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# Limnochemical Factors Influencing the Seasonal Population Density, Secondary Production, and Calcium-to-Tissue Ratio in the Freshwater Limpet *Septaria lineata* (Archaeogastropoda: Neritidae)

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**Abstract:** The distribution, secondary production, and calcium:tissue (Ca:tissue) ratio in relation to limno-chemical factors were studied in a freshwater limpet (*Septaria lineata*) population from the west bank of the River Hooghly, West Bengal, India, for 2 successive years. Total hardness, chloride, pH, and phosphate were shown to be significant in Pearson's correlations in influencing the abundance of the limpet population. Annual production for the 2 successive years was 10.3 g $m^{-2}$ and 9.5 g $m^{-2}$, respectively. The annual turnover ratios, the ratios of annual production (P) and annual standing crop (B), 'P/B', were calculated to be 2.06 and 1.67, while annual turnover times were 177 days and 219 days, respectively. The ratio of whole animal shell calcium content to tissue dry weight (shell Ca:tissue ratio) changed with age and size class, but these changes were largely restricted to early growth and did not significantly affect individuals over a particular size class (shell height 40-49 mm). Total hardness and chloride were the most important limnochemical factors influencing the shell Ca:tissue ratio.

**Key Words:** *Septaria*, freshwater limpet, secondary production, population density, Ganga

## Introduction

The diversity of freshwater molluscs is vast and their distributions depend on their abilities to colonise a habitat and survive there. Survival, in turn, is regulated by various physico-chemical factors that ultimately play a major role in determining the ecological traits associated with a particular species. Various detailed qualitative surveys since the 1930s have shown that hardness, pH, altitude, size of water bodies, temperature, vegetation, and pollution were among the significant aspects influencing the distribution and abundance of molluscs (Dillon, 2000). Secondary production, the role of environmental ions in tissue growth, and seasonal changes in shell Ca:tissue ratios were studied by Hunter and Lull (1977), Dussart (1976, 1979), Eleutheriadis and Lazaridou-Dimitriadou (1996, 2001), and Kobayashi and Wada (2004). Important ecological works on freshwater limpet were by Hunter (1953, 1961). Notable works on molluscan ecology from the Indian subcontinent are by Murti and Rao (1978), Khan and Chaudhuri (1984), Raut (1989), Subba Rao (1993), Jahan et al. (2001), and Aditya and Raut (2002). The present work is the first attempt to study the ecology of a rare freshwater limpet, *Septaria lineata* (Subba Rao, 1993), available only as a few metapopulation patches in Indian lotic situations. Seasonal changes in population density in relation to different physico-chemical factors, secondary production, and shell calcium-to-tissue ratios were studied.

