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INTERPOPULATION VARIATION IN CALCAREOUS AND PROTEINACEOUS SHELL COMPONENTS IN THE STREAM LIMPET, *FERRISSIA RIVULARIS*[1]

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ABSTRACT

Ten natural populations of the North American stream limpet, *Ferrissia rivularis,* were studied in upstate New York, in a set of localities whose waters have a 15-fold range of dissolved calcium (4.6 to 67.6 mg/liter) and also range from oligotrophy to eutrophy.

Shell component analyses (calcium carbonate, total organic carbon, and total nitrogen) are reported both as component mass-fractions (mg/g or μg/g dry weight) and as values for a "standard limpet" shell of 3.5 mm aperture length (AL). More than twofold differences occur between populations in all three components, with relatively little variation occurring within each population. Expressed "per standard limpet," $CaCO_3$ values for different populations range from 0.8 to 1.97 mg with no direct relationship to environmental dissolved calcium. Nominal "concentration ratios" of body calcium to environmental calcium range from 1,953:1 to 29,130:1. Values for total organic carbon (9.13 to 21.0 μg) and total nitrogen (2.7 to 6.69 μg) in the shells parallel each other, all C:N ratios being relatively uniform (3.0:1 to 3.4:1), and indicating that the non-calcareous components are largely proteinaceous. Although alternative hypotheses predict an inverse or a direct relationship between the organic and the calcareous components, neither is shown by these populations.

It appears that genetic controls of shell secretion for the two major components are independent, and that chance dispersal has resulted in some "rather inappropriate shells in certain habitats." This irregular variation in *Ferrissia* is first discussed in relation to other patterns of shell component relationships known for other freshwater molluscs, including direct relationship of the mass of shell calcium carbonate to the dissolved calcium available as in *Lymnaea peregra* and *Laevapex fuscus* and the apparent "regulation" producing standard shell weights in *Lymnaea palustris* and *Physa gyrina.* The results are then discussed in relation to assessment of radionuclide pollution using molluscan shells from fresh waters and in their more general relationship to modes and rates of evolutionary change in freshwater faunas.

INTRODUCTION

Natural populations of freshwater pulmonate snails show extensive infraspecific physiological variation between populations (Russell-Hunter, 1964, 1978). Aspects of this in growth, fecundity and respiration have been reported (Burky, 1970, 1971; Hunter, 1975a, b; McMahon, 1973, 1975a, b; Russell-Hunter, 1953, 1961, 1964) and its evolutionary significance discussed (Russell-Hunter, 1964, 1978). There can also be interpopulation variations in shell components in several species, and the present report concerns these in the freshwater limpet, *Ferrissia rivularis* (Say).

In freshwater pulmonates—as in the majority of molluscs—the secreted shells have two principal components: a meshwork of protein fibers (the organic matrix) and crystalline calcium carbonate (Degens, Spencer & Parker, 1967; Jones, 1969; Russell-Hunter, Meadows, Apley & Burky, 1968; Russell-Hunter, Burky & Hunter, 1970). The latter is secreted in greater part after active uptake directly from environmental water, and in a lesser fraction after assimilation from food. In the euryoecic species, *Lymnaea peregra,* the thickness (and mass) of the calcareous shell varies with the calcium available in the waters (Hubendick, 1947; Russell-Hunter, Burky & Hunter, 1970). Thus, it appears that *Lymnaea peregra* ex-

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pends about the same energy on shell-making no matter what the environmental hardness. A second pattern found in *Lymnaea palustris* (Hunter, 1975b) and in *Physa gyrina* (Hunter & Lull, 1977) involves somewhat more "regulation": over a considerable range of environmental calcium values, populations have shells of approximately "standard" weight at all growth stages. The case of the stream limpet, *Ferrissia rivularis,* in natural creek populations of upstate New York is strikingly different from both of these patterns. Although these creek waters vary over 15-fold in dissolved calcium, the highly significant differences in shell calcium found to exist between populations are not correlated (Russell-Hunter, Apley, Burky & Meadows, 1967; Russell-Hunter, Burky & Hunter, 1970). Anabolic concentration ratios appeared to range from 1,609:1 to 10,615:1 and there was other circumstantial evidence of physiological races. These data on shell calcium content (for limpets from six creeks and one lake) reported in these two earlier notes require some correction, as a result of the improved methods described below, but the significant differences and lack of environmental correlation remain as claimed. More recently, we have measured total organic carbon and total nitrogen in shells of limpet growth stages from ten natural populations, and published a preliminary abstract on seven of them (Russell-Hunter, Burky & Hunter, 1970). Subsequently, the shell calcium content for the same ten populations was redetermined. We now report environmental water conditions and the calcium, organic carbon and nitrogen contents of limpet shells for nine creeks and for Oneida Lake. After computing these values in terms of "standard" limpets to allow more direct comparisons, we discuss several hypothetical relationships which might be expected to affect variation in shell components, compare the available data for other species, and end by briefly reviewing the nature and significance of this kind of interpopulation physiological variation in freshwater molluscs.

