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THE RELATIONSHIP BETWEEN TROPHIC LEVEL AND STABLE ISOTOPES OF CARBON AND NITROGEN

It is useful for both scientific and management reasons to be able to describe nutrient and energy pathways in marine food webs. Ordinarily, this is done by assigning trophic levels based on biological knowledge as described by Mearns in the previous article. Animals at higher numbered levels are generally assumed to eat those at lower levels, but even with the direct proof of stomach contents, there are some uncertainties about whether the animals found in stomachs are normal or passing-fancy prey. Extensive direct observation of feeding habits is impractical. Therefore, reliance is increasingly placed on chemical indicators of trophic level. In the past this Project has utilized cesium-potassium ratios (Young 1980); in a previous paper in this report (Schafer *et al.*) methyl mercury and chlorinated hydrocarbons have been shown to give useful relative values.

Although it has been shown that the $^{13}C/^{12}C$ of animal tissue closely resembles the $^{13}C/^{12}C$ of the animal's diet, a small but significant elevation in animal $^{13}C/^{12}C$ relative to the $^{13}C/^{12}C$ of available food has generally been observed (DeNiro and Epstein 1978, Patelle *et al.* 1979, Teeri and Schoeller 1979, Haines and Montague 1979, McConnaughey and McRoy 1979a and b, Rau and Anderson 1981, Rau *et al.* 1981). Existing evidence also indicates significant trophic-level increases in marine animal $^{15}N/^{14}N$ (Miyake and Wada 1967, Wada and Hattori 1976, Rau 1981). Therefore, if a community of several animal species existed, such that one species was the sole food source, or prey, for a second species and this second species was the sole prey of a third species, and so on; then a progressive increase in animal $^{13}C/^{12}C$ and $^{15}N/^{14}N$ would be expected as these trophic levels were ascended.

The experiments described here extended that work by establishing the relationship between trophic level and its stable isotope abundance in muscle of pelagic macrofauna from a diverse food web. These measurements generally show that both animal $^{13}C/^{12}C$ and $^{15}N/^{14}N$ increase with trophic level because of the transfer and metabolism of carbon and nitrogen in food webs. Stable isotope ratios in fish, mammal, and molluscan muscle tissue all increase relative to trophic levels estimated from other data. These findings may prove to be of assistance in understanding the flow of C and N in marine ecosystems.

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MATERIALS AND METHODS

Specimens for this study were subsampled from a much larger collection of pelagic invertebrates, fish, and mammals taken between 1978 and 1981. Sampling was concentrated in two regions: 1) coastal waters of the Southern California Bight in an area roughly bounded by Port Hueneme, San Nicholas Island, San Clemente Island, and San Diego; and 2) pelagic waters of the Eastern Tropical Pacific Ocean in the area between mainland Costa Rica and Cocos Island (roughly 5°N to 12°N and 80°W to 90°W). In the Southern California Bight several sharks were taken from the San Pedro Channel area by commercial gill net fisherman, including a 5.49 m (10^3 kg) basking shark (*Cetorhinus maximus*), a 1.2 m (205 kg) mako shark (*Isurus oxyrhincus*), and a 5.5 m (1,400 kg) white shark (*Carcharodon carcharias*). A 17.4 m ($5x10^4$ kg) blue whale (*Balenoptera musculus*), struck and killed by a ship 140 km southwest of San Diego, was sampled when the ship arrived in Los Angeles Harbor and the carcass was discovered.

Samples from the eastern tropical Pacific Ocean were taken between 11 and 29 April 1980 during Cruise 1089 aboard the M/V SARAH ANN under charter to the Inter-American Tropical Tuna Commission (IATTC). The following animals were caught: yellowfin tuna (*Thunnus albacares*), skipjack tuna (*Katsuwonus pelamis*), frigate tuna (*Auxis thazard*), silky shark (*Carcharhinus falciformis*), flying fish (*Oxyporhamphus micropterus* and *Exocoetus* sp.) and squid (*Symplectoteuthis oualaniensis*). Additional samples of flying fish were collected aboard the M/V ENTERPRISE on IATTC charter Cruise 1083 during October and November of 1978. Plankton were collected by towing a 0.3 mm mesh net at depths of 15 m to the surface for 20-30 minutes at night.

