

ECOSYSTEMS NATURALLY LABELED WITH CARBON-13: APPLICATIONS TO THE  
STUDY OF CONSUMER FOOD-WEBS

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ECOSYSTEMS NATURALLY LABELED WITH CARBON-13: APPLICATIONS TO THE  
STUDY OF CONSUMER FOOD-WEBS

A  
THESIS

Presented to the Faculty of the  
University of Alaska in Partial Fulfillment  
of the Requirements  
for the Degree of

MASTER OF SCIENCE

by  
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May 1978

## ABSTRACT

Natural abundance  $^{13}\text{C}/^{12}\text{C}$  ratios provide a tracer for the origin of organic carbon in complex coastal marine food-webs and also appear to be useful for examining trophic organization and food transfer efficiencies in more strictly oceanic environments.

The tracer approach proved useful for analyzing the role of eelgrass (*Zostera marina*) in the food-web of Izembek Lagoon, Alaska. Both eelgrass and phytoplankton contribute to the productivity of that community.

That analysis was complicated by non-ideal tracer behavior, however. Animal  $^{13}\text{C}/^{12}\text{C}$  ratios appeared to depend on biochemical composition, and ways to deal with this were investigated. Furthermore, animal metabolism tended to retain  $^{13}\text{C}$  relative to  $^{12}\text{C}$ , resulting in progressive elevation of  $^{13}\text{C}/^{12}\text{C}$  ratios in higher trophic levels. By assuming a uniform relation between  $^{13}\text{C}$  enrichment and metabolic stoichiometry, it was possible to deduce animal "trophic positions" and food transfer efficiencies from  $^{13}\text{C}/^{12}\text{C}$  data taken from the Bering Sea.

## ACKNOWLEDGEMENTS

Even a relatively small scale graduate study, such as this one, requires the cooperation and assistance of a surprisingly large number of people. First of all come the members of the thesis committee whose time, money, effort, and insight contribute to the research program from its inception until its completion. I am especially grateful to Dr. C. Peter McRoy both for these concrete contributions and also for his enthusiasm (and occasional nagging) which helped me along. I also wish to thank Drs. Patrick L. Parker and Richard S. Scalen of the University of Texas Marine Science Institute for advice and analytical facilities extended to me.

The Izembek National Wildlife Range provided invaluable field support and a warm place to dry out between storms, but perhaps its greatest contribution was the inspiration I developed from the range and manager Robert D. Jones. I also wish to thank John Sarvis and other range personnel for their assistance and companionship, without which that rather remote part of the globe would have been quite inhospitable.

Shipboard work aboard the *Alpha Helix*, *Acona*, and *Discoverer* constituted a major part of this and related research, and I wish to acknowledge the help and support of the ships' crews and of the institutions which put these vessels to sea.

This thesis is a contribution of the Seagrass Ecosystem Study supported by grants from the office of the IDOE of the NSF (Grant Nos. GX-37852, OCE-74-13457 A02, and OCE-76-84257).

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## CHAPTER 1. STATEMENT OF THE PROBLEM

The food-web concept underlies much of current thinking about the structure and functioning of marine ecosystems. It contributes to the understanding of life cycles, productivities, and population dynamics of organisms, ecological stability and diversity, evolution, biogeochemical cycles, and the thermodynamics of living systems. Animal food-web relations are usually studied in terms of stomach contents and feeding behavior, with information on distribution, dentition, digestive physiology, etc. frequently contributing to the analyses. The structure of the food web is then reconstructed by piecing together information on the various organisms composing the ecosystem. Collecting sufficient data to accomplish this synthesis usually requires a major effort, and may be stymied by a failure to collect various key species or an inability to describe quantitatively the composition of animal diets. A partial solution to these difficulties may be found in the observation that certain biochemical and isotopic labels are transferred through the food web, and the resulting distributions of these parameters contains information on their sources and transfer pathways through the food web. Here the ratios of stable carbon isotopes ( $^{12}\text{C}$  and  $^{13}\text{C}$ ) in the biota of an eelgrass (*Zostera marina* L.) bed and in the eastern Bering Sea are used to examine plant inputs to the food webs, trophic organization, and the efficiency of carbon transfer through ecosystems.

In several respects, the biomass  $^{13}\text{C}/^{12}\text{C}$  ratio is an especially suitable parameter for such a study. Organic carbon is the major constituent of biomass, and can easily be extracted and analyzed. The

biochemistry of carbon metabolism is well known and is fundamentally similar at the biochemical level in most organisms. Carbon isotopes have also been studied extensively in connection with biogeochemical, physiological, chemical and carbon dating applications. The biological transformations of organic carbon parallel the transformations of energy for the most part, from its initial fixation by plants to its eventual release during respiration. Isotopic transfers through the biosphere can therefore be compared with energy and mass transfers. Two additional factors contribute to the desirability of using isotopic tracers: the high sensitivity with which they can be detected, and the fact that isotopes of an element behave very similarly in chemical and biological processes. Small isotope effects do occur however, and in fact tracer applications of the sort discussed here would be impossible were this not the case.

Because isotope effects exist, isotopes cannot be considered ideal tracers of chemical and biological processes. Ideal behavior is closely approximated in some reactions, while in others the isotope ratio is a semiconservative property. The magnitude of isotope fractionation during chemical transformations can be critical in specific applications. When the fate of an isotopically labeled substance, such as eelgrass, is being studied in a precursor-product context, zero isotope effect is usually desirable. However, with the exception of isotopes created by the decay of other elements (and we are not concerned with such sources here), isotopic labels exist only because certain reactions and processes have significant isotope effects.

Photosynthesis is one such process. Marine phytoplankton, utilizing dissolved molecular  $\text{CO}_2$  as their major carbon source, fix  $^{12}\text{C}$  from 6 to 19‰ faster (relative to the absolute abundance of each isotope) than  $^{13}\text{C}$ . The exact amount of isotope fractionation depends on temperature, pH, the concentration of dissolved  $\text{CO}_2$ , and the physiological state of the cell (Deuser and co-workers, 1968). In cool temperate oceans a typical value is close to 15‰. If dissolved molecular  $\text{CO}_2$  provides the major carbon source for eelgrass too, the extent of isotope fractionation is considerably less -- about 2‰. The resulting difference between the isotope ratios of eelgrass and phytoplankton -- about 13‰ -- is sufficiently large to distinguish the two plant types and derivatives of each. Similarly, the sediments and animals of an eelgrass bed or other environment receiving organic inputs from both plant types should have an intermediate  $^{13}\text{C}/^{12}\text{C}$  ratio given by the mixing equation:

$$R = R_e f + R_p (1-f)$$

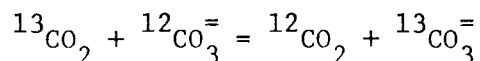
where  $R_e$  is the  $^{13}\text{C}/^{12}\text{C}$  ratio of eelgrass,  $R_p$  is the  $^{13}\text{C}/^{12}\text{C}$  ratio of phytoplankton, and  $f$  is the fraction eelgrass carbon in the sample. This ideal mixing equation will be realized if no other organic inputs are present and if isotope ratios are conserved in the mixture.

Not surprisingly, the real world defies such simplistic approximations. Plant isotope ratios vary according to external and internal conditions, and seagrass beds receive organic inputs from sources other

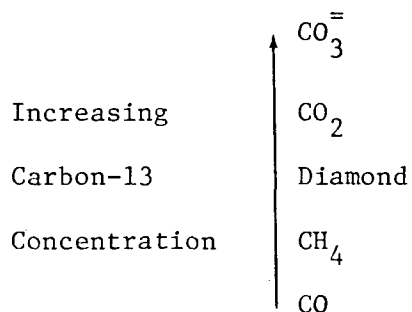
than just seagrass and phytoplankton. Post-photosynthetic metabolism in both plants and animals fractionates isotopes at many steps, making biomass a mixed bag of isotopically heterogeneous substances. Animals can also show net isotope enrichment relative to plants if isotope fractionation occurs in pathways leading to the uptake or loss of organic carbon.

### Isotope Fractionation in Chemical and Biochemical Systems

It is conventional to recognize two categories of isotope effects, those occurring in equilibrium exchange reactions and those associated with differential isotope reaction kinetics. For an isotopic exchange reaction of the sort



it is possible to estimate isotope fractionation factors from thermodynamic and spectroscopic data by methods pioneered by Urey (1947) and Bigeleisen and Mayer (1947). This has been done for a number of simple molecules containing carbon, yielding the following isotopic series (Craig, 1953):

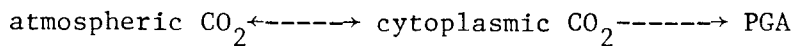


Lack of the appropriate spectroscopic data, particularly for large molecules and certainly for transition states, makes the exact calculation of isotope effects in most reactions impossible however. More frequently the observed isotope fractionation is used to deduce characteristics of the reaction mechanism and transition states. A common procedure in such an investigation is to examine isotope fractionation at specific positions of the reactant molecule. Large isotope effects may occur if bond rupture occurs at the isotopic atom (primary isotope effects), particularly if cleavage of the isotopic bond is rate determining. Isotopic substitution in substituent positions (secondary, etc. isotope effects) and in the solvent affect reaction kinetics much less.

More than a quarter of the literature concerning kinetic carbon isotope effects is probably devoted to decarboxylations. This is fortunate from the standpoint of understanding the effects of metabolism on biomass isotope ratios, since decarboxylation is the predominant means by which organic carbon is lost from the food web. In these reactions,  $^{12}\text{C}$  generally decarboxylates 20 to 40‰ faster than  $^{13}\text{C}$  (Dunn, 1977; Yankwich and Buddenbaum, 1967). Enzyme-catalyzed decarboxylations usually exhibit less isotope fractionation: for example, an enzyme isolated from *E. coli* shows a rate difference of 17‰ in the decarboxylation of glutamic acid (O'Leary, 1969) and the decarboxylation of oxalacetate by a *Micrococcus* enzyme shows no isotope effect (Seltzer *et al.*, 1959, cited in Simon and Palm, 1966). Enzymatic stabilization of the transition state and reduction of the activation energy required for C-C bond rupture probably contributes to the suppression of isotope effects.

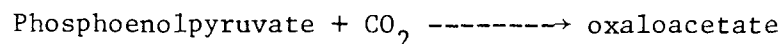
Isotope fractionation during respiratory decarboxylation is dramatically reduced in living systems, a phenomenon which will be further discussed in Chapter 2. If this were not the case, the approximately 13‰ difference in  $^{13}\text{C}/^{12}\text{C}$  ratios which separates eelgrass and phytoplankton would be completely useless as an isotopic tracer in food web studies.

Because of its importance to geochemistry and plant physiology, the isotope fractionation associated with photosynthetic carbon fixation has also received considerable attention. Craig (1953) was the first to attempt a large scale survey of plant  $^{13}\text{C}/^{12}\text{C}$  ratios, and with one exception (a grass) his samples of land plants displayed between 15‰ and 21‰ depletion in  $^{13}\text{C}$  compared to atmospheric  $\text{CO}_2$ . Park and Epstein (1960) developed a model which explained these fractionations in terms of the combined isotope effects occurring in the sequence



where PGA is 3-phosphoglyceric acid, a photosynthetic product in the so-called C-3 plants. This reaction is catalyzed by ribulose 1,5 diphosphate carboxylase. The model has been elaborated and extended to explain isotope fractionation in marine phytoplankton (Abelson and Hoering, 1961; Degens *et al.*, 1968; Deuser *et al.*, 1968).

However, plants utilizing the Hatch-Slack (1970) photosynthetic pathway (sometimes called C-4, Kranz-type, low compensation, tropical, "L", high  $^{13}\text{C}$ , and  $\beta$ -carboxylation plants) initially fix  $\text{CO}_2$  by a different mechanism:





and are depleted in  $^{13}\text{C}$  by only about 4 to 13‰ compared to air (Smith and Epstein, 1971; Bender, 1968, 1971; Tregunna *et al.*, 1970; Troughton, 1972). There is an additional group of plants which utilize both the phosphoenolpyruvate carboxylase and ribulose 1,5 diphosphate carboxylase photosynthetic pathways, and these CAM plants span most of the range of both C-3 and C-4 plants (Bender, 1971; Lerman and Queiroz, 1974). On the basis of isotopic evidence (Smith and Epstein, 1971; Craig, 1953; Wickman, 1952, Parker and Calder, 1970), it appears likely that seagrasses utilize the PEP carboxylase route, although the usual ultrastructural characteristics of C-4 and CAM plants are not well developed in seagrasses (Doohan and Newcomb, 1976).

Subsequent to the fixation of carbon dioxide, the isotopes of carbon are variously distributed among intermediary metabolites and structural components of biomass. The  $^{13}\text{C}/^{12}\text{C}$  compositions of selected biochemical fractions have been examined by many authors. In general, it has been found that isotope distributions correlate with metabolic pathways, and that  $^{13}\text{C}$  tends to become concentrated in oxidized carbon atoms while  $^{12}\text{C}$  is concentrated in reduced carbon atoms. As yet there is no systematic understanding of intermolecular and intramolecular carbon isotope distributions and isotope effects however.

The situation is similar at the ecosystem level. Degens *et al.*, (1968) and Sackett *et al.* (1965) concluded that phytoplankton and zooplankton are nearly equivalent in  $^{13}\text{C}/^{12}\text{C}$  ratio, although small differences are suggested in their data. Isotope enrichment in the food web becomes quite evident in fishes and benthic animals (Williams and Gordon, 1970; Eadie, 1972; Fry, 1977).

Figure 1 presents most of the isotopic data obtained in this study (excluding POC) in the form of a frequency histogram. In the Bering Sea, where phytoplankton average  $-24.4$  ‰ depleted in  $^{13}\text{C}$  compared to a carbonate standard, the animals range from  $-25.7$  to  $-16.3$ . Details concerning the measurements will be discussed later, but for now it is sufficient to recognize that almost all Bering Sea animals are  $^{13}\text{C}$  enriched (more positive in  $\delta^{13}\text{C}$ ) compared to phytoplankton. Benthic animals are most  $^{13}\text{C}$  enriched, averaging almost  $6$  ‰ higher in  $^{13}\text{C}/^{12}\text{C}$  ratio than phytoplankton. The obvious conclusion is that animal metabolism tends to retain  $^{13}\text{C}$  while respiring  $^{12}\text{C}$  preferentially, and that food-webs therefore concentrate  $^{13}\text{C}$  in the higher trophic levels. Some applications of this semiconservative tracer behavior will be discussed in Chapter 2. Once the gross behavior of the isotopic tracer has been established, it will be possible to apply the tracer to the analysis of more complex systems such as the eelgrass beds of Izembek Lagoon. From Figure 1 it is apparent that the distribution of animal  $^{13}\text{C}/^{12}\text{C}$  ratios in this environment is significantly different than in the Bering Sea. The impact of eelgrass on animal  $^{13}\text{C}/^{12}\text{C}$  ratios will be discussed in Chapters 3 and 4.

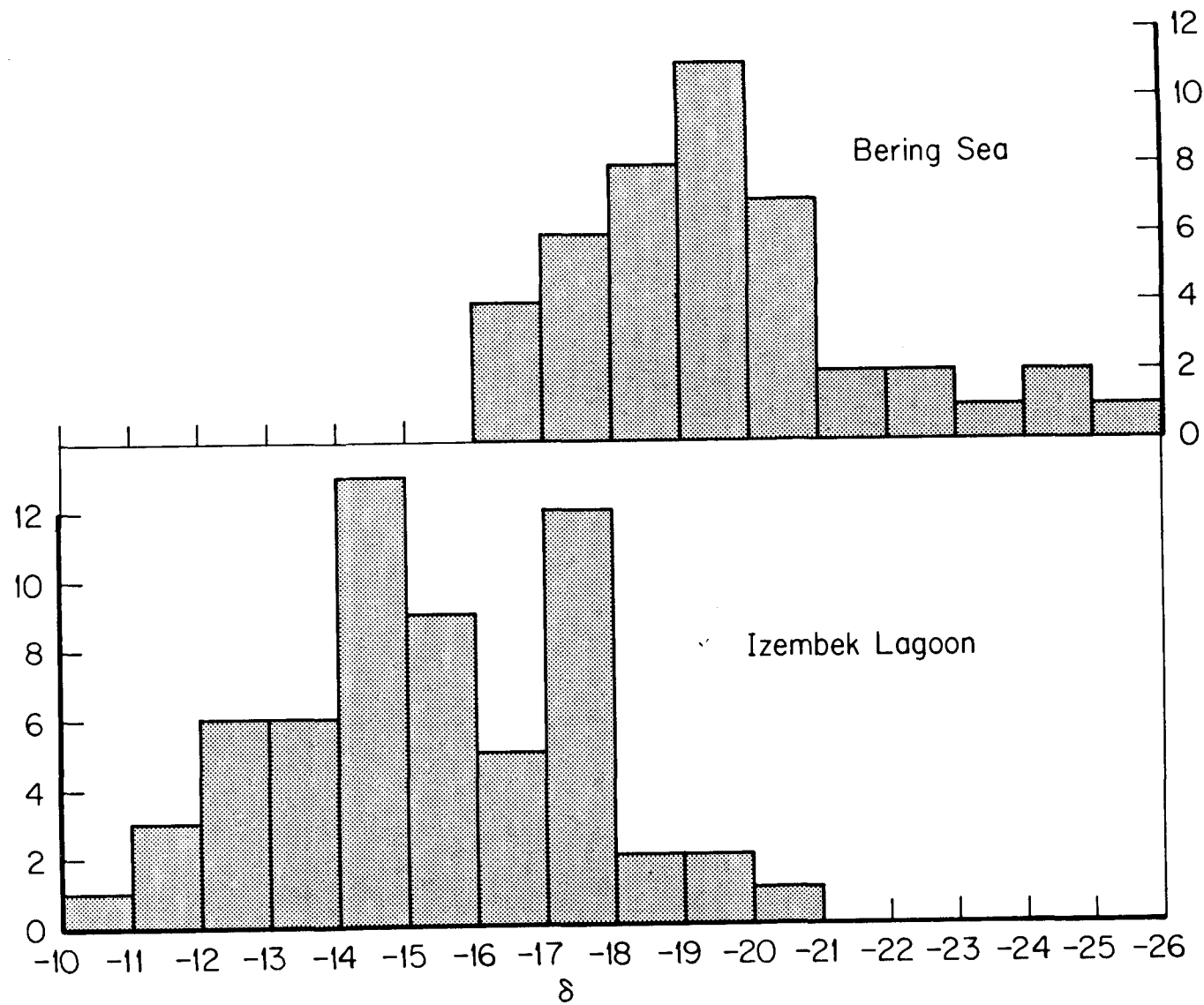


Figure 1. Frequency histogram of animal  $^{13}\text{C}/^{12}\text{C}$  ratios (as  $\delta^{13}\text{C}$ ) in the Bering Sea and Izembek Lagoon, Alaska.

CHAPTER 2. FOOD-WEB STRUCTURE AND FRACTIONATION OF CARBON ISOTOPES IN THE BERING SEA

ABSTRACT

Small variations in animal  $^{13}\text{C}/^{12}\text{C}$  ratios are observed among the biota of the Bering Sea. In general, higher trophic levels possess the highest  $^{13}\text{C}$  content, suggesting that respiratory isotope fractionation is primarily responsible for this isotopic enrichment. Both qualitative and quantitative aspects of carbon flow in the ecosystem can therefore be deduced from animal isotopic composition. Animal  $^{13}\text{C}/^{12}\text{C}$  ratios are also influenced by storage of isotopically light lipids, and means of accounting for this effect are offered.

## INTRODUCTION

The cycling of organic carbon is a cornerstone of life. Plants provide the major source of this carbon, packaged in a variety of biochemical forms. Animals assimilate these, rearrange their atoms, and oxidize the bulk of it back to  $\text{CO}_2$ . Both stable isotopes of carbon ( $^{12}\text{C}$  and  $^{13}\text{C}$ ) participate in these processes but at different specific rates. This isotope fractionation is evident when the  $^{13}\text{C}/^{12}\text{C}$  ratio of a metabolic product is different from that of its precursors or when the  $^{13}\text{C}/^{12}\text{C}$  ratio of an animal is different from that of its diet. Because metabolism at the biochemical level is similar in all animals, the effects of isotopic fractionation would logically accumulate in a food web, and the higher consumers would be expected to show the greatest isotopic enrichment relative to plants. If so, the distribution of carbon isotopes within a food web should provide a vehicle for examining the structure of a food web.

Because metabolism involves such a variety of reactions, any of which might potentially fractionate isotopes, biomass is quite heterogeneous in isotopic composition. The overall  $^{13}\text{C}/^{12}\text{C}$  ratio of an animal is not changed from that of the animal's diet unless the carbon exported from the animal is isotopically different than its food however. This can occur in two ways: either the reactions directly related to carbon export, such as respiration, may fractionate isotopes, or export processes may draw upon biochemical fractions already enriched in one isotope by previous reactions. This being the case, the  $^{13}\text{C}/^{12}\text{C}$  ratio of animals is likely to depend to some degree on

the peculiarities of their individual metabolisms as well as on their positions in the food web.

Isotope fractionation within a food web can be most readily studied in a food web based on a single type of primary producer. This enables the investigator to relate the isotopic content of animals directly to that of plants, without ambiguity regarding which plants are appropriate. The oceanic environment provides a good experimental system for such a study.

#### Oceanographic Setting

A suite of samples collected during the spring and early summer from the eastern Bering Sea supplied material for this study. With the exception of a few pelagic and bathypelagic specimens obtained south of the Pribiloff Islands, all samples came from the broad continental shelf region of the eastern Bering Sea.

The depth in this region is almost entirely less than 100 meters. The water column is therefore subject to mixing processes driven by the frequent storms in this area. Light varies annually from nearly complete darkness in winter to almost continuous light in mid-summer. Sea ice covers most of the shelf in winter and melts in the summer. The first algae blooms begin in late winter with the development of an under-ice diatom community, and are followed by larger planktonic blooms near the ice edge in the spring (McRoy and Goering, 1974). Summer plankton includes over 300 species, but large populations of a few species compose the great bulk of the plankton (Motoda and Minoda, 1974).

The benthic surface supports large numbers of infaunal filter and deposit feeders as well as other forms (Alton, 1964; Stoker, 1978). Proximity of the euphotic zone to the benthos facilitates the transfer and utilization of pelagic organisms and detritus by the benthos (Hargrave, 1973). The large demersal fisheries of the southeastern Bering Sea nevertheless depend primarily on pelagic sources for food (Alton, 1964). The most striking feature of the Bering Sea food web is its high production of animals in the upper trophic levels, including especially marine birds and mammals. With few exceptions, most notably the Pacific walrus, these also feed primarily on a pelagic food web.

Even a high latitude sea such as the Bering, which is dominated by a relatively small number of extremely abundant species, is difficult to characterize according to important inter-species relationships and interactions with the physical environment. One recourse is to lump the biota into general trophic groupings identified by representative organisms (Fig. 2).

#### METHODS

A variety of taxonomic groups and feeding types were captured for analysis. Plankton samples were collected in vertical and horizontal net tows (48-2000 micron mesh) and fractionated according to particle size using a graded series of Nitex screens. Phytoplankton were also obtained by filtering 2 liter water samples through glass fiber filters (Gelman A and Whatman GF/C precombusted 4 hours at 450°C) after preliminary removal of larger zooplankton. No attempt was made to remove

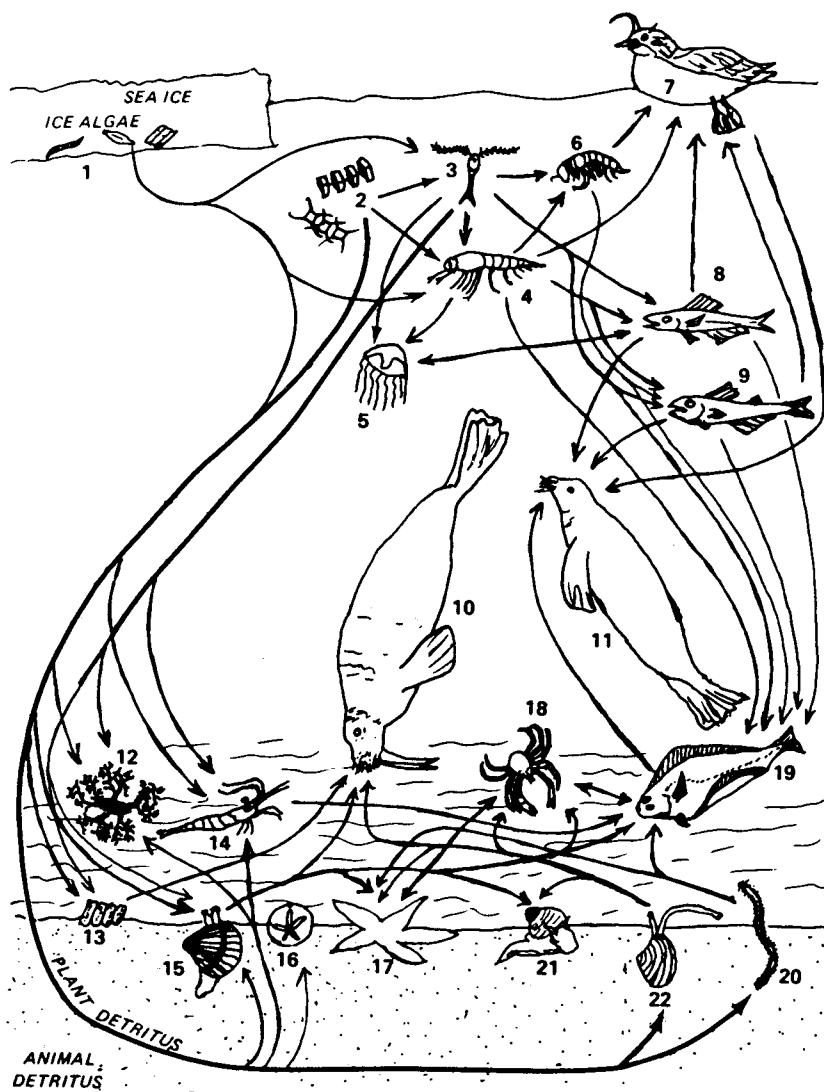


Figure 2. The Bering Sea food-web (a simplified version).