MATERIALS AND METHODS

The freshwater basommatophoran limpet, *Ferrissia rivularis* (Say), is ubiquitous in appropriate stream habitats in northeastern North America. In upstate New York, this species lives in waters ranging in calcium content from 4.6 to 67.6 mg per liter (total hardness values range from 25 to 243 mg calcium carbonate per liter), and we have collected regular population samples for other biometric studies from 53 localities. The ten localities providing populations of limpets for the present study are (in order of decreasing hardness): Limestone Creek (LC) near Manlius, Canandaigua Outlet (CO) at Alloway, Chittenango Creek (CC) at Cazenovia, a section of the shore of Oneida Lake (OL) at Shackleton's Point, Chenango River (CR) at Randallsville, Big Bay Creek (BBC) near Central Square, Fish Creek (FC) below Westvale, Slocum Creek (SC) at West Monroe, Black Creek (BC) above Cleveland, and Morgan's Hill Creek (MHC) near Truxton. All of these localities are in the central or "upstate" section of New York State (exact latitudes, longitudes, quadrangles and county references can be provided on request). CR and MHC are in the drainage system of the Susquehanna River which eventually empties into Chesapeake Bay. The waters at CO drain into the Clyde division, and LC, CC, BC, FC, SC, BBC and Oneida Lake (OL) itself into the Oneida division, of the Seneca-Clyde-Oneida drainage which passes by way of the Oswego River into Lake Ontario and then on to the St. Lawrence.

The environmental concentrations of dissolved calcium and magnesium were analyzed by an EDTA (ethylenediaminetetraacetate) titration, and total hardness also determined chemically at the same time. Independently the average total hardness was determined from conductivity measurements of samples made on every visit throughout the year.

The aperture length of each limpet shell (AL) was measured by stage micrometer in 0.1 mm class intervals (Russell Hunter, 1961). Weights of shell calcium carbonate (and of "ash-free" tissue dry weights) were determined on whole limpets (starved for 48 hours). Analyses of shell organic carbon and nitrogen were run on limpet shells from which the tissues had been removed. For dry weights and shell weights, two procedures were followed. Selected individual limpets were oven-dried at 98°C to constant weight, then transferred to a muffle-furnace at 475°C for 105 min. This provided an ash weight (almost entirely shell $CaCO_3$ in starved or laboratory-fed animals), a total dry weight, and by subtraction an ash-free dry weight (or tissue weight). Other individuals were oven dried at 98°C to constant weight and then treated with an ex-

cess of 12% nitric acid (8.5% HNO_3) and then washed and redried, giving two dry weights (whole limpet and tissue) and by subtraction a value for dissolved calcium carbonate. Earlier studies on limpets (Russell Hunter, Apley, Burky & Meadows, 1967) had utilized 3% nitric acid (2.2% HNO_3) which gives successful decalcification in other snails (Hunter & Lull, 1976, see also Richards & Richards, 1965). In *Ferrissia,* the results of muffle-furnace ashing could not be reconciled with those for decalcification with 3% nitric acid. A series of trials with limpets from BC and MHC (and with stocks of *Helisoma trivolvis,* see Russell-Hunter & Eversole, 1976) showed that significantly higher values for shell calcium resulted from decalcification with 8.5% HNO_3, and that these agreed with values obtained by ashing. [For Black Creek limpets, the following linear regressions of "total shell calcium carbonate" (S) in mg were computed: by 2.2% HNO_3, $S = -1.44 + 0.635$ AL (*r*-value of 0.95); by 8.5% HNO_3, $S = -1.95 + 0.927$ AL (*r*-value of 0.98); and by 475°C ashing, $S = -1.92 + 0.933$ AL (*r*-value of 0.99).] Two sets of calcium analyses (each on ten limpets from BC) by the chloranilic acid method gave values closely corresponding to the 8.5% HNO_3 regression (Dr. Christopher H. Price, unpublished). A series of additional tests revealed that a maximum of only 1.3% of the "total shell calcium carbonate" resulting from our standard 475°C ashing could not subsequently be dissolved by 8.5% HNO_3 (Dr. Jay S. Tashiro, unpublished). There were no significant systematic differences between ashing whole animals, and ashing shells alone. With larger limpets (approximately 4.0 mm AL and larger), less than 3% of the "ash" weight was attributable to the limpet bodies when these were separated from the shells; and, with smaller sizes of limpets suitably starved, there was no detectable difference between "ash" vales for whole limpets with shells and values for shells alone. Although we have data from ashing for three of the populations discussed here (BC, MHC, and CC), the results presented in detail below for the ten populations and used in subsequent computations are all derived from decalcification with 8.5% HNO_3.

