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Carbon and oxygen isotopic composition of mollusk shells from marine and fresh-water environments*

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Abstract—Results are given of a systematic survey of differences in the isotopic composition of carbon and oxygen of modern mollusk shells from marine and continental environments. Marine shells analysed show a range of δC^{13} (relative to Chicago PDB standard) from +4·2 to −1·7‰, whereas the fresh-water mollusk shells have relatively C^{13}-deficient carbon, in the range $\delta C^{13} = -0{\cdot}6$ to −15·2. There is a similar difference in O^{18} content.

Within the marine group, environmental sub-groups differ mainly in O^{18} content and the differences are consistent with the temperature dependence previously studied by other investigators. Within the fresh-water group, the most striking difference between sub-groups is in the C^{13} content of pelecypod shells from large lakes ($\delta C^{13} = -2{\cdot}4$ to 6·0‰), and from rivers ($\delta C^{13} = -8{\cdot}3$ to −15·2‰), a difference which is shown to be environment-controlled rather than species-controlled. The soft parts of pelecypods also show characteristic differences of carbon isotopic composition from marine to lacustrine to fluvial specimens. The ligament of pelecypods is found to consist partly of aragonite fibers which are isotopically different from shell carbonate.

It is concluded that the carbon isotope ratio in mollusk shells is considerably influenced by the proportional amount of land-plant derived carbon included in the food of the mollusks or contributed by humus decay to dissolved bicarbonate in the water. It appears likely that the observed isotopic differences can be applied to the environmental study of fossils and sedimentary carbonate rocks, and to the source identification of shell artifacts of archaeological interest.

INTRODUCTION

THE environment of deposition of sedimentary rocks and the extent of the oceans and continents during different geologic periods has been established by the study of fossils and of regional changes in lithology. In sections where fossils are scarce, or where continental and marine beds are intercalated in a cyclic or irregular way, the problem of determining depositional environments becomes complex and it is desirable to have criteria which will supplement those based on the fossil record.

Investigations of the use of isotopic criteria for differentiating marine and fresh-water carbonate rocks have been carried out at this laboratory for several years, beginning with the work of CLAYTON and DEGENS (1959). The present study of modern mollusk shells from known marine and continental sites was undertaken as a basis for understanding and interpreting variations in the carbon and oxygen isotopic composition of fossils and limestones in relation to their environment of deposition.

STRUCTURE AND FORMATION OF MOLLUSK SHELL

Aquatic mollusks occupy a wide variety of environments, from marine to brackish to fresh-water, and tropical to arctic, and they exhibit a wide variety of preferences

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for different bottom conditions; many live in clean sand, others are mud dwellers, still others prefer to attach themselves to rocks exposed at low tide.

Most bivalves feed on minute plants, diatoms and protozoa; some, such as oysters and venus clams are suspension feeders, while others, such as *Tellina* and *Macoma*, are deposit feeders. Among the gastropods a considerable proportion are carnivorous, some are deposit feeders, others live on aquatic plants (ABBOTT, 1953). The shell of the mollusks is deposited by the mantle in successive layers with a micro-architecture which is characteristic of the genus (BOGGILD, 1930). Most marine shells and all of the fresh-water shells examined consist mainly of aragonite. Some marine species deposit a calcite shell, and a few, such as *Mytilus* and *Chama*, deposit an outer layer of calcite and an inner pearly layer of aragonite.

Pelecypods probably deposit shell mainly during the warmer part of the year, above some minimum water temperature which varies with species. Many gastropods, on the other hand, show evidence of shell deposition over a wide range of water temperatures (EPSTEIN and LOWENSTAM, 1953).

OXYGEN AND CARBON AVAILABLE TO MOLLUSKS

(*a*) *Oxygen*

Variation of the $O^{18}:O^{16}$ ratio in the hydrosphere depends mainly on the fact that the vapor pressure of H_2O^{16} is greater than that of H_2O^{18}. Oxygen-18 is relatively concentrated in ocean water and varies with salinity over a range of about 0·6% (EPSTEIN and MAYEDA, 1953). In comparison with the ocean, continental waters are relatively deficient in oxygen-18 and isotopically more variable. EPSTEIN and MAYEDA found a depletion of oxygen-18 in continental waters at progressively higher altitude and higher latitude, over a total range of about 5% in O^{18} content. The temperature dependence of oxygen isotope fractionation in exchange reactions between calcium carbonate and water, based on calculations of UREY (1947) was determined experimentally for inorganic carbonate precipitation (MCCREA, 1950) and subsequently for organic precipitation by marine organisms (UREY *et al.*, 1951). The amount of isotope fractionation changes with water temperature so that for example, at 25°C the O^{18}/O^{16} ratio is higher by 2·85% in the calcium carbonate and at 7°C by 3·27% than it is in the water (CLAYTON, 1961).

(*b*) *Carbon*

The isotopic composition of carbon in the various sources available to mollusks, is pertinent to the present study. Omitting marine limestones, with δC^{13} near zero (Chicago PDB scale), the principal inorganic reservoirs or sources of carbon, in order of decreasing carbon-13 content are: (1) ocean-water bicarbonate, with δC^{13} about −2 per mil, (2) atmospheric carbon dioxide with δC^{13} about −7 per mil, and (3) fresh-water bicarbonate with widely variable δC^{13}, generally less than −8 per mil.

REVELLE and FAIRBRIDGE (1957) quote HARMON CRAIG to the effect that marine invertebrate tests appear to contain a mixture of carbon derived from metabolic activity and carbon derived from sea water bicarbonate. WILBUR (1960) concludes that the metabolic pathway is the more important one in mollusks.

Previous Isotopic Measurements on Mollusk Shells

Oxygen isotopic data for mollusk shells (fossil or modern) have been reported by Urey *et al.* (1951), Epstein *et al.* (1953), Epstein and Lowenstam (1953), Clayton and Degens (1959) and Keith, Eichler and Parker (1960). The first reference and the last two, above, include data on carbon as well as oxygen.

Investigations of the carbon isotope ratio alone, without reference to oxygen have been reported by Craig (1953, 1954), Jeffery *et al.* (1955) and Broecker and Olson (1961) for marine mollusk shells and fossils. Limited data for fresh-water shells are given by Broecker and Walton (1959), Broecker and Olson (1959), Oana and Deevey (1960) and Keith and Anderson (1963).

The data, summarized by Graf (1960), generally confirm the suggestion of Urey (1947) regarding oxygen, and the observation of Clayton and Degens (1959) to the effect that carbon and oxygen isotope ratios can be used with some caution, as supplementary criteria to differentiate marine and fresh-water carbonates. Understanding and use of the isotopic criteria for interpretation of sedimentary environments requires that a background be established by two additional stages of investigation: (1) detailed analysis and comparison of modern carbonate samples collected from known environments, and (2) statistical studies of fossils and limestones of different geologic ages. The present paper is a contribution to the first stage.

Sample Treatment and Analytical Procedure

With a few exceptions, notably the suite from Cape May, New Jersey, mollusk specimens were collected alive. Most of the shell samples were simply air dried after removal of soft parts. A few specimens were preserved in diluted isopropyl alcohol or in formalin for a few months. Remnants of soft parts were removed by scraping and by treatment in commercial Clorox (~5% solution of sodium hypochlorite). Formalin has a slight etching effect on shells; however, it was shown that preservation or treatment with isopropyl alcohol, formalin, or Clorox have no measurable effect on the carbon or oxygen isotopic composition of shells (Epstein and Lowenstam, 1953; Eichler, 1961).

In general, large shell specimens were sampled by sawing off a diagonal slice to represent a number of growth layers. The heavy hinge region of pelecypod shells was avoided, except for very small specimens, which were sampled *in toto*. Samples for investigation of within-shell variation were obtained by sawing or grinding off the desired portions.

Cleaned carbonate material was crushed to −80 mesh and heated for 20 min at 420°C in flowing helium, to remove or pyrolize organic compounds. The resulting carbonate residue was treated with 100% phosphoric acid in evacuated tubes, and carbon dioxide evolved over a 24 hour period. The carbon dioxide was purified and collected and was analysed with a 6 in., 60-degree sector mass spectrometer, following the procedure of McKinney *et al.* (1950), in which the isotopic ratio of the sample gas is compared with that of a carbon dioxide standard. Carbon isotopic compositions are expressed as δC^{13}, the difference, in parts per thousand, between the carbon-13 content of the sample gas and the Chicago PDB standard carbon dioxide.

$$\delta C^{13} = 1000 \left(\frac{C^{13}/C^{12}_{\text{sample}} - C^{13}/C^{12}_{\text{std.}}}{C^{13}/C^{12}_{\text{std.}}} \right)$$

Oxygen isotopic compositions are expressed similarly, as δO^{18}, in terms of the O^{18}/O^{16} ratios of samples and standard. Data are corrected for the effect of O^{17} on the carbon isotope measurement and for the effect of C^{13} on the oxygen isotope measurement (CRAIG, 1957).

Duplicate carbon dioxide sub-samples were prepared and analysed at different times with a total analytical random error of less than 0·2 per mil. Reproducibility of the measurement on a single carbon dioxide sample is somewhat better than ±0·1 per mil. Recorded δ-values are the means of replicate runs.

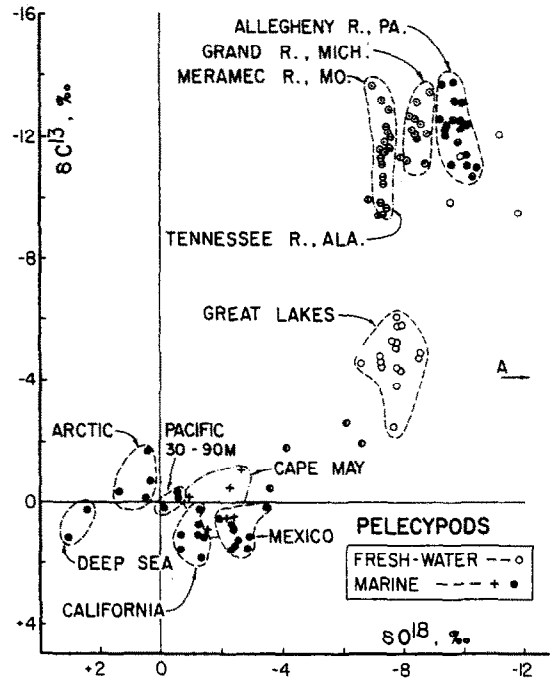

Fig. 1. Isotopic composition of marine and fresh-water pelecypod shells. Symbols within open circles indicate samples from river sites. Open circles without interior symbols indicate lacustrine samples, four of which on Fig. 1 and seven on Fig. 2 are from small lakes on the Canadian shield. Half-filled circles (Fig. 1) represent samples from intermediate salinity waters (Table 7). A = sub-Arctic samples 61-151, 152.