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## Materials and Methods

Samples were manually collected from the west bank of the River Ganga at Chinsurah (lat. 22°53'N; long. 88°27'E), West Bengal, India. The area was nearly 190 km up-stream from the river-mouth (Sand Head in the Bay of Bengal) and was under the influence of daily tidal cycles. Water samples were collected for chemical analyses once a month between January 1997 and December 1998. Recordings made at peak high tide and peak low tide were averaged to represent the concerned month. Population density was recorded once a week over the same period and the average population density per unit area per month was obtained. At each sampling time ten 1-$m^2$ quadrats were studied to estimate population density. For laboratory studies the necessary numbers of live specimens (at least 6 individuals from each size class) were collected, narcotised (in menthol), and preserved (in 70% ethyl alcohol). Usually the animals were studied in situ during their exposure at low tide. Size classes were recorded by measuring shell details with the aid of an electronic digital slide calliper (Digimac Japan). Representatives of the individual size classes were oven dried (at 60 °C for 30 h) and weighed using a Mettler AE 240 monopan electronic balance. Individuals were subsequently treated with 5 N HCl for 15 min to digest the shell and the tissues were further oven dried (at 60 °C for 30 h) and weighed again (Eleutheriadis and Lazaridou-Dimitriadou, 1995a). Secondary productivity and shell Ca to tissue ratios were measured following the procedures detailed by Hunter and Lull (1977) and Eleutheriadis and Lazaridou- Dimitriadou (2001). All units for Ca:tissue ratio are mg of Ca per mg of tissue dry weight (mg Ca $mg^{-1}$ dw). Taylor's (1961) power law was employed to comment on the spatial distribution of the limpet. The parameter *b* from Taylor's equation $s^2 = a X^b$ (where *a* and *b* are constants, $s^2$ = variance and X = mean) was used as an index of dispersion (Eleutheriadis and Lazaridou-Dimitriadou, 1995b). Annual secondary limpet production in 1997 and 1998 was calculated using the size frequency method described by Krueger and Martin (1980) with modifications and formulae suggested by Eleutheriadis and Lazaridou-Dimitriadou (2001). Collected water samples were analysed potentiometrically by Mettler Checkmate 90 Toledo (pH, and DO) and titrimetrically on the spot using E. Merck, Germany, Field Testing Aquamerck reagent Kits ($NO_3^{1-}$, $PO_4^{3-}$, $Cl^{1-}$, Total hardness). Principal component analysis (PCA), which is mathematically defined as an orthogonal linear transformation that transforms the data to a new coordinate system such that the greatest variance by any projection of the data comes to lie on the first coordinate (called the first principal component), the second greatest variance on the second coordinate, and so on, was performed on the limnochemical data applying the Varimax rotation with Kaiser normalisation. Statistical analyses were performed using the computer software *Statistica* for Windows, Version 5.1A, Statsoft Inc., 1996.

## Results

### Physico-Chemical Factors and Population Densities

Table 1 depicts the seasonal fluctuations in physico-chemical factors and population densities of *Septaria lineata* for 2 consecutive years. Seasonal pulses in pH, total hardness, phosphate, and chloride contents were recorded; variations in these parameters were 28%, 80%, 80%, and 85%, respectively. A maximum total hardness of 366.7 mg $l^{-1}$ $CaCO_3$ was recorded in March 1997 and 341.8 mg $l^{-1}$ $CaCO_3$ in March 1998. Minimum values of 76.5 and 97.9 mg $l^{-1}$ $CaCO_3$ were recorded in September 1997 and 1998, respectively. Chlorides were minimum in late monsoon and maximum in winter. Higher pH, nitrate, and phosphate values were recorded in winter and lower values in summer. Population size was fairly large during monsoon and post-monsoon months; minimum densities were observed in spring and early summer. Maximum densities of 14.4 $m^{-2}$ were recorded in October 1997 and 23.8 $m^{-2}$ in August 1998, respectively, while minimum densities of 3.0 $m^{-2}$ were recorded in May 1997 and 2.4 $m^{-2}$ in February 1998. Annual mean density was 9.29 ± 7.08 (mean ± SD) number $m^{-2}$. The spatial distribution was contagious, because parameter *b* of Taylor's power law ($s^2 = 1.15 X^{1.69}$) was equal to 1.69. Population density (n = 24) was negatively correlated with total hardness (r = -0.78; $P < 0.05$), chloride content (r = -0.79; $P < 0.05$), pH (r = -0.50; $P < 0.05$) and phosphate (r = -0.46; $P < 0.05$). Seasonal fluctuations in population density and total hardness and chloride content are shown in Figures 1 and 2. The correlation matrix for density and different physico-chemical factors is given in Table 2. Component-I of PCA appeared to correspond to total hardness (factor score weight 0.31) while dissolved oxygen formed the major constituent of Component-II (factor score weight 0.46). The less important Component-III was principally accounted for by phosphate (factor score weight 0.67).

