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CHARLES WRIGHT (1811-1885)

*Collectorum longe princeps*


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# CHARLES WRIGHT (1811-1885) IN CUBA AS REVEALED BY HIS LETTERS

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AMONG the best known collectors of Cuban land shells is the Connecticut-born botanist, Charles Wright. Ludwig Pfeiffer, who described and named most of the shells collected by Wright, credited him with discovering some 30 new species besides contributing much important ecological and distributional data. In addition, Rafael Arango frequently cites Wright as the source of much of the data which he published in his superb annotated catalog of Cuban shells (1878-1880).

Wright was an avid collector of natural history objects, especially plants. 'Princeps longe collectorum,' (by far the first among collectors) is what the German botanist A.H.R. Grisebach called him because of the wealth of new botanical material he sent from Cuba. Grisebach was one of several botanists who were associated with Wright, the most important of whom was Asa Gray of Harvard. Much of the data included in this report was derived from the large number of letters written by Wright to Gray which are preserved in the library of the Harvard Herbarium in Cambridge, Massachusetts.

Charles Wright was born on October 29, 1811, the second son in a family which was to consist, besides himself, of his older brother John and two younger sisters, Mary Ann and Abigail. Their father was a carpenter and farmer but their mother, Mary Goodrich, came from an early Connecticut family in comfortable circumstances and with some members notable in politics and the arts. In 1831 he was matriculated in Yale from which he was graduated in 1835 as a land surveyor. For a time he thought of pursuing a medical career, but he was unable to continue for lack of means. It was as an undergraduate in Yale that he developed a strong taste for botany, though apparently without benefit of formal instruction in that field. Botany as such was not taught at Yale in the 1830's. After a brief period as a tutor in Natchez, Mississippi—botanising at every opportunity—he migrated to the new Republic of Texas in 1837, earning his living as a surveyor and private tutor. He remained in Texas until 1852.

During the Mexican War he served in a company of volunteers raised by his friend Dr. Veitch, but there is nothing to show that this group saw any action. He later obtained leave to join a small company of troops which traversed the unexplored territory between San Antonio and El Paso. In 1851 he joined the surveying party under Col. J. D. Graham, one of the commissioners who established the boundary lines with Mexico from the Rio Grande to the Pacific Ocean. His name is included in the list of those who were 'attached to the Survey at various periods and made collections of natural history objects.' (Meisel, 1929, 3: 99). Wright left the expedition in the southwest corner of Arizona after it had traversed much unexplored territory. The plants collected on this trip were among those written up in PLANTAE WRIGHTIANAE (1852-1859) by Asa Gray of Harvard, who became Wright's warmest friend and chief patron. Their correspondence and personal contact began in 1844 and lasted until Wright's death.

In 1853 Wright was a member of the North Pacific Exploring Expedition under the command successively of Capt. Ringold and Commander John Rogers, which visited the Cape of Good Hope, Hong Kong, Borneo, the Loochoo (Ryuku, Bonin) Islands, Japan, Kamtschatka, Kiene or Arkamtschatschene Island in the Bering Straits, and California.

The expedition, which sailed from Norfolk on June 11, 1852, consisted of 5 ships, including the sloop 'Vincennes' on which, in addition to Wright, the zoologist William Stimp-

son and the botanist F.H. Storer were aboard. It was while Stimpson was studying the invertebrates, including 2359 mollusks, collected on this expedition that the disastrous fire of 1871 destroyed the museum in Chicago and all the work Stimpson had done. He never recovered from the blow and died soon after. Parenthetically it might be noticed that on November 5, 1865 Wright is credited with contributing to the Museum of Comparative Zoology at Harvard 955 specimens of shells from Hong Kong, China. These may well be part of the material collected on the expedition. Wright left the expedition when it reached San Francisco, California. He returned, via San Juan del Sur and Greytown, Nicaragua, to Cambridge, Massachusetts where, together with Gray, he worked on the plants he had collected.

In the autumn of 1856 he made his first trip to Cuba visiting the area around Santiago and other regions in the Province of Oriente. He stayed until the autumn of 1857 as a guest at the ranch of Federico Lescaille in Monteverde. On this trip, Wright collected in the El Cobre Mountains, Loma del Gato, Minamina, Saltadero and other areas to the west and north, as well as in the surroundings of Monteverde. During his excursions to the northern coast he stopped at various *hatos* or cattle ranches.

He returned briefly to the United States in 1857, but returned to Cuba at the end of 1858. Apparently he was no better a sailor than Charles Darwin on the Beagle, for he writes off St. Iago (Santiago) (November 25, 1856) '..... The babe's portion of food I ate would not stay eaten.' Between 1858 and 1861 he explored the vast region of the Sierra Maestra, as well as such areas as Monte Libano, Pico del Toro, Bayamo, the shores of the Río Cauto, Cuba's largest river, Punta de Maisi which is the extreme eastern tip of the island, Baracoa, and Mayari. Pfeiffer (1862:1) wrote that on these excursions Wright sent him many shells from areas previously visited by Gundlach, but 'he also went to places where G/undlach/ had not been, namely the region between Santiago and Mayari' (translated).

In the winter of 1861-62 accompanied by Gundlach, he traveled as far west as Camagüey Province and Sancti Spiritus in Las Villas Province where he paid a visit to the large swamp, the Cienaga de Zapata.

Wright made his first trip to the Vuelto Abajo in Pinar del Río Province in Western Cuba in the early summer of 1862. He stayed, among other places, at Balastena (Valastena) at the southern foot of the mountains near Bahia Honda, at the ranch of Don Jose Blaín, the son-in-law of the botanist Francisco Adolfo Sauvalle, who was to incorporate many of Wright's observations in his FLORA CUBANA (1873). Blaín was an enthusiastic botanist and naturalist in his own right. In his estates, here and at El Retiro, he was the open-hearted host to many naturalists such as Morelet—who received many of the Cuban land mollusks which he later described in TESTACEA NOVISSIMA INSULAE CUBANAE (1849, 1851) from Blaín—as well as Arango, Poey, Gundlach and many other botanists and entomologists. Wright visited the entire area of the Sierra de los Organos and even journeyed as far as the barren western tip of Cuba to Cabo San Antonio.

After a brief visit to Cambridge in 1864 he returned to Cuba and between 1865 and 1867, his last years in Cuba, he traveled by steamer from the Vuelto Abajo in Pinar del Río to Trinidad in Las Villas Province in central Cuba, and then to the east at Guantánamo and Santiago de Cuba. Here he found that his former host and good friend Lescaille had died.

In Cuba he early came in contact with Dr. Felipe Poey y Aloy the dean of Cuban naturalists and one of the most versatile and talented personalities of the New World who deserves to be much better known in our own country (Jacobson, 1972). He also met and became quite intimate with Juan (Johann) Gundlach, the gentle Cubanized German naturalist whom he adored. 'I shall consult with my friend Gundlach /about obtaining a new packhorse/, for a better man don't live on 'top o' ground,'" he wrote. 'He reminds me of the moral character, conscientiousness etc. of Cooper's 'Pathfinder.'" On Jan. 1, 1862, he writes: 'Gundlach knows all the good places about there and his name is a passport to go anywhere. He is a most zealous zoologist so exclusively given to science that he never looks at a newspaper. I have brought and sent him at times new shells and that even if he had any selfishness, which he has not, would have put my name in his books. I first made his acquaintance in Yateras /Oriente/.' Wright also set in motion a project to obtain contributions from each of the people who had received presents of natural history objects from Gundlach, the money destined for the purchase of a science library to be presented to Gundlach as a 'token of respect for his character as a man and for his industry in the cause of science.' Wright headed the subscription list with a donation of $50, a rather large sum for the day and for the usually financially straitened man from Wethersfield.

Finances seemed always to have been a problem for Wright during his stays in Cuba. Most of his collecting trips were financed, even if only partially, by Asa Gray. In his last letter to Gray, August 3, 1885—Wright died on August 11—he wrote: '.... it was always understood that the Herbarium Gray was to have as full a set as possible of everything of my gatherings, and even thus I can

never repay the many services you have rendered me.' Most of those services, as appears in frequent references in his letters, were financial. But Wright also tried to raise funds through the sale of duplicate sets of plants to other institutions as well as other natural history objects, such as birds, mammals, spiders, insects, shells etc. During the Civil War it was hard to transfer funds to Cuba and this added another source of worry. On October 13, 1862 he wrote that he had to take a 40 percent discount on U. S. dollars. It is likely that some of his money came from the family, for his older brother, John, farmed several plots of ground in Wethersfield. In 1899, eleven years after the death of John, the surviving sisters, Abigail and Mary Ann, were able to donate seven pieces of land in Wethersfield and neighboring Hartford to the Children's Aid Society, indicating that the family was never really destitute. None of the four Wright siblings ever married.

Years ago, when I was collecting in Cuba, my Cuban companions told me that Carlos Wright was a crazy old man with a wild white beard who travelled on foot all over Cuba with a pack on his back, collecting plants and shells, a sort of naturalist tramp. Henderson (1916: 36) writes that Wright had a 'long patriarchal beard' and that he was 'taciturn and unsocial,' and that 'without speaking a word of Spanish, he traversed the length of the island afoot leading a little donkey upon which he packed his precious collections.' Neither of these descriptions jibe with the man who wrote the warm, friendly letters to Asa Gray and other correspondents. Wright was always warmly received in the many *hatos* or ranches where he stayed, among which were such well known places as Monte Verde, Balastena, Filantropia, Retiro, Santa Catalina de Guasa and others. While at rest in these usually well-kept ranch houses he wrote most of his letters to Asa Gray. To these ranches he brought letters of introduction from his friend Gundlach. Of course, like all collectors, he had to rough it in the field, 'for weeks at a time /sleeping/ in caves /eating/ wild honey, and /living/ like an aborigine (sic)' to quote Henderson (1916: 37).

Once in the field, he stayed where he could most profitably collect. In a letter from the Vuelto Abajo (Dec. 10, 1862), he wrote that he preferred to rough it in a tumbledown *ingenio* (sugar mill) where the plants and shells were, rather than stay in more comfortable quarters in a pueblo or town nearby. Nevertheless he looked to his comfort whereever possible and usually had two horses with him, one to mount and one to pack.[1] His command of Spanish may not have been very great, but his letters show that he was not completely helpless in it. He probably knew enough to make his way among the peasantry. Besides, on some of his trips, he had the completely Cubanized Juan Gundlach with him as his companion.

His letters to Asa Gray written in a beautiful, strong, spencerian hand on thin blue or white letter paper, are full of accurate and detailed botanical observations which will in time, I have been informed, appear in a special botanical publication now under preparation.

Wright reveals himself to be a generous, indefatigable, humorous, extremely literate and eloquent personality who was, however, not without his peppery and explosive side. He is particularly irked by the inefficient and corrupt Spanish authorities who were slow and undependable in issuing the numerous 'licencias' needed for collecting, traveling, shipping his plants to America, and even the right to live in Cuba (permiso de domicilio). The lackadaisicalness and greed of the U. S. Consul in Santiago de Cuba brings forth a particularly bitter outburst (Jan. 10, 1860). 'Our purse-gathering gutter consul ain't worth a pinch of -----! (Wright's dash). He can't talk English much less Spanish. Nobody but the old bragging self-conceited Capt. Cochrane !!! Bah!!! He is a disgrace to our government!" He engaged in a long and lively duel with Asa Gray about the merits of the Civil War, to which he was bitterly opposed. On October 25, 1862, soon after a return to Cuba from the States, he writes: 'I don't care to take any part in the Kilkenny-cat scuffle. I hoped to see the Union restored. That hope has grown fainter every day till there is hardly a shadow left.' Four days later he writes: 'It is a well known fact that children often undo things their fathers did, put down what their fathers built up .... I don't mean to say that I approve the course of South Carolina. But I do say the war will not reestablish the Union. Hence it is useless, worse than useless --wicked!' On June 15, 1863 he chides Gray who apparently had become more bitterly anti-Rebel and pro-Union. 'At the beginning you were going to 'restore the Union' quicker than a cat could lick her ear. Now that you can't do this, it is 'wallop the rebels,' 'work them to utter desolation,' 'give em Hell and pay it down'." When Gray unwisely wrote that when Wright returned he should 'be a good and peaceable citizen (subject) of A. Lincoln's dominions,' Wright's letter sizzles with indignation. He had left a republic and now it was converted to a tyrant's dominions. 'Here /in Cuba/ I am free /even/ in a military despotism, and here I mean to stay until times become better at home.' On July 15, 1863 he accused Lincoln of trying to win the election by tricky means. 'Republican soldiers were sent home to vote while Democrats were retained to keep their posts and be killed off.' But after Gettysburg and the fall of New Or-

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[1] *Cf.* letter from Concordia, May 25, 1863: 'My packhorse is dead and all my plans upset. I can do little with one horse and have no means to buy another, even if I had the inclination.'

leans he writes, somewhat chastened, that he was glad the war was coming to an end.

Other things also infuriated him. His friend Gundlach had sent a new sphingid moth, apparently a paratypic specimen, to the German entomologist Grote, and thought the insect could not have reached Grote before late in December 1866, Grote dated his description November 27, 1866 to win priority over Gundlach's work. 'Don't,' added Wright in his letter to Gray, 'let him have any more of my insects.'

Nor was he much pleased with Grisebach's monumental works on his collections: PLANTAE WRIGHTIANAE E CUBA ORIENTALI (1866) and CATALOGUS PLANTARUM INSULA CUBA MISSAS (1866). He found in them 'many errors, more defects and a good many superfluities.' He noted that a revision would require immense labor. This revision, together with the description of many new species, appeared in Sauvelle's FLORA CUBANA (Havana, 1873). When Grisebach failed to acknowledge his shipments and letters promptly, Wright stated that he feels 'growly' about it (March 25, 1866).

Even Gray is not entirely free from the barbed bolts of Wright's fury. 'Pray,' he wrote on November 8, 1859, 'don't fill up your letters & waste valuable time in giving me advice as to what is best for me to do. If you were here & knew as much as I do about the weather, the roads, the state of vegetation etc. etc. I won't receive, very thankfully, any counsel you might offer.' And on July 14, 1860: 'Next time you are going to send me anything *presently* pray don't tell me about it. More than six or eight months before /you/ despatch it!' In a letter of August 15, 1863, he objected to the 'sneering' tone Gray takes toward Gundlach. 'Now just open your head wide enough,' he wrote, 'to admit the supposition that a Cuban is capable of doing a liberal or generous act while apparently engaged in a business transaction of all difficulty.'

There is no doubt that a good deal of this impatience was due to the frustrations Wright had to suffer—books, money, a microscope, even drying paper did not arrive on time; collecting conditions were difficult — 'Up hill & down mud! mud! mud! slip, slide, horses and men, yards at a time, dirty and wet & but few novelties.' Occasionally he was made irascible by ill health: at various times he suffered from fever, dysentery, piles, and wrenched limbs, besides once almost blinding himself with an acrid plant juice. Living conditions were also often provoking: he had to eat with his fingers, sleep with dogs and other animals, and suffer attack from fleas. Yet at times he can rise above the latter with some humor. 'The most exquisite pleasure I have nowadays is killing fleas—when I can catch them. Oh ain't it the height of felicity to have in possession the lifeless carcass of your enemy. Time was when I didn't care for a flea-bite. But *that time* isn't *now* & *those* fleas weren't *Monte Verde* fleas. I have been pretty well acquainted with the critters now for more than 20 years and till now I have always been content to give them breakfast dinner and supper if they would take it quietly and not gallop all over the most sensitive parts of my corporosity. The fact is these fleas are no locomotives. They are no tourists. They don't go prospecting. Whenever they alight at it they go & it's gouge, gouge gouge till they are forcibly geched (?) from their posture, when they take incontinently to another & gouge gouge away. And such venomous scamps. Every puncture raises a great knot such as I never get from any other insect.' (February 5, 1859). On January 21, 1867 he wrote that he had had a severe fainting spell. 'What if such an attack should come on me when scrambling on one of the cliffs? *I must quit this work!*' (his emphasis). He left Cuba for the last time soon after May 1867. The next year, while living in Wethersfield, he wrote to Gray 'I want to go to Cuba and I want to stay here. Ambition says go; prudence says stay.' Prudence won out.

Nevertheless in 1871 he was again a member of an exploratory expedition to Santo Domingo to investigate the possibility and advisability of annexing the island to the United States. (This was the epoch of unashamed and blatant 'Yankee imperialism'). But the winter season was unfavorable and the trip hurried and unsatisfactory. He returned, after a brief stay, with a smaller collection than usual. After a severe illness in 1875, he spent some time in Cambridge working as librarian at the Bussey Institution of Harvard with Professor Storer, his associate on the Pacific cruise of 1853. After 1876 he spent the remainder of his life in his old home in Wethersfield. He died of 'hypertrophy of the heart' on August 11, 1885, three years before the death of his older brother John.

Asa Gray (1886:17) left a short description of Wright. 'Mr. Wright was in person of low stature and well-knit frame, hardy rather than strong, scrupulously temperate, but direct and downright in expression, most amiable, trusty and religious. He accomplished a great amount of useful and excellent work for botany in the pure and simple love of it; and his memory is held in honorable and grateful remembrance by his surviving associates.'

In connection with the naming of *Carlowrightia*, an acanthaceous genus of the southwest, Gray wrote: 'Surely no botanist ever better earned such scientific remembrance by entire devotion, acute observation, severe exertion and perseverance under hardship and privation.' Though Wright wrote nothing on the Cuban molluscan fauna, Cuban malacologists honor his memory as one of theirs, and the

pomatiasid genus *Wrightudora* Torre & Bartsch, 1941 was established in his name. In addition his name was attached to many species.

His portraits show a man with a strong squarish face, a fine brow, firm nose and lips, and determined chin, covered in later years by a rather scraggly gray beard. His appearance was adversely affected by a strong internal strabismus in his right eye, clearly shown in his later portrait (frontispiece).

Wright was more a collector than a student or writer. In his chosen field of botany he published only two works in his lifetime, the revision of Grisebach's CATALOGUS mentioned above and a note on *Jassioea* in volume 10 of the Journal of the Linnaean Society. Hence it must be clear that the descriptions of new molluscan species credited to him were in all likelihood not his work. Pfeiffer ascribed about 30 species to him but it is apparent that at most Wright suggested the name alone,[2] and the description must be credited to Pfeiffer. Thus for the species *Choanopoma hystrix* Wright, 1862 (Mal. Blatt. 8: 221) Pfeiffer wrote: 'Diese herrlich Art wurde von dem Amerikanischen Botaniker Herrn Wright, entdeckt und *benannt*,' (my emphasis). In at least two cases (see below) all that Pfeiffer received from Wright was the manuscript names; he did not get to see the shells and hence, published two nude names. A list of the taxa in question is appended below.

Though strictly speaking Wright was no specialist in shells, he showed considerable interest and astuteness in them. As we have seen, it is quite likely that he collected shells on the North Pacific Exploring Expedition. There are several references to shell collecting in his letters. On April 17, 1860 he wrote from Monte Verde in Oriente Province: 'Lately I have been dipping into zoology—saving some birds for Bain and picking up now and then some shells.' On June 16 of the same year he wrote that he had wrenched his knee but 'I have been more or less all the intervening time collecting shells, insects, etc.' On September 2, 'I was mainly after shells and eminently successful,' and on the 9th 'while I remain here /near Holguin, Oriente/ I'll be likely to discover something new or desirable in zoology (conchology) if not in botany.' Gundlach egged him on. From San José in western Cuba (September 28, 1862): 'being with Gundlach I devoted considerable time to the collection of shells.' We have seen that he gave many shells to Gundlach.

Conde (1958: 287) wrote of Wright that 'he was the first North American naturalist to explore a tropical country extensively.' Malacologists can be grateful to Wright for having aided vigorously in the discovery of the superbly rich Cuban land molluscan fauna. Together with Poey, Gundlach, Arango, and Blaín, de la Torre, Bartsch, and a few others—his name—even if only as a collector—is bound forever to the exciting days when this rich field of natural history was first coming to the attention of the scientific world.

2 Even in botany he writes (May 3, 1859) that he was giving names only to new plants. 'So I have just put down what I thought would be a good name without any description.'

I am indebted to the library staff of the Gray Herbarium of Harvard University in Cambridge, Massachusetts, and of the Academy of Natural Sciences of Philadelphia for their kindness in permitting me to read the Wright correspondence in their files. The library of Wethersfield, Connecticut aided me in several important ways in my investigation of the history of the Wright family. Mr. John Collins, the principal of the Charles Wright School in Wethersfield, kindly permitted me to photograph the pictures of Wright hanging in the school. Dr. William K. Emerson of the American Museum of Natural History was kind enough to read and check the manuscript.

## MOLLUSCAN TAXA USUALLY CREDITED TO CHARLES WRIGHT

**NOTE:** The last nomen of each taxon is also provided. Abbreviations: MB, Malakozoologische Blätter; MPV, Monographia Pneumonopomorum Viventium.

alboviridis Wright 1864, Helicina. MB 11:108. *Semitrochatella alboviridis* (Pfeiffer).

angustior Wright 1864, Cylindrella. MB 11: 130. *Gongylostoma (Gongylostoma) angustior* (Pfeiffer)

arcustriata Wright 1864, Cylindrella. MB 11: 3. *Gongylostoma (Scopulospica) arcustriata* (Pfeiffer)

cirratum Wright 1867, Cyclostoma. MB 14: 210. *Hendersonia (Scobinopoma) cirrata* (Pfeiffer)

clara Wright 1865, Cylindrella. MB 12: 119. *Gongylostoma (Liocallonia) clara* (Pfeiffer)

cristallina Wright 1865, Cylindrella. MB 12: 120. *Hendersonina (Hendersonida) discolorans* (Pfeiffer)

echinulatum Wright 1863, Chondropoma. MB 10: 184. *Turrithyra (Turrithyretes) echinulata* (Pfeiffer)

echinus Wright 1864, Cyclostoma (Choanopoma). MB 11:102. *Blaesospira echinus* (Pfeiffer)

erythraea Wright 1866, Helicina. Thes. Conch. 3: 284, pl. 278, figs. 461-463. *Troschelviana (Troschelviana) erythraea* (Sowerby)

fusiformis Wright 1864, Cylindrella. MB 11: 12. *Gongylostoma (Gongylostoma) fusiformis* (Pfeiffer)

garciana Wright 1865, Cylindrella. /in/ Poey, F., Reportorio fisico-natural isla de Cuba, Habana, 1: 220. *Gongylostoma (Gongylostoma) garciana* (Presas)

hernandezi Wright 1876, Helicina. MPV supp. 3:287. Synonym of *Troschelviana (Troschelviana) chrysochasma* (Poey). Note: This taxon appeared as *Eutrochatella (Ustronia) chrysochasma hernandezi* 'Wright,' Wagner /in/ Martini & Chemnitz, Conchylien-Cabinet (2), 1: sect. 18, pt. 2, p. 128, pl. 25, figs. 3-4). It is surprising that Wagner took this to be *hernandezi* Wright, since Pfeiffer had not described it. Pfeiffer merely listed it as one of the 'Species quarum descriptiones non vidi.' It is also worthy of note that Wagner gave no bibliographical reference for the nomen.

hystrix Wright, 1862, Choanopoma. MB 8: 221. *Xenopoma hystrix* (Pfeiffer)

illamellata Wright 1864, Cylindrella. MB 11: 130. *Conchlodinella illamellata* (Pfeiffer)

incrassatum Wright 1863, Chondropoma. MB 10: 1863. *Chondrothyretes incrassata* (Pfeiffer)

infradenticulatus Wright 1864, Macroceramus. MB 11: 127. *Microceramus infradenticulatus* (Pfeiffer)

macra Wright 1867, Cylindrella. MB 14: 210. *Gongylostoma (Badiofaux) macra* (Pfeiffer)

maculatus Wright 1865, Macroceramus. MB 12: 119. *Microceramus maculatus* (Pfeiffer)

mixta Wright, 1865, Cylindrella. MB 12: 120. *Conchlodinella mixta* (Pfeiffer)

montana Wright 1864, Helicina. MB 11: 160. Synonym of *Alcadia (Penisoltia) minima* (d'Orbigny)

percrassa Wright 1864, Lincia? MB 11: 157. *Chondrothyra (Hendersonoma) percrassa* (Pfeiffer)

plumbea Wright 1864, Cylindrella. MB 11: 129. *Gongylostoma (Badiofaux) plumbea* (Pfeiffer)

pterostomum Wright 1865, Cyclostoma. MPV supp. 2, p. 106 (nomen nudum). NOTE: Pfeiffer refers to *Cyclostoma pterostomum* Gundlach 1858 /in/ Poey, Memorias historia natural isla de Cuba 2:405, a nomen nudum. Synonym of *Xenopoma hystrix* (Pfeiffer)

pulverulentum Wright 1864, Ctenopoma. MB 11: 103. *Eutudorops (Eutudorex) pulverulentum* (Pfeiffer)

rubella Wright 1864, Helicina. MB 11: 107. *non* J. Green 1833. Synonym of *Troschelviana (Troschelviana) erythraea* (Sowerby)

sinuosum Wright 1863, Chondropoma. MB 10:185. *Turrithyra (Turrithyretes) sinuosa* (Pfeiffer)

striatella Wright 1864, Cylindrella. MB 11: 2. *Gongylostoma (Pycnoptychia) striatella* (Pfeiffer)

subtussulcata Wright 1863, Helix. MB 10: 199. *Jeanneretia (Guladentia) subtussulcata* (Pfeiffer)

teneriensis Wright 1865, Cylindrella. MB 12: 121. *Gongylostoma (Esochara) teneriensis* (Pfeiffer)

vignalensis Wright 1863, Chondropoma. MB 10: 189. *Chondropometes (Chondropometes) vignalensis* (Pfeiffer)

vignalensis Wright 1864, Cylindrella. MB 11: 3. *Gongylostoma (Badiofaux) vignalensis* (Pfeiffer)

violacea Wright 1864, Cylindrella. MB 11:128. *Gongylostoma (Badiofaux) violacea* (Pfeiffer)

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# CULTIVATION OF *BULINUS (PHYSOPSIS) GLOBOSUS* (MORELET) AND *BIOMPHALARIA PFEIFFERI PFEIFFERI* (KRAUSS), SNAIL HOSTS OF SCHISTOSOMIASIS

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## ABSTRACT

Since laboratory cultivation of *Bulinus globosus* and *Biomphalaria pfeifferi* snails has been difficult, this study was designed to develop simpler, more efficient, more dependable and more readily reproducible culture methods; it also provides a better understanding of the ecological requirements necessary for raising these snails.

Information is provided on the optimum number of *Bulinus globosus* and *Biomphalaria pfeifferi* that can be accommodated in one tray. For best survivorship and growth, the method using 5 snails per tray is recommended, since the number surviving is highest and shell length is greatest. For maximum egg-laying the use of 10 snails per tray is recommended, since total number of eggs produced was highest.

The effects of different types of waters, such as distilled (or deionized) water, tap water and spring water, on snail growth and fecundity were measured. Growth was not affected by the types of waters used; however, egg-laying was affected. With *Bulinus globosus*, the largest number of eggs was laid in distilled water, followed by tap water, and the smallest was in spring water. With *Biomphalaria pfeifferi*, the largest number of eggs was laid in tap water, followed by both deionized and distilled waters, and smallest in spring water. These snails laid most eggs on the container wall and bottom when in distilled water (or deionized water), the fewest on those sites in spring water. In tap water the egg-laying pattern was intermediate. Further studies with chemical additives indicated that calcium ions reduced the egg production and the eggs laid on the container wall and bottom. The tray method was more efficient for both shell growth and egg production than the methods used by other investigators.

The effectiveness of various species of algae and various kinds of mud and mud extracts on growth of *Biomphalaria pfeifferi* was studied. Locally isolated blue-green algae, *Nostoc muscorum* and *Fischerella ambigua* were both found better than the mixture of blue-green algae which was used exclusively in the earlier parts of the study. *Nostoc muscorum* was used exclusively in later experiments because it was easily grown. With *Nostoc muscorum*, the trays always supported better snail growth than Petri dishes did; the addition of lettuce always enhanced growth. For practical purposes, *Biomphalaria pfeifferi* was efficiently cultured in Petri dishes with *Nostoc muscorum*. The organic substances in the mud were essential for snail growth. Mud without sufficient organic substances or mud extracts only partially supported snail growth; the 525° C-heated mud and 525° C-heated steamed extract did not support snail growth at all.

In studies concerning the formulation of a synthetic mud, two formulae were found most promising. One contained pottery clay, oyster shell, charcoal and agar; the other, had pottery clay, oyster shell and agar. In both an artificial water was used for making the paste and as an overlay. Pottery clay and oyster shell were essential to sustain the snails; the absence of charcoal was not critical. Agar helped to solidify the substance to assist snails in feeding. Artificial water provided enough chemicals for algal growth and was still not harmful to snail growth. The possible formulation of synthetic muds opens promising fields for further study.



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## INTRODUCTION

Information about the successful culture of medically important snails is difficult to find, and a great need exists to ascertain the conditions necessary to maintain some of the key species in culture. Successful snail host rearing methods in laboratory are required in such basic studies as: snail-host-parasite relationships, population dynamics, effects of molluscicides, snail control methods, among others. The almost universal use in laboratories of the American schistosome, *Schistosoma mansoni* Sambon, is a reflection of the ease with which its snail host, *Biomphalaria glabrata* (Say), is maintained in the laboratory. In contrast, the African schistosomes, *Schistosoma haematobium* (Bilharz) and *S. mansoni*, have not been similarly studied in laboratories, a situation mainly due to the fact that techniques for mass cultivation of the African snail vectors, *Bulinus (Physopsis) globosus* (Morelet) and *Biomphalaria pfeifferi pfeifferi* (Krauss), have not as yet been developed.

Some of the more important effects of environment on the growth of snails have been stressed in several publications. In his studies of *Lymnaea columella* Say, Colton (1908) emphasized the following significant factors: (1) food, (2) volume of water, (3) temperature, (4) snail interaction, (5) aeration, (6) room for crawling, (7) light and (8) chemical composition of the water. Boycott, in his scholarly paper (1936) on the ecology of freshwater snails stated that the most important features in favorable habitats were: (1) cleanliness of water, (2) absence of disturbance and (3) presence of lime. Deschiens (1957a) contributed the following information on some of the physico-chemical factors essential for conditioning the habitats of the *Biomphalaria glabrata* snails: water with a temperature of 22 to 26° C, a depth of less than 2 meters and less than 6 gm of NaCl per 1,000 ml of water. His biological factors were divided into two parts: nutrimental (i. e., elective phytophagy, microphagy and stability of the metabolic cycle of the nutrient material) and competitive (i.e., flora and fauna of the environment, natural enemies, predatory animals, and infectious and parasitic diseases). Malek (1958), who undertook extensive studies in North Africa, listed food, aquatic weeds, oxygen, sunlight, current, substratum, water quality and nuisance factors (parasites and predators) as conditions governing the suitability of habitats for planorbid snails serving as intermediate hosts of schistosomiasis. Claugher (1960), in studying *Schistosoma haematobium*, listed four essential requirements for establishing laboratory colonies of *Bulinus* snails: (1) all-glass tanks, (2) conditioned water, (3) clean aquatic plants and (4) a constant-temperature room. Berrie (1970), after extensive survey work in East Africa, listed (1) temperature, (2) snail density and (3) food as environmental factors important in studies involving snail population dynamics. For culturing snails in the laboratory, the several factors listed above should be taken into consideration and can be condensed further to include: temperature, light, water quality, food, population density, type of aquarium, substratum, and parasites and predators.

## LITERATURE REVIEW

### 1. Temperature

Gordon *et al.* (1934) observed that *Biomphalaria pfeifferi* was very sensitive to higher temperature. Although their lowest death rate was observed in the range of 25 to 33° C, the optimum temperature for oviposition was between 26 and 28 C. They also found that the optimum temperature for the oviposition of *Bulinus globosus* was between 25 and 26° C. Consequently, the following investigators raised their snails in temperature ranging between 20 and 30° C, as follows:

*Bulinus africanus africanus* (Krauss): 28°C (Wright & Bennett, 1967)

*Bulinus (Ph.) globosus* (Morelet): 25°C (Shiff, 1964a), 28° C (Wright & Bennett, 1967), 23-25° C and 26-30° C (Xavier *et al.*, 1968).

*Bulinus truncatus truncatus* (Audouin): 23± 1° C (Cowper, 1946), 25 -- 28° C (Standen, 1949), 28 - 30° C (Claugher, 1960), 15.5-25.5° C (Najarian, 1960a, 1960b), 23-25° C and 26-30° C (Xavier *et al.*, 1968).

*Biomphalaria alexandrina alexandrina* (Ehrenberg): 25-28° C (Standen, 1949)

*Biomphalaria angulosa* (Mandahl-Barth): 20-25° C (Sturrock, R. F., 1965).

*Biomphalaria glabrata* (Say): 23± 1° C (Cowper, 1946); 24.4° C (Chernin *et al.*, 1956); 25-30° C (Lee & Lewert, 1956); 24.4-25.6° C (Chernin, 1957b; Chernin & Michelson, 1957a, 1957b); 25° C (Michelson, 1961); 25-27° C (Hopf & Muller 1962), 21-24 C (Pan, 1963, 1965); 23-

25° C (Ritchie *et al.*, 1963a), 25 ± 1° C (Jobin & Michelson, 1967), 22-28° C (Sturrock, B.M. & Sturrock, R.F., 1970); Sturrock, R. F. & Sturrock, B.M., 1970); 23-25° C (Joy, 1971).

*Biomphalaria pfeifferi pfeifferi* (Krauss): 20-30° C (Frank, 1963); 20-25° C (Sturrock, B.M., 1966); 25° C (Harrison, 1968); 25 ± 1° C (Harrison *et al.*, 1970); 26° C (Jennings *et al.*, 1970).