Subsequently, the stomach contents of the captured macrofauna were analyzed to provide data for computing an Index of Relative Importance (IRI) for each predator species (Mearns *et al*. 1981) and the previous paper. In general, organisms and other material were removed from the stomachs, rough sorted, identified to the lowest possible taxonomic level, and weighed by category to the nearest 0.1 g (squid beaks and small items) or 1.0 g (whole organisms). This information was used to assign a trophic level to each of the consumer species analyzed, recognizing five such trophic levels as follows: (I) primary producers (autotrophs); (II) herbivores (feed on I); (III) primary carnivores (feed on II); (IV) secondary carnivores (feed on III); and (V) tertiary carnivores (feed on IV). As described by Mearns *et al*. (1981), prey organisms found in stomachs of the above predators were assigned a trophic level based on previous knowledge of the prey's feeding habits. These assignments were weighted with the IRI's of the prey found in the consumer's gut. A numerical estimate of the predator trophic level was then derived by adding 1 to the weighted average prey trophic level. Thus, the average number of feeding steps (trophic levels) the predator was removed from the food base of the community was estimated.

Sufficient quantities of clean white muscle were taken from the individual specimens and stored frozen. Subsequent to isotope analysis, subsamples of these tissues were dried at 60°C for several days. The zooplankton were submerged in 5 N HCL prior to this drying procedure. The organic carbon and nitrogen present in 5 to 10 mg subsamples of each of the above tissues was completely converted to CO_2 and N_2 respectively, using the method of Stump and Frazer (1973). These gas samples were then analyzed on a Varian MAT 250 ratio mass spectrometer. The resultant isotope ratios of each of these gas samples (representing the isotope ratio of the original source animal tissue) is reported using conventional delta (δ) values as follows:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3 \quad o/oo,$$
$$X = {}^{13}C \text{ or } {}^{15}N,$$
$$R = {}^{13}C/{}^{12}C \text{ or } {}^{15}N/{}^{14}N,$$

Analytical precision of these measurements is approximately ±0.2 o/oo for δ13C and ±0.5 o/oo for δ^{15}N.

RESULTS AND DISCUSSION

The δ^{13}C of the marine animal tissues analyzed ranged from -20.6 to -15.8 o/oo (Table 1). The macrofauna from the Eastern Tropical Pacific Ocean had significantly higher (less negative) isotope values than the net plankton collected from the same area. Excluding the measurements of the basking shark tissue, the whale, and shark δ^{13}C values from the California coastal pelagic region also were higher than previous reports of plankton δ^{13}C in this area (-22 to -18 o/oo: Degens *et al.* 1968a, Williams and Gordon 1970, Myers 1974, Rau *et al.* 1982). This general isotopic discrepancy between plankton and the higher animals we measured may be due in part to our analysis of bulk plankton biomass rather than the muscle tissue exclusively analyzed for the other animals. Muscle and other protein-rich tissues have been shown to exhibit somewhat greater δ^{13}C values relative to those of whole organisms, particularily lipid-rich tissues (Abelson and Hoering 1961, Sackett *et al.* 1965, Degens et al. 1968a, Smith and Epstein 1970, Gormly and Sacket 1977, DeNiro and Epstein 1978, Fry *et al.* 1978). Therefore, excluding the plankton values from the following interspecies comparison, mean muscle δ^{13}C for each consumer is highly correlated with estimated trophic level within each geographic area (correlation coefficient, r, ranges from 0.77 to 0.82). This indicates that a progressive increase in animal ^{13}C/^{12}C occurs from the food base of the community to its higher consumers. The average increase in δ^{13}C per trophic level ranges from 0.84 to 1.38 o/oo, well-approximating the δ^{13} C increases previously reported to occur within single trophic level steps (DeNiro and Epstein 1978a; Patelle *et al.* 1979, Teeri and Schoeller 1979, Haines and Montague 1979, Rau and Anderson 1981).