COMPARISON OF MARINE AND FRESH-WATER SHELLS

Carbon and oxygen isotopic data for marine and fresh-water shells are given in Table 7 and shown graphically in Figs. 1 and 2. Information regarding the collection sites is given in Table 8.

For the readers who may wish to compare the data with results reported relative to other standards, the oxygen data can be recalculated to "Mean Ocean Water" standard by the formula of CLAYTON and EPSTEIN (1958);

$$\delta_{OW} = 1{\cdot}0295\ (\delta_{PDB}) + 29{\cdot}5$$

Similarly, for recalculating to the NBS standard (Solenhofen limestone):

Carbon $\delta_{NBS\,20} = 1{\cdot}001\ \delta_{PDB} + 1{\cdot}06$ Oxygen $\delta_{NBS\,20} = 1{\cdot}004\ \delta_{PDB} + 4{\cdot}16$

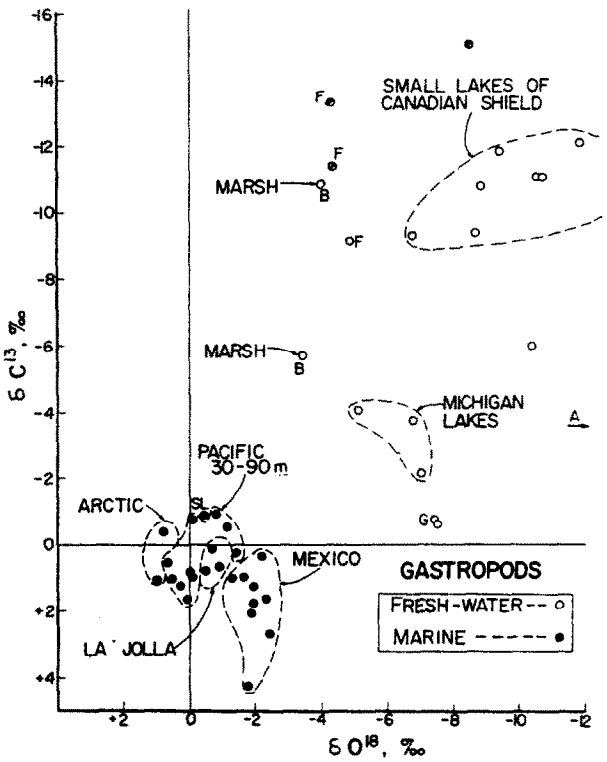

Fig. 2. Isotopic composition of marine and fresh-water gastropod shells. Symbols within open circles indicate samples from river sites. Open circles without interior symbols indicate lacustrine samples, four of which on Fig. 1 and seven on Fig. 2 are from small lakes on the Canadian shield. A = sub-Arctic samples 61–151, 152; B = Bermuda; F = Florida; SL = Scammon Lagoon, Mexico.

Table 1. Carbon isotopic composition of mollusk soft parts in comparison with shell

Sample number	Species	Soft parts δC^{13} (‰)	Shell δC^{13} (‰)	Difference (‰)
Marine samples				
62–132	*Tivela stultorum*	−17·0	+1·12	18·1
62–133	*Mytilus californianus*	−16·4	+0·24	16·6
	Mean	−16·7	+0·68	17·4
Lacustrine samples				
61–12	*Amblema costata plicata*	−22·6	−2·44	20·2
61–22	*Elliptio dilatatus*	−24·7	−4·87	19·8
61–24	*Elliptio complanatus*	−25·4	−4·52	20·9
	Mean	−24·2	−3·94	20·3
Fluvial samples				
61–202	*Actinonaias carinata*	−30·2	−11·50	18·7
61–239	*Strophitus rugosus*	−30·6	−11·12	19·5
61–240	*Strophitus rugosus*	−30·4	−11·74	18·7
61–241	*Strophitus rugosus*	−27·2	−11·44	15·8
61–242	*Strophitus rugosus*	−27·6	−11·76	15·8
	Mean	−29·2	−11·51	17·7

Combustion and measurement of soft parts by J. N. WEBER.

Pine wood, measured at the same time, for comparison, gave $\delta C^{13} = -24{\cdot}3$‰. NBS 21 (graphite) gave $\delta C^{13} = -26{\cdot}5$‰ (cf. −27·8‰ measured by CRAIG, 1957).

Table 2. Isotopic composition of interior and exterior layers of marine pelecypod shells

Sample number	"Species"	Locality	δO^{18}, ‰			δC^{13}, ‰		
			Interior	Exterior	DIFF.	Interior	Exterior	DIFF.
			In	Ex	In − Ex	In	Ex	In − Ex
62–52	*Ostrea*	Banderas, Mex.	−3·38	−3·96	+0·58	−0·20	+0·63	−0·83
62–132	*Tivela*	Balboa, Cal.	(−0·76)*	(−1·19)	+0·53	(+1·54)	(+0·66)	+0·88
62–17	*Chama*	Mazatlan, Mex.	(−1·85)	−2·39	+0·54	(+1·28)	+0·38	+0·90
62–42	*Spondylus*	Cleopha, Mex.	(−2·11)	−2·72	+0·61	(+1·02)	+0·71	+0·31
62–55	*Atrina*	G. of Mexico	(−1·33)	−1·21	−0·12	(+1·72)	+0·99	+0·73
62–73	*Atrina*	Breton Id., La.	(−2·24)	−1·37	−0·87	(+0·82)	+0·45	+0·37
62–62	*Mytilus*	La Jolla, Cal.	(−1·63)	−0·89	−0·74	(+0·16)	−0·38	+0·54
62–133	*Mytilus*	La Jolla, Cal.	(−1·40)	−0·90	−0·50	(+0·34)	+0·14	+0·20
58–262	*Mytilus*	Cape May, N. J.	(−1·46)	−0·60	−0·86	(+1·13)	−0·58	+1·71
62–211	*Mytilus*	Hudson Bay, Can.	(−3·97)	−3·17	−0·80	(−0·07)	−0·84	+0·77
	Mean Difference (without regard to sign)				0·65			0·73

* Parentheses indicate sample composed of aragonite; others are calcite.

Table 3. Isotopic composition of marine pelecypods: within-shell variation and comparison with ligament fibers

Sample number	Species	Site	Sub-sample distance from beak (mm)	δO^{18} (‰)	δC^{13} (‰)
62–62	*Mytilus californianus*	La Jolla, Calif.			
		Exterior (calcite):	30	−0·47	−0·31
			48	−1·12	−0·28
			67	−0·83	−0·06
			86	−0·94	−0·09
			99	−0·70	−0·97
			115	−1·28	−0·58
			Mean	−0·89	−0·38
		Interior (aragonite)		−1·63	+0·16
		Ligament fibers (aragonite)		−1·59	+1·69
62–132	*Tivela stultorum*	Balboa, Calif.			
		Exterior (aragonite)	18	−1·51	+0·54
			36	−0·84	+0·68
			54	−1·00	+0·67
			73	−1·13	+0·68
			93	−1·06	+0·75
			112	−1·60	+0·61
			Mean	−1·19	+0·66
		Interior (aragonite)		−0·76	+1·54
		Ligament fibers (aragonite)		−0·28	+2·42
62–52	*Ostrea iridescens*	Banderas Bay, Mexico			
		Shell (calcite and aragonite)		−3·67	+0·21
		Ligament fibers (aragonite)		−2·54	+0·25
62–133	*Mytilus californianus*	La Jolla, Cal.			
		Shell (calcite and aragonite)		−1·15	+0·24
		Ligament fibers (aragonite)		−0·76	+1·94
Average difference: (ligament fiber δ–mean shell δ)				0·47	1·21

Table 4. Isotopic composition of fluvial pelecypods: within-shell variation and comparison with ligament fibers

Sample number	Species	Site	Sub-sample distance from beak (mm)	δO^{18} (‰)	δC^{13} (‰)
61–101	*Megalonaias gigantea*	Tennessee R. Alabama			
		Exterior:	37	−7·39	−11·48
			50	−7·12	− 9·30
			58	−7·12	− 9·88
			66	−6·71	− 9·71
			83	−7·16	− 9·73
			91	−7·33	− 9·94
			97	−7·20	− 9·85
			104	−6·88	−10·20
			Mean	−7·11	−10·01
		Interior:			
		Muscle scar		−6·56	−8·48
		Middle		−7·08	−9·73
		Lip		−7·49	−9·92
			Mean	−7·04	−9·38
		Ligament fibers		−6·42	−8·10
61–127	*Actinonaias carinata*	French Creek, Pa.			
		Exterior:	20	−9·44	−11·92
			33	−9·22	−11·34
			49	−8·90	−11·69
			60	−9·60	−12·79
			73	−9·60	−14·61
			88	−9·33	−16·56
			Mean	−9·35	−13·15
		Interior:			
		Lip		−10·14	−16·25
		Hinge		−10·01	−15·62
			Mean	−10·08	−15·93
		Ligament fibers		−9·02	−13·79
61–2	*Amblema costata*	Grand R., Mich.			
		Shell		−8·55	−12·45
		Ligament Fibers		−8·12	−11·55
62–48	*Ligumia recta*	Meramec R., Missouri			
		Shell		−7·12	−13·91
		Ligament fibers		−6·72	−13·10
Average difference: (ligament fiber δ–mean shell δ)				0·54	1·01

Table 5. Variation of shell isotopic composition within communities of fresh-water pelecypods