Others interested in such values, as the 'intrinsic rate of natural increase' (=the population growth rate per snail under specified physical conditions, in an unlimited environment—space, food, other organisms not exerting a limiting effect) found the optimum temperatures for *Bulinus* and *Biomphalaria* to be:

*Bulinus globosus:* 25° C (Shiff, 1964a; Harrison & Shiff, 1966); 25 ± 0.5° C (Williams, 1970b).

*Biomphalaria pfeifferi:* 25° C (Harrison & Shiff, 1966; Sturrock, R. F., 1966); 20-25° C (Shiff & Garnett, 1967); 25 ± 0.5°C (Williams, 1970b).

In the World Health Organization report (1957) the optimum temperature for vector snails was given as between 22 and 26° C. In the field, Pimentel & White (1959a) observed that *Biomphalaria glabrata* was found in water bodies with a mean temperature of 26.7° C (range 21 to 37° C). Also in the field, Shiff (1964c) found that up to a limit of 25° C the egg-laying of *Bulinus globosus* increased rapidly and that maturation was faster with rising temperature. Similar observation was made on 3 species of planorbid snails, *Helisoma trivolvis* (Say), *H. anceps* (Menke) and *H. campanulatum* (Say). They found that these snails tended to grow larger as the temperature increased within the range of 20 to 30° C; the maximum egg production, however, occurred at temperatures between 25 and 26° C; at 30° C the reproduction of all the planorbids tested was inhibited. Shiff (1966) also found that *Bulinus globosus* was capable of detecting a temperature difference of 4° C within a habitat, and with a vertical gradient from 16 to 28° C the animals tended to cluster in the warmer water. Chernin (1967) observed that regardless of initial placement in a thermal gradient, well-fed *Biomphalaria glabrata* tended to accumulate horizontally with greatest frequency in zones between 27 and 32° C.

Ecologists seem to agree that temperature is among the most important of the physical influences in any biotope, especially in fresh water. From this brief review, it appears that a temperature of 25 ± 3° C represents the optimum temperature for both *Bulinus* and *Biomphalaria snails*.

## 2. Light

In contrast to temperature, the influence of light on snail behavior has been given too little attention. Boycott (1936) noted that snails were absent from densely shaded ponds and streams, having few or no plants and full of twigs and leaves. He contended that adequate light encouraged green plants which oxygenated the water and produced algae for snails to eat; this oxygenation also ensured decomposition of plant and animal remains at the bottom, thus making the habitats suitable for snails. Barlow (1937) stated that the optimum environments for both *Bulinus truncatus* and *Biomphalaria alexandrina* in Egypt were provided with both sunshine and shade. Malek (1958) noticed that the completely shaded forest pools in the Sudan did not harbor bilharziasis vectors, while pools partially shaded and provided with aquatic weeds and having grass on the banks were preferred by those vectors. The studies by van der Schalie and Davis (1965) indicated that the growth rate of the hydrobiid snail *Oncomelania hupensis formosana* was highest when the animals were maintained under a continuous, white, cool, fluorescent light (40 watt) suspended 10 inches above cultures. Similar results were obtained by Joy (1971) who showed that the rate of egg-laying of *Biomphalaria glabrata* was highest when the snails were maintained under continuous light with an intensity of 355 uw/cm$^2$.

While the beneficial effects of light, either direct or indirect, were emphasized as indicated above, others were of the opinion that light did not affect snails. Deschiens and Bijan (1956) found that *Biomphalaria glabrata* kept in total darkness still maintained normal activities. The absence of light did not depress the rhythm of egg-laying, the number of eggs laid or the growth of embryos, nor affect the forms of the newly hatched snails. Deschiens (1957b) found that *Bulinus truncatus* also could be maintained, and the snails reproduced for more than 1 year in total darkness. He also observed that *Biomphalaria glabrata* withdrew into its shell immediately when subjected to a strong direct light. He believed that *Biomphalaria glabrata* favored darkness or light of a low intensity. Watson and Al-Ali (1961) also found *Bulinus truncatus* could be raised under total darkness, and the snails completed their life-cycle from egg to egg in 70 days. Harry and Aldrich (1958) noticed that in the field *Biomphalaria glabrata* was little affected by the amount of light reaching the habitat, so that often the snails were found exposed to direct sunlight as well as in habitats partially or well shaded.

In summary, the effect of light on the snails was indirect. Light encouraged the growth of aquatic weeds and microflora which consumed carbon dioxide and produced carbohydrates and released oxygen. The light in the snail's habitat could have several beneficial influences. Light could also increase the rate of decomposition of the animal and plant remains on the bottom, so that the habitats could be rendered more favorable for snails.

### 3. Water Quality

Most investigators preferred to use biologically conditioned water. Cowper (1946) found that rain or tap water conditioned by using weeds or mud was best for culturing *Bulinus truncatus* and *Biomphalaria glabrata*. Standen (1949) had a more complicated way of conditioning the water; he used one or two small roots of several kinds of water plants such as wild celery (*Vallisneria spiralis*) water milfoil (*Myriophyllum japonicum*) and false loosestrife (*Ludwigia palustris*), 20 to 30 *Tubifex* or *Dero* oligochaete worms, and several hundred *Daphnia* to insure a water that would be well balanced for his *Bulinus truncatus* and *Biomphalaria alexandrina*. Moore *et al.* (1953) used *Elodea* (=water weed), *Sagittaria* (=arrowhead), *Vallisneria*, *Myriophyllum*, *Ludwigia* and hornwort to condition their aquaria for culturing *Bulinus truncatus*. Chernin *et al.* (1956) found that *Biomphalaria glabrata* did not grow well in freshly prepared aquaria even if the tap water utilized had previously been aerated to remove excess chlorine. Consequently, their new aquaria were either conditioned by permitting vegetation to take root in them for a week or longer, or by adding a sizeable volume of water from existing tanks several days before snails were introduced. Later Chernin (1957b) maintained *Biomphalaria glabrata* in distilled water to which an equal amount of water from an established aquarium was added. Chernin and Michelson (1957a) reported that watercress-conditioned tap water was necessary for *Biomphalaria glabrata*, since snails placed in pre-aerated laboratory tap water alone became inactive and frequently died within a day or two. Claugher (1960) discovered that tap water conditioned by maintaining guppies for two weeks was most suitable for *Bulinus* snails. He objected to introducing other live animals into aquaria for conditioning water, but he did recommend aquatic plants, such as *Vallisneria spiralis* and *Elodea*. Berrie and Visser (1963) found that lake water was suitable for *Biomphalaria sudanica sudanica* (Martens). Frank (1963) used stream water for *Biomphalaria pfeifferi*. McClelland (1964) recommended the use of lake water provided with a cow manure extract which seemed essential for the survival of young *Bulinus (Physopsis) nasutus* (Martens) during the first two weeks after hatching. Chu *et al.* (1966a, 1966b) found stored river water was most suitable for *Bulinus truncatus*. Kinoti (1968) used a water flea, *Daphnia*, and the coontail waterweed, *Ceratophyllum*, to condition the water for maintaining *Bulinus africanus*.

While many workers emphasized the importance of using biologically balanced aquaria, Chernin and Michelson (1957b) used aerated distilled water to observe the effects of water volume on the growth of *Biomphalaria glabrata* populations. Lee and Lewert (1956) reported that tap water dechlorinated by sand and activated charcoal was satisfactory for raising *Biomphalaria glabrata*. B. M. Sturrock (1966) also used dechlorinated tap water for culturing *Biomphalaria pfeifferi*. Joy (1971) used dechlorinated tap water, but he added plaster of Paris at the rate of 2.5 mg per liter of water (i. e., 2.5 ppm), for *Biomphalaria glabrata*. Najarian (1960a) even used non-aerated tap water with success in studies of *Bulinus truncatus*. Wright (1960) also used week-old non-aerated tap water for *Bulinus forskalii* (Ehrenberg). Ritchie *et al.* (1966) also succeeded in raising *Biomphalaria glabrata* with non-aerated tap water. Williams (1970b) used aged tap water for *Bulinus globosus* and *Biomphalaria pfeifferi*. Pellegrino and Gonçalves (1965) and R. F. and B. M. Sturrock (1970) employed spring water in their studies of *Biomphalaria glabrata*. Sodeman (1970) also succeeded by using spring water in his studies of *Biomphalaria pfeifferi*. Hopf and Muller (1962) prepared an artificial hard water (0.26 gm of $MgSO_4 \cdot 7H_2O$ and 0.104 gm of $CaCl_2$ in 1,000 ml of water) for breeding *Biomphalaria glabrata*.

Several investigators were concerned about water chemistry. In his study of *Lymnaea (Radix) caillaudi* Bourguignat, van Someren (1946) observed that total hardness had little effect on the distribution of snails provided that a hardness range of 8 - 200 ppm as $CaCO_3$ was maintained. De Meillon *et al.* (1958) concluded that chemical composition in natural water did not play even a minor part in determining snail habitats. Colton (1908), however, concluded that saturated solutions of certain calcium salts (particularly calcium sulfate) seemed beneficial to the growth of *Lymnaea* snails. Boycott (1936) reported that the most populous snail habitats were usually calcium-rich rivers, lakes and canals. Harry and Cumbie (1956a, 1956b) also observed that water quality accounted for the spotty distribution of *Biomphalaria glabrata* in nature. As an example, those snails did not seem to live in streams coming from limestone or some other formations. Harry *et al.* (1957) observed that the great excess of weak acid radicals ($CO_3$ $SO_4$. may be related to the absence of snails in water flowing over limestones. A similar observation was made by Harry and Aldrich (1958), who indicated that there was no significant correlation between the calcium-magnesium ratio and the distribution of *Biomphalaria glabrata* in the field. Instead, they found, in situations where those snails were present, the ratio (in ppm) of weak acids (carbonate and bicarbonate) to strong acids (chloride and sulfate) was less than 3:1; in places where the snails were not found (e.g., limestone streams) the ratio was usually between 4:1 and 6:1. In the laboratory, they found that zinc, copper, cadmium and silver with a concentration between 0.050 ppm and 0.100 ppm in distilled water produced a distinct distress syndrome among *Biomphalaria glabrata;* and when the concentration was more than 0.100 ppm, it killed snails rapidly.

Some of the more important observations relating to elements studied and in terms of the specific conditions tested are, as follows:

a) Calcium

Malek (1958) observed that snails may even thrive in water poor in calcium. Frank (1963) also demonstrated that *Biomphalaria pfeifferi* in the aquarium with lower concentration of calcium (13 ppm as $CaCO_3$) grew much faster and deposited more eggs than those in aquaria with higher concentration of calcium (22 ppm and 31 ppm as $CaCO_3$); and the mortality was found highest in aquaria with highest concentration of calcium (31 ppm as $CaCO_3$). However, usually *Bulinus (Physopsis)* and *Biomphalaria* habitats were found to be rich in calcium (Alves, 1958). Deschiens (1954b) found that both *Bulinus truncatus* and *Biomphalaria glabrata* could tolerate calcium ions (as $CaSO_4$) up to saturation concentration (i.e., 3,000 ppm). Harry *et al.* (1957) expressed the opinion that between 17 and 70 ppm (as calcium) was the optimum concentration range for *Biomphalaria glabrata* in nature. For *Bulinus nasutus*, the optimum concentration was 3.2 to 212.8 ppm (as calcium) (Webbe, 1962b); and for *Biomphalaria pfeifferi* 5 to 40 ppm (as calcium) (Harrison *et al.*, 1970). Harrison and Shiff (1966) and Williams (1970a, 1970b) observed that both *Bulinus globosus* and *Biomphalaria pfeifferi* showed their greatest intrinsic rate of natural increase in water with 5 to 40 ppm calcium.

b) Magnesium

As with calcium, Alves (1958) concluded that *Bulinus (Physopsis)* or *Biomphalaria* snail habitats were rich in magnesium but that if the magnesium content was greatly in excess of calcium, the habitat usuwlly was free of snails. This observation was verified by Harrison *et al.* (1966) in the field observation that aquatic snails were absent from streams with water high in dissolved magnesium but comparatively low in dissolved calcium, i. e., with a high magnesium-to-calcium ratio. In the laboratory they also found that egg-laying of *Biomphalaria pfeifferi* was adversely affected by maintaining a high value of this ratio. On the other hand, Schutte and Frank (1964) in field observations found that although calcium and magnesium were present roughly in equal amounts in most cases, in some places where the concentration of magnesium was disproportionately higher than that of calcium, e.g., calcium 54 ppm (as $CaCO_3$) and magnesium 467 ppm (as $CaCO_3$), the conditions apparently were not unfavorable to *Bulinus (Physopsis)* and *Biomphalaria* snails. Malek (1958) stated that only small amounts of magnesium were necessary for snails. Deschiens (1954b) also demonstrated that both *Bulinus truncatus* and *Biomphalaria glabrata* could tolerate magnesium ions (as $MgCl_2$) at a level as high as 510 ppm. The optimum range for *Bulinus (Ph.) nasutus*, however, was found to be between 1 and 90.3 ppm (as magnesium) (Webbe, 1962b); for *Biomphalaria glabrata* it was between 5 and 50 ppm (as magnesium) (Harry *et al.*, 1957).

c) Sodium

Deschiens (1954b) observed that *Bulinus truncatus* could tolerate sodium ions (as NaCl) up to 1374 ppm, and *Biomphalaria glabrata* up to 2359 ppm. Muirhead-Thomson (1958) indicated that *Bulinus* was not normally found where the salinity exceeded 5,500–6,000 ppm (as NaCl). Malek (1958) was of the opinion that water with a high concentration of sodium as compared to other cations, especially to calcium, was not favorable to snails; it appeared that the sodium ions tend to displace the beneficial calcium, so that less calcium would be absorbed by the snails. Frank (1963) concluded that water with a $CaCO_3$ concentration of approximately 18 ppm and with a sodium/calcium ratio of 1 was best for growth and fecundity of *Biomphalaria pfeifferi*. Schutte and Frank (1964) found that in the field both *Bulinus (Physopsis)* and *Biomphalaria* were always present in water with a sodium/calcium ratio between 0.5 and 2.0, whereas *Bulinus (Physopsis)* was always present and *Biomphalaria* most uncommon in waters with a ratio above 2.4.

d) Carbonate and Bicarbonate

Deschiens (1954b) showed that both *Bulinus truncatus* and *Biomphalaria glabrata* could tolerate up to 4,000 ppm of carbonate (as $NaHCO_3$). The optimum range of carbonate plus bicarbonate for *Biomphalaria glabrata* was between 70 and 120 ppm (as $HCO_3$ and $CO_3$) (Harry *et al.*, 1957). For *Biomphalaria pfeifferi* the optimum range of bicarbonate was 20 to 200 ppm (as $CaCO_3$) (Harrison *et al.*, 1970). Earlier Harrison and Shiff (1966) and Williams (1970a, 1970b) observed that both *Bulinus globosus* and *Biomphalaria pfeifferi* showed their greatest intrinsic rate of natural increase in water with 20 to 200 ppm bicarbonate as $CaCO_3$, and Harrison (1968) found that *Biomphalaria pfeifferi* oxygen uptake was highest in water with 35 ppm bicarbonate as $CaCO_3$. In both lower and higher concentrations the uptake rate was lower.

e) Iron

Deschiens (1954b) indicated that the maximum concentration of iron (as $FeSO_4$) tolerated by *Biomphalaria glabrata* was 140 ppm. Dechancé and Deschiens (1955) found that the lethal concentration of ferric chloride for *Bulinus truncatus* and *Biomphalaria glabrata* was 65 ppm and of ferric sulfate, 500 ppm. In the field Malek (1958) observed that a few marshes and ponds with a red precipitate, presumably iron compounds, were devoid of snails. In field studies Schutte and Frank (1964) found that adult *Bulinus (Physopsis)* were occasionally found in small streams fed by underground water with an iron concentration of 0.03 ppm (as Fe).

f) Chloride

Watson (1950) reported that the maximum salinity (dissolved chloride) found in natural habitats of *Bulinus* in Iraq was 1,010 ppm (as NaCl); his laboratory experiments showed that adult snails survived in water with as much as 1,500 ppm dissolved chlorides (as NaCl). Deschiens (1954b) showed that the maximum concentration of chlorides as NaCl tolerated by *Bulinus* was 2,123 ppm, and with *Biomphalaria glabrata* it was 3,641 ppm. In the field Harry *et al.* (1957) found the optimum chloride concentration for *Biomphalaria glabrata* was between 20 and 110 ppm (as Cl). Malek (1958) reviewed the literature and concluded that a high total salt content was lethal to freshwater snails; he observed that *Bulinus* was less tolerant of salinity than *Biomphalaria;* consequently the former was less common in the coastal habitats.

g) Sulfate

Deschiens (1954b) found that the maximum concentration of sulfate (as $Na_2SO_4$) tolerated by both *Bulinus truncatus* and *Biomphalaria glabrata* was 1,350 ppm. In the field Harry *et al.* (1957) found the optimum concentration for *Biomphalaria glabrata* was 10 to 80 ppm (as $SO_4$).

h) Nitrate and Nitrite

Deschiens (1954b) indicated that the lethal concentration of nitrate (as $NaNO_3$) for *Bulinus truncatus* was 1,000 ppm and for *Biomphalaria glabrata* 750 ppm. The lethal concentration of nitrite (as $NaNO_2$) on the other hand, was only 500 ppm for both of the species. Evidently, the nitrates seemed better tolerated by both species than nitrites; *Bulinus truncatus* had a greater resistance than *Biomphalaria glabrata* to nitrates and an equal resistance to nitrites. In field studies Schutte and Frank (1964) found these chemicals were usually absent or in low concentrations ranging between 0.06 and 1.2 ppm (as nitrogen).

i) pH Value

Ripsom (1949) reported that ovipositing among *Biomphalaria glabrata* slowed down when the pH in the aquaria exceeded 7.8, and at 8.2 egg-laying ceased. In the field Sioli (1953) concluded that the most important factor determining the presence of aquatic snails in bodies of water seemed to be water pH rather than a lack of dissolved salts (such as calcium ions). However, Boycott (1936) concluded that pH was not a major factor in snail distribution but that the calcium content of the water might be more important. In his studies of *Lymnaea caillaudi,* van Someren (1946) found the snails tolerated a pH range of 6.0 to 9.5, but that the snails were present more frequently in a range of 6.5 to 8.0. Standen (1949) observed that *Biomphalaria glabrata* tolerated a fairly wide range of pH and values between 7.3 and 8.0 were not unusual. Deschiens (1954b) observed in the laboratory that both *Bulinus truncatus* and *Biomphalaria glabrata* could tolerate a pH range of 4.0 to 10.0. The World Health Organization (1957) also reported that both for field and laboratory conditions intermediate host snails of schistosomiasis tolerated a wide pH range. Laboratory experiments verified that *Bulinus truncatus, Biomphalaria adowensis adowensis* (Bourguignat) and *Biomphalaria glabrata* could be bred between pH 4 and 10; within this range variation in pH seemed to have no effect on the density of snail populations. Harry *et al.* (1957) observed that, although a pH range of 6.0 to 9.1 was found in *Biomphalaria glabrata* habitats, the abundant populations tended to occur in waters with a range of 7.0 to 7.6. Similar observations were made by Harry and Aldrich (1958), who found snails were most abundant and persistent within the range of pH 7.0 to 8.0. They concluded that pH was probably not a limiting factor in the distribution of the snails in Puerto Rico. Malek (1958) also suggested that pH rarely was a limiting factor in the distribution of planorbid snails; he observed that pH between 6.0 and 9.0 was tolerable. He concluded that the combined effects of other factors correlated with pH (such as alkali reserve, $CO_2$ content, sunlight; photosynthesis with active removal of $CO_2$ and the production of $O_2$, as well as the character of the substratum) were more important than pH. Malek's view was also supported by Webbe (1962b). Schutte and Frank (1964) adequately buffered with bicarbonates; therefore few were found with a pH below 6.7 or above 8.4.

In field studies by Harrison (1966) and Harrison and Rattray (1966) the importance of water chemistry was especially stressed. They observed that recolonization of *Bulinus (Physopsis)* spp. and *Biomphalaria pfeifferi* after treatment with Bayluscid was related to water hardness. In hard water with bicarbonate 114 to 163 ppm as $CaCO_3$, and calcium 20 to 45 ppm as Ca, these snails reappeared 10 months later; in soft water with bicarbonate 15 to 40 ppm as $CaCO_3$, and calcium 2 to 6 ppm as Ca, 22 months were required for reappearance. In their field studies Pimentel and White (1959a) observed no differences in pH, alkalinity, $CaCO_3$ and electric conductance among habitats with or without *Biomphalaria glabrata.*

From the foregoing it is evident that clearcut conclusions on the water quality required by snails are rather difficult to draw. It may be stated generally that biologically conditioned water of 'medium hardness' (i.e., calcium 5 to 40 ppm as $CaCO_3$, and bicarbonate 20 to 200 ppm as $CaCO_3$) as classified by Williams (1970a), would be favorable for culturing snails.

4. Food

As indicated in the references that follow, most investigators used lettuce to feed their snails, either in its fresh condition or dried, boiled or dried-boiled (Gordon *et al.*, 1934; Cowper, 1946; Standen, 1949; Stirewalt,

1954; Shah and Gadgil, 1955; Chernin *et al.*, 1956; Malek, 1958; Claugher, 1960; Najarian, 1960a; Berrie and Visser, 1963; Frank, 1963; Ritchie *et al.*, 1963a; Sturrock, R. F., 1965; Chu *et al.*, 1966a, 1966b; Sturrock, B.M., 1966; Wright and Bennett, 1967; Shiff and Garnett, 1967; Harrison, 1968; Harrison *et al.*, 1970; Sodeman, 1970; Sturrock, B.M. and Sturrock, R.F., 1970; Sturrock, R.F. and Sturrock, B.M., 1970; Williams, 1970b and Joy, 1971). In addition to lettuce some recommend: dried maple leaves (Ward *et al.*, 1947; Stirewalt, 1954 and Malek, 1958), dried lucerne (Frank, 1963), decaying mango leaves (Shah and Gadgil, 1955), water cress (Chernin *et al.*, 1956; Chernin, 1957b; Chernin and Michelson, 1957a, 1957b; Michelson, 1961 and Jobin and Michelson, 1967); soft bean leaves (Chu *et al.*, 1966a) and raw carrot (Shiff, 1964a and Sturrock, R.F. 1966). In nature, Watson (1950) observed that *Bulinus truncatus* fed on the leaves, twigs and bark of eucalyptus, the leaves and twigs of oleander, and the leaves of palm, apricot and mulberry.

Semi-synthetic foods were also proved useful. Malek (1950) occasionally fed *Biomphalaria alexandrina* cooked wheat cereal supplied on cork floats, as advocated by Noland and Carriker (1946) for *Lymnaea stagnalis appressa* Say. Standen (1951) made an artificial food, which assisted *Biomphalaria* in passing the difficult stages immediately after hatching. His mixture contained Bemax (wheat germ preparation), dried milk, powdered dried lettuce and sodium alginate. Moore *et al.* (1953) substituted Cerophyl (dried grass cuttings) for the dried lettuce and added Glandex (fish food); this modified formula was satisfactory for maintaining *Bulinus truncatus* snails. Claugher (1960) omitted the dried milk used in Standen's formula and increased the amount of lettuce; his modified formula was also satisfactory for feeding *Bulinus truncatus*. Lee and Lewert (1956) and Chernin and Michelson (1957a) confirmed the value of Standen's formula as modified by Moore *et al.* (1953) for rearing *Biomphalaria glabrata*. Joy (1971) also found the sodium alginate was adequate as a supplemental food for *Biomphalaria glabrata*. Shah and Gadgil (1955), however, found that Standen's artificial food failed to attract any of the snails in their laboratory. Erickson *et al.* (1961) noticed that the formula modified by Moore *et al.* (1953) greatly reduced both body growth and egg production of *Biomphalaria glabrata* as compared with results obtained with the same species of snails fed a formula without alginate. Ritchie *et al.* (1963a, 1963b) modified the formula further to include only Cerophyl, Glandex, wheat germ and powdered milk in a ratio of 4 : 2 : 1. They reported that this new formula greatly shortened the maturation time of *Biomphalaria glabrata* to egg-laying and also improved their survival rates. Etges and Ritchie (1966) also used this new formula for raising *Biomphalaria glabrata*. In a comparative snail-diet study, Eveland and Ritchie (1972) found that when *Biomphalaria glabrata* were maintained on the modified formula (Ritchie *et al.*, 1963a, 1963b), Gaines meal (dog food in compressed pellets) or a commercial chicken food, the snails grew better than those fed Romaine lettuce, squash or the rhizome of a *Caladium* plant (elephant-ear plant).

Still others used fish food (Sodeman, 1970) or rat chow (Mecham and Holliman, 1972) for their snails. For his mass cultivation of *Biomphalaria glabrata* Rowan (1958) used a mixture of dog chow, dry silt, and calcium carbonate in a ratio of 50 : 50 : 1. Pelegrino and Gonçalves (1965) used Cerophyl, commercial guinea pig pellets, wheat germ and Pablum in a ratio of 4 : 2 : 2 : 1 to feed their newly hatched *Biomphalaria glabrata*.

Algae, either inadvertently or intentionally, have long been recommended as snail-food additives. Colton (1908) observed that the presence of algae greatly enhanced growth of *Lymnaea columella*. Boycott (1936) was of the opinion that in nature most snails were not dependent for food on any particular plant. According to him, snails liked vascular plants because while alive they oxygenated the water and provided surfaces for algal growth. The algae which grew on mud, stones and other surfaces, together with decayed remains, constituted the main source of food for snails. In his field studies van Someren (1946) found that a good habitat for *Lymnaea caillaudi* was characterized by a certain amount of macrophytic vegetation and a rich assortment of diatoms, desmids and other microscopic algae which usually covered the mud or plants. Similar studies by Taylor and Mozley (1948) culturing *Lymnaea truncatula* (Müller) found that the algae grown on a heavy clay soil collected from natural habitats of those snails served to stimulate rapid snail multiplication in their laboratory. Kendall (1953) used this same method and verified that *Lymnaea truncatula* grew rapidly and with low mortality in dishes provided with vigorous growth of green algae. Bitakaramire (1968) noticed that the algae grown in tanks were the main food source for *Lymnaea natalensis* (Krauss). With regard to aquatic schistosome-carrying snails, Scott (1942) appeared to be the first to observe that *Biomphalaria glabrata* in the field were found on a thin film of algae which grew on the sides and bottoms of canals. Standen (1949) observed that very young *Biomphalaria glabrata* snails fed almost exclusively upon unicellular algae until they were about two weeks old. Shah and Gadgil observed that algae grown on the sides of aquaria attracted both young and old *Ferrissia tenuis* (Bourguignat) snails. Hubendick (1958) also stated that the presence of algae was one of the basic necessities in providing suitable conditions for newly-hatched snails. Watson (1958) observed that *Bulinus truncatus* were feeding on algae and decaying vegetable matter in the field. Malek (1958) recommended the use of

green algae, blue-green algae and diatoms as suitable foods for *Biomphalaria sudanica* and *Bulinus (Physopsis) ugandae* (Mandahl-Barth). By examining the contents of the digestive tract he found that these snails showed no preference for any particular species of the microflora. As long as green and blue-green algae and diatoms were represented in the diet, that food was suitable for those snails. He considered it was not so much the type of diet but the quantitative composition which was important in the habitat of the snails. In the field, Pimentel and White (1959a) observed that areas with light to medium pollution supported more *Biomphalaria glabrata* than similar habitats elsewhere. Their observation strongly suggested that algae were important in providing the necessary foods for those snails. They (1959b) also noticed that in habitats where there was little macroscopic plant or animal life and no sewage, the snails were probably feeding on the dead plankton adhering to the substrate. Wright (1960) noticed that *Biomphalaria glabrata* in some of his experiments grew better when supplied with green algae. Gohar and El-Gindy (1962) observed the stomach contents of *Bulinus truncatus, Biomphalaria alexandrina* and *Lymnaea caillaudi* contained diatoms, unicellular algae, desmids, some filamentous algae, cysts of unicellular algae, protozoans, fragments of higher plants, sand and mud particles. There was no marked difference among those snails with respect to the selection of food. In the field Webbe (1962b) also found no apparent relationship between a particular species of alga or diatom and the aquatic snails present; as long as algae were there in appreciable quantities, thriving snail populations persisted. McClelland (1964) stated that green algae and diatoms, especially the green alga *Stigeoclonium*, seemed essential for the survival of young *Bulinus nasutus*, particularly during the first two weeks after hatching. Pellegrino and Gonçalves (1965) also observed that the newly hatched *Biomphalaria glabrata* snails were feeding on an algal film. Chu *et al.* (1966b) maintained newly hatched *Bulinus truncatus* in crystallizing dishes flourishing with algae. Kinoti (1968) observed that a unicellular alga was essential for feeding newly hatched *Bulinus africanus*. Jennings *et al.* (1970) observed newborn *Biomphalaria pfeifferi* feeding on algae in plastic dishes. Stiglingh and van Eeden (1970) found that the food of *Bulinus tropicus* (Krauss) consisted largely of unicellular algae and diatoms; filamentous green algae *Spirogyra* and other species were only rarely found in the stomach of these snails.

As indicated, many investigators observed the importance of algae for growing snails, but only a few deliberately inoculated cultures with known types of algae to improve their production. Ripsom (1949) reported that inoculating the blue-green algae predominantly *Oscillatoria*, into aquaria greatly shortened the time necessary for maturation of *Biomphalaria glabrata;* they oviposited 63 days after hatching as compared to 80 days when fed lettuce. Hopf and Muller (1962) based on the method advocated by Kendall (1953), found that young *Biomphalaria glabrata* bred well in dishes containing autoclaved river mud rich in organic matter and provided with an artificial hard water (as stated previously) into which the blue-green alga *Oscillatoria* sp. was inoculated. McClelland (1964) advocated the use of pure algal cultures as worth consideration for *Bulinus nasutus* in laboratory cultures. Xavier *et al.* (1968) also obtained significantly improved results with *Bulinus globosus* and *Bulinus truncatus* fed on a blue-green alga, *Oscillatoria formosa*, inoculated into the dishes. Besides this simple method of algal inoculation, Deschiens and Adetonah (1969) went further and demonstrated that satisfactory results were obtained with *Bulinus forskalii*, previously difficult to raise under the usual techniques, when a living microfauna *(Rhabditis macrocerca* Kreiss & Faust coprocultures) and a microflora *(Chlorella*, a unicellular alga) were added.

Although many authors have found algae useful as food for snails, Claugher (1960) reported that blue-green algae were quite harmful to *Bulinus* snails; also brown algae were the indicators of unfavorable conditions. Several investigators found that filamentous green algae often tend to become so thick as to choke the snails in the tank mechanically; as a consequence, snails often became trapped and died. Webbe (1962b) also stated that an appreciable quantity of both *Bulinus* and *Biomphalaria* spp. did thrive in habitats with blue-green algae, but these very algae can be considered inimical to those same species in laboratory cultures.

All of the above information leads to the basic conclusion that lettuce as a food additive has become firmly established; algae have become accepted as a food additive under certain conditions and especially in cultures of young snails.

### 5. Population Density

The stunting of snails due to population density has been observed for many years. Hogg (1854) found that *Lymnaea stagnalis* confined in small aquaria were much smaller than those from the same egg-mass raised in large aquaria. He then stated that the snail had the power of 'adapting itself to the necessities of its existence.' Semper (1874) first suggested that the snails in aquaria with overcrowding depleted some ingredient essential to their well-being; thus it was a lack and not the production of some substances that caused the reduction of growth and lowered fecundity. Colton (1908) also found that the number of *Lymnaea columella* in a jar affected their rate of growth and suggested it was probably due

to increased secretions or wastes, as well as diminished aeration. Turner (1927) showed a marked stunting of two species of *Lymnaea* due to crowding and attributed it largely to semi-starvation produced by intensive competition for food. Winsor and Winsor (1935), also studying *Lymnaea columella,* found, with but few exceptions, a regular increase in egg-laying per snail as the population density decreased. Forbes and Crampton (1942) found that isolated individuals of *Lymnaea palustris* Müller grew faster and attained larger sizes than individuals grown in aggregates. Noland and Carriker (1946) reviewed the previous *Lymnaea* studies and concluded there was little agreement as to the minimum volume of water required to enable a single snail to grow to full size; the range was from 10 ml to 5,000 ml. DeWitt (1954) found that as the number of *Physa gyrina* Say reared together increased, both their shell size at the time of oviposition and the mean number of eggs per snail decreased. Pereira and Deslandes (1954) found that both growth and reproduction of *Biomphalaria glabrata* were adversely affected by crowding. Wright (1956) suggested that overcrowding might cause dwarfing of snails through an accumulation of excretory products in the water. In the laboratory Chernin and Michelson (1957a, 1957b) found the fecundity of *Biomphalaria glabrata* decreased as snail density increased per unit volume of water; but when the water volume was reduced on constant numbers of snails, there was not a similar effect but rather an increase in growth and fecundity. They concluded that no one hypothesis was sufficient to explain the effects of crowding on the fecundity of *Biomphalaria glabrata.* Lagrange (1957) showed that the fecundity of *Biomphalaria glabrata* was strongly influenced by diet; he suggested that some possible vitamin complex was involved. Wright (1960) discussed the possibility of at least three factors involved in stunting; food, collisions and a chemical pollution produced by 'pheromones.' He particularly favored pheromones which acted in low concentrations to inhibit growth and fecundity. Testing this possibility in the field, Berrie and Visser (1963) found a substance, mono-hydroxy-tricarboxylic acid-mono-isodecyl-dimethyl ester with a molecular weight of 360 and a general formula $Cl_8H_{32}O_7$. They extracted this substance from the water of a pool in which *Biomphalaria sudanica* was stunted. They found that it was responsible for inhibiting the growth of this species in their laboratory experiment. Shiff (1964b), demonstrated a trend towards reduced mean growth rate and increased individual variation in size as snails were more crowded. He concluded that one *Bulinus globosus* per liter was optimal in the culture. Sturrock, R. F. (1965) observed that growth of *Biomphalaria angulosa* at different densities (i. e., 1, 2 and 4 snails per 250 ml of water) was linear until the 7th week; after that time the crowding effect causing stunting and growth ceased to be linear. Jobin and Michelson (1967) applied a mathematical approach and found the fecundity (E) of *Biomphalaria glabrata* was directly proportional to F/NV, where F was food in grams, N was the number of snails in the habitat and V was the volume of water in liters. Berrie (1968) observed that both food shortage and possibly mutual interference were factors causing growth inhibition in *Biomphalaria sudanica tanganyicensis* (Smith) in the field. Berrie (1969) again in the field observed that populations of *Bulinus* and *Biomphalaria* built up rapidly under favorable conditions. Then the rates of growth and fecundity soon slowed down and eventually ceased as the population increased. He suggested that the food requirements of those snails might be one of the important factors for triggering reproduction. Gazzinelli *et al.* (1970), by using $^{59}Fe$, demonstrated that crowding of *Biomphalaria glabrata* was related not only to population density but also to population size; they discovered an inhibitory factor in water and feces derived from water with crowded snail populations. Sturrock, R.F. and Sturrock, B. M. (1970) showed that the growth of solitary *Biomphalaria glabrata* was directly proportional to water volume.

