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Deep-Sea Food Web Analysis Using Immunological Methods: Results of a Feasibility Study

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TABLE OF CONTENTS

PAGE

List of Figures and Tables 3
Abstract 4
Introduction 5
Methods 12
Results 14
  RV Endeavor cruise 14
  Formalin preserved materials 14
  Immunodiffusion specificity tests 16
Summary 20
Recommendations 21
Acknowledgements 21
References 22

LIST OF TABLES AND FIGURES

FIGURES — PAGE

1. Schematic diagram of the immunological method of stomach content analysis 9

TABLES

1. Summary of methods for studying predator - prey interactions 7
3. List of organisms used in specificity tests 13
3. Bottom fauna collections from cruise EN-053, RV Endeavor.. 15
4. Precipitin reactions observed in specificity tests between antisera to shallow-water taxa and mid-water organisms extracts 17
5. Additional specificity test results 19

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ABSTRACT

Radioactive waste disposal sites used in the past have been found to be leaking low levels of radionuclides from containers placed on the sea bed. The potential exists for food chain transport of radionuclides from deep ocean regions to man, but the mechanisms by which such reverse transport upward can occur are largely unknown. Biologically mediated pathways could enhance dispersal rates of radionuclides from deep-sea sediments, but sampling difficulties in this remote environment render many potentially useful food web analysis methods useless. When biological samples are analyzed, it is frequently found that their stomachs contain visually unidentifiable remains. Immunological gut analysis methods are useful in identifying such remains.

The ability of antibodies to discriminate among proteins of different organisms depends on the degree to which a given antiserum cross-reacts with antigens from each organism. In low diversity shallow-water benthic communities, it is possible to make antisera specific to each target organism, but there are far too many species in the deep sea to ever produce highly specific antisera. Thus the ability of antisera to shallow-water taxa to discriminate among deep-sea taxa was tested in hopes that these antisera could discriminate among higher taxonomic levels of deep-sea organisms. Preliminary tests using protein extracts of mid-water planktonic animals were successful and revealed high affinities among shallow-water and mid-water species of the same taxon. It is concluded that the immunological method may provide higher-order taxon information for predator-prey interactions among deep-sea organisms. This level of discrimination may provide data which could not be gathered using traditional methodologies.

INTRODUCTION

The EPA Office of Radiation Programs has for the past several years been conducting comprehensive site specific oceanographic surveys at radioactive waste disposal sites used by the U.S. in the past. These survey activities have been conducted pursuant to the Marine Protection, Research and Sanctuaries Act of 1972, as Amended, which authorizes EPA to regulate all ocean disposal activities, including the disposal of radioactive waste not prohibited by law. Under the provision of the Act, EPA is also required to establish and apply criteria for reviewing and evaluating permit applications. To date, EPA has issued no permits for ocean disposal of low-level radioactive waste.

The Office of Radiation Programs has investigated the four major sites used for radioactive waste disposal in the past. These include two sites, at 900m and 1700m in the Pacific, and two at depths of 2800m and 3800m, in the Atlantic. In each of these sites, waste packages were located with the use of a submersible, and low levels of radionuclides were found to either be leaking or leaching from the containers.

One of the essential parameters yet to be addressed on a comprehensive basis is that of the potential for food chain transport of radionuclides from deep ocean regions upward to man. The primary focus of scientists in the past has been upon energy transfer downward through the water column. It is essential that an integrated approach be developed for identifying reverse transport mechanisms because of the complex interactions which take place between the organisms of the sea, their environment, and people. Research is needed to identify and evaluate:

a. The possible interrelationships among deep-sea, mid-water, and surface communities;
b. Factors which will assist in translating concentrations of radionuclides in seawater and bottom sediments into concentrations that will result in marine organisms; and,
c. Approaches to predict and analyze critical pathways to man.

This research may assist EPA in developing the technical basis and requirements for establishing regulations and evaluating permit applications for ocean dumping of other than high-level radioactive waste.

Given a point source of radionuclide leakage on the deep-sea floor, questions arise concerning the possible pathways by which these contaminants could reach man (Angino, 1977). Aside from diffusional and advective transport of radionuclides in soluble or fine particulate phase, it seems reasonable to suppose that biologically-mediated pathways also exist which could enhance dispersal rates.

The predominance of predatory, scavenging and deposit-feeding modes in the deep-sea increases the likelihood that radionuclides, if sorbed onto sediment particles, could enter the food web and be rapidly moved away from a leakage area (Sanders and Hessler, 1969; Hessler, 1974; Grassle et al., 1975). For example, numerically dominant deposit-feeding organisms such as polychaetes might ingest, assimilate, and thus bioconcentrate sediment-sorbed radionuclides. A highly motile or vertically migrating predator on polychaetes (fishes or amphipods, for example) could in turn translocate these materials a considerable distance from the site of ingestion (Hureau et al, 1979). In addition to such predator-prey relationships, biologically mediated mobilization of buried radioactive waste is possible as a consequence of simple sediment-moving activities of bottom-dwelling organisms. Burrowing behavior, subsurface deposit-feeding, and aqueous ventilation of burrow structures (for respiration and metabolite excretion) can increase the exposure of buried materials and increase their solubilization rates (Hessler and Jumars, 1977).

