\delta^{34}S$ values than those using organic matter of terrestrial origin.

Collection and Analysis For This Study

Samples of organisms, sediment and water column particulate matter were collected for stable isotope analysis. All samples were analysed for $\delta^{13}C$ and those samples which had sufficient material were also analysed for $\delta^{15}N$ and $\delta^{34}S$.

Epibenthic fishes and shrimps were collected near the vent and at a background site using an otter trawl (collection supervised by D. Stein and J. Fisher). Additionally, a single green-striped rockfish was caught near the vent using hook and line. For the fishes, only muscle tissues exclusive of scales, skin and internal organs were analysed. Whole shrimps minus their viscera and exoskeleton were analysed. Their stomach contents were analysed separately. Several large anemones which were attached to samples of carbonate rocks brought up from the vent were collected. Body wall muscles from these anemones were analysed. Sediment samples from the vent and a background site were provided from box cores which R. Collier and A. Ungerer had collected for chemical analysis. Samples of bacterial mats collected at the vent were also provided by R. Collier.

Water column particulate matter was isolated from the same bottle casts used for bacterial and microzooplankton counts. After subsampling for the microbial population, the contents of the hydrobottles at 10, 30, 90 and 115 m from stations 2 and 3 (vent) and stations 4 and 5 (background) were combined after prescreening thru a 200 μm nitex mesh. The resulting 20 ℓ sample was then sequentially filtered thru smaller and smaller meshes in order to isolate different size classes of particulate material. The material trapped on each mesh was then washed onto a Whatman GF/C filter. Due to financial constraints only one sample each from the vent and the background site could be analysed. Unfortunately, at all depths, insufficient material was collected in all size classes for reliable stable isotope

analysis. Consequently, for each of the two sites, the material from all size classes from the 90 m and 115 m casts was pooled into a single, <200 μm sample.

All samples collected for stable isotope analysis were immediately frozen upon collection. Later they were either freeze-dried or dried at 60°C in an oven. Where necessary, the samples were acidified to remove carbonate. All samples were analysed by Coastal Science Laboratories, Inc. $\delta\ ^{13}C$ for the methane gas and of the vent carbonates were provided by R. Collier, A. Ungerer and L. Kulm.

RESULTS

Only the results of work conducted by the author will be described below. Other project results will be presented in the discussion.

Microzooplankton Counts

The results of the five water column profiles from which microzooplankton were enumerated are presented in figures 4a-j. Microzooplankton were split into four size classes, >40 μm, 40 - 24 μm, 24 - 10 μm and <10 μm. The three largest classes were additionally separated into the 3 taxonomic categories of heterotrophic dinoflagellates, ciliates and other heterotrophic protists; the 24 - 10 μm size class had the additional taxonomic category of choanoflagellates. The smallest microzooplankton (the <10 μm size class) cannot be separated into even gross taxonomic groups without great difficulty, therefore they were all lumped together in a single category.

The general pattern is similar for organisms within the three largest size classes. Abundance is highest near the surface by as much as an order of magnitude greater than the deeper samples and then generally declines steadily with depth. Across depth comparisons, particularily at the deeper depths, would be the most straightforward way of finding differences between the vent and background sites. Only near the surface do large differences occur consistently between the two sites in several groups of organisms; the background abundance is higher than the vent stations in several cases. At the other depths one cannot distinguish between the vent and background stations based on these three largest size classes of microzooplankton.

Figure 4 a-j - Results of microscope counts of microzooplankton, taxa separated. Abundance in number per ml. Each graph corresponds to one taxon within one of the three size classes; a-c = >40 µm heterotrophs, d-f = 24-40 µm heterotrophs, and g-j = 10-24 µm heterotrophs. The 5 depths sampled were 10, 30, 60, 90 and 115m. The points were staggered slightly from these exact depths to minimize the degree to which points were obscured by others lying on top of them. Open symbols represent vent samples while closed symbols represent background samples. □ = station 1, O= station 2, Δ = station 3, ■= station 4, ● = station 5.

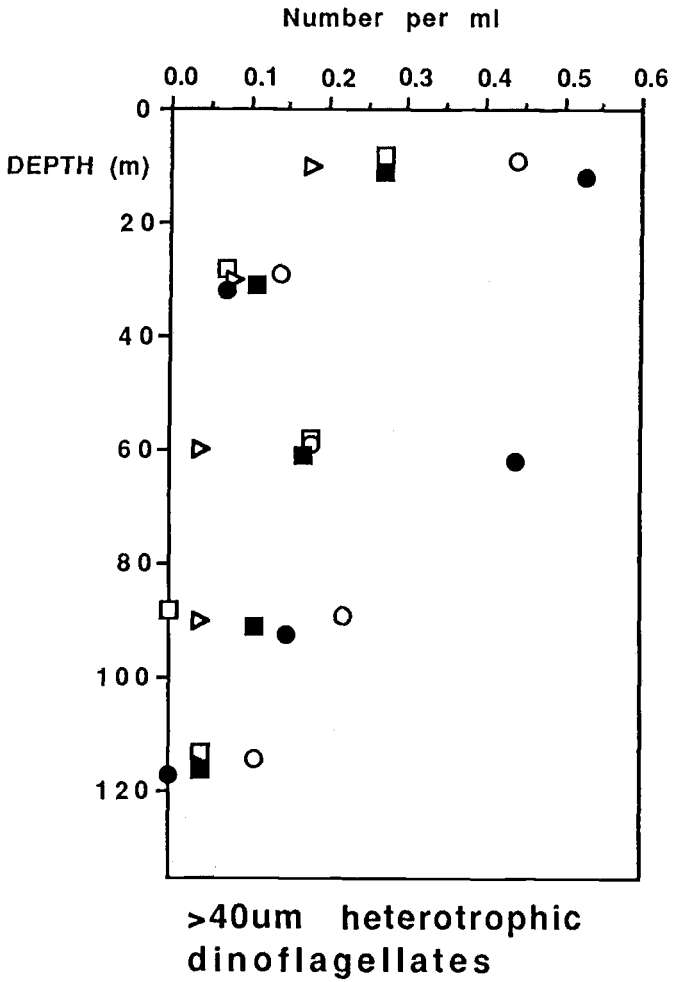


Figure 4a

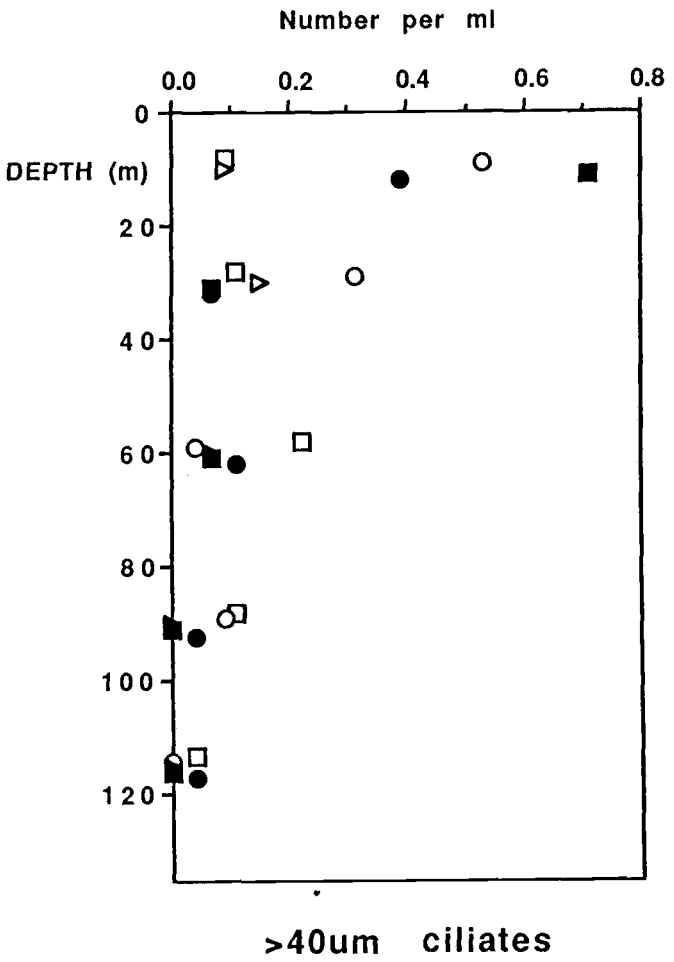
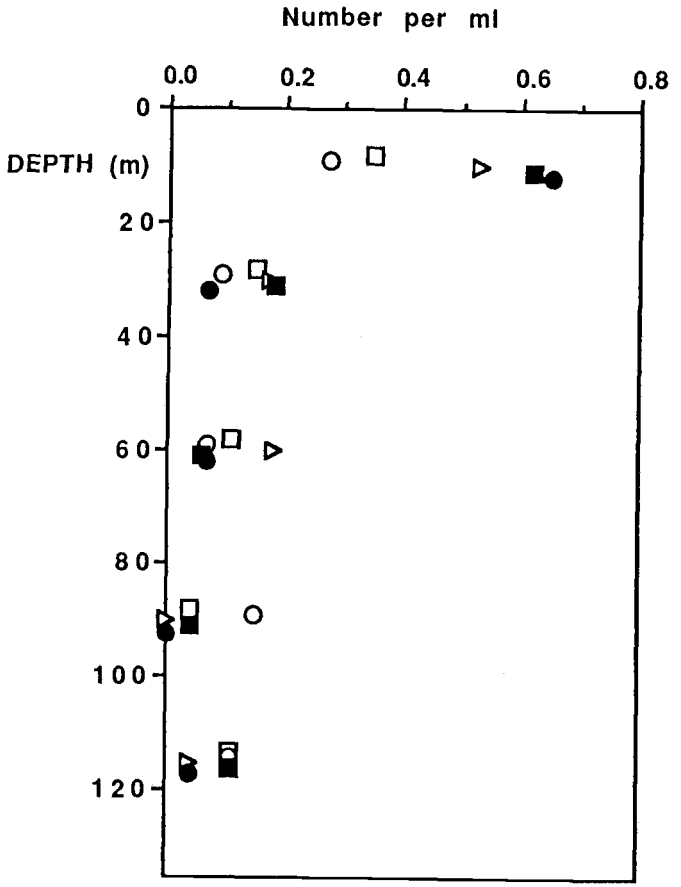
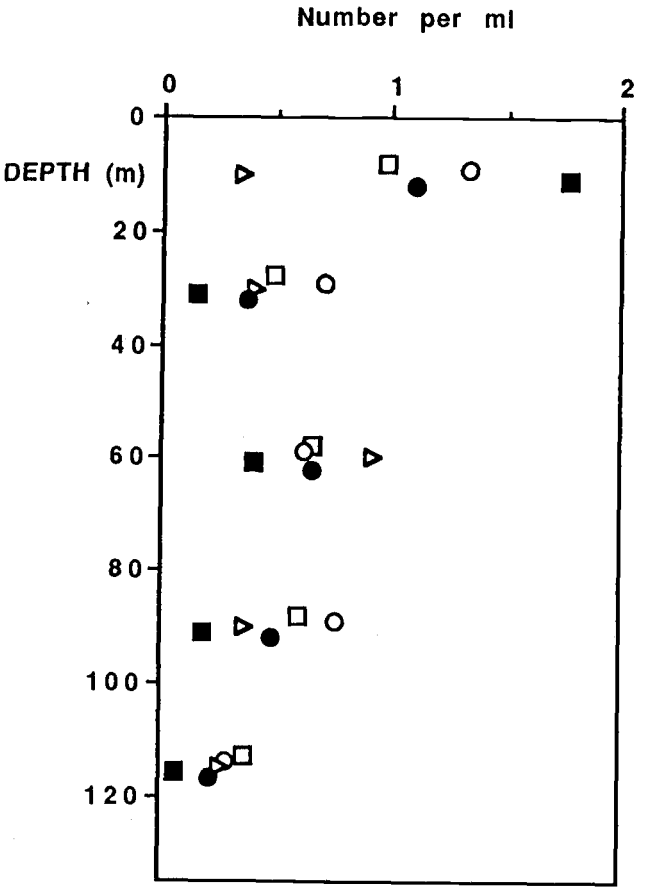


Figure 4b



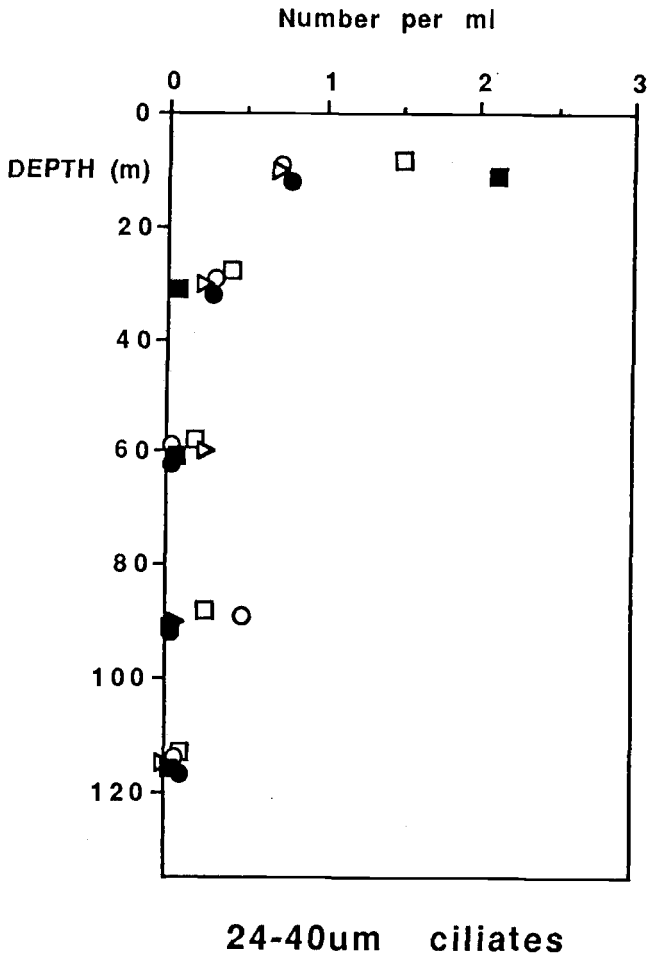
>40um unidentified heterotrophic protists