Total organic carbon was determined on batches (selected by aperture length) of limpet shells using a wet oxidation colorimetric method (Russell-Hunter, Meadows, Apley & Burky, 1968). Values for smaller limpet shells (e.g., at AL = 2.2 mm) had to be determined on batches of 24–27 individual shells of each cohort size. Analyses of total combined nitrogen on selected batches of shells were made on a Coleman model-29 semiautomatic nitrogen analyzer which employs a modified micro-Dumas method as described by Gustin (1960). Subsequent computational methods mostly utilized linear regressions. For some kinds of comparisons, shell $CaCO_3$, tissue dry weight, shell C, and shell N can be computed in terms of a "standard" limpet of modal size (3.5 mm AL), as read off from regressions for each of these components against shell size. Aperture length (AL) in limpets such as *Ferrissia rivularis* and *Laevapex fuscus* is a better measure of size (age) than similar shell measurements on other planorbid or turbinate snail species. Finally, all population samples used in the analyses were collected in early summer (thus avoiding any early spring complications from overwinter degrowth, see Russell-Hunter & Eversole, 1976) and, as already noted, all samples involving tissues had been starved for 48 hours (thus avoiding the complications of inorganic gut contents, see Hunter & Lull, 1976).

RESULTS

In Tables 1–4 and in Fig. 1, data are arranged from left to right in order of increasing calcium concentration of the habitat waters (from MHC to LC). The conditions of the abiotic environment are set out in Table 1, including concentrations of dissolved calcium and magnesium, the average pH, and the altitude. In Table 1 are also set out assessments of the trophic state of the habitats, of the limpet densities, and of the pattern of life-cycle involved. As with other freshwater molluscs, there can be infraspecific variation in the number of generations per year in different populations of *Ferrissia rivularis* (Burky, 1971), and in similar pulmonates including ancylid limpets (Russell-Hunter, 1964, 1978, and references therein). In the present set of ten populations, six have a simple annual life-cycle, two (CC and LC) have two generations with incomplete replacement (that is, representatives of both spring-born and late-summer born generations survive overwinter), and two (CR and CO) have two generations with complete replacement (that is, only the second or late-summer generation overwinters to breed in the next year).

The results of analyses of shell components

TABLE 1. Environmental conditions for ten localities in upstate New York where natural populations of *Ferrissia rivularis* were studied (the assessments of trophic productivity are: E = eutrophic, M = mesotrophic, O = oligotrophic; the limpet densities are H = high, M = medium, L = low and are given for two annual generations at CR and CC; and the limpet populations have either single annual generations, 1, or two generations per year with complete, 2C, or incomplete, 2I, replacement).

Population site	MHC	BC	SC	FC	BBC	CR	OL	CC	CO	LC
Dissolved calcium (mg/liter)	4.6	10.4	12.3	13.6	14.8	35.8	42.2	44.8	66.7	67.6
Dissolved magnesium (mg/liter)	0.9	3.2	2.9	4.9	4.5	5.4	7.2	11.0	18.7	14.6
Total hardness (mg $CaCO_3$/liter)	25	39	48	54	59	133	145	156	243	199
Average pH	7.23	7.11	7.29	7.19	7.29	8.23	8.30	8.01	8.07	8.06
Altitude (ft. above sea level)	1,175	465	385	535	380	1,110	370	1,180	410	605
Trophic assessment (E, M, O)	O	O	M	O	M	E	E	M	E	M
Comparative limpet density (H, M, L)	H	H	H	M	M	L/M	L	L	L/H	M
Generations of limpets per year (1, 2I, 2C)	1	1	1	1	1	2C	1	2I	2C	2I

TABLE 2. Shell calcium carbonate content for ten natural populations of *Ferrissia rivularis* in upstate New York. For further explanation, see text.