The various studies designed to evaluate the reason for growth inhibition under crowded conditions may be summarized by the statement that 'no one hypothesis was sufficient to explain the effects of crowding on the fecundity of *Biomphalaria glabrata.*'

### 6. Type of Aquarium

An unbelievably wide variation existed in the kinds of aquaria used by investigators in culturing snails. Their attitudes towards this matter were often so casual that some of them did not even bother to describe the aquaria they used in their experiments. Presumably most of them used so-called 'tanks' of variable capacities. In reviewing the literature the following were some types (other than the conventional ones) which were claimed to be efficient for culturing snails:

a) Porcelain-covered Metal Refrigerator tray (12.5 X 22.5 X 32.5 cm).

Lee and Lewert (1956) found this kind of container was most satisfactory for raising *Biomphalaria glabrata,* with each tray holding between 50 to 100 snails.

b) Enamel Tray

McClelland (1964) claimed these trays (30 X 25 X 5 cm) were efficient enough to produce a dependable supply of *Bulinus nasutus* of known age and standard size. These trays were also light, unbreakable, and could be stored compactly in tiers.

c) Battery Jar

Chernin (1957b) stated that a 3-liter jar was handy for handling small numbers of *Biomphalaria glabrata;* Olivier and Haskins (1960) used 2.5-liter jars to raise *Biomphalaria glabrata;* Michelson (1961) used 3-liter jars for

*Biomphalaria glabrata;* Ritchie *et al* (1966) used 6-liter jars for maintaining *Biomphalaria glabrata* in their flow system; Jobin and Michelson (1967) also used this same kind of jar for *Biomphalaria glabrata*.

d) Carboy

Ritchie *et al.* (1963a) found an inverted 20-liter carboy with the bottom removed by means of an electrically heated wire was convenient for mass culture of *Biomphalaria glabrata* in a continuous flow system.

e) Drinking Glass

Ritchie *et al.* (1966) maintained *Biomphalaria glabrata* singly in this type of aquaria containing 200 ml of unaerated tap water.

f) Crystallizing Dish

Wright (1960) used such a dish (10 X 5 cm) to raise *Bulinus forskalii;* Frank (1963) also used it (20 X 10 cm) to raise *Biomphalaria pfeifferi*. Sturrock, B. M. (1966, 1967) and Sturrock, R. F. (1965, 1966) also used a 250-ml dish for culturing *Bulinus nasutus, Biomphalaria angulosa* and *Biomphalaria pfeifferi*. Chu *et al.* (1966a, 1966b) used it for *Bulinus truncatus*. Xavier *et al.* (1968) used 180-ml and 400-ml dishes for *Bulinus globosus* and *Bulinus truncatus* cultures.

g) Baking Dish

Stirewalt (1954) maintained *Biomphalaria glabrata* in large Pyrex baking dishes.

h) Plastic Tank

Sodeman (1970) found such a tank (5 liter) more efficient than the conventional 32-liter glass aquaria for raising *Biomphalaria pfeifferi* in mass cultures.

i) Polyethylene Container

Jennings *et al.* (1970) used such a rectangular container (6.5 X 4.5 X 11.0 cm with a capacity of 300 ml) to raise *Biomphalaria pfeifferi*.

j) Polyethylene Bag

Pellegrino and Gonçalves (1965) found this bag insured a constant supply of egg-masses of *Biomphalaria glabrata* when lettuce was used as food for the adult snails.

Although Claugher (1960) indicated that the type of culture container was one of the essential requirements for establishing laboratory colonies of *Bulinus* snails and recommended all-glass tanks, it is clear from the above review of the various containers used that others were not so concerned. It remains an open question whether the type of aquarium in itself is a critical factor in culture work.

### 7. Substratum

Gordon *et al.* (1934) observed that some mud was necessary in containers used to culture *Bulinus globosus* and *Biomphalaria pfeifferi*. They indicated the particles in mud presumably were utilized in the crop for grinding food. In rearing *Bulinus truncatus* and *Biomphalaria glabrata,* the presence of grit was also observed by Cowper (1946). Ward *et al.* (1947) found that even a little sand in an aquarium was beneficial to planorbid snails. Pan (1958) also found that the many sand grains commonly encountered in the gizzards of *Biomphalaria glabrata* were necessary for grinding ingested foods. Chernin and Schork (1959) suggested that the growth of snails in axenic cultures was in part due to the small particle size of the dietary components. Firm mud bottoms composed of black cotton soils derived either from decomposed phonolites of Cretaceous age or from black soils of gneiss and schist origins, were observed by van Someren (1946) as preferred by *Lymnaea caillaudi* for crawling. Macnae (1956) indicated that the degree of compactness of the substrate was of more consequence to the snails than the particle size. De Meillon *et al.* (1958) doubted that snails would ingest mud in the same way as earthworms; they suggested that snails browsed on the surface microorganisms as often observed on the sides of concrete aquaria and emergent vegetation. They believed this was probably the reason that snails did not favor excessively soft and diffuse mud such as that found in many backwaters.

In the laboratory, Standen (1949) observed that both *Bulinus truncatus* and *Biomphalaria alexandrina* moved easily upon coarse soft mud. Therefore, he used sand (95 percent by volume) and air-dried pond mud (5 percent by volume) in aquaria for rearing those snails. Moore *et al.* (1953) mixed 90 percent sand and 10 percent potting soil for raising *Bulinus truncatus*. Chernin (1957b) used marble chips as a substrate for *Biomphalaria glabrata*. Chernin *et al.* (1956) and Chernin and Michelson (1957a) mixed autoclaved potting soil, sand and marble chips. While Claugher (1960) used sterilized silver sand as a substrate for *Bulinus* snails, his purpose was mainly to have support for rooted aquatic plants *(Vallisneria spiralis* and *Elodea)*. Chu *et al.* (1966a, 1966 b) used mud for his *Bulinus truncatus* cultures. Kinoti (1968) used mud and sand as substrate for *Bulinus africanus*. Xavier *et al.* (1968) had a sterilized soil as a substratum for raising *Bulinus globosus* and *Bulinus truncatus*. In the field, Watson (1958) observed that *Bulinus truncatus* preferred a substrate of mud rich in organic matter (*i.e.*, decayed aquatic plants or decaying leaves). Pimentel and White (1959a) also noticed that most *Biomphalaria glabrata* snails were found on substrates consisting of 42 percent clay (passed no. 220 sieve) and 41 percent sand (retained on no. 60 sieve). They found only a few *Biomphalaria glabrata* in streams with rock bottom and where the water was fast flowing or subjected to severe flushing action.

These observations indicate that the snails studied do best under conditions that comply with the nature of the soils observed in the field. Either mud or sand or a mixture of both, with a reasonable compactness, usually serve as satisfactory substratum.

### 8. Parasites and Predators

It was not unusual to find harmful conditions, either due to parasitism or to predation occurring in the habitats of fresh-water snails (Malek, 1958; Hubendick, 1958). Michelson (1957) gave a review of the various parasites and predators of fresh-water mollusks and he used the following group-headings: (a) microbial agents, including algae, fungi, bacteria and protozoa, (b) flat-worms and nematodes, (c) annelids, (d) rotifers, (e) arthropods, (f) mollusks, (g) amphibians and reptiles, (h) fish and (i) birds and mammals.

In the laboratory, Pringle (1960) observed that larvae of 4 species of chironomid midges, namely, *Polypedilum* sp., *P. (Pentapedilum) anale* Freeman, *P. (Polypedilum) kibatiense* Goetghebuer and *Chironomus (Cryptochironomus) acutus* Goetghebuer invaded and destroyed the contents of the egg-masses of *Bulinus globosus*. However, he did not consider that this type of predation exercised any restraint on the density of adult snails.

The threat by ostracodes (micro-crustaceans) on laboratory snail cultures has been known for some time. Deschiens (1954a) demonstrated that an ostracode, *Cypridopsis hartwigi* Müller actively attacked the bodies of *Bulinus truncatus* and *Biomphalaria glabrata*. Claugher (1960) also found that another ostracode, *Cypridopsis vidua* O. F. Müller was troublesome in Bulinus snail cultures. He found that this ostracode tended to cluster around the anal lobe of the snail, causing the snail to withdraw into its shell and thus preventing it from feeding. McClelland (1964) stated that *Bulinus nasutus* maintained in the laboratory frequently became infested with ostracodes which were very harmful to young snails. He found that frequent changes of water with subsequent removal of feces and decomposing lettuce would result in pest-free snail cultures.

Lo (1967) reported that two species of ostracodes, *Cypridopsis vidua* and *Cypricercus reticulatus* (Zaddach) caused *Biomphalaria glabrata* to withdraw into their shells. He also found that the snail egg-masses were destroyed when the concentration of ostracodes became high, or when there was insufficient food for the ostracodes. He suggested the surest way to eliminate ostracodes was to re-establish snail colonies using non-contaminated eggs or young. Recently, Sohn and Kornicker (1972) reported that the ostracode, *Cypretta kawatai* Sohn and Kornicker was an effective predator in laboratory experiments on 1- to 3-day old *Biomphalaria glabrata*.

The experience in several laboratories substantiates the suggestions made by McClelland (1964) and Lo (1967) as stated above, that pest-free snail cultures are necessary for successful laboratory snail cultures.

A few investigators tried to raise snails or snail organs axenically. By disinfecting snail eggs externally with a dilute sodium hypochloride solution (a solution of 'Clorox', a commercial liquid bleach), Chernin (1957a) was able to obtain bacteriologically sterile *Biomphalaria glabrata* and maintained them in culture tubes for 21 to 29 days in his HS(M) (=modified handling solution), or HS(M) plus glucose. Chernin and Schork (1959) found a basic diet consisting of autoclaved brewer's yeast and formalin-killed *Escherichia coli* sustained axenic growth of *Biomphalaria glabrata* in their HS(M). When maintained with this diet in sterile aquarium water, some snails reached adult size after prolonged periods. They concluded that this slower growth rate was probably due to the limitations in food supply. Nevertheless, the feasibility of securing axenic *Biomphalaria glabrata* would thus make possible a variety of studies requiring more carefully controlled conditions than have heretofore been attainable in the laboratory. Chernin (1959) also grew *Biomphalaria glabrata* axenically in culture tubes and bottles; these snails were fed with a mixture of autoclaved brewer's yeast and formalin-killed *Escherichia coli* as advocated by Chernin and Schork (1959). Some of these snails were maintained in that culture for 70 days and developed shells with diameters up to 6.4 mm. Chernin (1960) found those axenically reared *Biomphalaria glabrata* snails (2 to 7 mm in shell length) were highly susceptible to infection with *Schistosoma mansoni* in the absence of any evident bacteriological contamination; those snails produced normal infectious cercariae following the normal periods of incubation. Using his 'nutrient medium' Chernin (1963) was able to retain normal hearts of *Biomphalaria glabrata* in vitro, and heartbeat was observed for at least 47 days after explantation. Vieira (1967a) demonstrated that *Biomphalaria glabrata* could grow and oviposit under axenic conditions with the use of a semisynthetic diet. It took 6 to 7 months for snails to grow and produce eggs. Egg-laying occurred only if vitamin E was added. Later, Senna and Vieira (1970) devised a simplified and purified nutrient medium for culturing *Biomphalaria glabrata* axenically. Although snails eating this simplified food laid fewer eggs than those eating the casein-lettuce as described by Vieira (1967b), nevertheless, the study indicated that snails could be successfully raised axenically in a chemically defined medium.

In summary, while it has been established that snails can be cultured axenically and do produce eggs after a period of about 6 months, axenic culture is still far from feasible mass production, since by conventional methods these same snails need only a month or two to produce eggs and complete a normal cycle.

### PROPOSED STUDY

Cultivation of snail hosts of African

schistosomiasis in the laboratory is often undependable. For example, while *Biomphalaria glabrata* snails are relatively easy to maintain in our laboratory, cultivation of *Biomphalaria pfeifferi* is difficult and unreliable. Egg-laying was often unpredictable both in frequency and intensity; there was a low survival rate among the young; and large differences appeared in the rate of body growth. These were only some of the difficulties involved in raising this species of snail. Similar problems were also encountered with *Bulinus globosus*, which often declined at a steady rate. These observations were made when both species were cultured in one of the standard methods using an aquarium (20 X 25 X 35 cm), a corner filter containing charcoal and glass wool, crushed limestone over the bottom, a supply of spring water and constant aeration. The snails were fed lettuce. While the cultures were obviously inefficient, the reasons were not quite clear. In host-parasite relationship studies, Lo (1972) stated that *Bulinus globosus* snails were extremely susceptible to *Schistosoma haematobium* and produced a large number of cercariae subsequently. He was, however, reluctant to use this species of snail for maintaining the cycle because of great difficulty in producing a sufficient number of snails.

In a review of culture conditions, Harrison and Shiff (1966) concluded that water of 'medium' hardness, as determined by Williams (1970a), appeared to be most suitable for both *Bulinus globosus* and *Biomphalaria pfeifferi*. Yet, according to Williams' standard, the water used in our own laboratory was actually a relatively 'hard' water. A change to softer water also seemed desirable since the large amount of spring water used, which was obtained from a commercial dealer, was costly.

Many authors agreed that the food was of primary importance in snail growth. Yet, the great majority of these food additives, as mentioned in the review above, were subjected to putrefaction in the aquaria used. Although this putrefaction could be avoided in axenic cultures, the methods were still inefficient.

Concurrently, in a separate study of *Oncomelania* snails, the intermediate hosts of Oriental schistosomiasis, it was discovered that snail production could be increased several fold when the snails were raised in Petri dishes containing a mud mound with a pure culture of the blue-green alga *Phormidium luridum* var. *olivacea* Boresch. Later it was found that a mixture of blue-green algae, locally obtained, gave better production than a pure culture of *Phormidium luridum*. These observations, together with the information reviewed above, led to the decision that both water quality and food additives should be investigated further to determine their effects on the growth and fecundity of these planorbid snails.

The main aims of the present study are to develop simpler, more efficient, more dependable and more easily reproducible culture methods and to have better understanding of the ecological requirement of snails. As a partial approach the following questions were considered.

1. Optimum number of snails to be accommodated in one container (Part I).
2. Effect of various types of waters on the growth and fecundity of snails (Part II).
3. Effect of various species of algae (Part III).
4. Effect of various kinds of muds and mud extracts (Part III).
5. Possibility of formulating a mud with partially known compositions as an approach to make more reproducible conditions (Part IV).

## MATERIALS AND METHODS

### 1. Snails

This study dealt with both *Bulinus globosus* and *Biomphalaria pfeifferi*, snails belonging to the family Planorbidae. The former were stocks brought to our laboratory from Salisbury, Rhodesia in 1965; the latter came from Liberia in 1968. They were maintained in conventional types of aquaria, tanks, and only laboratory bred animals were used.

### 2. Methods to obtain newly hatched snails

To obtain eggs for hatching, about 50 egg masses, with embryos equally developed, were scraped from the wall of tanks (in Part I of the study) or from the wall of trays (Part II and thereafter) with a pair of blunt flat forceps and placed into Petri dishes with 40 ml of aerated tap water. Under constant cool-white fluorescent illumination at temperatures between 25 and 27° C, these eggs hatched 7 to 8 days after they were laid. In Part I and Part II of the study, the newly hatched young, about 0.78 mm shell length in *Bulinus globosus* and 0.61 mm in *Biomphalaria pfeifferi* were then transferred with a Pasteur capillary pipette to appropriate culturing trays. In Part III and Part IV of the study, only *Biomphalaria pfeifferi* were used. Newly hatched young were first maintained for one day in similar Petri dishes filled with 40 ml of aerated tap water and a small amount of a pure alga, *Fremyella tenera*, together with some mud until their shell length was about 0.70 mm. They were then transferred either to culturing trays or Petri dishes. Great care was taken to avoid damaging these young snails so early in their development. The age differences were not significant since 200 to 300 new born specimens of uniform size were easily obtained from any single dish at one time.

### 3. Water

The following types of water were used to make mud paste or used in the trays or dishes as overlay.

a) Distilled (Dis): Pyrex glass distilled.
b) Tap (Tap): Aerated for one day or more.
c) Spring (Spr): Locally obtained from the Arbor Springs Water Co.
d) Deionized (Dei): Barnstead Bantam Demineralizer deionized.
e) Calcium: calcium enriched.
f) Magnesium: magnesium enriched.
g) Calcium-magnesium: calcium and magnesium enriched.
h) Artificial (Art): water enriched with known amounts of chemicals. Called 'artificial' for simplicity.
i) Modified Artificial I - III (Mod Art I - III).
j) Bold's Basal Medium (BBM): See Bold, (1967).
k) BBM - Si - Urea (BBMSU)

The chemical analyses of the first 3 types of water were made with a HACH Chemical Company Kit. The results were shown in Table 1.

In the preparation of types e, f, and g, the following stock solutions were made first:

Calcium Stock:
$CaCl_2 \cdot 2H_2O$ 3.0 gm
$CaSO_4 \cdot 2H_2O$ 2.0 gm
Distilled ..... 2,000 ml

Magnesium Stock:
$MgCl_2 \cdot 6H_2O$ ..... 2.0 gm
$MgSO_4 \cdot 7H_2$ ..... 1.4 gm
Distilled ..... 2,000 ml

NaOH N/100 Solution:
NaOH ..... 0.4 gm
Distilled ..... 1,000 ml

The final solutions were then prepared according to the formulae shown in Table 2. The amounts of calcium, magnesium and calcium-magnesium ions for these 3 types of water were reasonably close to those found in the spring water which served as control in the chemical analyses shown in Table 3.

In the preparation of type h, the following stock solutions were made first; in use, appropriate amounts of these 3 stock solutions were added to 500 ml distilled water to make a pH of 7.2.

Stock Solution 1 (Osterhout's Medium Concentrate):
NaCl ... 5.22 gm
$MgCl_2$ .. 0.409 gm (=$MgCl_2 \cdot 6H_2O$ -0.978 gm)
$MgSO_4$ .. 0.198 gm (=$MgSO_4 \cdot 7H_2O$ - 0.405 gm)
KCl .... 0.111 gm
$CaCl_2$ .. 0.060 gm (=$CaCl_2 \cdot 2H_2O$ - 0.079 gm)
Distilled ..... 500 ml

Stock Solution 2 (=Phosphate Buffer):
$KH_2PO_4$ ..... 6.805 gm
Distilled ..... 1,000 ml

Table 1. Analyses of distilled, tap and spring waters

| Tests | Types of waters | | |
|---|---|---|---|
| | Distilled | Tap | Spring |
| Alkalinity (as ppm $CaCO_3$) | | | |
| Carbonate | 0 | 0 | 0 |
| Bicarbonate | 10 | 60 | 340 |
| Hardness (as ppm $CaCO_3$) | | | |
| Total | * | 75 | 465 |
| Calcium | * | 40 | 330 |
| Magnesium | * | 35 | 135 |
| Chloride (ppm) | 5 | 42.5 | 85 |
| Nitrate (ppm) | 0.03 | 0.60 | 0.25 |
| Sulphate (ppm) | 2 | 75 | 65 |
| pH | 6.6 | 7.4 | 7.4 |

* Concentration below the measurable level.

Stock Solution 3 (=Standard Potassium Hydroxide)
KOH ..... 2.805 gm
Distilled ..... 1,000 ml
When in use:
Solution 1 ..... 5.0 ml
Solution 2 ..... 16.7 ml
Solution 3 ..... 25.0 ml
Distilled ..... 500.0 ml

Table 2. Components of calcium, magnesium and calcium-magnesium waters

| Components (ml) | Types of waters | | |
|---|---|---|---|
| | Calcium | Magnesium | Calcium-magnesium |
| Calcium Stock | 300 | - | 300 |
| Magnesium Stock | - | 300 | 300 |
| Distilled | 1200 | 1200 | 900 |
| NaOH N/100 | 4.5 | 6.0 | 4.8 |
| pH | 7.25 | 7.25 | 7.25 |

Table 3. Analyses of calcium, magnesium and calcium-magnesium waters (Spring water served as control)

| Tests | Types of waters | | | |
|---|---|---|---|---|
| | Calcium | Magnesium | Calcium-magnesium | Spring |
| Alkalinity (as ppm $CaCO_3$) | | | | |
| Carbonate | 0 | 0 | 0 | 0 |
| Bicarbonate | 15 | 15 | 15 | 340 |
| Hardness (as ppm $CaCO_3$) | | | | |
| Total | 310 | 145 | 455 | 465 |
| Calcium | 310 | 0 | 310 | 330 |
| Magnesium | 0 | 145 | 145 | 135 |
| pH* | 6.55 | 6.45 | 6.50 | 7.40 |

* The pH determinations were made before the addition of an appropriate amount of N/100 NaOH.

Table 4. Components of modified artificial waters, I, II and III

| Components | Types of waters | | |
|---|---|---|---|
| | I | II | III |
| Artificial water | 98.92 ml | 200.0 ml | 1,250.0 ml |
| $NH_2CONH_2$ * | 0.50 ml | - | - |
| $Na_2SiO_3$ * | 0.50 ml | - | - |
| EDTA + KOH * | 0.02 ml | - | - |
| $FeSO_4.7H_2O$ * | 0.02 ml | - | - |
| $H_3BO_3$ * | 0.02 ml | - | - |
| Miscellaneous * | 0.02 ml | - | - |
| $CaCl_2.2H_2O$ | - | 150.0 mg | - |
| $CaSO_4.2H_2O$ | - | 100.0 mg | 250.0 mg |

*Stock solutions as used in the making of BBM and BBMSU

In the preparation of 3 types of modified artificial waters I through III, the formulae given in Table 4 were used.

To make BBM and BBMSU, both of which were primarily used as the media for algal growth, the following stock solutions were made first:

| | | | |
|---|---|---|---|
| $NaNO_3$ (in 2: | 10 gm | + Distilled | 400 ml |
| $CaCl_2.2H_2O$ | 1 gm | + " | " |
| $K_2HPO_4$ | 3 gm | + " | " |
| $KH_2PO_4$ | 7 gm | + " | " |
| $MgSO_4.7H_2O$ | 3 gm | + " | " |
| NaCl | 1 gm | + " | " |
| $NH_2CONH_2$ | 10 gm | + " | " |
| $Na_2SiO_3$ | 4 gm | + " | " |
| EDTA* | 50 gm | ) + " | 1,000 ml |
| KOH | 31 gm | " | " |
| $FESO_4.7H_2O$ | 4.98 gm | + Acidified** | 1,000 ml |
| $H_3BO_3$ | 11.42 gm | + Distilled | 1,000 ml |
| Miscellaneous | | | |
| $ZnSO_4.7H_2O$ | 8.82 gm | | |
| $MnCl_2.4H_2O$ | 1.44 gm | | |
| $MoO_3$ | 0.71 gm | + Acidified** | 1,000 ml |
| $CuSO_4.5H_2O$ | 1.57 gm | | |
| $Co(NO_3).6H_2O$ | 0.49 gm | | |
| NaOH | 40.01 gm | + Distilled | 1,000 ml |

* Ethylene-diamine-tetra-acetic acid

** Acidified water:
$H_2SO_4$ (concentrated) 1 ml Distilled 999 ml

Table 5. Components of BBM and BBMSU

| Components (ml) | Types of media | |
|---|---|---|
| | BBM | BBMSU |
| $NaNO_3$ | 10.0 | 10.0 |
| $CaCl_2.2H_2O$ | 10.0 | 10.0 |
| $K_2HPO_4$ | 10.0 | 10.0 |
| $KH_2PO_4$ | 10.0 | 10.0 |
| $MgSO_4.7H_2O$ | 10.0 | 10.0 |
| NaCl | 10.0 | 10.0 |
| $NH_2CONH_2$ (=Urea) | - | 10.0 |
| $Na_2SiO_3$ | - | 2.0 |
| EDTA + KOH | 1.0 | 1.0 |
| $FeSO_4.7H_2O$ | 1.0 | 1.0 |
| $H_3BO_3$ | 1.0 | 1.0 |
| Miscellaneous | 1.0 | 1.0 |
| NaOH (1 N) | - | 1.4 |
| Distilled | 940.0 | 928.0 |
| pH | - | 7.2 |

4. Algae

*a) Mixture of Blue-green Algae*

This mixture was first found in Petri dishes prepared for *Oncomelania* snail studies. The algae had been incubated under continuous illumination (40 watt cool-white fluorescent tube) 12 inches above the dishes at 25 to 27° C for a prolonged period. To maintain the cultures, a small amount of this mixture was ground with about 10 ml of autoclaved distilled water in a tissue grinder; the whole suspension was then inoculated into a 2,800 ml flask containing autoclaved BBMSU. The flask was then provided with continuous aeration and with the same illumination as above.

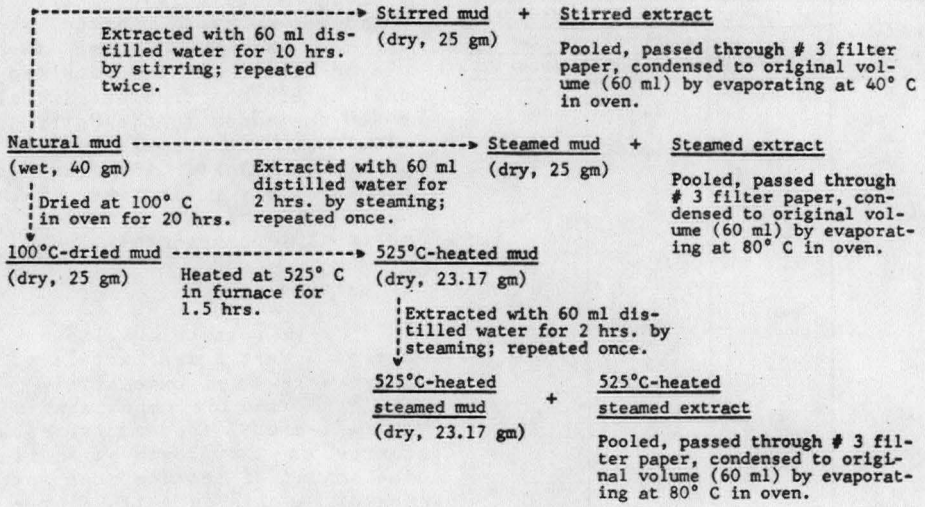
*b) Selected Species of Blue-green Algae*

An attempt was made to isolate unialgal cultures from the mixture of blue-green algae just mentioned above. The isolation was accomplished as follows: (1) with a pair of fine glass needles and under a dissecting microscope, candidate species (macromorphologically different in colony appearances) were removed from the Petri dishes in which this mixture of blue-green algae had grown for 3 weeks and more, (2) those selected were inoculated separately onto BBMSU agar plates (i.e., 100 ml of BBMSU + 1.5 gm of agar consolidated in small Petri dishes (60 X 15 mm). The Petri dishes were then placed into a simple humid chamber (using a plastic tray together with a couple of dishes, without cover, filled with distilled water for vaporization). The top of the trsy holding the Petri dishes was covered with a glass plate and the whole unit was then incubated under constant light using the same illumination as described previously, (3) two weeks later the algal colonies appearing on the agar plate preparations and (4) two to three selective removals of algae were sufficient for isolating unialgal cultures, although they were not bacteria-free. In this way 3 species of blue-green algae and 1 species of diatom were isolated. They were identified as:

Blue-green algae:
*Nostoc muscorum* Agardh
*Schizothrix calcicola* (Agardh) Gomont
(formerly *Phormidium* sp.)
*Fischerella ambigua* (Näg.) Gomont
Diatom:
*Nitzschia frustulum* (Kütz.) Grun.

For maintaining these algae, small amounts of the colonies were removed and processed in a way similar to that described for the mixture of blue-green algae.

Fig. 1. Procedures for preparing different kinds of muds and mud extracts




*c) Inoculation of Algae*

At the time of inoculating all of these algae onto Petri dish preparations (as will be described below), a small amount (about 10 mg in dry weight) of the respective alga was ground with 10 ml of autoclaved distilled water in a tissue grinder, and 30 ml more distilled water was then added to make a homogeneous algal suspension. A small amount of the suspension (0.5 ml) was then added to each Petri dish with the aid of a 1-ml disposable plastic syringe equipped with 20 G 1.5 in. disposable needle. Petri dishes were then incubated under the previously described constant source of illumination for 1 to 3 weeks.

In Part III and Part IV of the study it was frequently observed that the growth of *Nostoc muscorum* seen at the end of the incubation period was closely related to the growth of snails. For convenience, therefore, the growth of this alga was arbitrarily defined as follows:

3+: thick growth of algal film on the surface of water, bottom of Petri dish is hardly visible.
2+: thin growth of algal film on the surface of water, bottom of Petri dish is still visible.
1+: no growth of algal film on the surface of water only spotty growth of algae on the bottom.
0+: no growth of alga at all.

**5. Petri Dish Preparations for Algal Growth**

*a) Natural Mud*

The source of mud and procedures in preparation were as described by van der Schalie and Davis (1965) but with some minor modifications. Briefly, the river mud taken from the habitat of *Pomatiopsis cincinnatiensis* snails was mixed with a large quantity of distilled water to permit it to pass through a sieve of one-third inch mesh. The mud was then heated in an oven at 80° C for 8 hours per day for 3 consecutive days to eliminate unwanted animals such as annelids and other potential pests. On the last day of heating, the ventilation of the oven was so regulated that the softness of the mud would be equivalent to a mixture of 5 parts of 100° C-dried mud and 3 parts of distilled water, both by weight.

To make a suitable mud mound 40 gm of this wet mud was placed in the center of a Petri dish (100 X 20 mm) and stroked with a spatula to form a smooth and solid mound about 1.5 cm high at the center and 6.0 cm in basal diameter. About 60 ml of distilled water (not autoclaved) was then added as overlay. The suspension of the mixture of blue-green algae (as described above) was then inoculated. In some experiments in which unialgal cultures were used, the mud mound was autoclaved at 121° C for 1 hour to insure the mud free from other species of algae before overlying with 60 ml of autoclaved distilled water.

*b) Several Kinds of Mud and Mud Extracts*

The steps involved in the preparation of muds and their extracts were shown in Fig. 1. For sterilization, muds were autoclaved after they were placed into Petri dishes, and extracts were autoclaved in flasks before pouring into autoclaved Petri dishes. To each of the Petri dishes with muds, an appropriate amount of autoclaved distilled water (or in a particular case autoclaved 525° C-heated steamed extract) was added as overlay for algal inoculation. Mud mound making was not necessary (Table 6).

*c) Synthetic Mud*

The following components were used to make various types of synthetic mud.

Clay: Pottery clay. Purchased through a

Table 6. Mud and extract preparations

| Muds or mud extracts | Quantities per Petri dish | | |
|---|---|---|---|
| | Mud(gm) or extract(ml) | Distilled water | |
| | | Paste (ml) | Overlay (ml) |
| Natural mud | 25 | 15 | 60 |
| Steamed mud | 25 | 15 | 60 |
| Steamed extract | 60 | - | - |
| Stirred mud | 25 | 15 | 60 |
| Stirred extract | 60 | - | - |
| 100° C-dried mud | 25 | 15 | 60 |
| 100° C-dried mud | 25 | 15 | 60* |
| 525° C-heated mud | 23.17 | 14 | 60 |
| 525° C-heated steamed mud | 23.17 | 14 | 60 |
| 525° C-heated steamed extract(full-strength) | 60 | - | - |
| 525° C-heated steamed extract(half-strength) | 30 | - | 30 |

* 525° C-heated steamed extract in place of distilled water.

local pottery maker, from the Cedar Heights Clay Co., 50 Portsmouth Road, Oak Hill, Ohio.

Kaolin: From Merck & Co.

Sand: White sand. From local aquarium supply store. It was ground with pestle and mortar to form a powder.

Shell: Oyster shell. From local farm supply company. Finely ground to make a powder.

Charcoal: Bone charcoal. From Herman Brothers Sales Corp., 3005 Central Avenue, Detroit, Michigan, 48209. Again finely ground

Agar: Bacto-agar. From Difco Laboratories, Detroit, Michigan.

Bone: Bonemeal. From Agrico Chemical Co., Division of Continental Oil Co., Memphis, Tenn. 38101.

Fish: Fish meal. From Boston Feed Supply, Boston, Massachusetts.

$KNO_3$: From General Chemical Division, Allied Chemical & Dye Corporation, New York, N. Y.

Glucose: D-glucose. From B.D.H. Laboratory Chemicals Division, Poole, England.