Figure 4c



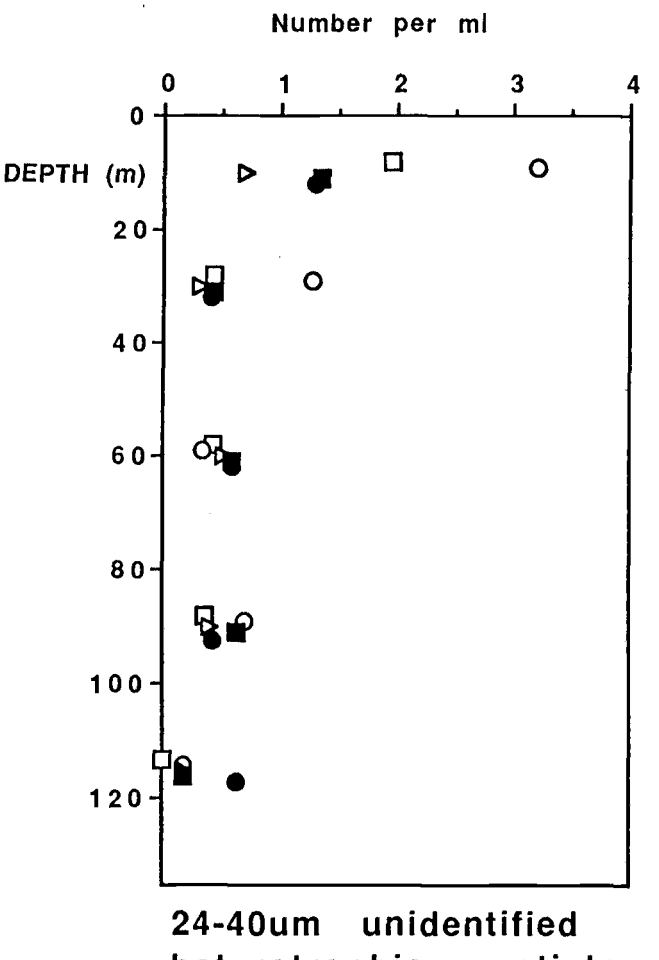
24-40um hetero-trophic dinoflagellates

Figure 4d



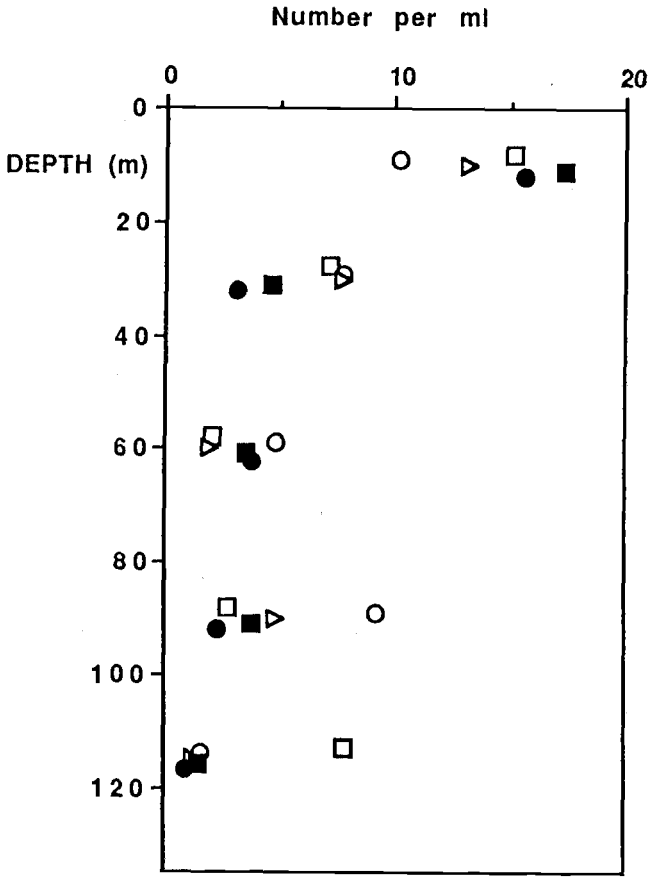
24-40um ciliates

Figure 4e



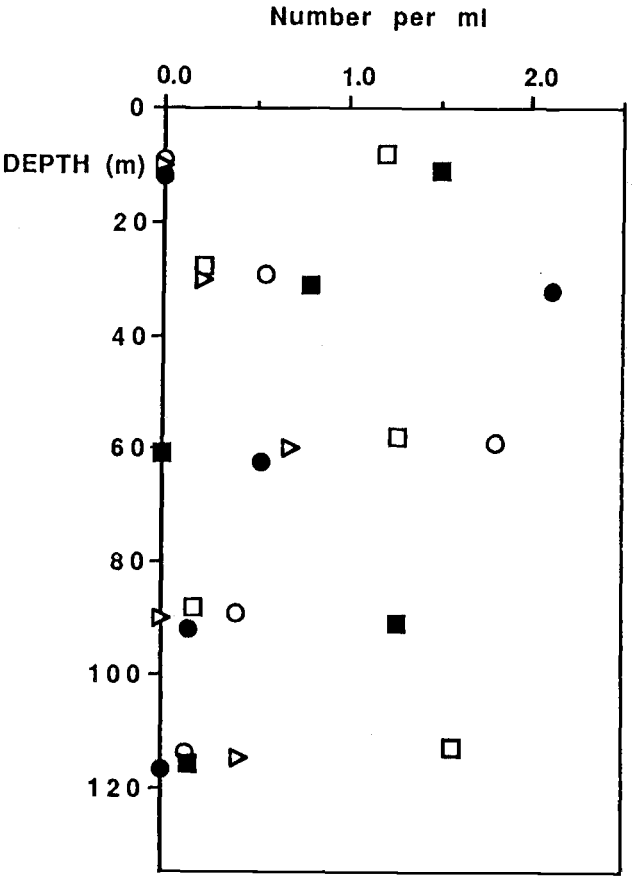
24-40um unidentified heterotrophic protists

Figure 4f



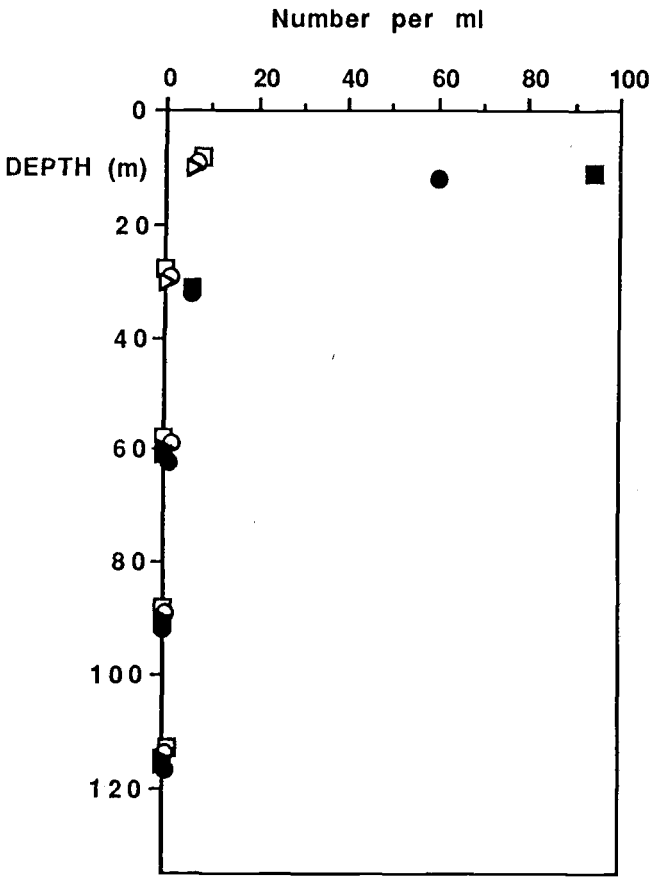
10-24um heterotrophic dinoflagellates

Figure 4g



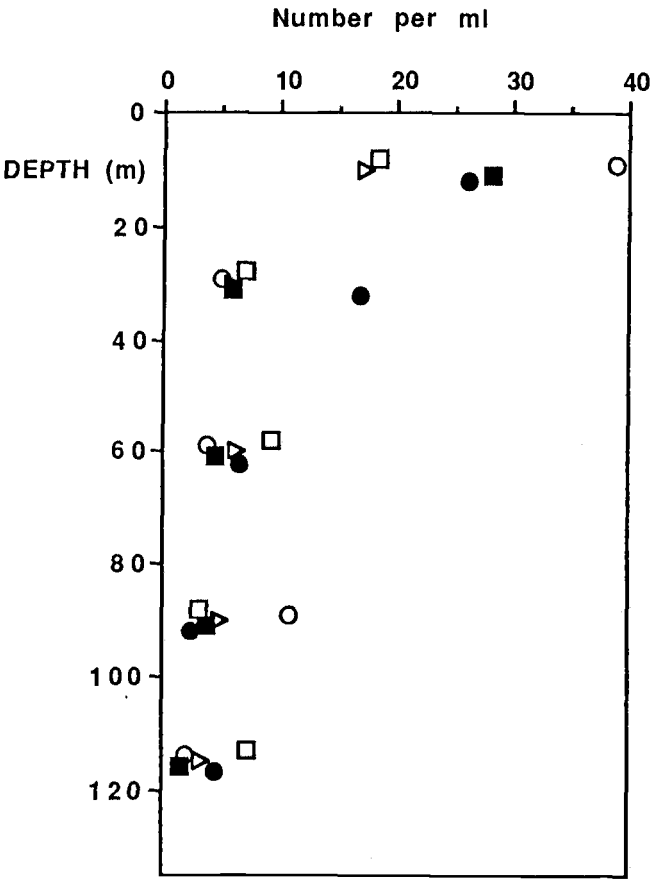
10-24um ciliates

Figure 4h



10-24um choano-flagellates

Figure 4i



10-24um unidentified heterotrophic protists

Figure 4j

Size structure within the planktonic community is important in determining carbon flow pathways (eg. Carpenter *et al.*, 1987). In order to more clearly see any potential changes within the size structure of the communities present at both sites, all organisms were separated into the same four size classes listed above but were not separated taxonomically. These data, with the organisms separated into four size classes but lumped together taxonomically are presented in figures 5a-c. The same patterns described for figures 4a-j are evident despite the lumping.

Because of the time lag between collection of the vent and background samples it is possible that the background samples do not truly represent the background microbial community at the time when the vent samples were collected. While this possibility cannot be excluded, the similarity, particularly at depth, between the 2 sets of counts of large microzooplankton leads one to believe that the comparison is appropriate.

The <10 μm size class consisted almost entirely of <4 μm heterotrophic flagellates. The data are presented graphically in figure 6. The abundance measurements reported for this size class are quite high at both sites compared to typical oceanic values. However, recounts and replicate counts have shown that these values are quite reproducible (see Appendix). Furthermore, it is the relative comparison of vent and background sites which is of interest to this study. At the background stations, the pattern of abundance for <10 μm heterotrophs is similar to the larger microbial size classes. Within the vent stations, there is a trend towards increased abundance at depth relative to the background values. This trend is not [statistically] significant for any of the stations except station 1. The mean abundance determined at the deepest depth (115 m) for station 1 is significantly greater than the combined mean of the background stations at the .01 level (t test).

Figure 5 a-c - Results of microscope counts of total heterotrophic protists. Abundance is given as number per ml. Same data as figure 4 except each all groups of organisms within a size class have been lumped together. Each graph depicts one size class; 5a = >40 µm heterotrophs, 5b = 24-40 µm heterotrophs, and 5c = 10-24 µm heterotrophs. Same symbols, depths, etc. as figure 4.