(*a*) *French Creek, north of Meadville, Pa.*

Species	Sample number	Weight of single valve (g)	δO^{18} (‰)	δC^{13} (‰)
Actinonaias carinata	61–192	1·4	−10·34	−10·96
	61–193	2·4	−10·24	−11·29
	61–194	4·9	−10·07	−10·53
	61–195	7·7	−10·13	−11·56
	61–126	15·0	−10·10	−11·18
	61–196	17·9	−10·03	−11·66
	61–197	21·0	−9·91	−12·33
	61–198	36·7	−10·05	−13·77
	61–199	42·8	−10·01	−12·26
	61–200	68·2	−10·07	−14·72
	61–202	82·4	−10·07	−12·26
	61–201	91·4	−9·76	−14·03
	61–127	122·0	−10·14	−16·25
	Mean		−10·07	−12·52
Elliptio dilatatus (purple shell)	61–177	6·5	−10·31	−12·41
	61–178	7·8	−10·11	−11·99
	61–181	13·9	−10·20	−11·97
	61–182	19·2	−10·18	−14·04
	61–183	19·8	−9·95	−14·96
	Mean		−10·15	−13·07
Lampsilis fasciola	61–184	13·4	−9·97	−13·39
	61–188	14·1	−9·73	−12·00
	61–185	14·6	−9·93	−12·37
	61–186	16·0	−9·81	−14·02
	61–187	22·4	−9·70	−13·57
	Mean		−9·83	−13·07
Pleurobema cordatum coccineum (pink shell)	61–189	4·4	−10·26	−10·43
	61–190	14·1	−10·29	−11·57
	Mean		−10·27	−11·00
Pleurobema cordatum coccineum (white shell)	61–124	16·0	−10·25	−10·67
	61–191	30·6	−10·20	−11·51
	Mean		−10·22	−11·09
Lasmigona costata	61–123B	25	−9·94	−13·07
	61–123A	50	−9·59	−13·18
	Mean		−9·77	−13·12
Lampsilis ovata ventricosa	61–125	65	−9·54	−11·03
	61–119	118	−9·36	−12·03
	Mean		−9·45	−11·53
French Creek, mean of six species			−9·92	−12·39

Table 5. *cont.*

Species	Sample number	Weight (g)	δO^{18} (‰)	δC^{13} (‰)
(*b*) *Grand River at Dunnville, Ontario*				
Quadrula quadrula	61–211	29·7	−10·62	−10·97
	61–212	32·4	−10·57	−11·85
	61–209	46·3	−10·63	−14·30
	61–210	47·3	−10·42	−13·63
	Mean		−10·56	−12·69
(*c*) *Clinch River at Kyle's Ford, Tenn.*				
Cumberlandia monodonta	61–232	1·5	−7·99	−12·27
	61–231	5·9	−7·99	−11·44
	61–229	7·2	−7·69	−11·48
	61–228	7·4	−7·68	−11·46
	61–230	7·5	−7·50	−10·80
	61–227	9·9	−7·51	−11·28
	61–226	13·0	−7·66	−12·03
	61–224	13·2	−7·77	−11·64
	61–223	19·8	−7·52	−11·34
	61–225	21·5	−7·35	−11·31
	61–222	32·8	−7·56	−11·15
	61–221	43·6	−7·43	−10·50
	Mean		−7·64	−11·39

The data above are for samples of recently deposited calcium carbonate, from the lip of each shell.

Table 6. Isotopic composition of aragonite fibers from the ligament of fluvial pelecypods*

Sample number	Weight of single valve (g)	δO^{18} (‰)	δC^{13} (‰)
61–192	1·4	−9·38	−11·66
61–193	2·4	−9·55	−11·04
61–195	7·7	−10·33	−12·15
61–196	17·9	−12·26	−11·37
61–197	21·0	−9·72	−11·02
61–198	36·7	−9·46	−12·10
61–199	42·8	−9·35	−11·57
61–200	68·2	−9·08	−12·20
61–202	82·4	−9·18	−11·50
61–201	91·4	−9·11	−12·70
61–127	122·0	−9·02	−13·79
	Mean	−9·68	−11·92
Mean difference from lip shell of the same specimens (Table 5), ligament δ-shell δ =		+0·39	+0·78

* Size sequence of *Actinonaias carinata* collected alive from a single community: French Creek, north of Meadville, Pa. (see Table 5 and Fig. 6).

Table 7. Isotopic composition of mollusk shells

Location	Sample number	Species	$CaCO_3$ Struct.	δO^{18} (‰)	δC^{13} (‰)
(a) MARINE SAMPLE		*Cape May, New Jersey (Atlantic)*			
(1)	56–641	*Spisula solidissima* Dillwyn	a*	−1·53	−0·91
	58–257	*Tagelus plebeius* Solander	a	−2·28	−0·47
	58–258	*Ensis directus* Conrad	a	−0·95	−0·23
	58–259	*Mactra* sp.	a	−2·36	−0·53
	58–260	*Noetia ponderosa* Say	a	−2·68	−1·03
	58–261	*Aequipecten irradians* Lamarck	c	−2·26	−0·64
	58–262	*Mytilus edulis* L.	c + a	−0·90	−0·10
	Cape May, mean			−1·85	−0·06
		Shallow Mexican Waters (Pacific)			
(2)	62–2†	*Turritella gonostoma* Valenciennes	a	−2·43	−2·72
	62–4	*Fissurella viriscens* Sowerby	a	−1·90	−2·05
	62–6	*Crucibulum scutellatum* Wood	a	−1·99	−1·26
	62–11	*Cerithium maculosum* Kiener	a	−2·31	−1·61
	62–13	*Trachycardium senticosum* Sowerby	a	−2·52	−1·25
	62–14	*Anadara esmeralda* Pilsbry and Olsson	a	−1·94	−0·55
	62–16	*Pseudochama corrugata* Broderip	a	−2·84	−1·55
	62–17	*Chama* sp. (piece of large shell)	a + c	−2·12	−0·83
(3)	62–28	*Strombus galeatus* Swainson	a	−1·96	−1·54
	62–32	*Cerithium maculosum* Kiener	a	−1·98	−1·74
	62–34	*Codakia distinguenda* Tryon	a	−2·45	−1·41
	62–38	*Pseudochama corrugata* Broderip	a	−2·32	−1·52
	62–40	*Fissurella viriscens* Sowerby	a	−1·90	−4·23
	62–42	*Spondylus princeps* Broderip	a + c	−2·42	−0·87
(4)	62–52	*Ostrea iridescens* Gray	a + c	−3·00	−0·04
(5)	62–122	*Crucibulum spinosum* Sowerby	a + c	−0·46	−0·89
	62–123	*Crepidula striolata* Menke	a	−1·50	−0·90
	Shallow Mexican Waters, mean			−2·12	−1·33
		Pacific Ocean, 30 to 90 meters			
(6)	62–18	*Cantharus* cf. *capitaneus* Berry	a	−0·80	−0·90
(7)	62–19	*Cantharus* cf. *capitaneus* Berry	a	−0·16	−0·74
	62–20	*Chione kelletti* Hinds	a	−0·62	−0·14
(8)	62–22	*Crucibulum* sp. nov.	a	−0·02	−0·89
(9)	62–23	*Murex (Hexaplex) brassica* Lamarck	a	−1·34	−0·29
	62–24	*Fusinus panamensis* Dall	a	−0·08	−0·96
(10)	62–43	*Bursa nana* Sowerby	a	−1·15	−0·52
	62–44	*Chlamys circularis* Sowerby	c	−0·63	−0·07
(11)	62–190	*Lunatia lewisi* Gould	a	+0·44	−0·30
	62–192	*Kelletia kelleti* Forbes	a	+0·24	−1·26
(12)	62–193	*Mytilus californianus* Conrad	a + c	−0·12	−0·20
	62–194	*Kelletia kelleti* Forbes	a	+0·65	−0·56
	62–195	*Bursa californica* Hinds	a	+0·08	−1·64
	Pacific Ocean, 30 to 90 meters, mean			−0·27	−0·47
		Deep Sea (Pacific, 3000 meters)			
(13)	62–25	*Dentalium megathyris* Dall (Scaphopod)	a	+2·98	−1·13
	62–26	*Limopsis compressus*	a	+2·47	−0·24
	Deep Sea, mean			−2·72	−0·69

* a = aragonite, c = calcite.

† Gastropoda indicated by sample number underline. Details regarding sites are given in Table 8.

Table 7 *cont.*

Location	Sample number	Species	$CaCO_3$ Struct.	δO^{18} (‰)	δC^{13} (‰)
		La Jolla and Newport, California (Pacific)			
(14)	62–49	*Donax gouldi* Dall	a	−1·39	+1·86
	62–61, 62, 133	*Mytilus californianis* Conrad (mean of 3)	a + c	−1·15	+0·33
	62–64	*Olivella biplicata* Sowerby	a	−0·87	+0·67
	62–74	*Macoma secta* Conrad	a	−0·69	+1·55
	62–184	*Leptopecten latiauratus* Conrad	c	−0·68	+1·05
	62–185	*Nassarius fossatus* Gould	a	−0·69	+0·10
	62–196	*Haliotis fulgens* Philippi (bulk sample)*	a	−0·60	+0·63
(15)	62–132	*Tivela stultorum* Mawe (bulk sample)*	a	−0·76	+1·12
	La Jolla and Newport, mean			−0·91	+0·80
		Canadian Arctic			
(17)	62–230	*Macoma calcarea* Gmelin	a	+1·36	−0·37
	62–231	*Clinocardium ciliatum* Fabricius	a	+0·45	−0·15
	62–232	*Hiatella arctica* L.	a	+0·94	+1·26
(18)	62–233	*Portlandia arctica*	a	+0·41	−1·67
(19)	62–214	*Colus* sp.	a	+0·86	−0·36
(20)	62–217	*Buccinum* sp.	a	+0·92	+1·07
(21)	62–215	*Nuculana* sp.	a	+0·35	−0·69
	Canadian Arctic, mean			+0·75	−0·14
	56 Marine samples, mean			−0·91	+0·68
(*b*)	SPECIMENS FROM INTERMEDIATE SALINITY WATERS†				
(16)	62–211	*Mytilus edulis* Linné	c + a	−3·57	−0·46
(22)	62–197	*Ostrea* sp.	c	−4·12	−1·83
(23)	62–212	*Mytilus edulis* L.	c + a	−6·64	−1·94
	62–213	*Macoma balthica* L.	a	−6·11	−2·60
(*c*)	FRESH-WATER SAMPLES				
		Small lakes, ponds and bays			
(25)	56–644	*Elliptio complanatus* Dillwyn	‡	−9·57	−9·82
(26)	61–40	*Australorbis bahiensis* Dunker		−3·45	−5·68
(27)	61–42	*Ampullaria paludosa* Say		−4·86	−9·14
(28)	61–46	*Physa parkeri* "Currier" de Camp		−7·04	−2·10
(29)	61–48	*Physa heterostropha* Say		−11·81	−12·07
(30)	61–49	*Lymnaea emarginata angulata* Sowerby		−6·75	−3·75
(31)	61–50	*Helisoma trivolvis trivolvis* Say		−5·13	−4·02
(32)	61–51	*Planorbula armigera* Say		−8·67	−9·40
(34)	61–53	*Hydrobia bermudaensis* Pilsbry		−3·95	−10·81
(35)	61–138	*Stagnicola palustris elodes* Say		−8·80	−10·79
	61–138A	*Aplexa hypnorum* L.		−9·39	−11·93
	61–139	*Helisoma trivolvis trivolvis* Say		−6·75	−9·28

* Uncorrected radiocarbon ages: *Tivela*, 40 ± 150 years; *Haliotis*, 125 ± 150 years (Michigan analyses M-1221 and 1222), KEITH and ANDERSON (1963).