Table 1. Seasonal fluctuation in hydrological factors, population density of *Septaria lineate*, and shell calcium during the study period.

| Months | Population Density Nos $m^{-2}$ | Dissolved Oxygen mg $l^{-1}$ | Water Temp. ºC | Total hardness mg $l^{-1}$ $CaCO_3$ | Nitrate mg $l^{-1}$ | Phosphate mg $l^{-1}$ | Chloride mg $l^{-1}$ | pH | Shell Calcium g/individual |
|---|---|---|---|---|---|---|---|---|---|
| Jan 97 | 4.40 | 7.8 | 22.3 | 254.5 | 45 | 1.25 | 26.3 | 8.4 | 0.0125 |
| Feb 97 | 4.40 | 5.3 | 27.6 | 235.0 | 40 | 1.25 | 24.3 | 8.6 | 0.0125 |
| Mar 97 | 4.60 | 7.4 | 28.3 | 366.7 | 56 | 0.75 | 35.1 | 8.5 | 0.0125 |
| Apr 97 | 3.20 | 7.5 | 25.6 | 343.5 | 35 | 0.50 | 29.3 | 7.8 | 0.0077 |
| May 97 | 3.00 | 6.2 | 29.8 | 217.2 | 36 | 0.50 | 34.7 | 7.3 | 0.0077 |
| Jun 97 | 7.20 | 6.8 | 24.6 | 242.1 | 46 | 0.25 | 12.3 | 6.5 | 0.0690 |
| Jul 97 | 14.2 | 6.2 | 23.1 | 99.7 | 35 | 0.50 | 8.60 | 6.7 | 0.1172 |
| Aug 97 | 12.4 | 6.0 | 24.6 | 121.0 | 30 | 0.25 | 5.20 | 6.6 | 0.0988 |
| Sep 97 | 27.0 | 8.0 | 27.3 | 76.5 | 65 | 0.25 | 6.20 | 6.7 | 0.1472 |
| Oct 97 | 14.4 | 8.2 | 26.0 | 192.2 | 55 | 0.50 | 12.8 | 6.8 | 0.1172 |
| Nov 97 | 8.20 | 7.9 | 22.1 | 227.8 | 68 | 0.50 | 16.6 | 6.8 | 0.0690 |
| Dec 97 | 6.80 | 7.3 | 22.3 | 331.1 | 70 | 0.50 | 18.9 | 6.5 | 0.0563 |
| Jan 98 | 4.20 | 6.3 | 26.2 | 218.9 | 57 | 1.00 | 25.2 | 8.8 | 0.0125 |
| Feb 98 | 2.40 | 5.3 | 27.6 | 224.3 | 38 | 1.20 | 20.2 | 8.5 | 0.0077 |
| Mar 98 | 2.60 | 7.4 | 28.6 | 341.8 | 54 | 0.50 | 31.0 | 8.5 | 0.0077 |
| Apr 98 | 2.60 | 5.4 | 24.4 | 220.7 | 35 | 0.50 | 25.2 | 7.5 | 0.0077 |
| May 98 | 3.40 | 5.6 | 29.3 | 218.9 | 38 | 0.50 | 34.2 | 7.0 | 0.0077 |
| Jun 98 | 4.80 | 6.3 | 29.8 | 210.0 | 40 | 0.25 | 13.6 | 6.4 | 0.0125 |
| Jul 98 | 16.8 | 5.5 | 25.6 | 129.9 | 35 | 0.50 | 9.30 | 6.8 | 0.1096 |
| Aug 98 | 23.8 | 5.2 | 28.6 | 110.4 | 60 | 0.50 | 6.10 | 7.2 | 0.2276 |
| Sep 98 | 21.1 | 6.3 | 29.0 | 97.9 | 66 | 0.30 | 6.10 | 6.7 | 0.1442 |
| Oct 98 | 12.6 | 5.8 | 30.6 | 108.6 | 75 | 0.25 | 6.20 | 6.4 | 0.1084 |
| Nov 98 | 13.6 | 7.4 | 22.6 | 126.4 | 70 | 0.50 | 14.8 | 6.8 | 0.0989 |
| Dec 98 | 5.20 | 8.0 | 23.1 | 226.1 | 105 | 0.75 | 16.5 | 6.7 | 0.0125 |
| Mean | 9.29 | 6.63 | 26.21 | 205.9 | 52.25 | 0.57 | 18.28 | 7.27 | 0.0619 |


Figure 1. Seasonal fluctuations in population density of *Septaria lineata* and total hardness.