- |                          |                              |
|--------------------------|------------------------------|
| 1. ice algae             | 12. basket stars             |
| 2. phytoplankton         | 13. ascideans                |
| 3. copepods              | 14. shrimps                  |
| 4. mysids and euphausids | 15. filter feeding bivalves  |
| 5. medusae               | 16. sand dollars             |
| 6. hyperiid amphipods    | 17. sea stars                |
| 7. sea birds             | 18. crabs                    |
| 8. pelagic fishes        | 19. bottom feeding fishes    |
| 9. pelagic fishes        | 20. polychaetes              |
| 10. walrus               | 21. predatory gastropods     |
| 11. seals                | 22. deposit feeding bivalves |



smaller zooplankton and detritus from these samples. Macrozooplankton and micronekton were captured in midwater Tucker trawls. Van Veen grabs and otter trawls were used to obtain benthic animals. A horned puffin (*Fraterecula corniculata*) was found dead at Round Island (Walrus Island group) and a harbor seal (*Phoca vitulina*) was taken at Southeast Cape, St. Lawrence Island from a colony believed to be resident there (R. Elsner, pers. commun.).

Samples were frozen in the field and maintained frozen except for brief intervals during shipping and taxonomic identification until they could be freeze-dried or oven dried (about 60°C for one day). Calcareous shells were removed and other calcareous parts (spicules, bones, shells, etc.) dissolved with 10% H<sub>3</sub>PO<sub>4</sub> if required. Dried samples were then pulverized.

Subsamples weighing 10-40 mg were converted to CO<sub>2</sub> for mass spectrometry in a modified Leco induction furnace connected to a gas collection line (Parker *et al.*, 1972). Oils, lignin, and plastic of known isotopic compositions were combusted similarly for standardization. <sup>13</sup>C/<sup>12</sup>C ratios were determined using a six inch 60° sector McKinney-Nier type mass spectrometer (Nuclide 6-60-RMS). All measurements were corrected for oxygen isotope abundance (Craig, 1957) and reported as ‰ deviations from the <sup>13</sup>C/<sup>12</sup>C ratio of Pee Dee belemnite (PDB) according to Craig's (1953) definition:

$$\delta(\text{in } \text{‰}) = \frac{R(\text{sample}) - R(\text{PDB})}{R(\text{PDB})} \times 1000$$

where R is the <sup>13</sup>C/<sup>12</sup>C ratio. Standard deviations in replicate specimens averaged 0.3 ‰.

The  $^{13}\text{C}/^{12}\text{C}$  ratio of PDB standard is 0.0112372 (Craig, 1957). Most organic materials, including all organisms analyzed in this study, have lower  $^{13}\text{C}/^{12}\text{C}$  ratios than PDB and therefore have negative  $\delta$  values by definition. When two materials are compared, the one with the lower (more negative)  $\delta$  is referred to as isotopically "light" since it contains relatively less of the heavy isotope  $^{13}\text{C}$ .

To facilitate isotopic comparisons between animals, a simplistic model was developed to estimate the effects of varying biochemical composition on  $\delta$ . It is well known that the biochemical fractions composing biomass are isotopically non-uniform, hence their proportions would be expected to affect  $\delta$ . Proteins and lipids are notable among animals biochemical constituents for both their abundance and the isotopic discrepancy between them, and are therefore the two most important constituents to consider. Protein, because of its nitrogen content and the structure of intermediary metabolism, cannot be quantitatively synthesized from lipids by animals. The converse synthesis of lipid from protein does occur. This conversion proceeds through 3 and 2 carbon intermediates which also enter into most other processes of intermediary metabolism. Lipid biosynthesis is similar in nearly all animals, hence the extent of protein-lipid isotope fractionation would also be similar under comparable conditions of metabolism.

The isotopic difference between lipids and protein ( $D = \delta_{\text{protein}} - \delta_{\text{lipid}}$ ) ranges from 3.8 to 15.5 ‰ for marine plankton (Degens *et al.*, 1968b) and intermediate values have been reported for a variety of other organisms. The measured value of D appears to depend on the

polarity of the solvent used for lipid extraction, and at least for plants, on the total lipid content (Park and Epstein, 1961). DeNiro and Epstein (1977) have shown that the relative rates of pyruvate conversion to acetyl CoA vs. other products is the primary determinant of lipid  $^{13}\text{C}$  content, and that the isotope effect is temperature sensitive. A value of 6.0 is representative of literature values and was used for calculations in this study when more direct estimates were unavailable.

A convenient means of estimating biochemical composition was then needed. C/N ratios, determined on a Hewlett-Packard gas chromatographic CHN analyzer were used because the value of the C/N ratio is not affected by the presence of ash and residual  $\text{H}_3\text{PO}_4$  (from carbonate dissolution) in the samples. Ash-free dry weight protein and lipid content were estimated using the equations:

$$\% \text{ Protein} = 93/[0.246(\text{C/N}) + 0.225]$$

$$\% \text{ Lipid} = 93/[1 + (0.246 (\text{C/N}) - 0.775)^{-1}]$$

The derivation of these equations assumes that (1) lipid, protein, and a third category of biochemicals having the overall carbon content of carbohydrate are the only major constituents of biomass, (2) protein is the only major nitrogenous component of biomass, and (3) protein plus lipid content is constant at 93%. This constant was determined by fitting the general equation to lipid-C/N data from midwater fishes and crustacea (Fig. 3, upper curve) analyzed by Childress and Nygaard (1973, 1974).

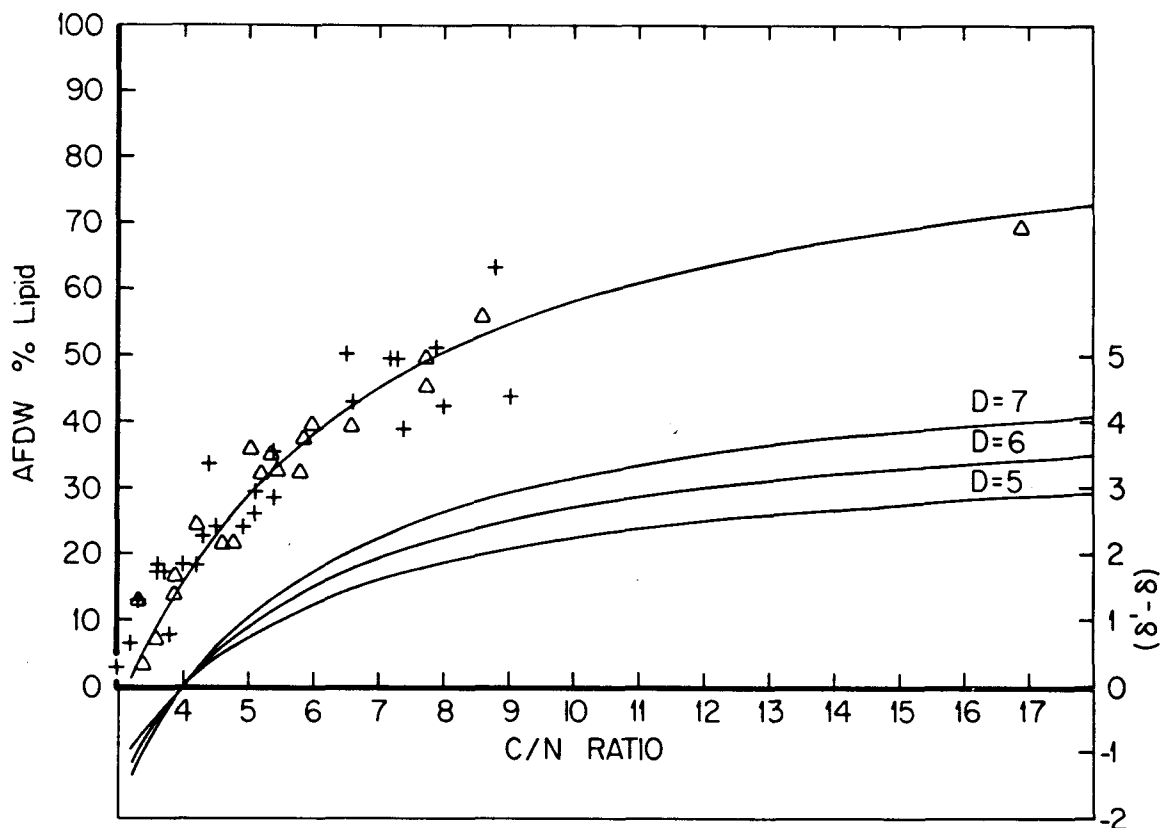


Figure 3. Relations used in the calculation of lipid-normalized  $^{13}C/^{12}C$  content of animal samples. Upper curve (use scale on left): lipid vs. C/N content. Data on mid-water fishes (+) and crustacea ( $\Delta$ ) reported by Childress and Nygaard (1973, 1974). Lower curves (use scale on right):  $^{13}C$  depletion in animal samples attributable to elevated lipid content.

A new parameter  $\delta'$ , called the lipid-normalized  $^{13}\text{C}$  content was then defined:

$$\delta' = \delta + D\left(-0.2068 + \frac{3.90}{1 + 287.1/L}\right)$$

where L is the AFDW % lipid content of the sample. Derivation of this equation assumes that  $\delta_{\text{protein}} = \delta_{\text{"carbohydrate"}}$ . Theoretically,  $\delta'$  is independent of the actual lipid content of the sample. The difference between  $\delta'$  and  $\delta$  is plotted against C/N ratio in the lower portion of Fig. 3 for 3 different values of D.

## RESULTS

Phytoplankton samples from the Bering Sea are isotopically similar ( $\delta = -24.5$  to  $-24.3$ ) to other cold water plankton (Sackett *et al.*, 1965; Williams and Gordon, 1970). As phytoplankton ultimately supply most of the organic input to the Bering Sea food web, their isotopic content represents a baseline against which the isotopic content of animals can be compared. With few exceptions, all animals have  $^{13}\text{C}/^{12}\text{C}$  ratios less than 8 ‰ different from that of the phytoplankton (Table 1). Furthermore, almost all animal tissues are isotopically heavy (i.e., relatively rich in  $^{13}\text{C}$ ) compared to the phytoplankton.

In general and extent of  $^{13}\text{C}$  enrichment correlates with known or probable food-web relations (Fig. 2). The smaller zooplankton (including mostly herbivorous calanoid copepods) are least  $^{13}\text{C}$  enriched ( $\delta = -24.5$  to  $-21.2$ ) while larger pelagic crustaceans ( $\delta = -19.7$  to  $-19.4$ ) and pelagic fishes ( $\delta = -24.1$  to  $-20.3$ ) are as much as 5 ‰ isotopically heavier than the phytoplankton. The puffin *Fratercula*

TABLE 1.  $^{13}\text{C}/^{12}\text{C}$  and C/N composition of Bering Sea organisms.  $^{13}\text{C}/^{12}\text{C}$  ratios are presented as  $\delta$  relative to PDB standard and as lipid-normalized  $\delta'$ , calculated from  $\delta$  and C/N ratios. The food-web relations of most of these animals are suggested in Figure 2. Individual measurements are listed in Appendix D.

Sample description	$\delta$	C/N	$\delta'$	Ref. # to Fig. 2
Plankton (average values)				
Predominantly phytoplankton	-24.4	5.3	-23.3	2
Zooplankton (mostly copepods)	-22.1	4.1	-22.0	3,5
Larger pelagic crustaceans				
<i>Parathemisto libellula</i>	-19.4	4.6	-18.8	6
<i>Neomysis rayii</i>	-19.4	4.1	-19.3	4
<i>Thysanoessa raschii</i>	-19.7	4.0	-19.7	4
Pelagic fishes				
<i>Mallotus villosus</i>	-20.3	3.7	-20.7	8
<i>Stenobrachius leucopsarus</i>	-24.1	9.2	-21.5	9
<i>Bathylagus stilbius schmidti</i>	-20.5	3.7	-20.9	9
Ascideans				
solitary ascidean	-20.8	5.3	-19.7	13
social ascidean	-20.9	4.3	-20.6	13
Benthic molluscs				
<i>Cyclocardia tertiolecta</i>	-19.0	4.8	-18.2	15
<i>Cardium cilliatum</i>	-19.5	5.5	-18.3	15
<i>Macoma calcarea</i>	-18.7	5.7	-17.3	22
<i>Yoldia limatulata</i>	-17.3	4.0	-17.3	22
<i>Natica clausa</i>	-16.6	3.9	-16.7	21
<i>Polinices pallida</i>	-16.3	4.1	-16.2	21
Benthic polychaetes				
<i>Nephtys caeca</i>	-17.8	3.8	-18.0	20
Benthic crustaceans				
<i>Orangon dalli</i>	-16.9	3.5	-17.6	14
<i>Erimacrus isenbeckii</i>	-16.6	3.2	-17.7	18
<i>Pandalus goniurus</i>	-17.5	3.9	-17.6	14
<i>Pandalus</i> sp.	-19.1	4.0	-19.1	14
<i>Hyas coarctatus</i>	-18.9	4.7	-18.2	18
<i>Chionoecetes bairdi</i>	-17.5	4.7	-16.8	18
Benthic echinoderms				
<i>Echinarachnius parma</i>	-20.5	5.8	-19.1	16
<i>Jorgonocephalus</i> sp.	-19.8	3.5	-20.5	12
<i>Evasterias</i> sp.	-18.3	4.6	-17.7	17

TABLE 1. Continued

Sample description	$\delta$	C/N	$\delta'$	Ref. # to Fig. 2
Bottom fishes				
<i>Lepidopsetta bilineata</i>	-19.9	3.7	-20.3	19
<i>Hippoglossus stenolepis</i>	-17.9	3.5	-18.6	19
<i>Podothecus</i> sp.	-17.5	3.6	-18.0	19
<i>Gymnoacanthus tricuspis</i>	-18.1	4.6	-17.5	19
Mammals and birds				
<i>Odobenus rosmarus</i> , bristle	-17.3	-	-	-
" " muscle	-16.9	3.6	-	-
" " blubber	-22.2	2264.0	-	-
" " total	-20.5	8.6	-18.1	10
<i>Phoca vitulina</i> , muscle	-19.4	3.2	-	-
" " blood	-19.9	3.4	-	-
" " liver	-20.0	3.4	-	-
" " blubber oil	-28.7	2077.0	-	-
" " total animal	-25.7	8.6	-23.3	11
<i>Fratercula corniculata</i>	-19.5	-	-	7

( $\delta = -19.5$ ) is also about 1 ‰ heavier than the nektonic and macrozooplanktonic animals upon which it feeds.

Benthic animals are isotopically heavier than pelagic ones for the most part. Least  $^{13}\text{C}$  enriched among them are ascideans ( $\delta = -20.8$  and  $-20.9$ ), which probably feed on the smaller plankton fractions and suspended detritus. Filter-feeding bivalves (*Cardium*,  $-19.5$ ; *Cyclocardia*,  $-19.0$ ) are also less  $^{13}\text{C}$  enriched than deposit or detritus feeders (*Macoma*,  $-18.7$ ; *Yoldia*,  $-17.3$ ). Isotopically heaviest of all animals analyzed are two gastropods (*Natica*,  $-16.6$ ; *Polinices*,  $-16.3$ ) which prey on bivalves. Benthic polychaetes, crustaceans, echinoderms, and fishes have  $\delta$  values ranging from  $-20.5$  to  $-16.6$ .

$\delta$  values of animals having similar diets usually fall close together. For example, the particle-feeding pelagic crustaceans *Neomysis* and *Thysanoessa* are 0.3 ‰ different, as are the predatory gastropods *Natica* and *Polinices*. Larger differences do occur, for example, the filter feeding bivalves, ascideans, and net zooplankton (predominantly calanoid copepods) span a range of about 3 ‰ even though their diets are presumably similar. Such large differences may be partially an artifact of sampling, but probably also reflect differences in animal feeding ecology and physiology. Incomplete knowledge about such factors hampers the interpretation of isotopic data, and it must also be realized that only a small fraction of the Bering Sea fauna was actually analyzed in this study. Spatial and temporal variability were also not examined. Despite these uncertainties it appears clear that  $^{13}\text{C}/^{12}\text{C}$  ratios are strongly related to food-web structure.



### Biochemical Composition of the Samples

As described in the methods section, C/N ratios can be used to estimate the lipid/protein content of animals. The lowest C/N ratios measured here approach the value for pure protein (2.9) while the highest values (>2000 for seal and walrus blubber oils) approach the value for pure lipid. Plankton have C/N ratios between 3.7 and 6.2 with the highest ratios occurring among the smaller size fractions of the net plankton samples. Shipboard microscopic observations revealed the presence of lipid globbules in some diatoms, copepods, nauplii, and pelagic eggs, confirming that these C/N values do indeed reflect an elevated lipid content. Most fishes and benthic invertebrates have C/N ratios between 3.3 and 5.0. The highest C/N ratio recorded for a whole animal was 9.2 for the lantern fish *Stenobrachius*, which has the rather high lipid content of 43% AFDW (Childress and Nygaard, 1973).

Because most organisms have C/N ratios between 3.3 and 5.1, lipid-normalized  $^{13}\text{C}$  content ( $\delta'$ ) usually differs from  $\delta$  by less than 1 ‰. Where the differences are larger,  $\delta'$  values group species with apparently similar feeding relations closer together in several cases. The range among pelagic planktivorous fishes, for example, shrinks from 3.6 to 0.8 ‰ and the difference between two filter feeding bivalves drops from 0.5 to 0.1 ‰. This is not always the case, however.

The accuracy of normalization would be increased by direct measurement of D for each species, and it is also likely that comparison of protein  $\delta$  values for different animals would yield the desired result

more easily and with less ambiguity. The approach used here is a first approximation.

#### DISCUSSION

Carbon loss from the food web occurs mainly through respiration. In laboratory experiments, the decarboxylation of organic molecules generally favors the loss of  $^{12}\text{C}$ , resulting in a corresponding increase in the  $^{13}\text{C}/^{12}\text{C}$  ratio of the remaining substrate material (Dunn, 1977). Biological respiration obeys the same kinetic principles but the observed isotope fractionation is generally smaller than laboratory results by perhaps an order of magnitude. The cause of this apparently lies in the organization of intermediary metabolism: molecules become committed to reaction along metabolic pathways such as the TCA cycle, allowing little opportunity for net isotope fractionation.

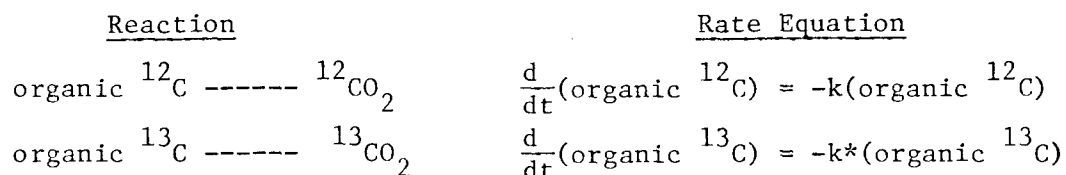
Unfortunately, few studies have simultaneously examined the stoichiometry of respiration and the isotopic relations between substrates and products. The best available estimates suggest that  $\text{CO}_2$  is enriched in  $^{12}\text{C}$  by less than 2 ‰ compared to respiratory substrates, except perhaps in bacteria. Because metabolism switches between substrates having  $\delta$ 's differing by as much as 40 ‰ (the range among different atom positions in amino acids, sugars, and lipids combined) the product  $\text{CO}_2$  can be grossly different from biomass or food on a short time basis. This effect has frequently been observed for living organisms (Jacobson *et al.*, 1970a,b; Lacroix *et al.*, 1973; Jacobson *et al.*, 1972; Smith, 1971; Mosora *et al.*, 1971, 1972; Krouse and Sasaki, 1968;

Smejkal *et al.*, 1971) and probably contributes most of the variability in measurements of respiratory isotope fractionation.

As mentioned above, bacteria may present an exception to this generalization. Anaerobic bacterial cultures may respire  $\text{CO}_2$  which is more than 5 ‰ enriched with  $^{12}\text{C}$  compared to food-substrate (Smejkal *et al.*, 1971; Kaplan and Rittenburg, 1964; Krouse and Sasaki, 1968). Barghoorn *et al.* (1977) have shown that aerobic bacterial cultures are sometimes notably enriched in  $^{13}\text{C}$  compared to their carbon sources, and Abelson and Hoering (1964) have observed  $^{13}\text{C}$  depletion in  $\text{CO}_2$  respired by bacteria. Bacterial isotope fractionation during respiration is probably the major cause of sediment  $^{13}\text{C}$  enrichment relative to POC in overlying waters (Eadie and Jeffrey, 1973; Sackett *et al.*, 1965). The lesser compartmentalization of metabolism and greater mixing of metabolites within a bacterium, or simply more complete oxidation of the food substrates could contribute larger isotope effects in bacterial respiration.

#### Modeling the Food-Web Distribution of Carbon Isotopes

Kinetic models are useful for examining isotope fractionation in chemical systems and can be applied to biological systems as well. Without going into detail about biochemical reaction sequences and mechanisms, we can start by assuming that metabolism oxidizes both stable carbon isotopes at rates proportional to their concentrations:



Integrating these rate equations and substituting  $a = k^*/k$  and  $R = {}^{13}\text{C}/{}^{12}\text{C}$  we obtain

$$\text{organic } R/R_0 = e^{-kt(a-1)} = f^{(a-1)}$$

where  $R_0$  is the initial  ${}^{13}\text{C}/{}^{12}\text{C}$  ratio of the organic material and  $f$  is the fraction of the original organic  ${}^{12}\text{C}$  remaining unoxidized. Because the natural abundance of  ${}^{13}\text{C}$  is low and "a" is close to unity,  $f$  is an excellent approximation for the fraction of the total organic carbon ( ${}^{12}\text{C} + {}^{13}\text{C}$ ) remaining. "a" is called the instantaneous isotope fractionation factor because it describes the isotopic differences between the remaining organic substrate and the  $\text{CO}_2$  being generated at any time during the reaction:

$$a = k^*/k = R(\text{CO}_2 \text{ being generated})/R \text{ (remaining organics)}$$

The exponential increase in  $R$  with respect to  $f$  stated above was proposed by Craig (1954) to predict isotope enrichments in decomposing materials such as wood. It is even more applicable to living organisms because the carbon atoms in living organisms undergo more thorough intermixing and recombination prior to their oxidation to  $\text{CO}_2$ .

In a food chain context however, animals constantly dilute old  ${}^{13}\text{C}$  enriched carbon with new food carbon and therefore show little  ${}^{13}\text{C}$  enrichment. Their  ${}^{13}\text{C}/{}^{12}\text{C}$  ratios may be conveniently expressed relative to the isotope ratios of their foods and metabolic products generated by metabolism, including respired  $\text{CO}_2$  excretions, molts, etc. Mass balance requires that the food input of an animal equal the product output for both isotopes:

$$^{12}\text{C}(\text{food}) = ^{12}\text{C}(\text{growth}) + ^{12}\text{C}(\text{CO}_2) + ^{12}\text{C}(\text{detritus})$$

$$^{13}\text{C}(\text{food}) = ^{13}\text{C}(\text{growth}) + ^{13}\text{C}(\text{CO}_2) + ^{13}\text{C}(\text{detritus})$$

Letting R be the  $^{13}\text{C}/^{12}\text{C}$  ratio, and representing the fractions of the  $^{12}\text{C}$  input converted into growth,  $\text{CO}_2$ , and organic detritus be represented by G, E, and D respectively, we get

$$\text{for } ^{12}\text{C}: \quad 1 = G + E + D$$

$$\text{for } ^{13}\text{C}: \quad 1 = \frac{R(\text{growth})}{R(\text{food})}G + \frac{R(\text{CO}_2)}{R(\text{food})}E + \frac{R(\text{detritus})}{R(\text{food})}D$$

The lower case letters g, e, and d will be used for the isotope fractionation factors  $R(\text{growth})/R(\text{food})$ ,  $R(\text{CO}_2)/R(\text{food})$ , and  $R(\text{detritus})/R(\text{food})$  respectively. Assuming that detritus includes some fraction F of fecal wastes having the same  $^{13}\text{C}/^{12}\text{C}$  ratio as food and some fraction (1-F) of metabolic wastes having the same  $^{13}\text{C}/^{12}\text{C}$  ratio as the consumer biomass, we can write

$$d = F + g(1-F)$$

From these assumptions and relations it follows that

$$g = \frac{1 - eY}{1 - Y} = \frac{1}{1 - Y(1-a)} \quad \text{where } Y = \frac{E}{1 - DF}$$

In these equations,  $e = R(\text{CO}_2)/R(\text{food})$  and  $a = R(\text{CO}_2)/R(\text{consumer}) = e/g$  provide two alternative means of expressing respiratory isotope fractionation. A third possibility,  $b = R(\text{CO}_2)/R(\text{respiratory substrate})$  would describe isotope fractionation at the biochemical level where the isotope fractionation originates. Because respiratory metabolism is similar in most animals, "b" is presumably also similar for most animals. "b" is more difficult to measure than "a" or "e", but its

value should lie between those of "a" and "e" and should approach "a" with thorough mixing of food and body carbon.