† Salinity ranges at collection sites:

Loc. (16) Hudson Bay 22‰ to 27‰ Loc. (22) Hood Canal 5‰ to 30‰ Loc. (23) James Bay 10‰ to 20‰

‡ All of the fresh-water mollusk shells are composed of aragonite, therefore crystal structure is not indicated for individual samples.

Table 7 *cont.*

Location	Sample number	Species	δO^{18}	δC^{13}
		Small lakes, ponds and bays cont.		
(36)	61–140A	*Helisoma trivolvis trivolvis* Say	−10·54	−11·04
	61–140B	*Helisoma anceps* Menke	−10·43	−11·14
	61–141	*Valvata tricarinata* Say	−10·66	−11·08
	61–142	*Anodonta grandis* Say	−11·15	−12·04
(37)	61–143	*Anodonta grandis* Say	−11·79	−9·48
(38)	61–149	*Stagnicola* cf. *palustris elodes* Say	−10·40	−6·05
	61–150	*Stagnicola* cf. *emarginata ontariensis* Küster	−9·84	−9·35
(39)	61–151	*Sphaerium nitidum* Clessin	−15·21	−4·11
(40)	61–152	*Discus* or *Zonitoides* sp.	−17·50	−3·91
(41)	61–174	*Goniobasis livescens* Menke	−10·05	−7·42
(42)	62–201	*Elliptio complanatus* Dillwyn	−9·86	−11·33
	Small lakes, ponds and bays, mean		−9·29	−8·51
		Great Lakes Suite		
(43)	56–639,640	*Goniobasis livescens* Menke, mean of 2	−7·42	−0·64
(44)	61–12	*Amblema costata plicata* Say	−7·66	−2·44
	61–14	*Proptera alata* Say	−7·94	−5·77
	61–15	*Lampsilis ventricosa* Barnes	−7·74	−3·78
	61–16	*Lampsilis siliquoidea* Barnes, (bulk sample)*	−7·59	−5·23
	61–17	*Pleurobema cordatum coccineum* Conrad	−7·74	−4·37
	61–18	*Elliptio dilatatus* Raf.	−7·75	−5·06
	61–20	*Fusconaia flava* Raf.	−7·90	−5·77
(45)	61–21	*Lampsilis siliquoidea* Barnes	−7·20	−4·75
(46)	61–22	*Elliptio dilatatus* Raf.	−8·52	−4·87
	61–23	*Lampsilis radiata* Gmelin	−8·49	−4·76
	61–24	*Elliptio complanatus* Dillwyn	−7·20	−4·52
	61–25	*Anodonta grandis* Say	−6·56	−4·53
(47)	61–85	*Lampsilis radiata* Gmelin	−7·80	−4·33
(48)	61–176	*Pleurocera acuta* Raf.	−7·27	−3·76
(49)	62–200	*Proptera alata* Say	−7·20	−4·70
(50)	62–202, 203	*Lampsilis siliquoidea rosacea*, mean of 2	−7·64	−5·21
	Great Lakes suite, Mean		−7·62	−4·23
		Rivers and Streams		
(51)	61–1	*Elliptio dilatus* Raf.	−8·37	−12·06
	61–2	*Amblema costata* Raf.	−8·55	−12·45
	61–3	*Cyclonaias tuberculata* Raf.	−8·23	−12·18
	61–4	*Fusconaia flava* Raf.	−8·42	−13·11
	61–5	*Quadrula pustulosa* Lea	−8·69	−11·06
	61–6	*Lampsilis ventricosa* Barnes	−8·10	−11·18
(52)	61–7	*Fusconaia flava* Raf.	−8·86	−13·44
	61–8	*Stroplitus rugosus* Swainson	−8·17	−12·64
	61–9	*Lasmigona costata* Raf.	−8·31	−12·61
	61–10	*Quadrula pustulosa* Lea	−8·44	−11·97
	61–11	*Elliptio dilatatus* Raf. (bulk sample)†	−8·61	−11·45

* Uncorrected radiocarbon age = 440 ± 150 years. (Michigan analysis M-1223), KEITH and ANDERSON (1963).

† Uncorrected radiocarbon age = 1890 ± 200 years (Michigan analysis M-1224), KEITH and ANDERSON (1963).

Table 7 *cont.*

Location	Sample number	Species	δO^{18}	δC^{13}
		Rivers and Streams cont.		
(53)	61–41	*Viviparus georgianus* Lea	−4·32	−11·33
(54)	61–43	*Ampullaria paludosa* Say	−4·29	−13·25
(55)	61–47	*Aplexa hypnorum* L.	−8·52	−15·17
(56)	61–101	*Megalonaias gigantea* Barnes	−7·37	−9·71
	61–102	*Amblema costata* Raf.	−7·17	−9·33
	61–103	*Quadrula quadrula* Raf.	−7·32	−10·57
	61–104	*Cyclonaias tuberculata granifera* Lea	−7·22	−9·81
	61–105	*Plagiola lineolata* Raf.	−7·35	−11·67
	61–106	*Elliptio dilatatus* Raf.	−7·23	−11·49
	61–107	*Elliptio crassidens* Lamarck	−7·35	−12·34
	61–108	*Pleurobema cordatum* Raf.	−7·20	−11·14
	61–109	*Proptera alata* Say	−7·27	−10·76
	61–110	*Quadrula pustulosa* Lea	−7·40	−12·12
	61–111	*Corbicula fluminea* Müller	−7·43	−9·61
(57)	61–113	*Lasmigona costata* Raf.	−9·40	−12·35
	61–114, 116, 118	*Actinonaias carinata* Barnes, mean of 3	−9·31	−12·52
	61–117, 119	*Lampsilis ovata ventricosa* Barnes, mean of 2	−9·24	−12·84
(58)	61–120, 1, 2	*Actinonaias carinata* Barnes, mean of 3	−10·07	−12·53
(59)	*	*Actinonaias carinata* Barnes	−10·07	−12·52
	*	*Elliptio dilatatus* Raf.	−10·15	−13·07
	*	*Lampsilis fasciola* Raf.	−9·83	−13·07
	*	*Pleurobema cordatum coccineum* Conrad	−10·24	−11·04
	*	*Lasmigona costata* Raf.	−9·77	−13·12
	*	*Lampsilis ovata ventricosa* Barnes	−9·45	−11·53
	61–127	*Actinonaias carinata* Barnes, (bulk sample)†	−9·65	−13·83
(60)	61–128, 29, 30	*Actinonaias carinata* Barnes, mean of 3	−9·97	−11·55
(61)	61–132–137	*Lampsilis siliquoidea* Barnes, mean of 6	−10·20	−11·51
(62)	61–173	*Goniobasis livescens* Menke	−7·85	−9·09
(63)	61–175	*Pleurocera acuta* Raf.	−6·69	−8·32
(64)	62–45	*Lampsilis siliquoidea* Barnes	−6·97	−13·63
	62–46	*Amblema costata* Raf.	−7·39	−11·80
	62–47	*Cyclonaias tuberculata* Raf.	−7·37	−11·42
	62–48	*Ligumia recta latissima* Raf. (bulk sample)†	−7·12	−13·91
	62–198	*Quadrula pustulosa* Lea	−7·52	−12·86
	62–199	*Actinonaias carinata* Barnes	−7·48	−12·13
(65)	56–636	*Anodonta grandis footiana* (Lea)	−7·18	−9·34
	56–637	*Lampsilis radiata* Gmelin	−6·82	−9·88
	56–638	*Elliptio complanatus* Dillwyn	−7·90	−11·30
	Rivers and streams, Mean		−8·45	−11·80
102 Fresh-water samples, mean			−8·46	−9·68

* See means of analyses in Table 5.

† Uncorrected radiocarbon ages: *Actinonaias* 1010 ± 150 years; *Ligumia* 2300 ± 200 years (Michigan analyses M-1225 and M-1226), KEITH and ANDERSON (1963).

Table 8. Collection sites of the analysed samples

Location number	Collection site	Depth and temperature	
1	Atlantic at Cape May, New Jersey	S*	(4–17°)
2	Mazatlan, W. coast of Mexico	S	(18–31°)
3	Maria Cleophas Island, Tres Marias Islands 60 miles W. of San Blas, Mexico at 21° 14′ N.	S	(21–31°)
4	Pacific coast of Mexico, N. of Banderas Bay, 20° 50′ N., 105° 30′ W.	S	(18–31°)
5	Scammon Lagoon (Ojo de Liebre), Baja California, Mexico, 27° 45′ N., 114° 15′ W.	1·8	(18–24°)
6	Pacific, off Rio San Lorenzo, Mexico, at 23° 56·2′ N., 107° 19·6′ W. (Scripps Sta. VS–BII–25)†	53	(14–28°)
7	Pacific, 23° 50·5′ N., 107° 18·2′ W. (Scripps Sta. VS–BII–26)	84	(14–22°)
8	Pacific, off Robilar, Sinaloa, Mexico, at 24° 9·0′ N., 107° 50·5′ W. (Scripps Sta. VS–BII–30)	88	(13–23°)
9	Pacific, off Puenta Piaxtla, Mexico, at 24° 35·0′ N., 106° 53·5′ W. (Scripps Sta. VS–BII–33)	46	(14–27°)
10	Pacific, E. of Tres Marias Islands, Mexico, at 21° 51′ N., 106° 10′ W. (Scripps Sta. VS–BII–39)	55	(14–28°)
11	Todos Santos Bay, Punta Banda, Baja California, 50 mi. S. of Ensenada, Mexico	31	
12	Arbeletos Cove, Punta Banda, Baja California, Mexico	33	
13	Pacific, N. W. of Tres Marias Islands, Mexico, at 22° 18′ N., 107° 48′ W. (Scripps Sta. VS–BII–35)	3000	(1·8°)
14	Pacific Ocean at La Jolla, California	S	(16–21°)
15	Pacific, off Balboa Pier, Newport, California	3·7	(15–20°)
16§	Beach near Cape Jones, Hudson Bay, Canada, at 54° 30′ N., 79° 20′ W.	S	(2–8°)
17	N. E. Hudson Bay, Canada, F. R. B.‡ Sta. 549 (1953), 63° 36′ N., 82°W.	73	(−0·8°)
18	S. E. Hudson Bay, Canada F. R. B.‡ Sta. 59–72, at 56° 11′ N., 80° 14′ W.	93	(−1·3°)
19	Hole—in Fog Bay, Ellef Ringnes Island, N. W. Territories, Canada, Lat. approx. 78°N.		(−1 to 0·5°)
20	Arctic Ocean at Mould Bay, Prince Patrick Island N. W. Territories, Canada, 76° N., 119° 45′ W.	25	(−1 to 0·5°)
21	Slide Fjord, near Alert, N. coast Ellesmere Island, N. W. T., Canada, Lat. approx. 82° N.		(−1 to 0·5°)
22§	Hood Canal near Union, Mason County, Washington State	S	(5–25°)
23§	Charlton Island, James Bay, N. W. Territories, 70 miles N. E. of Moosonee, Canada, at 51° 45′ N., 80° W.	S	(0·4 to 10·4°)
24	Shallow bay and stream inlet, Lake Ontario near Jordan, Ontario		