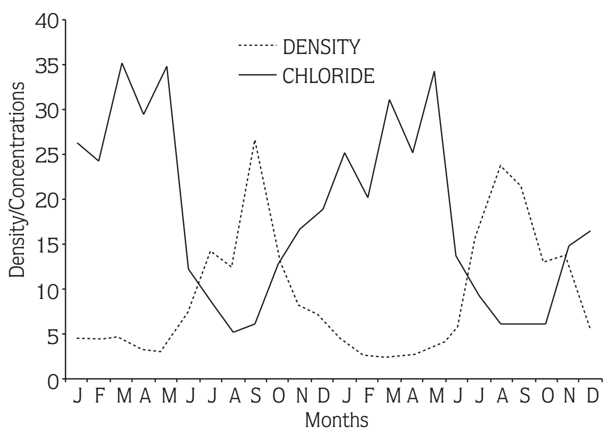


Figure 2. Seasonal fluctuations in the population density of *Septaria lineata* and chloride content of the water.

Table 2. Correlation matrix of the different hydrological factors, population density, and shell calcium.

| Variables | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Density | Dissolved Oxygen | Temp. | Carbonate Hardness | Nitrate | Phosphate | Chlorides | pH |
| Dissolved Oxygen | 0.02 | | | | | | | |
| Temp. | 0.06 | -0.44* | | | | | | |
| Carbonate Hardness | -0.78* | 0.32 | -0.09 | | | | | |
| Nitrate | 0.24 | 0.50* | -0.16 | -0.06 | | | | |
| Phosphate | -0.46* | -0.08 | -0.16 | 0.35 | -0.07 | | | |
| Chlorides | -0.79* | 0.08 | 0.12 | 0.78* | -0.24 | 0.47* | | |
| pH | -0.50* | -0.09 | 0.13 | 0.53* | -0.25 | 0.80* | 0.67* | |
| Shell Calcium | 0.93* | -0.07 | -0.01 | -0.73* | 0.20 | -0.47* | -0.81* | -0.53* |

* Marked correlations are significant at $P < 0.05$; N = 24.

## Secondary Production and Calcium to Tissue Ratio

Table 3 depicts different parameters for the calculation of annual secondary production of *Septaria lineata* using the size frequency method. Mean biomass of the size classes is expressed as dry weight. With the suitable modifications of Benke (1979), the values of annual standing crop (B) were calculated as 5.01 g $m^{-2}$ for 1997 and 5.70 g $m^{-2}$ for 1998, while the annual production (P) values for those years were 10.33 and 9.50 g $m^{-2}$, respectively. The annual turnover ratios (P/B) for 1997 and 1998 were 2.06 and 1.67, respectively. Turnover times were 177 days and 219 days, again respectively. The correlation matrix (Table 2) identifies the environmental factors that might have influenced shell calcium values. Seasonal fluctuations in shell calcium along with water phosphate, hardness, and chloride are depicted in Figures 3-5, respectively. The maximum Ca:tissue ratio was 2.09 (mean dry weight 27.0 mg) in 1997 and 2.98 (mean dry weight 36.4 mg) in 1998 for the 40-49 mm size class (Table 4).

Table 3. Calculation of secondary production of *Septaria lineata* by size-frequency method. Annual production based on sets of samples from 2 successive years, 1997 and 1998.