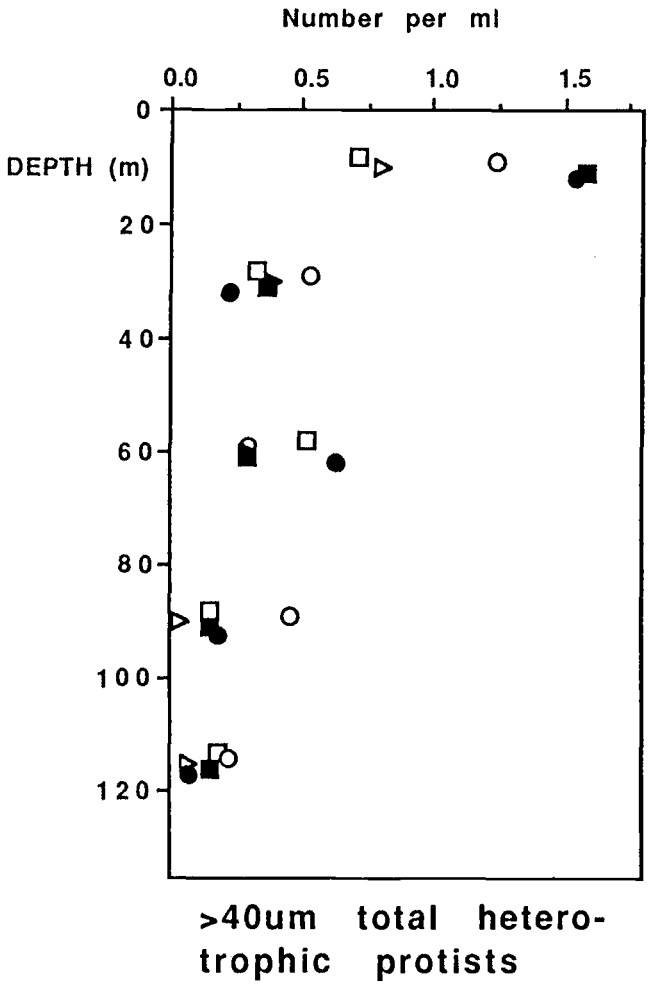


Figure 5a

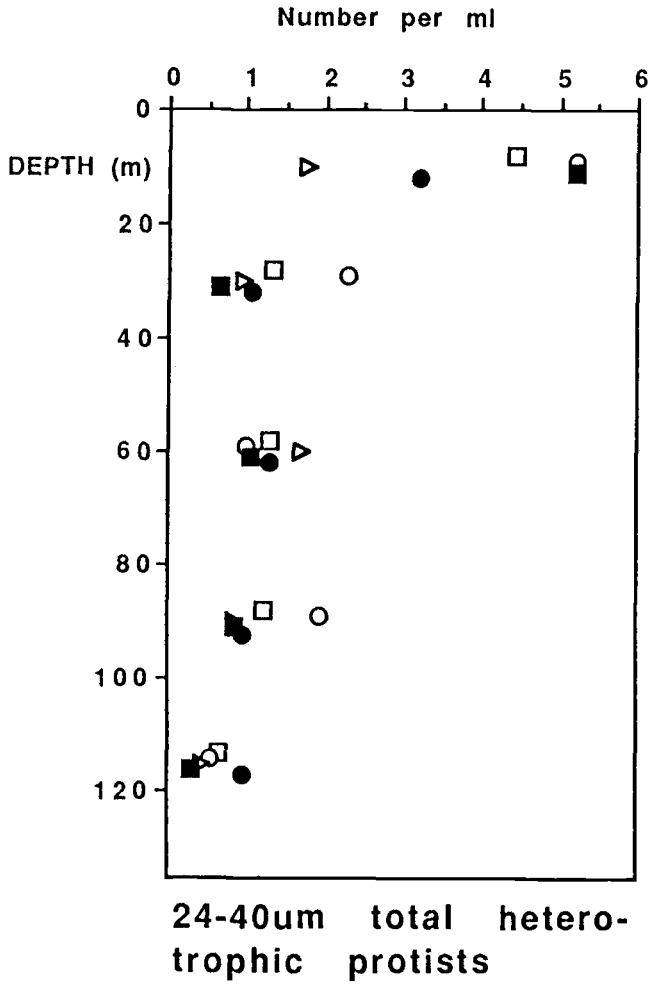


Figure 5b

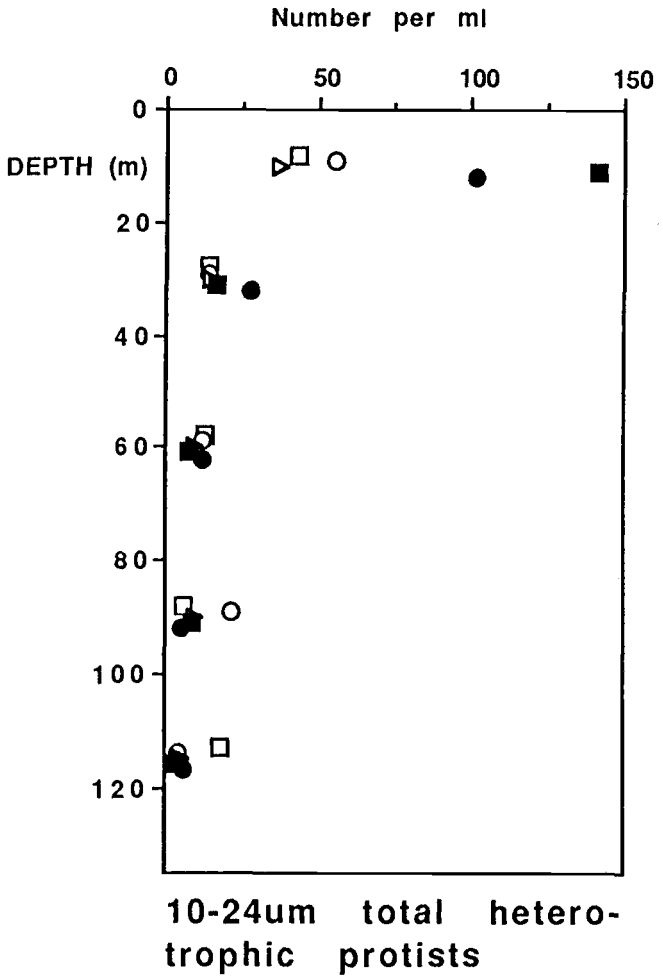

Number per ml
0
50
100
150
DEPTH (m)
0
20
40
60
80
100
120
10-24um total hetero-
trophic protists

Figure 5c

Figure 6 - Results of microscope counts of <10 µm heterotrophic protists. Symbols, depths, etc. as in figure 4. Where present, error bars represent one standard deviation from the mean count on several (2 or 3) replicate slides made from the same seawater sample. See appendix for more information.

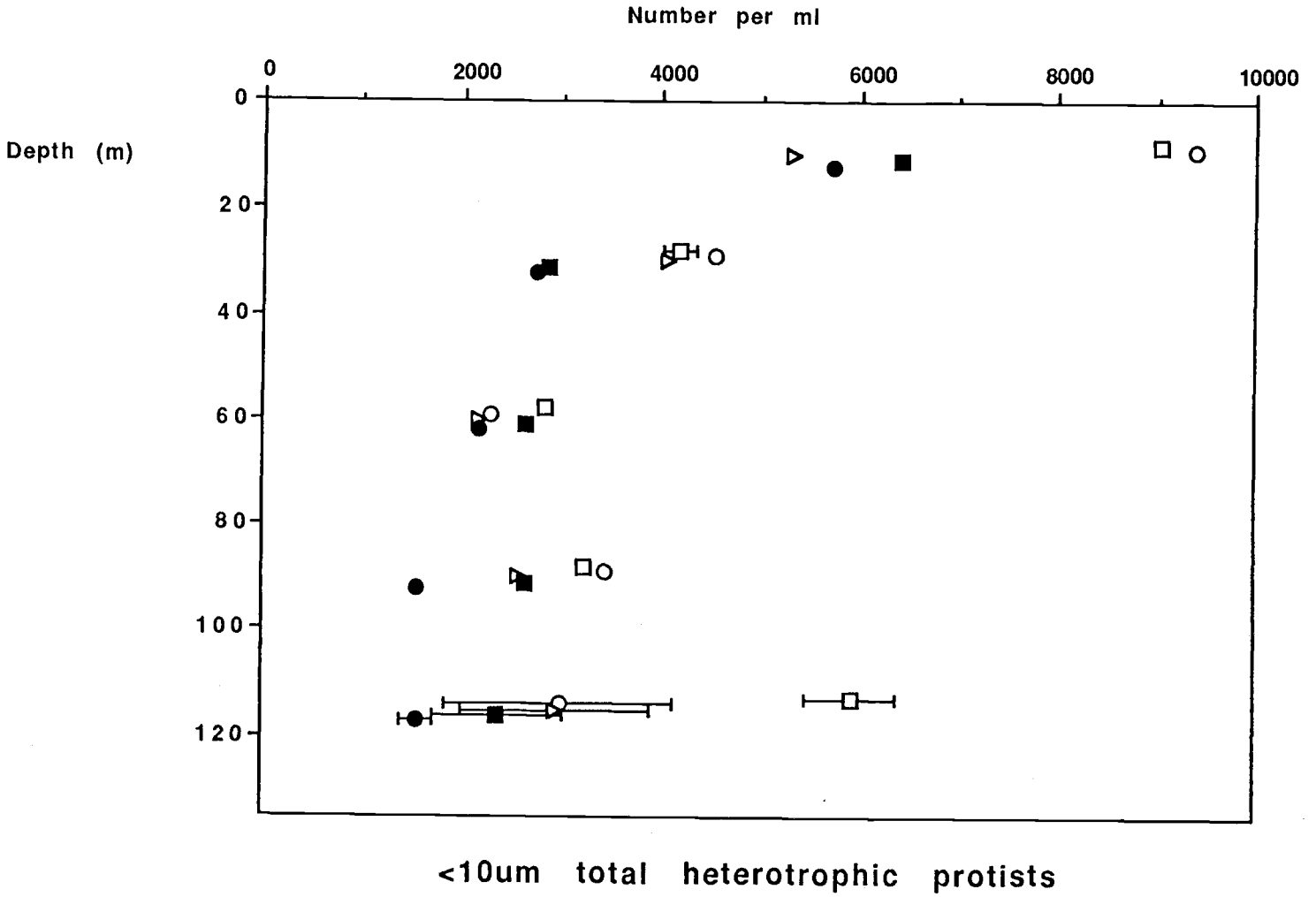

Number per ml
0
2000
4000
6000
8000
10000
Depth (m)
0
20
40
60
80
100
120
<10um total heterotrophic protists

Figure 6

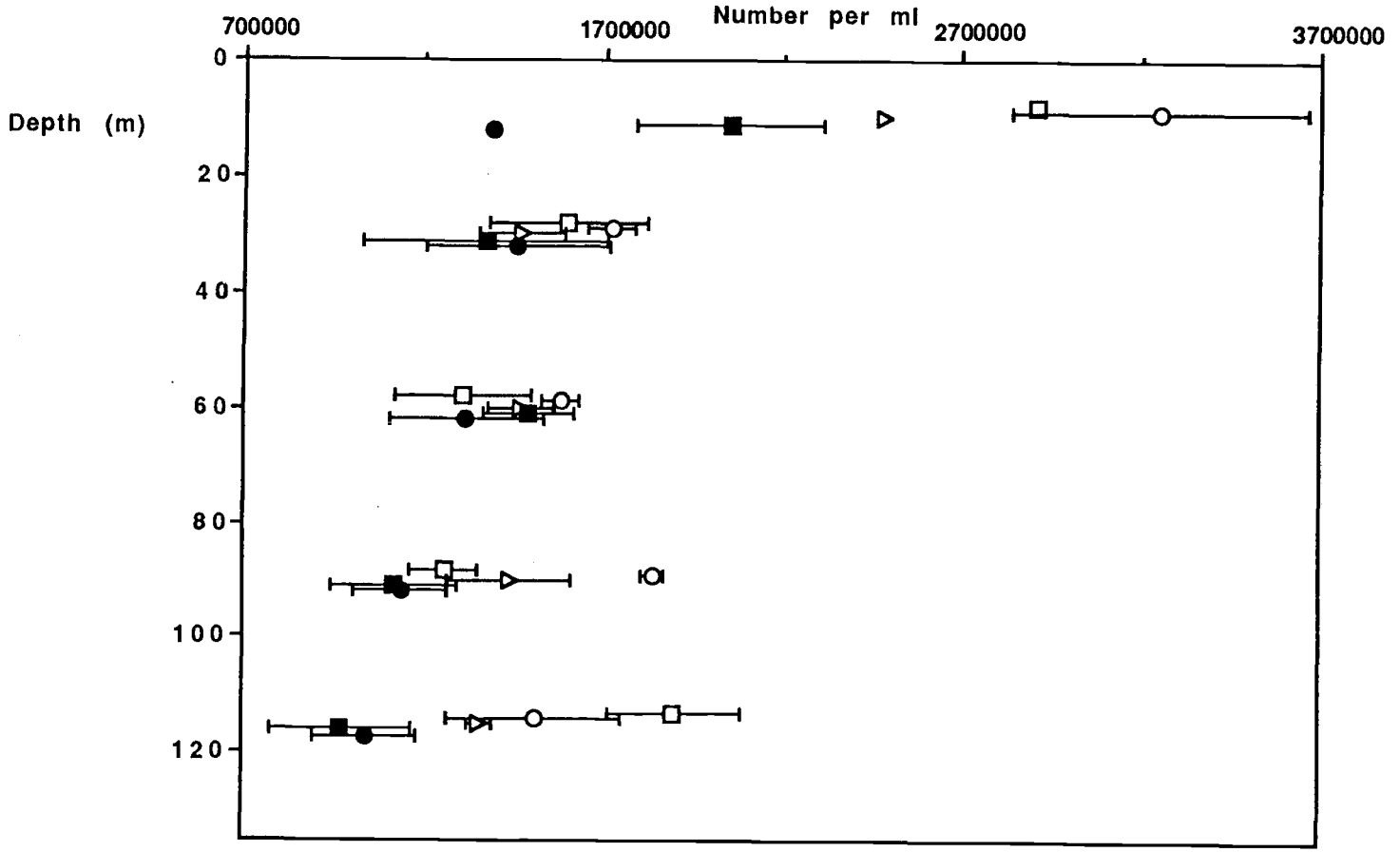
Water Column Bacteria Counts

The results of water column bacterial cell counts are shown in figure 7. Abundance at the background stations is highest at the surface and decreases steadily with depth with the nearsurface value approximately twice that of the deepest sample. Similar to the <10 μm heterotrophs, the abundance of water column bacteria at the vent is high relative to background at the deepest depth. In this case though, the relationship is stronger; at the deepest depth (115 m) the combined mean of all of the vent samples is [statistically] significantly greater than the combined mean of the background samples (at the .01 level, t test).