Assuming, then, that deposit-feeders and motile predators can mobilize buried or leaked radionuclides, transfer pathways involving these types of organisms must be identified in order to predict transfer rates. Numerous methods exist for identifying predator-prey relationships (Kiritani and Dempster, 1973), but the deep-sea environment constrains the application of many of them, especially observational and "tracer" or labelling methods. Stomach contents analysis of deep-sea organisms might then seem to be a prime candidate for application to the problem, but, unfortunately, many of the same difficulties encountered in the visual analysis of deposit-feeders and motile predators in shallow waters apply to deep-sea taxa as well. That is, large portions of the gut contents are recognizable only as fluidized amorphous masses. Fish stomach contents are more easily identified than those of deposit-feeders (prey are typically ground up), but deep-sea fishes often regurgitate or otherwise lose their ingesta upon capture and retrieval to the surface. Examination of such specimens rarely reveals the presence of intact, identifiable prey. Deep-sea scavengers' stomachs are frequently found full or even distended with unidentifiable "meat" (Dahl, 1979).

Alternatives to visual analysis of stomach contents are few (Table 1). Chemical analysis for specific elements or measurement of bioaccumulations of specific elements is possible, of course, and can provide valuable information on the distribution routes of target elements. The collection of fresh specimens for analysis is very expensive, however, and the effects of biological fixatives may render chemical tracer methods useless. Recent application of serological methods for analysis of food web connections in benthic organisms by Feller et al. (1979) may hold promise for tracing biomass fluxes among deep-sea taxa. The methods are particularly useful in cases where stomach contents are morphologically unrecognizable. The basic concepts of the immunological method are shown in Figure 1.

TABLE 1. An abbreviated summary of methods for the analysis of predator-prey food web interactions and their potential use in the deep-sea.

METHOD	COMMENTS	POTENTIAL
Direct observation	Possible, but very expensive, as remote sensing devices or deep submersibles required; not likely to yield even qualitative data on predation processes; visual observation will be size-biased; data for epifauna difficult to extrapolate to infauna.	Low
Predator exclusion	Usually do not provide unequivocal results on soft-bottoms; out of the question for deep-sea where predators are generally not well-identified.	Very low
Laboratory prey offerings	We cannot reliably collect and/or maintain deep-sea fauna in the lab; extrapolation to field difficult.	Low
Tracer or label experiments	Recovery of labelled prey would be essentially zero in the deep-sea.	Very Low
Chemical analyses of stomach contents	Could provide qualitative data on food sources, but such data are generally unspecific; requires elaborate equipment and technical skills; biased by whatever types of animals are examined (true for any method).	Moderate
Fluorescence analyses	May work for pelagic species at mid-depths, but not likely to work for benthic species as method requires presence of chlorophyll.	Low
Bioaccumulation studies	Variable in quality and difficult to interpret, but possible to follow gross patterns of biomass flux; correct choice of target elements or compounds not easy.	High
Carbon isotope ratios	Useful where plant or detrital material serves as food; poor choice in deep-sea because ratios are unknown for most taxa.	Low
Hydrogen isotope ratios	Untested in marine environment and susceptible to gut content contamination; less sensitive than carbon isotope ratio method.	Low

TABLE 1. (cont'd)

METHOD	COMMENTS	POTENTIAL
Mouth part morphology	Allows only broad classification of animals into feeding types; useless for tracing fluxes.	Low
Visual stomach content analyses	Probably the most reliable technique in spite of its potential biases; stomachs of ingested prey should also be examined.	Very high
Immunological methods	Worth testing on fresh-frozen specimens to see if cross-reactions are phylogenetically faithful across taxa from shallow to deep habitats.	Unknown