The factor  $Y = E/(1-DF)$  appearing in the above expressions is the rate of respiration relative to the rate of assimilation. This proportion is obviously important in determining how much  $^{13}\text{C}$  enrichment will occur in the food chain. Higher values of  $Y$  (and consequently lower growth efficiencies) should produce greater  $^{13}\text{C}$  enrichments. Despite the great effort which has been expended studying the energy and material budgets of animals, it remains difficult to evaluate this parameter. Variations in experimental procedure, artifacts induced by handling and laboratory rearing, environmental and ontogenetic changes, and differences between animals contribute to the measured variations in  $Y$ . Some values from the literature are as follows: 0.5-0.8 for pelagic crustaceans (Corner *et al.*, 1967; Lasker, 1966; Clutter and Thielacker, 1971; Dagg, 1977), 0.2-0.9 for harpacticoid copepods (Lasker *et al.*, 1970; Harris, 1973), 0.5 for an amphipod feeding on sediments (Hargrave, 1971), 0.3-0.4 for carnivorous polychaetes (Ivleva, 1970), 0.4 for a predatory snail and a sea star (Birkett, 1970), 0.6-1.0 for fishes (Phillips, 1969; Lasker, 1970; Pandian, 1967, 1970; Edwards *et al.*, 1972), and 0.7-0.8 for a herbivorous gastropod (Paine, 1971).

By comparing the isotope ratios of consumers with those of their foods under natural and laboratory conditions (Eadie, 1972; Jacobson and co-workers, 1970; Fry, 1977; Minson and co-workers, 1975; this study) it is possible to arrive at an approximate value of 1.0015 for  $g$ . Assuming that  $G = 20\%$ ,  $E = 50\%$ ,  $D = 30\%$ , and  $F = 67\%$  ( $Y = 0.63$ ),  $e$  and  $d$  then have values of 0.9991 and 1.0005 respectively. In terms of isotope

ratios, consumer biomass, respired  $\text{CO}_2$ , and detritus are 1.5, -0.9, and 0.5 ‰ enriched in  $^{13}\text{C}$  compared to food carbon (Fig. 4).

As carbon progresses through the food web from primary producers to higher consumers, these  $^{13}\text{C}$  enrichments will be passed on and accumulated in the higher consumers (Fig. 5). Because the isotope fractionation occurs mainly during respiration the total  $^{13}\text{C}$  enrichment of any animal relative to primary producers is determined by the cumulative respiration by the food chain at that point. The remaining flux to unrespired organic carbon is equivalent to the productivity of the food web, and can be calculated from isotope ratios (Fig. 6). Assuming that metabolism has the same stoichiometry for all animals in a strictly carnivorous linear food chain, the resulting  $^{13}\text{C}/^{12}\text{C}$  ratio and productivity of the  $n^{\text{th}}$  trophic level will be given by:

$$R = R_o \cdot g^n$$

$$\delta = (\delta_o + 1000)g^n - 1000 \approx \delta_o + n(g-1)1000$$

$$P = P_o G^n (1 + g^n R_o) / (1 + R_o) \approx P_o G^n$$

where the subscript "o" refers to primary production, and the effects of variable biochemical composition have been removed by normalization or other procedures as discussed.

The detritus produced by living animals is, of course, utilized by other heterotrophs, and may thereby be returned to the food web. The proportion of organic  $^{12}\text{C}$  productivity available as food to the  $n^{\text{th}}$  trophic level (compared to  $^{12}\text{C}$  primary productivity) is therefore  $(G + D)^{n-1}$ , of which  $G(G + D)^{n-1}$  is transformed into consumer biomass,

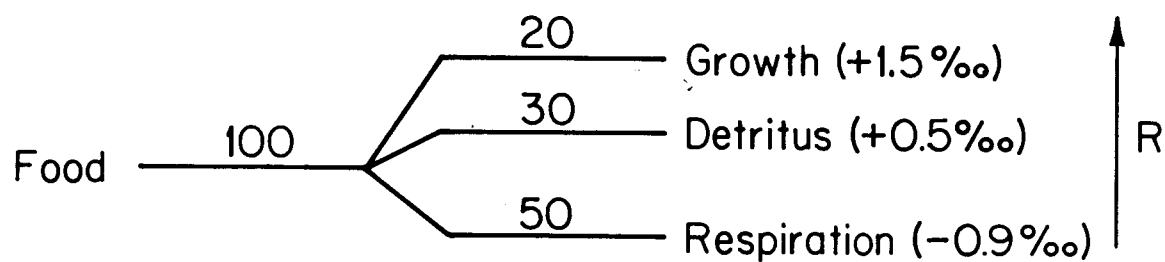


Figure 4. The partitioning of carbon and fractionation of carbon isotopes by animal metabolism assumed in marine food-web model. Here, 100 grams of food carbon is divided among growth, organic by-products, and respiration in a 2:3:5 ratio. The  $^{13}\text{C}$  enrichment in each product relative to food carbon is given in brackets, and is plotted on the vertical scale.



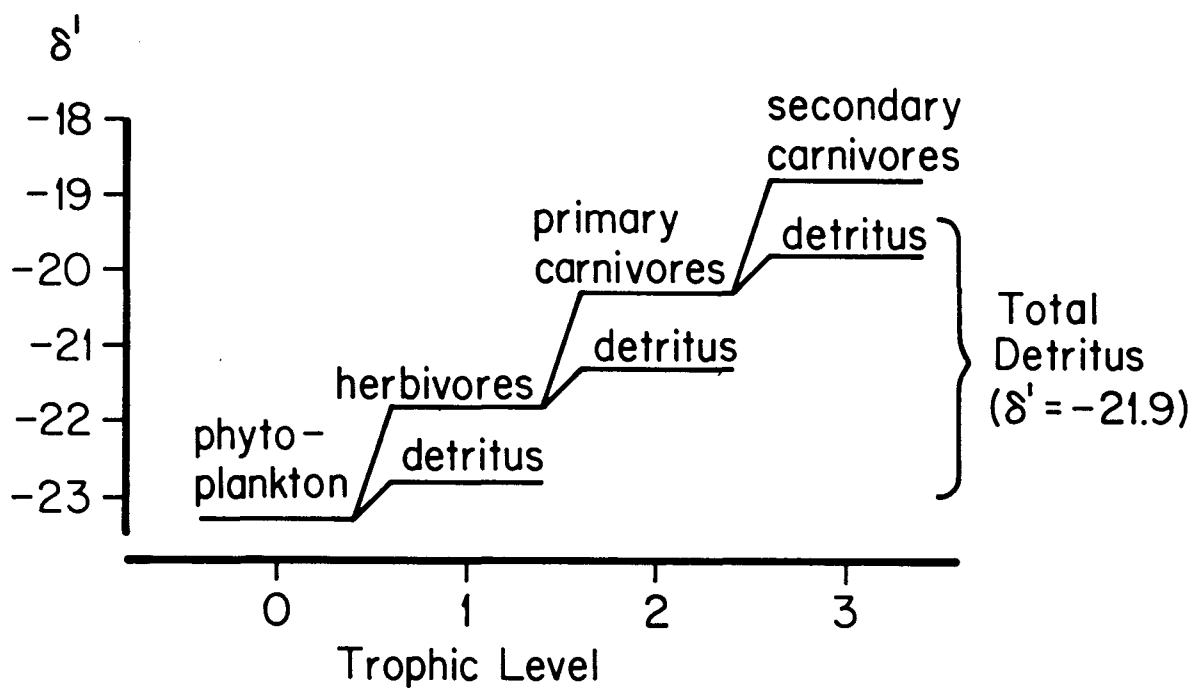


Figure 5. Progressive enrichment of  $^{13}\text{C}$  in a food-chain resulting from model calculations.

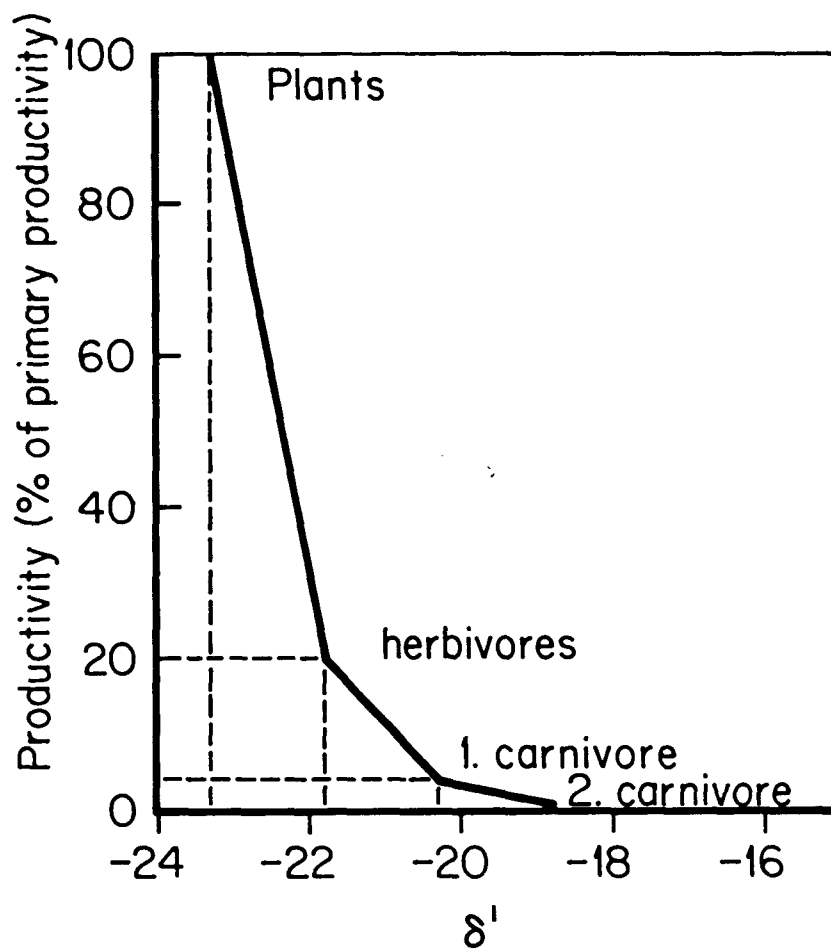


Figure 6. Simultaneous dependence of animal productivity and  $^{13}C/^{12}C$  ratio on animal trophic position in a model marine food-web.

$D(G + D)^{n-1}$  is transformed into detritus by the consumer, and  $E(G + D)^{n-1}$  is transformed into  $\text{CO}_2$ . Similar equations can be written for  $^{13}\text{C}$ .

The total organic  $^{12}\text{C}$  production (excluding primary producers) in a web of  $n$  steps is therefore distributed as follows:

$$\text{Heterotrophic biomass: } \sum_{k=1}^n G(G+D)^{k-1} = G/E \text{ as } n \rightarrow \infty$$

$$\text{Detritus: } \sum_{k=1}^n D(G+D)^{k-1} = D/E \text{ as } n \rightarrow \infty$$

$$\text{Total organics: } \sum_{k=1}^n (G+D)(G+D)^{k-1} = (1-E)/E \text{ as } n \rightarrow \infty$$

Similar expressions can be written for  $^{13}\text{C}$ .  $^{13}\text{C}/^{12}\text{C}$  ratios can be calculated by dividing the latter by the former:

	$R/R_0$	$\delta$	Total $^{12}\text{C}+^{13}\text{C}$ production
Heterotrophic biomass:	$g/e$	-21.0	40
Detritus:	$d/e$	-21.9	60
Total organic matter:	$\frac{1-eE}{e-eE}$	-21.5	100

Here it is assumed that  $\delta_0$  has the value -23.3 relative to PDB and production is expressed as a percentage of net primary production of the system. These values should be independent of which animals compose the food web and of their feeding habits. The notion of "trophic level" is simply a convenience referring to the number of organisms through which a parcel of carbon passes before reaching any one consumer (or detrital condition), and is independent of the route by which it gets there. Isaacs (1972, 1973) developed models for this sort of food web

and termed it "unstructured" to emphasize the fact that discrete trophic levels and feeding habits are not required of the model.

For animals feeding on more than one food, the calculations may assume that some fraction  $C_{ij}$  of the diet of consumer  $j$  comes from food  $i$ . Then there are three sets of variables in the system, the coefficients  $C_{ij}$ , the  $^{13}\text{C}/^{12}\text{C}$  ratios  $R_j$ , and the productivities  $P_j$ . The values of any one of these sets of variables can be calculated from the values of the other two by solving a series of simultaneous equations of the sort

$$R_j = g \sum_i R_i C_{ij}$$

$$P_j = G \sum_i P_i C_{ij} \cdot \frac{(1 + gR_i)}{(1 + R_i)}$$

for each animal in the system.

The unstructured food-web model provides a convenient framework for examining the interrelations between animal production, feeding habits, and isotope ratios. Values of  $\delta$  and production can be calculated for any point in a hypothetical food web, and these values compared against observed values. Large differences should imply inadequacies in the model, and suggest appropriate measures for correcting it. Even when minimal data on animal production and feeding habits exists, isotopic data can place upper limits on the potential production of any species and help to determine its trophic position. The model used can be as complex as desired and incorporate any available qualitative or quantitative data on animal abundance and feeding habits.

For example, it is generally thought that the bivalve *Yoldia* ( $\delta = -17.3$ ) feeds on detrital material, microbes, and or meiofauna. If detritus has a  $\delta$  of  $-21.9$ , as calculated, its consumers would presumably be  $g(-21.9 + 1000) - 1000 = -20.4$  which is  $^{13}\text{C}$  - poor compared to *Yoldia*. The following hypothetical food-chain would produce the observed  $\delta$  for *Yoldia*:

<u>Component:</u>	detritus	bacteria	protozoa & meiofauna	<i>Yoldia</i>
$\delta$ :	-21.9	-20.4	-19.0	-17.5
Production:	60	12	2.4	.48

The production figures above represent the maximum production of this food-chain since there are no competing sinks for detritus and other food-web intermediates. For each 0.48 g of the ecosystem carbon production harnessed by *Yoldia*, the food web must also produce 2.4 g carbon in the form of protozoa and meiofauna, 12 g bacterial-C, and 60 g detrital-C, as well as 100 g phytoplankton carbon. There are of course alternate sinks for carbon at each step in the food web, for example *Yoldia* competes with *Macoma* for food. "Production" might more accurately be called food-web production efficiency calculations. Thus for every 100 g phytoplankton carbon contributing to the production of *Yoldia*, only 0.48 g can actually be realized as *Yoldia* production.

Using such reasoning, the isotope ratios and production efficiencies for several hypothetical animal types were calculated for comparison with observed values (Fig. 7). For animals feeding on more than one food source it was assumed that the contribution of each source was

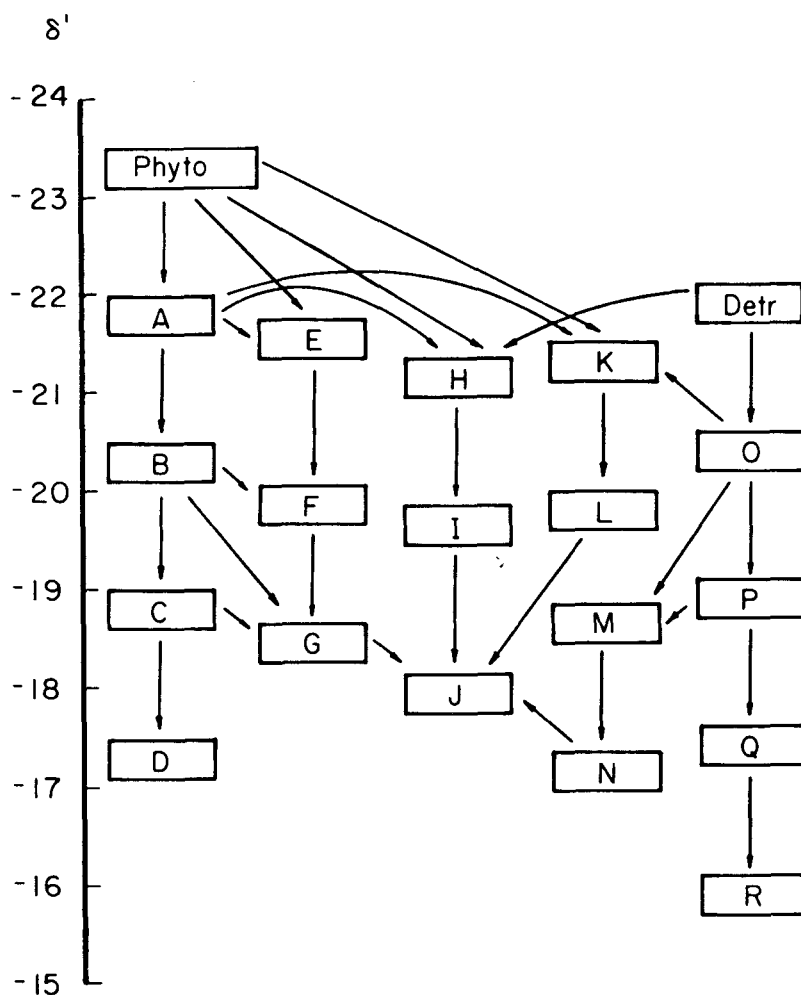


Figure 7. Carbon isotope ratio distribution in a model marine food-web. The efficiency with which the ecosystem produces each component from phytoplankton carbon are as follows:

Component	Production efficiency	Component	Production efficiency
PHYTOPLANKTON	100.0	J Generalized predator	3.0
A Herbivore	20.0	K Particle feeder #2	26.4
B Primary carnivore	4.0	L Benthic feeder #1	5.3
C Secondary carnivore	0.8	M Deposit feeder #1	2.9
D Tertiary carnivore	0.2	N Benthic predator #2	0.6
E Plankton feeder	24.0	Detritus	60.0
F Pelagic predator #1	5.6	O Bacteria	20.4
G Pelagic predator #2	2.1	P Meiofauna	2.4
H Particle feeder #1	36.0	Q Deposit feeder #2	0.5
I Predator	7.2	R Benthic predator #3	0.1

proportional to the production efficiency of that food. This strongly biases animal diets in favor of the shortest possible food webs. Different assumptions about feeding habits and the fate of ingested carbon change the calculated efficiency and  $\delta$  values somewhat, but the pattern which emerges is substantially unchanged. Higher predators have higher  $^{13}\text{C}/^{12}\text{C}$  ratios, and recycling of detritus by the food web raises the  $^{13}\text{C}$  content of the recipients of recycled detrital carbon, particularly if bacteria, protozoa, etc. process the detritus prior to its utilization by metazoa. Also, if bacteria fractionate carbon isotopes more strongly than other organisms, then the food web can be considerably more efficient than indicated and still produce the same distribution of isotopes.

Comparing the model against the isotope distribution in Bering Sea organisms, it appears that the benthic food web must rely heavily on detritus, and/or animal material for its input, and that microbial intermediates must play an important role in the recycling of detritus. Deposit-feeding animals naturally utilize detritus and small heterotrophs animals more extensively than do the suspension feeders, but no benthic suspension feeders appear to rely entirely on phytoplankton for food. The diets of benthic shrimps (*Crangon dalli* and *Pandalus* spp.) probably contain somewhat more animal material than the deposit feeding clams *Macoma calcarea* and *Yoldia limatulata*, which in turn contain more highly metabolized carbon than the filter feeders *Cylocardia tertiolecta*, *Cardium cilliatum*, or the ascideans. Benthic fishes evidently depend on the pelagic food web and/or benthic filter feeders to a greater degree than crabs or sea stars. In the pelagic system,

it appears that some of the larger pelagic crustaceans, including euphausiids and mysids, consume significant quantities of animal material as well as phytoplankton, and that the pelagic food web includes more major steps than simply phytoplankton-zooplankton-fish.

It also appears that some animals fractionate isotopes more or less than others. Some, such as the seal *Phoca vitulina* and some fishes, even appear to be  $^{13}\text{C}$  depleted compared to their probable prey. For the most part these apparent anomalies occur among animals with high lipid content so it is possible that the  $^{13}\text{C}$  normalization of biochemical variability was inadequate. Many of the problems of the model undoubtedly derive from its assumptions of identical partitioning of carbon by animal metabolism for all animals, as well as uniform fractionation of carbon isotopes.

Some factors which may contribute to the observed  $^{13}\text{C}$  enrichment in the benthos include the fact that most of the benthic animals sampled are relatively long lived and probably have lower growth efficiencies than pelagic animals sampled. Also, since sampling was carried out in the spring, it is possible that winter carbon derived more from detritus and animals still made up most of their bodies, even though diets at the time of sampling would likely contain more phytoplankton and pelagic organisms. Furthermore, plankton available as food to the benthos during early spring may be relatively depleted of isotopically light lipids due to respiration at depth (Lee, 1974; Conover and Corner, 1968) and a greater tendency to sink. The benthos also tends to return its  $^{12}\text{C}$  to the pelagic system in the form of lipid-rich eggs and larvae while retaining  $^{13}\text{C}$  in the lipid-poor parent. Biochemical effects such



as these should not affect the distribution of  $\delta'$  in the food web however.

Phytoplankton  $^{13}\text{C}/^{12}\text{C}$  ratios may also show seasonal dependence due to changes in temperature and  $\text{CO}_2$  system conditions (Deuser *et al.*, 1968; Degens *et al.*, 1968a), biochemical changes in the algae related to nutrient availability (Werner, 1970; Pugh, 1975; Strickland *et al.*, 1969), and perhaps other physiological and ecological factors.

#### CONCLUSIONS

Isotopic results obtained here extend the coverage for the world's oceans and food webs, but retain the basic patterns reported elsewhere. The data of Degens *et al.* (1968b), Eadie (1972), and Sackett *et al.*, (1965) show samples containing predominantly zooplankton to be about 1 ‰ isotopically heavier than those containing predominantly phytoplankton. A similar figure appears likely from the data of Williams and Gordon (1970). The slightly larger difference observed in the Bering Sea may be related to techniques of separating phyto- from zooplankton, biochemical differences, or differences in plankton composition with respect to trophic levels.

Published data are sparse for other oceanic organisms. Williams and Gordon (1970) analyzed a suite of bathypelagic fishes and crustaceans, and these appear to be about 3 to 6 ‰  $^{13}\text{C}$  enriched compared to plankton. Eadie (1972), and Rafter (1973) give data for several antarctic animals, ranging in  $\delta$  from about -2 to +6 relative to plankton. Fry (1977) observed  $^{13}\text{C}$  enrichments in Texas nearshore fishes ranging from +1.6 to +6.5 relative to POC. In these studies, benthic animals

and large fishes show the greatest  $^{13}\text{C}$  enrichments, while planktonic animals are more similar to POC. The direction and magnitude of isotope enrichment is similar in all food webs studied, suggesting that the number of trophic levels and trophic organization are similar.

The phenomenon of metabolic isotope fractionation provided the key to this study. Fractionation affects the isotopes of other elements besides carbon, as well as various molecular species, and their distributions may provide insight into food-web structure and functioning. In some cases the effect of the food web on the tracer parameter may be most interesting in itself. For example, hydrocarbon transfers through the food web are poorly understood at present, largely because of the difficulty in measuring absolute concentrations exactly and relating absolute concentration to processes of uptake, degradation, and biosynthesis. Changes in the abundance of hydrocarbons with respect to other hydrocarbons may be more useful for pinpointing important metabolic sources and sinks or, conversely, identifying parts of the food web where certain hydrocarbons are neither synthesized nor degraded.

In this study, the distribution of  $^{13}\text{C}/^{12}\text{C}$  ratios lent support to the following assertions: (1) Most animals are fairly similar with respect to their effects on organic  $^{13}\text{C}/^{12}\text{C}$  ratios. These effects were grouped under two categories: those related to the storage of  $^{12}\text{C}$ -rich lipids, and those related to respiration. A few species apparently do not follow the general patterns of isotope fractionation however, and it may be interesting to find out why. (2) Detritus supplies much of the organic input to the Bering Sea benthos, but

bacteria and other microbes must process it prior to its utilization by macrofauna. Among the benthic molluscs, those recognized as deposit feeders utilize the detrital milieu more than those usually considered to be plankton feeders. Ascideans depend more on phytoplankton than do the benthic molluscs, but no benthic filter feeders utilize phytoplankton exclusively. (3) The food webs producing the macrobenthos are probably much less efficient than the pelagic food web for producing large animals. The high productivity of the Bering Sea macrobenthos must therefore result from a large proportion of the ecosystem productivity being channeled into the benthos. The inefficiency of the benthic food web is largely due to the requirement for microbial intermediates in the detrital food web and the stronger dependence on animal, rather than plant foods. An alternative interpretation of the isotopic data, dependent on the hypothetical capacity of bacteria to fractionate isotopes more strongly than other organisms, would place the efficiency of the benthic food web much higher, possibly as high as for the pelagic system. (4) Food webs are generally not more than five or six "trophic levels" long, probably because of the relatively small amounts of carbon available to such higher trophic levels.

As yet the techniques for interpreting isotopic data are rather tentative, but as the behavior of isotopes in biological systems becomes better understood, it may provide a very powerful tool. The information to be gained from isotopic data include estimations of the efficiency with which ecosystems can produce individual species and identification of species position within a food web.

CHAPTER 3. EELGRASS HELPS TO FEED A MARINE COMMUNITY:  
AN EVALUATION INCORPORATING NATURAL ISOTOPIC  
EVIDENCE

ABSTRACT

A stable isotope tracer method was used to distinguish between carbon derived from two plant sources -- eelgrass (*Zostera marina*) and phytoplankton -- in the tissues of animals inhabiting the eelgrass beds of Izembek Lagoon, Alaska. Isotopic data is also compared with food-web data obtained through gut content analysis and feeding observations. The two techniques complement and reinforce each other, and together they provide information on the sources of primary production in food-webs and the routes by which it is distributed to animals.