The increase in number of bacteria is small but there could be a large difference in biomass if the cells at the two sites were of different size. For this reason, the average relative cell size was determined for bacteria from the deepest depth (115 m) for all stations (table 1). A relative size of 1.0 approximately equals 0.8 μm. However, absolute values were not given because DAPI staining can give misleading size measurements (E. Sherr, pers. comm.) and also because of the potential for cell shrinkage during preservation. Moreover, the question at hand concerned only the comparison between the vent and background site. From this analysis, the average relative size of vent and background cells is essentially the same.

See Appendix I for an analysis of the different levels of variability and the reliability of the microzooplankton and bacteria counts.

Figure 7 - Results of microscope counts of heterotrophic, free-living water column bacteria. Symbols, depths, etc. as in figure 4. Where present, error bars represent one standard deviation from the mean count on several (usually 3) replicate slides made from the same seawater sample. See appendix for more information. All blanks were <<1% of count.



total heterotrophic, free-living, water column bacteria

Figure 7

Table 1 - Average relative size of bacterial cells from all stations at 115 m. Procedure used to determine cell size described in text. Average relative size refers to longest diameter across cell, most cells were coccoid.

Station	Ave. Rel. Size	n (# of fields)	SD	Range	CV
1 (vent)	1.36	44 (5)	0.43	0.79 - 3.16	32%
2 (vent)	1.0	47 (6)	0.28	0.53 - 1.84	28%
3 (vent)	1.38	37 (4)	0.45	0.92 - 2.63	32%
4 (bkgd)	1.18	38 (7)	0.29	0.79 - 1.84	24%
5 (bkgd)	1.26	60 (7)	0.34	0.66 - 2.24	27%

Stable Isotopes

$^{13}C/^{12}C$

The results of the $\delta^{13}C$ measurements are given in table 2a, they are depicted graphically in figure 8a. Consistent with an influx of vent-derived carbon (see discussion), most samples from the vent area are slightly more negative (lighter) than the corresponding background samples. The difference is very slight and is only intriguing because it occurs fairly consistently. Some other interesting findings become apparent upon closer inspection. Water column particulate organic matter (POM) from the vent is lighter than background POM to approximately the same degree as the vent sedimentary organic matter is lighter relative to its background counterpart. However, the absolute value of $\delta^{13}C$ for each of these pools of organic matter is different. The bacterial mat sample is the lightest of all of the samples. The bodies and stomach contents of the two species of shrimps collected were analysed separately on the presumption that body isotope values would reflect the long term average composition of their diet while stomach contents would reflect their most recent meal. There is a large discrepancy between body and stomach content values for both species at both sites. Additionally, while the body values of both shrimp species are lighter than background in vent samples, the values for their stomach contents do not differ depending on site. At least two species of adult fishes have lighter $\delta^{13}C$ values at the vent relative to the background site. However, this result is not seen in the juvenile fishes sampled. Finally, the anemone which was collected from a carbonate slab near the vent has a body $\delta^{13}C$ value which is similar to the other vent organisms but which is considerably heavier than the POM.

Table 2 a-c - Results of stable isotope analysis, 2a = $\delta^{13}C$ PDB, 2b = $\delta^{15}N$ Air, and 2c = $\delta^{34}S$ CDT. All values given as ‰. Each vent/background pair of samples was given a number and each organism was assigned a letter, v for vent and b for background. Thus, for example, pink shrimp stomach 2v represents the stomach of that *Pandalus jordani* collected near the vent which is in the second pair of *P. jordani*.

Table 2a - Results of stable isotope analysis, del ^{13}C.

Sample	Vent	Background
water column POM	-24.2, -24.4	-23.5
sediment OM	-23.7, -23.7	-22.7
bacterial mat	-24.8, -24.6	---
small shrimp (*Crangon communis*) 1v, 1b	-17.7, -17.6	-17.1
small shrimp 2v, 2b	-17.2, -17.3	-16.7
small shrimp stomach 3v, 3b	-22.4	-22.4
pink shrimp (*Pandalus jordani)* 1v, 1b	-18.8	-18.6
pink shrimp 2v, 2b	-18.9	-17.7, -17.9
pink shrimp stomach 1v	-22.7, -22.8	---
pink shrimp stomach 2v	-23.6, -23.6	---
pink shrimp stomach 3b	---	-23.2
flounder adult (*Atheresthes stomias*) 1v	-19.6	---
rockfish adult (*Sebastes elongatus*) 1v, 1b	-19.5	-19.1
slender sole adult (*Lyopsetta exilis*) 1v, 1b	-19.5	-17.6
slender sole juvenile 1v, 1b	-19.7	-19.7
rex sole juvenile (*Glyptocephalus zachirus*) 1v, 1b	-18.4	-18.4
anemone (?) 1v	-19.2	---

Table 2b - Results of stable isotope analysis, del 15N.

Sample	Vent	Background
water column POM	---	---
sediment OM	---	---
bacterial mat	---	---
small shrimp (*Crangon communis*) 1v, 1b	14.0	14.5
small shrimp 2v, 2b	13.4	14.3
small shrimp stomach 3v, 3b	12.0, 12.4	12.7
pink shrimp (*Pandalus jordani*) 1v, 1b	13.0, 12.7	13.1
pink shrimp 2v, 2b	12.9	13.7
pink shrimp stomach 1v	11.5	---
pink shrimp stomach 2v	12.0	---
pink shrimp stomach 3b	---	12.6
flounder adult (*Atheresthes stomias*) 1v	13.8, 13.9	---
rockfish adult (*Sebastes elongatus*) 1v, 1b	15.0, 14.9	15.0
slender sole adult (*Lyopsetta exilis*) 1v, 1b	13.8	15.1, 15.6
slender sole juvenile 1v, 1b	12.0, 12.2	12.0
rex sole juvenile (*Glyptocephalus zachirus*) 1v, 1b	15.9, 15.2	15.3
anemone (?) 1v	14.9	---

Table 2c - Results of stable isotope analysis, del ^{34}S.

Sample	Vent	Background
water column POM	---	---
sediment OM	---	---
bacterial mat	---	---
small shrimp (*Crangon communis*) 1v, 1b	8.0	11.1
small shrimp 2v, 2b	7.7	11.1
small shrimp stomach 3v, 3b	---	---
pink shrimp (*Pandalus jordani*) 1v, 1b	13.6	13.5
pink shrimp 2v, 2b	14.6	13.9, 13.9
pink shrimp stomach 1v	13.6	---
pink shrimp stomach 2v	13.7	---
pink shrimp stomach 3b	---	---
flounder adult (*Atheresthes stomias*) 1v	11.5	---
rockfish adult (*Sebastes elongatus*) 1v, 1b	13.8	14.9
slender sole adult (*Lyopsetta exilis*) 1v, 1b	14.1	8.5
slender sole juvenile 1v, 1b	14.2, 14.4	15.3, 15.3
rex sole juvenile (*Glyptocephalus zachirus*) 1v, 1b	6.1, 6.4	11.1
anemone (?) 1v	18.0, 17.9	---

$^{15}N/^{14}N$

As in the $\delta^{13}C$ analyses, most of the samples from the vent have lighter $\delta^{15}N$ values than the respective background sample (table 2b, figure 8b). Again, the difference is slight and can only be considered important because it occurs consistently. Interestingly, the two exceptions are from juvenile fishes. These were among the samples which did not have different $\delta^{13}C$ values at the vent and background sites. The lowest values are from the shrimp stomach contents and the highest values are from the adult fishes but the range of values is smaller than was expected.

$^{34}S/^{32}S$

The values of $\delta^{34}S$ range from a low of +6.1 for one of the *C. communis* bodies (small shrimp) to a high of +17.9 for the anemone (see table 2c, figure 8c). Even this high value is lower than would be predicted from the $\delta^{34}S$ of typical oceanic sulfate (approximately +21 ‰; Peterson and Fry, 1987). Unlike the results for carbon and nitrogen, there is not a consistent difference between the vent and background sites in sulfur isotope ratios. The bodies of the small shrimp species, as well as the two species of juvenile fishes and the adult rockfish which were collected near the vent, have lighter $\delta^{34}S$ values than the corresponding background samples. The other shrimp species and the adult slender sole show the opposite trend.

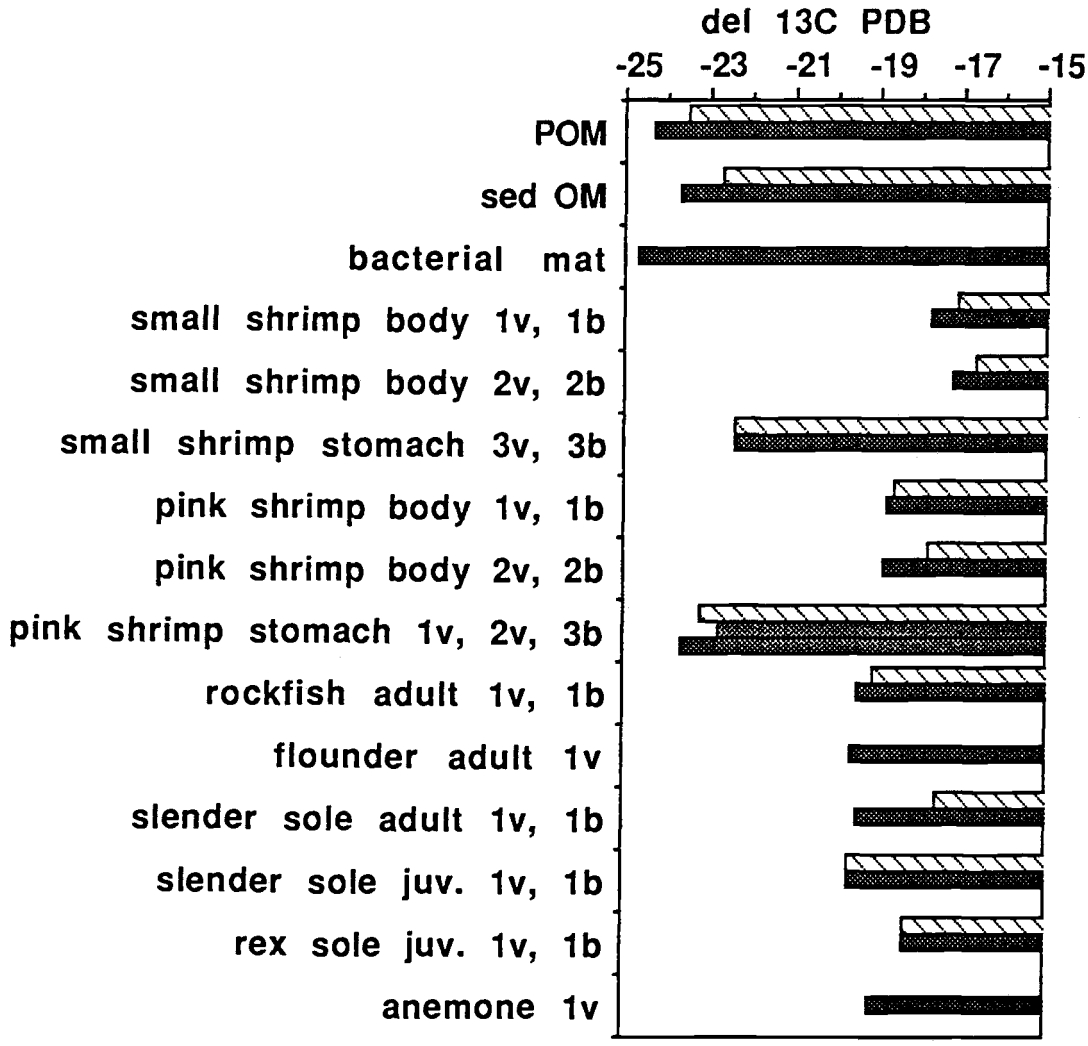


Figure 8 a-c - Results of stable isotope analysis, data from table 2. Sample numbers correspond to table 2. ▩ = vent, ▨ =background.