The actual formulae for making quantities of synthetic mud sufficient to prepare 4 Petri dishes each were individually indicated in Table 7. In each formulation certain kinds and amounts of components just described above were mixed well with 70 ml of an appropriate type of water, distilled water, artificial water, modified artificial water I, modified artificial water II BBM or BBMSU, in a 150-ml beaker before autoclaving. An appropriate amount (about ¼) of this synthetic mud was then placed in an autoclaved Petri dish and the mud mound was made with an autoclaved spatula while the mud was still warm. The 60 ml of autoclaved water, either distilled water, artificial water, modified artificial water III or BBMSU was added to the Petri dish as overlay after the whole preparation was cool. The suspension of *Nostoc muscorum* was inoculated in the usual way. The time of incubation spent under constant illumination varied from experiment to experiment.

### 6. Lettuce

Fresh lettuce was also used as a food additive in Part I and Part II of the study when trays were used extensively as aquaria. It was also used for comparison in Part III, when the effects of food additives and types of aquaria on the growth of snails were studied. The amount of lettuce used varied. To feed 5 newborn snails in a tray (or a Petri dish) up to the 2-week old stage, about one-fourth leaf of lettuce was torn into small pieces and scattered on the water surface. For the 3- to 4-week old snails, one-half leaf was provided; for the 5-week and older snails, a whole leaf. This amount was usually sufficient to last the entire period until the tray (or Petri dish) was changed. For more than 5 snails per aquarium, the amount of the lettuce used was increased accordingly.

### 7. Methods of Preparing Aquaria and Initiating Snail Cultures

*a) Tray Method*

Unbreakable commercial green plastic trays (7.5 X 20 X 30 cm), designed for plant seedlings were employed as aquaria since they were easy to handle and reasonable in price. They were used in Parts I – III of the study. They were prepared as follows: (1) each tray was overlaid with 1,500 ml of either deionized water, distilled water, tap water, spring water, calcium water, magnesium water or calcium-magnesium water, (2) a mud mound, on which the mixture of blue-green algae or a unialgal culture of *Nostoc muscorum* was growing, was transferred from the Petri dish into the tray with the aid of a spatula, (3) an appropriate amount of lettuce was then added to the tray, (4) the newly-hatched (in Parts I and II) or 1-day old (in Part III) snails, 5 per tray, were introduced with the aid of a Pasteur capillary pipette. It was crucial that snails be dropped on the top of the algal mass to insure their immediate access to the food, since the fresh lettuce leaf was too hard for newborn snails to ingest, and (5) the tray was covered with a glass plate and incubated under the standard lighting system.

To study the effect of crowding on the growth of snails, 5, 10, 20 or 40 snails were cultured per tray. The number of replicate aquaria made and other specific details for each experiment are described under Results.

Table 7. Synthetic mud preparations

| Expt. No. | Formula No. | Quantities to make 4 Petri dishes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Solid (gm) | | | | | | | | Liquid (ml) | |
| | | Clay | Shell | Charco | Agar | Bone | Fish | $KNO_3$ | Glucos | Paste (70) | Overlay (240) |
| 1 | 1 | 100 | 1 | 2 | 2 | 1 | 1 | .05 | 1 | Dis | Dis |
| | 2 | 100 | 1 | 2 | 2 | 1 | 1 | .05 | 1 | Dis | BBMSU |
| | 3 | 100 | 1 | 2 | 2 | 1 | 1 | .05 | 1 | BBMSU | Dis |
| | 4 | 100 | 1 | 2 | 2 | 1 | 1 | .05 | 1 | BBMSU | BBMSU |
| 2 | 1 | 100 | .5 | 2 | 2 | .5 | .5 | - | - | Dis | Dis |
| | 2 | 100 | .5 | 2 | 2 | .5 | .5 | - | - | Dis | Art |
| | 3 | 100 | .5 | 2 | 2 | .5 | .5 | - | - | BBMSU | Dis |
| | 4 | 100 | .5 | 2 | 2 | .5 | .5 | - | - | BBMSU | Art |
| 3 | 1 | 100 | 1 | 3 | .5 | 1 | 1 | .05 | - | Dis | Dis |
| | 2 | 100 | 1 | 3 | .5 | 1 | 1 | .05 | - | Dis | Art |
| 4 | 1 | 100 | .5 | 2 | .3 | - | .5 | - | - | Dis | Dis |
| | 2 | 100 | .5 | 2 | .3 | - | .5 | - | - | Dis | Art |
| | 3 | 100 | .5 | 2 | .3 | - | .5 | - | - | Mod Art I | Dis |
| | 4 | 100 | .5 | 2 | .3 | - | .5 | - | - | Mod Art I | Art |
| 5 | 1 | 100 | 1 | 4 | .3 | 1 | 1.5 | .05 | - | Dis | Dis |
| | 2 | 100 | 1 | 4 | .3 | 1 | 1.5 | .05 | - | Dis | Art |
| | 3 | 100 | 1 | 4 | .3 | 1 | 1.5 | .05 | - | Mod Art II | Dis |
| | 4 | 100 | 1 | 4 | .3 | 1 | 1.5 | .05 | - | Mod Art II | Art |
| 6 | 1 | 100 | 1 | 4 | .3 | 1 | 1.5 | - | - | Dis | Mod Art III |
| | 2 | 100 | 1 | 4 | .3 | 1 | - | - | - | Dis | Mod Art III |
| | 3 | Ka 100 | 1 | 4 | .3 | 1 | 1.5 | - | - | Dis | Mod Art III |
| | 4 | Sa 140 | 1 | 4 | .3 | 1 | 1.5 | - | - | Dis | Mod Art III |
| 7 | 1 | 100 | 1 | 3 | .5 | .5 | 1 | .05 | - | Dis | Art |
| | 2 | 100 | 1 | 3 | .5 | 2 | 1 | .05 | - | Dis | Art |
| | 3 | 100 | 1 | 3 | .5 | 1 | - | .05 | - | Dis | Art |
| | 4 | 100 | 1 | 3 | .5 | 1 | .5 | .05 | - | Dis | Art |
| | 5 | 100 | .5 | 3 | .5 | 1 | 1 | .05 | - | Dis | Art |
| | 6 | 100 | 2 | 3 | .5 | 1 | 1 | .05 | - | Dis | Art |
| | 7 | 100 | 1 | 1 | .5 | 1 | 1 | .05 | - | Dis | Art |
| | 8 | 100 | 1 | 6 | .5 | 1 | 1 | .05 | - | Dis | Art |
| | 9 | 100 | 1 | 3 | .3 | 1 | 1 | .05 | - | Dis | Art |
| | 10 | 100 | 1 | 3 | 2 | 1 | 1 | .05 | - | Dis | Art |
| | 11 | 100 | 1 | 3 | .5 | 1 | 1 | - | - | Dis | Art |
| | 12 | 100 | 1 | 3 | .5 | 1 | 1 | .1 | - | Dis | Art |
| | 13 | 100 | 1 | 3 | .5 | 1 | 1 | .05 | - | Dis | Art |
| | 14 | 100 | .5 | 2 | 2 | .5 | .5 | - | - | BBMSU | Art |

Table 7. Continued

| Expt. No. | Formula No. | Quantities to make 4 Petri dishes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Solid (gm) | | | | | | | | Liquid (ml) | |
| | | Clay | Shell | Charco | Agar | Bone | Fish | $KNO_3$ | Glucos | Paste (70) | Overlay (240) |
| 8 | 1 | 100 | 1 | 3 | .5 | 1 | - | - | - | Dis | Art |
| | 2 | 100 | 1 | 3 | .5 | 1 | - | - | - | BBM | Art |
| | 3 | 100 | 1 | 3 | .5 | 1 | - | - | - | BBMSU | Art |
| | 4 | 100 | 1 | 3 | .5 | 2 | - | - | - | BBM | Art |
| | 5 | 100 | 1 | 3 | .5 | 4 | - | - | - | BBM | Art |
| | 6 | 100 | 2 | 3 | .5 | 1 | - | - | - | BBM | Art |
| | 7 | 100 | 4 | 3 | .5 | 1 | - | - | - | BBM | Art |
| | 8 | 100 | 2 | 3 | .5 | 2 | - | - | - | BBM | Art |
| | 9 | 100 | 4 | 3 | .5 | 2 | - | - | - | BBM | Art |
| | 10 | 100 | 2 | 3 | .5 | 4 | - | - | - | BBM | Art |
| | 11 | 100 | 4 | 3 | .5 | 4 | - | - | - | BBM | Art |
| | 12 | 100 | - | 3 | .5 | 2 | - | - | - | BBM | Art |
| | 13 | 100 | 2 | 3 | .5 | - | - | - | - | BBM | Art |
| | 14 | 100 | - | 3 | .5 | - | - | - | - | BBM | Art |
| | 15 | Sa 140 | 1 | 3 | .5 | 1 | - | - | - | BBM | Art |
| 10 | 1 | 100 | 1 | 3 | .5 | - | - | - | - | Art | Art |
| | 2 | 100 | 1 | 3 | .5 | - | - | - | - | BBM | Art |
| | 3 | - | 1 | 3 | .5 | - | - | - | - | Art | Art |
| | 4 | - | 1 | 3 | .5 | - | - | - | - | BBM | Art |
| 11 | 1 | - | 1 | 3 | .5 | - | - | - | - | Art | Art |
| | 2 | - | 1 | - | .5 | - | - | - | - | Art | Art |
| 12 | 1 | 100 | 1 | 3 | .5 | - | - | - | - | Art | Art |
| | 2 | 100 | 1 | - | .5 | - | - | - | - | Art | Art |
| | 3 | 100 | - | 3 | .5 | - | - | - | - | Art | Art |
| | 4 | 100 | - | - | .5 | - | - | - | - | Art | Art |
| | 5 | - | 1 | 3 | .5 | - | - | - | - | Art | Art |
| | 6 | - | 1 | - | .5 | - | - | - | - | Art | Art |
| | 7 | - | - | 3 | .5 | - | - | - | - | Art | Art |
| | 8 | - | - | - | .5 | - | - | - | - | Art | Art |
| | 9 | 100 | 1 | 3 | .5 | - | - | - | - | BBM | Art |
| | 10 | Nm 100 | - | - | - | - | - | - | - | Dis | Dis |

Ka = Kaolin; Sa = Sand; Nm = Natural mud.

*b) Petri Dish Method*

Petri dishes (100 X 20 mm), containing mixture of blue-green algae, several species of unialgal cultures, or *Nostoc muscorum* were employed as aquaria. They were used in Parts III and IV of the study. They were prepared as follows: (1) an appropriate amount of distilled water was added to each Petri dish to restore its original water level, (2) the algal mass was torn open in such a way that a small area of mud surface was exposed, (3) an appropriate amount of lettuce was added whenever it was required and (4) one-day old snails, 5 per dish, were introduced with a pipette as above. In this case, the snails were delivered to the exposed area as mentioned above, so that they could easily and immediately ingest both algae and mud.

The effect of crowding on the growth of snails was studied using *Nostoc muscorum* as a food additive with 1, 2, 5, 10, 20 or 40 snails per Petri dish. The number of replicate aquaria made and other specific details for each experiment are described under Results.

**8. Measuring Survival, Growth and Fecundity**

In Parts I and II of the study, snails were first collected at the end of the second week, and their shell lengths measured under a dissecting microscope with an ocular micrometer. Following oviposition, egg-masses were scraped from the container, the lettuce leaves and the shells of individual snails with blunt-tipped forceps. These egg masses were then placed in a Petri dish filled with tap water. Egg masses and eggs were counted, using a dissecting microscope, and recorded according to the sites on which they were laid. Parent snails were placed in freshly prepared trays filled with either the same or different kinds of water. These measurements provided data necessary for an understanding of growth, survival and fecundity.

In Parts III and IV of the study, snails were collected at the end of either the first or second week and measured in the same way. In many experiments, snails were cultured for only a 2-week period; therefore, measurements were taken only once at the end of the period. Fecundity could not be observed in such a short period. For those experiments in which snails were cultured more than 2 weeks, fresh aquaria (either trays or Petri dishes) were supplied only in one of the experiments of Part III in which the effect of kinds of aquaria on the growth of snails were being studied. Specific details for each experiment will be given under Results.

## RESULTS

### Part I. Effect of Crowding on Survivorship, Growth and Fecundity

The object of this study was really two-fold; to determine if snails could be successfully cultured in trays, and if so, the optimum number of snails to be placed in each tray.

*1. Bulinus globosus*

Altogether 8 trays supplied with spring water were used; two with 5 snails per tray, two with 10, two with 20 and the last two with 40. Measurements and water changes were made 2, 4, 6, 7, 8, 9 and 11 weeks after adding the snails. As judged by survivorship, these snails were efficiently raised with this tray method. In trays with 5 snails per tray all of the snails survived; those with 10 snails, 80% (=16/20); those with 20 snails, 92.5% (=37/40); and with 40 snails, 83.8% (=67/80) (Table 8).

As indicated (Fig. 2) the mean shell lengths of the snails were almost the same for all 4 groups in the first two weeks. But at the end of the fourth week 3 growth patterns developed, and by the end of the eleventh week there were 4 distinct growth curves which clearly demonstrated stunting in growth through crowding, so that individuals in trays with 5 snails per tray were largest and those in trays with 40 snails per tray were smallest. The subtle relationships between values of standard deviation and the population size were evident by the end of the period of logarithmic growth (about the end of 6th week). Although the mean shell length became smaller as population size increased, the values of standard deviations became greater, as follows: 0.3 to 0.4 mm in the 5-snail group, 0.3 to 0.5 mm with 10 snails, 0.4 to 0.6 mm with 20 snails and 0.6 to 0.7 mm with 40 snails. These differences indicated that the more the animals were crowded, the less the uniformity in the size of the snails.

There were also striking differences in egg-laying in that eggs first appeared in both trays with 5 snails per tray and in only one with 10 snails per tray at the end of the sixth week. It should be stressed that under crowded conditions, as found in trays with 20 and 40 snails per tray, no eggs were laid throughout the experiment. The number of eggs laid per snail was 45.7 for the 5-snail group and 27.8 for the 10-snail group (Table 9 and Fig. 3).

Table 8. Effect of crowding on survivorship and shell length ($\bar{x} \pm$ S.D.) (mm) of B. globosus reared in spring water for 11 weeks

| Snails/tray | Tray No. | Weeks 0 S | 0 SL | 2 S | 2 SL | 4 S | 4 SL | 6 S | 6 SL | 7 S | 7 SL | 8 S | 8 SL | 9 S | 9 SL | 11 S | 11 SL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 1 | 5 | 0.8 0.0 | 5 | 3.0 0.7 | 5 | 5.7 0.9 | 5 | 8.1 0.5 | 5 | 8.4 0.4 | 5 | 9.1 0.3 | 5 | 9.3 0.3 | 5 | 9.8 0.4 |
| | 2 | 5 | 0.8 0.0 | 5 | 2.6 0.6 | 5 | 5.6 0.8 | 5 | 8.3 0.3 | 5 | 9.0 0.1 | 5 | 9.2 0.3 | 5 | 9.3 0.2 | 5 | 9.8 0.3 |
| | TM | 10 | 0.8±0.0 | 10 | 2.8±0.7 | 10 | 5.7±0.8 | 10 | 8.2±0.4 | 10 | 8.7±0.4 | 10 | 9.2±0.3 | 10 | 9.3±0.3 | 10 | 9.8±0.3 |
| 10 | 1 | 10 | 0.8 0.0 | 10 | 2.7 0.4 | 10 | 5.6 0.5 | 9 | 7.5 0.4 | 9 | 8.3 0.2 | 9 | 8.6 0.2 | 9 | 8.6 0.3 | 9 | 9.1 0.3 |
| | 2 | 10 | 0.8 0.0 | 8 | 2.0 0.2 | 8 | 5.3 0.7 | 7 | 8.2 0.4 | 7 | 8.9 0.3 | 7 | 8.9 0.2 | 7 | 9.1 0.3 | 7 | 9.4 0.3 |
| | TM | 20 | 0.8±0.0 | 18 | 2.4±0.4 | 18 | 5.5±0.6 | 16 | 7.8±0.5 | 16 | 8.5±0.4 | 16 | 8.7±0.3 | 16 | 8.8±0.4 | 16 | 9.2±0.3 |
| 20 | 1 | 20 | 0.8 0.0 | 20 | 2.3 0.4 | 20 | 4.7 0.8 | 20 | 6.7 0.7 | 18 | 7.2 0.4 | 18 | 7.3 0.3 | 18 | 7.3 0.3 | 18 | 7.5 0.3 |
| | 2 | 20 | 0.8 0.0 | 19 | 2.6 0.3 | 19 | 4.5 0.5 | 19 | 6.7 0.6 | 19 | 6.8 0.4 | 19 | 7.3 0.5 | 19 | 7.3 0.5 | 19 | 7.9 0.5 |
| | TM | 40 | 0.8±0.0 | 39 | 2.5±0.4 | 39 | 4.6±0.7 | 39 | 6.7±0.6 | 37 | 7.0±0.5 | 37 | 7.3±0.4 | 37 | 7.3±0.4 | 37 | 7.7±0.5 |
| 40 | 1 | 40 | 0.8 0.0 | 36 | 2.2 0.2 | 36 | 3.3 0.4 | 36 | 5.8 0.7 | 36 | 6.0 0.6 | 36 | 6.2 0.6 | 36 | 6.2 0.6 | 36 | 6.8 0.7 |
| | 2 | 40 | 0.8 0.0 | 36 | 2.7 0.4 | 35 | 3.8 0.5 | 35 | 5.5 0.6 | 33 | 5.8 0.6 | 32 | 6.1 0.6 | 32 | 6.2 0.6 | 31 | 7.1 0.5 |
| | TM | 80 | 0.8±0.0 | 72 | 2.4±0.4 | 71 | 3.6±0.5 | 71 | 5.7±0.6 | 69 | 6.0±0.6 | 68 | 6.1±0.6 | 68 | 6.2±0.6 | 67 | 6.9±0.7 |

S = No. of survivors; SL = Shell length; TM = Total and mean.

When those eggs were recorded according to the sites from which they were harvested, an interesting pattern appeared (Table 10). In both 5-snail and 10-snail groups, most of the eggs (90.6% and 39.6%) were on the lettuce (usually underneath the leaf); next (5.0% and 31.0%) were on the container (either the wall or the bottom of the tray) and fewest (4.4% and 29.4%) were on the shells of individual snails. This particular pattern of egg-laying sites was found to be persistent in all of the experiments in which spring water was used in the trays.

2. *Biomphalaria pfeifferi*

The data presented above demonstrated that some reliable methods for culturing *Bulinus globosus* were developed with the use of trays. A need still exister for similar studies with *Biomphalaria pfeifferi*, which was hitherto maintained only in an unreliable way. To determine the optimum number of these snails to be cultured per aquarium, 12 trays were established and arranged essentially the same as those previously described except that they were supplied with aerated tap water instead of spring water. Previous experiments indicated that if the snails were to die, death would occur before the end of the second week. Consequently, extra snails were put into the trays at the beginning to allow for individuals which may have died during this critical period. For trays with 5 and 10 snails per tray, one extra snail was added to each tray; trays with 20 snails, two extra; and trays with 40 snails, four extra. At the end of the second week, the extra snail(s) (smallest ones) were removed so that three trays had 5 snails per tray, three had 10, three had 20 and the

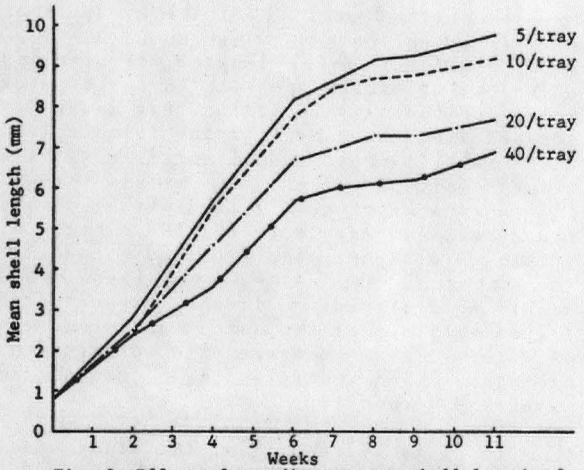



Fig. 2. Effect of crowding on mean shell length of B. globosus reared in spring water for 11 weeks

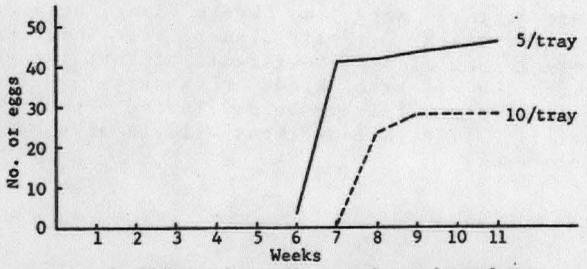



Fig. 3. Effect of crowding on the number of eggs (cumulated) laid per B. globosus reared in spring water for 11 weeks

Table 9. Effect of crowding on fecundity of B. globosus reared in spring water for 11 weeks

| Snails/ tray | Tray No. | Weeks | | | | | | | | | | Tot. & Mean | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | | 7 | | 8 | | 9 | | 11 | | | |
| | | T. eggs by ( #) | Eggs/ snail | T. eggs by ( #) | Eggs/ snail | T. eggs by ( #) | Eggs/ snail | T. eggs by ( #) | Eggs/ snail | T. eggs by ( #) | Eggs/ snail | T. eggs by ( #) | Eggs/ snail |
| 5 | 1 | 15( 5) | 3.0 | 191( 5) | 38.2 | 3( 5) | 0.6 | 12( 5) | 2.4 | 5( 5) | 1.0 | 226( 5) | 45.2 |
| | 2 | 21( 5) | 4.2 | 181( 5) | 36.2 | 3( 5) | 0.6 | 5( 5) | 1.0 | 21( 5) | 4.2 | 231( 5) | 46.2 |
| | TM | 36(10) | 3.6 | 372(10) | 37.2 | 6(10) | 0.6 | 17(10) | 1.7 | 26(10) | 2.6 | 457(10) | 45.7 |
| 10 | 1 | 0( 9) | 0.0 | 150( 9) | 16.7 | 15( 9) | 1.7 | 19( 9) | 2.1 | 0( 9) | 0.0 | 184( 9) | 20.5 |
| | 2 | 11( 7) | 1.6 | 217( 7) | 31.0 | 33( 7) | 4.7 | 0( 7) | 0.0 | 0( 7) | 0.0 | 261( 7) | 37.3 |
| | TM | 11(16) | 0.7 | 367(16) | 22.9 | 48(16) | 3.0 | 19(16) | 1.2 | 0(16) | 0.0 | 445(16) | 27.8 |
| 20 | TM | 0(39) | 0.0 | 0(37) | 0.0 | 0(37) | 0.0 | 0(37) | 0.0 | 0(37) | 0.0 | 0(37) | 0.0 |
| 40 | TM | 0(71) | 0.0 | 0(69) | 0.0 | 0(68) | 0.0 | 0(68) | 0.0 | 0(67) | 0.0 | 0(67) | 0.0 |

T. = Total; # = No. of survivors; TM = Total and mean.

Table 10. Egg-laying sites of B. globosus reared in spring water for 11 weeks

| Snails /tray | Wks | Tray No. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | | | 2 | | | | Total | | | |
| | | Tot. eggs | Egg-laying site | | | Tot. eggs | Egg-laying site | | | Tot. eggs | Egg-laying site | | |
| | | | C | L | S | | C | L | S | | C | L | S |
| 5 | 6 | 15 | 15 | 0 | 0 | 21 | 0 | 21 | 0 | 36 | 15 | 21 | 0 |
| | 7 | 191 | 8 | 183 | 0 | 181 | 0 | 181 | 0 | 372 | 8 | 364 | 0 |
| | 8 | 3 | 0 | 0 | 3 | 3 | 0 | 3 | 0 | 6 | 0 | 3 | 3 |
| | 9 | 12 | 0 | 12 | 0 | 5 | 0 | 0 | 5 | 17 | 0 | 12 | 5 |
| | 11 | 5 | 0 | 0 | 5 | 21 | 0 | 14 | 7 | 26 | 0 | 14 | 12 |
| | Tot. | 226 | 23 | 195 | 8 | 231 | 0 | 219 | 12 | 457 | 23 | 414 | 20 |
| | % | | 10.2 | 86.3 | 3.5 | | 0.0 | 94.8 | 5.2 | | 5.0 | 90.6 | 4.4 |
| 10 | 6 | 0 | - | - | - | 11 | 0 | 11 | - | 11 | 0 | 11 | 0 |
| | 7 | 150 | 22 | 119 | 9 | 217 | 106 | 37 | 74 | 367 | 128 | 156 | 83 |
| | 8 | 15 | 0 | 0 | 15 | 33 | 0 | 0 | 33 | 48 | 0 | 0 | 48 |
| | 9 | 19 | 10 | 9 | 0 | 0 | - | - | - | 19 | 10 | 9 | 0 |
| | 11 | 0 | - | - | - | 0 | - | - | - | 0 | - | - | - |
| | Tot. | 184 | 32 | 128 | 24 | 261 | 106 | 48 | 107 | 445 | 138 | 176 | 131 |
| | % | | 17.4 | 69.6 | 13.0 | | 40.6 | 18.4 | 41.0 | | 31.0 | 39.6 | 29.4 |
| 20 | Tot. | 0 | - | - | - | 0 | - | - | - | 0 | - | - | - |
| 40 | Tot. | 0 | - | - | - | 0 | - | - | - | 0 | - | - | - |

C = Container; L = Lettuce; S = Shell.

Table 11. Effect of crowding on shell length($\bar{x}$ ± S.D.)(mm) of B. pfeifferi reared in tap water for 12 weeks

| * | ** | Weeks | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 2 | | 3 | | 4 | | 6 | | 8 | | 10 | | 12 | |
| 5 | 1 | 0.6 | 0.0 | 5.0 | 0.5 | 6.4 | 0.2 | 7.8 | 0.1 | 8.2 | 0.2 | 9.8 | 0.2 | 10.5 | 0.1 | 11.2 | 0.2 |
| | 2 | 0.6 | 0.0 | 5.9 | 0.5 | 7.8 | 0.3 | 8.4 | 0.3 | 9.0 | 0.2 | 10.6 | 0.2 | 11.4 | 0.3 | 11.6 | 0.2 |
| | 3 | 0.6 | 0.0 | 4.4 | 0.3 | 6.4 | 0.4 | 7.6 | 0.2 | 8.2 | 0.3 | 9.9 | 0.2 | 10.7 | 0.3 | 11.0 | 0.2 |
| | M | 0.6 ± 0.0 | | 5.1 ± 0.7 | | 6.9 ± 0.7 | | 7.9 ± 0.4 | | 8.5 ± 0.4 | | 10.1 ± 0.4 | | 10.9 ± 0.5 | | 11.3 ± 0.3 | |
| 10 | 1 | 0.6 | 0.0 | 4.5 | 0.6 | 5.8 | 0.5 | 6.7 | 0.4 | 7.5 | 0.4 | 8.9 | 0.5 | 9.6 | 0.4 | 9.8 | 0.4 |
| | 2 | 0.6 | 0.0 | 3.9 | 0.5 | 6.2 | 0.5 | 7.5 | 0.3 | 7.9 | 0.2 | 9.4 | 0.2 | 9.9 | 0.2 | 10.3 | 0.3 |
| | 3 | 0.6 | 0.0 | 4.4 | 0.3 | 6.2 | 0.3 | 7.4 | 0.2 | 8.1 | 0.2 | 9.5 | 0.4 | 10.0 | 0.3 | 10.5 | 0.2 |
| | M | 0.6 ± 0.0 | | 4.3 ± 0.6 | | 6.1 ± 0.5 | | 7.2 ± 0.5 | | 7.8 ± 0.4 | | 9.3 ± 0.5 | | 9.8 ± 0.4 | | 10.2 ± 0.4 | |
| 20 | 1 | 0.6 | 0.0 | 3.3 | 0.4 | 5.6 | 0.4 | 6.1 | 0.4 | 6.9 | 0.3 | 7.8 | 0.3 | 8.4 | 0.4 | 8.8 | 0.3 |
| | 2 | 0.6 | 0.0 | 3.3 | 0.3 | 5.0 | 0.3 | 6.2 | 0.2 | 7.1 | 0.1 | 8.2 | 0.2 | 8.7 | 0.2 | 9.2 | 0.2 |
| | 3 | 0.6 | 0.0 | 3.7 | 0.9 | 5.4 | 0.8 | 6.4 | 0.4 | 7.0 | 0.4 | 8.0 | 0.3 | 8.6 | 0.4 | 9.0 | 0.2 |
| | M | 0.6 ± 0.0 | | 3.4 ± 0.6 | | 5.3 ± 0.6 | | 6.2 ± 0.3 | | 7.0 ± 0.4 | | 8.0 ± 0.3 | | 8.6 ± 0.3 | | 9.0 ± 0.3 | |
| 40 | 1 | 0.6 | 0.0 | 2.6 | 0.3 | 4.2 | 0.5 | 5.5 | 0.4 | 6.4 | 0.3 | 7.1 | 0.2 | 7.6 | 0.3 | 8.0 | 0.3 |
| | 2 | 0.6 | 0.0 | 2.9 | 0.4 | 4.4 | 0.6 | 5.5 | 0.5 | 6.4 | 0.3 | 7.4 | 0.3 | 7.9 | 0.3 | 8.3 | 0.2 |
| | 3 | 0.6 | 0.0 | 3.1 | 0.3 | 4.4 | 0.3 | 5.5 | 0.2 | 6.5 | 0.2 | 7.4 | 0.2 | 7.7 | 0.2 | 8.1 | 0.2 |
| | M | 0.6 ± 0.0 | | 2.8 ± 0.4 | | 4.3 ± 0.5 | | 5.5 ± 0.4 | | 6.4 ± 0.3 | | 7.3 ± 0.3 | | 7.7 ± 0.3 | | 8.1 ± 0.3 | |

* = Snails/tray; ** = Tray No.; M = Mean.

last three had 40 snails. Measurements were then made weekly and tabulations were made bi-weekly until the end of the twelfth week.

None of the snails died during the entire observation period following the initial arrangement as established at the end of the second week. As shown (Table 11) the largest snails developed in the aquaria with 5 snails per tray and had a mean shell length (=diameter) of 11.3 mm at the end of the twelfth week; somewhat smaller specimens (10.2mm) appeared in the trays with 10 per tray; still smaller ones (9.0 mm) developed in those with 20; and the smallest (8.1 mm) grew in the trays with 40 per tray. When these data were plotted (Fig. 4) the effect of crowding on growth was shown more clearly. These differences became noticeable at the end of the second week. Following the logarithmic phase of growth at the end of the fourth week, growth in all 4 groups was maintained at similar rates until the end of observation period, as revealed by the parallel growth curves. However, the correlation between the value of standard deviation and population size which was clearly evident in *Bulinus globosus*, was not observed in these data. The values for snails in all 4 groups were very similar beginning at the end of the logarithmic phase of growth (the end of the fourth week) and thereafter. The differences ranged between 0.3 and 0.5 mm for 5-snail group; 0.4 and 0.5 mm for 10; 0.3 and 0.4 mm for 20; and 9.3 and 0.4 mm for 40 snail groups. Nevertheless, although the mean shell length value became smaller as the population size increased, yet the values of the standard deviation were essentially the same, therefore, the statement 'the more crowded the conditions, the less uniform the size of the snails' still holds true.

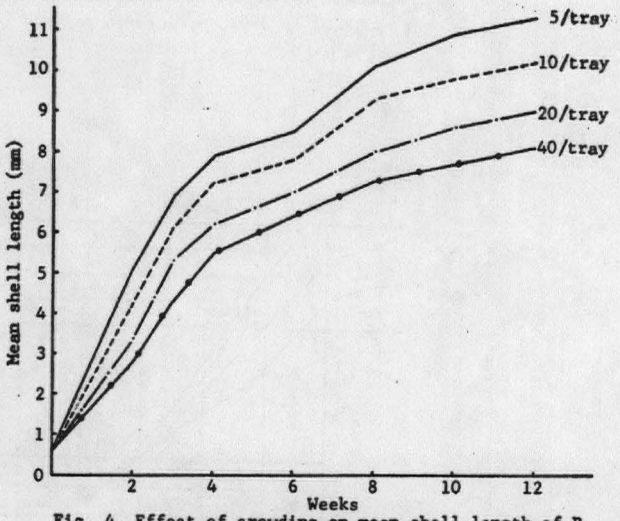



Fig. 4. Effect of crowding on mean shell length of B. pfeifferi reared in tap water for 12 weeks

With relation to egg laying, all snails started to lay eggs 3 weeks after hatching except those in the trays containing 40 specimens per tray. They started to lay eggs between the third and fourth weeks, which again clearly indicated the effect of crowding on the fecundity of these snails (Table 12). The number of eggs laid per snail was also very much influenced by crowding. It was highest in the 5-snail group with 2072 eggs produced over an 8-week period; somewhat lower in the 10-snail group with 1282; still lower in the 20-snail group with 548; and lowest in the 40-snail group with 225. When the number of eggs laid per snail was tallied and plotted in a graph, 4 distinct curves appeared, as shown in Fig. 5.