Population site	MCH	BC	SC	FC	BBC	CR	OL	CC	CO	LC
Number of limpets	18	55	41	32	60	28	46	41	35	49
Number of batches	6	8	7	4	6	5	6	5	6	6
Limpet length range (mm)	3.1-4.9	2.0-4.6	2.0-5.9	1.9-4.7	1.9-4.2	2.4-4.3	2.3-4.8	2.1-4.6	1.8-4.8	2.3-4.2
Mean limpet length (mm)	3.68	3.17	3.01	2.92	2.86	3.13	3.18	2.86	3.20	3.02
Mean shell $CaCO_3$ (mg)	1.62	.993	.704	.547	.464	.656	.760	.578	1.30	.603
Shell $CaCO_3$/limpet DW (mg/g)	670	649	698	621	636	696	699	721	749	674
Shell $CaCO_3$/standard limpet (mg)	1.34	1.29	1.18	.800	.801	.938	.998	1.97	1.65	1.32

TABLE 3. Shell organic carbon content for ten natural populations of *Ferrissia rivularis* in upstate New York. For further explanation, see text.

Population site	MHC	BC	SC	FC	BBC	CR	OL	CC	CO	LC
Number of limpets	56	129	86	77	69	77	55	75	81	114
Number of batches	4	8	6	6	6	5	4	5	5	6
Limpet length range (mm)	2.5-4.6	2.1-5.1	1.6-5.6	1.4-4.9	1.7-4.9	2.1-4.9	2.4-4.8	2.0-5.5	1.5-4.9	1.6-5.4
Mean limpet length (mm)	3.29	3.46	3.21	3.07	2.98	3.42	3.46	3.68	3.11	3.64
Mean shell organic carbon (μg)	10.6	21.2	9.02	8.78	8.56	10.7	11.7	14.4	7.09	10.9
Shell C/Shell DW (μg/mg)	9.51	17.7	10.2	12.4	11.7	8.04	11.1	6.88	8.50	5.40
Shell C/standard limpet (μg)	12.6	21.0	10.9	11.7	12.3	11.3	12.1	12.3	9.13	10.1

TABLE 4. Shell nitrogen content and C:N ratio for ten natural populatons of *Ferrissia rivularis* in upstate New York. For further explanation, see text.

Population site	MHC	BC	SC	FC	BBC	CR	OL	CC	CO	LC
Number of limpets	59	119	85	76	58	92	46	52	81	110
Number of batches	5	8	6	5	5	6	4	4	5	6
Limpet length range (mm)	2.3-4.6	2.0-4.8	1.4-5.3	1.3-4.6	1.9-5.1	1.7-5.0	2.3-5.0	1.6-5.0	1.7-5.0	2.5-5.4
Mean limpet length (mm)	3.45	3.47	3.16	2.96	3.08	3.39	3.52	2.77	3.11	3.99
Mean shell nitrogen (μg)	3.79	6.62	2.95	2.50	2.99	3.07	4.11	2.11	2.34	4.42
Shell N/Shell DW (μg/mg)	2.98	5.41	3.35	3.92	3.66	2.36	3.55	2.12	2.80	1.82
Shell C:N ratio	3.2	3.3	3.0	3.2	3.2	3.4	3.2	3.3	3.0	3.0
Shell N/standard limpet (μg)	4.02	6.69	3.64	3.47	4.18	3.28	3.96	4.72	2.89	2.70