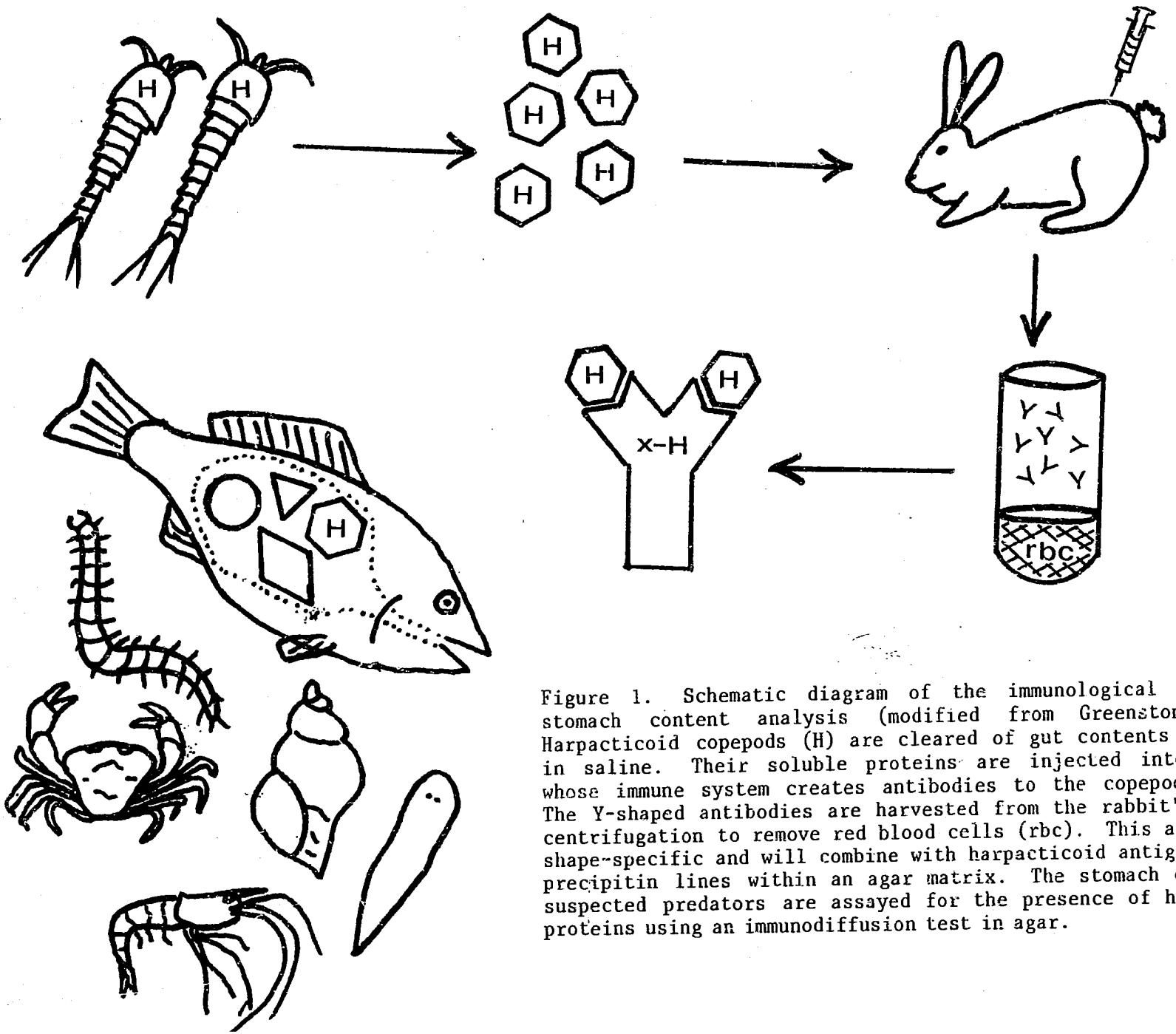
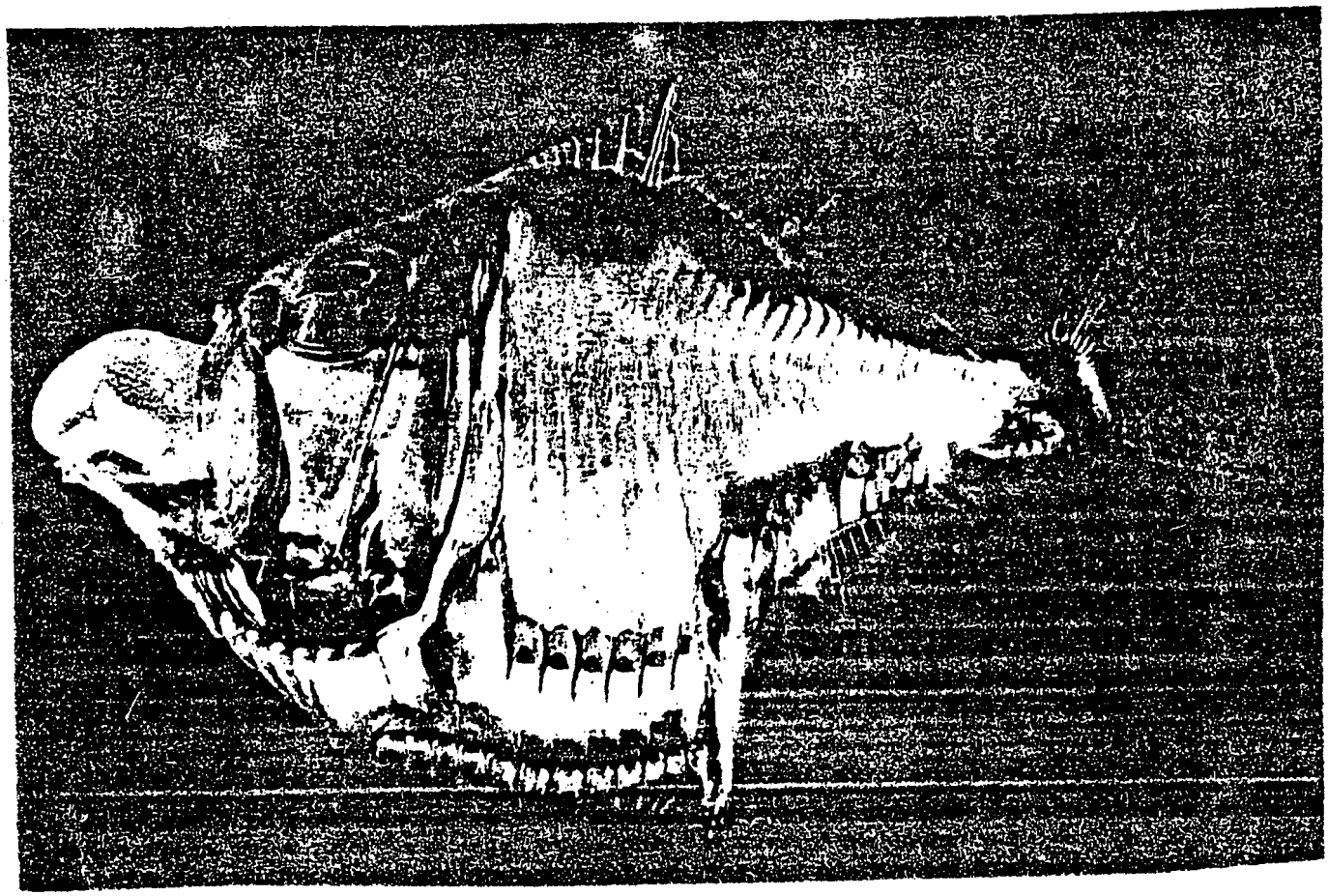


Figure 1. Schematic diagram of the immunological method for stomach content analysis (modified from Greenstone, 1979). Harpacticoid copepods (H) are cleared of gut contents and ground in saline. Their soluble proteins are injected into a rabbit whose immune system creates antibodies to the copepod proteins. The Y-shaped antibodies are harvested from the rabbit's blood by centrifugation to remove red blood cells (rbc). This antiserum is shape-specific and will combine with harpacticoid antigens to form precipitin lines within an agar matrix. The stomach contents of suspected predators are assayed for the presence of harpacticoid proteins using an immunodiffusion test in agar.