Table 12. Effect of crowding on fecundity (eggs/snail) of B. pfeifferi reared in tap water for 12 weeks

| Snails /tray | Tray No. | Weeks | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 3 | 4 | 6 | 8 | 10 | 12 | Total |
| 5 | 1 | 35.4 | 208.0 | 229.4 | 353.6 | 519.4 | 583.0 | 1928.8 |
| | 2 | 55.2 | 219.0 | 338.2 | 674.4 | 619.8 | 556.2 | 2462.8 |
| | 3 | 6.6 | 159.8 | 245.2 | 459.0 | 446.0 | 510.4 | 1827.0 |
| | Mean | 32.4 | 195.6 | 270.9 | 495.7 | 528.4 | 549.9 | 2072.9 |
| 10 | 1 | 9.9 | 93.1 | 156.7 | 238.3 | 376.1 | 312.4 | 1186.5 |
| | 2 | 7.0 | 111.2 | 153.1 | 264.8 | 360.0 | 288.8 | 1184.9 |
| | 3 | 24.6 | 130.0 | 204.5 | 372.1 | 397.5 | 347.4 | 1476.1 |
| | Mean | 13.8 | 111.4 | 171.4 | 291.7 | 377.9 | 316.2 | 1282.4 |
| 20 | 1 | 3.6 | 26.3 | 58.2 | 94.3 | 131.9 | 148.5 | 462.8 |
| | 2 | 0.1 | 30.6 | 60.5 | 82.7 | 175.2 | 187.7 | 536.8 |
| | 3 | 5.5 | 42.5 | 60.3 | 121.9 | 195.9 | 218.1 | 544.2 |
| | Mean | 3.1 | 33.1 | 59.7 | 99.6 | 167.7 | 184.8 | 548.0 |
| 40 | 1 | 0.0 | 8.0 | 24.9 | 46.0 | 60.7 | 85.6 | 225.2 |
| | 2 | 0.0 | 6.4 | 19.1 | 64.3 | 68.4 | 78.1 | 236.3 |
| | 3 | 0.0 | 10.7 | 28.9 | 34.1 | 70.3 | 69.8 | 213.8 |
| | Mean | 0.0 | 8.4 | 24.3 | 48.1 | 66.5 | 77.8 | 225.1 |

When those eggs were recorded according to the laying sites as described previously, another interesting pattern, different from that found in *Bulinus globosus*, developed (Table 13). In all of these 5-, 10-, 20- and 40-snail groups, most eggs (i.e., 72.6%, 76.3%, 71.5% and 74.8%, respectively) were on container; some (i. e., 27.2%, 23.6%, 28.4% and 25.1%, respectively) were on lettuce; and the least (i.e.,0.2%, 0.1%, 0.1%, and 0.1% respectively) were on shell. This same pattern of egg-laying sites was again found to be rather consistent in most experiments using aerated tap water in the trays. It was also interesting to note that while in *Bulinus globosus* snails, some 4.4% to 29.4% of the eggs were laid on the shell of individual snails, in the present experiment with *Biomphalaria pfeifferi*, however, only few eggs, about 0.1% to 0.2% were laid on the shell of individual snails.

The number of egg-masses laid was also influenced by crowding. The total egg-masses were 1,894, 2,821, 3,646 and 3,828 respectively as the densities increased from 5 to 40 (Table 14). When the size of egg-masses were grouped according to the number of eggs per egg-mass, some interesting results appeared as shown in Table 14 and Fig. 6. In trays with 5 snails per tray, 90.9% (12.1 + 28.2 + 30.3 + 20.3) of the egg-masses were in the group of 6–25 eggs/mass; with 10 snails, 86.8% (24,2 + 34.2 + 28.4) of the egg-masses were in the group of 6–20 eggs/mass; with 20 snails, 97.2% (17.4 + 48.7 + 31.1) of the egg masses were in the group of 1–15 eggs/mass; and finally with 40 snails, 95.2% (24.7 + 70.5) of the egg-masses were in the group of 1–10 eggs/

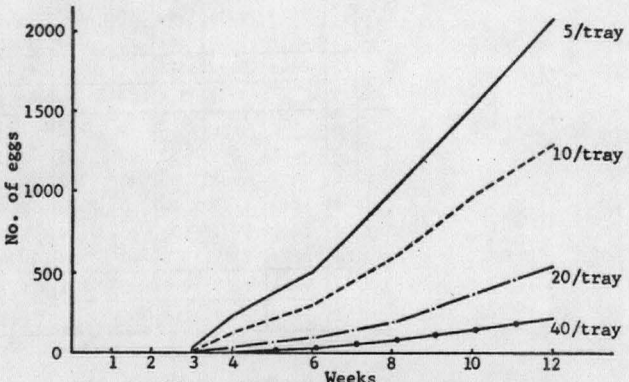



Fig. 5. Effect of crowding on the number of eggs (cumulated) laid per B. pfeifferi reared in tap water for 12 weeks

mass. From these data it appears that the more the snails are crowded in each tray the smaller the mean number of eggs/mass. In the 5-snail group the number was 16.4; in the 10-snail 13.6; in the 20-snail, 9.0; and in the 40-snail, only 7.1 (Table 14). When these mean numbers of eggs/mass were plotted against elapsed time, 4 non-overlapping curves appeared as shown in Fig. 7. The mean number of eggs/mass increased as the snails grew larger and also were older.

Table 13. Egg-laying sites of B. pfeifferi reared in tap water for 12 weeks.

| Snails/ tray | Weeks | Tray No. 1 Tot. eggs | 1 Egg-lay. site C | 1 L | 1 S | Tray No. 2 Tot. eggs | 2 Egg-lay. site C | 2 L | 2 S | Tray No. 3 Tot. eggs | 3 Egg-lay. site C | 3 L | 3 S | Total Tot. eggs | Total Egg-lay. site C | Total L | Total S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 3 | 177 | 148 | 29 | 0 | 276 | 268 | 8 | 0 | 33 | 9 | 24 | 0 | 486 | 425 | 61 | 0 |
| | 4 | 1040 | 834 | 206 | 0 | 1095 | 824 | 271 | 0 | 799 | 432 | 367 | 0 | 2934 | 2090 | 844 | 0 |
| | 6 | 1147 | 1013 | 134 | 0 | 1691 | 968 | 723 | 0 | 1226 | 326 | 900 | 0 | 4064 | 2307 | 1757 | 0 |
| | 8 | 1768 | 1561 | 207 | 0 | 3372 | 2962 | 410 | 0 | 2295 | 1853 | 442 | 0 | 7435 | 6376 | 1059 | 0 |
| | 10 | 2597 | 1849 | 731 | 17 | 3099 | 1966 | 1074 | 59 | 2230 | 1646 | 584 | 0 | 7926 | 5461 | 2389 | 76 |
| | 12 | 2915 | 1894 | 1021 | 0 | 2781 | 2110 | 671 | 0 | 2552 | 1912 | 640 | 0 | 8248 | 5916 | 2332 | 0 |
| | T. | 9644 | 7299 | 2328 | 17 | 12314 | 9098 | 3157 | 59 | 9135 | 6178 | 2957 | 0 | 31093 | 22575 | 8442 | 76 |
| | % | | 75.7 | 24.1 | 0.2 | | 73.9 | 25.6 | 0.5 | | 67.6 | 32.4 | 0.0 | | 72.6 | 27.2 | 0.2 |
| 10 | 3 | 99 | 74 | 25 | 0 | 70 | 0 | 70 | 0 | 246 | 41 | 205 | 0 | 415 | 115 | 300 | 0 |
| | 4 | 931 | 779 | 152 | 0 | 1112 | 98 | 1014 | 0 | 1300 | 1006 | 294 | 0 | 3343 | 1883 | 1460 | 0 |
| | 6 | 1567 | 939 | 628 | 0 | 1531 | 922 | 609 | 0 | 2045 | 1927 | 118 | 0 | 5143 | 3788 | 1355 | 0 |
| | 8 | 2383 | 2237 | 146 | 0 | 2648 | 2485 | 163 | 0 | 3721 | 3458 | 263 | 0 | 8752 | 8180 | 572 | 0 |
| | 10 | 3761 | 2789 | 972 | 0 | 3600 | 2348 | 1240 | 12 | 3976 | 2466 | 1510 | 0 | 11337 | 7603 | 3722 | 12 |
| | 12 | 3124 | 2266 | 858 | 0 | 2888 | 2283 | 605 | 0 | 3474 | 3234 | 240 | 0 | 9486 | 7783 | 1703 | 0 |
| | T. | 11865 | 9084 | 2781 | 0 | 11849 | 8136 | 3701 | 12 | 14762 | 12132 | 2630 | 0 | 38476 | 29352 | 9112 | 12 |
| | % | | 76.6 | 23.4 | 0.0 | | 68.7 | 31.2 | 0.1 | | 82.2 | 17.8 | 0.0 | | 76.3 | 23.6 | 0.1 |
| 20 | 3 | 72 | 0 | 72 | 0 | 2 | 0 | 2 | 0 | 110 | 110 | 0 | 0 | 184 | 110 | 74 | 0 |
| | 4 | 525 | 435 | 90 | 0 | 612 | 468 | 144 | 0 | 849 | 720 | 129 | 0 | 1986 | 1623 | 363 | 0 |
| | 6 | 1162 | 858 | 304 | 0 | 1208 | 1011 | 197 | 0 | 1205 | 956 | 249 | 0 | 3575 | 2825 | 750 | 0 |
| | 8 | 1884 | 1616 | 268 | 0 | 1653 | 1569 | 84 | 0 | 2438 | 2284 | 154 | 0 | 5975 | 5469 | 506 | 0 |
| | 10 | 2636 | 1456 | 1180 | 0 | 3503 | 1804 | 1694 | 5 | 3917 | 2908 | 1009 | 0 | 10056 | 6168 | 3883 | 5 |
| | 12 | 2969 | 2308 | 661 | 0 | 3753 | 1810 | 1921 | 22 | 4361 | 3195 | 1166 | 0 | 11083 | 7313 | 3748 | 22 |
| | T. | 9248 | 6673 | 2575 | 0 | 10731 | 6662 | 4042 | 27 | 12880 | 10173 | 2707 | 0 | 32859 | 23508 | 9324 | 27 |
| | % | | 72.2 | 27.8 | 0.0 | | 62.1 | 37.7 | 0.2 | | 79.0 | 21.0 | 0.0 | | 71.5 | 28.4 | 0.1 |
| 40 | 3 | 0 | - | - | - | 0 | - | - | - | 0 | - | - | - | 0 | - | - | - |
| | 4 | 321 | 269 | 52 | 0 | 257 | 243 | 14 | 0 | 429 | 420 | 9 | 0 | 1007 | 932 | 75 | 0 |
| | 6 | 997 | 938 | 59 | 0 | 762 | 715 | 47 | 0 | 1156 | 1031 | 125 | 0 | 2915 | 2684 | 231 | 0 |
| | 8 | 1840 | 1814 | 26 | 0 | 2572 | 2318 | 245 | 9 | 1363 | 1030 | 333 | 0 | 5775 | 5162 | 604 | 9 |
| | 10 | 2426 | 1971 | 455 | 0 | 2734 | 2226 | 501 | 7 | 2811 | 1428 | 1383 | 0 | 7971 | 5625 | 2339 | 7 |
| | 12 | 3423 | 2118 | 1305 | 0 | 3123 | 1730 | 1393 | 0 | 2793 | 1953 | 840 | 0 | 9339 | 5801 | 3538 | 0 |
| | T. | 9007 | 7110 | 1897 | 0 | 9448 | 7232 | 2200 | 16 | 8552 | 5862 | 2690 | 0 | 27007 | 20204 | 6787 | 16 |
| | % | | 78.9 | 21.1 | 0.0 | | 76.5 | 23.3 | 0.2 | | 68.5 | 31.5 | 0.0 | | 74.8 | 25.1 | 0.1 |

C = Container; L = Lettuce; S = Shell; T. = Total.

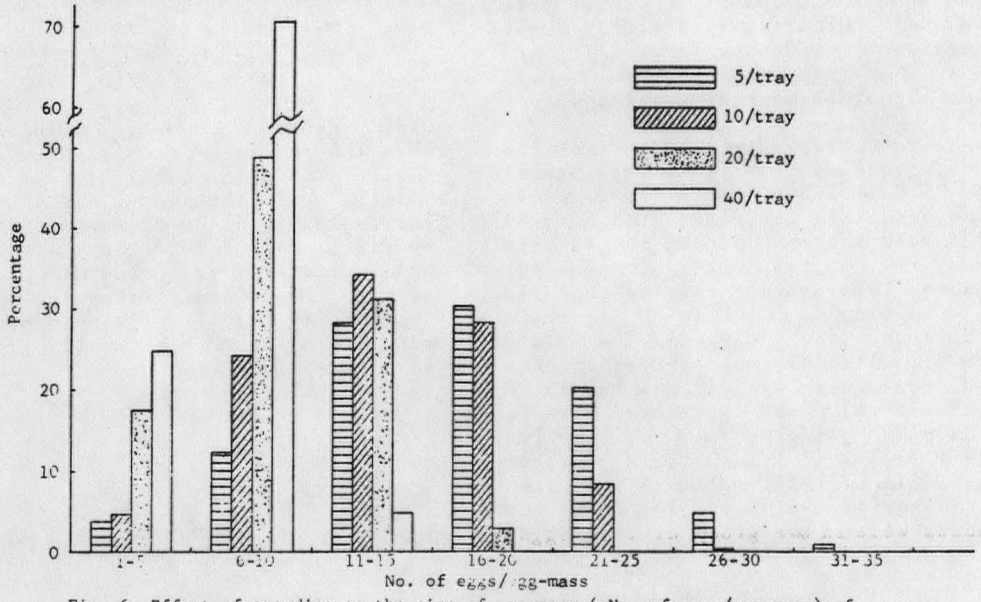



Fig. 6. Effect of crowding on the size of egg-mass (=No. of eggs/egg-mass) of B. pfeifferi reared in tap water for 12 weeks

Table 14. Effect of crowding on the number and the size of egg-masses of B. pfeifferi reared in tap water for 12 weeks

| Snails/ tray | Wks | Total eggs | No. of egg-masses In the range of (# eggs) 1-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 | 31-35 | Total | Mean egg-mass size (eggs/mass) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 3 | 486 | 34 | 29 | 11 | 1 | | | | 75 | 6.5 |
| | 4 | 2934 | 19 | 84 | 97 | 42 | 7 | | | 249 | 11.8 |
| | 6 | 4064 | 8 | 57 | 182 | 58 | 7 | | | 312 | 13.0 |
| | 8 | 7435 | 6 | 24 | 103 | 146 | 103 | 26 | 4 | 412 | 18.0 |
| | 10 | 7926 | 1 | 15 | 62 | 148 | 142 | 35 | 4 | 407 | 19.5 |
| | 12 | 8248 | 2 | 21 | 79 | 179 | 126 | 27 | 5 | 439 | 18.8 |
| | Tot. | 31093 | 70 | 230 | 534 | 574 | 385 | 88 | 13 | 1894 | 16.4 |
| | % | | 3.7 | 12.1 | 28.2 | 30.3 | 20.3 | 4.7 | 0.7 | | |
| 10 | 3 | 415 | 31 | 26 | 7 | 1 | | | | 65 | 6.4 |
| | 4 | 3343 | 59 | 244 | 87 | 2 | | | | 392 | 8.5 |
| | 6 | 5143 | 24 | 237 | 214 | 20 | | | | 495 | 10.4 |
| | 8 | 8752 | 11 | 88 | 218 | 237 | 41 | 1 | | 596 | 14.7 |
| | 10 | 11337 | 4 | 46 | 219 | 310 | 103 | 5 | | 687 | 16.5 |
| | 12 | 9486 | 2 | 41 | 221 | 231 | 89 | 2 | | 586 | 16.2 |
| | Tot. | 38476 | 131 | 682 | 966 | 801 | 233 | 8 | | 2821 | 13.6 |
| | % | | 4.6 | 24.2 | 34.2 | 28.4 | 8.3 | 0.3 | | | |

| Snails/ tray | Wks | Total eggs | No. of egg-masses In the range of (# eggs) 1-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 | 31-35 | Total | Mean egg-mass size (eggs/mass) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 3 | 184 | 9 | 21 | | | | | | 30 | 6.1 |
| | 4 | 1986 | 210 | 149 | 6 | | | | | 365 | 5.4 |
| | 6 | 3575 | 198 | 369 | 10 | | | | | 577 | 6.2 |
| | 8 | 5975 | 102 | 366 | 200 | 6 | | | | 674 | 8.9 |
| | 10 | 10056 | 78 | 498 | 413 | 17 | | | | 1006 | 10.0 |
| | 12 | 11083 | 38 | 374 | 505 | 77 | | | | 994 | 11.1 |
| | Tot. | 32859 | 635 | 1777 | 1134 | 100 | | | | 3646 | 9.0 |
| | % | | 17.4 | 48.7 | 31.1 | 2.8 | | | | | |
| 40 | 3 | 0 | | | | | | | | 0 | 0.0 |
| | 4 | 1007 | 242 | 18 | | | | | | 260 | 3.9 |
| | 6 | 2915 | 233 | 292 | | | | | | 525 | 5.6 |
| | 8 | 5775 | 160 | 595 | 42 | | | | | 797 | 7.2 |
| | 10 | 7971 | 177 | 879 | 33 | | | | | 1089 | 7.3 |
| | 12 | 9339 | 133 | 914 | 110 | | | | | 1157 | 8.1 |
| | Tot. | 27007 | 945 | 2698 | 185 | | | | | 3828 | 7.1 |
| | % | | 24.7 | 70.5 | 4.8 | | | | | | |

## Part II. Effect of Types of Waters on Survivorship, Growth and Fecundity

### *A. Culturing Snails in Deionized Distilled, Tap or Spring Water*

*1. Bulinus globosus*

Distilled water, tap water and spring water were used, and for each kind, three tray cultures were tested on two separate occasions. The data were tabulated biweekly through the 44th week. As shown in Table 15, survivorship was generally good in all 3 waters used. In the first two weeks after hatching, often considered as the critical period of life for young snails, only 2 snails in each culture died in both the distilled water and spring water groups; none was lost in the tap water group during this period. Beyond this critical period none died in any of the groups until the end of the twelfth week. Between the thirteenth and 44th weeks 2 snails died in both the distilled water and spring water groups, with 4 in the tap water group. Therefore, at the end of the 44th week, survivorship was the same with 73.3% (=11/15) of snails surviving.

There were no significant differences in growth rate as shown by the shell lengths among all 3 groups (Table 15 and Fig. 8). The logarithmic phase in growth was complete at the end of the fourth week; rather steep parallel and linear growth curves were observed in all 3 groups throughout the rest of the observation period with a mean shell length of 16.9 mm, 16.8 mm and 18.1 mm in distilled water, tap water and spring water, respectively.

In terms of the number of eggs produced during the 44 week period, there were significant differences in the types of waters used as shown in Table 16. It was obvious that trays with distilled water were most suitable for egg production; trays with tap water were less so; and those with spring water were least suitable. Altogether 57,231 eggs, or 4,480 eggs per snail, were laid in trays with distilled water; 46,321 eggs, or 3,391 eggs per snail, in trays with tap water; and 24,384 eggs, or 2,053 eggs per snail in trays with spring water. When the cumulative number of eggs laid per snail was plotted, 3 distinct groups appeared, as in Fig. 9. In all groups the snails started to oviposit at the end of the fourth week. The number of eggs laid by snails in distilled water throughout the period was always twice or more the number laid by snails in spring water. The number laid in tap water up to the end of the eighth week was more or less the same as in spring water;

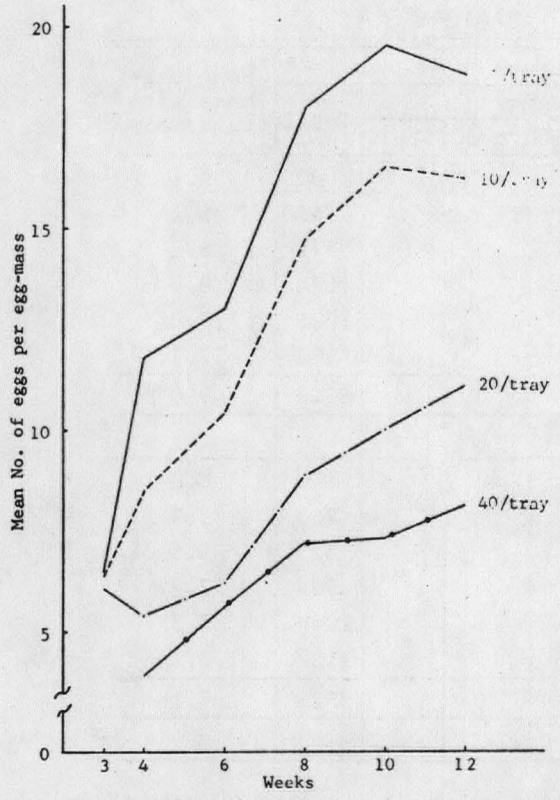



Fig. 7. Effect of crowding on the mean egg-mass size of B. pfeifferi reared in tap water for 12 weeks

Table 15. Effect of distilled, tap and spring waters on survivorship and shell length (x̄) (mm) of B. globosus reared for 44 weeks

| Weeks | Types of waters | | | | | |
|---|---|---|---|---|---|---|
| | Distilled | | Tap | | Spring | |
| | # survived | Shell length | # survived | Shell length | # survived | Shell length |
| 0 | 15 | 0.8 | 15 | 0.8 | 15 | 0.8 |
| 2 | 13 | 5.0 | 15 | 4.5 | 13 | 4.6 |
| 4 | 13 | 9.0 | 15 | 7.7 | 13 | 8.7 |
| 6 | 13 | 10.2 | 15 | 9.3 | 13 | 10.3 |
| 8 | 13 | 11.2 | 15 | 10.5 | 13 | 10.9 |
| 10 | 13 | 11.7 | 15 | 11.2 | 13 | 11.6 |
| 12 | 13 | 12.2 | 15 | 11.6 | 13 | 12.0 |
| 14 | 13 | 13.1 | 15 | 12.1 | 12 | 12.8 |
| 16 | 13 | 13.4 | 15 | 12.6 | 12 | 13.4 |
| 18 | 13 | 13.7 | 15 | 12.9 | 12 | 13.6 |
| 20 | 13 | 14.0 | 15 | 13.1 | 12 | 14.1 |
| 22 | 13 | 14.2 | 15 | 13.4 | 12 | 14.5 |
| 24 | 13 | 14.5 | 15 | 13.5 | 12 | 14.8 |
| 26 | 13 | 14.9 | 15 | 14.0 | 12 | 15.2 |
| 28 | 13 | 15.3 | 14 | 14.3 | 12 | 15.6 |
| 30 | 13 | 15.6 | 14 | 14.8 | 12 | 15.7 |
| 32 | 13 | 15.8 | 13 | 15.3 | 12 | 16.2 |
| 34 | 13 | 16.0 | 13 | 15.4 | 12 | 16.4 |
| 36 | 13 | 16.2 | 13 | 15.8 | 12 | 16.6 |
| 38 | 12 | 16.4 | 13 | 16.0 | 12 | 16.9 |
| 40 | 12 | 16.7 | 13 | 16.3 | 11 | 17.2 |
| 42 | 12 | 16.9 | 11 | 16.7 | 11 | 17.8 |
| 44 | 11 | 16.9 | 11 | 16.8 | 11 | 18.1 |

Table 16. Effect of distilled, tap and spring waters on fecundity of B. globosus reared for 44 weeks

| Wks | Types of waters | | | | | |
|---|---|---|---|---|---|---|
| | Distilled | | Tap | | Spring | |
| | Tot eggs by (#) | Eggs/ snail | Tot eggs by (#) | Eggs/ snail | Tot eggs by (#) | Eggs/ snail |
| 3 | 0 (13) | | 0 (15) | | 0 (13) | |
| 4 | 426 (13) | 32.8 | 49 (15) | 3.3 | 269 (13) | 20.7 |
| 6 | 1671 (13) | 128.5 | 1016 (15) | 67.7 | 1119 (13) | 86.1 |
| 8 | 2635 (13) | 202.7 | 2145 (15) | 143.0 | 983 (13) | 75.6 |
| 10 | 1044 (13) | 80.3 | 1327 (15) | 88.5 | 541 (13) | 41.6 |
| 12 | 1527 (13) | 117.5 | 1325 (15) | 88.3 | 415 (13) | 31.9 |
| 14 | 2926 (13) | 225.1 | 1416 (15) | 94.4 | 1121 (12) | 93.4 |
| 16 | 2290 (13) | 176.2 | 2265 (15) | 151.0 | 661 (12) | 55.1 |
| 18 | 2265 (13) | 174.2 | 1586 (15) | 105.7 | 631 (12) | 52.6 |
| 20 | 2191 (13) | 168.5 | 1980 (15) | 132.0 | 654 (12) | 54.5 |
| 22 | 2000 (13) | 153.8 | 1355 (15) | 90.3 | 1088 (12) | 90.7 |
| 24 | 3591 (13) | 276.2 | 1932 (15) | 128.8 | 1134 (12) | 94.5 |
| 26 | 3936 (13) | 302.8 | 1894 (15) | 126.3 | 872 (12) | 72.7 |
| 28 | 4510 (13) | 346.9 | 3463(14*5) | 238.8 | 1414 (12) | 117.8 |
| 30 | 4538 (13) | 349.1 | 4091 (14) | 292.2 | 817 (12) | 68.1 |
| 32 | 4227 (13) | 325.2 | 3710 (13) | 285.4 | 1714 (12) | 142.8 |
| 34 | 3104 (13) | 238.8 | 3033 (13) | 233.3 | 1246 (12) | 103.8 |
| 36 | 3518 (13) | 270.6 | 3095 (13) | 238.1 | 977 (12) | 81.4 |
| 38 | 2929(12*5) | 234.3 | 3077 (13) | 236.7 | 1916 (12) | 159.7 |
| 40 | 2452 (12) | 204.3 | 2052 (13) | 157.8 | 2250(11*5) | 195.7 |
| 42 | 3038 (12) | 253.2 | 2919(11*5) | 253.8 | 2206 (11) | 200.5 |
| 44 | 2413 (11) | 219.4 | 2591 (11) | 235.5 | 2356 (11) | 214.2 |
| Tot. | 57231 | 4480.4 | 46321 | 3390.9 | 24384 | 2053.4 |

# = No. of survivors.

* The No. of survivors was not an integer, because in each of 4 cases, one individual died during the first half of the 2-week period.

then there was a trend with 1.3 times or more than laid by the latter until the end of the whole period.

In the crowding effect experiments with *Bulinus globosus* and *Biomphalaria pfeifferi* the different types of waters appeared to regulate the sites for egg-laying. Consequently, in this study all eggs laid were also determined as to where they were laid. Data (Table 17) indicated some significant differences among the types of waters used. In distilled water 67.4% of the eggs were laid on the container; in tap water 50.8%; and in spring water, only 28.9%. On the other hand, the percentage laid on lettuce was 23.8% in distilled water, 36.3% in tap water and 57.1% in spring water. Finally, the percentage of eggs laid on shells of individual snails was always smallest among the 3 sites observed, with 8.8% in distilled water, 12.9% in tap water and 14.0% in spring water.

Since in the foregoing observations the types of water used affected the numbers of eggs laid and the sites at which they were laid, these three kinds of water from trays with 24-week old snails were sampled to determine some of the minerals present in the cour-

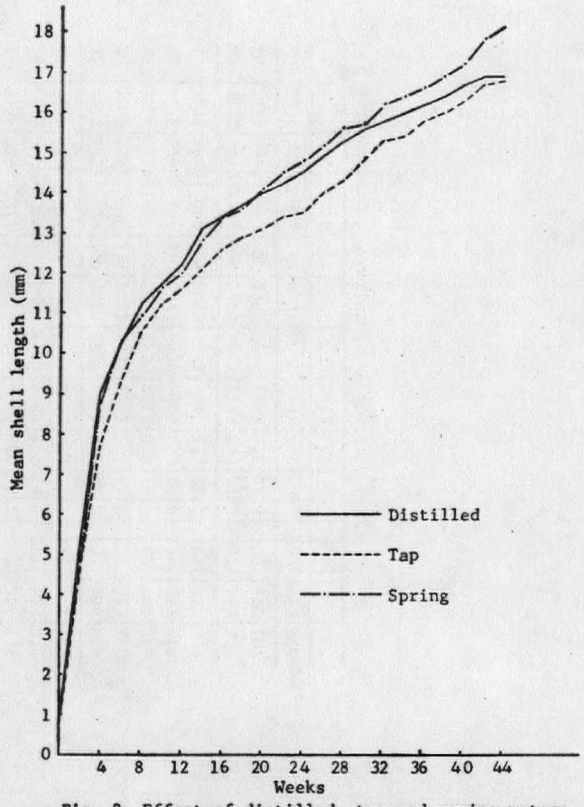



Fig. 8. Effect of distilled, tap and spring waters on mean shell length of B. globosus reared for 44 weeks

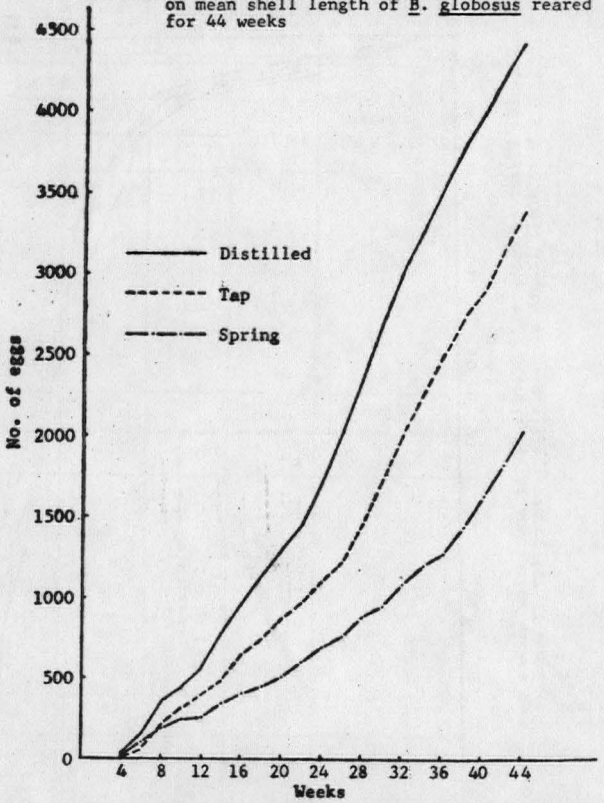



Fig. 9. Effect of distilled, tap and spring waters on the number of eggs (cumulated) laid per B. globosus reared for 44 weeks

Table 17. Effect of distilled, tap and spring waters on egg-laying sites of B. globosus reared for 44 weeks

| Weeks | Types of waters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Distilled | | | | Tap | | | | Spring | | | |
| | Tot. eggs | Egg-lay. site | | | Tot. eggs | Egg-lay. site | | | Tot. eggs | Egg-lay. site | | |
| | | C | L | S | | C | L | S | | C | L | S |
| 3 | 0 | | | | 0 | | | | 0 | | | |
| 4 | 426 | 401 | 0 | 25 | 49 | 49 | 0 | 0 | 269 | 253 | 16 | 0 |
| 6 | 1671 | 1273 | 259 | 139 | 1016 | 832 | 47 | 137 | 1119 | 644 | 331 | 144 |
| 8 | 2635 | 2278 | 139 | 218 | 2145 | 1408 | 438 | 299 | 983 | 638 | 190 | 155 |
| 10 | 1044 | 906 | 12 | 126 | 1327 | 888 | 184 | 255 | 541 | 260 | 101 | 180 |
| 12 | 1527 | 1210 | 179 | 138 | 1325 | 848 | 107 | 370 | 415 | 196 | 62 | 157 |
| 14 | 2926 | 2679 | 167 | 80 | 1416 | 1079 | 92 | 245 | 1121 | 658 | 371 | 92 |
| 16 | 2290 | 1655 | 342 | 293 | 2265 | 1634 | 396 | 235 | 661 | 354 | 236 | 71 |
| 18 | 2265 | 1844 | 277 | 144 | 1586 | 938 | 456 | 192 | 631 | 72 | 460 | 99 |
| 20 | 2191 | 1873 | 164 | 154 | 1980 | 1050 | 699 | 231 | 654 | 267 | 328 | 59 |
| 22 | 2000 | 1516 | 227 | 257 | 1355 | 931 | 369 | 55 | 1088 | 658 | 207 | 223 |
| 24 | 3591 | 2369 | 769 | 453 | 1932 | 1037 | 458 | 437 | 1134 | 247 | 568 | 319 |
| 26 | 3936 | 3096 | 311 | 529 | 1894 | 1165 | 249 | 480 | 872 | 472 | 266 | 134 |
| 28 | 4510 | 2958 | 1300 | 252 | 3463 | 1402 | 1489 | 572 | 1414 | 321 | 814 | 279 |
| 30 | 4538 | 2207 | 1774 | 557 | 4091 | 1989 | 1679 | 423 | 817 | 128 | 521 | 168 |
| 32 | 4227 | 2753 | 774 | 700 | 3710 | 1878 | 1301 | 531 | 1714 | 231 | 1194 | 289 |
| 34 | 3104 | 1274 | 1596 | 234 | 3033 | 1634 | 1101 | 298 | 1246 | 128 | 923 | 195 |
| 36 | 3518 | 1752 | 1626 | 140 | 3095 | 1130 | 1567 | 398 | 977 | 17 | 744 | 216 |
| 38 | 2929 | 1764 | 955 | 210 | 3077 | 932 | 1695 | 450 | 1916 | 279 | 1512 | 125 |
| 40 | 2452 | 1963 | 461 | 28 | 2052 | 973 | 944 | 135 | 2250 | 401 | 1750 | 99 |
| 42 | 3038 | 1113 | 1631 | 294 | 2919 | 986 | 1793 | 140 | 2206 | 337 | 1562 | 307 |
| 44 | 2413 | 1669 | 653 | 91 | 2591 | 746 | 1767 | 78 | 2356 | 479 | 1770 | 107 |
| Tot | 57231 | 38553 | 13616 | 5062 | 46321 | 23529 | 16831 | 5961 | 24384 | 7040 | 13926 | 3418 |
| % | | 67.4 | 23.8 | 8.8 | | 50.8 | 36.3 | 12.9 | | 28.9 | 57.1 | 14.0 |

C = Container; L = Lettuce; S = Shell.