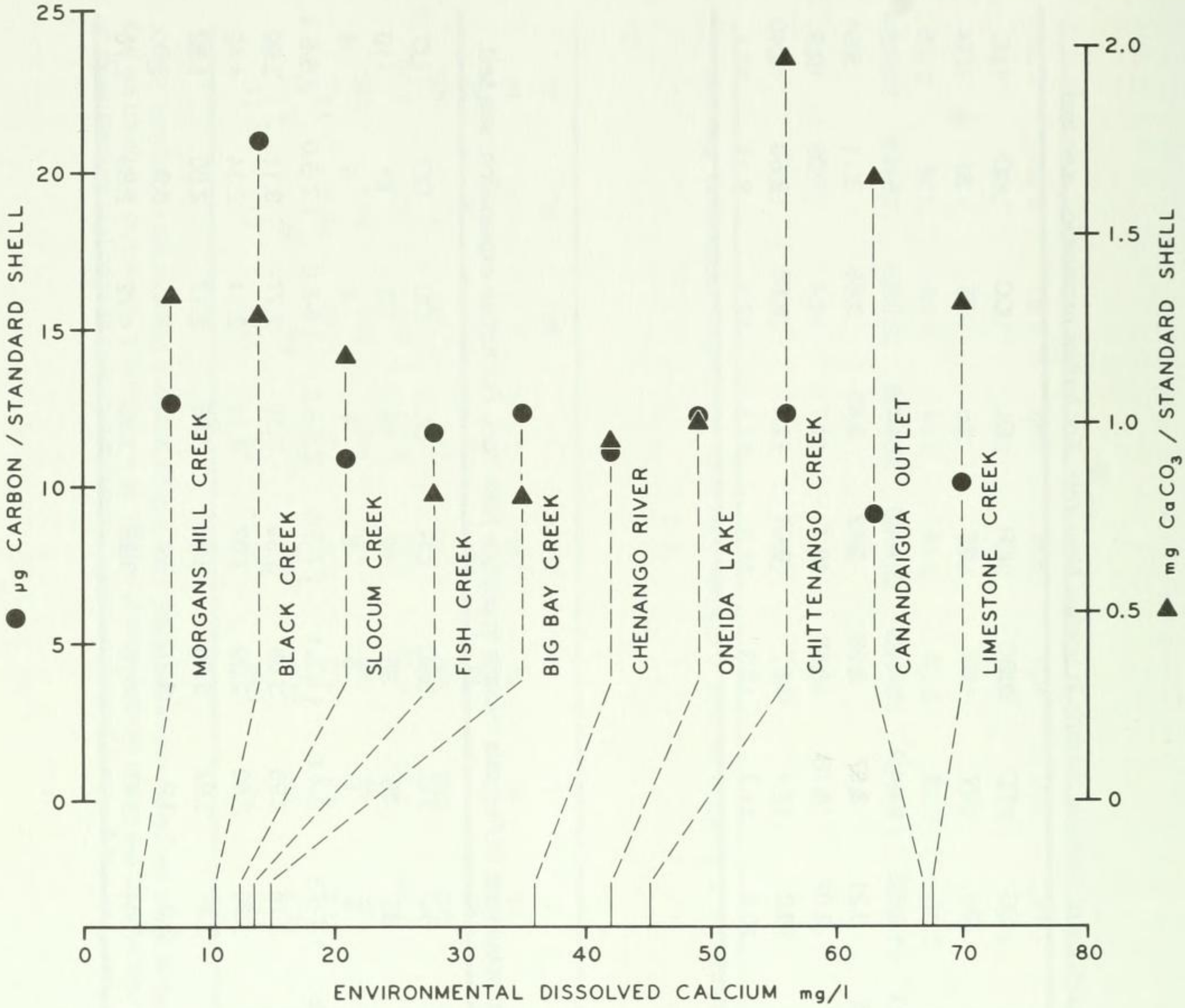

FIG. 1. Summary of shell component data for ten natural populations of *Ferrissia rivularis* in upstate New York. Shell carbon values (filled circles) are shown in µg organic carbon per standard shell (of 3.5 mm limpet derived from regression values). Shell nitrogen values closely parallel these (the noncalcareous component appears to be pure protein). Shell $CaCO_3$ values (filled triangles) are shown in mg per standard shell. Note that the values are only *ranked* in order of increasing concentrations of environmental dissolved calcium; actual values for calcium content (mg/liter) being shown to scale only on the abscissa.

(shell calcium carbonate, total organic carbon, and total nitrogen) are set out in Tables 2–4. They are shown in the lower lines of the tables computed both as component fractions (mg/g) of dry weight (of limpets for $CaCO_3$, and of shell for organic C and for total N), and as values for the "standard limpet" of 3.5 mm AL. The 32 regression equations of components against shell size are not set out here, but can be made available to any interested investigator.

As shown in Table 2, there can be a twofold difference in mass of calcium carbonate (FC at 0.80 and CC at 1.97 mg) in the standard limpet. There is clearly no direct relationship to environmental dissolved calcium. Further, computaton of (somewhat arbitrary) "concentration ratios" between environmental calcium content and the calcium contents of whole limpet tissues (as wet weights—not shown in Table 2) show that these can range from 1,953 (LC) to 29,130 (MHC). This is clearly an interpopulation variable of considerable bioenergetic significance.

As shown in Table 3, the organic carbon content of *Ferrissia* shells also shows a more than twofold range, whether expressed as dry weight component fractions (mg/g) or as values for the "standard" limpet. The highest carbon content is found in the shells of the BC population, the lowest in CO (or LC depending on method of computation). Carbon val-

ues show no direct relationship with environmental dissolved calcium, and neither a direct nor an inverse relationship to shell calcium. Further, there is no obvious relationship of shell organic carbon content with the assessed productivity of the environment, or even with the productivity of the limpets themselves (which can be crudely assessed in terms of generations times densities, rather than simple densities). However, organic carbon content does correlate rather closely with total shell nitrogen.