Argyropelecus sp., a common mesopelagic hatchet fish, is shown with stomach everted. The reduction in pressure as the animal was brought to the surface has caused the stomach to balloon out of its mouth. Incomplete eversion often leaves a fluid residue in the stomach which would be amenable to immunological analysis. (from Fig. 227, Deep Ocean, P. J. Herring and M. R. Clarke, eds., Praeger Publishers, New York, 1971)

Serological assays require a modest stock of taxon-specific antibodies with which to test for the presence of prey antigens (proteins, carbohydrates, fatty acids, etc.) in predators stomachs. Antibody specificity is highest whenever an antibody recognizes and reacts only with its target antigen - this is seldom the case when antibodies are made by injecting whole-organism extracts into a mammalian host such as a rabbit. That is, an antiserum prepared to recognize antigenic proteins from a species of bivalve may also (and usually does) recognize and react to some extent with antigens from another species of bivalve and to a lesser degree with other more distantly related molluscs. Such cross-reactions may be used to advantage in the stomach analysis of deep-sea organisms under the assumption that similar taxa (phylum, order, family, etc.) from shallow water share antigenic components with their deep-sea relatives. It is obviously too expensive to collect a sufficient diversity or quantity of live specimens from abyssal depths with which to prepare antibodies to test the assumption on a grand scale. But the existence of a variety of antibodies to shallow water benthic invertebrate taxa (e.g., Annelida, Mollusca, Arthropoda, and many other lower-order taxa) allows alternative approaches to this otherwise expensive problem.

In conjunction with investigations of predator-prey interactions among shallow-water marine organisms, an antiserum was successfully prepared in rabbits by injecting them with whole-organism extracts of adult grass shrimp, Palaemonetes pugio, which had been preserved in a 5% formalin-seawater solution for nearly five months. This was a somewhat surprising discovery, especially since formaldehyde polymerizes antigenic proteins so readily (Jones, 1976). The anti-P. pugio antiserum was of course not as sensitive and specific as antiserum prepared using fresh or fresh-frozen shrimp extract, but it retained sufficient specificity for recognition of higher order taxa. That is, the antiserum cross-reacted with several other crustaceans but not with the annelids or bivalve molluscs tested. Further, antiserum prepared with fresh shrimp also appeared to retain the ability to discriminate among higher order taxa when tested against formalin-preserved material. This suggested that it might be possible to examine the stomachs of formalin-preserved deep-sea specimens using an extant battery of antisera prepared with extracts from fresh, shallow-water organisms. Results obtained might provide predator-prey data at only a high order taxonomic level, but even this type of information is sorely lacking for abyssal animals. Such coarse data still might identify key predator-prey links worthy of more detailed study in the future. Some fraction of the large number of deep-sea specimens reposited in various oceanographic institutions and museums would have to be made available for serological examination towards this end.

It was thus proposed to examine the nature of serological cross-reactions among organisms collected from the deep-sea with antibodies prepared against whole-organism saline extracts of shallow-water benthic invertebrates. These studies were designed

to specifically test the feasibility of using immunological methods to examine the stomach contents of deep-sea predators, scavengers, and deposit-feeding organisms.

A cruise aboard RV Endeavor, University of Rhode Island, to collect deep-sea fauna for immunological testing was unsuccessful in this regard, as little time was available for biological sampling under the prevailing weather and scheduling of other worker's tasks (Laine et al., 1980). However, through the cooperation of Dr. Bruce Robison, University of California at Santa Barbara, a variety of mid-water organisms were donated for testing. This report concerns results of specificity tests using antisera to shallow-water benthic invertebrates from both Puget Sound, Washington, and North Inlet, South Carolina, and whole-organism saline extracts of the mid-water specimens donated by Dr. Robison. Comments are also directed towards the feasibility of analysing the stomach contents of formalin preserved specimens, the applicability of other methods of food web analysis in the deep-sea, and recommendations for further research.

METHODS

Antisera to shallow-water organisms were prepared by injecting whole-organism extracts of a given invertebrate species into white, female, New Zealand rabbits. The extracts were prepared by grinding freshly-collected animals (whose guts had been cleared for 24 hr) in 5 mM TES [N - tris (hydroxymethyl) methyl-2-aminoethane sulfonic acid], 30 mM NaOH, and 150 mM NaCl at pH 7.3. The TES-saline protein mixture was centrifuged at 1000 x g and the supernate stored at -20°C. This soluble protein extract served as antigen for the injection series following the protocol of Feller et al. (1979). Serum collected from the rabbits was stored at -20°C until use.

Mid-water animals donated by Dr. Robison were sorted from trawl catches in the Santa Cruz basin at depths of about 1200 m and frozen intact on board ship. They were mailed air freight to Columbia, S.C., and arrived still frozen on dry ice. No thawing was known to have occurred during handling or shipment. The numbers of each organism solubilized in TES-saline, the volumes of TES-saline used, and other comments regarding preparation of the mid-water organism extracts for testing are very similar to procedures used in preparing extracts of the shallow-water organisms (Table 2). Total protein concentrations of the various extracts were not measured but probably ranged from 1 to 10 mg total protein per ml. Each species was ground with glass beads in a cold mortar and pestle for approximately 1 min. The soluble protein slurry was then centrifuged at 1000 x g for 10 min and the supernate stored at -20°C until tested.