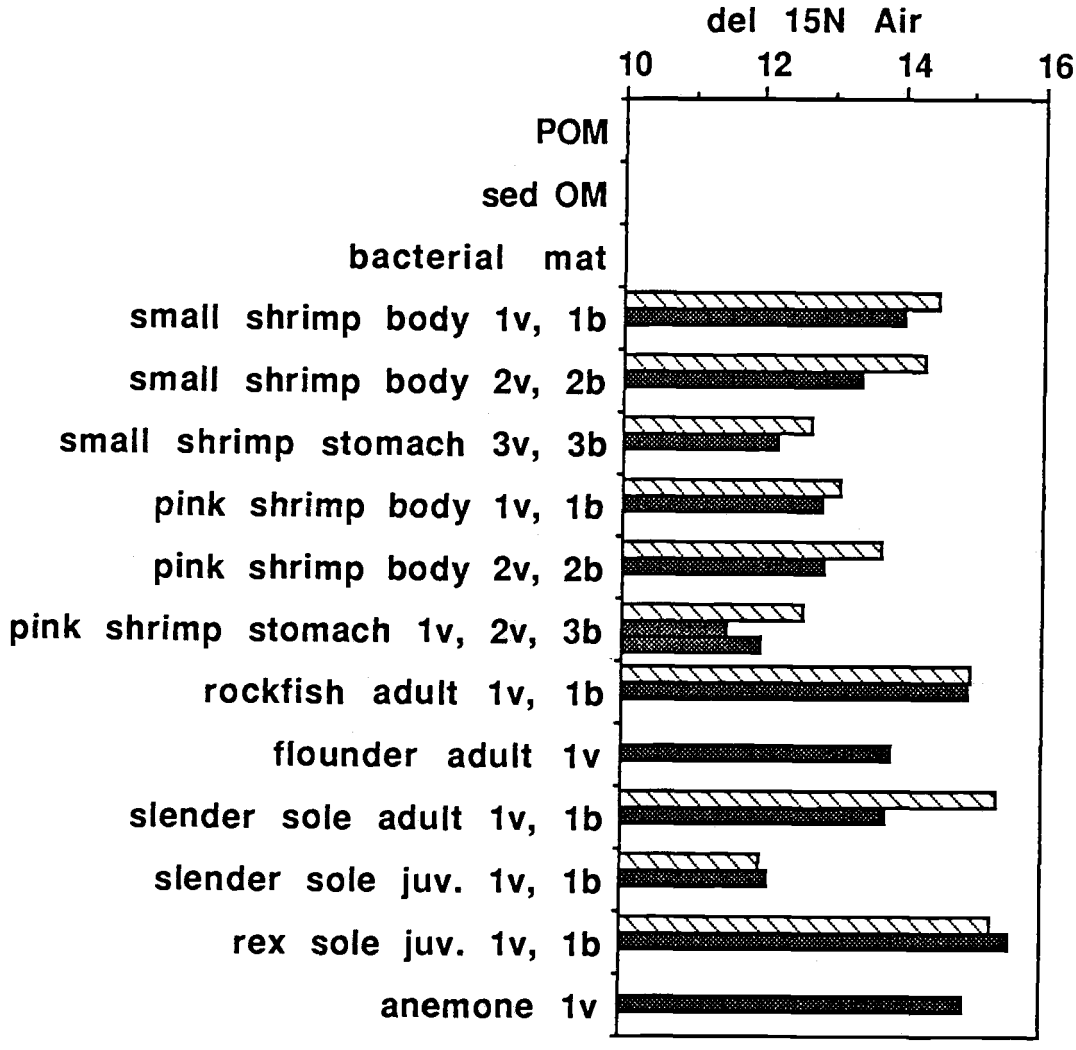

del 15N Air
10
12
14
16
POM
sed OM
bacterial mat
small shrimp body 1v, 1b
small shrimp body 2v, 2b
small shrimp stomach 3v, 3b
pink shrimp body 1v, 1b
pink shrimp body 2v, 2b
pink shrimp stomach 1v, 2v, 3b
rockfish adult 1v, 1b
flounder adult 1v
slender sole adult 1v, 1b
slender sole juv. 1v, 1b
rex sole juv. 1v, 1b
anemone 1v

Figure 8b

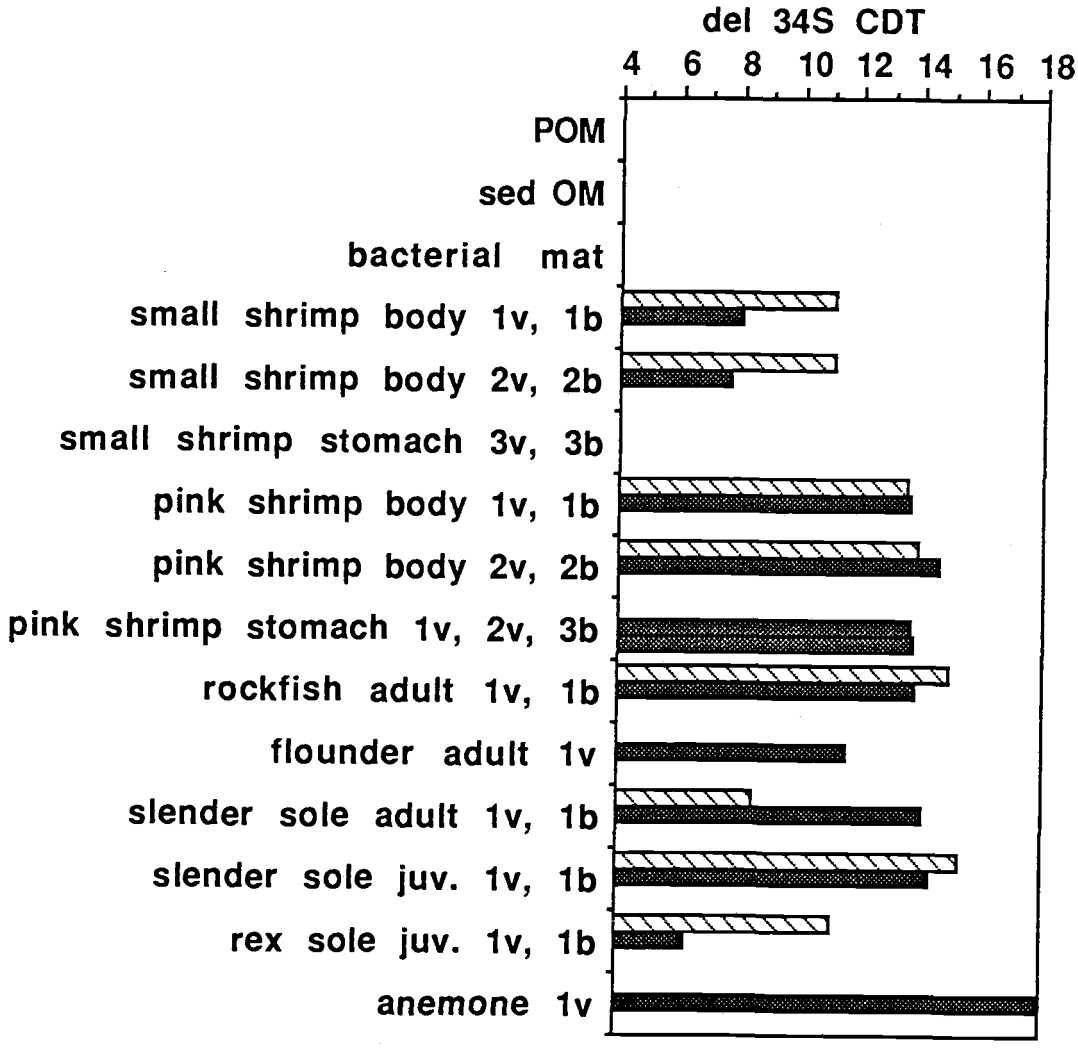

del 34S CDT
4
6
8
10
12
14
16
18
POM
sed OM
bacterial mat
small shrimp body 1v, 1b
small shrimp body 2v, 2b
small shrimp stomach 3v, 3b
pink shrimp body 1v, 1b
pink shrimp body 2v, 2b
pink shrimp stomach 1v, 2v, 3b
rockfish adult 1v, 1b
flounder adult 1v
slender sole adult 1v, 1b
slender sole juv. 1v, 1b
rex sole juv. 1v, 1b
anemone 1v

Figure 8c

DISCUSSION

The subjective impression of investigators who participated in both the 1989 and 1990 cruises, was that the Greenie Spot was less active during 1990, both in terms of ebullition rate and abundance of organisms, than it had been the previous year. Nothing is known about the temporal variability of pockmark venting, although one can assume it to be a critical factor in determining the importance of pockmark venting to continental shelf processes. The fisherman who revealed the location of the Greenie Spot to the investigators in 1989 claimed that the vent was active every time he had been to the site over the previous 7 years. He had not noticed any variation in the degree of activity of the vent over that time. However, variation in ebullition rate may not be detectable on fish finding sonar.

The 1990 Cruise

Water Column - Gases

The Greenie Spot was located again in September of 1990 and was found to be still actively venting. At times when the seas were calm, small bubbles could be seen bursting at the surface directly above the vent. While bubbles seen at the surface in 1989 were reportedly up to 20 cm across, those seen in 1990 were, at best, only a few cm across. Analysis of bubbles collected at the surface and of gas collected directly from the vent show that the gas is almost 96% methane with the remainder being primarily nitrogen (Collier and Lilley, unpublished manuscript).

A series of bottlecasts were made to determine water column profiles of methane

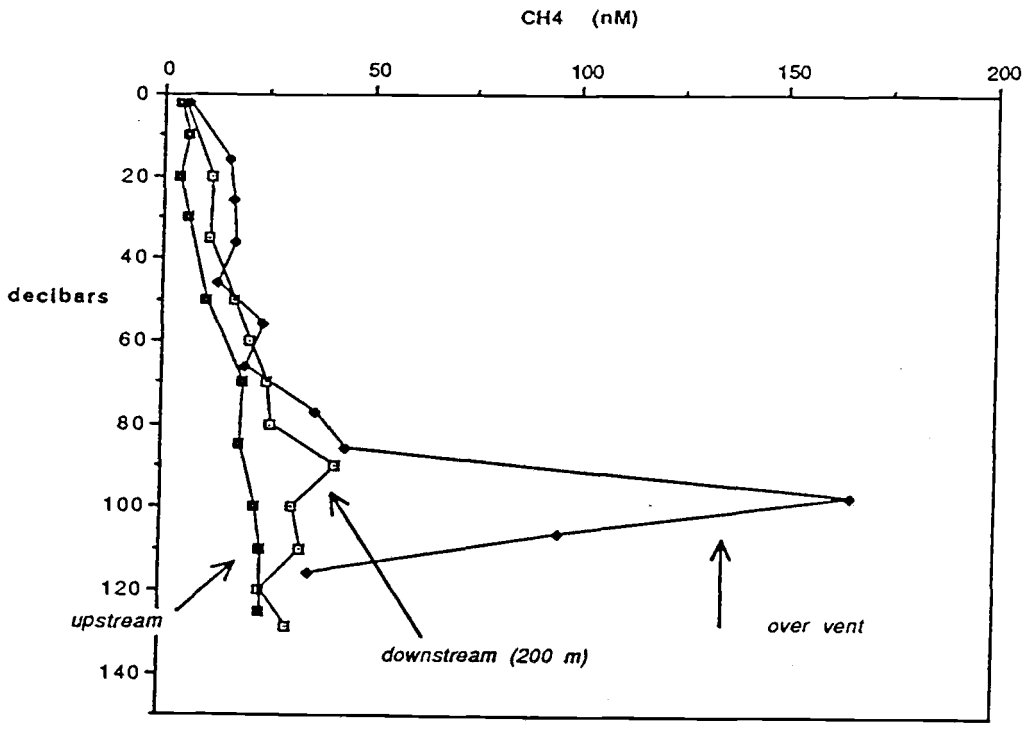


Figure 9 - Vertical profile of methane concentration from water samples collected upstream of the vent in the prevailing current (background), above the vent, and 200 m downstream of the pockmark. Water samples collected by CTD/rosette from the RV *Wecoma*. From Collier and Lilley (unpublished manuscript). Decibars roughly equal meters in depth.

concentration, the results are shown in figure 9. Not surprisingly, the highest methane concentrations were found directly over the vent. However, methane concentration was also considerably higher than background at the downstream site. At both the vent and downstream site, elevated methane concentration persisted in the water column for at least 50 m above the bottom. The highest concentration of methane in the water column was approximately 175 nM, roughly 8 times background but much less than a value >720 nM measured in 1989. This corroborates the observation of those who had been on the 1989 cruise, that the vent was less active in 1990.

Water column - Biology

The results of the microscope counts do not indicate that the presence of the vent enhances local standing stocks of large microzooplankton. The only noticable difference between the vent and background sites is a trend towards higher abundance at the background stations in the nearsurface samples. This trend is seen in several but not all groups of organisms in the three largest microzooplankton size classes. This trend is probably not due to the presence of the vent but rather is a consequence of the elapsed time between collection of the two sets of samples and the more dynamic nature and greater variability of surface waters relative to deeper waters.