As shown in Table 4, the total shell nitrogen content can vary over nearly a threefold range. The highest nitrogen content is again at BC while the lowest (by both computations) is at LC. The dry weight component values for nitrogen (line 7, Table 4) can be used with those for organic carbon (line 7, Table 3) to produce carbon:nitrogen (C:N) ratios for the limpet shells. These are all relatively uniform in the range 3.0:1 to 3.4:1 (an average value for pure animal protein would be C:N = 3.25:1, Brody, 1945; Russell-Hunter, 1970), and we can conclude that the noncalcareous component of these limpet shells is largely proteinaceous. In fact, the individual shell nitrogen values are closely parallel to those for shell organic carbon, and require no further separate discussion. Accordingly, Fig. 1 presents the relationship between only three variables for the ten population-sites: the mean values for dissolved calcium in the environmental waters, the noncalcareous components of the limpet shells as micrograms organic carbon per standard shell, and the calcareous component as milligrams $CaCO_3$ per standard shell.

DISCUSSION

Infraspecific (interpopulation) physiological variation in growth, fecundity, life-cycle pattern and respiration, as reported for many freshwater molluscs, appears in a number of cases to be based on distinct genotypes ("physiological races"). In such cases, transfer experiments between population-sites have shown that particular patterns of fecundity or ratios of shell dimensions are retained by "foreign" stocks introduced to other sites where the "native" snails have different characteristics. In other cases, notably of growth rates and size at maturity, stocks transferred to waters of markedly different hardness or trophic conditions take on similar external characteristics to those shown by the natural population at the sites.

Apart from genetic controls of shell secretion, variation in the two principal components of the shells of freshwater snails—the crystalline calcium carbonate and the organic matrix—could be seen as depending upon available energy for shell-making (reflecting trophic conditions) and available precursors for components (reflecting on the one hand, available environmental calcium, and on the other hand, trophic conditions once again). A starving snail would not secrete much shell protein. Our present studies cannot discriminate among the noncalcareous components of the limpet shell, all shell protein (including both organic matrix fibers and periostracal sheets, and perhaps encompassing a polysaccharide fraction) being expressed as total organic carbon or as total combined nitrogens. Future work may allow both finer biochemical discrimination and more specific structural allocation. Recent X-ray diffraction studies (Weiner & Traub, 1980) have confirmed that the fibers of the organic matrix are a silk-like β-fibroin protein, as earlier suggested by studies on amino acid residues by Degens and his associates (Degens, Spencer & Parker, 1967; Ghiselin, Degens, Spencer & Parker, 1967; Degens, 1976), and by ultrastructural studies (Jones, 1969). Similarly, it is almost certain that the polysaccharide fraction found in certain mollusc shells is chitin. For the limited purposes of this discussion, secretion of *all* components of the organic fraction of the shell can be regarded as energy-consuming and dependent on trophic input.

Populations of molluscs with calcareous shells are found in fresh waters with more than 100-fold range in concentrations of dissolved calcium (Boycott, 1936; Russell-Hunter, 1964, 1978). Several workers have presented clear experimental evidence from laboratory cultures of direct effects of calcium concentration on the growth and fecundity of snails (Williams, 1970b; Harrison, Williams & Greig, 1970; Thomas, Benjamin, Lough & Aram, 1974). Correlations of environmental calcium with field distribution patterns and abundance have been demonstrated for several snail species (Williams, 1970a; McKillop & Harrison, 1972; Dussart, 1976, 1979). In temperate regions of the world, extremely soft waters (calcium concentrations <3 mg/liter) can support only about 5% of the molluscan species of the region, moderately soft waters (Ca $<$ 10 mg/liter) can support about 40%,

intermediate waters (10 to 25 mg/liter) can support up to 55%, with hard waters (Ca >25 mg/liter) being required for the rest (Boycott, 1936; Macan, 1950; Russell-Hunter, 1957, 1964, 1978). However, it is noteworthy that most of those species tolerant of low calcium could survive in, and are sometimes found in, harder waters (Russell-Hunter, 1964). Although Dussart (1976) had claimed that environmental calcium level was a major determinant of field abundance for several molluscan species, his more recent multiple regression analyses (Dussart, 1979) suggest that in some species, correlation is with other cations associated with water hardness rather than with calcium itself. Future experimental work may show that exclusion of "soft-water species" of molluscs from certain waters of high mineral content does not result from high calcium content as such.

The present paper reports populations of the freshwater limpet *Ferrissia rivularis* in waters with a nearly fifteen-fold range (4.6 to 67.6 mg/liter) of calcium content. The extremely euryoecic freshwater snail *Lymnaea peregra* can undoubtedly colonize an even wider range. In terms of organic productivity, waters supporting freshwater snails can again vary widely. *Lymnaea peregra* is found in the most oligotrophic mountain lakes, but also can occur in eutrophic (even hypertrophic, or mildly polluted) waters. Again the range of *Ferrissia rivularis* (Table 1) is somewhat less but still extensive. Direct environmental effects on variation in shell components in *F. rivularis* are not apparent (Fig. 1). The lightest shells in terms of calcium occur not in the three populations from the softest waters but at the two somewhat harder sites, the heaviest shells in waters of intermediate hardness (CC), and the overall range of apparent concentration ratios runs from 1,953:1 to 29,130:1.