Table 18. Analyses of distilled, tap and spring waters taken from trays with 24-week old B. globosus

| Tests | Types of waters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Distilled | | | Tap | | | Spring | | |
| | * | | | * | | | * | | |
| | 0 | 4 | 7 | 0 | 4 | 7 | 0 | 4 | 7 |
| Alkalinity (as ppm $CaCO_3$) | | | | | | | | | |
| Carbonate | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Bicarbonate | 10.0 | 100.0 | 110.0 | 60.0 | 140.0 | 155.0 | 340.0 | 290.0 | 310.0 |
| Chloride (as ppm Cl) | 5.0 | 20.0 | 22.5 | 42.5 | 57.5 | 60.0 | 85.0 | 100.0 | 107.5 |
| Hardness (as ppm $CaCO_3$) | | | | | | | | | |
| Total | ** | 115.0 | 120.0 | 75.0 | 145.0 | 145.0 | 465.0 | 445.0 | 435.0 |
| Calcium | ** | 95.0 | 100.0 | 40.0 | 105.0 | 105.0 | 330.0 | 330.0 | 320.0 |
| Magnesium | ** | 20.0 | 20.0 | 35.0 | 40.0 | 40.0 | 135.0 | 115.0 | 115.0 |
| Nitrate & nitrite nitrogen (as ppm nitrogen) | 0.03 | 0.48 | 0.52 | 0.60 | 0.36 | 0.22 | 0.25 | 0.38 | 0.50 |
| pH value | 6.60 | 7.40 | 7.25 | 7.40 | 7.25 | 7.50 | 7.40 | 7.80 | 7.75 |
| Sulphate (ppm) | 2.0 | 16.0 | 17.0 | 75.0 | 88.0 | 96.0 | 65.0 | 89.0 | 97.0 |

* = Days after introduction of snails. Data on day 0 were same as those shown in Table 1.

** Concentration below the measurable level.

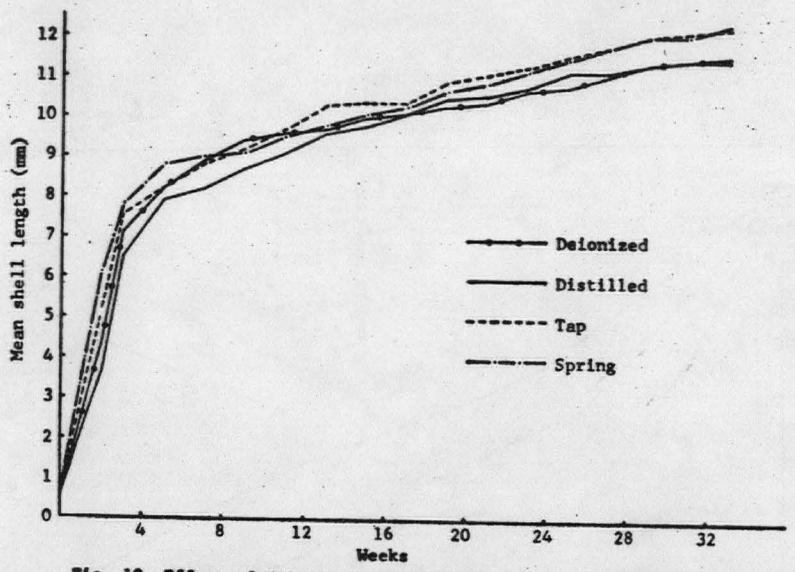



Fig. 10. Effect of deionized, distilled, tap and spring waters on mean shell length of B. pfeifferi reared for 33 weeks

se of snail development, as shown in Table 18. The data indicate that stability was reached in all three waters by day 4. The ratio of bicarbonate alkalinity in distilled, tap and spring waters was 1 : 1.4 : 2.9 for the 4-day period; of chloride, 1 : 2.9 : 5; for total hardness, 1 : 1.3 : 3.9; for nitrate and nitrite nitrogen, 1 : 0.8 : 0.8; and for sulphate, 1 : 5.5 : 5.5. While the pH values in distilled water and tap water were about the same for 4-day period, in the spring water it increased to 7.8 over the same period. This difference suggested that bicarbonate alkalinity, chloride, total hardness, or a combination of them, was influencing both the number of eggs laid and the sites at which they appeared.

2. *Biomphalaria pfeifferi*

For this study the following four kinds of water were used: deionized water, distilled water, tap water and spring water. One tray was used for each kind of water, the data were tabulated biweekly through the 33rd week. Survivorship is shown in Table 19 In both the deionized and the distilled water all snails survived throughout the entire period; in tap water 4 out of 5 snails survived; and in spring water only 3 out of 5 survived. Since only 5 snails were tested in each group, the significance of these differences remains questionable.

No differences appeared in shell length among those 4 kinds of water tested (Table 19 and Fig. 10). The logarithmic growth was attained at the end of the third week; parallel linear growth curves, which were less steep than those found in *Bulinus globosus*, continued until the end of the 33rd week; the final means in shell length were 11.6 mm, 11.5 mm, 12.3 mm and 12.4 mm in deionized water, distilled water, tap water and spring water, respectively.

In terms of the number of eggs laid by these snails during the 33 week period, the pattern was slightly different from that found in *Bulinus globosus*. Snails in both the deionized water and the distilled water had similar patterns in reproduction, with 4,352 eggs per snail in the former and 4,689 eggs per snail in the latter. In tap water there were 5,454 eggs per snail, and in spring water only 3,792 eggs per snail (Table 20). These differences were shown even more clearly when the number of eggs laid per snail was plotted on a cumulative basis as shown in Fig. 11. The steepest slope occurred with tap water; a medium slope appeared with both deionized and

Table 19. Effect of deionized, distilled, tap and spring waters on survivorship and shell length (x̄) (mm) of B. pfeifferi reared for 33 weeks

| Weeks | Types of waters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Deionized | | Distilled | | Tap | | Spring | |
| | S | SL | S | SL | S | SL | S | SL |
| 0 | 5 | 0.6 | 5 | 0.6 | 5 | 0.6 | 5 | 0.6 |
| 2 | 5 | 4.4 | 5 | 3.6 | 5 | 5.1 | 5 | 6.1 |
| 3 | 5 | 7.1 | 5 | 6.5 | 4 | 7.5 | 5 | 7.8 |
| 5 | 5 | 8.2 | 5 | 7.9 | 4 | 8.2 | 5 | 8.8 |
| 7 | 5 | 8.9 | 5 | 8.2 | 4 | 8.8 | 5 | 9.0 |
| 9 | 5 | 9.4 | 5 | 8.7 | 4 | 9.2 | 5 | 9.1 |
| 11 | 5 | 9.6 | 5 | 9.1 | 4 | 9.7 | 5 | 9.5 |
| 13 | 5 | 9.7 | 5 | 9.6 | 4 | 10.3 | 5 | 9.8 |
| 15 | 5 | 10.0 | 5 | 9.8 | 4 | 10.4 | 3 | 10.1 |
| 17 | 5 | 10.1 | 5 | 10.1 | 4 | 10.4 | 3 | 10.3 |
| 19 | 5 | 10.3 | 5 | 10.5 | 4 | 10.9 | 3 | 10.7 |
| 21 | 5 | 10.4 | 5 | 10.6 | 4 | 11.1 | 3 | 10.9 |
| 23 | 5 | 10.7 | 5 | 10.8 | 4 | 11.3 | 3 | 11.2 |
| 25 | 5 | 10.8 | 5 | 11.2 | 4 | 11.6 | 3 | 11.5 |
| 27 | 5 | 11.1 | 5 | 11.2 | 4 | 11.8 | 3 | 11.8 |
| 29 | 5 | 11.4 | 5 | 11.4 | 4 | 12.1 | 3 | 12.1 |
| 31 | 5 | 11.5 | 5 | 11.5 | 4 | 12.2 | 3 | 12.1 |
| 33 | 5 | 11.6 | 5 | 11.5 | 4 | 12.3 | 3 | 12.4 |

S = No. of survivors; SL = Shell length.

Table 20. Effect of deionized, distilled, tap and spring waters on fecundity of B. pfeifferi reared for 33 weeks

| Wks | Types of waters | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Deionized | | Distilled | | Tap | | Spring | |
| | T. eggs by (#) | Eggs/ snail | T. eggs by (#) | Eggs/ snail | T. eggs by (#) | Eggs/ snail | T. eggs by (#) | Eggs/ snail |
| 2 | 0(5) | | 0(5) | | 0(5) | | 0(5) | |
| 3 | 143(5) | 28.6 | 104(5) | 20.8 | 367(4) | 91.8 | 168(5) | 33.6 |
| 5 | 1150(5) | 230.0 | 1134(5) | 226.8 | 1199(4) | 299.8 | 812(5) | 162.4 |
| 7 | 1532(5) | 306.4 | 1274(5) | 254.8 | 1212(4) | 303.0 | 867(5) | 173.4 |
| 9 | 1717(5) | 343.4 | 1309(5) | 261.8 | 1108(4) | 277.0 | 642(5) | 128.4 |
| 11 | 1280(5) | 256.0 | 2064(5) | 412.8 | 1804(4) | 451.0 | 1288(5) | 257.6 |
| 13 | 1192(5) | 238.4 | 1689(5) | 337.8 | 1181(4) | 295.3 | 1103(5) | 220.6 |
| 15 | 1534(5) | 306.8 | 1534(5) | 306.8 | 1028(4) | 257.0 | 1148(3) | 382.7 |
| 17 | 1712(5) | 342.4 | 1617(5) | 323.4 | 1256(4) | 314.0 | 590(3) | 196.7 |
| 19 | 1767(5) | 353.4 | 1951(5) | 390.2 | 1818(4) | 454.5 | 906(3) | 302.0 |
| 21 | 1551(5) | 310.2 | 1378(5) | 275.6 | 1653(4) | 413.3 | 752(3) | 250.7 |
| 23 | 1726(5) | 345.2 | 1774(5) | 354.8 | 1998(4) | 499.5 | 922(3) | 307.3 |
| 25 | 1697(5) | 339.4 | 2012(5) | 402.4 | 1836(4) | 459.0 | 1234(3) | 411.3 |
| 27 | 1296(5) | 259.2 | 980(5) | 196.0 | 1116(4) | 279.0 | 1039(3) | 346.3 |
| 29 | 1446(5) | 289.2 | 1736(5) | 347.2 | 1481(4) | 370.3 | 875(3) | 291.7 |
| 31 | 1616(5) | 323.2 | 1786(5) | 357.2 | 1534(4) | 383.5 | 513(3) | 171.0 |
| 33 | 402(5) | 80.4 | 1105(5) | 221.0 | 1225(4) | 306.3 | 470(3) | 156.7 |
| Tot | 21761 | 4352.2 | 23447 | 4689.4 | 21816 | 5454.3 | 13329 | 3792.4 |

T. = Total; # = No. of survivors.

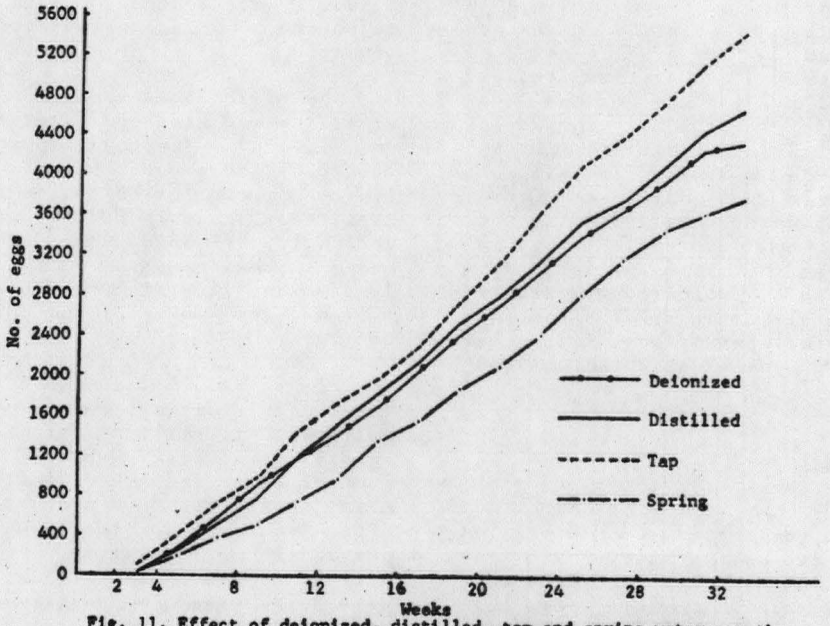



Fig. 11. Effect of deionized, distilled, tap and spring waters on the number of eggs (cumulated ) laid per B. pfeifferi reared for 33 weeks

Table 21. Effect of deionized, distilled, tap and spring waters on egg-laying sites of B. pfeifferi reared for 33 weeks

| Wks | Types of waters | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Deionized | | | | Distilled | | | | Tap | | | | Spring | | | |
| | Tot. eggs | Egg-laying site | | | Tot. eggs | Egg-laying site | | | Tot. eggs | Egg-laying site | | | Tot. eggs | Egg-laying site | | |
| | | C | L | S | | C | L | S | | C | L | S | | C | L | S |
| 2 | 0 | | | | 0 | | | | 0 | | | | 0 | | | |
| 3 | 143 | 83 | 60 | 0 | 104 | 104 | 0 | 0 | 367 | 209 | 158 | 0 | 168 | 168 | 0 | 0 |
| 5 | 1150 | 1056 | 94 | 0 | 1134 | 778 | 356 | 0 | 1199 | 583 | 616 | 0 | 812 | 159 | 653 | 0 |
| 7 | 1532 | 1286 | 226 | 20 | 1274 | 1115 | 159 | 0 | 1212 | 850 | 362 | 0 | 867 | 650 | 217 | 0 |
| 9 | 1717 | 1339 | 378 | 0 | 1309 | 1293 | 16 | 0 | 1108 | 723 | 385 | 0 | 642 | 237 | 405 | 0 |
| 11 | 1280 | 1067 | 213 | 0 | 2064 | 1698 | 366 | 0 | 1804 | 1011 | 793 | 0 | 1288 | 178 | 1110 | 0 |
| 13 | 1192 | 1040 | 152 | 0 | 1689 | 1689 | 0 | 0 | 1181 | 682 | 468 | 31 | 1103 | 581 | 501 | 21 |
| 15 | 1534 | 1088 | 446 | 0 | 1534 | 892 | 642 | 0 | 1028 | 229 | 799 | 0 | 1148 | 257 | 891 | 0 |
| 17 | 1712 | 1049 | 663 | 0 | 1617 | 1073 | 544 | 0 | 1256 | 548 | 708 | 0 | 590 | 0 | 590 | 0 |
| 19 | 1767 | 1042 | 725 | 0 | 1951 | 1174 | 777 | 0 | 1818 | 992 | 826 | 0 | 906 | 324 | 582 | 0 |
| 21 | 1551 | 669 | 882 | 0 | 1378 | 834 | 544 | 0 | 1653 | 214 | 1439 | 0 | 752 | 54 | 698 | 0 |
| 23 | 1726 | 1170 | 556 | 0 | 1774 | 968 | 806 | 0 | 1998 | 526 | 1472 | 0 | 922 | 285 | 637 | 0 |
| 25 | 1697 | 1074 | 623 | 0 | 2012 | 1348 | 664 | 0 | 1836 | 787 | 1049 | 0 | 1234 | 154 | 1080 | 0 |
| 27 | 1296 | 797 | 499 | 0 | 980 | 600 | 380 | 0 | 1116 | 400 | 716 | 0 | 1039 | 615 | 424 | 0 |
| 29 | 1446 | 376 | 1070 | 0 | 1736 | 636 | 1093 | 7 | 1481 | 221 | 1260 | 0 | 875 | 190 | 685 | 0 |
| 31 | 1616 | 282 | 1334 | 0 | 1786 | 567 | 1199 | 20 | 1534 | 137 | 1397 | 0 | 513 | 0 | 488 | 25 |
| 33 | 402 | 99 | 303 | 0 | 1105 | 505 | 583 | 17 | 1225 | 643 | 563 | 19 | 470 | 80 | 364 | 26 |
| Tot | 21761 | 13517 | 8224 | 20 | 23447 | 15274 | 8129 | 44 | 21816 | 8755 | 13011 | 50 | 13329 | 3932 | 9325 | 72 |
| % | | 62.1 | 37.8 | 0.1 | | 65.1 | 34.7 | 0.2 | | 40.1 | 59.7 | 0.2 | | 29.5 | 70.0 | 0.5 |

C = Container; L = Lettuce; S = Shell.

Table 22. Effect of alternation of waters (Dis→Spr→Tap→Dis) on egg-laying sites of B. globosus

| Types of water at start | Weeks | Experimental trays: $H_2O$ used | Total eggs | Egg-laying site: Container No. | Container % | Lettuce No. | Lettuce % | Shell No. | Shell % | Control trays: $H_2O$ used | Total eggs | Egg-laying site: Container No. | Container % | Lettuce No. | Lettuce % | Shell No. | Shell % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Distilled | 0-3 | Dis | 0 | | | | | | | Dis | 0 | | | | | | |
| | 4-12 | " | 5024 | 3844 | 76.5 | 771 | 15.4 | 409 | 8.1 | " | 5674 | 4903 | 86.4 | 467 | 8.2 | 304 | 5.4 |
| | 13-17 | Spr | 3342 | 1707 | 51.1 | 1277 | 38.2 | 358 | 10.7 | " | 5597 | 4742 | 84.7 | 490 | 8.8 | 365 | 6.5 |
| | 18-22 | Tap | 2703 | 1031 | 38.1 | 1108 | 41.0 | 564 | 20.9 | " | 4538 | 3273 | 72.1 | 815 | 18.0 | 450 | 9.9 |
| | 23-27 | Dis | 6713 | 4200 | 62.6 | 1926 | 28.7 | 587 | 8.7 | " | 7864 | 4633 | 58.9 | 1953 | 24.8 | 1278 | 16.3 |
| | Total | | 17782 | * | * | * | * | * | * | | 23673 | 17551 | 74.2 | 3725 | 15.7 | 2397 | 10.1 |
| Tap | 0-3 | Tap | 0 | | | | | | | Tap | 0 | | | | | | |
| | 4-12 | " | 5818 | 3851 | 66.2 | 1725 | 29.6 | 242 | 4.2 | " | 4654 | 3263 | 70.1 | 696 | 15.0 | 695 | 14.9 |
| | 13-17 | Dis | 4755 | 3883 | 81.7 | 737 | 15.5 | 135 | 2.8 | " | 4331 | 3181 | 73.4 | 662 | 15.3 | 488 | 11.3 |
| | 18-22 | Spr | 1967 | 455 | 23.1 | 1415 | 72.0 | 97 | 4.9 | " | 3112 | 1863 | 59.9 | 766 | 24.6 | 483 | 15.5 |
| | 23-27 | Tap | 6791 | 3371 | 49.6 | 3022 | 44.5 | 398 | 5.9 | " | 5651 | 3125 | 55.3 | 765 | 13.5 | 1761 | 31.2 |
| | Total | | 19331 | * | * | * | * | * | * | | 17748 | 11432 | 64.4 | 2889 | 16.3 | 3427 | 19.3 |
| Spring | 0-3 | Spr | 0 | | | | | | | Spr | 0 | | | | | | |
| | 4-12 | " | 1020 | 482 | 47.3 | 489 | 47.9 | 49 | 4.8 | " | 2086 | 1159 | 55.5 | 652 | 31.3 | 275 | 13.2 |
| | 13-17 | Tap | 4563 | 3296 | 72.2 | 701 | 15.4 | 566 | 12.4 | " | 2443 | 839 | 34.3 | 1160 | 47.5 | 444 | 18.2 |
| | 18-22 | Dis | 4866 | 3684 | 75.7 | 719 | 14.8 | 463 | 9.5 | " | 700 | 107 | 15.3 | 404 | 57.7 | 189 | 27.0 |
| | 23-27 | Spr | 4226 | 1326 | 31.4 | 2175 | 51.5 | 725 | 17.1 | " | 2952 | 638 | 21.6 | 1625 | 55.1 | 689 | 23.3 |
| | Total | | 14675 | * | * | * | * | * | * | | 8181 | 2743 | 33.5 | 3841 | 47.0 | 1597 | 19.5 |

* = Not applicable.

distilled water; and the lowest slope with spring water.

The sites of egg-laying were also closely related to the kinds of water used (Table 21). The patterns observed were essentially the same as those observed in *Bulinus globosus* described earlier. The eggs laid on container in both deionized and distilled water were about the same, with 62.1% in the former and 65.1% in the latter; in tap water it was 40.1% and in spring water only 29.5%. The eggs laid on or under lettuce were, therefore, the other way around, with 37.8%, 34.7%, 59.7%, and 70.0% for deionized, distilled, tap and spring, respectively. Only a small percentage of eggs, ranging from 0.1% to 0.5%, were laid on the shells of individual snails in all 4 kinds of water.

### *B. Culturing Snails in Trays while Alternating the Types of Waters*

Previous studies (see Tables 17 and 21) indicated that snails demonstrated specific behavior patterns of egg-laying in response to the types of waters used. To confirm this relationship, snails were cultured in trays in which the types of wwters were periodically altered.

#### *1. Bulinus globosus*

The three kinds of water used were alternated in two different sequential orders, as follows:

a) Dis → Spr → Tap → Dis

Distilled water, tap water and spring water were used, 2 trays for each type of water, to make a total of 6 trays. Newly hatched snails were cultured until the end of the twelfth week, at which time their respective patterns of egg-laying sites were determined. In the distilled water most of the eggs were laid on the container; in the tap water there was a moderate amount; and the spring water had the least. At the beginning of the thirteenth week, snails in half the trays (=3) were designated as 'experimental group' thereafter, and they were subjected to a change of water with weekly renewal of the same kind of water for the next 5 weeks, at which time the next type of water was used. Snails in the remaining three trays, designated as 'control group' thereafter, were also furnished with weekly change of water but the same type of water was used throughout the whole study period. Altogether two such experiments were made on two separate occasions and the data are combined as shown in Table 22.

In groups started with distilled water, the percentages of eggs laid on container in both of the experimental group and the control group were in the same order to the end of the twelfth week, with 76.5% for the former and 86.4% for the latter. While the overall percentage of eggs laid on container in the control group remained at 74.2% (with a range of 58.9% to 86.4%) for the entire period, these percentages in the experimental group varied according to the types of water used; 51.1% in spring water, 38.1% in tap water and 62.6% in distilled water. Similarly in groups started

Table 23. Effect of alternation of waters (Dis→Tap→Spr→Dis) on egg-laying sites of B. globosus

| Types of waters at start | Weeks | Experimental trays: $H_2O$ used | Total eggs | Egg-laying site: Container No. | Container % | Lettuce No. | Lettuce % | Shell No. | Shell % | Control trays: $H_2O$ used | Total eggs | Egg-laying site: Container No. | Container % | Lettuce No. | Lettuce % | Shell No. | Shell % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Distilled | 0-3 | Dis | 0 | | | | | | | Dis | 0 | | | | | | |
| | 4-12 | " | 2523 | 1976 | 78.3 | 311 | 12.3 | 236 | 9.4 | " | 1961 | 1551 | 79.1 | 267 | 13.6 | 143 | 7.3 |
| | 13-17 | Tap | 1637 | 518 | 31.6 | 772 | 47.2 | 347 | 21.2 | " | 2741 | 1846 | 67.4 | 716 | 26.1 | 179 | 6.5 |
| | 18-22 | Spr | 1441 | 816 | 56.6 | 452 | 31.4 | 173 | 12.0 | " | 2683 | 2054 | 76.6 | 323 | 12.0 | 306 | 11.4 |
| | 23-27 | Dis | 3483 | 2027 | 58.2 | 1267 | 36.4 | 189 | 5.4 | " | 3792 | 1927 | 50.8 | 1694 | 44.7 | 171 | 4.5 |
| | Total | | 9084 | * | * | * | * | * | * | | 11177 | 7378 | 66.0 | 3000 | 26.8 | 799 | 7.2 |
| Tap | 0-3 | Tap | 0 | | | | | | | Tap | 0 | | | | | | |
| | 4-12 | " | 1272 | 1158 | 91.1 | 83 | 6.5 | 31 | 2.4 | " | 1877 | 1481 | 78.9 | 340 | 18.1 | 56 | 3.0 |
| | 13-17 | Spr | 1332 | 606 | 45.5 | 542 | 40.7 | 184 | 13.8 | " | 2007 | 1152 | 57.4 | 736 | 36.7 | 119 | 5.9 |
| | 18-22 | Dis | 3826 | 3138 | 82.0 | 497 | 13.0 | 191 | 5.0 | " | 1266 | 741 | 58.5 | 474 | 37.5 | 51 | 4.0 |
| | 23-27 | Tap | 4234 | 2735 | 64.6 | 926 | 21.9 | 573 | 13.5 | " | 4550 | 3674 | 80.8 | 548 | 12.0 | 328 | 7.2 |
| | Total | | 10664 | * | * | * | * | * | * | | 9700 | 7048 | 72.7 | 2098 | 21.6 | 554 | 5.7 |
| Spring | 0-3 | Spr | 0 | | | | | | | Spr | 0 | | | | | | |
| | 4-12 | " | 580 | 135 | 23.3 | 295 | 50.8 | 150 | 25.9 | " | 973 | 343 | 35.2 | 494 | 50.8 | 136 | 14.0 |
| | 13-17 | Dis | 2222 | 1590 | 71.6 | 430 | 19.3 | 202 | 9.1 | " | 1740 | 962 | 55.3 | 682 | 39.2 | 96 | 5.5 |
| | 18-22 | Tap | 2457 | 1429 | 58.2 | 347 | 14.1 | 681 | 27.7 | " | 2295 | 1449 | 63.1 | 658 | 28.7 | 188 | 8.2 |
| | 23-27 | Spr | 1023 | 54 | 5.3 | 853 | 83.4 | 116 | 11.3 | " | 2751 | 785 | 28.6 | 1635 | 59.4 | 331 | 12.0 |
| | Total | | 6282 | * | * | * | * | * | * | | 7759 | 3539 | 45.6 | 3469 | 44.7 | 751 | 9.7 |

* = not applicable.

with tap water, the percentage of eggs laid on container in the experimental group (66.2%) at the end of the twelfth week matched well with that of the control group (70.1%). The overall percentage of eggs laid on container in the control group was 64.4% (with a range of 55.3% to 73.4%) for the entire period. In the experimental group, the percentages also varied according to the types of water used; i.e., 81.7% in distilled water, 23.1% in spring water, 23.1% in spring water and 49.6% in tap water. Finally, in the groups started with spring water, the percentages on container again were similar to the end of the twelfth week as between the experimental group (47.3%) and the control group (55.5%). The overall percentage in the latter was 33.5% (with a range of 15.3% to 55.5%) for the whole period; while in the former, the percentages again varied according to the types of water used: 72.2% in tap water, 75.7% in distilled water and 31.4% in spring water.

b) Dis ⟶ Tap ⟶ Spr ⟶ Dis

Snails were also cultured in distilled, tap or spring water, and for each type of water two trays (a total of 6) were used. Half of the trays (3) were designated as 'experimental group' and subjected to experiment, and the remaining 3 trays served as 'control group.' Only one set of observations was made, and the results, which were similar to the ones found just above, are shown in Table 23. Up to the end of the twelfth week, in groups started with distilled water, the percentage of eggs laid on container was 78.3% in the experimental group and 79.1% in the control group. While the overall percentage in the control group for the entire period was maintained at 66.0% (with a range of 50.8% to 79.1%), the percentages in the experimental group varied according to the types of waters used; 31.6% in tap water, 56.6% in spring water and 58.2% in distilled water.

In groups started with tap water, up to the end of the twelfth week, the percentage on container was 91.1% for the experimental group and 78.9% for the control group. The overall percentage for the control group was maintained at 72.7% (with a range of 57.4% to 80.8%), but the percentages for the experimental group again varied according to the types of waters used: 45.5% in spring water, 82.0% in distilled water and 64.6% in tap water.

In groups started with spring water, the percentage on container up to the end of the twelfth week was 23.3% in the experimental group and 35.2% in the control group. The overall percentage in the control group was 45.6% (with a range of 28.6% to 63.1%) for the entire period of time but the percentages in the experimental group again varied according to the types of waters used: with 71.6% in distilled water, 58.2% in tap water and 5.3% in spring water.

In summary, it was found that, regardless of the order in which waters were used, almost without exception snails laid most of their eggs on container when they remained in distilled water and they laid the least on that same site when they were placed in spring water. In tap water, the patterns of egg-laying were somewhat irregular.

2. *Biomphalaria pfeifferi*

Newly hatched snails were cultured in deionized, distilled, tap and spring waters, two

Table 24. Effect of alternation of waters (Dei→Dis→Tap→Spr→Dei) on egg-laying sites of B. pfeifferi

| Types of waters at start | Weeks | Experimental trays: $H_2O$ used | Total eggs | Egg-laying site: Container No. | Container % | Lettuce No. | Lettuce % | Shell No. | Shell % | Control trays: $H_2O$ used | Total eggs | Egg-laying site: Container No. | Container % | Lettuce No. | Lettuce % | Shell No. | Shell % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Deionized | 0-2 | Dei | 0 | | | | | | | Dei | 0 | | | | | | |
| | 3-9 | " | 3817 | 2794 | 73.2 | 1023 | 26.8 | 0 | 0.0 | " | 4542 | 3764 | 82.9 | 758 | 16.7 | 20 | 0.4 |
| | 10-11 | Tap | 1344 | 475 | 35.3 | 869 | 64.7 | 0 | 0.0 | " | 1280 | 1067 | 83.4 | 213 | 16.6 | 0 | 0.0 |
| | 12-13 | Dis | 1774 | 1774 | 100.0 | 0 | 0.0 | 0 | 0.0 | " | 1192 | 1040 | 87.2 | 152 | 12.8 | 0 | 0.0 |
| | 14-15 | Spr | 1812 | 529 | 29.2 | 1283 | 70.8 | 0 | 0.0 | " | 1534 | 1088 | 70.9 | 446 | 29.1 | 0 | 0.0 |
| | 16-17 | Dei | 1344 | 1020 | 75.9 | 324 | 24.1 | 0 | 0.0 | " | 1712 | 1049 | 61.3 | 663 | 38.7 | 0 | 0.0 |
| | 18-19 | Tap | 1961 | 1691 | 86.2 | 270 | 13.8 | 0 | 0.0 | " | 1767 | 1042 | 59.0 | 725 | 41.0 | 0 | 0.0 |
| | 20-21 | Dis | 1538 | 613 | 39.9 | 925 | 60.1 | 0 | 0.0 | " | 1551 | 669 | 43.1 | 882 | 56.9 | 0 | 0.0 |
| | 22-23 | Spr | 1299 | 59 | 4.5 | 1241 | 95.5 | 0 | 0.0 | " | 1726 | 1170 | 67.8 | 556 | 32.2 | 0 | 0.0 |
| | 24-25 | Dei | 1920 | 1467 | 76.4 | 453 | 23.6 | 0 | 0.0 | " | 1697 | 1074 | 63.3 | 623 | 36.7 | 0 | 0.0 |
| | Total | | 16809 | * | * | * | * | * | * | | 17001 | 11963 | 70.4 | 5018 | 29.5 | 20 | 0.1 |
| Distilled | 0-2 | Dis | 0 | | | | | | | Dis | 0 | | | | | | |
| | 3-9 | " | 3354 | 2578 | 76.9 | 776 | 23.1 | 0 | 0.0 | " | 3821 | 3290 | 86.1 | 531 | 13.9 | 0 | 0.0 |
| | 10-11 | Spr | 804 | 40 | 5.0 | 764 | 95.0 | 0 | 0.0 | " | 2064 | 1698 | 82.3 | 366 | 17.7 | 0 | 0.0 |
| | 12-13 | Dei | 1205 | 943 | 78.3 | 262 | 21.7 | 0 | 0.0 | " | 1689 | 1689 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| | 14-15 | Tap | 1783 | 944 | 52.9 | 839 | 47.1 | 0 | 0.0 | " | 1534 | 892 | 58.1 | 642 | 41.9 | 0 | 0.0 |
| | 16-17 | Dis | 1276 | 855 | 67.0 | 421 | 33.0 | 0 | 0.0 | " | 1617 | 1073 | 66.4 | 544 | 33.6 | 0 | 0.0 |
| | 18-19 | Spr | 1072 | 178 | 16.6 | 894 | 83.4 | 0 | 0.0 | " | 1951 | 1174 | 60.2 | 777 | 39.8 | 0 | 0.0 |
| | 20-21 | Dei | 1488 | 388 | 26.1 | 1100 | 73.9 | 0 | 0.0 | " | 1378 | 834 | 60.5 | 544 | 39.5 | 0 | 0.0 |
| | 22-23 | Tap | 1556 | 143 | 9.2 | 1413 | 90.8 | 0 | 0.0 | " | 1774 | 968 | 54.6 | 806 | 45.4 | 0 | 0.0 |
| | 24-25 | Dis | 1800 | 1387 | 77.1 | 413 | 22.9 | 0 | 0.0 | " | 2012 | 1348 | 67.0 | 664 | 33.0 | 0 | 0.0 |
| | Total | | 14338 | * | * | * | * | * | * | | 17840 | 12966 | 72.7 | 4874 | 27.3 | 0 | 0.0 |
| Tap | 0-2 | Tap | 0 | | | | | | | Tap | 0 | | | | | | |
| | 3-9 | " | 4416 | 2528 | 57.2 | 1888 | 42.8 | 0 | 0.0 | " | 3886 | 2365 | 60.9 | 1521 | 39.1 | 0 | 0.0 |
| | 10-11 | Dei | 1572 | 1195 | 76.0 | 377 | 24.0 | 0 | 0.0 | " | 1804 | 1011 | 56.0 | 793 | 44.0 | 0 | 0.0 |
| | 12-13 | Spr | 1086 | 501 | 46.1 | 585 | 53.9 | 0 | 0.0 | " | 1181 | 682 | 57.8 | 468 | 39.6 | 31 | 2.6 |
| | 14-15 | Dis | 1854 | 1242 | 67.0 | 612 | 33.0 | 0 | 0.0 | " | 1028 | 229 | 22.3 | 799 | 77.7 | 0 | 0.0 |
| | 16-17 | Tap | 1988 | 697 | 35.1 | 1291 | 64.9 | 0 | 0.0 | " | 1256 | 548 | 43.6 | 708 | 56.4 | 0 | 0.0 |
| | 18-19 | Dei | 2450 | 1731 | 70.7 | 719 | 29.3 | 0 | 0.0 | " | 1818 | 992 | 54.6 | 826 | 45.4 | 0 | 0.0 |
| | 20-21 | Spr | 1462 | 320 | 21.9 | 1142 | 78.1 | 0 | 0.0 | " | 1653 | 214 | 12.9 | 1439 | 87.1 | 0 | 0.0 |
| | 22-23 | Dis | 1609 | 967 | 60.1 | 642 | 39.9 | 0 | 0.0 | " | 1998 | 526 | 26.3 | 1472 | 73.7 | 0 | 0.0 |
| | 24-25 | Tap | 2025 | 235 | 11.6 | 1790 | 88.4 | 0 | 0.0 | " | 1836 | 787 | 42.9 | 1049 | 57.1 | 0 | 0.0 |
| | Total | | 18462 | * | * | * | * | * | * | | 16460 | 7354 | 44.7 | 9075 | 55.1 | 31 | 0.2 |
| Spring | 0-2 | Spr | 0 | | | | | | | Spr | 0 | | | | | | |
| | 3-9 | " | 3275 | 693 | 21.2 | 2582 | 78.8 | 0 | 0.0 | " | 2489 | 1214 | 48.8 | 1275 | 51.2 | 0 | 0.0 |
| | 10-11 | Dis | 2717 | 2127 | 78.3 | 590 | 21.7 | 0 | 0.0 | " | 1288 | 178 | 13.8 | 1110 | 86.2 | 0 | 0.0 |
| | 12-13 | Tap | 1839 | 1451 | 78.9 | 369 | 20.1 | 19 | 1.0 | " | 1103 | 581 | 52.7 | 501 | 45.4 | 21 | 1.9 |
| | 14-15 | Dei | 2361 | 1844 | 78.1 | 517 | 21.9 | 0 | 0.0 | " | 1148 | 257 | 22.4 | 891 | 77.6 | 0 | 0.0 |
| | 16-17 | Spr | 1375 | 307 | 22.3 | 1068 | 77.7 | 0 | 0.0 | " | 590 | 0 | 0.0 | 590 | 100.0 | 0 | 0.0 |
| | 18-19 | Dis | 1999 | 1809 | 90.5 | 168 | 8.4 | 22 | 1.1 | " | 906 | 324 | 35.8 | 582 | 64.2 | 0 | 0.0 |
| | 20-21 | Tap | 1466 | 848 | 57.8 | 618 | 42.2 | 0 | 0.0 | " | 752 | 54 | 7.2 | 698 | 92.8 | 0 | 0.0 |
| | 22-23 | Dei | 2250 | 1335 | 59.3 | 915 | 40.7 | 0 | 0.0 | " | 922 | 285 | 30.9 | 637 | 69.1 | 0 | 0.0 |
| | 24-25 | Spr | 1465 | 213 | 14.5 | 1252 | 85.5 | 0 | 0.0 | " | 1234 | 154 | 12.5 | 1080 | 87.5 | 0 | 0.0 |
| | Total | | 18747 | * | * | * | * | * | * | | 10432 | 3047 | 29.2 | 7364 | 70.6 | 21 | 0.2 |

* = Not applicable.

trays for each type of water, totaling 8 trays. Snails were cultured in this way until the end of the ninth week, when the specific patterns of egg-laying sites were observed in each tray. That is, in two trays started with deionized water, the percentages of eggs laid on container were 73.2% and 82.9%; in two trays started with distilled water, they were 76.9% and 86.1%; with tap water 57.2% and 60.9%; and finally with spring water 21.2% and 48.8% (Table 24).