| Year 1997 | Height Class (cm) | $n_j$ $m^{-2}$ | $n_j - n_{j+1}$ | $W_j$ (g) | $G_j$ in mg $(W_j W_j+1)^{0.5}$ | B in g $m^{-2}$ $(n_j W_j)$ | P in mg $m^{-2}$ $(n_j - n_{j+1}) G_j$ |
|---|---|---|---|---|---|---|---|
| 1 | 0.02-0.29 | 1.00 | -1.00 | 0.0312 | 39.34 | 0.0312 | -39.34 |
| 2 | 0.30-0.39 | 2.00 | -0.09 | 0.0496 | 91.23 | 0.0992 | -8.21 |
| 3 | 0.40-0.49 | 2.09 | -1.92 | 0.1678 | 190.12 | 0.3507 | -365.03 |
| 4 | 0.50-0.59 | 4.01 | -0.08 | 0.2154 | 269.54 | 0.8638 | -21.56 |
| 5 | 0.60-0.69 | 4.09 | 1.08 | 0.3373 | 374.81 | 1.3796 | 404.79 |
| 6 | 0.70-0.79 | 3.01 | 1.01 | 0.4165 | 464.35 | 1.2537 | 468.99 |
| 7 | 0.80-0.90 | 2.00 | 2.00 | 0.5177 | 517.70 | 1.0354 | 1035.40 |
| Total | | 18.20 | | | | 5.0136 | 1475.04 |
| Year1998 | | | | | | | |
| 1 | 0.30-0.39 | 1.00 | -0.01 | 0.0777 | 154.55 | 0.0777 | -1.55 |
| 2 | 0.40-0.49 | 1.01 | -3.04 | 0.3074 | 310.61 | 0.3105 | -944.25 |
| 3 | 0.50-0.59 | 4.05 | 0.05 | 0.3139 | 364.77 | 1.2713 | 18.24 |
| 4 | 0.60-0.69 | 4.00 | 0.95 | 0.4240 | 469.12 | 1.6960 | 445.66 |
| 5 | 0.70-0.79 | 3.05 | 2.05 | 0.5191 | 632.35 | 1.5833 | 1296.32 |
| 6 | 0.80-0.90 | 1.00 | 1.00 | 0.7703 | 770.30 | 0.7703 | 770.30 |
| Total | | 14.11 | | | | 5.7091 | 1584.72 |

$n_j$ = number of snails at the size class j in number; $W_j$ = mean individual dry body weight + mean dry shell of organic mass (in mg); $G_j$ = geometric mean of weight of pairs of successive size classes; B = mean standing crop or population biomass in mg; P = annual production in mg; P/B = annual turnover ratio; a = number of size classes; CPI = Cohort Production Interval). P = a* P'*365/CPI = 10.32 g $m^{-2}$ and 9.50 g $m^{-2}$ (for 1997 and 1998 respectively), P/B = 2.06 and 1.67 (for 1997 and 1998 respectively), Turnover time = 177 days and 219 days (for 1997 and 1998 respectively).

Table 4. Calculation of calcium-to-tissue ratio as mg calcium per mg tissue dry weight.

| Year 1997 | Density Nosm$^{-2}$ | Height Class (cm) | H (cm) | A.W.W. (g) | $W_1$ (g) | $W_2$ (g) | $W_3$ (g) | T.C.C. (g) | Ca:Tissue (g/g) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 0.20-0.29 | 0.212 | 0.079 | 0.031 | 0.012 | 0.019 | 0.008 | 0.640 |
| 2 | 2 | 0.30-0.39 | 0.308 | 0.091 | 0.050 | 0.018 | 0.031 | 0.012 | 0.678 |
| 3 | 2 | 0.40-0.49 | 0.404 | 0.370 | 0.168 | 0.027 | 0.141 | 0.056 | 2.086 |
| 4 | 4 | 0.50-0.59 | 0.504 | 0.399 | 0.215 | 0.043 | 0.172 | 0.069 | 1.603 |
| 5 | 4 | 0.60-0.69 | 0.602 | 0.496 | 0.337 | 0.090 | 0.247 | 0.099 | 1.092 |
| 6 | 3 | 0.70-0.79 | 0.718 | 0.723 | 0.416 | 0.124 | 0.293 | 0.117 | 0.948 |
| 7 | 2 | 0.80-0.90 | 0.810 | 0.911 | 0.518 | 0.151 | 0.368 | 0.147 | 0.982 |
| Total | | | | | | | | | 1.147 |
| Year 1997 | Density Nosm$^{-2}$ | Height Class (cm) | H (cm) | A.W.W. (g) | $W_1$ (g) | $W_2$ (g) | $W_3$ (g) | T.C.C. (g) | Ca:Tissue (g/g) |
| 1 | 1 | 0.3-0.39 | 0.356 | 0.101 | 0.077 | 0.009 | 0.068 | 0.027 | 2.390 |
| 2 | 1 | 0.4-0.49 | 0.459 | 0.492 | 0.307 | 0.036 | 0.271 | 0.108 | 2.978 |
| 3 | 4 | 0.5-0.59 | 0.557 | 0.510 | 0.314 | 0.066 | 0.247 | 0.099 | 1.488 |
| 4 | 4 | 0.6-0.69 | 0.630 | 0.719 | 0.424 | 0.150 | 0.274 | 0.110 | 0.731 |
| 5 | 3 | 0.7-0.79 | 0.720 | 0.959 | 0.519 | 0.159 | 0.360 | 0.144 | 0.909 |
| 6 | 1 | 0.8-0.90 | 0.801 | 1.207 | 0.770 | 0.201 | 0.569 | 0.227 | 1.130 |
| Total | | | | | | | | | 1.662 |