## INTRODUCTION

Macrophytes form conspicuous zones of high primary productivity in most shallow aquatic and marine environments. Various animals utilize the macrophytes or organic derivatives of the macrophytes (including detritus and animals) as food, but seldom is this the only major source of primary production available to the animal communities. This study examines the utilization of eelgrass, *Zostera marina* L., by the community inhabiting a subarctic marine lagoon, against the background of a generally plankton-based food-web. Stable carbon isotope ratios were used to identify the sources of carbon in the food-web.

The stable isotope tracer technique capitalizes on the observation that different plants have different ratios of the two stable carbon isotopes,  $^{12}\text{C}$  and  $^{13}\text{C}$ , and relies on the fact that animal  $^{13}\text{C}/^{12}\text{C}$  ratios depend primarily on the  $^{13}\text{C}/^{12}\text{C}$  ratios of their diets (Minson *et al.*, 1975; Degens *et al.*, 1968; Eadie, 1972; Fry, 1977). The  $^{13}\text{C}/^{12}\text{C}$  ratios of animals are consequently close to the weighted averages of the  $^{13}\text{C}/^{12}\text{C}$  ratios of the plants which contribute to their diets. This reasoning has been applied in recent studies by Haines (1976a,b), who questioned the dogma that organic detritus derived from *Spartina* supports most of the animal productivity of the Georgia salt marshes, and by Fry (1977), who examined both marine and terrestrial food-webs from an isotopic perspective.

The results of such studies illustrate some constraints which affect investigations of this type. First, the carbon inputs to the food-web must possess distinct isotopic labels. The greater the isotopic

difference between plants, the greater the sensitivity with which their derivatives can be distinguished. Second, in a study utilizing only one label parameter, such as the  $^{13}\text{C}/^{12}\text{C}$  ratio, two isotopically distinguishable plant types must be of preponderant importance in order to permit the unique determination of the contribution of each. Third, processes which alter the  $^{13}\text{C}/^{12}\text{C}$  ratio of organic materials should be identified and their magnitudes known, although it is sufficient to know the gross relationship between the isotope ratios of consumers and their foods.

These constraints were addressed through selection of an appropriate study site and concomitant studies of isotope fractionation by a model food-web (Chapter 2). The results of that investigation suggest that metabolic alterations of organic  $^{13}\text{C}/^{12}\text{C}$  ratios result primarily from processes common to all animals, and include two processes of particular significance. The first of these is the preferential respiration of  $^{12}\text{CO}_2$ , which leaves the remaining biomass enriched in  $^{13}\text{C}$ . As a result, consumers become  $^{13}\text{C}$  enriched compared to their foods, and this enrichment may be about 1.5 ‰ per trophic level. This increase in  $^{13}\text{C}/^{12}\text{C}$  ratio is cumulative in the food-web. Second is the metabolic redistribution of isotopes within organisms. Preferential accumulation of certain products (and destruction or loss of others) produces net isotope enrichment relative to food. Lipids are particularly notable for their variable contribution to biomass and for the isotopic difference which sets them apart from other major biochemicals. It was estimated that the accumulation of lipids lowers the overall  $^{13}\text{C}/^{12}\text{C}$  content of biomass by about 0.06 ‰ per 1% increase in body lipid content, regardless of

whether the lipid is synthesized *de novo* or acquired from food.

A procedure was developed to estimate animal lipid content from C/N ratios. The isotopic effects of lipid accumulation were thought to be more or less constant for all species and not cumulative within the food-web.

Izembek Lagoon, Alaska (Fig. 8) was selected as study site because it approximates the two-plant ecosystem unusually well. The lagoon measures approximately 41 by 3 to 12 km and communicates with the Bering Sea through 3 passes. Approximately 78% of its 218 km<sup>2</sup> area is intertidal, of which 68% is vegetated by eelgrass (Barsdate *et al.*, 1974). Eelgrass standing stocks average about 1200 g/m<sup>2</sup> or about 430 gC/m<sup>2</sup> and productivity is estimated at 1-8 gC/m<sup>2</sup> day. Apart from eelgrass, the other important source of organic carbon is thought to be phytoplankton and organic carbon (including phytoplankton) advected into the lagoon from the Bering Sea. Summer phytoplankton productivity within Izembek Lagoon is estimated at 0.079 gC/m<sup>2</sup>-day (McRoy *et al.*, 1972) assuming an average depth of 1 m. Assuming 68% of the lagoon volume is exchanged with the Bering Sea twice daily (Barsdate *et al.*, 1974) and that surface DOC and POC values measured "upstream" of Izembek near Unimak Pass can represent those found offshore of Izembek, gross carbon imports may include 0.82-2.58 g DOC/m<sup>2</sup>-day and 0.05-1.10 g POC/m<sup>2</sup>-day (Loder, 1971). Other sources of organic input not considered separately in the following analysis include macrophytic algae, terrestrial exports, and epiphytic and epibenthic microalgae. Seaweeds commonly found in the eelgrass meadows include *Chaetomorpha* sp., *Chorda fillum*, *Fucus* sp., and an unidentified filamentous green algae. The biomass of these algae

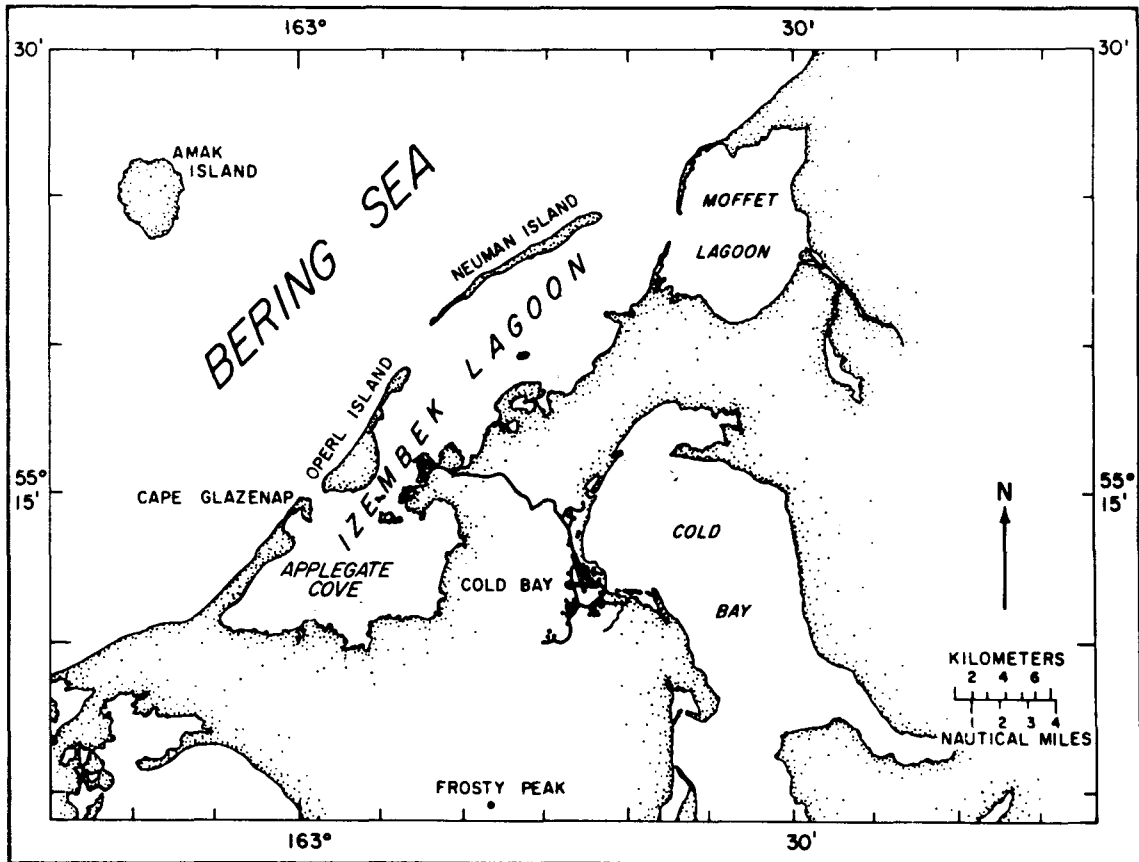


Figure 8. Izembek Lagoon, Alaska.



seldom exceeds 5% of the eelgrass biomass, although in other habitats algae may be more abundant. Terrestrial inputs are thought to be minor since streams are relatively small and few, and terrestrial habitats are not normally inundated by tidal waters. Several species (including amphipods, isopods, and fishes) are known to move between tundra fresh water systems and the lagoon. Benthic microalgae are thought to be unimportant within the eelgrass beds simply because of the strong shading under the eelgrass canopy, and are observable as a bottom felt only in rather low energy environments such as those occurring between the upper margin of eelgrass growth and the high tide line. Visible infestations of epiphytic microalgae occasionally occur in the eelgrass meadows, but for the most part, Izembek eelgrass is fairly clean compared to eelgrass observed in Southeast Alaska, Washington, Oregon, Massachusetts, and North Carolina. Of the various "third" plants contributing to Izembek Lagoon, these epiphytic algae may be the most important and need further investigation.

#### METHODS

Sampling in Izembek Lagoon was designed to provide quantitative data on animal abundance and food habits as well as specimens for isotopic analysis. Phytoplankton were collected by filtering 2 liters of sea water onto glass fiber pads (Whatman GF/C precombusted at 450°C for 4 hrs). Zooplankton were collected in vertical and horizontal net tows (216  $\mu$  net) and separated into size groups by graded screens. Fishes were captured by otter trawl, push net, and gill net. Shrimps and crabs were collected by hand or nets. Eelgrass epibiota were obtained in

epibenthic net towed and by collection within a hand-held screen basket attached to a 0.06 m<sup>2</sup> metal frame placed over the grass. Infauna were sampled using a 0.11 m<sup>2</sup> plastic core pushed at least 20 cm into the mud, and collected by hand after washing the core sample. Gut content observations were made with the aid of microscopes on animals immediately after capture, or later on animals which had been injected (if large) and pickled with 10% sea water formalin.

Specimens were converted to CO<sub>2</sub> for mass spectrometry in a modified Leco induction furnace mated to a vacuum line (Parker *et al.*, 1972). Calcareous spicules and shells were first dissolved with dilute H<sub>3</sub>PO<sub>4</sub> if required, so only organic carbon contributed to the sample. Oils, lignin, and plastic of known isotopic compositions were combusted similarly using the same oxygen source for internal standardization, and compared against isotopic results obtained on the standard Craig (1954) combustion line.

<sup>13</sup>C/<sup>12</sup>C ratios were determined using a 6 inch, 50° sector Neir-type mass spectrometer (model 6-60-RMS) equipped with dual inlet and magnetic valve assembly located at the University of Texas Marine Science Institute. Precision on duplicate samples was better than 1 part per thousand for most materials. All measurements were made relative to mean values of standards and converted to part per thousand deviations from the mean value of PDB standard according to the methods of Craig (1954). These deviations are expressed as δ, defined as

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{PDB}}} - 1 \right) \times 1000$$

where R is the  $^{13}\text{C}/^{12}\text{C}$  ratio.

The C/N ratios of most samples were measured using a Hewlett-Packard gas chromatographic CHN analyzer. Using equations derived from the elemental composition of midwater crustaceans and fish (Childress and Nygaard, 1973, 1974), the biochemical (lipid/protein) composition of animal samples was then estimated. The equations used, derived in a companion study of carbon isotope fractionation in the Bering Sea food-web (McConnaughey and McRoy, in prep.) are as follows:

$$\frac{L}{P} = (C/N) \times 0.2460 - 0.7753$$

$$L + P + 7 = 100$$

where L and P are the ash free dry weight percentages of lipid and protein in animal tissues respectively. This procedure is thought to be reasonably accurate for animal materials, but cannot be applied to plants or detritus due to their much higher contents of non-protein, non-lipid biochemicals.

The effects of protein/lipid content on  $^{13}\text{C}/^{12}\text{C}$  content was then estimated using the equation

$$\delta - \delta' = D(-0.2068 + \frac{3.90}{1 + 287.1/L})$$

where D, the  $^{13}\text{C}$  depletion in lipids compared to protein (in ‰), is estimated to have the value 6.0‰.

Primed symbols above indicate the values these variables will take if the C/N ratio is that of a "standard" animal, i.e. C/N = 4.0. Values of  $\delta'$  (calculated from C/N ratios and  $^{13}\text{C}/^{12}\text{C}$  ratios) are presumably more useful than  $\delta$  for isotopic comparisons between animals, since  $\delta'$  is relatively independent of animal biochemical composition.

## RESULTS AND DISCUSSION

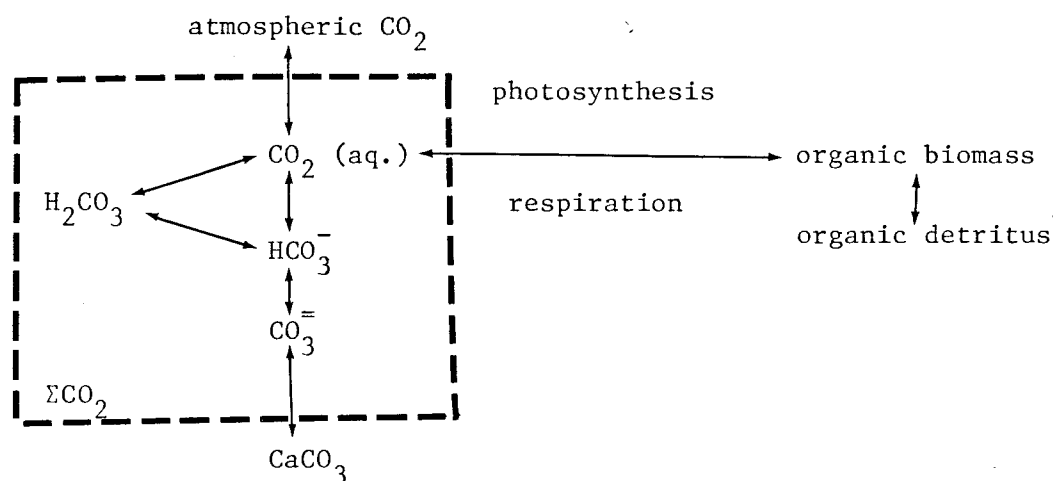
Primary Production

Eelgrass and phytoplankton produce most of the organic carbon which enters the food-webs of Izembek Lagoon. In order to study the mixing of carbon from these sources from an isotopic perspective, it is therefore particularly important to know the  $^{13}\text{C}/^{12}\text{C}$  ratios of these plants and have some appreciation for the factors which can influence plant  $^{13}\text{C}/^{12}\text{C}$  ratios.

Eelgrass leaves collected in Izembek Lagoon during the summer months have  $\delta$  values ranging from -11.6 (one sample, intertidal) to  $-7.9 \pm 0.1$  (2 samples, tidepool). The large subtidal eelgrass meadows have an intermediate value of  $-10.3 \pm 0.4$  (5 samples). These variations appear to reflect differences in plant environments. Eelgrass leaves from other bays gave the following  $\delta$  values: Intertidal plants from Cold Bay, Alaska -10.4, intertidal (winter) plants from Coos Bay, Oregon -12.6, and subtidal plants from El Infiernillo, Mexico -11.6. Roots and rhizomes are approximately 0.6 ‰ more negative than leaves. Previously recorded  $^{13}\text{C}/^{12}\text{C}$  ratios for eelgrass include measurements of  $\delta$  equal to -10.0 (Smith and Epstein, 1970, 1971), and -12.9 and -7.2 (calculated from Wickman, 1952).

These variations in eelgrass  $^{13}\text{C}/^{12}\text{C}$  ratio are similar in magnitude to variations observed in other plants (Craig, 1953; Sutton *et al.*, 1976; Lowdon and Dyck, 1974; Deevey and Stuiver, 1961; Fry, 1977). Physiological variables may be involved although it is also likely that differences in the  $^{13}\text{C}/^{12}\text{C}$  ratio of assimilated dissolved inorganic carbon

are equally important. Various mixes of sediment  $\text{CO}_2$ , atmospheric  $\text{CO}_2$ , aqueous  $\text{HCO}_3^-$ , and dissolved molecular  $\text{CO}_2$ , each bearing its own  $^{13}\text{C}/^{12}\text{C}$  ratio, may contribute to this variability. However, it is more likely that isotopic variations in dissolved molecular  $\text{CO}_2$ , which is probably the major carbon source, have greater impacts. This variation is a direct consequence of intense photosynthesis in environments where the  $\text{CO}_2$  reservoir is limited, and can be understood within the context of the overall  $\text{CO}_2$  system, as diagrammed below:



Under conditions of isotopic equilibrium between dissolved  $\text{CO}_2$  and air, the components of the  $\text{CO}_2$  system have the following approximate  $\delta$  values: atmospheric  $\text{CO}_2$ , -7;  $\text{CO}_2$ (aq.), -7;  $\text{HCO}_3^-$  and  $\text{CO}_3^{=}$ , 0 (Deuser and Degens, 1967; Wendt, 1968). Since  $\text{CO}_2$ (aq.) comprises less than 1% of the total dissolved  $\text{CO}_2$ ,  $\delta(\Sigma\text{CO}_2) \cong 0$ .

Plant photosynthesis preferentially consumes  $^{12}\text{CO}_2$  over  $^{13}\text{CO}_2$ , causing residual dissolved  $\text{CO}_2$  to become enriched in  $^{13}\text{C}$ . In the open ocean this enrichment is negligible because the  $\Sigma\text{CO}_2$  reservoir is

approximately 3 orders of magnitude larger than the daily photosynthetic uptake of carbon and 2 orders of magnitude larger than particulate plant carbon. In contrast, the  $\Sigma\text{CO}_2$  reservoir in Izembek Lagoon is at least an order of magnitude smaller than the plant biomass and of the same order as daily plant production. If the lagoon were a closed system (carbon exchange with the Bering Sea and with the atmosphere are neglected) and assuming that exchange between  $\text{CO}_2(\text{aq.})$ ,  $\text{HCO}_3^-$ , and  $\text{CO}_3^{=}$  is sufficiently rapid to maintain approximate isotopic equilibrium between the dissolved  $\text{CO}_2$  species, it would be possible to calculate the potential  $^{13}\text{C}$  enrichment which might result from intense photosynthesis using the equation (adapted from Craig, 1954):

$$\Delta = (\delta_o + 1000) f^{-\left(\frac{\alpha-1}{\alpha}\right)} - 1000 - \delta_o$$

Here,  $\Delta$  is the difference between initial and final  $\delta$  of  $\Sigma\text{CO}_2$ ,  $f$  is the fraction of the  $\Sigma\text{CO}_2$  remaining after photosynthesis,  $\alpha$  is the isotope fractionation factor for photosynthesis, and the subscripts  $o$  and  $f$  refer to initial and final conditions. For eelgrass ( $\delta = -10.3$ ), which is the predominant primary producer in Izembek Lagoon,  $\alpha$  can be calculated:

$$\alpha = \frac{\delta(\Sigma\text{CO}_2) + 1000}{\delta(\text{plants}) + 1000} = 1.0104$$

For values of  $f = 0.90$ ,  $0.80$ , and  $0.70$  (10, 20, and 30% of  $\Sigma\text{CO}_2$  is fixed by plants in one day), the  $\Sigma\text{CO}_2$  reservoir would attain  $^{13}\text{C}$  enrichments of 1.09, 2.30, and 3.68‰ respectively. Opening the model lagoon to exchange with the Bering Sea and the atmosphere will reduce these estimates

by an unknown, but probably substantial amount. Weber and Woodhead (1971) observed  $^{13}\text{C}$  enrichments in  $\Sigma\text{CO}_2$  of up to 3 ‰ over the course of a day in some tropical lagoons and Parker and Calder (1970) have measured slightly greater diurnal changes in a Texas lagoon. Plants should display half the total diurnal enrichment seen in the  $\Sigma\text{CO}_2$ .

Plant isotope ratios are influenced by the pH, temperature, and other characteristics of the water which affect the  $\text{CO}_2$  system, as well as by the  $\delta$  of  $\text{CO}_2(\text{aq.})$ . The reasons for this apparently lie in the kinetic isotope fractionation which accompanies  $\text{CO}_2$  transport across the plant-water interface (Dueser *et al.*, 1968), and to a lesser extent with the temperature dependence of equilibrium isotope fractionation between  $\text{CO}_2(\text{aq.})$  and  $\text{HCO}_3^-$  (Dueser and Degens, 1967; Wendt, 1968). These effects may be summarized as follows: plant  $^{13}\text{C}/^{12}\text{C}$  ratios will increase with increasing temperature and pH. Light absorption and photosynthesis in shallow basins raises the temperature and pH, and plant  $\delta$  values should be correspondingly more positive in a lagoon than in the open sea.

Within the lagoon, these processes produce maximal effects in densely vegetated tidepools, resulting in higher  $^{13}\text{C}/^{12}\text{C}$  ratios there. Kinetic isotope effects associated with  $\text{CO}_2$  transport into water and leaves would pull isotope ratios in the opposite direction.

Phytoplankton growing in the lagoon is affected by the same environmental parameters (temperature and the state of the dissolved  $\text{CO}_2$  system) as affects eelgrass. Compared to marine phytoplankton, lagoon phytoplankton are therefore likely to be somewhat enriched in  $^{13}\text{C}$ . Due to the ubiquitous presence of eelgrass detritus and animals in lagoon waters,

as well as the exchange of suspended particulates between the lagoon and the Bering Sea, the  $^{13}\text{C}/^{12}\text{C}$  ratio of lagoon POC (including phytoplankton) is rather variable (Fig. 9).

The major components of lagoon POC are microalgae grown in the lagoon, Bering Sea POC ( $\delta = -23$  to  $-24$ , average  $-23.3$  in the smaller size fractions), detritus derived largely from eelgrass ( $\delta = -10.3$ ), and small heterotrophs (ranging in  $\delta$  from  $-23$  to  $-10$ ). The larger POC size fractions have  $^{13}\text{C}/^{12}\text{C}$  ratios up to  $6$  ‰ higher than the smaller POC fractions even after removal of macroscopic detrital eelgrass particles. POC leaving the lagoon at ebb tide is also  $^{13}\text{C}$  enriched compared to POC entering on the flood tide. In flowing water is usually turbid and discolored and has an elevated  $^{13}\text{C}/^{12}\text{C}$  ratio compared to offshore waters, indicating that it is not completely free of lagoonal influences.

Assuming that lagoon dissolved  $\text{CO}_2$  is not more than  $4$  ‰ enriched in  $^{13}\text{C}$  compared to oceanic dissolved  $\text{CO}_2$  and that the doubling time of phytoplankton is less than one day, it is possible to calculate a maximum  $^{13}\text{C}$  enrichment of  $1$  ‰ for lagoon phytoplankton relative to Bering Sea phytoplankton. Comparisons between Izembek and Bering Sea filter feeders appear to give similar differences. Because subsequent calculations made in this paper depend on the  $^{13}\text{C}/^{12}\text{C}$  ratio adopted for lagoon phytoplankton, it was decided to assume a value of  $-22.3$  ( $1$  ‰ enriched in  $^{13}\text{C}$  compared to average Bering Sea phytoplankton) so that estimations of eelgrass contributions to food-webs would not be exaggerated by this uncertainty.



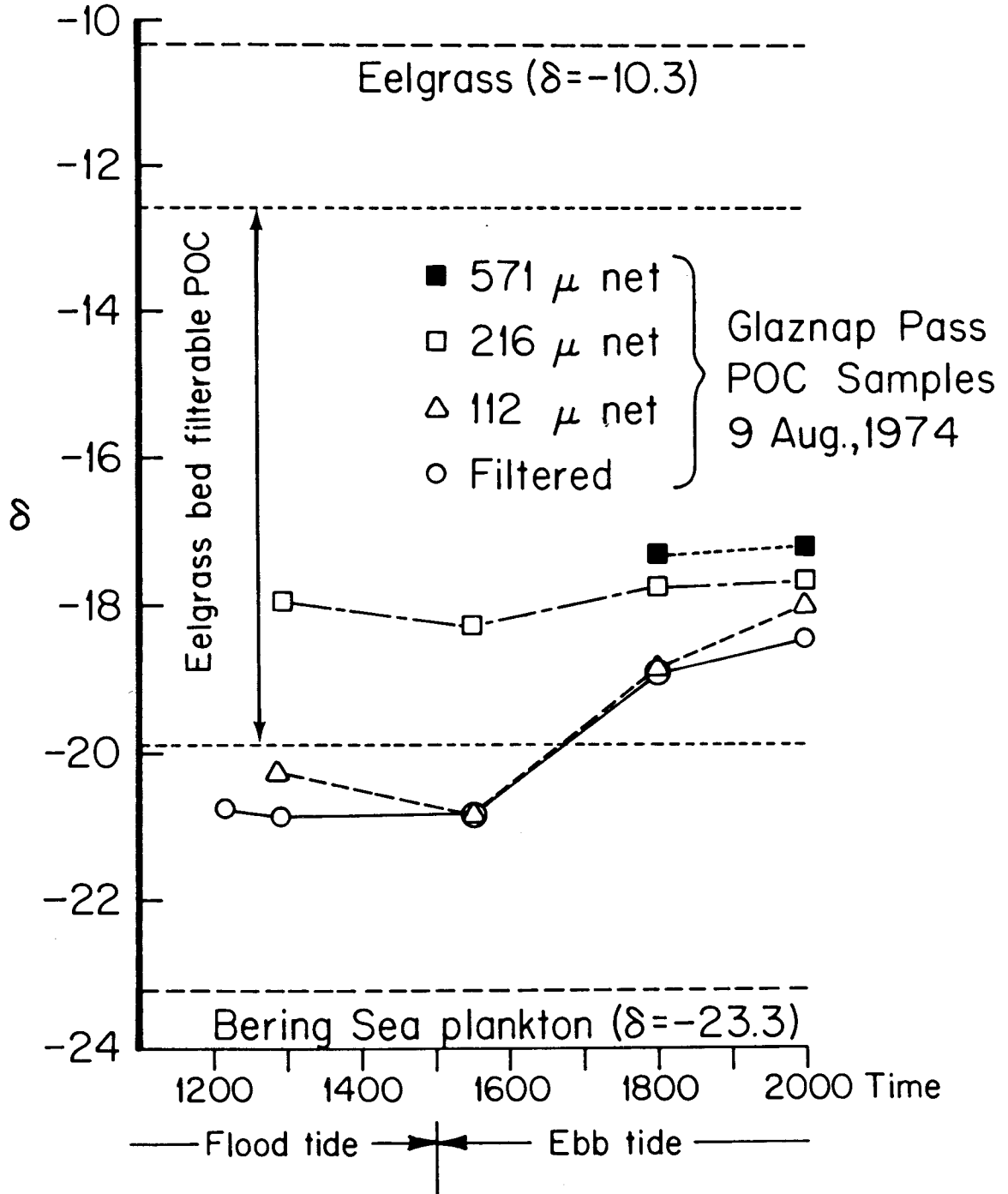


Figure 9.  $\delta^{13}C$  of Particulate Organic Carbon (POC) samples obtained inside Izembek Lagoon, at the mouth of the lagoon, and in offshore waters of the Bering Sea.