* Depth in meters, temperature in degrees C.
S = shallow water biologic community, intertidal to sub-littoral.

† Station of Scripps Vermilion Sea Expedition, 1959.

‡ F. R. B. = Fisheries Research Board of Canada.

§ Intermediate salinity ranges, 5 to 30 ‰.

Table 8 *cont.*

Location number	Collection site
25	Piseco Lake, 37 miles N. W. of Amsterdam, Hamilton County, New York
26	Pembroke Marsh, near Hamilton, Bermuda
27	Pond, Eastern Florida
28	Houghton Lake, Roscommon County, Michigan
29	Crystal Lake, Benzie County, N. W. Michigan
30	Higgins Lake, Roscommon County, Michigan
31	Reeds Lake, Michigan
32	Pond near Thomasburg, Hasting's County, Ontario
34	Paget Marsh, Paget Parish, Bermuda
35	Kenosee Lake, near Carlyle, S. E. Saskatchewan, Canada
36	Crooked Lake, Saskatchewan, Canada
37	Midway Lake, N. W. of Lac La Ronge, Saskatchewan
38	Belcher Islands, Hudson Bay, Canada F. R. B.* Sta. 58–34
39†	Whitefish Lake, N. W. Territories, Canada, 62° 37′ N, 106° 44′ W.
40†	Pellatt Lake, N. W. Territories, Canada, 65° 10′ N, 109° 40′ W.
41	Burt Lake, Cheboygan County, Michigan
42	Long Lake, Adirondacks, New York
43	Lake Erie, Beach at Evans Center, S. W. of Buffalo, N. Y.
44	Lake Erie at Put-in Bay, Ohio
45	Lake Erie at Athol Springs, S. of Buffalo, N. Y.
46	Lake Ontario at Sodus Bay, Monroe County, N. Y.
47	Lake Huron at Bruce Beach, near Kincardine, Ontario
48	Lake Erie at Sterling State Park, near Monroe, Michigan
49	Lake Erie at East Harbour Beach, near Sandusky, Ohio
50	Georgian Bay, Lake Huron, near Midland, Ontario
51	Grand River, Michigan, at 2 miles E. of Ionia
52	Grand River, Michigan, at 1 mile W. of Saranac
53	Silver Spring, Marion County, Florida
54	Wakulla River, near Wakulla Springs, Florida
55	Shallow ditch near Higgins Lake, Michigan
56	Tennessee River at mile 303, near Decatur, Alabama
57	French Creek at Cochranton, Pennsylvania
58	French Creek at 2 miles N. of Meadville, Pennsylvania
59	French Creek at 5 miles N. of Meadville, Pennsylvania
60	Allegheny River between Warren and Kinzua, Pennsylvania
61	Black Creek, Monroe County, New York
62	Stream, inlet to Zukey Lake, Lakeland, Livingston County, Michigan
63	Little Portage River, Washtenaw, on Washtenaw–Livingston County line, Michigan
64	Meramec River, Meramec State Forest Park, Franklin County, Missouri

* F. R. B. = Fisheries Research Board of Canada.

† Specimens from locations 39 and 40 are from stomach samples of a fish: *Coreganus clupeaformis.*

Complete salinity data are not available for most of the collection sites. Samples included in the marine group are from normal marine waters with salinities in the range of 30 to 36‰. Data at hand show a salinity range of 34·4‰ to 35·0‰ for Pacific Ocean sites (2) to (13) off the Mexican coast, 33·3‰ to 33·7‰ for locations (14) and (15) in the near coastal Pacific off southern California, and 30‰ to 32·5‰ for locations (17) to (21) in the Canadian Arctic.

The fresh-water species which were analysed are from relatively shallow lake and river sites (less than 2 meters deep); no data were obtained regarding the range of water temperatures. Three sites, locations 16, 22 and 23 are in waters of intermediate salinity, between 5 and 30‰.

The shells of modern fresh-water mollusca, including pelecypods (Fig. 1) and gastropods (Fig. 2) have oxygen and carbon isotope ratios different from those of marine shells; the fresh-water shells are relatively deficient in both oxygen-18 and carbon-13.

Oxygen

Within the marine group, the principal differences from one environmental sub-group to another are in the oxygen isotope ratio. It is evident that the differences are in accord with the well-known temperature dependent oxygen isotope fractionation between calcium carbonate and ocean water. The highest oxygen-18 content is found in the shells from deep sea and Arctic Ocean sites, the lowest in the shells from warm Pacific waters off the coast of Mexico.

The fresh-water shell samples are O^{18}-deficient, relative to the marine samples, an expectable consequence of the H_2O^{18}:H_2O^{16} fractionation which takes place during evaporation and precipitation in the weather cycle (EPSTEIN and MAYEDA, 1953). The latitude effect observed by those authors, i.e. a progressive depletion of oxygen-18 in rain and surface waters from higher latitudes, is observable in the fresh-water shell samples, for example, in the sequence from the Meramec and Tennessee River shells (lat. 35° to 38°N.) to those from the more northerly Grand River and Allegheny River (lat. 42° to 43°N.) A similar latitude-dependent variation of oxygen isotopic composition can be seen in the gastropod shells (Fig. 2); the highest oxygen-18 content is found in the fresh-water shells from Bermuda and Florida, (indicated by "B" and "F" in Fig. 2) and the lowest in gastropod shells from northern lakes of the Canadian Shield. Two of the specimens from sub-arctic lakes,* marked "A" in Figs. 1 and 2, have extreme oxygen isotopic composition, (δO^{18} = −15·2 and −17 per mil), well beyond the scale of the figures.

Carbon

The analysed marine and fresh-water shells have different ranges of carbon isotopic composition; the marine shells fall between +4·2 and −1·7 per mil,† and the fresh-water shells between −2·1 and −15·2 per mil, with two exceptions.

* Samples 61–151, 152,

† The compositional range of the marine shells is similar to that reported by CRAIG (1954). He records one extreme analysis (δC^{13} = −3·8 per mil) for a pecten shell specimen from Apalachee Bay, Florida, a marginal marine environment in which some continental carbon contribution from river waters is to be expected.

Two fresh-water gastropod shells ("G" on Fig. 2), both *Goniobasis* from eastern Lake Erie, have carbon isotope compositions which overlap the range of marine shells. H. VAN DER SCHALIE of the University of Michigan reports (personal communication) that *Goniobasis* feeds on the aquatic plants growing on boulders in shallow water. It is possible that they may ingest some calcium carbonate from the rocks, or more probably, from the carbonate precipitated on some types of aquatic plants, which is relatively enriched in carbon-13 (CRAIG, 1953).

Among fresh-water shells, the most striking difference between environmental sub-groups is in the carbon isotope ratio. Shells from rivers (Table 7 and Figs. 1 and 2) are relatively deficient in carbon-13, with a mean δC^{13} of $-11{\cdot}9$ per mil, in comparison with those from large clear lakes, such as the Great Lakes (mean $\delta C^{13} = -4{\cdot}3$ per mil). The shells from brown bog-water lakes* have carbon isotopic compositions which overlap the range of river specimens.

The observed carbon-13 deficiency of fresh-water shells, and the relative carbon-13 deficiency of river shells in comparison with those from large lakes, probably are due mainly to a variable land plant and humus contribution to fresh-water systems. C^{13}-deficient carbon resulting from carbon fractionation by land plants can be contributed in two principal ways: by the pathway of carbon dioxide from plant respiration and humus decay added to ground water and streams, and by the incorporation of humus particles, soil microbes and derived material in the food web of aquatic animals.

BAERTSCHI (1951), CRAIG (1954) and CLAYTON and DEGENS (1959) referred to the expected effect on fresh-water carbonates of CO_2 from decaying organic matter. KEITH, EICHLER and PARKER (1960) proposed that the food web, rather than dissolved bicarbonate in the water, may be the main direct source of carbon in shell carbonate. The problem of environment-controlled differences in carbon isotope ratios was re-examined by KEITH and ANDERSON (1963), utilizing data on C^{14} content as well as C^{13} content of marine and fresh-water shells; their results support the probability that the land plant and humus reservoir of carbon has an important effect on continental waters and on the carbon isotopic composition of fresh-water shells.

CONSIDERATION OF POSSIBLE VITAL EFFECTS

A firm conclusion regarding environmental control of carbon isotope ratios of shells requires that we examine an alternative explanation: the possibility that the observed differences may be due primarily to vital effects, differing from one species to another, rather than to environmental differences. The analysed marine and fresh-water specimens are of different genera of course, and most of those from river sites are different genera and species from those collected in lakes. The question is whether different species may fractionate carbon isotopes to different degrees and thus exhibit differences which could erroneously be attributed to environment.