The results for the <10 μm heterotrophs and the water column bacteria show similar trends. At depth, the vent samples tend to have increased abundance relative to background. Such a trend is exactly what one would predict if the vent methane was promoting microbial activity. Although this trend is statistically significant for one station in the case of the <10 μm heterotrophs and for all vent stations in the case of the bacterial counts, these results are <u>not</u> good evidence for a major enhancement of biological activity

due to the vent. This is a situation where statistical significance does not indicate biological significance. First of all, the increase in abundance at the vent stations is small compared to the range of abundance seen in natural microbial populations. Estimates of natural abundance of small marine flagellates and free-living water column bacteria vary over several orders of magnitude (flagellates, Porter *et al.*, 1985; bacteria, Ducklow, 1983). Bacterial abundance may reach 10^9 cells/ml near hydrothermal vents (Corliss *et al.*, 1979) Compared to this potential, the observed increase is minor. Second, even if the increased abundance were due to the presence of the vent, as described above, there is no corresponding enhancement of higher trophic level organisms even within the microbial loop, not to mention metazoans (see below).

Bacterial productivity was not measured. It is possible that relative productivity at the vent and background sites is not accurately reflected in the comparison of their standing stocks. Bacterial standing stock near the vent could be near background levels despite high production rates if predation was high. One would predict that this would result in higher standing stocks of small heterotrophs which are primarily bactivorous, unless they were also being cropped down, and so on up the food chain. While this scenario is possible, it seems unlikely. The microorganisms enumerated vary in size over three orders of magnitude, span several trophic levels and almost certainly display a huge range of growth rates. This being the case, if bacterial productivity were greatly enhanced at the vent, one would expect to see some prey organisms within the microbial food chain outstrip their predators' ability to crop them down. In other words, if bacterial productivity were greatly enhanced one would expect to find an increase in standing stock of at least some higher trophic level organisms. This is not seen in the data.

Apparently average cell size is approximately the same at the vent and background

sites. Had the sizes differed, the small abundance increase could have meant a large difference in biomass. However, since the average sizes are similar, bacterial biomass at the vent site is only greater than the background site to the same proportion as the numerical abundance. Absolute biomass has not been calculated because the conversion factors for planktonic organisms are controversial and because such a calculation would not convey any additional information.

Numerous horizontal plankton tows were made with a 500 μm mesh net near the vent and at a background site. The organisms in these collections have not been enumerated, however, the results of these tows given as settled volume of plankton (see table 3) do not indicate a difference in the abundance of large plankters between the two sites. Hydroacoustic surveys were also conducted from the ship to detect macrozooplankton distribution and abundance patterns around the pockmark. Submersible transects were made to ground truth the acoustic data. No significant difference between the area near the vent and the surrounding area could be detected in these data (R. Starr, pers. comm.)

Taken together, these data indicate that the pelagic food web is not altered by energy transfer from the pockmark vent. It is possible that a minor enhancement of bacterial standing stock occurs near the vent. However, this effect does not seem to extend to the higher trophic levels within the pelagic food web. Considering the high-energy nature of coastal waters this result is perhaps not surprising. Acoustic doppler current profiler surveys at the vent showed variable horizontal currents with a net shear of up to 10-30 cm/sec. Bottom currents of several cm/sec were observed from the submarine (Collier and Lilley, unpublished manuscript). Planktonic organisms and vent gases are subject to rapid dispersion from the vent area. Only organisms with the highest growth rates would be capable of taking advantage of the vent-derived energy.

Table 3 - Results of horizontal plankton net tows with a 500 μm mesh plankton net. Plankton volume expressed as settled volume of plankton per volume of water passing through the net. Net was towed at 50 m depth. From W. Pearcy (unpublished data).

Date and time	Location	Length of tow (m)	Plankton volume (mL m^{-3})
9/12/90, 0408	Control site	420	2.5
9/12/90, 0428	Control site	369	2.6
9/12/90, 0556	Control site	525	2.2
9/12/90, 0633	Control site	518	1.5
9/12/90, 2320	Control site	369	2.1
9/13/90, 0047	Control site	501	1.8
9/13/90, 0112	Control site	308	2.1
9/13/90, 0139	Control site	405	2.2
9/12/90, 0443	At vent	392	1.8
9/12/90, 0454	North of vent	354	2.2
9/12/90, 0518	North of vent	354	1.6
9/12/90, 0618	At vent	531	1.8
9/13/90, 0000	At vent	341	2.3
9/13/90, 0018	North of vent	325	2.4
9/13/90, 0102	Start at vent	310	2.3
9/13/90, 0149	Past vent	404	2.3
9/13/90, 0214	Near vent	435	2.1

Benthos

The benthic environment may be the most likely place to find an influence of the vent since benthic organisms are usually sessile and because porewaters of vent sediments contain several orders of magnitude higher methane concentration than the water column (up to 1300 μM in 1990, Collier and Lilley, unpublished manuscript).

There are several other interesting chemical features which distinguish the vent sediment porewaters from background porewaters. The vent porewaters are of low salinity and are also depleted in SO_4^{-2}, Mg, SiO_2 and CO_2. They are enriched with Ca^{+2} and highly enriched with NH_4^+, despite the depleted CO_2. The vent porewaters are also anomalously warm. A temperature probe placed approximately 5 cm into the vent sediments read 15°C while surrounding bottom waters were 7.5°C (Collier and Lilley, unpublished manuscript).

Two types of bacterial mat are found near the pockmark; thick, rugose, salmon-colored mats, and thin white mats. Samples of the thicker mat were collected. Under the microscope, they were revealed to consist of an almost pure culture of a very large (50 μm diameter) *Beggiatoa* species, similar to those found at the Guaymas Basin hydrothermal vents (Nelson *et al.*, 1989). These bacteria are most probably sulfide-oxidizers (Collier and Lilley, unpublished manuscript).

Unfortunately, due to equipment failure, no samples of benthic infauna were collected. However, samples of benthic epifauna were collected for stable isotope analysis (see below) and collections of epibenthic fishes were made with an otter trawl. The results from two trawls near the vent and two background trawls are compared in table 4. There is no obvious influence of the pockmark on the abundance of any particular fish species or on total abundance. This contrasts with the reports from the 1989 cruise.

Table 4 - Results of otter trawls for epibenthic fishes. Four otter trawls, varying in length from 300 to 420 m, were made. Two trawls were at background sites away from the vent and two traversed the area of the pockmark. The abundances of animals are given per 100 m of trawl length. From D. Stein (unpublished data).

Species	Control site		Near pockmark	
	Trawl 1	Trawl 2	Trawl 3	Trawl 4
Atheresthes stomias (Arrowtooth flounder)	-	0.24	0.74	-
Citharichthys sordidus (Pacific sanddab)	0.63	-	0.49	
Eptatretus stoutii (Pacific hagfish)	-	0.71	-	-
Glyptocephalus zachirus (Rex sole)	3.48	0.48	1.72	0.66
Hagfish	0.32	1.19	2.45	0.33
Icelinus filamentosus (Threadfin sculpin)	0.32	-	-	-
Juvenile rockfish	0.32	-	0.25	0.33
Liparus pulchellus (Showy sailfish)	-	-	-	0.66
Lycodes pacificus (Blackbelly eelpout)	4.43	12.1	19.1	5.65
Lyopsetta exilis (Slender sole)	27.2	30.0	38.0	25.2
Merluccius productus (Hake)	-	-	-	0.66
Microstomus pacificus (Dover sole)	2.53	0.71	0.74	0.33
Plectobranchus evides (Bluebarred prickleback)	-	-	-	0.66
Radulinus asprellus (Slim sculpin)	0.63	1.67	-	-
Raja binoculata (Big skate)	0.32	-	0.74	1.00
Raja kincaidii (Sandpaper skate)	-	-	-	0.33
Ronquilus jordanii (Northern ronquil)	0.32	-	-	-
Sebastes diploproa (Splitnose rockfish)	0.32	-	-	-
Sebastes elongatus (Greenstriped rockfish)	2.53	0.48	-	-
Sebastes wilsoni (Pygmy rockfish)	0.32	-	-	-
Xeneretmus latifrons (Blackedge poacher)	3.16	0.48	0.74	0.66
TOTAL	**46.9**	**48.1**	**65.0**	**36.5**

Stable Isotopes - Gases and Carbonates

Methane gas collected from the vent had an average $\delta^{13}C$ value of -28.6‰, this is near to the heaviest that methane ever gets suggesting that the gas is thermogenic in origin. Naturally occuring methane ranges from approximately -25 to -80‰. Biogenic methane (produced by bacteria) generally is much more negative than thermogenic methane (produced by geologic processes) Another possible explanation for this value is that the gas is the leftover from a large biogenic pool which has nearly all been oxidized. The gas contains only small amounts of higher order hydrocarbons. This is typical for biogenic but not for thermogenic gas (Collier and Lilley, unpublished manuscript).

Besides the gas itself, and the bacterial mats, the most obvious feature of the vent is the presence of carbonate slabs and stacks. Similar carbonate formations have been found at sites of deep-water methane vents on the Oregon slope (Kulm *et al.*, 1984; Ritger *et al.*, 1987; Suess and Whiticar, 1989). Isotopic evidence has confirmed that formation of the deep-water carbonates is related to bacterial methane oxidation (Kulm *et al.*, 1984; Ritger *et al.*, 1987; Suess and Whiticar, 1989). Samples of carbonates from the Greenie Spot are depleted in ^{13}C relative to typical marine carbonates (normal marine carbonate $\delta^{13}C$ = -5 to +5‰; Hoefs, 1987; see figure 10) indicating that the same process apparently occurs near shallow water vents. In this scenario, sediment bacteria oxidize the vent methane to CO_2 which reacts with Ca^{2+} and other ions in the porewaters and precipitates out as carbonate. Thus the carbon in the carbonates is vent-derived and reflects the isotopic signature of the methane.

Figure 10 - Del ^{13}C values of several inorganic carbon reservoirs, vent methane, vent carbonates and typical marine organic matter. Arrows point from carbon source to product.

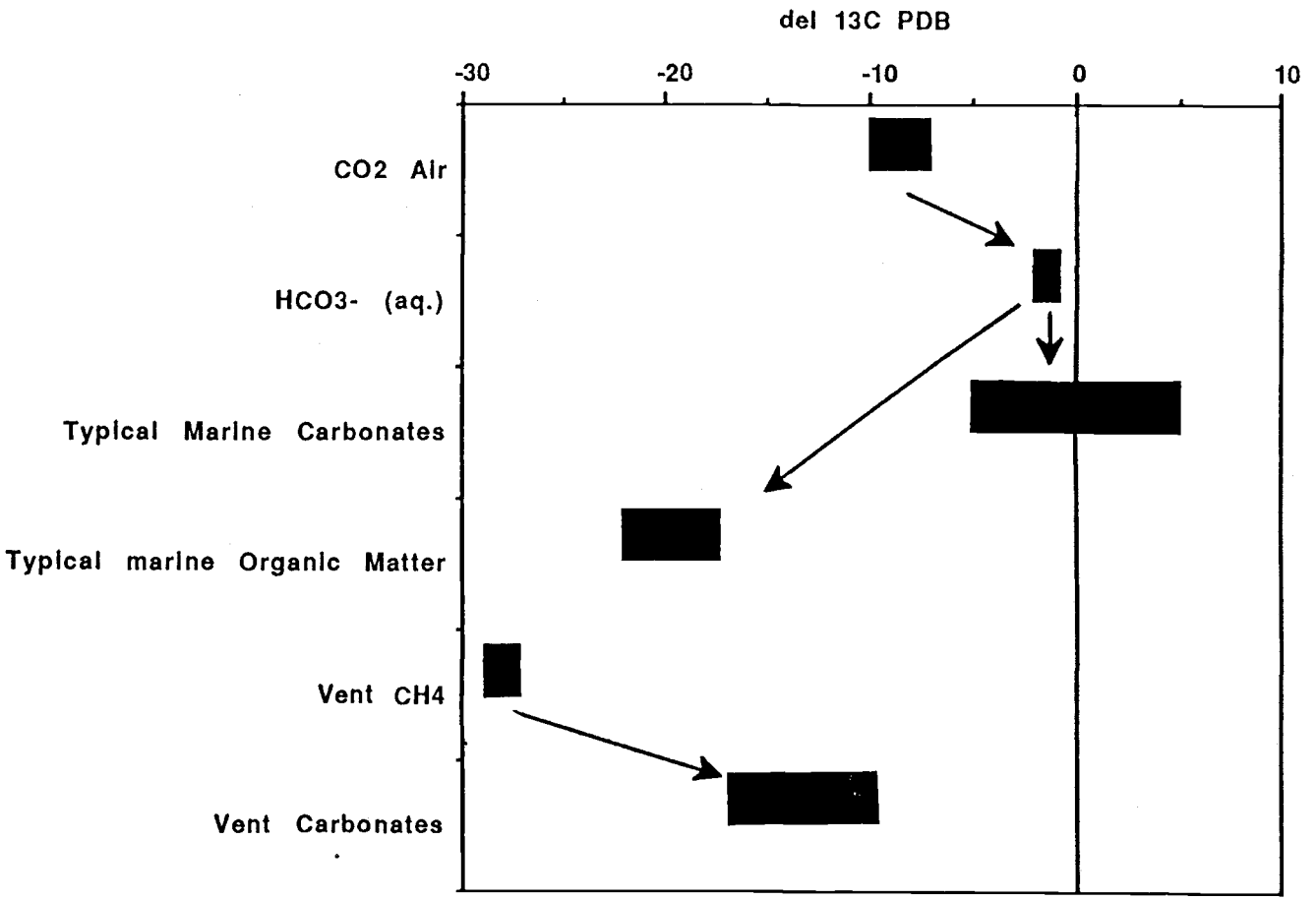

del 13C PDB
-30
-20
-10
0
10
CO2 Air
HCO3- (aq.)
Typical Marine Carbonates
Typical marine Organic Matter
Vent CH4
Vent Carbonates

Figure 10

Stable Isotopes - Biology

Typically, marine organic matter has a $\delta^{13}C$ range of -18 to -24‰ (Fry and Sherr, 1984), reflecting the $^{13}C/^{12}C$ ratio of phytoplankton. Recalling that $^{13}C/^{12}C$ changes little as carbon cycles through an ecosystem and therefore $\delta^{13}C$ preserves information about carbon source, if vent methane of approximately -29‰ were entering the food web, one would expect the $\delta^{13}C$ value of locally produced organic matter to be more negative than background organic matter in proportion to the amount of methane-derived carbon present.