### Food-Web Effects

If the  $^{13}\text{C}/^{12}\text{C}$  ratios of organic matter were strictly conserved as it progresses through the food-web, then the  $^{13}\text{C}/^{12}\text{C}$  ratios of the animal community would be bounded by the isotopic contents of the primary producers.  $\delta$  values of lagoon animals should lie between -10.3 and -22.3. (The only exceptions would be recent immigrants from habitats with isotopically different primary producers, such as the open ocean or terrestrial regions.) Animal  $\delta$  values could be used to calculate the proportions of their body carbon derived from eelgrass and phytoplankton, using the equation:

$$\text{Maximum \% eelgrass carbon} = \frac{\delta'_p - \delta'_s}{\delta'_p - \delta'_e} \times 100$$

where the subscripts p, e, and s refer to phytoplankton, eelgrass, and the animal sample respectively.  $\delta'$  values are primed to indicate that the dependence of  $\delta$  on the biochemical composition of the samples has been taken into consideration. This calculation yields a maximum value for the contributions of eelgrass carbon because animal metabolism generally eliminates  $^{12}\text{C}$  in slight preference to  $^{13}\text{C}$ , causing biomass  $\delta$  values to become more positive as metabolism proceeds.

The total increase in  $^{13}\text{C}/^{12}\text{C}$  ratio occurring along a food-web between plant and consumer will be designated as  $I_n$ , where the subscript n identifies the plant i.e. eelgrass or phytoplankton. The above equation can then be modified to take food-web isotope fractionation into account:

$$\% \text{ eelgrass carbon} = \frac{(\delta'_p + I_p) - \delta'_s}{(\delta'_p + I_p) - (\delta'_e + I_e)} \times 100$$

If  $I_p \approx I_e$  this can be further simplified:

$$\% \text{ eelgrass carbon} = \frac{\delta'_p + I - \delta'_s}{\delta'_p - \delta'_e} \times 100$$

Values of  $I$  as high as 7 have been observed for benthic predators of the Bering Sea. In general, it appears that  $I$  increases up to 1.5 ‰ per trophic level, although the increase varies somewhat among animals. Since there is considerable uncertainty with regard to appropriate values of  $I_p$  and  $I_e$  as well as uncertainty associated with values of  $\delta'$ , estimates of % eelgrass carbon are probably no better than  $\pm 20\%$ .

#### Isotopic Comparisons Between Lagoon and Ocean Animals

Several animals found in Izembek Lagoon were also collected offshore in the Bering Sea. Assuming equal isotope enrichments in both plankton and eelgrass food-webs, the plant origins of their carbon were analyzed (Table 2). Isotopic evidence suggests that the halibut *Hippoglossus* in Table 2 had recently immigrated to the lagoon from the Bering Sea, and the assumption that its carbon was derived strictly from a combination of carbon from eelgrass and lagoon phytoplankton was wrong. For this reason, the calculations of % eelgrass carbon in animal tissues tend to underestimate the importance of eelgrass carbon to mobile animals. The calculations may underestimate the importance of eelgrass for another reason as well. With the partial exception of the seal *Phoca*, the above animals are all benthic feeders and therefore likely to depend largely on a detrital food-web in the offshore environment. The  $^{13}\text{C}$  enrichments in such food-webs are maximal. Lagoon detritus, in contrast to open sea

TABLE 2.  $^{13}\text{C}/^{12}\text{C}$  ratios (as  $\delta'$ ) of animals collected in Izembek Lagoon and in offshore waters:  $^{13}\text{C}$  enrichments attributable to food-web effects and eelgrass input to lagoon food-webs.

Species	Bering Sea $\delta'_A$	I*	Izembek Lagoon $\delta'_S$	Eelgrass carbon** %
<i>Nephtys caeca</i>	-18.1	5.2	-16.3	7
<i>Crangon dalli</i>	-17.6	5.7	-14.6	17
<i>Macoma</i> spp.	-17.3	6.0	-13.4	24
<i>Phoca vitulina</i>	-23.3	0.0	-19.7	20
<i>Lepidopsetta bilineata</i>	-19.9	3.4	-14.8	34
<i>Hippoglossus stenolepis</i>	-17.8	5.5	-18.1	-11 #
<i>Evasterias</i> sp.	-17.7	5.6	-13.7	25

$$* I = \delta'_A - (-23.3)$$

$$**\% E = \frac{\delta'_p + I - \delta'_s}{\delta'_p - \delta'_e} \times 100$$

# Sample variance or incorrect assumptions concerning carbon source could produce negative values. See text.

detritus, consists mostly of dead plant material and comparatively less fecal material and carrion. Particle sizes are larger and the food-webs which carry the detrital carbon to macrofauna can be comparatively short and efficient. The  $^{13}\text{C}$  enrichments (I) occurring in the detritus food-web of Izembek Lagoon would therefore be less than in the offshore environment. If I is effectively less by 1 ‰, this would raise the estimates of eelgrass carbon contribution to lagoonal samples by 8%.

But taking the calculations as they stand, it can be seen that eelgrass carbon composes 10 to 40% of a shrimp, a clam, a sea star, a seal, and a fish in Izembek Lagoon. These values are plausible when the habitats and food habits of the animals are considered. The clam *Macoma* and the shrimp *Crangon* both feed on bottom deposits and are known to consume eelgrass detritus. Both dominate their respective taxonomic classes with respect to number and biomass abundance in the lagoon, indicating that they successfully exploit the food resources in the lagoon. *Crangon*, moreover, migrates between the lagoon and the Bering Sea, so like the halibut, its body carbon may not be in isotopic "equilibrium" with lagoon foods.

The diet of the seal *Phoca* is not known with certainty. The sample analyzed, consisting of the umbilicus of a newborn pup, can be taken as representative of the mother's diet during the spring months. Otter trawls taken inside the lagoon among the feeding adults in the summer netted crangonid shrimps almost to the exclusion of everything else. Seals are known to feed in offshore waters as well as the lagoon.

The flatfish *Lepidopsetta* feeds primarily on polychaetes, gammarid amphipods, bivalves, and gastropods in Izembek Lagoon. It is therefore a probable recipient of eelgrass carbon. Most fish are thought to migrate offshore during the winter (R. Smith, personal communication) so the  $^{13}\text{C}/^{12}\text{C}$  ratio of the fish probably represents a transient value approaching isotopic "equilibrium" with lagoon foods.

The sea star *Evasterias* feeds mainly on bivalves, especially *Macoma*, hence it is reasonable that *Evasterias* contains the same percentage of eelgrass carbon as the clam. Other prey include polychaetes and the crab *Telmessus*. The diet of the polychaete *Nephtys* is not well known. Dissected specimens often contained sediment, but this polychaete also forages in the water column.

#### $^{13}\text{C}/^{12}\text{C}$ Ratios in the Lagoon Food-Web

Several other lagoon animals were also analyzed for  $^{13}\text{C}$  content (Table 3). Their  $^{13}\text{C}/^{12}\text{C}$  ratios span nearly the entire range from phytoplankton to eelgrass. Animal groupings based on gross habitat differences and functional morphology related to feeding show some differences in  $^{13}\text{C}$  content, but the differences are not pronounced, indicating that eelgrass and phytoplankton carbon become mixed early in the food-web by a wide variety of animals.

The lowest  $^{13}\text{C}/^{12}\text{C}$  ratios were of course found among plankton feeders, including a common sponge ( $\delta = -18.5$ ,  $\delta' = -17.6$ ) and a copepod ( $\delta = -18.8$ ) indigenous to the lagoon. Filter feeding bivalves had somewhat higher  $^{13}\text{C}/^{12}\text{C}$  ratios. Assuming again that lagoon phytoplankton

TABLE 3.  $^{13}\text{C}/^{12}\text{C}$  ratios (measured and corrected for lipid effects), C/N ratios, and feeding relations of animals collected in Izembek Lagoon.

	$\delta$	C/N	$\delta'$	Diet*
ZOOPLANKTON AND MICRONEKTON				
Copepod "A"	-18.8	-	-	-
Amphipod <i>Metopella nasuta</i>	-17.6	-	-	-
Copepods and amphipods	-17.4	-	-	-
Total zooplankton	-17.3	5.3	-16.2	-
Medusae <i>Goneionemus vertens</i>	-15.9	-	-	Z,A
Amphipod <i>Anisogammarus</i> sp.	-14.7	4.8	-13.9	-
Amphipod <i>Oradarea</i> sp.	-14.7	-	-	-
Amphipod <i>Calliopius laevisculus</i>	-14.5	-	-	-
Small copepods	-13.1	4.8	-12.3	-
Amphipod <i>Ampithoe</i> sp.	-12.4	-	-	-
EELGRASS EPIBIOTA				
Amphipod <i>Caprella alaskana</i>	-16.0	4.8	-15.2	Q,P,K,D,A
Bivalve <i>Turtonia minuta</i>	-15.6	4.3	-15.3	P,Q
Asteroid <i>Lepasterias</i> sp.	-13.9	4.6	-13.3	S,B
Gastropod <i>Littorina sitkana</i>	-12.7	5.4	-11.5	-
Gastropod <i>Margarites helycinus</i>	-12.8	4.3	-12.5	-
Eelgrass blade scrapings	-11.6	9.7	-	-
Gastropod <i>Lacuna variegata</i>	-10.7	4.9	- 9.9	Q,E,D
BENTHOS AND EPIBENTHOS				
Sponge	-18.5	4.9	-17.7	P
Polychaete <i>Maldanidae</i> sp.	-18.5	-	-	-
Polychaete <i>Rhynchospio</i> sp.	-17.6	-	-	-
Decapod <i>Pagurus hirsutiusculus</i>	-17.6	-	-	D,Q,W
Bivalve <i>Mya arenaria</i>	-16.6	5.5	-15.4	P,Z
Polychaete <i>Nephtys caeca</i>	-15.9	3.7	-16.3	R,D,Q
Polychaete <i>Arenicola glacialis</i>	-15.2	3.5	-15.9	R,J
Asteroid <i>Evasterias</i> sp.	-14.9	5.4	-13.7	B,T,W
Echiuroid <i>Echiurus echiurus alask.</i>	-14.6	3.5	-15.3	D,R
Bivalve <i>Macoma inconspicua</i>	-14.4	5.0	-13.5	D,P,Q,J,
Decapod <i>Telmessus chieragonus</i>	-12.2	3.4	-13.0	E,D,S,K,W,B
NEKTOBENTHOS AND NEKTON				
Fish <i>Hippoglossus stenolepis</i>	-17.4	3.7	-17.8	W,A,C
Fish <i>Pallasina barbata</i>	-17.1	3.7	-17.5	Z,A,C
Fish <i>Theragra chalcogramma</i>	-15.6	4.2	-15.4	A,C,V,Z
Fish <i>Hexagrammos octogrammus</i>	-15.1	3.8	-15.4	C,A,W,Z,S
Fish <i>Hexagrammos stelleri</i>	-14.8	3.7	-15.2	A,C,Q,N,Z,T
Fish <i>Lepidopsetta bilineata</i>	-14.4	3.7	-14.8	W,A,Z
Decapod <i>Crangon septemspinosa</i>	-14.1	3.4	-14.9	D,Q,R,G,A,K
Decapod <i>Crangon dalli</i>	-14.1	3.6	-14.6	D,R,Q,G,P
Fish <i>Pholis laeta</i>	-13.8	3.8	-14.0	C,A,Z,I,F
Fish <i>Myoxocephalus polyacanthocephalus</i>	-13.5	3.8	-13.7	W,G,I

TABLE 3. Continued

	$\delta$	C/N	$\delta'$	Diet*
MISCELLANEOUS ANIMALS OBTAINED OUTSIDE THE EELGRASS BEDS				
Bear <i>Ursus arctos</i>	-23.1	3.3	-24.1	L,O,T,F,H
Fish <i>Gasterosteus aculeatus</i>	-19.4	3.7	-19.8	L,A,Z
Seal <i>Phoca vitulina</i>	-19.4	3.8	-19.6	O,S,F
Fish <i>Pungitius pungitius</i>	-19.1	-	-	L,A,Z
Sea Otter <i>Enhydra lutris</i>	-17.2	3.1	-18.5	O,T
Gastropod <i>Acmæa testudinialis</i>	-17.1	-	-	K,Q
Barnacle <i>Balanus</i> sp.	-15.7	-	-	P,Z
Gastropod <i>Thais lamellosa</i>	-14.6	-	-	M
MACROPHYTES				
Algae <i>Chorda filum</i>	-14.3	22.6	-	-
Algae <i>Fucus</i> sp.	-14.1	62.7	-	-
Seagrass <i>Zostera marina</i>	-10.3	27.8	-	-

\*Symbols used in the description of animal diets:

A. Amphipods	M. <i>Balanus</i> sp.
B. Bivalves	N. Shrimp
C. <i>Caprella</i>	O. Offshore animals
D. Detritus	P. Planktonic algae
E. Eelgrass	Q. Epiphytic algae
F. Fish	R. Sediment
H. Humans	S. Snails
I. Isopods	T. <i>Telmessus</i>
J. Protozoa	V. Cumaceans
K. Seaweeds	W. Worms
L. Land plants and animals	Z. Zooplankton



( $\delta = -22.3$ ) and eelgrass ( $\delta = -10.3$ ) constitute the primary carbon sources, and comparing these filter feeders to their taxonomic and ecological counterparts in the Bering Sea, it appears that eelgrass carbon can make up as much as 15% of the total for these species. The value of  $I$  must be higher for eelgrass carbon than for phytoplankton carbon, since decomposers and microbes must act on eelgrass detritus before it becomes available to filter feeders.

Microalgal and eelgrass carbon quickly become further mixed in the food-web, making isotopic distinctions between planktonic and benthic food-webs impossible. Gammarid amphipods usually dominate the zooplankton samples, and these range in  $\delta$  from  $-12.4$  to  $-17.6$  among the species analyzed. One sample of copepods strongly dominated by a single benthic species had a  $\delta$  of  $-13.1$  ( $\delta' = -12.3$ ), indicating that phytoplankton contribute relatively little to their nutrition.

Eelgrass blades provide substratum for two very abundant filter feeders, the amphipod *Caprella alaskana* ( $\delta' = -15.2$ ) and the bivalve *Turtonia minuta* ( $\delta' = -15.3$ ). *Caprella* moves along the grass blades, stopping frequently to sweep diatoms, protozoa, and detritus from the water using the setations of its second antenna as a filter. *Caprella* less frequently combs epiphytic diatoms, detritus, fungi, filamentous algae, and detritus from the grass blades and occasionally catches small crustaceans. *Turtonia's* diet is not known, but is probably similar. Eelgrass carbon apparently contributes 30-40% to the diets of *Caprella* and *Turtonia*, the remainder coming from microalgae.

Zooplankton and eelgrass epibiota are favored foods for fishes and birds. Copepods furnish nutrition for most juvenile fishes and adults

of *Pallasina barbata* ( $\delta' = -17.5$ ) and *Pholis laeta* ( $\delta' = -14.0$ ) in particular. Various gammarid amphipods are eaten by almost every fish and bird feeding in Izembek Lagoon. Caprellids fall prey to most fishes and constitute the major prey of *Hexagrammos octogrammus* ( $\delta' = -15.8$ ) and *Pholis laeta*. *Turtonia* is an important food for juvenile sea stars ( $\delta' = -13.3$ ), sculpins, and several birds (Jones, 1976). The larger filter feeding bivalves fall prey to sea stars, fishes, gulls, and sea otters.

The isotopic implication that filter feeders (and their predators) receive substantial proportions of eelgrass carbon is realistic in light of the fact that lagoon POC also contains high proportions of eelgrass carbon (sometimes greater than 50% for filterable POC by  $^{13}\text{C}$  calculations). The variability between filter feeders undoubtedly reflects differences in their respective microhabitats, particle selection, and utilization of supplemental foods. Somewhat paradoxical is the implication that some filter feeders utilize eelgrass carbon as extensively as do benthic polychaetes and deposit feeding bivalves. While this may in part be an artifact of sampling locations and the life histories of individual species, it is probable that bacteria, fungi, protozoa, and micrometazoans subsisting primarily off of eelgrass detritus are readily available to and utilized by filter feeders. Their common association with detrital particles might also increase their catchability. The importance of plankton carbon to detritus feeders should not be underrated, since their densities can be equally high in regions where eelgrass carbon is unavailable.

Fenchel (1970, 1977) has drawn an analogy between phytoplankton and the microscopic organisms involved in the breakdown of detritus. Detritus, Fenchel argues, cannot be utilized directly by metazoans, but must first be processed by bacteria or fungi, followed (optionally) by protozoa, nematodes, and other micrometazoans. What is important here is the fact that these food-web intermediates are available to filter-feeders and are of comparable sizes and digestibility to phyto- and zooplankton.

Larger detrital particles can be ingested by particle feeders, deposit feeders, sediment ingestors, and even animals which are usually predacious. Through these modes of feeding, eelgrass carbon becomes available to a wide variety of amphipods, isopods, copepods, shrimps, crabs, bivalves, gastropods, polychaetes, echiuroids, and possibly some fishes. The predators on these species include most of the benthic and demersal fishes, sea stars, crabs, birds, and mammals inhabiting or frequenting Izembek Lagoon.

The detritus food-web encompasses such a wide variety of animals that tracing the flow of carbon by feeding observation studies gets hopelessly involved. Furthermore, the detrital foods are difficult to characterize in terms of plant origin and nutritional value. This leaves the isotope tracer method as the only simple, and perhaps the most accurate way for evaluating the contributions of eelgrass and phytoplankton to the productivity of detritus feeders and their predators.

The contributions of eelgrass detritus to animal productivity are undoubtedly highest in regions where detritus accumulates as beach

wracks or submerged mats. Classical detritus feeders inhabit these regions. Amphipods ( $\delta' = -12.4$  to  $-13.9$ ) are particularly abundant in beach deposits, while bivalves ( $\delta' = -13.5$ ) and polychaetes dominate intertidal areas of heavy eelgrass accumulation. Along the upper margin of the tidepool, in an area often overlain by mats of decaying eelgrass, *Macoma inconspicua* attains densities of up to  $1555 \pm 481$  per square meter (flesh dry weight =  $28 \text{ g m}^{-2}$ ) and *Arenicola* sp. reaches densities of  $29 \pm 43$  per square meter (dry weight  $4.0 \text{ g m}^{-2}$ ). These animals are present in the main eelgrass beds, but usually number fewer than 1 per square meter. Beach amphipods, *Macoma* and *Arenicola* are favored prey for shorebirds, which descend on the lagoon in such great numbers during migrations that their flocks resemble swarms of bees or smoke clouds when they fly from the tide flats.

#### Eelgrass Grazers

Three seagrass species are named after prominent grazers (turtlegrass, widgeon grass, and manatee grass), and eelgrass has sometimes been called goose grass, widgeon grass, and crab grass. Jones (1976) lists seven species of migratory birds as predominately or partially herbivorous on eelgrass in Izembek Lagoon. These include the black brant (*Branta bernicla orientalis*), lesser Canada goose (*Branta canadensis parvipes*), emperor goose (*Anser canagica*), mallard (*Anas platyrhynchos*) Pintail (*Anas acuta*), green-winged teal (*Anas carolinensis*), and the whistling swan (*Olor columbianus*). McRoy (1966, 1970b) estimated that black brant, which feeds almost exclusively on eelgrass,

consume 2% of the annual eelgrass crop, while Canada and Emperor geese bring the total to somewhat less than 3%. Some ducks feed only on the eelgrass seeds.

No mammals or fish are known to feed on eelgrass in Izembek Lagoon, and urchins are rare in the grass beds. Some amphipods have been observed to nibble at the edges of eelgrass blades and eelgrass has been seen in the guts of polychaetes, but the importance of herbivory to these species is not known. Three species of gastropod (*Lacuna variegata*, *Margarites helicinus*, and *Littorina sitkana*) commonly inhabit the eelgrass blades, and *Lacuna* can be seen rasping away eelgrass tissues and thereby damaging the leaves. While these snails consume a small fraction of the plant crop, they nevertheless provide a significant channel by which eelgrass carbon becomes available to other animals. Snails, especially *Lacuna*, make up approximately 30% of the diet of the fish *Microcottus sellaris*, which is one of the most abundant fishes in the eelgrass beds and is sometimes taken by birds and foxes due to its habit of remaining in the grass beds at low tide. Several other fishes prey on *Lacuna* and the other snails as does the crab *Telmessus cheiragonus* and several species of birds.

Perhaps the most significant, and certainly the most surprising herbivore is the crab *Telmessus cheiragonus*, which consumes some 37% live eelgrass and 22% eelgrass detritus (Table 4). Its herbivorous behavior was unknown until an effort was made to investigate its surprisingly high  $^{13}\text{C}/^{12}\text{C}$  ratio ( $\delta' = -13.0$ ). *Telmessus* captured in several other Alaskan bays supporting eelgrass seldom fed on the grass,

TABLE 4. Stomach contents of the crab *Telmessus chieragonus* collected in Izembek Lagoon (n = 44).

Food Item	% Volume
Eelgrass leaves	9.8
Eelgrass stems	15.3
Eelgrass roots and rhizomes	12.0
Eelgrass detritus	21.9
Seaweeds ( <i>Chaetomorpha</i> sp.)	11.0
Gastropods ( <i>Lacuna</i> and <i>Margarites</i> )	13.0
Polychaetes	6.1
Bivalves ( <i>Macoma</i> spp.)	4.0
<i>Caprella</i>	0.8
Asteroids	0.6
Sponges	0.5
Miscellaneous and unidentified	3.7
TOTAL	100.0

suggesting that eelgrass is not always a preferred food, but in Izembek Lagoon it is probably the predominant dietary item. The crab's preference for eelgrass stems and rhizomes may indicate a taste for the sweetest and juiciest portions of the plant.

Four species of crabs collected in the Bering Sea showed an average of 5.2 ‰  $^{13}\text{C}$  enrichment compared to the primary producers in the Bering Sea. If this is accepted as applicable to *Telmessus*, it would imply that 34% of *Telmessus* carbon derives from eelgrass. 5.2‰ is an unrealistically high estimate of food-web  $^{13}\text{C}$  enrichment however, since *Telmessus* feeds lower on the food-web than do the Bering Sea crabs. A more reasonable estimate of 3‰ leads to an estimate of 53% eelgrass carbon and 47% phytoplankton carbon in the tissues of *Telmessus*. A few percent must be subtracted from each of these figures because of the seaweeds (mainly *Chaetomorpha*) eaten by the crab. This estimate is consistent with stomach contents data, since the major prey of Izembek crabs (*Lacuna* and *Macoma* especially) are known to utilize eelgrass-derived carbon. However, it should be remembered that gut contents studies cannot accurately compare utilization of foods as different as eelgrass and clams. *Telmessus* does not assimilate eelgrass efficiently, as evidenced by the substantially undigested appearance of eelgrass chunks in the crabs' hindguts, and animal materials are digested more rapidly causing their relative volumetric importance to be underestimated.

*Telmessus* mobilizes considerable quantities of eelgrass carbon into the food-web. This crab is by far the most abundant brachyuran in Izembek

Lagoon, numbering approximately  $0.8 \pm 0.2$  (dry weight  $10.7 \pm 2.6$  g) per square meter in the intertidal eelgrass beds. Sea stars, several species of fishes, sea otters, foxes, brown bears, ravens, diving birds, waterfowl, sandpipers, and gulls prey on the crab. *Telmessus* often burries itself in the sand, at which times it may be trapped by marauding sea stars (*Evasterias* and *Lepasterias*). Congregations of several sea stars can sometimes be found sharring a hapless crab, surrounded by clouds of amphipods (especially *Metopella nasuta*) scavenging stray particles from the water. At low tide, ravens (*Corvus corax*) and gulls (*Larus glaucescens*) stalk the grass flats and sand bars searching for crabs as well as the invertebrates and fishes. Sometimes they will carry the quarry off to the tundra before eating it, but gulls usually flip a crab over on the spot and devour its insides and legs. The empty crab carapaces float like hundreds of tiny boats above the eelgrass beds as the tide rises on calm days. On one favorable low tide, 0.6 and 2.8% of the crabs on two grass beds were so consumed by gulls. This represents a transfer of carbon via crabs to gulls of about  $20 \text{ mg C/m}^2$ -day. This carbon is roughly half derived from eelgrass and half from phytoplankton. These fluxes amount to somewhat less than 1% of the rate of eelgrass production, but about 10% of the *in situ* rate of plankton production. Bird predation this intense occurs infrequently, but these crude calculations have important implications on the functioning of the Izembek ecosystem.