In order to resolve that question, we have made a study of isotopic variability within and between communities of mollusks. A comparison of three mollusk species

* Four open circles clustered around the Allegheny River data (Fig. 1) represent pelecypod shells from Precambrian shield lakes of Canada and the Adirondack area; their carbon isotopic compositions are similar to those of gastropods (Fig. 2) from small lakes of the Canadian Shield.

from a river community with the same three species from a lake community* is presented graphically in Fig. 3. In each case, several samples of exterior shell carbonate were analysed, in a progression from older to younger shell, in order to show within-shell variability.

It is evident that the largest and most significant difference between the analysed lake and river shells is environment-controlled rather than species-controlled. The three species from the Grand River in Michigan have shells with a mean δC^{13} of

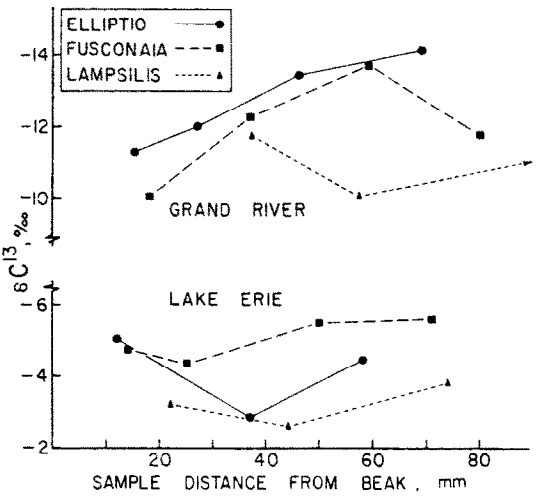

Fig. 3. Carbon isotopic composition of three pelecypod species from a lake and a river. Mean δO^{18} values of exterior shell subsamples of *Elliptio*, *Fusconaia* and *Lampsilis* are −8·68, −8·80 and −8·31 per mil for specimens from Grand River, Michigan; −7·71, −7·94 and −7·55 per mil for the Lake Erie specimens.

−12·0 per mil, whereas the same three species from Lake Erie have shells with a mean δC^{13} of −4·1 per mil, with no overlap between the lacustrine and fluvial shell data.

CARBON ISOTOPIC COMPOSITION OF SOFT PARTS

Carbon isotopic composition of the soft parts of invertebrates have previously been reported, for example by CRAIG (1953), BROECKER and OLSON (1961), but the data on fresh-water mollusks are very limited and it appeared desirable to compare lake and river specimens with marine specimens. Two marine, three lacustrine and five fluvial specimens of air dried pelecypod flesh were oxidized to yield carbon dioxide samples following the procedure of CRAIG (1953). Isotopic analyses, compared with carbon dioxide from shells of the same specimens, are presented in Table 1.

Two observations appear to be worthy of mention: firstly, the carbon isotopic composition of pelecypod flesh samples are characteristic, as are the shell compositions, of the environment in which the animals lived. There are three distinctive groups with δC^{13} decreasing in sequence from marine to lacustrine to fluvial samples. Secondly, the mean isotopic difference between shell and soft parts is about the same, 17 to 20 per mil, regardless of environment. In view of the limited number of samples,

* Collected with the advice of HENRY VAN DER SCHALIE of the University of Michigan.

the greater difference between shell and soft parts of lacustrine specimens is not regarded as significant.*

It is concluded that vital effects are less important than environmental effects in controlling the carbon isotopic composition of pelecypod soft parts and shell.

Fibrous Aragonite in Ligaments

In connection with the study of mollusk shells, an examination was made of the external ligament of several species of marine and fresh-water pelecypods. The ligaments consist of closely-packed parallel fibers embedded in a brown organic matrix. Fibers make up the bulk of the interior portion of the ligaments examined but are absent in the exterior portion and adjacent to the attached shell surfaces. If the brown organic matrix is removed by solution in Clorox (5% solution of sodium hypochlorite), the fibers separate from one another; they are silky and flexible, give the X-ray diffraction pattern of aragonite, and dissolve with effervescence in cold dilute HCl.

It is of interest that the ligament fibers are composed of the aragonite form of calcium carbonate in all specimens examined, regardless of whether the shell also is aragonite, as in *Tivela* (Table 3) and fresh-water pelecypods (Tables 4 and 6), or whether the shell is wholly or in part composed of calcite, as in *Chlamys* (No. 62–44) or the *Ostrea* and *Mytilus* specimens examined (Table 3).

Carbon and oxygen isotopic compositions of ligament fiber samples are given in Tables 3, 4 and 6. With the exception of the oxygen in one *Mytilus* specimen (62–62), the ligament fibers are enriched in both oxygen-18 and carbon 13, in comparison with average shell isotopic composition of the same specimens. The average difference of isotopic composition between shell and ligament (8 specimens) is about 0·5 per mil for δO^{18} and about 1 per mil for δC^{13}.

Invertebrate ligaments probably deserve further study. They may contribute appreciable quantities of fine-grained acicular aragonite to detrital carbonate sediments. For the present investigation, the significant observation is simply that shell carbonate and ligament fiber carbonate are isotopically different. It follows that the calcium carbonate precipitated by pelecypods probably is laid down in equilibrium with local environments created by the animal, and does not necessarily attain isotopic equilibrium with the external environment. Isotopic differences between exterior and interior shell layers, discussed in the succeeding section, lead to the same conclusion.

Within-Shell Variation of Isotopic Composition

The variability of carbon and oxygen isotopic composition within single shells was investigated as a necessary basis for choosing a method of sampling and for understanding the differences within and between biologic communities.

Exterior versus interior shell of marine pelecypods

As a first step, exterior and interior shell samples were analysed (Table 2), beginning with an oyster shell (62–52) in which interior and exterior are calcite, and a

* Broecker and Olson (1961) give $\delta C^{13} = -22$ per mil for *Margaritifera* flesh, $\delta C^{13} = -4{\cdot}8$ per mil for the shell, i.e. a shell-flesh difference of 17·2 per mil for a pelecypod from the outlet of Lake Tahoe.

clam shell (62–132) in which both are aragonite. The remaining data in Table 2 are for specimens in which the exterior layer is calcite and the interior pearly layer is aragonite.

The difference $\delta O^{18}_{interior} - \delta O^{18}_{exterior}$ is negative for specimens of *Atrina* and *Mytilus*, positive for single specimens of four other pelecypod species analysed. It would, of course, require more extensive sampling to establish a generalization regarding species-controlled differences.

The difference: $\delta C^{13}_{interior} - \delta C^{13}_{exterior}$ has a positive sign in all of the analysed marine pelecypod shells except one, an oyster (62–52) with complicated shell structure possibly due to extreme changes of environment during its growth. Although the exterior and interior layers are calcite, several irregular intermediate layers are composed of aragonite.*

Neither the oxygen nor the carbon isotopic data of Table 2 show any consistent difference which can be correlated with the aragonite or calcite form of shell carbonate.

Exterior shell layers of marine mollusks

A second stage of the investigation of isotopic variability within single shells involved analyses of exterior shell sub-samples in sequences from older to younger shells. For individual marine pelecypods, discussed above, the variation of oxygen and carbon isotopic composition of exterior shell is less than one per mil and the range within any one specimen does not overlap the composition of interior-shell. This is not the case for marine gastropods.

Two large marine gastropod shells were studied in some detail. One of them, *Lunatia* from 34 meter depth off the Pacific coast of Mexico, shows a relatively small changes of isotopic composition in successive samples of exterior shell, possibly due to a nearly constant environment. The exterior shell is relatively deficient in carbon-13 ($\delta = -0{\cdot}44‰$), as compared with the interior pearly shell ($\delta = -1{\cdot}04‰$), a difference similar to that observed in marine pelecypod shells.

The other large gastropod, a 9 in. long conch shell (*Strombus*) from 1·5 meter depth in the Florida Keys, shows systematic changes of both oxygen and carbon isotopic composition in a sequence of exterior shell sub-samples ground to less than 1 mm depth from the tips of successive spines. The data are shown graphically in Fig. 4. *Oxygen* isotopic composition exhibits a cyclical variation which almost certainly is related to seasonal changes of water temperature, and is analogous to the cyclical change observed by UREY *et al.* (1951) in a belemnite. There are two complete cycles of change of δO^{18} (Fig. 4), which leads to the rather surprising conclusion that this large specimen of *Strombus gigas* was only two years old.†

The conclusion that *Strombus* grows and deposits shell carbonate at a very rapid rate is supported by the observation of the University of Miami Marine Laboratory,

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* A separated aragonite sub-sample gave $\delta O^{18} = -2{\cdot}63$ per mil (appreciably different from exterior and interior shell) and gave $\delta C^{13} = +0{\cdot}12$ per mil, intermediate between the carbon isotopic compositions of exterior and interior layers.

† Previous estimates give a much longer life span; for example, ABBOTT (1953, p. 21) states that large specimens probably represent ten to twenty-five years of growth.

that tank cultured *Strombus* grow from the larval stage to a 2 in. length in about 5 weeks (R. JOHNSON and L. GREENFIELD personal communication).

It should be noted that our method of sampling to obtain an ordered sequence of growth increments of conch shells is entirely different from that adopted by EPSTEIN and LOWENSTAM (1953), who sampled *Strombus* shells by cutting out a 5 × 5 mm rectangular column from the lip shell. They analysed successive layers ground from that columnar piece, beginning at the inside, and interpreted the resultant sequence of oxygen isotopic analyses as representing seasonal growth temperatures over a

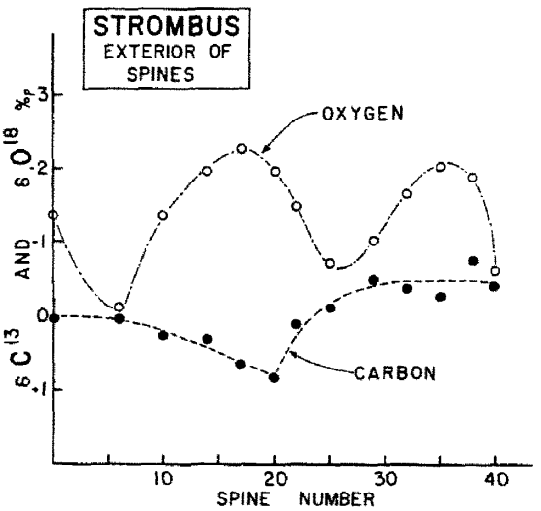

Fig. 4. Within-shell isotopic variation of a marine gastropod: *Strombus gigas*.

period of years. It now appears that their sampling procedure and interpretation should be applied only to mature specimens. *Strombus* individuals which have reached maximum size add to the thickness of lip shell by depositing new layers on the inside; however, if specimens grow to a nine-inch length in about two years, then a small segment of lip shell would represent, for most specimens, growth of only a part of one season. In that connection, EPSTEIN and LOWENSTAM recognized that the calculated range of about 8°C in shell growth temperature of a *Strombus* specimen from Bermuda was considerably less than the 14° annual range of water temperatures. By contrast, the range of 2·16 per mil in δO^{18}, which we find by sampling the exterior spines of a *Strombus* from the Florida Keys, is equivalent to a shell growth temperature range of about 10°C, very close to the actual range of water temperatures at our collection site.