To test for the presence of cross-reactions between soluble protein extracts of mid-water species and antisera to shallow-water organisms, an immunodiffusion technique was used. Microscope slides (25 x 75 mm) were coated with 1.2 ml of 0.5%

TABLE 2. Mid-water organisms utilized in specificity test.

ORGANISM	NO.	ml TES	COMMENTS
Gausia princeps (copepod)	35	3.0	intact adult females
Euphausia pacifica (euphausiid)	14	2.0	intact adults; 15mm total length each
Sergestes similis (decapod)	8	2.0	Tail meat only, no exoskeleton; 1.2-2.5 cm total length
Cystisoma sp. (amphipod)	5	1.0	intact animals with visually empty guts seawater frozen inside exoskeleton
Phronima sp. (amphipod)	4	1.0	intact animals plus barrel; visually empty guts
Cranchiid squid	1	2.0	4.5 cm total length; intact, empty gut
Cyclothone acclindens (pisces)	4	3.0	tail meat only
Stenobrachius leucopsaurus (pisces)	1	3.0	6.5 cm total length; tail meat only
Triphoturus mexicanus (pisces)	1	4.0	6.8 cm total length; tail meat only
Eucopia sp. (decapod)	10	5.0	intact animals; 2.0-2.5 cm total length
Pasiphaea emarginata (amphipod)	4	8.0	tail meat only; 7.0-7.8 cm total length
Hymenodora debilis (decapod)	4	6.0	tail meat only; 4.0-7.0 cm total length

agarose (in 8 mM veronal, 40 mM sodium veronal, 0.25% Triton X-100, 0.01% sodium azide). A plastic template with 4 wells surrounding a central well served as point sources for the diffusion of extracts and antisera through the agarose. Typically, 10-15 µl of extract was added to the center well with four different antisera (15 µl per well) in the surrounding wells. Each test was duplicated. Diffusion proceeded at room temperature for 48 hr. Slides were washed in saline to remove unprecipitated proteins and in distilled water to remove salts. After washing, each slide was dried and stained with Coomassie Brilliant Blue R. Precipitin lines were examined and counted using back-lighting through opaque glass.

RESULTS

RV Endeavor cruise

Despite time and equipment limitations, two bottom samples were collected with a geologist's sphincter core during cruise EN-053, 11 August 1980. Geologists operated both cores which, upon retrieval, were routinely siphoned to remove overlying water (25-30 cm deep). Most of this water was collected and examined for fauna. It is unknown to what extent this material was contaminated by surface waters during the 1.5 hr retrieval period. The surface 1 cm of sediment was collected from each core, with one-third frozen on dry ice, one-third preserved in 0.5% formalin-seawater (v/v), and one-third preserved in 0.5% gluteraldehyde-seawater (v/v). All sediments were washed through a 44 micrometer mesh. The cores were severely winnowed when they reached deck, and therefore, any quantification on an areal basis will be underestimated by an unknown amount.

Abundances recorded fell within the range reported for meiofauna in the area (Coull *et al.*, 1977), and harpacticoid copepod diversity was also high as expected (e.g., Thistle, 1978). An insufficient biomass of any taxon was collected in the frozen fraction to test with shallow-water antisera.

Notable aspects of the two core samples examined were the dominance of agglutinated foraminifera (though very few if any may have been alive), absence of macrofauna (not particularly surprising for such small samples at abyssal depths), the presence of a molt of *Microsetella*, a surface-dwelling planktonic harpacticoid copepod (if it was not due to surface water contamination, it would have taken weeks to reach the bottom), and the absence of any obvious macrofaunal-scale features on the sediment surface (no tubes, tracks, or biogenic structures).

Formalin preserved materials

Attempts to utilize antisera prepared against fresh or fresh-frozen organism protein extracts to detect specific proteins in formaldehyde-preserved specimens were initially encouraging. However, sample size was too small, and when additional and more extensive tests were performed, the immuno-assay became an

TABLE 3. Summary of bottom fauna collections using 1 m long, 21.6 cm inside diameter, sphincter core (366 cm^2), RV Endeavor cruise EN-053, 11 August 1980, location 1.

SAMPLE NO.	LATITUDE	LONGITUDE	TIME (Z)	DEPTH (m)
SC #4	32 44.6N	70 43.2W	0134	5348
SC #6	32 46.5N	70 44.1W	0730	5346

OVERLYING WATER:	SC #4	SC #6
Nematoda	2***	2***
Harpacticoida	4	1
Calanoida	2	3

SEDIMENT (upper 1 cm):	SC #4	SC #6
Nematoda	3***	0***
Harpacticoida	3**	1**
Radiolaria	128*	15*
Foraminifera tests	3322*	5540*

* Nearly all were bits and pieces of tests, with fewer than 0.1% intact; unable to determine if any were alive when collected since no vital stains were used; sieving was too gentle to have broken intact specimens, thus likely that much fewer than 0.1% were alive.

** Most were nearly intact or easily recognized pieces.

*** No two organisms were the same species; mostly from family Cletodidae (Eurycletodes spp.) and Ameridae; a single molt of Microsetella sp. occurred in SC #4 sediment; SC #6 contained a gravid female from family Cletodidae.

unreliable methodology. Although formalin preservation effectively cross-links proteins and prevents their denaturation, the immunoreactive sites on the proteins may retain a conformation which is still recognizable to antisera. However, the extent to which this conformation remains constant is essentially unknown, and even slight shifts in the pH of the preservative medium may alter the shape of the protein molecules. It is thus difficult to ascertain whether reactions observed are due to true antigen-antibody interactions or to reactions caused by alterations in molecular shape. The lack of standardized preservation methods among different reseachers complicates the picture considerably, for proteins may obtain varying degrees of immunoreactivity depending upon formalin strength, buffering capacity, and preservation temperature. It is tempting to think that preserved materials from the deep-sea may retain enough reactivity for use with antibody recognition, but reliability is too low for any practical application in food web or taxonomic studies. I have abandoned any further testing of preserved material.