### Migratory Animals

A major value of Izembek Lagoon lies in the abundant stocks of plant and animal foods which it offers to migratory birds and other animals which exploit Izembek Lagoon on a transient or occasional basis. Such species include most of the fishes, all the birds, and all the mammals which feed in Izembek Lagoon. Can  $^{13}\text{C}/^{12}\text{C}$  ratios be useful in studying their feeding ecology while they utilize lagoon foods?

Immigrants arrive with  $^{13}\text{C}/^{12}\text{C}$  ratios significantly lower than typical lagoon fauna, whether they arrive from terrestrial or marine environments. As lagoon carbon accumulates in their tissues and dilutes or replaces outside carbon, tissue  $^{13}\text{C}/^{12}\text{C}$  ratios will increase in some complex manner. Data from several bird species (Table 5) illustrates this point.

The isotopic differences between bird muscle, liver, and fat tissues are interesting here. Lipids are normally at least 4 ‰ depleted in  $^{13}\text{C}$  compared to proteins, so the relatively high  $^{13}\text{C}$  content in bird fat compared to muscle stands out as anomalous. The obvious interpretation of these results is as follows: These birds, having arrived from terrestrial and/or marine habitats, initially had lower  $^{13}\text{C}/^{12}\text{C}$  ratios in all body tissues, particularly fat deposits. While feeding in Izembek Lagoon, the birds accumulate fat deposits to provide energy for their southern migrations. Fat tissues are therefore among the first to show pronounced  $^{13}\text{C}$  enrichment from the isotopically "heavy" lagoon carbon. The liver is one of the most metabolically active tissues in the body and is the site of most fat biosynthesis. This organ

TABLE 5.  $^{13}\text{C}/^{12}\text{C}$  ratios in the tissues of migratory birds feeding in Izembek Lagoon

Species		Tissue	$\delta^{13}\text{C}$	Diet
<i>Branta canadensis</i>	#1	muscle	-25.9	terrestrial
Canada goose	#1	fat	-28.3	plants,
	#1	liver	-24.2	eelgrass
	#2	muscle	-21.6	
	#3	muscle	-21.3	
		liver	-14.6	
<i>Philacte canagica</i>		muscle	-17.0	eelgrass
Emperor goose				terrestrial
				plants,
				invertebrates
<i>Anas acuta</i>		muscle	-17.8	eelgrass
Pintail duck		liver	-12.6	seeds,
				eelgrass
				epibiota
<i>Anas carolinensis</i>		muscle	-18.3	eelgrass seeds
Green-winged teal				and epibiota
<i>Polysticta stelleri</i>		muscle	-19.7	eelgrass
Steller's eider				epibiota
				other invertebrates
<i>Larus glaucescens</i>		muscle	-18.7	invertebrates,
Glaucous-winged gull		fat	-16.2	fish, carrion
Unidentified sandpiper*		muscle	-20.8	invertebrates
		fat	-22.5	

\*probably least sandpiper, *Calidris minutilla*, some of which nest in the Izembek area.

consequently shows the effects of new, heavy carbon also. Muscle  $^{13}\text{C}/^{12}\text{C}$  ratios change more slowly because the turnover rate for structural proteins is low, as is the rate of muscle growth during migrations. These results suggest that  $^{13}\text{C}/^{12}\text{C}$  ratios may prove useful for investigating not only the foods taken during migration, but also the phenomenon of metabolic turnover rates.

### CONCLUSIONS

Conventional techniques of food-web analysis can, in principle, supply all the necessary data for estimating the relative contributions of different plants to higher levels of the food-web. The data required include quantitative knowledge of what every animal eats and how efficiently it assimilates each food item. Normally much of these data are unavailable and only crude estimations fill in the gaps. These deficiencies are generally acute at lower trophic levels and especially among detritus feeders, which leaves any food-web model on uncertain foundations.

By capitalizing on the fact that different plants can have easily distinguishable  $^{13}\text{C}/^{12}\text{C}$  ratios, it is possible to examine the mixing of carbon from different plants in terms of isotopic mixing by the food-web. This approach has been used here. The stable isotope tracer methodology complements conventional techniques particularly well at the lower trophic levels where it is most badly needed. With the higher trophic levels the isotopic tracer method remains useful, but is less accurate due to the tendency of metabolism to alter the

isotopic composition of organic materials. This phenomenon was examined in a companion study on the transfer of carbon isotopes through the food-web of the Bering Sea, but is not yet adequately understood.

Animal  $^{13}\text{C}/^{12}\text{C}$  content should be viewed as a complex progressive average of the  $^{13}\text{C}/^{12}\text{C}$  ratios of diet. Isotopic content is therefore a more stable property of animals than is diet, which can change radically in response to changes in prey abundance, etc. This lowers the variability of  $^{13}\text{C}/^{12}\text{C}$  ratios over time and between individuals, and reduces the sample size required for adequate characterization of a given animal species. The relatively slow response of biomass  $^{13}\text{C}/^{12}\text{C}$  content to dietary shifts can prove either useful or frustrating when dealing with transient species which crop a food-web for short periods only.

The animal community of Izembek Lagoon, Alaska draws upon two major sources of primary production: eelgrass and phytoplankton. Both plant types can be seen to contribute to the productivity of most lagoon animals, although most animals are of course dependent on one more than the other. Eelgrass herbivores, including a crab, some snails, and perhaps some amphipods show the greatest isotopic similarity to eelgrass. Plankton feeders, including a sponge and some copepods resemble phytoplankton most closely in  $^{13}\text{C}/^{12}\text{C}$  ratio. Most of the macrobenthos, which includes many detritus feeders and animals with complex feeding habits, lie intermediate along the  $^{13}\text{C}/^{12}\text{C}$  ratio gradient, as do their predators. In general, it appears that phytoplankton supply the greater part of their carbon.

Eelgrass stands out as an unusually productive and ecologically unique member of the marine ecosystem, hence this study focused on the mechanisms by which it contributes to macrofaunal food-webs. Animal utilization of detritus undoubtedly provides the major mechanism. However, significant quantities of eelgrass carbon do enter the food-web in Izembek Lagoon through herbivory, primarily via a small number of key species. These animals include the crab *Telmessus cheiragonus*, the snail *Lacuna variegata* (and possibly other snails), and several species of birds. Amphipods and polychaetes may be added to this list as their feeding habits become better understood. The transfer of eelgrass carbon to birds, mammals, and large fishes generally requires 0 to 2 intermediates by the grazing food-web and perhaps 2 to 4 intermediates by the detrital route. As far as larger marine vertebrates are concerned, these are reasonably short food-webs and therefore capable of supporting high vertebrate productivity per unit primary productivity. Combined with the accessibility and concentrated productivity of the lagoon environment, this accounts for the attractiveness of Izembek Lagoon as a migratory stopover for birds and contributes to the productivity of the region for resident birds and mammals. Lagoon exports are expected to enhance animal productivity in the nearby Bering Sea as well. Most of the  $1.66 \times 10^8$  g C of eelgrass produced annually in Izembek Lagoon (Barsdate *et al.*, 1974) washes out into the Bering Sea, and during the collection of samples for these isotopic studies large amounts of green eelgrass detritus were recovered more than 150 km from Izembek Lagoon. Investigations into food-web utilization of eelgrass

detritus in the sea date back to the turn of the century (Petersen, 1918) but its importance remains unquantified although some utilization obviously must exist. Ellson *et al.* (1949) reported that the natives of St. Lawrence Island (in the Bering Sea) knew that good cod fishing could be found over detrital seagrass bottoms. Even in the deep oceanic trenches, seagrasses have been shown to contribute to animal productivity (Wolff, 1976, 1977).

Natural abundance tracer studies have many potential food-web applications. When aquatic weed removal projects, dredging, wetlands development, pollution, and other manipulations of aquatic or marine environments threaten to alter the relative abundance of macrophytic plants, the probable effects on animal food-webs can be more accurately predicted. The nursery function of these areas might also be examined in light of food-web transfers of tracer parameters. Animal migrations and dietary changes may be studied by watching changes in tracer parameters. The value of these tracers for geochemical and other applications will also be enhanced as their behavior in biological systems becomes better understood.

#### CHAPTER 4. FEEDING RELATIONS OF THE FISH COMMUNITY IN IZEMBEK LAGOON

##### ABSTRACT

The eelgrass (*Zostera marina*) beds of Izembek Lagoon support an abundant summer fish fauna consisting mainly of small demersal and benthic species. Transient and occasional species are usually represented by juveniles, while most of the abundant and resident fishes are present both as juveniles and adults. The distributions and feeding habits of fishes are related to their interactions with the eelgrass beds. Smaller individuals usually inhabit the eelgrass beds, where they feed on the abundant epiphytic and semi-planktonic invertebrates. Larger fishes gravitate toward the channels or swim above the eelgrass canopy, although some, particularly the sculpins, also remain in the eelgrass beds. Infaunal, epifaunal, and nektobenthonic animals constitute their major prey. Although it is unimportant as a fish food, eelgrass contributes to fish productivity by way of herbivorous and detritus-based invertebrate food-webs and through its effects on the habitat.

## INTRODUCTION

Predatory fishes generally dominate the higher trophic levels of the seas. Consequently, their feeding activities are particularly likely to affect ecosystem composition and to play an important role in redistributing biological energy reserves. This chapter examines the feeding habits of the major fish species inhabiting the eelgrass beds of Izembek Lagoon and attempts to elucidate some of their interactions with the seagrass ecosystem.

Because of their relatively great mobility, fishes display more complex distributional and behavioral patterns than most invertebrates. Juvenile fishes generally inhabit the eelgrass beds where appropriate foods are plentiful, while benefitting from the reduced currents and shelter from predators offered by the seagrass. This nursery function of seagrass beds has frequently been noted (Kikuchi, 1966, 1977; Kikuchi and Peres, 1977; Thayer *et al.*, 1975a,b; Adams, 1976a,b; Kitamori *et al.*, 1959; Fuse, 1962; Carr and Adams, 1973). Migration from the seagrass beds to other habitats, usually in deeper water, is common for adults of many species. Some return to the seagrass beds for feeding and spawning on a diel, seasonal, or occasional basis. Fishes which reside in the seagrass beds in both juvenile and adult stages are predominantly small, noncommercial species (Kikuchi, 1966; Adams, 1976a).

These distributional patterns are influenced by short and long term fluctuations in temperature, light, and other environmental parameters, as well as developmental changes in the fishes. These factors



are of course interrelated, since fish responses to environmental variables change as they mature just as do their requirements for shelter and food.

The types of fish foods available inside seagrass beds are often markedly different from those outside the beds, and the habitat is also sufficiently different that the same foraging techniques are not necessarily successful inside and outside the seagrass beds. Kikuchi (1977) and Kikuchi and Peres (1977) have reviewed the faunas of seagrass beds, while other studies have compared the faunas of seagrass and non-seagrass habitats (Barnard, 1970; Ledoyer, 1964a,b; Rasmussen, 1977). On the basis of these studies it is possible to speak of typical seagrass faunas and to categorize them according to how fishes might see them:

1. biota of the green leaves, including epiphytic micro- and meio-fauna, small sessile animals, and animals clinging, crawling, creeping, walking, or resting on the grass blades;
2. animals attached to stems and rhizomes;
3. highly mobile animals living below the leaf canopy;
4. sediment epifauna;
5. sediment infauna;
6. zooplankton; and
7. large transient animals including birds and mammals.

Epiphytic algae and detritus are usually abundant in seagrass beds and also provide food to some fishes. The above classification identifies several categories of potential fish foods which bear obvious relationships to the seagrass, while other groups are not so obviously

related. Infauna, epifauna, zooplankton, and large transient species may be influenced by the seagrass through feeding relationships or because of the modifications of the environment resulting from the growth of seagrass. These include the accumulation of organic matter and the development of highly reducing sediments bound together by the seagrass roots and rhizomes, the shaded benthic zone interrupted by numerous vertical seagrass shoots, reduced water turbulence, and the likelihood of pronounced temperature, salinity, and oxygen fluctuations in the water.

#### STUDY SITE

Izembek Lagoon (55°15'N, 163°05'W) is an embayment of the Bering Sea located near the tip of the Alaska peninsula. This lagoon measures approximately 41 km long by 3 to 12 km wide and has a surface area of approximately 218 km<sup>2</sup>, of which about 78% is tidal and 22% is channels. Eelgrass beds cover roughly 68% of the tideflats plus limited subtidal areas, making this one of the largest eelgrass beds in the world (Barsdate *et al.*, 1974). The density of eelgrass is relatively high - approximately 5000 shoots per square meter (McRoy, 1970b) and the leaves float to a height of about 50 cm above the sediment surface. The grass blades are usually free from obvious microalgal epiphytism, although outbreaks have been observed. Eelgrass growth occurs primarily during the summer, followed by senescence and sloughing of the leaves in autumn. Winter ice gouges and pits the grass beds and uproots plants frozen into the ice. Temperatures in the eelgrass beds are

generally less than 10°C in summer, although shallow waters may be heated above 20° on calm, sunny days. Storms are frequent throughout the year.

The importance of these factors to fishes is easy to appreciate. Many species must leave the intertidal eelgrass beds at low tide to avoid stranding, asphyxiation, and predation by birds, and many leave the lagoon during winter because of temperature and ice conditions. The high plant density obstructs swimming for larger fishes, but provides shelter and concentrations of prey to smaller individuals. The general paucity of microalgal epiphytes on the eelgrass would also be expected to influence the composition and trophic structure of Izembek grassbeds, as compared to other seagrass systems.

#### METHODS

Collections of grass bed fishes were made in the vicinity of Grant Pt., Izembek Lagoon during the months of June through August. Most fishes were captured in a small otter trawl (3.05 m head and food ropes with 38 mm stretch mesh netting over the body of the net and 31 mm stretch mesh netting over the cod end) towed by skiff. A 1 m wide, 6 mm mesh push net and 10 m variable panel gill net were occasionally employed. Larval and small juvenile fishes were captured in a 1 mm mesh net mounted on an epibenthic sled. These nets appear to be fairly effective for capturing most fishes, but were not effective for salmonids (and perhaps other fast swimmers) and some bottom fishes inhabiting the eelgrass beds.

Specimens were brought to the Izembek field station as quickly as practicable for processing, or injected and preserved with 10% sea water formalin immediately after capture. Following identification and measurement, the fishes were dissected and their stomach contents (or the contents of the entire gut for those not possessing well defined stomachs) examined under a dissecting microscope. Prey fragments were separated according to taxon, and their original volumes (before partial digestion) estimated. Fish lengths are expressed as standard length (in mm) and diets expressed as the average volumetric percent of the stomach contents. Empty stomachs were seldom encountered among most species, and are not included in the data. Many captured fishes were not analyzed for stomach contents, although an effort was made to sample all species and all sizes captured.

For the sake of comparison with fish stomach contents, eelgrass infauna, epiphytic fauna, and sediment epifauna were identified and their densities estimated. Infauna were sampled using a  $0.021 \text{ m}^2$  plastic core pushed by hand into the sediment to a depth of about 30 cm. Mud was washed away using a seive bucket, and the animals and eelgrass returned to the laboratory for analysis. Eelgrass epiphytic animals were enclosed in a screen bag attached to a  $0.64 \text{ m}^2$  metal quadrat frame, and removed on the grass with minimal disturbance after clipping the grass at the sediment surface with shears. The densities of crabs and other large epibenthic invertebrates was estimated at low tide by feeling by hand through  $50 \text{ m}^2$  plots of eelgrass and collecting the enclosed crabs. Biomass estimates were made for most grass bed fauna after drying at about  $60^\circ\text{C}$  until weights stabilized.

## RESULTS

Twenty-two species of fish, 18 of which have been previously recorded in Izembek Lagoon (McRoy and Peden, 1964; Tack, 1970), contributed stomach contents data to this study. An additional 20 species have been identified in the lagoon, but stomach contents data for these were not obtained. Of the species analyzed here, which includes all of the most abundant grass bed fishes, all are considered either benthic (morphologically adapted to life on the bottom) or demersal (associated with the bottom). Although somewhat artificial, this distinction is generally descriptive of fish behavior, distribution, and feeding habits as well as morphology.

Of the ten demersal fishes analyzed, six inhabit the eelgrass beds in their adult as well as juvenile stages. These include the poacher *Pallasina barbata*, the greenlings *Hexagrammos octogrammus* and *H. stelleri*, the gunnel *Pholis laeta*, and the sticklebacks *Gasterosteus aculeatus* and *Pungitius pungitius*. *P. barbata* is the commonest fish in the eelgrass beds, while hexagrammids dominate the grass bed fish fauna in biomass (*H. octogrammus* in the intertidal beds and *H. stelleri* in the subtidal beds and adjacent channels). Both hexagrammids tend to move from the dense intertidal eelgrass beds to the sparser subtidal beds and channel fringe zone as they mature, and *H. stelleri* ultimately comes to live primarily in the channels. *Pholis laeta* generally appears resting on the bottom beneath the eelgrass canopy, or curled around several grass blades within 30 cm of the bottom. The sticklebacks move freely between tundra freshwater systems and the lagoon, and are

generally captured near the upper edge of the intertidal or tide pool eelgrass beds. The remaining four demersal fishes analyzed in this study include 3 species of cod (*Gadus macrocephalus*, *Theragra chalcogramma*, and *Eleginus gracilis*) and one prickleback (*Lumpenus* sp.). Juvenile cods school at the top of the eelgrass canopy but will retreat within the canopy at the approach of a diver. Larger subadults swim along the lower margins of the eelgrass beds and above the canopy. No adults were seen or captured. The prickleback *Lumpenus* sp. was captured in the channels.

Most of the benthic fishes captured in this study are either sculpins (4 species) or flatfishes (6 species). Additional types include the sand lance *Ammodytes hexapterus* and the poacher *Agonus acipenserinus*. *Agonus* adults and juveniles are occasionally captured in the eelgrass beds but more commonly inhabit the channels, as does *Ammodytes*. The six flatfishes inhabit unvegetated bottom areas, and when disturbed they will usually swim over an eelgrass bed rather than land in it. The close spacing of eelgrass plants appears to be incompatible with their requirements for swimming, resting, and feeding on the bottom. The sculpins, in contrast, most frequently inhabit the eelgrass beds in juvenile and frequently adult stages. Two common species, *Microcottus sellaris* and *Myoxocephalus polyacanthocephalus* often remain in the intertidal eelgrass beds at low tide, and direct counts of stranded fish (hidden in the eelgrass) give densities of over 100/ha for each. This is about an order of magnitude above the estimates Tack (1970) made by trawling at high tide. *Leptocottus armatus* apparently feeds

primarily in the sand flats at high tide, but retreats to the eelgrass beds and channels at low tide.

### The Diets of Fishes

The proportions of different prey observed in fish stomachs (Table 6) are, for the most part, loosely determined by behavioral and morphological characteristics of the fishes. The six pleuronectids (*Limanda aspera*, *L. proboscidea*, *Isopsetta isolepis*, *Lepidopsetta bilineata*, *Platichthys stellatus*, and *Hippoglossus stenolepis*) feed primarily on infaunal and epifaunal benthic invertebrates, including clams, polychaetes, echiuroids, crabs, cumaceans, and gammarid amphipods. The sculpins, which share the unvegetated habitats with the flatfishes but also feed in the grass beds, have correspondingly enlarged diets. *Microcottus sellaris* is unique for its intense utilization of the epiphytic snails *Lacuna variegata* and *Margarites helycinus*, and is also the only fish which was observed to eat the epiphytic sea star *Lepasterias* sp. *Myoxocephalus polyacanthocephalus* frequently eats shrimps, while the smaller sculpins *Leptocottus armatus* and *Myoxocephalus scorpiodes* concentrate on amphipods, isopods, polychaetes, and echiuroids. The snout of the tubenose poacher *Pallasina barbata* is modified for slurping up the small swimming crustaceans which constitute its major prey, although it will occasionally take the caprellid amphipod *Caprella alaskana* too. This amphipod clings tenaciously to the eelgrass blades by its grasping posterior pereopods, making it difficult to capture by this method. The other poacher, *Agonus acipenserinus*, possesses

TABLE 6. Volumetric stomach contents of fishes collected less than 1% stomach contents volume.

<u>Fish Species</u>	<u>Number analyzed</u>	<u>Standard lengths (mm)</u>	<u>Copepods</u>	<u>Gammarid amphipods</u>	<u>Caprellid amphipods</u>
<u>Demersal Fishes</u>					
<i>Eleginus gracilis</i>	2	121-245	0	20	0
<i>Gadus macrocephalus</i>	14	81-199	4	53	6
<i>Theragra chalcogrammus</i>	3	76- 81	7	65	12
<i>Gasterosteus aculeatus</i>	3	66- 85	+	+	+
<i>Pungutius pungitius</i>	5	nd	+	+	+
<i>Hexagrammos octogrammus</i>	188	45-195	3	27	44
<i>Hexagrammos stelleri</i>	81	57-218	2	42	26
<i>Pallasina barbata</i>	92	16-139	58	35	2
<i>Pholis laeta</i>	12	70-159	12	33	41
<i>Lumpenus</i> sp. ( <i>mackayi</i> ?)	1	124	85	10	0



in Izembek Lagoon, Alaska. "+" indicates

PREY TAXON

Isopods	Shrimps	Other crustaceans	Polychaetes + Echiurids	Clams	Snails	Fishes	eelgrass + detritus
0	20	0	15	35	0	0	0
+	17	7	1	0	0	14	+
0	15	0	0	0	0	0	0
+	0	+	+	0	0	0	+
+	0	0	+	0	0	0	+
+	+	2	20	1	1	+	+
+	4	3	17	+	+	+	1
+	1	1	+	+	+	0	+
5	0	1	+	1	1	3	+
0	0	5	0	0	0	0	0

TABLE 6. Continued

Fish Species	Number analyzed	Standard lengths (mm)	Copepods	Gammarid amphipods
<u>Benthic Fishes</u>				
<i>Leptocottus armatus</i>	1	86	0	50
<i>Myoxocephalus polyacanthocephalus</i>	5	75-155	0	32
<i>Myoxocephalus scorpioides</i>	2	82- 99	0	30
<i>Microcottus sellaris</i>	12	74-121	+	30
<i>Agonus acipenserinus</i>	1	69	70	30
<i>Ammodytes hexapterus</i>	4	79-133	50	10
<i>Hippoglossus stenolepis</i>	2	107-111	0	35
<i>Iopsetta isolepis</i>	1	170	0	20
<i>Lepidopsetta bilineata</i>	5	45-152	1	30
<i>Limanda aspera</i>	3	97-105	0	63
<i>Limanda proboscidea</i>	1	100	0	50
<i>Platichthys stellatus</i>	1	112	0	10

PREY TAXON

Caprellid amphipods	Isopods	Shrimps	Other crustaceans	Polychaetes + Echiurids	Clams	Snails	Fishes	Seagrass + detritus
0	10	0	0	40	0	0	0	0
2	2	38	+	18	0	+	0	+
0	5	0	+	65	0	0	0	0
2	1	+	+	30	+	27	+	6
0	0	0	0	0	0	0	0	0
0	0	0	34	0	0	5	0	0
5	0	0	0	40	0	0	0	0
0	0	0	60	20	0	0	0	0
+	0	+	2	63	2	+	0	+
3	+	0	13	20	0	0	0	0
0	0	0	0	50	0	0	0	0
0	0	0	0	30	60	0	0	0

a ventrally located mouth equipped with sensory barbules, suggesting that its prey are normally associated with the benthic surface.

Invertebrates plucked from the eelgrass blades and from the bottom constitute the major prey of the greenlings, cods, sticklebacks, and gunnels. Those species commonly feeding below the eelgrass canopy (including *Hexagrammos octogrammus*, *Pholis laeta*, and juveniles of *H. stelleri* and *Theragra chalcogrammus*) take proportionally more epiphytic animals than other fishes.

Eelgrass is ingested rather frequently by large sculpins and hexagrammids in particular, but seldom contributes much to the overall stomach contents volume. Because the grass never shows evidence of successful digestion, and because no fish fed predominantly on eelgrass, it was concluded that eelgrass is nutritionally unimportant to the fishes to Izembek Lagoon. Most frequently, fishes probably ingest the grass incidental to its epiphytes. Similar behavior has been previously noted in other seagrass beds (Carr and Adams, 1973; Odgen, 1977). Sticklebacks and possibly other species may deliberately ingest eelgrass detritus however.

While most of the fishes are relatively nonspecific predators, most do exhibit some degree of prey selectivity. It is common for different individuals of the same species, same size, and from the same haul to contain completely different prey, suggesting that fishes may follow prey clusters or feed preferentially on certain prey at various times. The major prey taxon in the stomachs of individual fishes contributed an average of 91% to the stomach contents volume of

*Pallasina barbata*, 83% for *Gadus macrocephalus*, 75% for *Ammodytes hexapterus*, 68% for *Hexagrammos stelleri*, 66% for *Hexagrammos octogrammus*, 60% for *Microcottus sellaris*, and 53% for *Pholis laeta*. In most cases, individual prey were small compared to total stomach contents, so a predominance of one prey taxon indicates repeated feeding on that prey rather than cessation of feeding after ingesting one individual.

Variations in the relative abundances of prey species would be expected to influence the dietary composition of fishes. Kikuchi (1966), Marsh (1970), Nagle (1968), and others have documented pronounced seasonal and shorter term population cycles in a variety of grass bed invertebrates. Although invertebrates were not counted on a regular basis for this study, population fluctuations were fairly apparent among gammarid amphipods, *Caprella*, shrimps, and epitokous polychaetes. It is suspected that these changes precipitated shifts observed in the diets of *Hexagrammos octogrammus* (Fig. 10) and other fishes over short time intervals. The statistical unpredictability of fish stomach contents (Fig. 1), is largely a reflection of the all-or-none prey selectivity noted above.