At the beginning of the tenth week, trays with each type of water were divided into two parts so that four trays were subjected to experiment (=experimental group) and the remaining four trays served as control (=control group). In the experimental group, the types of water were alternated in such a way that snails from a tray with one type of water were maintained in a tray with another type of water for the next two weeks with weekly water replacement before the snails were transferred to a tray with the still next type of water. Altogether, there were 32 counts of water alternations; 8 with deionized, 8 with distilled, 8 with tap and 8 with spring. In the control group, the weekly replacement was made with the same kind of water throughout the whole study period.

While in the control group the overall percentages of eggs laid on container were 70.4%, 72.7%, 44.7% and 29.2% in deionized, distilled, tap and spring waters, respectively; those percentages in the experimental group

Table 25. Effect of calcium, magnesium and calcium-magnesium waters on shell length ($\bar{x}$) (mm) of B. globosus reared for 16 weeks (Spring and distilled waters served as control)

| Types of waters | Weeks | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
| Calcium | 0.8 | 2.1 | 3.2 | 7.4 | 8.5 | 9.5 | 11.0 | 12.3 | 12.6 |
| Magnesium | 0.8 | 1.9 | 5.7 | 8.0 | 9.5 | 10.2 | 11.9 | 12.5 | 12.8 |
| Calcium-magnesium | 0.8 | 2.0 | 3.4 | 7.8 | 9.2 | 10.3 | 11.9 | 12.8 | 13.1 |
| Spring | 0.8 | 3.2 | 5.2 | 8.5 | 9.4 | 10.1 | 11.5 | 12.4 | 12.9 |
| Distilled | 0.8 | 2.2 | 4.0 | 8.7 | 9.9 | 10.5 | 11.1 | 11.7 | 12.2 |

varied according to the types of waters used. On the whole, when the trays were provided with deionized water, 7 out of 8 counts had 59.3% to 78.3% of the eggs laid on container; and the last count, 26.1%, was found during the period of the twentieth to twenty-first week in the tray initially started with distilled water. When the trays were provided with distilled water, again 7 out of 8 counts had 60.1% to 100.0% of eggs laid on container and among them 5 counts were between 60.1% and 78.3%; and the last count was found as low as 39.9% during the period of the twentieth to twenty-first week in the tray initially started with deionized water. When trays had tap water, the patterns of egg-laying were so variable as to be hard to follow; the range was between 9.2% and 86.2%. The trays with spring water developed patterns of egg-laying which were most consistent; all 8 counts were found below 50% with a range between 4.5% and 46.1%.

In summary, the eggs in both the deionized and the distilled water had most eggs deposited on container, but in spring water the smallest amounts were laid at this same site. In tap water, the patterns of egg-laying were quite inconsistent.

### *C. Culturing Snails in Calcium, Magnesium or Calcium-Magnesium Water*

Previous chemical analyses (see Table 18) suggested that the total hardness, composed of calcium hardness and magnesium hardness, might be a factor affecting the number of eggs laid by snails and the sites on which they were laid. Consequently, snails were cultured in calcium, magnesium, as well as a combined calcium-magnesium waters. Spring and distilled water served as controls.

#### *1. Bulinus globosus*

Altogether 5 trays were used, one for each kind of water. Each tray contained 5 snails, and none died in any of the trays during the 16 week period. Judging from the excellent survivorship it appeared that neither the calcium nor the magnesium in the water adversely affected the snails. There were also no significant differences in the rate of growth among the 5 groups tested (Table 25 and Fig. 12). Although to the end of the fourth week slower growth appeared in snails in both the calcium water and the calcium-magnesium water groups (perhaps also in the distilled water group), these differences became insignificant after the sixth week. The snails in all five groups completed their logarithmic growth at the end of the sixth week, which was about two weeks later than was observed in a previous experiment with the same species of snails (see Fig. 8). These growth phases were again followed by parallel linear growths among all snails until the end of the sixteenth week with a mean shell length 12.6 mm, 12.8 mm, 13.1 mm, 12 9 mm and 12.2 mm in calcium, magnesium, calcium-magnesium, spring and distilled waters, respectively.

With regard to egg production, there were striking differences among snails in the different types of water used (Table 26). Snails in magnesium, spring and distilled waters started to lay eggs at the end of the sixth week, two weeks later than in previous groups studied (see Table 16). Snails in calcium water and calcium-magnesium water did not oviposit until the seventh week.

When the cumulative numbers of eggs laid per snail were plotted as shown in Fig. 13, it was found that the number of eggs laid by snails in calcium water was very much less than for the rest of the snails in the four

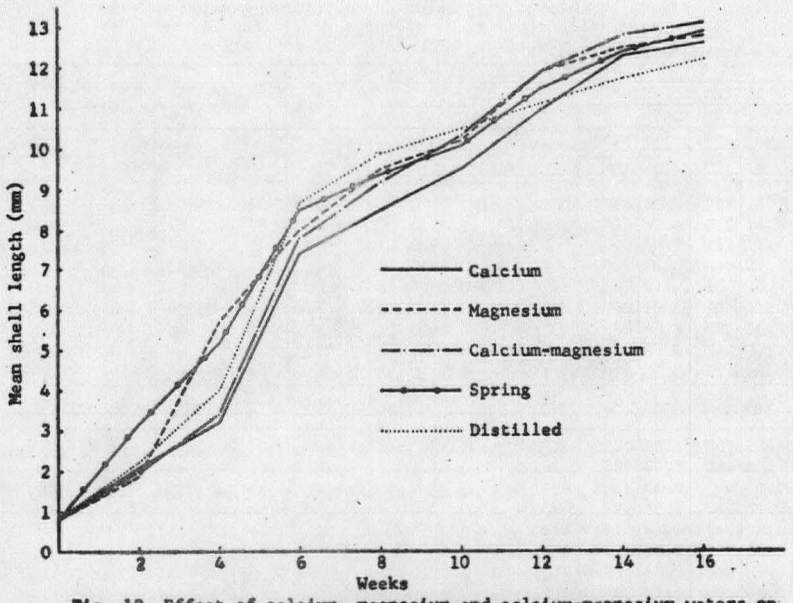



Fig. 12. Effect of calcium, magnesium and calcium-magnesium waters on mean shell length of B. globosus reared for 16 weeks (spring and distilled waters served as control)

Table 26. Effect of calcium, magnesium and calcium-magnesium waters on fecundity (eggs/snail) of B. globosus reared for 16 weeks (Spring and distilled waters served as control)

| Types of waters | Weeks | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 6 | 7 | 8 | 10 | 12 | 14 | 16 | Total |
| Calcium | 0.0 | 18.6 | 5.6 | 56.0 | 229.6 | 301.8 | 53.6 | 665.2 |
| Magnesium | 13.6 | 59.4 | 58.6 | 147.4 | 389.6 | 275.8 | 136.6 | 1081.0 |
| Calcium-magnesium | 0.0 | 41.6 | 77.0 | 198.8 | 318.8 | 228.4 | 30.6 | 895.2 |
| Spring | 39.6 | 25.4 | 71.8 | 120.0 | 298.2 | 164.0 | 59.8 | 778.8 |
| Distilled | 33.0 | 105.6 | 70.8 | 148.0 | 201.6 | 348.0 | 118.8 | 1025.8 |

groups. Among snails in those four groups, egg-laying was more or less similar until the end of the twelfth week. Between the thirteenth and fourteenth weeks, however, three patterns of egg-laying developed. Snails in both magnesium water and distilled water had the highest egg production, snails in calcium-magnesium water were in the middle, and the snails in spring water the lowest.

The total number of eggs laid per snail in calcium water was only 665; in magnesium water, 1,081; in calcium-magnesium-water, 895, which was a mean value for those in the calcium water and the magnesium water. In spring water the number was 778, or about the same amount as laid by snails in the calcium-magnesium water. Finally in distilled water the number was 1,025, which was again similar to those laid in the magnesium water. The conclusion seems warranted that it was the calcium, not the magnesium water which had some adverse effect on potential egg-laying.

The sites of egg-laying were also again

Table 27. Effect of calcium, magnesium and calcium-magnesium waters on egg-laying sites of B. globosus reared for 16 weeks (Spring and distilled waters served as control)

| Wks | Types of waters | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Calcium | | | | Magnesium | | | | Calcium-magnesium | | | | Spring | | | | Distilled | | | |
| | Tot. eggs | Egg-lay site | | | Tot. eggs | Egg-lay site | | | Tot. eggs | Egg lay site | | | Tot. eggs | Egg-lay site | | | Tot. eggs | Egg-lay site | | |
| | | C | L | S | | C | L | S | | C | L | S | | C | L | S | | C | L | S |
| 6 | 0 | | | | 68 | 57 | 11 | 0 | 0 | | | | 198 | 0 | 198 | 0 | 165 | 115 | 50 | 0 |
| 7 | 93 | 44 | 49 | 0 | 297 | 256 | 29 | 12 | 208 | 202 | 6 | 0 | 127 | 66 | 48 | 13 | 528 | 506 | 11 | 11 |
| 8 | 28 | 0 | 28 | 0 | 293 | 25 | 259 | 9 | 385 | 55 | 330 | 0 | 359 | 0 | 359 | 0 | 354 | 160 | 164 | 30 |
| 10 | 280 | 0 | 259 | 21 | 737 | 352 | 346 | 39 | 994 | 89 | 905 | 0 | 600 | 3 | 513 | 84 | 740 | 175 | 565 | 0 |
| 12 | 1148 | 114 | 1034 | 0 | 1948 | 1139 | 523 | 286 | 1594 | 68 | 1490 | 36 | 1491 | 39 | 1378 | 74 | 1008 | 473 | 518 | 17 |
| 14 | 1509 | 673 | 836 | 0 | 1379 | 388 | 891 | 100 | 1142 | 184 | 916 | 42 | 820 | 9 | 800 | 11 | 1740 | 607 | 1116 | 17 |
| 16 | 268 | 133 | 109 | 26 | 683 | 589 | 73 | 21 | 153 | 59 | 84 | 10 | 299 | 108 | 173 | 18 | 594 | 422 | 141 | 31 |
| Tot | 3326 | 964 | 2315 | 47 | 5405 | 2806 | 2132 | 467 | 4476 | 657 | 3731 | 88 | 3894 | 225 | 3469 | 200 | 5129 | 2458 | 2565 | 106 |
| % | | 29.0 | 69.6 | 1.4 | | 51.9 | 39.5 | 8.6 | | 14.7 | 83.3 | 2.0 | | 5.8 | 89.1 | 5.1 | | 47.9 | 50.0 | 2.1 |

C = Container; L = Lettuce; S = Shell.

Table 28. Effect of calcium, magnesium and calcium-magnesium waters on shell length ($\bar{x}$) (mm) of B. pfeifferi reared for 16 weeks (Spring and distilled waters served as control)

| Types of waters | Weeks | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 2 | 3 | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
| Calcium | 0.6 | 3.0 | 4.1 | 5.1 | 6.5 | 7.1 | 7.6 | 8.6 | 8.9 | 9.4 |
| Magnesium | 0.6 | 3.2 | 4.4 | 5.5 | 8.0 | 8.9 | 9.1 | 9.7 | 10.2 | 10.4 |
| Calcium-magnesium | 0.6 | 2.2 | 3.7 | 5.3 | 7.1 | 8.2 | 8.7 | 9.7 | 10.3 | 10.5 |
| Spring | 0.6 | 3.6 | 5.0 | 6.2 | 8.4 | 9.0 | 9.4 | 10.0 | 10.4 | 11.0 |
| Distilled | 0.6 | 2.8 | 4.3 | 6.0 | 7.9 | 8.5 | 9.2 | 9.9 | 10.5 | 10.6 |

closely related to the types of water used (Table 27). In calcium water, only 29.0% of the eggs was laid on container; in magnesium water, 51.9%; in calcium-magnesium water, 14.7%; in spring water, 5.8%; and in distilled water, 47.9%. In other words, snails in calcium water, calcium-magnesium water and spring water produced fewer eggs (a range of 5.8% to 29.0% on container; the snails in both of magnesium water and distilled water laid more eggs (a range of 47.9% to 51.9%) on the same sites as mentioned above. These results imply that it was again calcium, not magnesium, which influenced snails to lay fewer eggs on container.

2. *Biomphalaria pfeifferi*

As with *Bulinus globosus*, five trays were used, one for each type of water, and each tray contained 5 snails. None of the snails died during the 16 weeks in culture. The results on growth were shown in Table 28 and Fig. 14. Snails in all of the five groups completed logarithmic growth at the end of the sixth week, which was three weeks longer than previously indicated (see Fig. 10). In this series there were no significant differences in the rate of growth among all of these snails except for those placed in calcium water. The growth of snails in the calcium water slowed down at the end of the sixth week and continued to maintain the same degree of difference as compared to the rest of the snails until the end of the experiment (Fig. 14). The data show that the mean shell length at the end of the period was 9.4 mm, 10.4 mm, 10.5 mm, 11.0 mm and 10.6 mm in that order with calcium water, magnesium water, calcium-magnesium water, spring water and distilled water, respectively. Since only one experiment was made, the significance of these differences may be questionable.

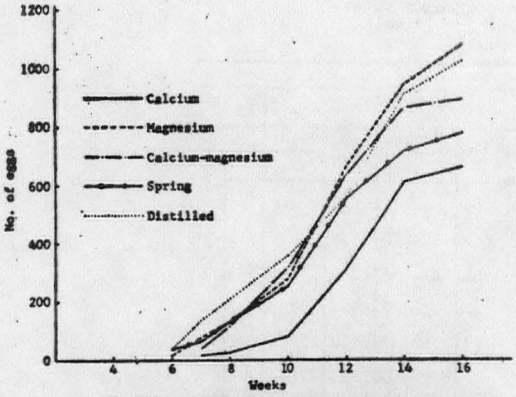



Fig. 13. Effect of calcium, magnesium and calcium-magnesium waters on the number of eggs (cumulated) laid per B. globosus reared for 16 weeks (spring and distilled waters served as control)

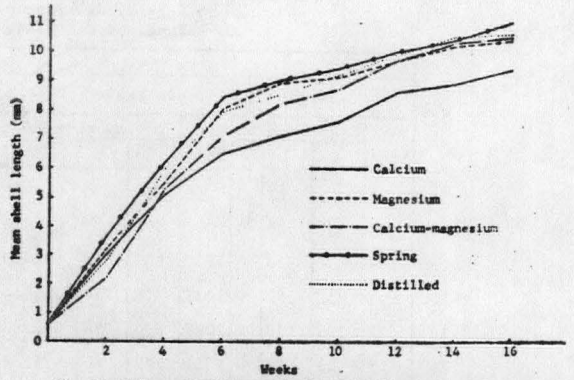



Fig. 14. Effect of calcium, magnesium and calcium-magnesium waters on mean shell length of B. pfeifferi reared for 16 weeks (spring and distilled waters served as control)

Table 29. Effect of calcium, magnesium and calcium-magnesium waters on fecundity (eggs/snail) of B. pfeifferi reared for 16 weeks (Spring and distilled waters served as control)

| Types of waters | Weeks | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 | 6 | 8 | 10 | 12 | 14 | 16 | Total |
| Calcium | 5.4 | 107.2 | 72.8 | 120.6 | 282.6 | 35.8 | 15.0 | 639.4 |
| Magnesium | 12.2 | 217.4 | 344.2 | 266.4 | 417.6 | 267.4 | 334.4 | 1859.6 |
| Calcium-magnesium | 6.4 | 129.2 | 294.0 | 192.8 | 472.6 | 247.6 | 320.6 | 1663.2 |
| Spring | 31.6 | 194.0 | 251.0 | 234.6 | 431.8 | 35.4 | 260.2 | 1438.6 |
| Distilled | 16.4 | 188.2 | 274.0 | 272.8 | 420.4 | 302.2 | 269.2 | 1743.2 |

The snails in all five groups started laying eggs at the end of the fourth week (Table 29), which was one week later than previously observed (see Table 20). However, the differences in the numbers of eggs produced among the snails in these 5 groups were quite similar to those found in *Bulinus globosus* described earlier. The data are summarized as follows: in calcium water only 639 eggs per snail were produced; in magnesium water, 1,859; in calcium-magnesium water, 1,663—a number intermediate between calcium water and magnesium water, in spring water, 1,438—a number of about the same magnitude as laid by snails in calcium magnesium water; and in distilled water, 1,743—similar to the production of snails in magnesium water.

When the cumulative number of eggs laid was plotted, as in Fig. 15, the number produced by snails in calcium water was strikingly smaller than for the snails in the remaining four groups. Among those four groups, egg production was more or less the same throughout the entire period, except that in the spring water, production slowed down once between the twelfth and fourteenth weeks. Again, the data indicate that calcium had an adverse effect on egg production.

As to the sites of egg-laying it was found that they, too, were closely related to the types of waters used (Table 30). In calcium water only 12.9% of the eggs were laid on container; in magnesium water 55.3%; in calcium-magnesium water 51.3%; in spring water 28.6%; and in distilled water 52.4%. Again calcium affected the snails so that they had fewer eggs on container.

## Part III. Effect of Various Species of Algae and Various Kinds of Muds and Mud Extracts on the Growth of Biomphalaria pfeifferi

### *A. Culturing Snails in Unialgal Preparations*

While the mixture of blue-green algae as reported previously was found satisfactory for culturing *Bulinus globosus* and *Biomphalaria*

Table 30. Effect of calcium, magnesium and calcium-magnesium waters on egg-laying sites of B. pfeifferi reared for 16 weeks (Spring and distilled waters served as control)

| Wks | Types of waters | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Calcium | | | | Magnesium | | | | Calcium-magnesium | | | | Spring | | | | Distilled | | | |
| | Tot. eggs | Egg-lay site | | | Tot. eggs | Egg-lay site | | | Tot. eggs | Egg-lay site | | | Tot. eggs | Egg-lay site | | | Tot. eggs | Egg-lay site | | |
| | | C | L | S | | C | L | S | | C | L | S | | C | L | S | | C | L | S |
| 4 | 27 | 0 | 27 | 0 | 61 | 51 | 10 | 0 | 32 | 24 | 8 | 0 | 158 | 14 | 144 | 0 | 82 | 37 | 45 | 0 |
| 6 | 536 | 31 | 505 | 0 | 1087 | 494 | 593 | 0 | 646 | 237 | 409 | 0 | 970 | 467 | 503 | 0 | 941 | 297 | 644 | 0 |
| 8 | 364 | 51 | 313 | 0 | 1721 | 656 | 1065 | 0 | 1470 | 624 | 836 | 10 | 1255 | 81 | 1174 | 0 | 1370 | 565 | 805 | 0 |
| 10 | 603 | 53 | 550 | 0 | 1332 | 558 | 774 | 0 | 964 | 664 | 300 | 0 | 1173 | 47 | 1126 | 0 | 1364 | 332 | 1032 | 0 |
| 12 | 1413 | 214 | 1199 | 0 | 2088 | 690 | 1398 | 0 | 2363 | 465 | 1898 | 0 | 2159 | 207 | 1952 | 0 | 2102 | 1081 | 1021 | 0 |
| 14 | 179 | 53 | 126 | 0 | 1337 | 1249 | 69 | 19 | 1238 | 1030 | 208 | 0 | 177 | 92 | 85 | 0 | 1511 | 1204 | 307 | 0 |
| 16 | 75 | 11 | 64 | 0 | 1672 | 1448 | 224 | 0 | 1603 | 1223 | 308 | 0 | 1301 | 1152 | 149 | 0 | 1346 | 1051 | 295 | 0 |
| Tot | 3197 | 413 | 2784 | 0 | 9298 | 5146 | 4133 | 19 | 8316 | 4267 | 4039 | 10 | 7193 | 2060 | 5133 | 0 | 8716 | 4567 | 4149 | 0 |
| % | | 12.9 | 87.1 | 0.0 | | 55.3 | 44.5 | 0.2 | | 51.3 | 48.6 | 0.1 | | 28.6 | 71.4 | 0.0 | | 52.4 | 47.6 | 0.0 |

C = Container; L = Lettuce; S = Shell.

*pfeifferi*, it seemed to be desirable to prepare unialgal cultures to evaluate more accurately the precise effect of each algal species on snail growth. Only *Biomphalaria pfeifferi* were tried, and altogether three species of blue-green algae and one species of diatom were tested on three different occasions. For convenience, Petri dishes containing algal cultures served as aquaria.

*1. With 10-day old algae*

Since unialgal cultures were not all available when this experiment was started, only two species of blue-green algae, *Nostoc muscorum* and *Fischerella ambigua* and one species of diatom, *Nitschia frustulum*, were used. Each was inoculated into three Petri dishes. As one control, three Petri dishes were inoculated with the mixture of blue-green algae; as another control, three Petri dishes were left without any inoculation. All dishes were incubated for 10 days before introducing snails. Five snails were then introduced per dish, and measurements were made 14 days later. Results are shown in the first part of Table 31.

The best survivorship was found in Petri dishes with *Nostoc muscorum* and the mixture of blue-green algae; in each of these 14 out of 15 individuals survived. Next highest was in Petri dishes with *Nitzschia frustulum*, in which 13 out of 15 survived. Fewest survived, 12 out of 15, in the Petri dishes with *Fischerella ambigua*. In the Petri dishes in which no algae were added (one of the controls) it was not surprising to find that no snails were alive in 2 out of 3 dishes and in the third only two snails survived. It was understandable that the dishes without algal food would not support growth. The low survivorship in one dish with *Fischerella ambigua* is not considered significant, since in this case the other two dishes showed 100% survival.

*Nostoc muscorum* supported growth very well; the mean shell length in this culture at the end of the second week was 5.9 mm. *Fischerella ambigus* and the mixture of blue-green algae produced only moderate growth; these two cultures had a mean shell length of 4.0 mm and 3.7 mm respectively. With the diatom, *Nitzschia frustulum*, the snails grew poorly and had a mean shell length of only 3.1 mm. This poor growth may have been more a matter of quantity of food rather than quality, since macroscopically the shell length of the snails in this group at the end of the first week was about the same as that in the other groups.

*2. With 14-day old algae*

Since in the 10-day old algal cultures the diatoms did not support growth as well as the other algae, the diatoms were eliminated in this experiment. Altogether three species of blue-green algae: *Nostoc muscorum*, *Schizothrix calcicola* and *Fischerella ambigua*, were tried. The mixture of blue-green algae again served as one control and the mud without algae served as the other. Again three Petri dishes were prepared for each group, and the snails were introduced 14 days after algal inoculation at 5 snails per dish. Measurements were made 14 days later (see second part of Table 31). Survivorship was good in all of the groups, with 93% (14/15) to 100% (15/15) surviving, except in the cultures without algae.

When the rate of growth was measured, both cultures, *Nostoc muscorum* and *Fischerella ambigua* supported growth equally well, and the mean shell lengths were 5.5 mm and 5.7 mm, respectively. The cultures with *Schizothrix calcicola* and the mixture of blue-green algae produced only moderate growth, with mean shell lengths of 4.2 mm and 4.5 mm respectively.

Table 31. Effect of species of algae on survivorship and shell length ($\bar{x}$) (mm) of B. pfeifferi reared for 2 weeks (mixture of blue-green algae and mud served as control)

| Species of algae | Age of algae (days) | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | | | | | | | | 14 | | | | | | | | 24 | | | | | | | |
| | Dish No. | | | | | | Mean & Total | | Dish No. | | | | | | Mean & Total | | Dish No. | | | | | | Mean & Total | |
| | 1 | | 2 | | 3 | | | | 1 | | 2 | | 3 | | | | 1 | | 2 | | 3 | | | |
| | S | SL | S | SL | S | SI | S | SL | S | SL | S | SL | S | SL | S | SL | S | SL | S | SL | S | SL | S | SL |
| Nos. muscorum | 4 | 5.8 | 5 | 5.9 | 5 | 6.0 | 14 | 5.9 | 5 | 6.1 | 5 | 4.8 | 5 | 5.6 | 15 | 5.5 | 5 | 5.1 | 5 | 4.9 | 5 | 5.1 | 15 | 5.0 |
| Sch. calcicola | * | * | * | * | * | * | * | * | 5 | 3.9 | 5 | 4.2 | 5 | 4.6 | 15 | 4.2 | * | * | * | * | * | * | * | * |
| Fis. ambigua | 5 | 4.3 | 2 | 2.5 | 5 | 4.4 | 12 | 4.0 | 5 | 5.5 | 5 | 5.9 | 5 | 5.8 | 15 | 5.7 | 5 | 5.2 | 5 | 5.1 | 5 | 5.1 | 15 | 5.1 |
| Nit. frustulum | 5 | 3.2 | 4 | 2.8 | 4 | 3.2 | 13 | 3.1 | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| Mix. b.-green | 5 | 3.6 | 5 | 3.7 | 4 | 4.0 | 14 | 3.7 | 5 | 4.9 | 5 | 3.7 | 4 | 5.0 | 14 | 4.5 | 5 | 3.2 | 5 | 5.3 | 5 | 5.1 | 15 | 4.5 |
| Mud (no algae) | 2 | 1.9 | 0 | | 0 | | 2 | 1.9 | 0 | | 0 | | 0 | | 0 | | * | * | * | * | * | * | * | * |

S = No. of survivors; SL = Shell length; * = Not tested.

Table 32. Effect of types of containers and food additives on survivorship and shell length ($\bar{x}$) (mm) of B. pfeifferi reared for 3 weeks

| Types of containers | | Types of food additives | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mix. blue-green + Lettuce | | | | Nos. muscorum + Lettuce | | | | Nos. muscorum (no lettuce) | | | |
| | | 2 wks | | 3 wks | | 2 wks | | 3 wks | | 2 wks | | 3 wks | |
| | | S | SL | S | SL | S | SL | S | SL | S | SL | S | SL |
| Tray | 1 | 5 | 4.3 | 5 | 7.1 | 5 | 8.5 | 5 | 10.0 | 5 | 7.5 | 5 | 8.7 |
| | 2 | 5 | 4.3 | 5 | 6.5 | 5 | 8.1 | 5 | 9.7 | 5 | 7.7 | 5 | 9.2 |
| | 3 | 5 | 5.3 | 5 | 7.2 | 5 | 8.0 | 5 | 9.9 | 5 | 8.0 | 5 | 9.0 |
| | Total & Mean | 15 | 4.7 | 15 | 7.0 | 15 | 8.2 | 15 | 9.9 | 15 | 7.7 | 15 | 9.0 |
| Petri dish | 1 | 5 | 4.9 | 5 | 7.4 | 5 | 6.4 | 5 | 8.9 | 5 | 6.4 | 5 | 8.5 |
| | 2 | 5 | 4.6 | 5 | 7.5 | 5 | 6.4 | 5 | 8.8 | 5 | 6.4 | 5 | 8.0 |
| | 3 | 5 | 4.8 | 5 | 7.1 | 5 | 6.2 | 5 | 8.5 | 4 | 6.8 | 4 | 8.4 |
| | Total & Mean | 15 | 4.7 | 15 | 7.3 | 15 | 6.3 | 15 | 8.7 | 14 | 6.5 | 14 | 8.3 |

S = No. of survivors; SL = Shell length.

*3. With 24-day old algae*

For this experiment, only *Nostoc muscorum* and *Fischerella ambigua* were tested, and the mixture of blue-green algae again served as control. For each group 3 Petri dishes were prepared. All algae in the dishes were cultured for 24 days to allow more algal growth. The shell lengths were measured 14 days after the snails were introduced (see last part of Table 31). There were no dead snails in any of the groups tested, and both cultures, *Nostoc muscorum* and *Fischerella ambigua*, supported growth equally well, with themean shell lengths 5.0 mm and 5.1 mm, respectively. The mixture of blue-green algae supported a moderate degree of growth, and the mean shell length was 4.5 mm.

In summary, in 3 experiments *Nostoc muscorum* and *Fischerella ambigua* both supported growth equally well. It appears advisable to provide a sufficiently long algal growing period prior to the introduction of the snails. In the experiments that follow, however, *Nostoc muscorum* was used exclusively because it is easily grown in Petri dish preparations.

*B. Culturing Snails in various kinds of Tray and Petri Dish Preparations*

Earlier (Part I) *Biomphalaria pfeifferi* was reported to grow 5.1 ± 0.7 mm in 2 weeks when cultured in trays with tap water and supplied with the mixture of blue-green algae and lettuce (See Table 11, 5 snails per tray). Later (Part II) these same species grew to 6.1 mm in the same period when placed in trays with spring water and supplied with the same mix-

Table 33. Effect of types of containers and food additives on fecundity of B. pfeifferi reared for 3 weeks *

| Types of containers | | Types of food additives | | | | | |
|---|---|---|---|---|---|---|---|
| | | Mix. blue-green + Lettuce | | Nos. muscorum + Lettuce | | Nos. muscorum (no lettuce) | |
| | | Tot. eggs by ( #) | Eggs/ snail | Tot. eggs by ( #) | Eggs/ snail | Tot. eggs by ( #) | Eggs/ snail |
| Tray | 1 | 405 ( 5) | 81.0 | 1049 ( 5) | 209.8 | 920** ( 5) | 184.0 |
| | 2 | 127 ( 5) | 25.4 | 921 ( 5) | 184.2 | 639 ( 5) | 127.8 |
| | 3 | 2 ( 5) | 0.4 | 607 ( 5) | 121.4 | 933*** ( 5) | 186.6 |
| | Total & Mean | 534 (15) | 35.6 | 2577 (15) | 171.8 | 2492 (15) | 166.2 |
| Petri dish | 1 | 49 ( 5) | 9.8 | 235 ( 5) | 47.0 | 23 ( 5) | 4.6 |
| | 2 | 0 ( 5) | 0.0 | 22 ( 5) | 4.4 | 0 ( 5) | 0.0 |
| | 3 | 2 ( 5) | 0.4 | 173 ( 5) | 34.6 | 117 ( 4) | 29.3 |
| | Total & Mean | 51 (15) | 3.4 | 430 (15) | 28.7 | 140 (14) | 10.0 |

# = No. of survivors; * = Egg-laying began during the period of 2.5 - 3.0 weeks unless otherwise indicated; ** = Including 624 eggs laid during the period of 2.0 - 2.5 weeks; *** = Including 67 eggs laid during the period of 2.0 - 2.5 weeks.