Immunodiffusion specificity tests

The cross-reaction tests between soluble protein extracts of the mid-water organisms and antisera to shallow-water benthic invertebrates were very successful. They revealed the following relevant features (Table 4):

1) antisera to shallow-water organisms recognize similar antigenic proteins in mid-water animals;
2) this recognition, as measured by the numbers of precipitin lines formed, was always less intense than the respective self-reactions;
3) many of the mid-water animal extracts did not react with some of the antisera, i.e., no precipitin lines formed in the agarose;
4) those mid-water animals whose extracts did cross-react did so along classical phylogenetic lines.

Thus, the antisera were predominately taxon-specific, the only strong exception being the Hobsonia antiserum which recognized antigenic proteins from mid-water crustaceans. This specificity at higher taxonomic levels coupled with broad cross-reactivity within a given taxonomic level is an ideal property for antisera which might be used as a gross assay tool in deep-sea food web studies. Furthermore, many of the antisera tested were prepared using whole-organism extracts of species from the west coast of the United States (Puget Sound, Washington), so that cross-reactivity and taxon specificity was apparently independent of whether the antisera were from widely separated geographic areas. More detailed evidence along these lines is presented by Feller and Gallagher (in preparation).

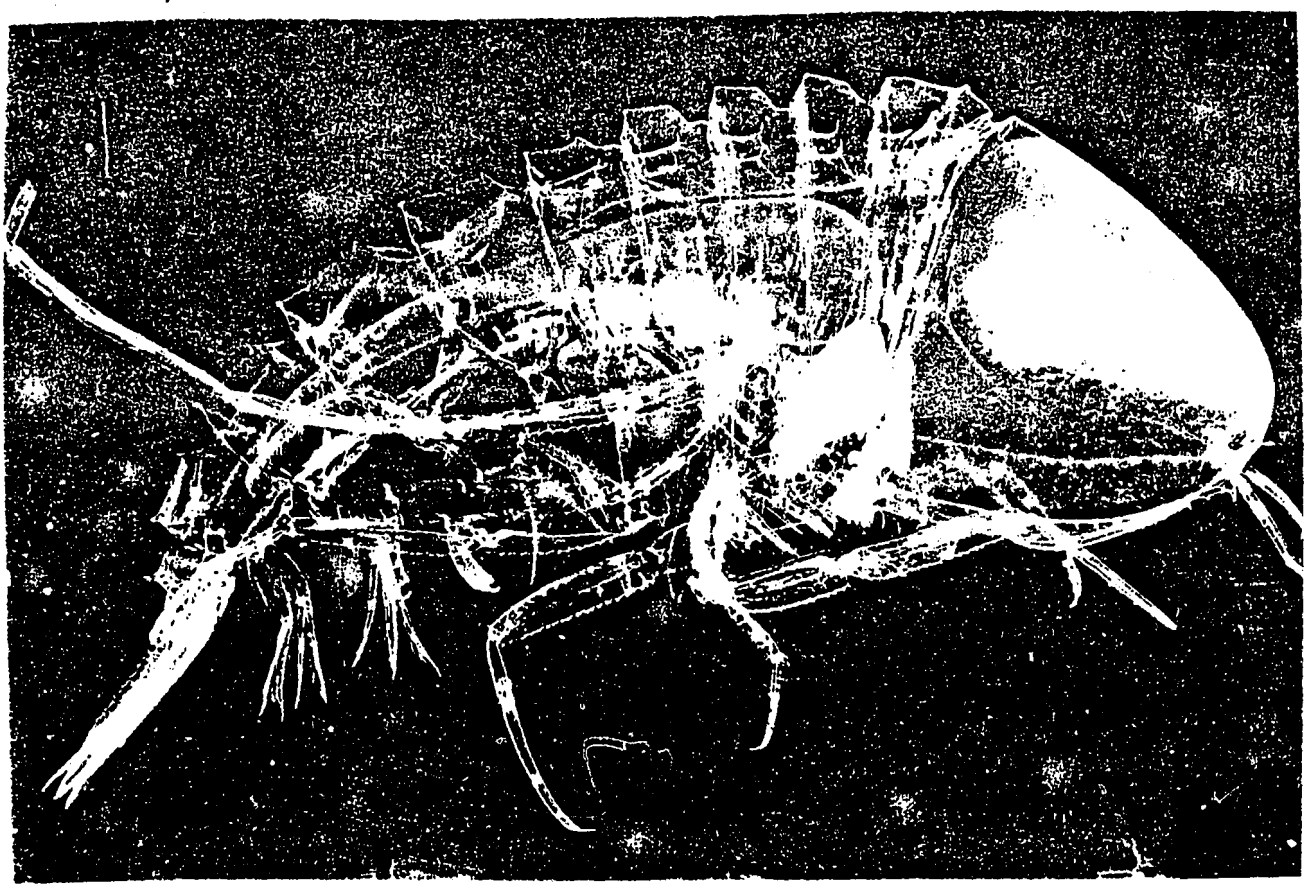
Additional but less extensive tests were performed using only nine of the nineteen antisera in Table 4 with extracts of T. mexicanus, Eucopia sp., P. emarginata, and H. debilis (Table 5). These tests also revealed the same features as outlined in points 1 through 4 above. This further enhances the generality of the

TABLE 4. Maximum number of precipitin lines observed in antigen-antibody reactions between whole-organism extracts of mid-water organisms and antibodies prepared against extracts of shallow-water taxa. Few if any cross-reactions are extensive when compared to the number of lines observed in self-reactions.