Size is another obvious determinant of fish diets. Larval fishes prey on small animals, and progress to larger things as they grow. In approximate order of increasing size, the principle foods available in the eelgrass beds of Izembek Lagoon are: planktonic, epibenthic, and epiphytic microalgae and detritus; zooplankton; caprellids and small isopods, gammarids and polychaetes; cumaceans, mysids, epiphytic molluscs, and somewhat larger gammarids and polychaetes; and finally shrimps,

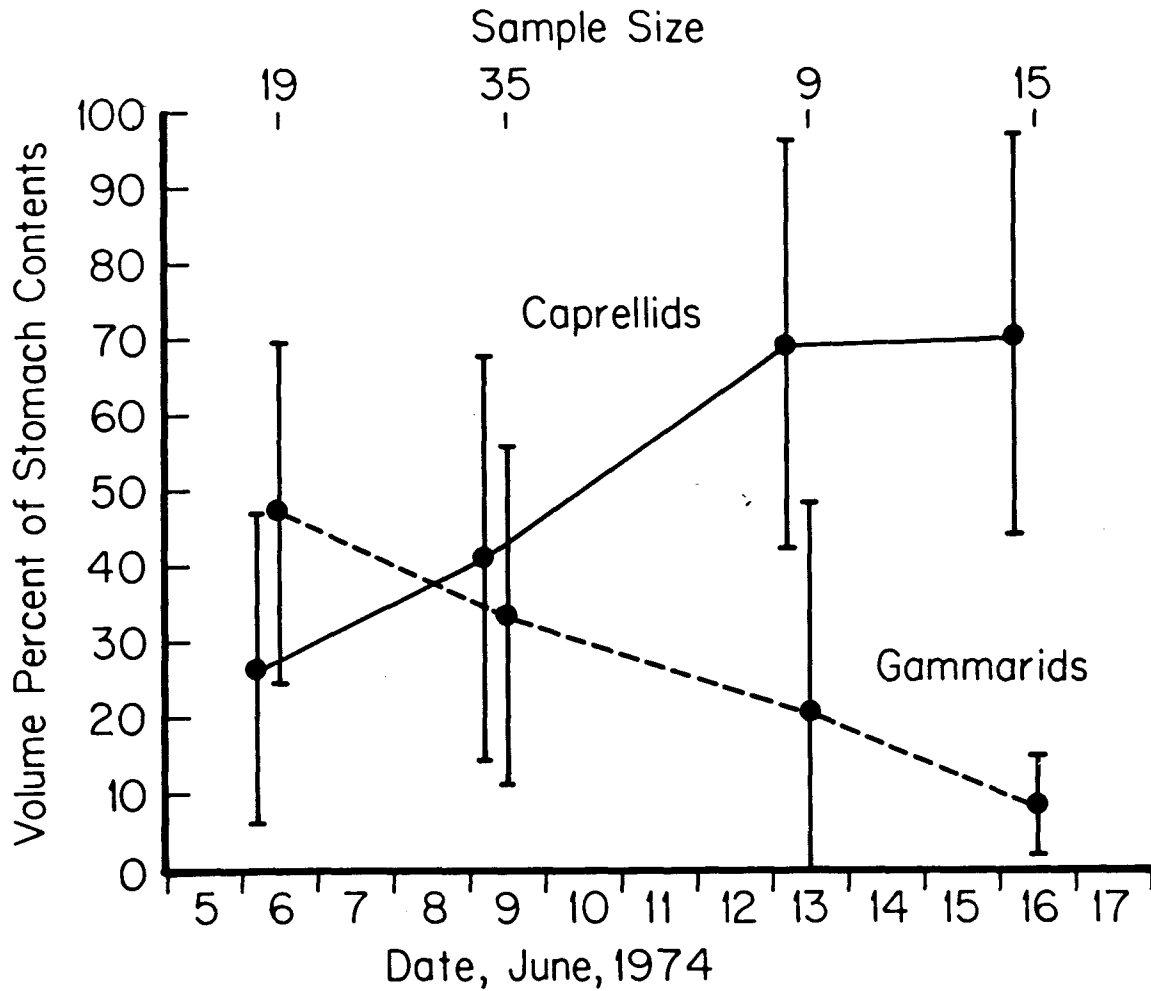


Figure 10. Short term changes in the diet of masked greenling, *Hexagrammos octogrammus*. Variations in the importance of amphipods to fishes 90-109 mm (standard length) observed during June 1975. Error bars represent 1 standard deviation.

crabs, clams, large polychaetes, echiurids, and fishes. These prey tend to be distributed with the smaller categories in the eelgrass beds and the larger ones in the channels. In fact, it is found that smaller fishes usually feed inside the grass beds while larger fishes feed above or around the grass beds, and that the size and species composition of fish diets progresses more or less according to the sequence outlined above. This is illustrated for the two hexagrammids (Figs. 11 and 12) and *Pallasina barbata* (Fig. 13). Fishes having small adult sizes, such as *Pallasina*, may continue to eat relatively small animals in the grass beds as adults. Others outgrow the small, primarily epiphytic animals or become too large to maneuver amidst the closely spaced plants. These larger fishes gravitate toward the channels and other unobstructed environments offering larger epibenthic, infaunal, and nektonic prey. Of course changes in size occur simultaneously with increased swimming ability, development of the digestive system, etc. These factors contribute to changes in diet and habitat.

#### Eelgrass as a Primary Food Source and Carbon-13 Studies

In order to help determine the extent to which eelgrass contributes to the nutrition of the food-web, an isotope tracer study was conducted in Izembek Lagoon (Chapter 3). The procedure relied on the fact that eelgrass and planktonic microalgae have recognizably different ratios of the stable carbon isotopes,  $^{12}\text{C}$  and  $^{13}\text{C}$ . These ratios, expressed relative to the isotopic standard PDB (Craig, 1953), are:

eelgrass	$\delta = -10.3$
phytoplankton	$\delta = -22.3$

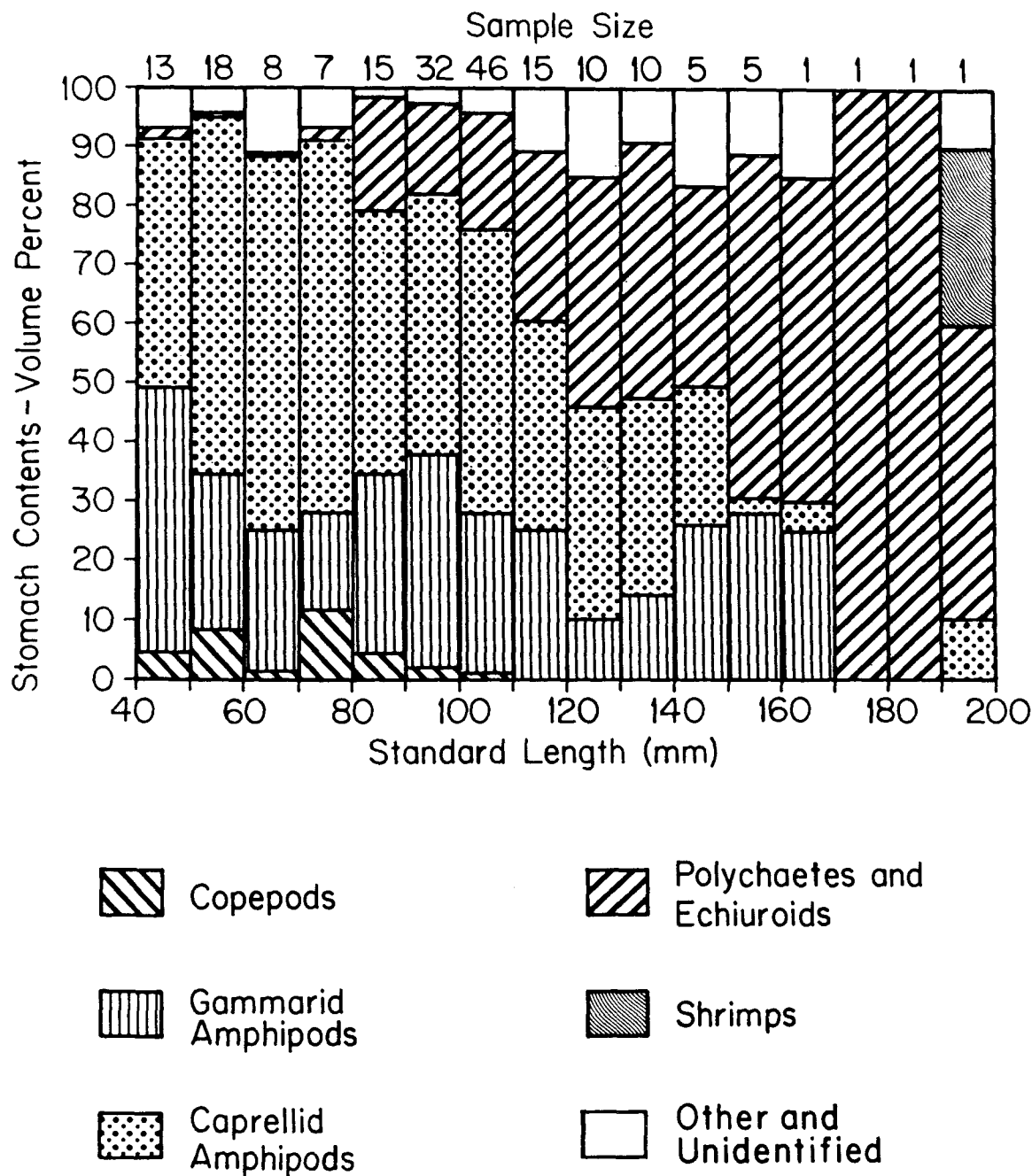


Figure 11. The summer diet of *Hexagrammos octogrammus* in IZembek Lagoon.



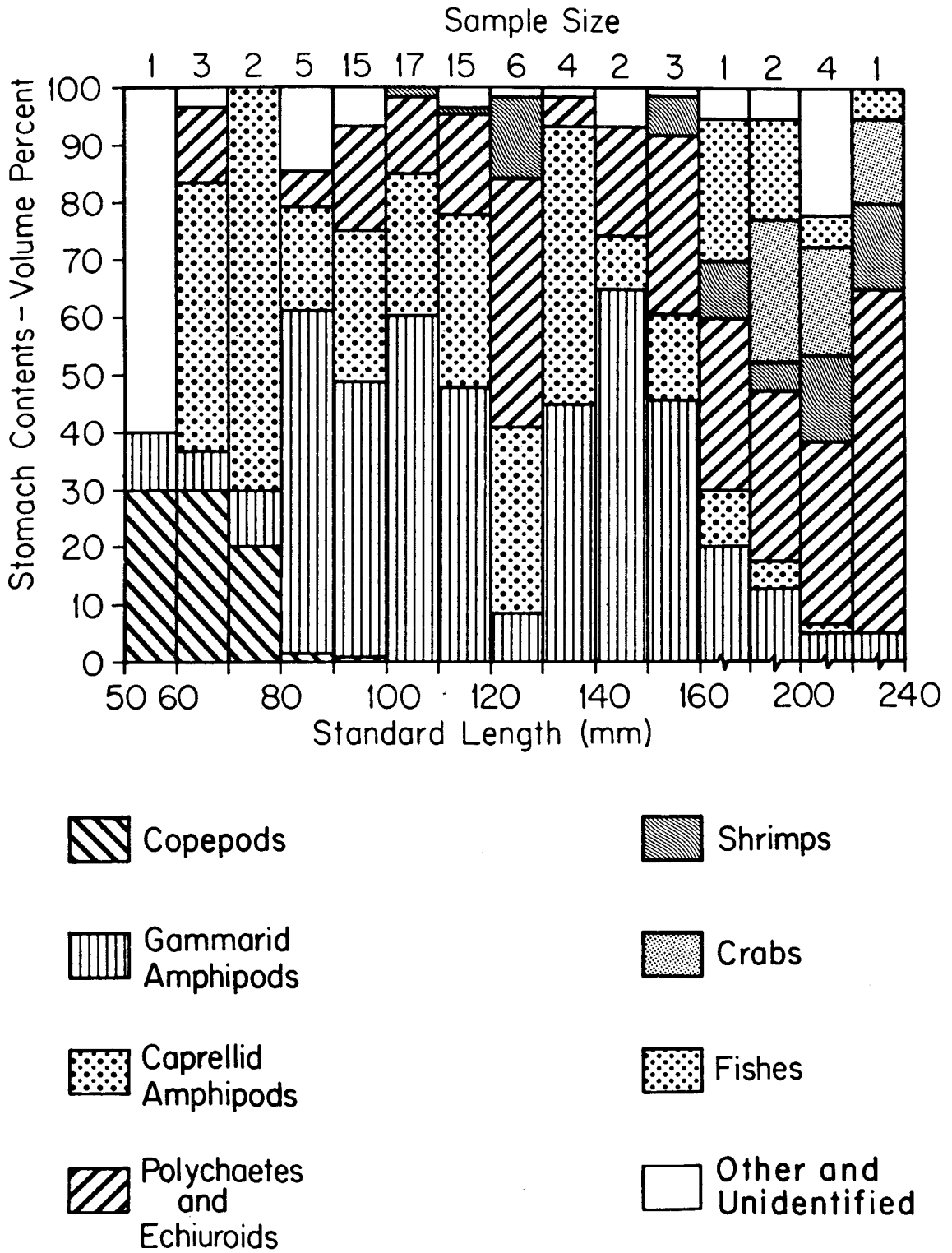


Figure 12. The summer diet of *Hexagrammos stelleri* in Izbek Lagoon.

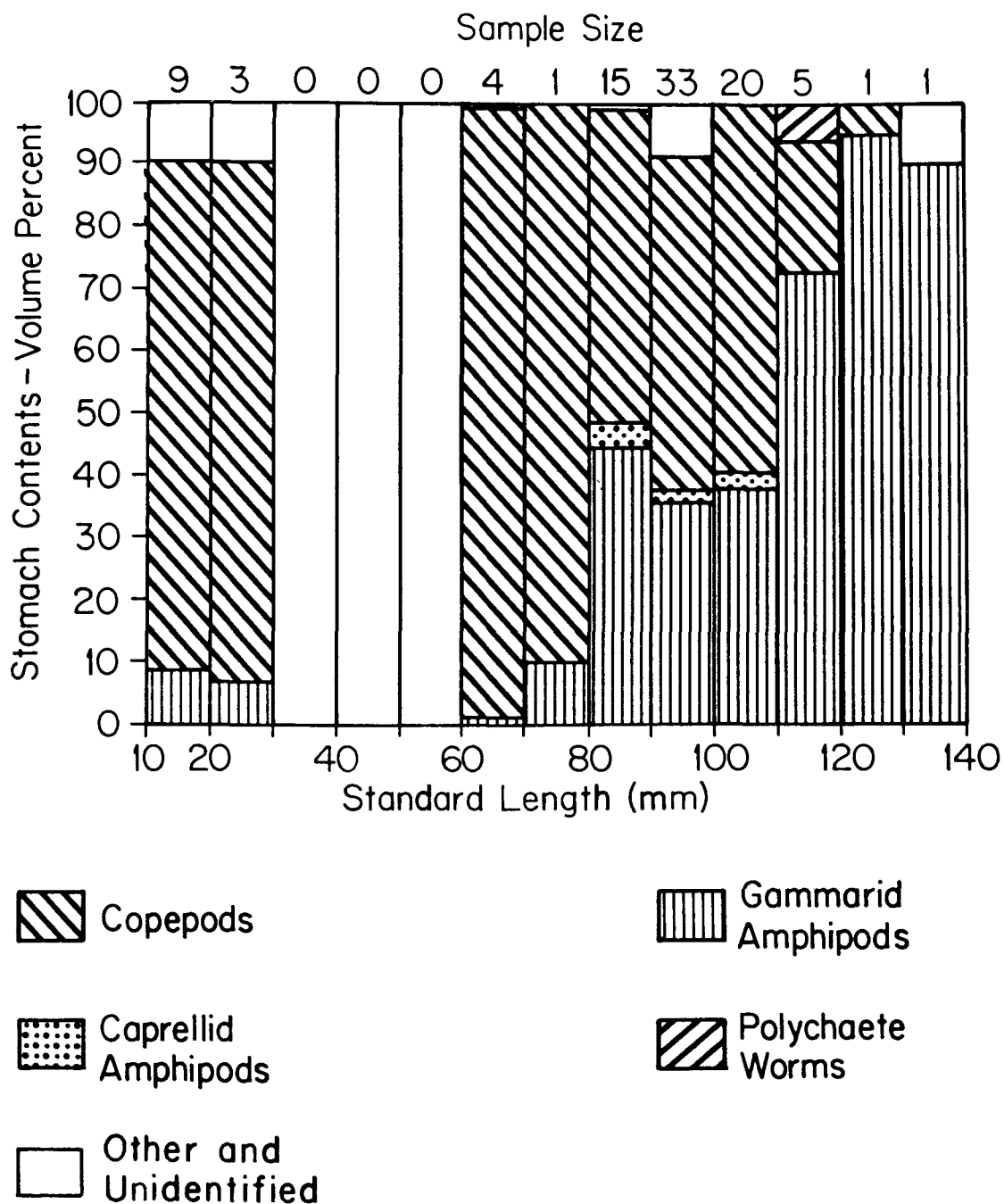


Figure 13. The summer diet of *Pallasina barbata* in Izembek Lagoon.

where

$$\delta = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{PDB}}} - 1 \times 1000$$

Carbon entering the lagoon food-web from primary producers carries with it the isotopic signatures of the plants, and the  $^{13}\text{C}/^{12}\text{C}$  ratios of animals therefore depend on the proportions of carbon in their tissues derived from each plant. These proportions can be only approximately calculated however, due to the input of carbon derived from plants other than eelgrass and phytoplankton, and small but significant changes in body  $^{13}\text{C}/^{12}\text{C}$  content caused by metabolic isotope fractionation. This latter process tends to enrich animals in  $^{13}\text{C}$  compared to their foods. These uncertainties, and some ways to reduce them have been discussed in Chapters 2 and 3.

$^{13}\text{C}/^{12}\text{C}$  ratios (expressed as  $\delta$ ) were measured for the following Izembek fishes (adult or subadult unless otherwise specified): *Theragra chalcogrammus* ( $\delta = -15.6$ ), *Gasterosteus aculeatus* (-19.4), *Pungitius pungitius* (-19.1), *Hexagrammos octogrammus* (-15.0, juveniles -17.7), *H. stelleri* (-14.8, juveniles -17.8), *Pallasina barbata* (-17.1), *Pholis laeta* (-13.8), *Microcottus sellaris* (-14.0), *Hippoglossus stenolepis* (-17.4) and *Lepidopsetta bilineata* (-14.4, larva -16.6). Higher (less negative) values of  $\delta$  are presumed to reflect relatively larger contributions of eelgrass carbon to fish tissues, while lower  $\delta$  values reflect relatively less eelgrass carbon and more microalgal carbon (and perhaps terrestrial and aquatic carbon in the case of the sticklebacks).

Accordingly, *Pholis laeta* ( $\delta = -13.8$ ,  $n=1$ ) and *Microcottus sellaris* ( $\delta = -14.0 \pm 0.1$ ,  $n=3$ ) appear to contain proportionately more eelgrass carbon than the other fishes analyzed. This is reasonable in view of the fact that these species live and feed under the eelgrass canopy. *Pholis* preys primarily on crustaceans living on or associated with the eelgrass plus lesser amounts of fish larvae and epiphytic molluscs. *Microcottus* takes primarily gammarid amphipods, polychaetes, and epiphytic snails. In both cases, probable and demonstrated routes of carbon transfer through the food web from eelgrass to fishes are evident. The epiphytic snails and some gammarid amphipods are largely herbivorous on eelgrass. Detritus, much of which is derived from eelgrass, contributes to the diets of several amphipods, copepods, cumaceans, polychaetes, and clams. Based on isotopic evidence, the best estimate for the contribution of eelgrass carbon to *Pholis* and *Microcottus* is 50% or less.

Conversely, some of the lowest  $\delta$  values came from *Pallasina barbata* ( $\delta = -17.1 \pm 0.0$ ,  $n=2$ ) and juveniles of *Hexagrammos octogrammus* ( $\delta = -17.7 \pm 0.3$ ,  $n=3$ ) and *H. stelleri* ( $\delta = -17.8$ ,  $n=1$ ). These fishes feed on copepods and small filter feeding crustaceans for the most part, and as such are likely to receive predominantly microalgal carbon with only lesser amounts (probably less than 20%) of eelgrass carbon. *Hippoglossus stenolepis* and the sticklebacks also possess relatively low  $\delta$  values, but because these fishes migrate between the lagoon and other environments (the Bering Sea for the halibut and tundra freshwater systems for the sticklebacks), the isotopic data are more difficult to interpret.

Changes in diet related to growth and development affect fish  $^{13}\text{C}/^{12}\text{C}$  ratios in the manner which would be expected. As juveniles mature, they switch from a diet dominated by filter feeding crustaceans to a more diverse assortment including more detritus feeders and herbivores. These prey contain proportionately more eelgrass carbon, which raises the  $\delta$  values of the fishes.

## DISCUSSION

### Food-Web Relations

Petersen (1918) attempted to quantify the fauna of Danish eelgrass beds and relate the invertebrate stocks to fish production. He felt that Rissoid snails, which numbered up to  $10^5/\text{m}^2$ , were particularly important as fish food. Subsequent observations on the stomach contents of fishes have shown that crustaceans and polychaetes are generally far more important as fish food, despite their lower densities. The early Danish investigations (Blegvad, 1916; Boysen-Jensen, 1914; Petersen, 1918) made major contributions to the study of seagrass utilization by marine food-webs through their identification of the detritus food-web pathway and habitation of eelgrass beds for feeding and shelter by fishes.

The detritus connection remains poorly quantified despite the recognition of its general importance, due to the difficulty in characterizing the nutritional value and composition of detrital materials and their uptake rates by marine food-webs. Radioisotope uptake and transfer studies (Sibert *et al.*, 1977) and stable isotope

studies of the type attempted here should help to elucidate this problem.

Direct utilization of seagrasses through grazing by fishes assumes more importance in the tropics than in temperate and subarctic regions (Ogden, in press). The same is probably true for grazing invertebrates, although recent observations by myself and others reveal considerable grazing activities by invertebrates in temperate and subarctic waters.

Fishes frequently ingest seagrass incidentally to the epiphytic flora and fauna which it supports however. The main eelgrass beds at Izembek Lagoon are usually almost free of visible algal epiphytism, although outbreaks (most often of fungi) have been observed. McRoy (personal communication) reports epiphyte biomasses which equaled those of eelgrass leaves, but the maximum which I have seen is about 20% of the eelgrass leaf weight. The average biomass of epiphytes in Izembek Lagoon is estimated at 0.5% of the dry weight eelgrass leaf weight, or about  $4 \text{ g/m}^2$ . Selected values for comparison are  $24.7 \text{ g/m}^2$  in North Carolina (Penhale, 1977),  $42\text{--}360 \text{ g/m}^2$  in the Seto Inland Sea, Japan (calculated from Kita and Harada, 1962) and  $57\text{--}183 \text{ g/m}^2$  of algae consisting mainly of epiphytes in Rhode Island (Nixon and Oviatt, 1972). Epiphytic animals (excluding protozoa, nematodes, and other microscopic forms) are estimated to have a dry weight biomass of about  $21 \text{ g/m}^2$ , or about 3% of the eelgrass leaf biomass in Izembek Lagoon. Snails (mostly *Lacuna variegata* and *Margarites helicinus*) contribute the largest fraction of this biomass, while the animals more generally favored as foods by fishes make up 7-14% of

the epiphytic animal biomass. What little is known of the feeding habits of these epiphytic animals is discussed in Chapter 3.

#### Fish Standing Stocks

Tack (1970) estimated the wet weight biomass of fishes in Izembek Lagoon during the summer of 1968. His figures have been recalculated to dry weight biomass in Table 7. From these data it is apparent that eelgrass beds contain about twice the fish biomass found in non-vegetated habitats. The total fish biomass in Izembek eelgrass beds, about  $3 \text{ g/m}^2$ , compares favorably with biomass estimated from temperate seagrass beds. Adams (1976a) reported an average annual fish biomass of about  $1.5 \text{ g/m}^2$  in a North Carolina estuary and a monthly maximum biomass of about twice that amount. Thayer *et al.*, (1975b) give similar figures. Lower values were obtained in Rhode Island eelgrass beds (Nixon and Oviatt, 1972) and Texas turtle grass beds (Hoesse and Jones, 1963; Hellier, 1962). The type of fishing gear employed in Tack's Izembek study is probably less efficient than that used in the other studies (Kjelson and Colby, 1977) and was constructed of coarser mesh netting, so the actual differences may be larger than indicated.

The high fish biomass in Izembek Lagoon is probably related to the abundance of eelgrass. Compared to most other seagrass beds (McRoy and McMillan, 1977; Thayer *et al.*, 1975a,b) the eelgrass biomass, productivity, density and bed size are larger in Izembek Lagoon. From the isotopic data presented here and what is known or suspected concerning the feeding relations of the animals, there are important food-web channels which link the fishes to eelgrass productivity.