This is in fact what one sees in the data. The difference in any individual comparison between a vent and background sample is slight but since the same result is seen in nearly all of the paired samples, the result becomes significant. Furthur corroborative evidence comes from the nitrogen isotopes. The $\delta^{15}N$ values of the majority of vent samples are lighter than the corresponding background samples. Nitrogen isotopes are primarily used as information on trophic status and it cannot be predicted ahead of time what absolute values of $\delta^{15}N$ one might find in a system. Therefore the $\delta^{15}N$ discrepancy can be interpreted in two ways; either the organisms near the vent are feeding slightly lower in the food web, or more likely, there is a different nitrogen end-member near the vent. This may be related to the porewater NH_4^+ anomaly refered to above. For example, a decrease in the rate of denitrification (NH_4^+ --> NO_2^-) near the vent would result in a smaller $\delta^{15}N$ value for the local nitrate reservoir. Although this does not relate directly to the question of whether vent-derived energy is entering the local food web it does indicate that there is a difference between the vent and background sites.

Assuming that, had the vent not been present, the $\delta^{13}C$ values of the samples collected at the vent would have been equivalent to the corresponding background samples and

Table 5 - Proportion of vent-derived carbon in each vent sample. If the proportion of vent-derived carbon = Q; Q = [$\delta^{13}C$ of vent sample - $\delta^{13}C$ of background sample] / [$\delta^{13}C$ of vent endmember - $\delta^{13}C$ of background sample] . Del ^{13}C of vent endmember was assumed to be -28.6‰. Sample numbers correspond to table 2 and figure 8.

Sample	Proportion vent carbon (%)
water column POM	16%
sediment OM	17%
small shrimp (*C. communis*) 1v	5%
small shrimp 2v	5%
pink shrimp (*P. jordani*) 1v	2%
pink shrimp 2v	10%
rockfish adult (*S. elongatus*) 1v	4%
slender sole adult (*L. exilis*) 1v	17%
slender sole juvenile 1v	0%
rex sole juvenile (*G. zachirus*) 1v	0%

assuming that the methane endmember is -28.6‰, one can calculate the proportion of vent-derived carbon present in each of the samples. The proportion ranges from 0% for the juvenile fishes to 17% for the sediment OM and the adult slender sole (see table 5). Since $\delta^{13}C$ increases by around 1‰ per trophic step, this estimate is probably conservative especially for organisms which are high in the food web.

There are a number of details in the carbon and nitrogen isotope data which need to be addressed here. The bacterial mat sample is the lightest of all the biological samples collected. This may indicate that the bacteria utilise methane derived carbon to a greater extent than do the other organisms but it could also be due to contamination by sediment, since the sedimentary organic matter is also very light. Due to the similarity in $\delta^{13}C$ of the POM, sedimentary organic matter and the stomach contents of both species of shrimps at both sites, it appears that the shrimps are detritivores. The reason for the lack of a vent/background difference in stomach content $\delta^{13}C$ remains a mystery. The large discrepancy between the $\delta^{13}C$ values of stomach contents and bodies of shrimps also remains a mystery. In a similar analysis, the difference between stomach contents and body $\delta^{13}C$ values of benthic crustaceans from the Gulf of Mexico measured by Fry *et al.* (1984) was only 0.9‰, which is the normal increase one sees per trophic level (Fry and Sherr, 1984; Peterson and Fry, 1987). One may assume that the anemone found at the vent feeds on POM. Interestingly, there is a large $\delta^{13}C$ difference between the body and putative diet of this organism as well.

There are differences in $\delta^{34}S$ between the vent and background samples but since the differences are not consistent it is impossible to interpret their meaning without furthur information. It would be most helpful to have information on the sulfate end-members at each site. All of the samples are depleted in ^{34}S relative to what would be expected from

typical values of seawater sulfate. One possible explanation is that this shallow nearshore environment is influenced by organic matter of terrestrial origin.

Methane Oxidation Rates

Very high rates of aerobic methane oxidation, corresponding to methane turnover times of only 1.5 to 7 days, were measured in the sediments of the pockmark. By comparison the rates measured in bacterial mat sample and in the water column were much lower, corresponding to turnover rates of 100 days and 1.5 to 90 years, respectively. Background rates were not measured for comparison (Collier and Lilley, unpublished manuscript). However, the lowest of the water column rates measured are typical for the open ocean (de Angelis, 1989).

Conclusions

The methane of the pockmark vent suffuses the local sediments which promotes very high rates of methane oxidation, this also leads indirectly to the formation of massive carbonate structures. Carbon isotope evidence indicates that some methane is incorporated into the organic matter in the sediments and water column. Isotopic evidence furthermore indicates that some vent-derived carbon is incorporated into the tissues of organisms collected near the vent. Despite this, there is no evidence that the vent enhances standing stocks of organisms above the lowest microbial trophic levels. The effect of venting on benthic infauna and the importance of the bacterial mats is not known. The conservative conclusion must be that pockmark venting does not enhance standing stocks or change the structure of any part of the community which was sampled.

This is essentially the same conclusion which was reached by Dando *et al.* (1991) in their study of a pockmark methane vent in the North Sea. In light of what is known about other vent and seep phenomena, it is somewhat surprising that two studies of pockmark vents would arrive at this result. The comparison of the Greenie Spot with the deep methane vents on the Oregon slope is especially intriguing. As described earlier, these deep-water vents support communities of organisms which are remarkably similar to hydrothermal vent communities. However, water column methane concentrations directly above these vents were, in comparison to the Greenie Spot, 2 orders of magnitude lower (Kulm *et al.*, 1984), implying that the deep-water vents release much less methane.

There are at least three hypotheses which could explain the apparent lack of response of the local community beyond the bacterial level to the venting of methane from the Greenie Spot. First, the shallow, nearshore environment of the Greenie Spot is much more physically dynamic than that of deeper vents. Such strong physical forcing would rapidly disperse vent gases and locally produced organic matter and may preclude the long-term settlement of organisms with growth rates slower than bacteria at the site. By itself, this hypothesis cannot explain the isotope results and is therefore unconvincing.

Second, there may be considerable variation in ebullition rate of the vent. Since the background organic loading in shallow, nearshore waters is very high, it may be that only during periods of highest activity does the vent constitute a source of energy which is large compared to background. Thus at times of high venting activity, extremely high bacterial productivity in the sediments and water column would attract and enhance metazoan populations. During periods of lowered activity, bacterial productivity would not be significantly higher than background and most metazoans would disperse to near background levels. This scenario could explain the reports of high abundances of fish and

zooplankton near the vent in 1989. It could additionally explain the finding in the carbon isotopes that the bodies of both species of shrimp collected near the vent were lighter than background while their most recent meal had the same $\delta^{13}C$ value as background.

The third possibility is that organisms of the shelf environment may not be nutrient (or energy) limited. For example, shelf populations could be limited by high predation rates and/or physical factors. If this were the case, vent-derived energy would not impact local populations because it would be superimposed on an already excessive energy supply. The presence of methane would cause a shift from typical heterotrophic bacteria to methanotrophic bacteria but there would not be a major change in the overall rate of bacterioproduction. Local metazoans would incorporate vent-derived carbon into body tissues in proportion to its presence in local organic matter, resulting in the observed stable isotope ratios. However their production rates would not be enhanced.

These hypotheses have bearing on the general relationship between shallow- and deep-water venting phenomena. It is most remarkable that despite the wide range of geochemical conditions at the various deep-water vents and seeps, the biological communities are so similar. Shallow-water vents and seeps appear to be geologically and chemically identical to deep-water phenomena but they do not even superficially resemble them biologically. The following hypothesis is advanced.

There is strong evidence that deep ocean communities are highly nutrient limited (eg. Turner, 1981; Stockton and DeLaca, 1982; Hecker, 1985; Smith, 1987). The contrast between the typical deep-sea environment and the high organic loading near deep-water vents and seeps has led to the development of specialized deep-water animals. By contrast, typical shallow-water organisms are preadapted to conditions of high organic

loading and will take advantage of vent-derived energy themselves but only if physical and/or biological factors (such as predation) allow.

Finally, it should be reiterated that this was a pilot project of limited scope and even more limited funding. The initial "selling point" of the project had been the potential for enhanced commercial fishery production due to vent-derived energy. While there has been no evidence to support this contention, the system is still deserving of furthur study on other grounds. From a biological perspective, it provides an interesting comparison to other vents and seeps and it provides an opportunity to observe the effect of nutrient enhancement on shelf communities. Furthermore, if pockmark venting is as common as fishermen claim, the phenomena is also interesting from a geochemical viewpoint as a potentially large source of methane, which is a greenhouse gas.

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APPENDIX

APPENDIX I

Statistical Analysis of the Direct Count Method.

The microscope counts completed in this study were conducted with the purpose of investigating the ecology of the methane vent. Additionally, because of the design of the field plan, these data may also be used to examine the accuracy and precision of the epifluorescence direct count method. In this analysis I have made the assumptions that there is no large count bias due to the sampling method, that I have not made any major systematic counting errors, and that the average of several recounts or replicates most closely approaches the true value of the sample and therefore variation from that mean is an estimate of error.

When sampling a natural population of microbes there are several discrete types of variation in the estimate of abundance. Small scale patchiness will lead to different estimates of abundance in replicate bottle casts, I will call this within-site variation. Counts of replicate slides made from a single bottle cast will vary, this will be termed between-slide variation. Finally, due to subsampling and counting errors, multiple counts of the same slide will also vary, this will be known as within-slide variation. I am able to quantify the coefficient of variation (CV, = SD/mean of counts x 100%) at each step within this hierarchy of variation using the samples which I collected for the methane vent study. Specifically, I assessed within-slide variation by recounting a number of slides. I counted replicate slides which I had made from a single bottle cast to determine between-slide variation. I compared the counts from stations 2 and 3, and stations 4 and 5 to quantify within-site variation. The individual casts of each of these two pairs were completed as close in time and space to each other as possible and are therefore considered replicates.

Stations 2 and 3 are located near the vent while 4 and 5 are background sites.

I determined the CV for each size class (>40 μm, 24-40 μm, 10-24 μm, <10 μm) and each taxon (heterotrophic dinoflagellates, ciliates, choanoflagellates and unidentified heterotrophic protists) which I had enumerated for the methane vent study. Because of the time involved, I conducted only 2-3 recounts or replicate counts per sample for only 5-7 samples from each size class except in the case of bacteria, for which I have counted 3 replicates for every sample (see table 6). I did not detect any consistent trends in variation depending on site (vent vs. background), depth or taxon (see table 7). There were two obvious trends in the data. These may indicate inherent properties of planktonic populations or may be artifacts of the data set. First, there was a consistent decrease in all three types of variation as organism size decreased. Second, there was an increase from within-slide variation to between-slide variation, to within-site variation.

Ideally when interested in variation in abundance estimates of water-column organisms, one would design a sampling program which would control for all conceivable sources of variation. These sources of variation are confounded when one examines data after the fact, as in this case. I have attempted to isolate and determine the importance of the possible sources of variation by conducting some simple data manipulations after making a few assumptions.

Regardless of the comparison being made, small sample size will increase variation. I have examined this effect by graphing CV vs. the number of organisms counted for within-slide, between-slide and within-site comparisons (figure 11 a-c).

For within-slide and between-slide variation the CV appears to continually decrease with

Table 6 - Description of how variation statistics were calculated. Column 1 lists the 5 size classes enumerated. Column 2 lists the taxa which each size class was separated into; dinoflagellates = heterotrophic dinoflagellates, unidentified = unidentified heterotrophic protists, total = total heterotrophic protists. The remaining 3 columns describe the samples used to determine the 3 different types of variation.