ANTISERA TO:			WHOLE-ORGANISM EXTRACTS OF:								
			A	B	C	D	E	F	G	H	Self
Crustacea											
Amphipoda	:	Corophium salmonis	2	1	1		1				7
		Eogammarus confervicolous	3	2			2				8
Decapoda	:	Callinectes sapidus	4	2	4	2	6	2			12
		Penaeus setiferus	6	5	9	2	8	3			15
		Palaemonetes pugio	2	3	5	1	3	1		1	13
		Crangon franciscorum	1		1	1					12
		Uca pugnax	1					1			11
		Uca pugilator	4	2		2	3				14
Copepods	:	Huntemannia jadensis			1	1	1				12
Ostracoda	:	Ostracoda spp.	5	2	5	3	2				7
Mollusca											
Bivalvia	:	Mercenaria mercenaria	2	2		1	1	2			13
		Crassostrea virginica	1					1			12
		Tagelus plebius	1								12
		Genkensia demissa									15
Gastropoda	:	Littorina irrorata									12
Annelida											
Polychaeta	:	Diopatra cuprea		2		1		3			11
		Hobsonia florida	3	2	5	2		2			8
Oligochaeta	:	Oligochaeta spp.									12
Nematoda	:	Diplolaimella chitwoodi	2								6

A - Phronima sp. (amphipod)
B - Cystosoma sp. (amphipod)
C - sergestes similis (decapod)
D - Gausia princeps (copepod)
E - Euphausia pacifica (euphausiid)
F - Cranchiid squid (cephalapod)
G - Stenobrachius Leucopsaurus (pisces)
H - Cyclothone acclindens (pisces)

A)



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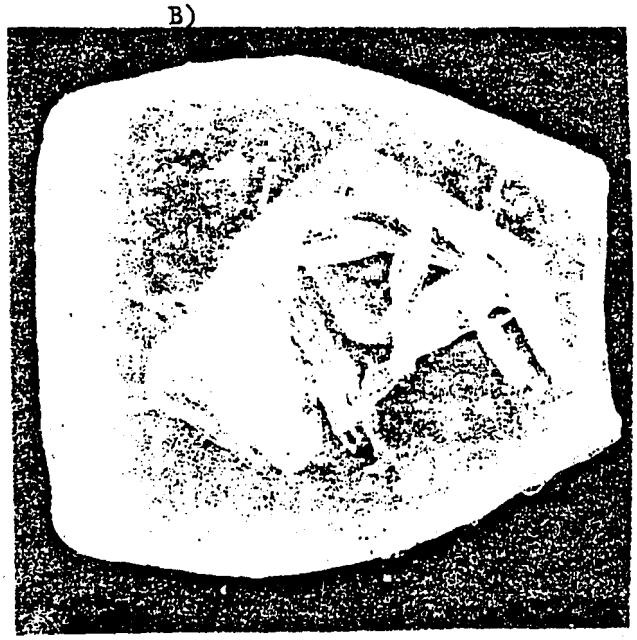

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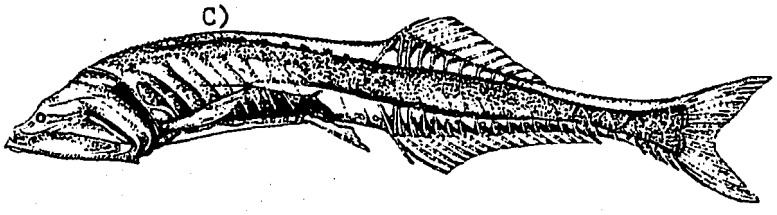

Representative taxa used for cross-reaction tests:

A) Cystisoma sp., a large (10 cm) hyperiid amphipod;
B) Phronima sp., a pelagic amphipod that lives within the empty barrel of a siphonophore;
C) Cyclothone sp., a numerically dominant meso - and bathypelagic fish genus.

(from Figs. 171, 172, 185, Deep Oceans, P. J. Herring and M. R. Clarke, eds, Prager Publishers, New York, 1971)

TABLE 5. Maximum number of precipitin lines observed in cross-reaction tests between antibodies to shallow-water benthic invertebrates and whole-organism extracts of mid-water organisms.

ANTISERA TO:		WHOLE-ORGANISM EXTRACTS OF: I	J	K	L	Self
Crustacea						
Decapods :	*Callinectes sapidus*	6	6	6		12
	Penaeus setiferus	7	8	9	1	15
	Palaemonetes pugio	5	5	6		13
	Uca pugilator	4	6	8		14
Ostracoda :	Ostracoda spp.					7
Mollusca						
Bivalvia :	*Mercenaria mercenaria*	4	2	1		13
	Crassostrea virginica	2	2	3		12
Gastropods :	*Littorina irrorata*	4	1			12
Annelida						
Polychaeta :	*Diopatra cuprea*	1	1	1		11

I - *Eucopia* sp. (decapod)
J - *Hymenodora debilis* (decapod)
K - *Pasiphaea emarginata* (amphipod)
L - *Triphoturus mexicanus* (pisces)

observed antiserum specificity at higher taxonomic levels with broad cross-reactivity within taxonomic levels.