TABLE 7. Standing stocks (in grams dry weight per m<sup>2</sup>) of fishes in Izembek Lagoon, Alaska, calculated from the data of Tack (1970)

Fish taxon	HABITAT			
	shallow eelgrass	deep eelgrass	channels	sand flats
<i>Hexagrammos stelleri</i>	0.15±0.44	0.48±0.80	1.16±2.50	0.00±0.00
<i>H. octogrammus</i>	1.00±1.18	1.44±2.70	0.03±0.09	0.00±0.01
<i>Pallasina barbata</i>	0.27±0.27	0.23±0.22	0.12±0.03	0.00±0.00
Sculpins	1.08±5.89	0.38±1.20	0.15±0.53	0.39±0.44
Flat Fishes	0.10±0.87	0.01±0.07	0.08±0.29	0.00±0.00
Total Fishes	3.00±3.64	2.72±2.79	1.55±2.68	0.44±0.54



Paradoxically, the biomass and productivity of microalgae (including phytoplankton, eelgrass epiphytes, and benthic algae) appear to be relatively low, and these primary producers are believed to be quantitatively as important as the eelgrass in the fish food-webs.

Part of the explanation for the high fish biomass in Izembek Lagoon may lie in the fact that the eelgrass beds are unusually extensive and fishes cannot readily migrate between the eelgrass beds and other habitats. The standing stocks observed in and around the eelgrass beds therefore give a fairly good measure of what they can support. Where the seagrass beds are smaller and bordered by other, usually deeper habitats, many fishes, particularly large ones, move into the seagrass beds to feed and then leave. The standing stocks within the beds are therefore smaller, even though the seagrass beds might actually support comparable or larger fish stocks. The relatively low dependence of fish stocks on time of day observed by Tack (1970) in Izembek Lagoon, compared to other sites (Adams, 1976a; Kikuchi, 1966; Ogden, personal communication) probably reflect this difference in bed size and situation.

#### Composition of the Fish Fauna

Comparison of fish taxa from Izembek Lagoon and more southerly eelgrass beds reveals only limited overlap. Of the 13 families and 40 species found in Izembek Lagoon (McRoy and Peden, 1964; Tack, 1970; this study), 12 families and 10 species were reported in Oregon (Pearcy and Meyers, 1974; McConnaughey *et al.*, 1971; U.S. Dept. of Interior, 1971); 9 families and 2 species in the Mystic River estuary, Connecticut

(Pearcy and Richards, 1962); 7 families and 0 species in Humboldt Bay, California (Eldridge and Bryan, 1972); 5 families and 0 species in Tomioka Bay, Japan (Kikuchi, 1966); and 2 families and 0 species in Newport Estuary, North Carolina (Adams, 1976). Differences in collection techniques etc. undoubtedly contribute to the apparent faunistic differences, but these differences are nevertheless noteworthy. Estuarine fishes of the Northeast Pacific show the greatest taxonomic similarity to the Izembek fauna, as would be expected from geographical and hydrographical considerations. Kikuchi's (1966) and Adams' (1976) eelgrass fishes (taken from near the southern extremes of eelgrass growth) show the least taxonomic overlap compared to the Izembek roster. 25 of 56 families inhabiting seven Japanese eelgrass beds are tropical or warm-temperate, and 51 of the Japanese families are absent in the Alaskan bed. The comparison with North Carolina is similar. Only 2 of the 28 families captured there were also captured in Izembek. Clearly eelgrass provides habitat for quite diverse fish faunas within its large geographic range.

Regardless of the particular species of fish inhabiting an eelgrass bed, certain general patterns tend to emerge. One of these is the "nursery" role which eelgrass beds play in the rearing of many fishes. A nursery requires two things to be successful: abundant food and safety. Eelgrass beds are well suited to provide these. Foods of appropriate sizes are abundant and available both in the water and on the grass blades. The comparatively warm water of the grass bed speeds larval development and the attenuated currents benefit weak swimmers.

The eelgrass blades both hide the young fish from their predators, and turn the predators advantage of speed and size into liabilities.

#### CONCLUSIONS

Dense eelgrass vegetation exerts profound effects on the faunistic composition and trophic relations of associated animals. The grass promotes vigorous nutrient and energy cycling through the ecosystem, while the greatly expanded benthic surface and relatively quiet waters provide the habitat for epiphytic and semi-planktonic algae and invertebrates. These attributes provide an excellent environment for small fishes, but the physical characteristics of an eelgrass bed simultaneously discourage habitation by large fishes. Several factors apparently important in this regard are: (1) infauna and benthic epifauna are probably harder to locate and capture in an eelgrass bed due to the presence of eelgrass and detritus on the bottom and the tangled mats of eelgrass rhizomes, (2) fishes are hindered in swimming or resting on the bottom by the close spacing of the plants, and (3) potential prey are mostly smaller than the inhabitants of non-vegetated areas, and are hidden by the grass blades.

What results is the recurrent pattern of inhabitation of the grass beds by small and young fishes. Inside the bed they find abundant foods, favorable current and temperature conditions, and refuge from predators. Larger fishes forage around and above the grass canopy, feeding on infaunal, epifaunal, and nektonic forms and picking off exposed inhabitants of the grass beds.

While the role of eelgrass appears to be primarily one of structure in the environment, it also makes a significant contribution as a food source. Direct grazing by fishes is almost absent in Izembek and in other seagrass systems grazing is probably never as important as predation, but invertebrate grazers and detritus food-webs make large quantities of eelgrass production available to fishes.

Because seagrass beds are the sole habitat for few, if any fishes and serve largely as nurseries or feeding areas instead, there is considerable taxonomic variability among the fish faunas of different seagrass beds. The physical and biological similarities of seagrass beds therefore dictate similar patterns of utilization by fishes despite differences in faunistic composition.

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## APPENDIX A

### Equations Used in the Calculation of Biochemical Composition

Assume:

- (1) Biomass contains 3 organic fractions, protein, lipid and carbohydrate
- (2) Biomass contains one nitrogenous fraction, protein
- (3) The carbohydrate content is constant

Define P, L, and Q to be protein, lipid, and carbohydrate content (on an ash-free dry wt basis)

Define C, N to indicate carbon and nitrogen content (AFDW). Subscripts b, p, l, and q refer to biomass, protein, lipid, carbohydrate.

Assign the following numerical values:

	<u>% carbon</u>	<u>% nitrogen</u>
protein	$C_p = 51.3$	$N_p = 17.8$
lipid	$C_l = 69.0$	$N_l = 0$
carbohydrate	$C_q = 44.4$	$N_q = 0$

then  $100 C_b = C_p P + C_l L + C_q Q$   
 $= C_p P + C_l L + C_q r(P+L)$  where  $r = \frac{Q}{P+L} = \frac{Q}{100-Q}$

$$100 N_b = N_p P$$

$$\frac{C_b}{N_b} = \frac{C_p + C_q r}{N_p} + \frac{L}{P} \frac{C_l + C_q r}{N_p}$$

rearranging,  $\frac{L}{P} = \frac{C_b}{N_b} \frac{N_p}{C_l + C_q r} - \frac{C_p + C_q r}{C_l + C_q r}$



Use the following identities to solve for L and P:

$$P = \frac{100-Q}{1 + \frac{L}{P}} \quad Q = r(P+L) \quad L = \frac{100-Q}{1 + \frac{1}{L/P}} .$$

Inserting the appropriate constants and setting

$$Q = 7 \quad (r = 0.0753)$$

then: 
$$P = 93 / (0.246 \frac{C}{N} + 0.225)$$

$$L = 93 / (1 + (0.246 \frac{C}{N} - 0.775)^{-1}).$$

APPENDIX B

Derivation of equations for calculating the lipid-normalized  $^{13}\text{C}/^{12}\text{C}$  ratio ( $\delta'$ ) of animal materials.

Symbols: as defined in Appendix A. The addition of a prime (') indicates the variable pertains to the lipid-normalized transformation.

Distribution of biochemicals in sample

$$P + L + Q = 100 \quad \text{for sample}$$

$$P' + L' + Q' = 100 \quad \text{for lipid-normalized (LN) sample}$$

Distribution of carbon in sample

$$C_p P + C_1 L + C_q Q = 100 C_b \quad \text{for sample}$$

$$C_p P' + C_1 L' + C_q Q' = 100 C'_b \quad \text{for LN sample}$$

Distribution of isotopes in sample

$$C_p P \delta_p + C_1 L \delta_1 + C_q Q \delta_q = 100 C_b \delta \quad \text{for sample}$$

$$C_p P' \delta_p + C_1 L' \delta_1 + C_q Q' \delta_q = 100 C'_b \delta' \quad \text{for LN sample}$$

$$\delta = \delta_p \frac{C_p}{100} \frac{P}{C_b} + \delta_1 \frac{C_1}{100} \frac{L}{C_b} + \delta_q \frac{C_q}{100} \frac{Q}{C_b}$$

$$\delta' = \delta_p \frac{C_p}{100} \frac{P'}{C'_b} + \delta_1 \frac{C_1}{100} \frac{L'}{C'_b} + \delta_q \frac{C_q}{100} \frac{Q'}{C'_b}$$

Since  $\delta_q \approx \delta_p = \delta_1 + D$

$$\delta = (\delta_1 + D) \left( \frac{C_p}{100} \frac{P}{C_b} + \frac{C_q}{100} \frac{Q}{C_b} \right) + \delta_1 \left( \frac{C_1}{100} \frac{L}{C_b} \right)$$

$$\delta' = (\delta_1 + D) \left( \frac{C_p}{100} \frac{P'}{C'_b} + \frac{C_q}{100} \frac{Q'}{C'_b} \right) + \delta_1 \left( \frac{C_1}{100} \frac{L'}{C'_b} \right)$$

$$\delta' - \delta = (\delta_1 + D) \left( \frac{C_p}{100} \left( \frac{P'}{C'_b} - \frac{P}{C_b} \right) + \frac{C_q}{100} \left( \frac{Q'}{C'_b} - \frac{Q}{C_b} \right) \right) + \delta_1 \frac{C_1}{100} \left( \frac{L'}{C'_b} - \frac{L}{C_b} \right)$$

$$= \delta \frac{C_p P' + C_q Q' + C_1 L'}{100 C_b'} - \frac{C_p P + C_q Q + C_1 L}{100 C_b} + D \frac{C_p P' + C_q Q'}{100 C_b'} + \frac{C_p P + C_q Q}{100 C_b}$$

$$= \delta \quad 1 - 1 \quad + D \left( - \frac{C_1 L'}{100 C_b'} + \frac{C_1 L}{100 C_b} \right)$$

substitute in  $C_b = (C_p P + C_q Q + C_1 L)/100$

$$P + Q + L = 100$$

$$Q = 7$$

$$\begin{aligned} (\delta' - \delta) &= D \left( - \frac{C_1 L'}{100 C_b'} + \frac{C_1 L}{(C_1 - C_p)L + 93C_p + 7C_q} \right) \\ &= D \left( - 0.2068 + \frac{3.90}{1 + 287.1/L} \right) \end{aligned}$$

APPENDIX C

$\delta^{13}\text{C}$  of Izembek Lagoon Organisms. Replicate analyses of single samples listed together, different individuals or samples on separate lines.

Sample description	Preparation*	$\delta^{13}\text{C}$
POC samples, Glaznap Pass 8 Aug. 74		
Filtered, flood tide 12:10 pm, 9.7°C	FD	-20.8
Filtered, flood tide 12:55 pm, 10.0°C	FD	-20.9
112 $\mu$ net, flood tide 12:55 pm, 10.0°C	LPR, FD	-20.2
216 $\mu$ net, flood tide 12:55 pm, 10.0°C	LPR, FD	-18.0
Filtered, high tide 15:30	FD	-20.5 -21.1
112 $\mu$ net, high tide 15:30	LPR, FD	-20.9
216 $\mu$ net, high tide 15:30	LPR, FD	-18.3
Filtered, ebb tide 18:00, 12.5°C	FD	-18.7 -19.2
112 $\mu$ net, ebb tide 18:00, 12.5°C	LPR, FD	-18.8
216 $\mu$ net, ebb tide 18:00, 12.5°C	LPR, FD	-17.5 -17.9
571 $\mu$ net, ebb tide 18:00, 12.5°C	LPR, FD	-17.3
Filtered, ebb tide 20:00, 11.0°C	FD	-18.3 -18.6
112 $\mu$ net, ebb tide 20:00, 11.0°C	LPR, FD	-18.0
216 $\mu$ net, ebb tide 20:00, 11.0°C	LPR, FD	-17.7
571 $\mu$ net, ebb tide 20:00, 11.0°C	LPR, FD	-17.2
Filtered POC samples from eelgrass beds		
High tide, calm sunny day	FD	-19.9
Low tide, calm sunny day	FD	-18.5 -18.7
Low tide, calm sunny day	FD	-18.2 -18.4
Ebb tide, windy day	FD	-17.2 -17.2
Ebb tide, windy day	FD	-12.6 -14.3
Eelgrass microepibiota, washed off	FD	-11.4
Eelgrass microepibiota, scraped off	FD	-11.8
Eelgrass leaves, meadow	FD	- 9.8 -10.3 -10.5 -10.6
Eelgrass leaves, tidepool	FD	- 7.9 - 8.0 - 9.7
Eelgrass leaves, intertidal	FD	-11.6
Eelgrass roots, meadow		-10.1

\* FD=Freeze dried; LPR=large particles removed; Ac=acidified, OD=oven dried  
DS=deshelled; M=muscle.

## APPENDIX C

(Continued)

Sample description	Preparation*	$\delta^{13}\text{C}$
Zooplankton, 216-571 $\mu$	FD	-17.3
Small copepods, 216-308 $\mu$	FD	-13.1
Copepods and amphipods, 308-571 $\mu$	FD	-17.4
Copepod sp.	FD	-18.8
Amphipod, <i>Anisogammarus</i> sp.	FD	-15.8
Amphipod, <i>Anisogammarus</i> (beach wrack)	FD	-13.6
Amphipod, <i>Metopella nasuta</i>	FD	-17.6
Amphipod, <i>Oradarea</i> sp.	FD	-14.7
Amphipod, <i>Ampithoe</i> sp.	FD	-12.4
Amphipod, <i>Calliopius laevisculus</i>	FD	-14.5
Amphipod, <i>Caprella alaskana</i> #1	FD	-14.5 -14.8
Amphipod, <i>Caprella alaskana</i> #2	FD	-16.3 -16.5
Amphipod, <i>Caprella alaskana</i> (mixture)	FD	-16.8 -17.0
Sponge	FD, Ac, OD	-18.5
Barnacle, <i>Balanus</i> sp.	FD, Ac, OD	-15.7
Shrimp, <i>Crangon dalli</i>	FD, DS	-14.1
Shrimp, <i>Crangon septemspinosa</i>	FD, DS	-14.1
Shrimp, <i>Haptacarpus camtschatica</i>	FD, DS	-12.4 -13.0
Crab, <i>Telmessus chieragonus</i>	FD, DS, M	-11.7 -11.9
Crab, <i>Telmessus chieragonus</i>	FD, DS, M	-12.6 -12.6
Crab, <i>Pagurus hirsutiussculus</i>	FD, DS, M	-17.6
Medusae, <i>Gonionemus vertens</i>	FD	-15.9

## APPENDIX C

(Continued)

Sample description	Preparation*	$\delta^{13}\text{C}$
Snail, <i>Lacuna variegata</i>	FD, Ac, OD	-10.7
Snail, <i>Margarites helycinus</i>	FD, Ac, OD	-12.8
Snail, <i>Littorina sitkana</i>	FD, Ac, OD	-12.7
Snail, <i>Thais lamellosa</i>	FD, DS	-14.6
Limpet, <i>Acmaea testudinalis</i>	FD, DS	-17.1
Clam, <i>Macoma inconspicua</i>	FD, DS	-14.4
Clam, <i>Macoma inconspicua</i>	FD, DS	
Clam, <i>Turtonia minuta</i>	FD, Ac, OD	-15.6
Clam, <i>Mya arenaria</i>	FD, DS	-16.6
Echiuroid, <i>Echiurus echiurus</i>	FD	-14.6
Polychaete, <i>Nephtys caeca</i>	FD	-15.9
Polychaete, <i>Maldanidae</i> sp.	FD	
Polychaete, <i>Arenicola glacialis</i>	FD	-15.2 -15.2
Polychaete, <i>Rhynchospio</i>	FD	-17.6
Polychaete, <i>Haploscoloplos</i> sp.	FD	-20.4
Asteroid, <i>Lepasterias</i> sp.	FD, Ac, OD	-13.9
Asteroid, <i>Evasterias</i> sp.	FD, Ac, OD	-14.9
Fish, <i>Hexagrammos octogrammus</i>	FD, M	-14.5
Fish, <i>Hexagrammos octogrammus</i>	FD, M	-14.9 -15.1
Fish, <i>Hexagrammos octogrammus</i>	FD, M	-15.9
Fish, <i>Hexagrammos octogrammus</i> (juv.)	FD, M	-17.8 -17.9
Fish, <i>Hexagrammos octogrammus</i> (juv.)	FD, M	-17.4

## APPENDIX C

(Continued)

Sample description	Preparation*	$\delta^{13}\text{C}$
Fish, <i>Hexagrammos stelleri</i>	FD, M	-14.8
Fish, <i>Hexagrammos stelleri</i> (juv.)	FD, M	-17.8
Fish, <i>Pholis laeta</i>	FD, M	-13.8
Fish, <i>Pallasina barbata</i>	FD, M	-17.1
Fish, <i>Pallasina barbata</i>	FD, M	-17.2
Fish, <i>Pallasina barbata</i>	Scales	-16.3
Fish, <i>Hippoglossus stenolepis</i>	FD, M	-17.4
Fish, <i>Lepidopsetta bilineata</i>	FD, M	-14.4
Fish, <i>Lepidopsetta bilineata</i> (juv.)	FD, M	-16.6
Fish, <i>Gasterosteus aculeatus</i>	FD, M	-19.4
Fish, <i>Pungitius pungitius</i>	FD, M	-19.1
Fish, <i>Myoxocephalus polyacanthocephalus</i>	FD, M	-13.5
Fish, <i>Microcottus sellaris</i>	FD, M	-13.8
Fish, <i>Microcottus sellaris</i>	FD, M	-14.0
Fish, <i>Microcottus sellaris</i>	FD, M	-14.1
Fish, <i>Theragra chalcogrammus</i>	FD, M	-15.6
Bird, <i>Branta canadensis</i> #1, muscle	FD	-25.9
Bird, <i>Branta canadensis</i> #11, fat	FD	-28.3
Bird, <i>Branta canadensis</i> #1, liver	FD	-24.2
Bird, <i>Branta canadensis</i> #2 muscle	FD	-21.6
Bird, <i>Branta canadensis</i> #3 muscle	FD	-21.3
Bird, <i>Branta canadensis</i> liver	FD	-14.6

## APPENDIX C

(Continued)

Sample description	Preparation*	$\delta^{13}\text{C}$
Bird, <i>Philacte canagica</i> , muscle	FD	-17.0
Bird, <i>Anas acuta</i> , muscle	FD	-17.8
Bird, <i>Anas acuta</i> , liver	FD	-12.6
Bird, <i>Anas carolinensis</i> , muscle	FD	-18.3
Bird, <i>Polysticta stelleri</i> , muscle	FD	-19.7
Bird, <i>Larus glaucescens</i> , muscle	FD	-18.7
Bird, <i>Larus glaucescens</i> , fat	FD	-16.2
Bird, sandpiper, muscle	FD	-20.8
Bird, sandpiper, fat	FD	-22.5
Mammal, <i>Ursus arctos</i> , muscle	FD	-23.1
Mammal, <i>Phoca vitulina</i> , umbilicus	FD	-19.4
Mammal, <i>Enhydra lutris</i> , muscle	FD	-17.2 -17.2
Seaweed, <i>Chorda filum</i>	FD	-14.3
Seaweed, <i>Fucus</i> sp.	FD	-14.1



## APPENDIX D

$\delta^{13}\text{C}$  of Bering Sea organisms. Replicate analyses of single samples listed together, different individuals on separate lines.

Sample	Station	Preparation*	$\delta^{13}\text{C}$
Plankton			
48 $\mu$ net "Phyto"	A-101	WS, FD	-24.5
48-216 $\mu$ "Phyto"	A-101	WS, FD	-24.3
48-216 $\mu$ "Phyto"	A- 61	WS, FD	-24.5
48-216 $\mu$ "Phyto"	D- 8	WS, FD	-24.3
112 $\mu$ net "Phyto"	A-101	WS, FD	-23.8
112 $\mu$ net "Phyto"	A-127	WS, FD	-23.7, -23.8 -24.3, -24.3
112 $\mu$ net "Zoo"	A-101	WS, FD	-24.4
216 $\mu$ net "Zoo"	A-127	WS, FD	-21.2
216-308 $\mu$	A-113	WS, FD	-22.6
216-571 $\mu$	A- 61	WS, FD	-23.0
308-571 $\mu$	A-113	WS, FD	-22.2
571-2000 $\mu$	A-113	WS, FD	-21.9
571-2000 $\mu$	A- 61	WS, FD	-19.2
Small Pelagic Crustaceans and Fishes			
<i>Parathimisto libellula</i> #1	D- 8	WS, FD	-19.9, -20.5
<i>Parathimisto libellula</i> #2	D- 8	WS, FD	-18.9, -19.3
<i>Parathimisto libellula</i> #3	D- 8	WS, FD	-19.2
<i>Parathimisto libellula</i> (7 comb.)	D- 8	WS, FD	-18.7
<i>Neomysis rayii</i> #1	D- 8	WS, FD	-20.0
<i>Neomysis rayii</i> #2	D- 8	WS, FD	-18.8
<i>Thysanoessa raschii</i> #1	D- 8	WS, FD	-19.7
<i>Thysanoessa raschii</i> #2	D- 8	WS, FD	-19.7
<i>Thysanoessa raschii</i> #3	D- 8	WS, FD	-19.6, -19.8
<i>Thysanoessa raschii</i> (mix.)	D- 8	WS, Ac, OD	-19.3, -19.4
<i>Mallotus villosus</i>	D- 40	WS, FD	-19.6, -21.0
<i>Stenobrachius leucopsanus</i>	D- 21	WS, Ac, OD	-23.9, -24.3
<i>Bathylagus stilbius schmidti</i>	D- 21	WS, FD	-20.0, -20.1
Benthic Organisms			
Unidentified ascidean	A-127	WS, FD	-20.9
Unidentified ascidean	A-127	WS, FD	-20.8
<i>Cardium ciliatum</i>	A- 61	DS, WS, FD	-19.5
<i>Cyclocardia tertiolecta</i>	A-127	DS, WS, FD	-19.0
<i>Macoma calcarea</i>	A- 61	DS, WS, FD	-18.7

\*WS=whole specimen, FD=freeze dried, Ac=acid treated, OD=oven dried, M=muscle, DS=deshelled.

## APPENDIX D

(Continued)

Sample	Station	Preparation*	$\delta^{13}\text{C}$
Benthic Organisms (cont'd)			
<i>Yoldia limatulata</i>	A-127	DS, WS, FD	-17.3
<i>Natica clausa</i>	A- 61	DS, WS, FD	-16.6
<i>Polinices pallida</i>	A- 61	DS, WS, FD	-16.3
<i>Crangon dalli</i>	D- 8	DS, WS, FD	-17.3, -17.3
<i>Crangon dalli</i>	A-127	DS, WS, FD	-17.4, -18.5
<i>Crangon dalli</i>	A-127	DS, WS, FD	-16.9
<i>Pandalus</i> sp.	A-113	DS, WS, FD	-19.1
<i>Pandalus goniurus</i>	D- 8	DS, WS, FD	-17.4, -17.6
<i>Erimacrus isenbeckii</i>	A-127	DS, WS, FD	-16.6
<i>Hyas coarctacus</i>	A-101	DS, WS, FD	-18.9
<i>Chionoecetes bairdi</i>	A-127	DS, WS, FD	-17.5
<i>Paralithodes camtschatica</i>	A-123	DS, WS, FD	-18.4
<i>Echinarchnius parma</i>	A-127	Ac, WS, OD	
<i>Evasterias</i> sp.	A- 61	Ac, WS, OD	-18.3
<i>Gorgonocephalus</i> sp.	A-101	Ac, WS, OD	-19.8
<i>Nephtys caeca</i>	A-127	WS, FD	-17.8
Bottom Fishes			
<i>Lepidopsetta bilineata</i>	A-101	M, FD	-19.9
<i>Hippoglossus stenolepis</i>	A-127	M, FD	-17.9, -18.0
<i>Gymnocanthus tricuspis</i>	A- 61	M, FD	-18.1
<i>Podotheucus</i> sp.	D- 8	M, FD	-17.5
Mammals and Birds			
<i>Odobenus rosmarus</i> , bristle	BS	(none)	-17.3
<i>Odobenus rosmarus</i> , muscle		FD	-16.9
<i>Odobenus rosmarus</i> , blubber		FD	-22.2
<i>Odobenus rosmarus</i> , (total)		(estimated)	-20.5
<i>Phoca vitulina</i> , muscle		FD	-19.4
<i>Phoca vitulina</i> , blood		FD	-19.9
<i>Phoca vitulina</i> , liver		FD	-20.0
<i>Phoca vitulina</i> , blubber oil		FD	-28.7
<i>Phoca vitulina</i> , (total)		(estimated)	-25.7
<i>Fratercula corniculata</i>			

## APPENDIX D

(Continued)

## Station Data

Station Number	Date	N. Lat.	W. Long.	Temperature °C	
				Surface	Bottom
A- 61	30/ 6/74	63°35'	172°31'	-	-
A-101	12/ 7/74	58°26'	164°22'	5.91	5.09
A-113	17/ 7/74	58°37'	159°59'	10.40	10.28
A-123	23/ 7/74	56°39'	161°54'	8.70	1.18
A-127	24/ 7/74	55°18'	163°16'	8.13	6.49
D- 8	20/ 5/75	57°48'	167°08'	-	-
D- 21	23/ 5/75	57°48'	167°25'	-	-
D- 40	25/ 5/75	58°24'	169°20'	-	-