The carbon isotope ratio of the exterior spine carbonate changes within a narrower range (Fig. 4). There is an abrupt change of trend at about mid-point in the sequence of spine samples, i.e. at the point taken to represent the end of the first year of the animal's life span. Possibly a migration or change of feeding habits took place at that time.

Exterior shell layers of fresh-water pelecypods

The average range of oxygen-18 content (minimum to maximum) within the external layers of single fresh-water pelecypod shells (Table 4) is only 0·65 per mil,

similar to the range of variation in two marine pelecypod shells (Table 3), and probably reflecting a general tendency for pelecypods to deposit shell carbonate only within a limited range of water temperature (cf. EPSTEIN and LOWENSTAM, 1953).

The carbon-13 content of fresh-water pelecypod shells varies over a much wider range; external shell sub-samples (Table 4) show an average within-shell variation of 2·6 per mil in δC^{13}. The largest within-shell variation of carbon-13 content encountered (5·2 per mil) is that of a fluvial pelecypod, *Actinonaias* (Table 4 and Fig. 5).

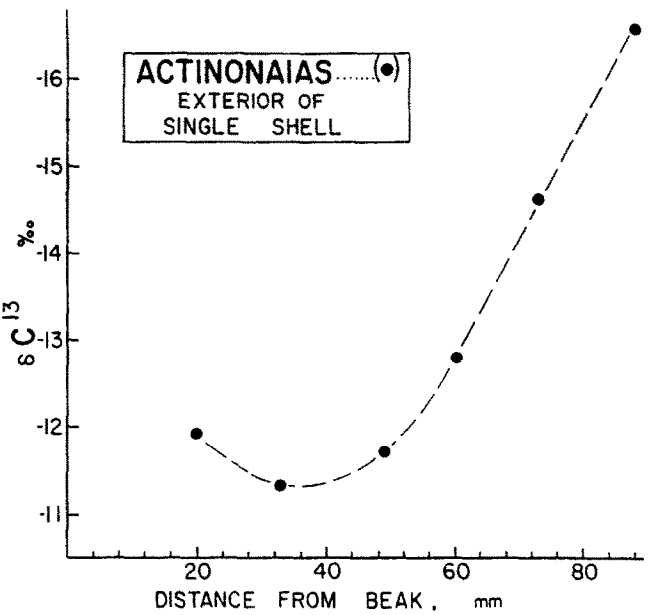

Fig. 5. Within-shell carbon isotopic variation of a fluvial pelecypod: *Actinonaias carinata*.

The sequence of change in that shell appears to be quite regular; δC^{13} increases slightly at first, and then decreases from −11·34 to −16·56 per mil in the most recently deposited shell carbonate.

The observed within-shell changes of carbon isotope ratio could be due either to a change of carbon isotopic composition in the local carbon cycle of the river environment or to a change of feeding habits of the pelecypods. Evidence from an examination of within-community variation, discussed in a succeeding section, leads one to choose the second alternative: a change in feeding habits.

The sampling problem

The largest within-shell variation of δO^{18} is in a marine gastropod, whereas the largest variation of δC^{13} is found in fresh-water pelecypods. The most extreme ranges of variation are such that sampling of a whole shell or a small portion of shell would not influence the isotopic differentiation of marine from fresh-water shells nor the differentiation of lacustrine and fluvial pelecypod shells. Our practice of sampling larger shells by cutting a representative slice across growth bands and including both inner and outer shell layers, appears to be a defensible compromise.

However, it is clear that investigations of within-species or within-community variability can best be made by sampling the entire shell of specimens to be compared, or by careful selection of comparable samples taken from a particular part of each shell.

Within-community Variation of Shell Isotopic Composition

The present work includes studies of three marine and seven fresh-water mollusk communities,* from each of which six or more cohabitant specimens were analysed. It is considered that the other sites listed in Table 7 have not been sampled adequately to permit evaluation of within-community variation. Data reported in Table 7 are for samples obtained by grinding an entire shell of small specimens or a representative cross section of large shells.

Oxygen

The within-community range of δO^{18} is less than one per mil for shells from each of the above noted communities, both marine and fresh-water. The average *within-community* range is 0·59 per mil, essentially the same as the average *within-shell* range of δO^{18} for pelecypods. Our data show, on the average, a slight enrichment of oxygen-18 in marine gastropod shells, relative to the shell of cohabitant pelecypods.† The differences are small but they are consistent and in the same direction as those observed by Epstein and Lowenstam (1953), who attributed the difference to a tendency of pelecypods to deposit shell only during the warmer part of the year. The sampling problem is particularly difficult for gastropods, in which within-sheil variation may be considerably larger (Fig. 4) than the mean differences between gastropods and pelecypods.

Our data do not show any pronounced differences in oxygen-18 content among pelecypod shells of different cohabitant species. Detailed study of recently deposited lip shell from river clams of the French Creek community (Table 5), shows some differences of δO^{18}, for example between cohabitant *Lampsilis* and *Pleurobema*, but the differences are small and possibly are due to differences in the temperature range over which the animals actively deposit shell carbonate. At a more southerly site, in the Tennessee River (Loc. 56, Table 7), shells of eleven different species of pelecypods gave δO^{18} between −7·17 and −7·43 per mil, i.e. nearly identical within the limits of measurement.

Carbon

Within-community variation of δC^{13}, based on analyses of representative cross sections of shells, is much larger than that of δO^{18}. The average within-community range of δC^{13} for three marine collection sites (2·45 per mil) is about the same as the average range for seven fresh-water sites (2·21 per mil).

The observation that carbon isotopic composition may vary widely within a single shell of some fresh-water pelecypods (e.g. Fig. 5) prompted us to modify

* Table 7, marine locations 2, 3 and 14, fresh-water locations 44, 51, 56, 57, 59, 61 and 64. Marine location 1 is not considered here because the specimens are dead shell from the beach and represent an unknown range of offshore depths.

† Mean δO^{18} (in per mil) of gastropods (G) and pelecypods (P)....Loc. 2: G(−2·16), P(−2·71); Loc. 3: G(−1·95), P(−2·40); Loc. 14: G(−0·72), P(−1·04).

our sampling procedure to allow a better comparison from one specimen to another. Data in Table 5 are analyses of rectangular pieces cut from the lip of each shell outside of the palial line, to represent shell laid down during approximately the same period of time.

The data show some differences in carbon isotopic composition from one species to another. For example, lip shell samples of *Elliptio*, *Lasmigona* and *Lampsilis fasciola* from the French Creek community (Table 5) have average δC^{13} assays near

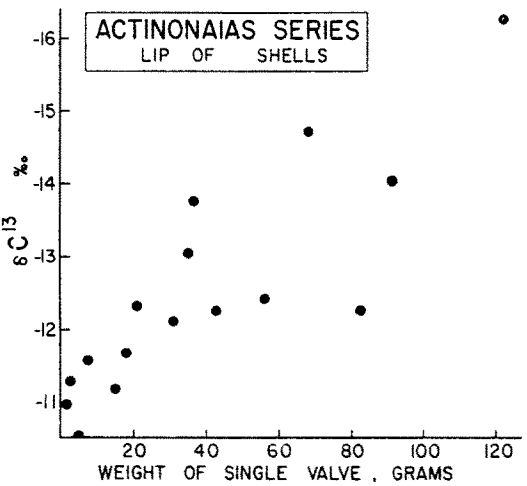

Fig. 6. Variation of shell carbon isotopic composition within a community of fresh-water pelecypods: *Actinonaias carinata*.

−13 per mil, whereas lip shell samples of cohabitant pink and white *Pleurobema* have an average δC^{13} near −11 per mil, with no overlap into the range of δC^{13} of the other three species.

The maximum observed within-community differences of δC^{13} are between small and large individuals of the same species rather than between one species and another. The isotopic composition of a size sequence of *Actinonaias* from one biologic community* was investigated in some detail. The analytical results (Table 5 and Fig. 6) show a progressive decrease in carbon-13 content of lip shell samples with increasing size of the animal, over a range of δC^{13} from −10·5 per mil to −16·3 per mil. A similar tendency, i.e. for lip shell deposited by large individuals to be depleted in carbon-13 relative to shell concurrently deposited by small individuals, can be observed in data for other species from French Creek (Table 5) and also in the *Quadrula* sequence from the Grand River at Dunnville, Ontario. The size sequence of Cumberlandia from the Clinch River is quite different from the others and exhibits an irregular contrary trend, toward increase of δC^{13} in shell laid down by larger individuals.

It is noteworthy that the range of δC^{13} of lip shell concurrently deposited by a size sequence of cohabitant *Actinonaias* individuals (Fig. 6) is essentially the same as the

* Specimens collected alive from the French Creek, 5 miles north of Meadville, Pa.

range of δC^{13} observed within the exterior layers of a single large shell (Fig. 5). Therefore, the differences cannot be attributed to a change of carbon isotopic composition in the environment. We propose that the variation is probably a consequence of a change of feeding habits, which takes place gradually as the animal gets older. It is known from tank culture of oysters that they reject some food particles and digest others (H. J. TURNER, Woods Hole, personal communication) and a similar selective ability probably is a general attribute of pelecypods.

CARBON ISOTOPIC COMPOSITION IN RELATION TO MOLLUSK METABOLISM AND TO SOURCES OF CARBON

It has been shown that there are wide differences of carbon-13 content between marine and fresh-water mollusks and between lake and river mollusks, and the differences between environmental groups can be observed by comparing δC^{13} of shells, or equally well by comparing δC^{13} of ligament fiber aragonite or mollusk flesh. It has been shown that there are systematic differences of δC^{13} between exterior and interior shell layers and between shell and ligament fiber carbonate. In addition, there are differences of carbon-13 content among the shells of cohabitant mollusks of different species, and even larger differences between small and large individuals within a biologic community.