SUMMARY

The immunological approach to food web analysis in the deep-sea merits further testing for the following reasons:

a) the method works for terrestrial and/or aquatic communities (Boreham and Ohiagu, 1978);
b) the method is extremely sensitive and can detect very low concentrations of protein (µg to mg per ml);
c) cross-reactions among shallow-water antigens and their homologous antibodies reflect traditional taxonomic similarities;
d) preliminary tests utilizing antisera to shallow-water species were successful in detecting antigenic proteins from taxonomically related mid-water planktonic organisms;
e) antibody affinities are highest between shallow-water and mid-water species of the same taxa;
f) cross-reactions among similar taxa from the west coast and east coast also reflect traditional taxonomic similarities (manuscript in preparation).

These preliminary findings could not be more encouraging. They indicate that it may be possible to analyse the stomachs of deep-sea predators and easily determine which taxonomic groups they had been eating. With some refinement it may be possible to determine that, for instance, a crustacean meal was amphipod and not decapod. Many marine fish predators contain visible masses of organic material or "meat" which cannot be identified. The development of the immunological method now has a high probability of offering a means by which such stomach material may be identified. Although the deep-sea biological community is too diverse to ever hope that specific identifications could be made, this higher-order taxon information will be invaluable in providing direct evidence for predator-prey interactions that could perhaps never be determined using traditional methodology.

Visual analysis of stomach contents should, of course, always be performed in conjunction with any immunological analysis. But because this technique is so sensitive, we may also be able to examine the stomach contents of the ingested prey themselves and determine secondary or second-order predator-prey interactions.

Relative to other's efforts at deep-sea food web analysis, the immunological method thus offers not simply an alternative approach but a complimentary technique which can give information when other methods fail. The major disadvantage, however, is that specimens must be examined in either the fresh or fresh-frozen state. Other methods, especially visual ones, can utilize formalin preserved material. However, since most deep-sea

collections are made from ships of substantial size, most are likely to have sufficient cold-storage capacity to preserve catches in the frozen state.

RECOMMENDATIONS

Deep-sea food chain transfer studies should begin, of course, with a competent review of published and "gray" literature pertaining to stomach content analysis in deep-sea organisms. There are very few of these. A review of literature pertaining to estimates of biomass and abundance of deep-sea fauna (there are considerably more of these and they vary in quality to such an extent that many are uninterpretable and useless) will provide a baseline against which future estimates from proposed dump sites may be compared. No estimate of biomass, however, can provide anything more than a broad, subjective indication that certain taxa may or may not enter food webs. Direct evidence is necessary.

The interests of EPA would be best served at this time by interacting with active deep-sea researchers and providing them nominal support to report their food-web findings from various deep-sea oceanic provinces. I believe EPA is now doing this very well, with the exception that, to my knowledge, no strictly biologically oriented cruises to the dump sites have been organized. Samples should be taken from the areas of interest utilizing box cores for sediment biota, bottom trawls, bait-trapping, microbial, and bioenergetic studies (e.g., oxygen consumption). These are all expensive propositions. Stomach content analysis of specimens continues to provide the most informative and reliable data for food-web studies. Hyslop (1980) reviews these visual methods with emphasis on their quantitation. Such traditional methodology suffers the typical limitations imposed when organisms sampled contain no visually recognizable remains in their stomachs.

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REFERENCES

Angino, E. E. 1977. High-level and long-lived radioactive waste disposal. Science 198: 885-890.

Boreham, P. F. L., and C.E. Ohiagu. 1978. The use of serology in evaluating invertebrate prey-predator relationships: a review. Bull. ent. Res. 68: 171-194.

Coull, B. C., R. L. Ellison, J. W. Fleeger, R. P. Higgins, W. D. Hope, W. D. Hummon, R. M. Rieger, W. E. Sterrer, H. Thiel, and J. H. Tietjen, 1977. Quantitative estimates of the meiofauna from the deep sea off North Carolina, USA. Marine Biology 39: 233-240.

Dahl, E. 1979. Deep-sea carrion feeding amphipods: evolutionary patterns in niche adaptation. Oikos 33: 167-175.

Feller, R. J., G. L. Taghon, E. D. Gallagher, G. E. Kenny, and P. A. Jumars. 1979. Immunological methods for food web analysis in a soft-bottom benthic community. Mar. Biol. 54: 61-74.

Grassle, J. F., H. L. Sanders, R. R. Hessler, G. T. Rowe, and T. McLellan. 1975. Pattern and zonation: a study of the bathyal megafauna using the research submersible Alvin. Deep-Sea Res. 22: 457-481.

Greenstone, M. H. 1979. Passive hemagglutination inhibition: a powerful new tool for field studies of entomophagous insects. Misc. Publ. Ent. Soc. Amer. 11: 69-78.

Hessler, R. R. 1974. The structure of deep benthic communities from central oceanic waters. In, Biology of the Oceanic Pacific, edited by C. Miller. Oregon State Univ. Proc. 33rd Ann. Biol. Colloq., pp. 79-93.

Hessler, R. R. and P. A. Jumars. 1977. Abyssal communities and radioactive waste disposal. Oceanus 20: 41-46.

Hureau, J. -C., P. Geistdoerfer, and M. Rannou. 1979. The ecology of deep-sea benthic fishes. Sarsia 64: 103-108.

Hyslop, E. J. 1980. Stomach contents analysis - a review of methods and their application. J. Fish. Biol. 17: 411-429.

Jones, D. 1976. Chemistry of fixation and preservation with aldehydes. In, Zoolplankton fixation and preservation, edited by H. F. Steedman. The UNESCO press, Paris. pp. 155-171.

Kiritani, K. and J. P. Dempster. 1973. Different approaches to the quantitative evaluation of natural enemies. J. appl. Ecol. 10: 323-330.

Sanders, H. L. and R. R. Hessler. 1969. Ecology of the deep-sea benthos. Science 163: 1419-1424.