In both the Eastern Tropical Pacific and the California coastal pelagic samples the δ^{15}N of high-trophic-level biomass is significantly larger than the δ^{15}N of the lower consumers (Table 1). However, consistent δ^{15}N increases with trophic level are evident only in the coastal pelagic animals. The smaller and more uneven trophic level ^{15}N enrichment in the eastern tropical Pacific food web may reflect spatial heterogeneity in the type and availability of inorganic nitrogen for phytoplankton production previously reported in this area (Thomas 1969).

By what mechanisms do animal δ^{13}C and δ^{15}N increase with increasing trophic level? Relative to the isotope abundance in its food, for any consumer there must be 1) a preferential assimilation of the heavier isotope into its tissues, 2) a preferential loss of the lighter isotope from its tissues, or 3) some combination of 1 and 2. In the case of carbon, selective uptake of dietary ^{13}C by animals was not indicated by the isotopic comparison between food and feces of a number of invertebrates under controlled laboratory conditions (DeNiro and Epstein 1978). As previously mentioned, significant differences in δ^{13}C have been found among animal tissues and among their biochemical constituents. These tissue and biochemical effects, however, must be a small part of the isotopic trends we are reporting because a common tissue type was analyzed from each of the consumers studied. Lastly, as effective way of enriching animal biomass in ^{13}C would be for the preferential loss of $^{12}CO_2$ during respiration. Existing data from direct and indirect measurements generally (but not always) show the ^{13}C/^{12}C of respiration CO_2 to be slightly lower than the ^{13}C/^{12}C of the respiring organism's tissues (Park and Epstein 1961, Degens *et al.* 1968b, Mosora *et al.* 1971, Smith 1971, DeNiro and Epstein 1978, Lyon and Baxter 1978). Similarly, selective metabolism and excretions of ^{14}N relative to ^{15}N (Steele and Daniel 1978, DeNiro and Epstein 1981b) may explain the trophic-level increases in animal δ^{15}N reported here and elsewhere.

TABLE 1. Carbon and Nitrogen Isotopes Data from two food webs: Eastern Tropical Pacific and Coastal Pelagic Samples

Species	Lab Number	Trophic* Level	$\delta^{13}C^o/oo$ Rep 1	Rep 2	$\delta^{15}N^o/oo$ Rep 1	Rep 2
EASTERN TROPICAL PACIFIC						
Zooplankton	586	2.0	-19.9	-20.1	9.8	9.1
	588		—	-20.6	7.9	7.6
Flying fish	534	3.0	-16.7	-16.6	10.3	11.5
	532		-16.7	-16.7	—	12.7
Squid	573	3.51	-17.6	-17.7	13.6	13.3
Frigate tuna	714	3.9	-16.6	-16.5	9.1	8.5
	715		-16.3	-16.3	7.1	8.0
Skipjack tuna	528	4.5	-16.1	—	10.4	11.2
Yellowfin tuna	523	4.3	-16.1	-16.2	13.6	13.5
Silky shark	519	4.8	-15.8	-15.8	12.6	12.7
	520		-15.8	-15.9	12.2	—
COASTAL PELAGIC						
Plankton		2.0	-18.	-22	—	—
Basking shark		3.0	-20.2	-20.4	7.9	7.4
Blue whale		3.0	-17.3	-17.9	12.4	12.7
Mako shark		4.4	-17.8	-17.4	16.5	16.8
White shark		5.0	-16.0	-15.8	17.1	17.4

*data from Mearns *et al.*, 1981.

Whatever the underlying cause(s), our observations further substantiate the existence of progressive increases in animal tissue $^{13}C/^{13}C$ and $^{15}N/^{14}N$ with increasing trophic level. Therefore, natural abundance of isotopes in animal tissues appears to be useful for determining the trophic structure and food base of animal communities.

ACKNOWLEDGEMENTS

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