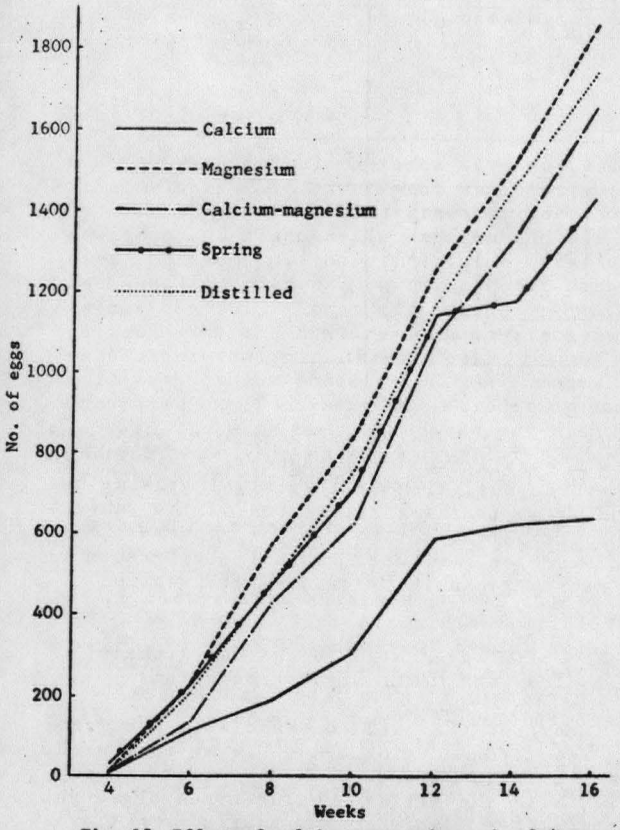



Fig. 15. Effect of calcium, magnesium and calcium-magnesium waters on the number of eggs (cumulated) laid per B. pfeifferi reared for 16 weeks (spring and distilled waters served as control)

ture of blue-green algae and lettuce (see Table 19, spring water). Finally (Part III 'A'), this same species grew to 5.9 mm when cultured in Petri dishes with *Nostoc muscorum* (see Table 31, algae aged 10 days).

These observations suggested the need for comparisons between trays and Petri dishes, the use of mixture of blue-green algae and *Nostoc muscorum*, and feeding with and without lettuce over a 3 week period (until oviposition began) to determine the optimal conditions for the culturing of *B. pfeifferi* snails. Consequently, 6 cultures were established, 3 in trays and 3 in Petri dishes, with 5 snails per tray or dish. At the end of the second week they were measured and reset in new trays or dishes with same preparation for another week, when they were remeasured. The number of eggs laid and egg-laying sites were also recorded. The results are shown in Tables 32-34 and Figs. 16 and 17. Survival was very good, and no snails died in any of the cultures during the observation period, except in dish 3 with *Nostoc muscorum* (without lettuce), where one snail was dead at the end of the second week (Table 32).

In general, the trays supported growth better than the Petri dishes (Table 32, Figs. 16 and 17). However, when a mixture of blue-green algae with lettuce was used as a food additive, this was not the case. At the end of the second week Petri dish cultures supported growth as well as trays with similar food additive, with a mean shell length of 4.7 mm. By the end of the third week, even better growth was observed in Petri dishes, with a mean shell length of 7.3 mm, while the mean in trays was 7.0 mm. This difference in shell length is of doubtful significance. *Nostoc*

Table 34. Effect of types of containers and food additives on egg-laying sites of B. pfeifferi reared for 3 weeks

| Types of containers | | Types of food additives: Mix. blue-green + Lettuce | | | | Nos. muscorum + Lettuce | | | | Nos. muscorum (no lettuce) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total eggs | Egg-laying site C | L | S | Total eggs | Egg-laying site C | L | S | Total eggs | Egg-laying site C | I | S |
| Tray | 1 | 405 | 181 | 224 | 0 | 1049 | 814 | 235 | 0 | 920 | 920 | * | 0 |
| | 2 | 127 | 90 | 37 | 0 | 921 | 834 | 87 | 0 | 639 | 639 | * | 0 |
| | 3 | 2 | 0 | 2 | 0 | 607 | 518 | 89 | 0 | 933 | 933 | * | 0 |
| | Total | 534 | 271 | 263 | 0 | 2577 | 2166 | 411 | 0 | 2492 | 2492 | * | 0 |
| | % | | 50.7 | 49.3 | 0.0 | | 84.1 | 15.9 | 0.0 | | 100.0 | * | 0.0 |
| Petri dish | 1 | 49 | 37 | 12 | 0 | 235 | 0 | 235 | 0 | 23 | 19 | * | 4 |
| | 2 | 0 | | | | 22 | 0 | 22 | 0 | 0 | | | |
| | 3 | 2 | 2 | 0 | 0 | 173 | 2 | 171 | 0 | 117 | 107 | * | 10 |
| | Total | 51 | 39 | 12 | 0 | 430 | 2 | 428 | 0 | 140 | 126 | * | 14 |
| | % | | 76.5 | 23.5 | 0.0 | | 0.5 | 99.5 | 0.0 | | 90.0 | * | 10.0 |

C = Container; L = Lettuce; S = Shell; * = Not applicable.

*muscorum*, either alone or combined with lettuce, was always better than the mixture of blue-green algae and lettuce.

The effect of adding lettuce to *Nostoc muscorum* cultures appeared earlier in the tray group, where at the end of the second week the mean shell length was 8.2 mm in trays with lettuce but only 7.7 mm in trays to which lettuce was not added. At the end of the third week, the effect of lettuce was even more significant, with 9.9 mm in the former but only 9.0 in the latter. In the Petri dish cultures the beneficial effect of lettuce was not observed until the end of the third week. At the end of the second week, the mean shell length was 6.3 mm in dishes with lettuce and 6.5 mm in dishes without lettuce. At the end of the third week, the mean length was 8.7 mm in dishes with lettuce but only 8.3 mm in dishes without lettuce. The best growth was in trays with *Nostoc muscorum* and lettuce; second best in trays with *Nostoc muscorum* but no lettuce; third in Petri dishes with *Nostoc muscorum* and lettuce; fourth in Petri dishes with *Nostoc muscorum* but no lettuce; and last in trays with the mixture of blue-green algae and lettuce or in Petri dishes with the same mixture of blue-green algae and lettuce.

When fecundity is expressed in terms of number of eggs laid per snail as in Table 33, some consistent patterns appear. Those in trays produced more eggs than those in Petri dishes; cultures with *Nostoc muscorum* produced more eggs than with the mixture of blue-green algae; and adding lettuce resulted in little or no increase of egg production. Most snails did not start to lay eggs until they were 2.5 to 3 weeks old. Snails in trays with *Nostoc muscorum* (without lettuce) produced eggs as early as 2 to 2.5 weeks. Since this experiment was done only once, its significance is uncertain.

Sites of egg-laying are shown in Table 34. In trays or Petri dishes containing *Nostoc muscorum* but no lettuce, 100 or 90% of the eggs were found on the container. In the other trays or dishes, all supplied with lettuce, 50.7 to 84.1% of the eggs were found on the container except in the Petri dishes with both *Nostoc muscorum* and lettuce, in which only 0.5% of the eggs were found on the container. The significance of these differences in egg-laying sites remains uncertain, since the period covered by present experiment was too short to permit defining patterns of egg-laying sites; it was usually some 10 weeks before these were stabilized.

### *C. Culturing snails under crowded conditions*

The foregoing experiment showed that the Petri dishes with *Nostoc muscorum* (without lettuce) supported snail growth better than trays with the mixture of blue-green algae and lettuce (see Table 32). Consequently, it seemed important to determine the optimum number of snails per Petri dish should *Nostoc muscorum* be used as an additive in routine culturing.

Using the standard mud mounds inoculated with *Nostoc muscorum*, 18 Petri dishes were incubated one week before the snails were introduced. At the end of that incubation period, 1-day old snails were placed in the Petri dishes, as follows: 3 dishes each with 1, 2, 5, 10, 20 and 40 snails. The survivors were checked and measured for shell length at the end of the first, second, third and fourth weeks. The numbers of eggs laid were also recorded. No replacements of Petri dishes were made, and no distilled water nor algae was added during the entire period. The results are tabulated in Table 35.

Table 35. Effect of crowding on survivorship, shell length ($\bar{x} \pm$ S.D.) (mm) and fecundity of B. pfeifferi reared for 4 weeks

| Snails/tray | Dish No. | Weeks 1 S | 1 SL | 1 E | 2 S | 2 SL | 2 E | 3 S | 3 SL | 3 E | 4 S | 4 SL | 4 E |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 2.3 0.0 | 0 | 1 | 5.2 0.0 | 0 | 1 | 7.4 0.0 | 0 | 1 | 7.7 0.0 | 50 |
| | 2 | 1 | 2.3 0.0 | 0 | 1 | 6.2 0.0 | 0 | 1 | 7.7 0.0 | 0 | 1 | 8.0 0.0 | 12 |
| | 3 | 1 | 2.3 0.0 | 0 | 1 | 5.9 0.0 | 0 | 1 | 6.3 0.0 | 0 | 1 | 6.3 0.0 | 0 |
| | TM | 3 | 2.3±0.0 | 0 | 3 | 5.8±0.4 | 0 | 3 | 7.1±0.6 | 0 | 3 | 7.3±0.7 | 62 |
| 2 | 1 | 2 | 2.3 0.0 | 0 | 2 | 5.5 0.5 | 0 | 2 | 5.9 0.7 | 0 | 2 | 6.0 0.8 | 0 |
| | 2 | 2 | 2.3 0.0 | 0 | 2 | 5.5 0.2 | 0 | 2 | 6.1 0.5 | 0 | 2 | 6.4 0.7 | 8 |
| | 3 | 2 | 2.3 0.0 | 0 | 2 | 5.9 0.3 | 0 | 2 | 6.9 0.1 | 0 | 2 | 6.9 0.2 | 6 |
| | TM | 6 | 2.3±0.0 | 0 | 6 | 5.7±0.4 | 0 | 6 | 6.3±0.6 | 0 | 6 | 6.4±0.7 | 14 |
| 5 | 1 | 5 | 2.3 0.2 | 0 | 5 | 5.3 0.7 | 0 | 5 | 5.9 0.5 | 0 | 5 | 6.0 0.6 | 17 |
| | 2 | 5 | 2.3 0.0 | 0 | 5 | 5.9 0.3 | 0 | 5 | 6.2 0.3 | 0 | 5 | 6.3 0.3 | 0 |
| | 3 | 5 | 2.4 0.1 | 0 | 5 | 6.0 0.6 | 0 | 5 | 6.5 0.4 | 0 | 5 | 6.7 0.3 | 0 |
| | TM | 15 | 2.3±0.1 | 0 | 15 | 5.8±0.6 | 0 | 15 | 6.2±0.6 | 0 | 15 | 6.4±0.6 | 17 |
| 10 | 1 | 10 | 2.3 0.1 | 0 | 10 | 4.7 0.2 | 0 | 10 | 5.2 0.4 | 0 | 10 | 5.3 0.4 | 6 |
| | 2 | 10 | 2.5 0.1 | 0 | 10 | 6.0 0.4 | 0 | 10 | 6.1 0.5 | 0 | 10 | 6.1 0.5 | 3 |
| | 3 | 9 | 2.4 0.2 | 0 | 9 | 5.6 0.5 | 0 | 9 | 6.0 0.5 | 0 | 9 | 6.0 0.5 | 3 |
| | TM | 29 | 2.4±0.2 | 0 | 29 | 5 4±0.7 | 0 | 29 | 5.8±0.6 | 0 | 29 | 5.8±0.6 | 12 |
| 20 | 1 | 19 | 2.3 0.2 | 0 | 19 | 4.1 0.2 | 0 | 19 | 4.6 0.3 | 0 | 19 | 4.6 0.3 | 0 |
| | 2 | 20 | 2.4 0.1 | 0 | 20 | 4.4 0.3 | 0 | 20 | 4.8 0.4 | 0 | 20 | 4.8 0.4 | 0 |
| | 3 | 18 | 2.3 0.3 | 0 | 18 | 4.7 1.4 | 0 | 14 | 5.4 0.5 | 0 | 14 | 5.4 0.5 | 0 |
| | TM | 57 | 2.3±0.2 | 0 | 57 | 4 4±0.9 | 0 | 53 | 4.9±0.5 | 0 | 53 | 4.9±0.5 | 0 |
| 40 | 1 | 39 | 2.2 0.3 | 0 | 36 | 3.4 0.4 | 0 | 33 | 3.4 0.3 | 0 | 15 | 3.7 0.2 | 0 |
| | 2 | 34 | 2.2 0.4 | 0 | 32 | 3.9 0.9 | 0 | 26 | 4.3 0.5 | 0 | 24 | 4.4 0.3 | 0 |
| | 3 | 40 | 2.3 0.1 | 0 | 40 | 3.6 0.2 | 0 | 40 | 3.6 0.2 | 0 | 37 | 3.6 0.2 | 0 |
| | TM | 113 | 2.2±0.3 | 0 | 108 | 3.6±0.6 | 0 | 99 | 3.7±0.5 | 0 | 76 | 3.9±0.4 | 0 |

S = No. of survivors; SL = Shell length; E = Total eggs; TM = Total and mean.

None of the snails died in the dishes with 1, 2 or 5 snails throughout the entire period; in dish 3 with 10 snails one died at the end of the first week. In both the 20-snail and 40-snail groups, however, snails were dying at a steady rate from the beginning of the experiment. At the end of the fourth week, 7 out of 60 snails had died in the dishes with 20 snails and 44 out of 120 in the dishes with 40 snails.

With regard to growth, at the end of the first week the mean shell lengths were about the same regardless of the number of snails per dish, with a range between 2.2 and 2.4 mm (Fig. 18). At the end of the second week, the mean shell lengths fell into three groups: (a) the values in the dishes with 1, 2, 5 and 10 snails were about the same, with a range of 5.4 to 5.8 mm; (b) in the dishes with 20 snails the mean was 4.4 mm; and (c) in the dishes with 40 snails, 3.6 mm. All snails completed logarithmic body growth by the end of the second week except those in dishes with 1 snail, which still exhibited logarithmic growth until the end of the third week. At that time the mean shell lengths fell into 5 groups: (a) the dishes with 1 snail had a mean of 7.1 mm; (b) the dishes with 2 and 5 snails, 6.3 and 6.2 mm respectively; (c) the dishes with 10 snails, 5.8 mm; (d) the dishes with 20 snails, 4.9 mm; and (e) the dishes with 40 snails, 3.7 mm. By the end of the fourth week, the patterns remained the same as those found at the end of the third week.

Egg-laying took place before the end of the fourth week in two dishes with one snail/dish; two with two snails/dish; one with 5 snails/dish; and all dishes with 10 snails-dish (Table 35). No eggs were found in any of the dishes with 20 or 40 snails per dish.

When the presence of algal food was examined, sufficient algae remained in all dishes with 1 or 2 snails throughout the entire period. In the dishes with 5 snails the algae remained in all 3 Petri dishes until the end of the second week; at the end of the third week no algae were left in any of the dishes. In the dishes with 10 snails the algae remained only to the end of the first week. At the end of the second week algae were left in only one dish; none was left at the end of the third week. In the dishes with 20 snails the algae remained until the end of the first week, but no algae remained in any of the dishes thereafter. Finally, in the dishes with 40 snails no algae remained even at the end of the first week. The possible correlation between the presence of algal food and the rate of growth will be discussed later.

### *D. Culturing snails in various kinds of muds and mud extracts.*

Previous experiments demonstrated that algae were necessary to sustain the snails (see Table 31) and that the *Nostoc muscorum* grown on mud supported growth better than the mixture of blue-green algae grown on the same mud (see Tables 31 and 32). Accordingly, experiments were designed to determine which components of the mud contribute to the growth of *Nostoc muscorum* and, in turn, to the growth of the snails. Various kinds of muds and

*(Text continued p. 51)*

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Figure 16, page 49:

Fig. 16. Growth differences in *B. pfeifferi* reared in various kinds of tray and Petri dish preparations for two weeks.

Tray
- 1-3 Mixture of b-green algae plus lettuce
- 4-6 *Nostoc muscorum* plus lettuce
- 7-9 *Nostoc muscorum* alone

Petri dish
- 10-12 Mixture of b-green algae plus lettuce
- 13-15 *Nostoc muscorum* plus lettuce
- 16-18 *Nostoc muscorum* alone

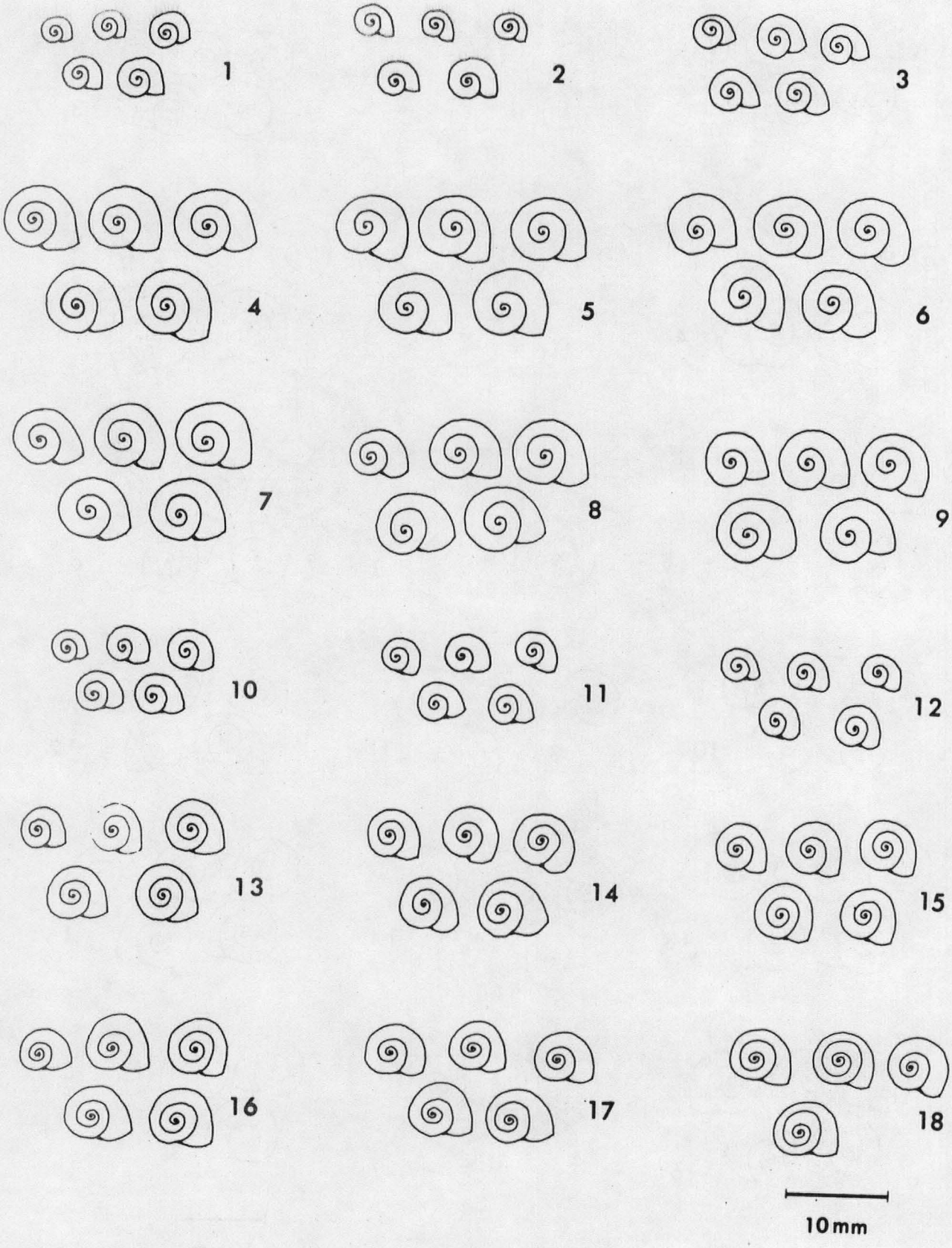
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FIGURE 17

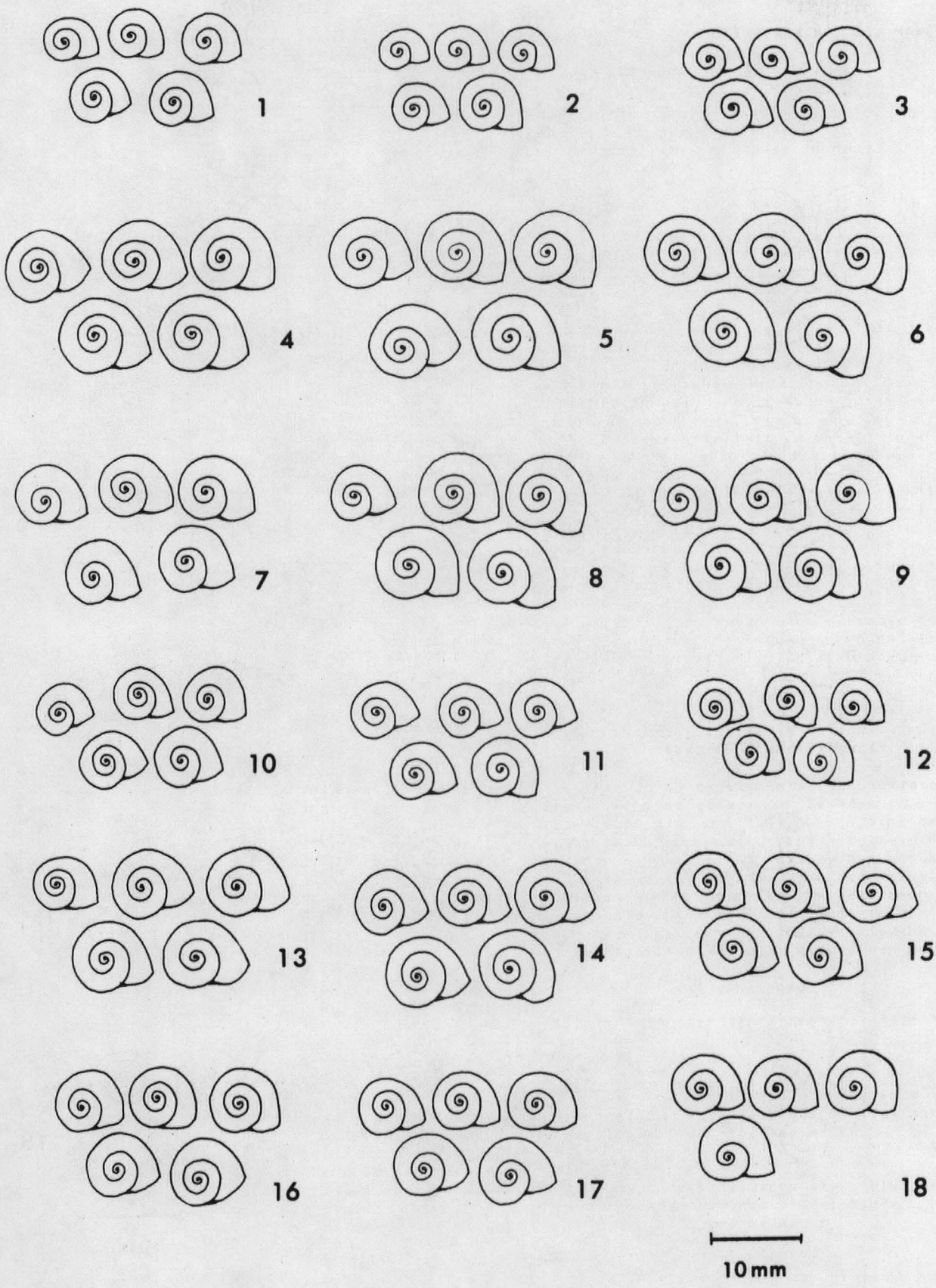

Table 36. Effect of kinds of muds and mud extracts on survivorship and shell length ($\bar{x}$) (mm) of B. pfeifferi reared for 2 weeks (Expt. 1)

| Algae inoculated on day * | Dish No. | Kinds of muds and mud extracts | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Natural mud | | Steamed mud | | Steamed extract | | 100°C-dried mud | | 525°C-heat. mud | | 525°C-heat. steamed mud | | 525°c-heat. steamed extract | |
| | | S | SL | S | SL | S | SL | S | SL | S | SL | S | SL | S | SL |
| -10 | 1 | 4 | 5.8 | 5 | 6.2 | 4 | 2.2 | 5 | 5.2 | 0 | | 5 | 1.8 | 0 | |
| | 2 | 5 | 5.8 | 5 | 5.9 | 3 | 1.9 | 5 | 6.0 | 0 | | 4 | 1.5 | 0 | |
| | 3 | 5 | 5.6 | 5 | 5.9 | 4 | 2.0 | 4 | 6.2 | 0 | | 4 | 1.9 | 0 | |
| | TM | 14 | 5.7 | 15 | 6.0 | 11 | 2.0 | 14 | 5.8 | 0 | | 13 | 1.7 | 0 | |
| | # | 3+ | | 3+ | | 2+ | | 3+ | | 0 | | 2+ | | 1+ | |
| 0, 3, & 11 | 1 | 4 | 3.6 | 5 | 6.0 | 5 | 3.2 | 3 | 3.9 | 0 | | 5 | 3.2 | 0 | |
| | 2 | 5 | 5.2 | 5 | 5.2 | 4 | 2.4 | 5 | 5.2 | 0 | | 5 | 4.0 | 0 | |
| | 3 | 4 | 5.7 | 4 | 5.7 | 5 | 3.1 | 5 | 4.7 | 0 | | 4 | 2.7 | 0 | |
| | TM | 13 | 4.9 | 14 | 5.7 | 14 | 2.9 | 13 | 4.7 | 0 | | 14 | 3.3 | 0 | |
| | # | ** | | ** | | ** | | ** | | ** | | ** | | ** | |

S = No. of survivors; SL = Shell length; TM = Total and mean; * = Day 0 refers to the day snails were introduced; # = Growth of algae on day 0; ** = Not applicable.

their extracts were prepared (see Table 6). These preparations were then tested in two experiments; chemical analyses on the extracts were also made.

*1. Experiment 1*

The purpose here was twofold: (a) to determine whether mud without organic components or a mud extract alone could support algal growth needed to grow snails; and (b) to determine whether the algae had to be grown for a certain period of time before snail introduction or could be added at the time the snails were introduced into a culture. For each kind of mud or mud extract 6 Petri dishes were prepared. To half (3) of these dishes a *Nostoc muscorum* suspension was inoculated (designated '-10 group'), to the other half of the dishes the alga was not inoculated (designated '0-3-11 group'). All dishes, including those not inoculated with algae, were incubated for 10 days before snails were introduced. On the day of snail introduction (day 0), the degree of algal growth in the -10 group was determined; to the 0-3-11 group the same kind of *Nostoc muscorum* suspension, in an excess amount, was inoculated. One-day old snails, 5 per dish, were then introduced into the dishes of the -10 group and the 0-3-11 group. Both groups were then incubated for 2 weeks before the shell lengths were measured. Meanwhile, more *Nostoc muscorum* suspension was added to the 0-3-11 group on days 3 and 11. The results are shown in Table 36.

On the whole, three kinds of preparations in the -10 group, i.e., natural mud, steamed mud and 100° C-dried mud, supported snail growth better than the similar preparations in the 0-3-11 group. On the other hand, two kinds of preparations in the -10 group, i.e., steamed extract and 525° C-heated steamed mud, were less effective in supporting snail growth than similar preparations in the 0-3-11 group. Both the 525° C-heated mud and the 525° C-heated steamed extract did not support snail growth at all, regardless of when the algae were introduced.

Within each group steamed mud supported snail growth best; natural mud and 100° C-dried mud supported growth only to a moderate degree; and steamed extract and 525° C-heated steamed mud were least effective in promoting snail growth. When the relative amount of algal growth was determined macroscopically, there was a close relation between algal growth and snail growth. In general, snails grew well in dishes with 3+ algal growth, were somewhat stunted with 2+ growth, and did not grow at all with 1+ or 0 growth.

*2. Experiment 2*

This experiment essentially repeated Ex-

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Figure 17, page 50:

Fig. 17. Growth differences in *B. pfeifferi* reared in various kinds of tray and Petri dish preparations for 3 weeks.

Tray
1-3 Mixture of b-green algae plus lettuce
4-6 *Nostoc muscorum* plus lettuce
7-9 *Nostoc muscorum* alone.
Petri dish
10-12 Mixture of b-green algae plus lettuce
13-15 *Nostoc muscorum* plus lettuce
16-18 *Nostoc muscorum alone*

Table 37. Effect of kinds of muds and mud extracts on survivorship and shell length ($\bar{x}$) (mm) of B. pfeifferi reared for 2 weeks (Expt. 2)

| Dish No. | Kinds of muds and mud extracts | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Natural mud | | Steamed mud | | Steamed extract | | Stirred mud | | Stirred extract | | 100°C-dried mud | | 100°C-dried mud + 525°C-h. s. extract | | 525°C-h. mud | | 525°C-h. s. mud | | 525°C-h. s. extract (full-stren-gth) | | 525°C-h. s. extract (half-stren-gth) | |
| | S | SL | S | SL | S | SL | S | SL | S | SI | S | SL | S | SL | S | SL | S | SL | S | SL | S | SL |
| 1 | 5 | 4.1 | 4 | 4.6 | 5 | 2.0 | 5 | 5.1 | 5 | 1.7 | 4 | 4.1 | 4 | 3.8 | 0 | | 0 | | 0 | | 0 | |
| 2 | 3 | 4.1 | 5 | 4.2 | 5 | 1.6 | 3 | 4.3 | 5 | 1.7 | 5 | 4.5 | 5 | 3.9 | 0 | | 0 | | 0 | | 0 | |
| 3 | 5 | 4.4 | 5 | 4.9 | 5 | 1.9 | 5 | 4.9 | 3 | 1.2 | 5 | 4.5 | 4 | 4.0 | 0 | | 0 | | 0 | | 0 | |
| TM | 13 | 4.2 | 14 | 4.5 | 15 | 1.8 | 13 | 4.8 | 13 | 1.6 | 14 | 4.4 | 13 | 3.9 | 0 | | 0 | | 0 | | 0 | |
| # | 3+ | | 3+ | | 2+ | | 3+ | | 2+ | | 3+ | | 3+ | | 0 | | 0 | | 1+ | | 0 | |

S = No. of survivors; SL = Shell length; TM = Total and mean; h. = heated; s. = steamed;
# = Growth of algae on the day snails were introduced (= day 0).

periment 1 except that some additional preparations were added: (a) stirred mud and stirred extract were added on the assumption that they could contain some essential components needed for the growth of algae, hence, also the growth of snails, components which might have been destroyed during the process of steaming: (b) there was also a suspicion that 525° C-heated mud and the extract from it might have contained some harmful chemicals as a result of the heat treatment. Therefore, a new preparation was made to examine this possibility; i. e., to the 100° C-dried mud, 525° C-heated steamed extract was added instead of distilled water; (c) on the chance that full-strength 525° C-heated steamed extract might be toxic because of its high concentration of certain chemicals, a half-strength extract was prepared. All Petri dishes were inoculated with the algae 10 days before snail introduction. The results are shown in Table 37.

All natural mud, steamed mud, stirred mud and 100° C-dried mud showed favorable snail growth. In this series the stirred mud best supported snail growth and the shells had a mean length of 4.8 mm; both the steamed mud and the 100° C-dried mud supported the growth to about the same degree, and the mean shell length was 4.5 mm and 4.4 mm, respectively; the natural mud supported growth least among those four preparations tested, with the mean shell length of 4.2 mm. Although the significance of these differences in shell length is uncertain, it appears that stirred mud was better than steamed mud, and that the steamed mud was, in turn, better than the 100° C-dried mud or the natural mud.

The combination of 100° C-dried mud and 525° C-heated steamed extract supported snail growth to a moderate degree with the mean shell length 3.9 mm. Both the steamed extract and the stirred extract were least effective in supporting growth, with the mean shell lengths of only 1.8 mm and 1.6 mm, respectively. None of the 525° C-heated mud, 525° C-heated steamed extract (full-strength) or 525° C-heated steamed extract (half-strength) supported snail growth at all.

There was also a close correlation between algal growth and snail growth, i.e. snails grew well in 3+, were stunted in 2+ and did not grow at all in 1+ and 0.

### *3. Chemical Analyses of Mud Extracts*

In both experiments above (see Tables 36 and 37) the steamed extract and the stirred extract supported snail growth to a certain degree, and the 525° C-heated steamed extract did not support growth at all. These differences suggested that chemical analyses should be made of these three extracts to determine the differences in their chemical compositions; results are shown in Table 38.

The bicarbonate alkalinity was highest in stirred extract, less in steamed extract, and lowest in 525° C-heated steamed extract. Calcium hardness was highest in 525° C-heated steamed extract with 845 ppm (=890 + 800/2), followed by the stirred extract with 540 ppm and lowest in steamed extract with only 422.5 ppm (=405 + 440/2), or exactly half that found