It appears from the evidence, as recapitulated above, that the observed broad scale differences of carbon isotope ratio are controlled mainly by the isotopic composition of the external environment. Smaller scale within-mollusk and within-community differences probably are dependent upon internal environments maintained by the mollusk, and can best be explained by the hypothesis that the organic carbonate is not deposited in carbon-isotopic equilibrium with dissolved bicarbonate in the water (CRAIG, 1953). The present evidence appears to favor an additional or corollary hypothesis, namely that the carbonate carbon in shell and ligament is mainly metabolic and is derived from the food web rather than directly from dissolved bicarbonate.

The land plant and humus reservoirs of carbon affect the bicarbonate, the food web and the biologic communities of continental waters in several stages, with consequent C^{13}-deficiency in those of continental waters relative to those of the ocean and in those of river waters relative to those of lakes. In a first stage, a part of the CO_2 from terrestrial plant respiration and from oxidation of humus in soils will be added to ground water and thence to rivers and to lakes. That CO_2, with δC^{13} of about -26 per mil, will lower the average carbon-13 content of bicarbonate in continental waters, more in rivers than in lakes, and may be regarded as opposing the effect of exchange of atmospheric CO_2($\delta C^{13} = -7$ per mil) across the air–water interface (BROECKER and WALTON, 1959) as well as the more local effects of dissolution of marine limestones (δC^{13} close to zero) and of fermentative CO_2 from bottom muds (OANA and DEEVEY, 1960).

Atmospheric CO_2, with δC^{13} about -7 per mil, is an important contributor to the carbon cycle in lakes, but probably plays a minor part in the formation of bicarbonate in ground waters contributing to rivers, due to the low CO_2-content of the atmosphere (0·03 vol%), too low to account for the solution of carbonates and silicates in the

amounts necessary to produce hard ground waters. In contrast with the above figure, soil gases may contain about 0·3 vol. % of CO_2 within the upper 10 cm and up to 10% or more at a depth of 1 or 2 meters (VOGEL, 1959).

A second stage, additional to the ground water effect, will result from the oxidation of humus within the waters and sediments of rivers and lakes. The net effect of the two stages or paths of addition of land plant carbon will vary according to the nature of vegetation and soils in a drainage basin and may be very small in barren areas. In general the land plant effect and consequent lowering of C^{13} content in bicarbonate and dependent food web will be large in rivers because of large proportional amounts of suspended humus and of ground water,* and smaller in lakes, where much of the humus has been removed by settling and where the long residence time of water permits prolonged exchange with atmospheric CO_2.

A third stage humus effect is suggested, based upon the hypothesis that mollusk shell carbonate is mainly derived from metabolic CO_2, and therefore is affected by the carbon isotopic composition of the food web and by food selectivity of the mollusks. The change of shell δC^{13} of some fluvial pelecypods with age (Fig. 6) is in a direction to be expected if they adapt themselves gradually to utilize a larger proportion of humus and humus-derived materials.† It is to be expected that the food web in humus-laden streams will be less homogeneous than that of large clear lakes. If fluvial pelecypods adapt themselves to select and digest humus-derived components in preference to bicarbonate-dependent components of the food web, the selective process will accentuate the carbon isotopic differences between pelecypods from lakes and from rivers.

Radiocarbon assays of mollusk shells, discussed briefly in a succeeding section, are consistent with the hypothesis that the C^{14} content as well as the C^{13} content of bicarbonate, food web and mollusks, is affected by contributions of carbon from the humus reservoir to continental waters.

The major differences of δC^{13} in mollusk shells are between environmental groups (see Figs. 1, 2 and 3) and are clearly environment controlled; the smaller scale differences attributed to metabolism and food selectivity of mollusks do not result in appreciable overlap in the range of carbon isotopic composition of marine, lacustrine and fluvial shells.

ARCHAEOLOGICAL CONSIDERATIONS AND RADIOCARBON DATING

The present work has a bearing on archaeology in two respects. Firstly, it is apparent that measurement of carbon and oxygen isotopic composition of shell fragments or artifacts provides a means of determining whether they came originally from the ocean, from lakes or from rivers (Fig. 1). In some instances it may be possible to go a step further and to differentiate between alternative river sources of

* Most streams are fed mainly by ground water, except during the short run-off period following a rain; in addition, they are continually re-working soil materials from run-off and river bank erosion.

† If the observed change of δC^{13} with age is the result of a change of feeding habits, it would appear that *Cumberlandia* (Table 5) differs from *Actinonaias* and from the other fluvial pelecypods studied in size sequence, and does not adapt itself to utilize increasing amounts of humics. Perhaps there is a difference in the origin and evolutionary adaption of *Cumberlandia* compared to the other species.

shell material. The data show, for example, that one can differentiate Tennessee River shells (latitude 34°N) from Allegheny River shells (latitude 42°N).

Secondly, measurement of both carbon-13 and carbon-14 content of modern shell samples provides some insight regarding the assumptions upon which radiocarbon dating is based. Three river shells, three marine shells and one lacustrine shell,* all from specimens collected alive, were submitted to the Michigan Radiocarbon Laboratory for measurement of radiocarbon age.

Radiocarbon assays reported separately (KEITH and ANDERSON, 1963) show a variation of C^{14} which parallels that of C^{13} and is apparently a dilution effect rather than a fractionation effect; fresh-water shells are C^{14}-deficient relative to marine shells and river shells are C^{14}-deficient relative to lake shells. The three fluvial specimens, with a mean δC^{13} of $-13{\cdot}06$ per mil gave radiocarbon ages of 1010 to 2300 years, attributed mainly to incorporation of inactive carbon from humus, probably via the food web as well as by the pathway of carbon dioxide from humus decay.

Local C^{14} deficiency of a fresh-water system may be due to incorporation of inactive carbon from limestones, as suggested by GODWIN (1951). The effect was demonstrated by DEEVEY *et al.* (1958) and the idea was extended by BROECKER and WALTON (1959), who attributed variations in the C^{14}/C^{12} ratio of fresh-water bicarbonate to two factors: (a) the relative amounts of carbonate and silicate dissolved, and (b) the rate of exchange with atmospheric CO_2. In view of the present results, including the widespread characteristic difference of δC^{13} between lake shells and river shells, it appears more logical to attribute the differences of both C^{13} and C^{14} content mainly to a variable humus contribution, in competition with the atmospheric CO_2 contribution, and to regard dissolving carbonate-bearing rocks as having a superimposed local effect in drainage basins where limestones are abundant. On this basis, appreciable errors of radiocarbon ages are to be expected for fresh-water shell samples, whether or not they are from a limestone area. Maximum errors probably will be encountered in dating shells from mollusks which lived in streams which were actively eroding old flood plains or well developed soil profiles.

APPLICATION TO FOSSILS

The existence of a characteristic range of carbon isotopic composition for modern fresh-water mollusk shells and the conclusion that the difference between them and marine shells is due mainly to a land plant and humus effect in continental waters, leads to the expectation that the difference should be diagnostic in well-preserved molluscan fossils from rocks formed as far back as the Carboniferous, when land plants had reached a stage of extensive development. That conclusion is in accord with the data of CLAYTON and DEGENS (1959), whose fossil and limestone samples were mainly of Carboniferous age, and in accord with a recently completed extension of their work to samples of different geologic ages (KEITH and WEBER, in press).

A schematic representation of depositional environments in relation to variation of carbon and oxygen isotope ratios of pelecypod shells is presented in Fig. 7, as a means of summarizing the various effects which have been discussed. Figure 7 is

* Marine specimens 61–155, 62–132, 62–196; Fluvial specimens 61–11, 61–127, 62–48; Lacustrine specimen 61–16 (see Table 7).

partly conjectural but is based mainly on present and previous work, including the ocean paleotemperature studies at Chicago and elsewhere, the work of EPSTEIN and MAYEDA (1953) on oxygen-18 in continental waters and the work of CRAIG (1953, 1954), CLAYTON and DEGENS (1959) and others on variation in the carbon isotope ratio. Data on mollusk shells from transitional environments will be presented in a separate paper (KEITH and PARKER, in preparation). The figure is presented with overlapping environmental fields and without numerical scales because

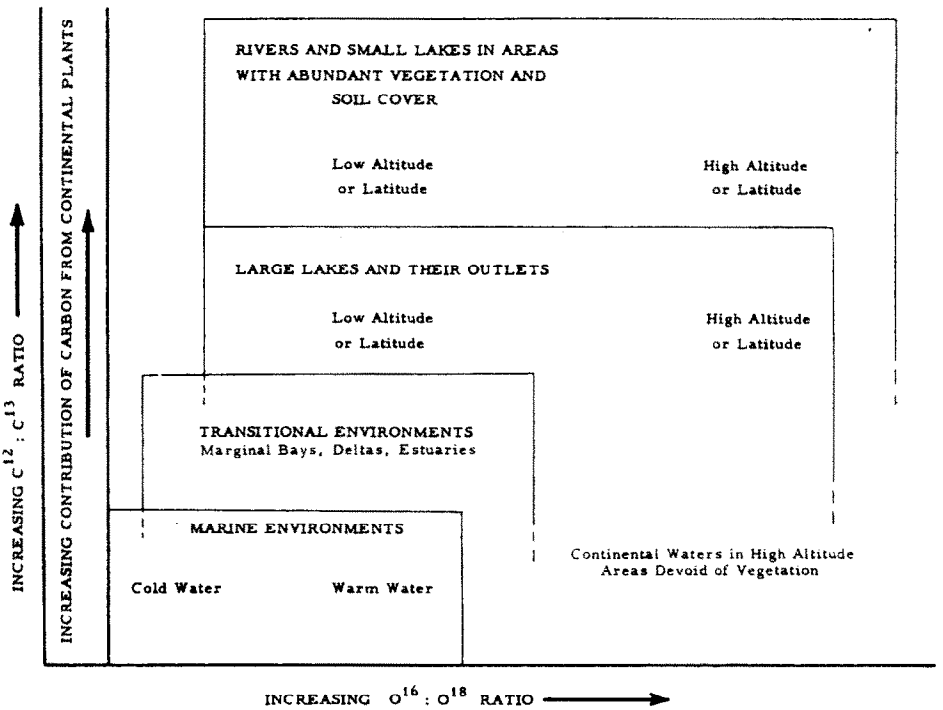

Fig. 7. Idealized relationships between environment and shell isotopic composition of mollusks.

it is intended to show only directions of change. There are many complicating factors and many exceptions are to be expected.

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