If ratios of secretion of shell calcium and shell protein both depend upon levels of energy turnover, one might have expected a *direct* relationship between the two components (and possibly some relationship to the general trophic state of each habitat). Our ten limpet populations do not show this (Fig. 1). On the other hand, one could hypothesize that the adaptive need for a certain level of mechanical protection by the shell could result in an *inverse* relationship between shell calcium and shell protein. [In certain land snails of the tropical rain forest, relatively uncalcified shells have unusually massive proteinaceous layers, and in certain freshwater sphaeriid clams there are supportive data for a similar inverse relationship (Burky, Benjamin, Catalano & Hornbach, 1979)]. Again, our ten natural populations of *F. rivularis* show no evidence of such an inverse relationship (Fig. 1). It should be noted that in content of shell protein and of shell calcium (as even more clearly in other measurable characters), the variation *within* the majority of single populations is very much *less* than the range of variation for the species as a whole.

It seems that genetic controls of shell secretion for the two major components are independent, and that the chances of genetic dispersal among the isolated creek populations of this limpet have resulted in an irregular distribution of shell forms. Obviously this anomalous variation in components found in *Ferrissia rivularis* differs from the patterns found in other freshwater snails.

In *Lymnaea peregra,* the mass of calcium carbonate in the shell varies directly with calcium available. The shell component differences in another North American freshwater limpet, *Laevapex fuscus,* are markedly less than those discussed for *Ferrissia,* but shell calcium content increases with calcium concentration (McMahon, 1973, 1975a). As regards the calcareous component then, in *Laevapex* and *Lymnaea peregra,* the shells of variable mass in different populations could result from similar energy expenditures in shell-making. A third pattern of relationship between shell calcium and the environment has been demonstrated for *Lymnaea palustris* (Hunter, 1972, 1975b), where a survey of fourteen population-sites showed that the ratio of shell calcium to whole animal dry weight changes little throughout growth, and does not vary greatly between populations. This proved true over a wide range of calcium concentrations of environmental water, and represents a "regulation" unusual in a species which shows great interpopulation variation in other respects. *Physa gyrina* (Hunter & Lull, 1977) appears to show similar "regulation." Hunter & Lull (1976, 1977) also studied natural populations of *Physa integra* and *Helisoma anceps,* and for these two species, there was no relationship between shell calcium to tissue ratios (which varied greatly from population to population) and the calcium concentrations of their environmental waters. In this "irregular" variation these species resembled the populations of *Ferrissia rivularis* described above. However, Hunter &

Lull (1977) claim that a similar ranking between these two species in seven population-sites where they co-exist is evidence for a possible relationship to trophic conditions. Unfortunately, there are no data on shell protein from these species-populations of the sort we have presented for *Ferrissia.*

Thus, at least four "patterns" of shell calcium relationship can be discerned in the data already available on freshwater pulmonates. These are: first, a direct relationship as in *Lymnaea peregra* and *Laevapex fuscus* between shell calcium and environmental hardness; secondly, a seeming "regulation" of calcareous shell secretion, as in *Lymnaea palustris* and *Physa gyrina,* resulting in shells of standard weight for size categories with each species; thirdly, a relationship between shell calcium secretion and general bioenergetic turnover (or trophic) rates, as was claimed for *Physa integra* and *Helisoma anceps*; and fourthly, great variation between (but not within) populations, as in *Ferrissia rivularis*, reflecting an irregular distribution of genetic "forms" neither obviously clinal nor adaptive (as claimed here). The fifth possible relationship—an inverse relationship between shell calcium and environmental calcium—has never been found in a pulmonate nor in other freshwater gastropods. However, Agrell (1949) found, in populations of the freshwater bivalve *Unio tumidus* in Sweden, that shell weights decreased from oligotrophic to eutrophic waters. For two other species of *Unio* and one of *Anodonta,* he found increasing shell weights with "rising trophic degree," essentially as claimed for *Physa integra* and *Helisoma anceps.*

"Over-compensation" in the form of increased calcium storage in populations in low calcium environments is better documented in plants, where pairs of closely-related species and subspecies have long been known to occur (Tansley, 1917; Salisbury, 1920; De Silva, 1934). Closer parallels to our possible physiological races in the limpet *Ferrissia,* irregularly distributed with no obvious geographic clines, are provided by the economically important grass, *Festuca ovina* (Bradshaw and Snaydon, 1959), and the ecologically important microorganism, *Azotobacter* (Bullock, Bush & Wilson, 1960). In the case of *Festuca,* population differentiation has resulted in "races," termed edaphic ecotypes, which when cultivated in a range of calcium levels show significantly different responses in growth rates and patterns.