Table 6 - Description of how variation statistics were calculated

Size Class	Taxa	Within-slide	Between-slide	Within-site
>40µm	dinoflagellates ciliates unidentified total	single recounts of 6 slides	5 counts of paired replicates	compare all counts from sta. 2 + 3 and from sta. 4 + 5 = 10 pr., of slides
24-40µm	dinoflagellates ciliates unidentified total	single recounts of 5 slides	5 counts of paired replicates	compare all counts from sta. 2 + 3 and from sta. 4 + 5 = 10 pr. of slides
10-24µm	dinoflagellates ciliates choanoflagellates unidentified total	single recounts of 5 slides	5 counts of paired replicates	compare all counts from sta. 2 + 3 and from sta. 4 + 5 = 10 pr. of slides
<10µm	total	single recounts of 6 slides	7 counts of paired or triplicated replicates	compare all counts from sta. 2 + 3 and from sta. 4 + 5 = 10 pr. of slides
bacteria	bacteria	triple recounts of 6 slides	22 counts of 3 replicate slides	compare all counts from sta. 2 + 3 and from sta. 4 + 5 =10 pr. of slides

Table 7 - Summary of variation statistics with taxa separated. N is greater than the number of slides counted because the CV was determined for each taxon within a size class separately and therefore each taxon is considered an independent sample. Cases where zero or only one organism of a particular group was counted have been excluded from the analysis. Size class refers to size in µm.

A - Within-slide variation; recounts of the same slide.

Size Class	Ave CV	Range	n	# of recounts
>40	18%	0 - 73%	14	2
24-40	13%	0 - 46%	15	2
10-24	13%	0 - 46%	15	2
<10	14%	2.0 - 25%	6	2
bacteria	11%	6.3 - 14%	6	3

B - Between-slide variation; replicates from one bottle cast.

Size Class	Ave CV	Range	n	# of recounts
>40	28%	2.7 - 74%	4	2-3
24-40	31%	13 - 62%	13	2-3
10-24	22%	1.7 - 63%	14	2
<10	17%	1.0 - 39%	7	2-3
bacteria	12%	1.7 - 25%	22	3

C - Within-site variation; replicate bottle casts

Size Class	Ave CV	Range	n
>40	33%	0 - 64%	15
24-40	44%	0 - 103%	25
10-24	36%	0 - 77%	27
<10	21%	2.4 - 51%	12
bacteria	see table 9		

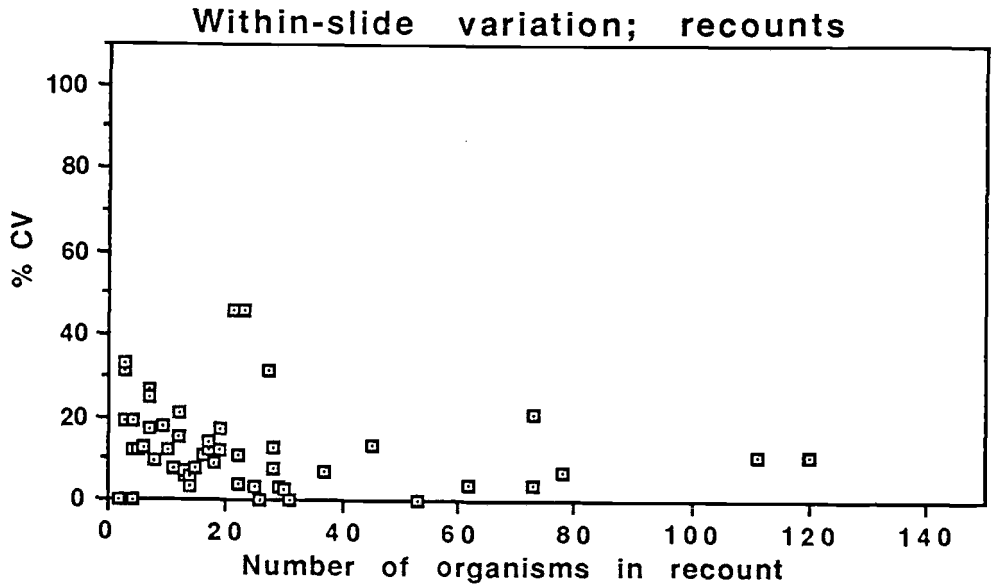

Figure 11 a-c - The effect of sample size on CV. A = within-slide variation; b = between-slide variation; c = within-site variation. All 3 graphs are to the same scale. Counts of bacteria were not included in these graphs since >200 cells were counted on each slide.

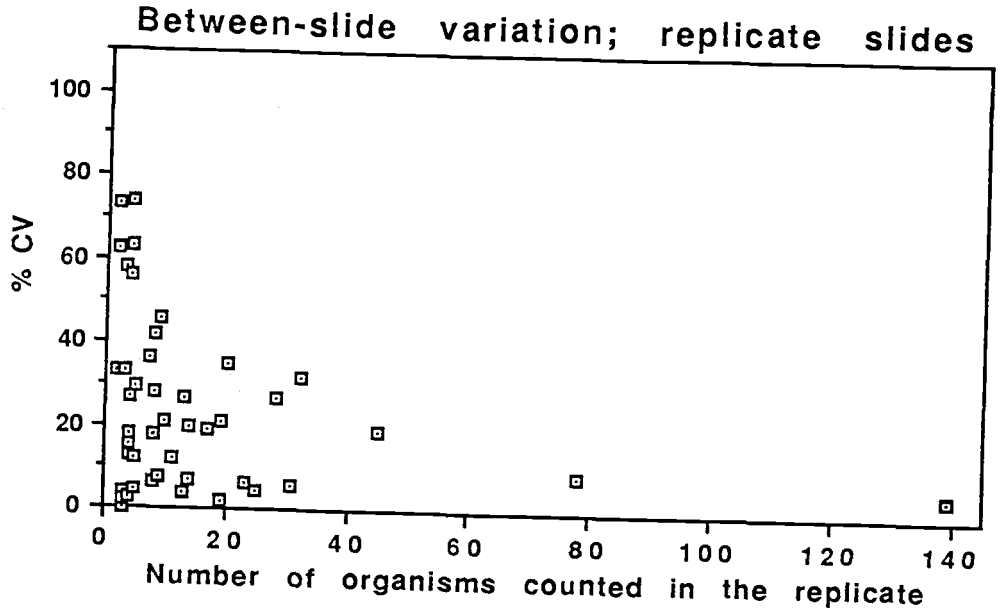

Figure 11c

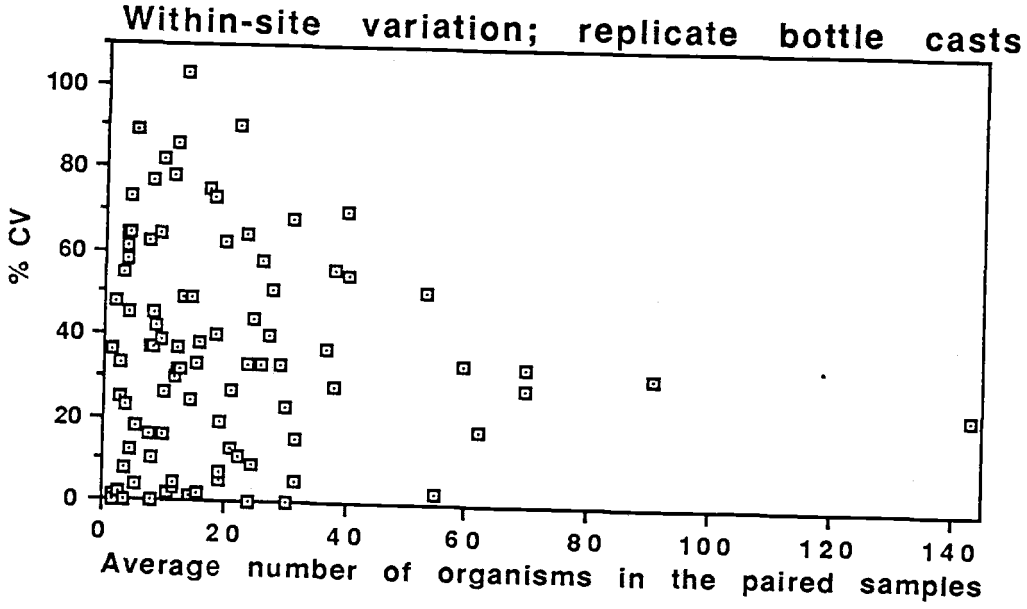

Figure 11b

increased sample size. In both cases, however, there is little decrease in the range of CV after about 30 organisms have been counted. There does not appear to be a relationship between CV and sample size in the within-site case.

Another potentially major source of variation is counting error; placing organisms in the wrong taxon or size class. I have made the assumption that I can reduce counting error by lumping organisms together and reanalysing the data as total heterotrophic protists (table 8). The effect has been to reduce the average and especially the range of the within-slide CV. The effect is not as great on between-slide variation and even less on the within-site variation.

A final problem is the accumulation of variation within the hierarchy I have described. For example, the within-site variation may be impacted by counting errors on individual slides, poor estimates of abundance within a bottle as well as the inherent patchiness in the plankton. The subset of the data which best allows me to examine this problem is the bacterial counts. Counting error is uniformly low in the bacterial counts due to the large number of organisms counted per slide (>200). Additionally, I have made three replicate counts for each sample. Presumably the average of three replicates is a better estimate of abundance within a bottle than the individual counts. On this assumption, I calculated the summary statistics for the within-site variation of the bacterial counts using one count of a single slide and then recalculated these same statistics using the average of 3 replicates (table 9a-b). There is no discernible difference in the estimate of variance between these two treatments.

Table 8 - Summary of variation statistics with all taxa in a size class lumped as heterotrophic protists. Size class column refers to size in µm. Values for <10 and bacteria are the same as in table 7 since these size classes could not be separated into multiple taxa. For a summary of within-site variation for bacteria see table 9.

A - Within-slide variation; recounts of the same slide.

Size Class	Ave CV	Range	n	# of recounts
>40	17%	6.5 - 25%	6	2
24-40	5.4%	0 - 21%	5	2
10-24	7.0%	2.6 - 11%	5	2
<10	14%	2 - 25%	6	2
bacteria	11%	6.3 - 14%	6	3

B - Between-slide variation; replicates from one bottle cast.

Size Class	Ave CV	Range	n	# of recounts
>40	27%	4.3 - 73%	5	2-3
24-40	29%	21 - 42%	5	2-3
10-24	8.1%	4.6 - 19%	5	2
<10	17%	1.0 - 39%	7	2-3
bacteria	12%	1.7 - 25%	22	3

C - Within-site variation; replicate bottle casts

Size Class	Ave CV	Range	n
>40	30%	0 - 73%	9
24-40	40%	8.8 - 75%	10
10-24	28%	2.4 - 51%	10
<10	21%	2.4 - 51%	12
bacteria	see table 9		

Table 9 - Summary of within-site variation for bacteria. In 9a statistics were calculated using the number of bacteria counted on individual slides. In 9b the same statistics were recalculated using the averages of 3 replicate slides instead of individual slides. N refers to the number of sets of paired slides.

A - Using paired single counts

Ave CV	Range	n
13%	0.5 - 39%	18

B - Using pairs of the averages of three replicates

Ave CV	Range	n
10.5%	1.2 - 27%	10

Conclusions

My estimates of variation compare well with the few other studies which have approached this question. Kirchman *et al.* (1982) found CVs of within- and between-slide variation of 9.99% and 6.88%, respectively for epifluorescence counts of marine and freshwater bacteria. Using epifluorescence and Ütermohl counts, Kuosa (1988) found between slide variation which ranged from 8.5% - 42.3% (ave. CV = 23.5) for several communities of heterotrophic nanoflagellates and small algae from the Baltic Sea and from cultures.

Much of the variation in the recounts (within-slide variation) is due to counting error and to small sample size. Under the best circumstances though, any single count of a slide will be within approx. ±10% of the true value on the slide. Between-slide variation was less influenced by counting errors but still heavily influenced by small sample size. Again, given the best circumstances, any single count will usually be within about 10% of the true value in the bottle. Of course, rarely does one find the best circumstances.

The evidence from the bacterial counts indicates that the large within-site variation is not caused by the accumulation of errors from lower levels in the hierarchy. Within-site variation is also not majorly influenced by taxonomic lumping or small sample size. It thus appears that this level of variation is an inherent property of the plankton.

Since small sample sizes are most likely for larger organisms, the possibility that the trend of increasing variation with body size is merely an artifact of the data cannot be rejected. However, within-site variation is not impacted by small sample size and still shows this trend. Therefore it is also possible that the abundance of larger organisms is more

variable at all levels.

Under the best circumstances, within- and between-slide variation are about the same value and are reasonably small. Within site variation is considerably larger (especially with large organisms) and reflects real variability in the system rather than counting problems.

Lund *et al.* (1958), refering to the Ütermohl counting method, claim that ecological questions generally deal with changes in population size of 100% or more and therefore single counts are sufficient to determine field abundance of organisms. If only this level of accuracy is required, the same claim can certainly be made for epifluorescence counts. Accuracy can be made much better than ±100% with the following recommendations. One should filter sufficient water that at least 30 organisms of any relevant type will be present. If this condition is met, recounts or replicate slides are unnecessary (but at least one replicate slide should probably be made in case something should go wrong with the primary slide). It may be necessary to collect >10 μm organisms on a separate filter from bacteria and nanoflagellates. Collecting >10 μm organisms on filters with 1 or 2 μm pore diameter allows one to filter sufficient water in a reasonable time.

The greatest increase in accuracy of the abundance estimate comes from making replicate bottlecasts. This will also allow one to quantify the small scale patchiness of the system, which may be a relevant statistic for the question being studied.