H = average height (cm); A.W.W. = average wet weight (g); $W_1$ = soft organic part weight + shell dry weight (g); $W_2$ = soft organic part weight (g); $W_3$ = calcium carbonate weight (g); T.C.C. = total calcium content ($W_3$ * 0.04) (g).

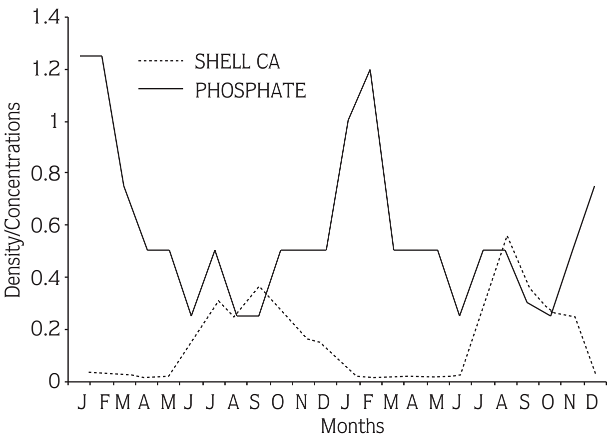


Figure 3. Seasonal fluctuations in the shell calcium of Septaria lineata and phosphate content of water.

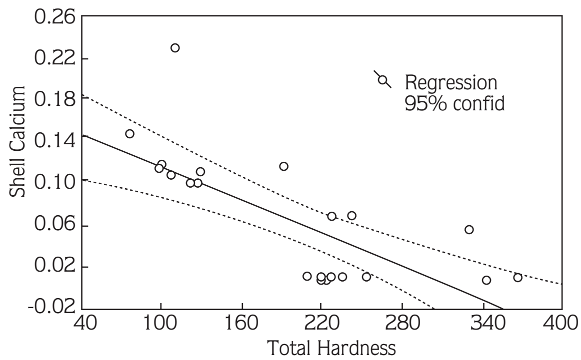


Figure 4. Regression plotto show there relationship between shell calcium and total hardness of the water (Shell calcium = 0.16816-0.0005 *hardness; Correlation: $r = -0.7316$; $P < 0.05$).

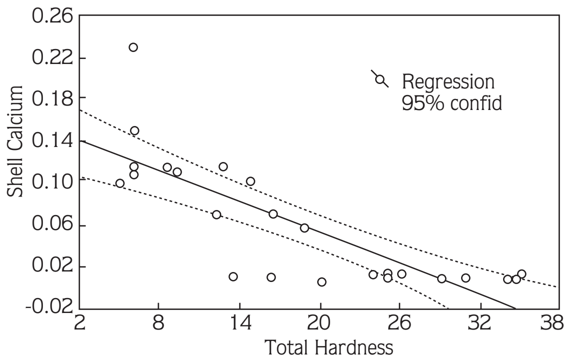


Figure 5. Regression plotto show there relation between shell calcium andchloride content of the water (Chlorides = 26.454 - 1350 *Shell calcium; Correlation: r = -0.8074; P < 0.05).