In many pulmonates, shell shape is another infraspecific variable. Extensive data have been collected on shell biometrics in *Ferrissia rivularis* from a set of 53 populations in a variety of localities in upstate New York of varying trophic conditions and water hardness, and with different degrees of isolation from each other (Russell-Hunter and Nickerson, unpublished; Russell-Hunter, 1978). Certain shell-ratios (including isometric "roundness" in marginal growth) seem to be rather rigidly genetically determined, while others (allometric "steepness" of the cone) reflect local growth patterns as modified by trophic conditions. None of the present data on interpopulation variation in shell components can be directly related to *either* kind of shell-ratios as determined for this set of populations.

Despite all this, it seems reasonable to hypothesize that, in *Ferrissia,* differences between populations in both shell-calcium secretion and shell-protein synthesis and secretion are under independent genetic control, and that the different genomes are "irregularly" (not necessarily adaptively, nor in geographic clines) distributed among the isolated creek populations as a result of the stochastic element introduced by passive dispersal of single propagule individuals. Short reviews of evidence on passive dispersal of freshwater molluscs are provided by Rees (1965) and Russell-Hunter (1978). Two consequences of this kind of infraspecific variation remain to be discussed: one related to assessments of radionuclide pollution in fresh waters, and the other to broader aspects of evolutionary processes in freshwater animals.

Molluscs of various sorts have been proposed as indicators of environmental radiocontamination with strontium-90 (Nelson, 1964; Rosenthal, Nelson & Gardiner, 1965) which they found to be accumulated indiscriminately with calcium. Likins, Berry & Posner (1963) claimed relative discrimination against strontium in *Australorbis glabratus,* but Van Der Borght and Van Puymbroeck (1964, 1966) demonstrated active uptake direct from environmental water of both calcium and strontium in pulmonates such as *Lymnaea stagnalis, L. auricularia* and *Planorbarius corneus.* Further, their work showed that 80% of shell calcium gained by growing snails comes directly from the water, and only 20% indirectly through their food. Thus, it becomes important that some freshwater mollusc species have populations which differ significantly from each other in

the extent to which they *do* concentrate calcium from the environment. It is worth noting that the range of apparent concentration ratios (1,953:1 to 29,130:1) for *Ferrissia* populations noted above do not necessarily measure calcium transport costs through successive physiological "compartments" on the way to the shell, nor do they assess relative bioenergetic expenditures by the stocks in any actuarial framework of assimilation and growth. They are sufficiently different, however, to suggest that any proposed use of a freshwater mollusc species in a biological assay of strontium-90 pollution would have to be based upon a set of stocks known to be genetically uniform in such aspects of calcium metabolism.

Finally, these data on interpopulation variation in the calcareous and proteinaceous shell components in *Ferrissia,* and the subsequent deductions regarding their bases in an "irregular" distribution of genetic units by the chances of passive dispersal in the establishment of isolated creek populations can be added to lists of other types of physiological variation already documented for freshwater molluscs. All provide evidence that the rates and modes of evolutionary change which have worked to produce present (and past) freshwater molluscan faunas, differ markedly from those which have operated for similar animals living in the sea or on land. As more fully discussed elsewhere (see, for example, Russell-Hunter, 1961, 1964, 1978), a dominant characteristic of the freshwater environment is that the transience of most freshwater habitats in time, along with their spatial limitations and discontinuity, results in animal species distributed with much small-scale and short-term isolation of populations. Comparatively little full speciation occurs in freshwater animals like gastropod molluscs, since this is prevented by sufficient gene exchange resulting from limited and rare transfers of individuals between populations by passive dispersal. In contrast, much infraspecific interpopulation variation can and does occur because of the short-term, smaller scale isolation of discretely panmictic population-units. Where the high levels of interpopulation variation in some physiological or morphological characteristic have been investigated in detail there is always one consistent feature. The amount of variation found within any single population is always much less than the range of that character's variation for the species as a whole. Considering certain other features of the physiology of freshwater molluscs (particularly respiration), one also encounters stocks with exceptionally high levels of adaptive plasticity. All of these features of freshwater molluscs reflect the environmental discontinuities of time and space during their evolution. The irregular distribution of shell components found in these creek populations of *Ferrissia* again reflects the peculiarities of dispersal, gene-flow, and evolutionary rates in such freshwater habitats.

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