## Discussion

The importance of water chemistry in relation to the distribution and abundance of freshwater gastropods has often been emphasised in the literature (Yipp, 1990). However, relatively scanty information is available regarding seasonal changes in population density in relation to limno-chemical factors. The results show that physico-chemical variables such as pH, total hardness, chloride, and phosphate have statistically significant negative correlations with population density over the seasonal scale. Hardness and pH are important factors that both directly and indirectly influence metabolic activities and thereby the growth and abundance of freshwater molluscs (Eleutheriadis and Lazaridou-Dimitriadou, 1995b). Low pH values (<6.0) were reported to be unfavourable for mollusc growth (Dussart, 1976).

In the present study, however, maximum population densities were observed over the pH range of 6.5 to 7.2. Higher population densities were recorded during monsoon and post-monsoon months when the ionic concentrations of the water were much lower than those in winter and summer. Dillon (2000) summarised the possible causes for this, such as rainfall, producing dilution or changing the volume of influents that could influence ionic fluctuations. It should also be pointed out that the present study location was under the influence of a daily cycle of tidal flow and thereby the volume of influents beside the effects of dilution during heavy monsoon precipitation could well change the physico-chemical conditions of the lotic water both over diel and seasonal scales. It appears from the present study that pH, total hardness, phosphate, and chloride contents exhibited an almost identical seasonal pulse, and low concentrations of these factors were noted when limpet population densities were maximal. Therefore, such low concentrations could never be unfavourable for limpet growth and development. Although hard water is considered better than soft for molluscan development, it is also emphasised that it would be difficult to dissociate the effects of limno-chemistry from factors such as lithology, geography, and other physical water body characteristics (Dussart, 1976).

According to Taylor's law, the spatial distribution of the limpet was contagious. Freshwater snails aggregate on filamentous algae and diatom clusters (Eleutheriadis and Lazaridou-Dimitriadou, 1995b) and a similar algal dependence by *S. lineata* might result in their contagious distribution. The comparison of annual limpet production was undertaken using turnover time. The turnover time for *S. lineata* was short (177 days) for the first year, reflecting a high level of productivity. However, in the second year it was longer (219 days), with comparatively low levels of productivity. Much higher turnover times have been reported for some freshwater prosobranchs: *Viviparus georgianus* – 399 to 510 days (Browne 1978); *Leptoxis carinata* – 303 to 372 days (Aldridge, 1982); *Bithynia tentaculata* – 314 to 337 days (Russell-Hunter and Buckley, 1983). Lack of competition for food and space could account for the short turnover time and high productivity of *S. lineata.*

Calcium salts in both food and water are important for growth of the molluscan shell, which is largely controlled by genetic makeup (Russell-Hunter and Eversole, 1976). Calcium once added to the shell cannot be resorbed whereas the dry weight of contained tissue can fluctuate in response to seasonal changes in nutritional support, physiological stress, and rhythms in metabolic functions (Hunter and Lull, 1977). Shell calcium-to-tissue ratio, therefore, is indicative of pulses in the metabolic activities of molluscs (Hunter and Lull, 1977). The hardness and pH of water, which were thought to be important in influencing shell Ca, did not show any direct influence on the total shell Ca of *S.*

*lineate* in the present study. Changes in Ca content with the size class were evident in the present study and such changes were more pronounced during early growth and did not affect individuals over a particular size class (shell height 40-49 mm). Similar observations were made by Hunter and Lull (1977) while studying 3 species of freshwater pulmonates. The present work indicates that more detailed research is required of the physiology, growth, and secondary production of this freshwater limpet thriving in isolated patches at low population densities.

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