Heavy Metal Concentrations and Year-Class Structure of a Venus Clam *Meretrix lusoria*

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The study was carried out to demonstrate the relationship between metal concentrations in soft bodies at the pre-spawning stage and year-class structure determined by shell length. The specimens for the determination of metal concentrations were divided into groups at the interval of 2 mm in shell length, and 26 analytical groups were formed. The concentrations of Fe, Zn, Cu, and Mn in soft bodies were determined by atomic absorption spectrophotometry.

In the sample population collected in May, 1984, the seven groups, by shell length, were equivalent to the seven normal distributions, respectively. They were converted into the respective year-classes on the basis of analyses for shell lengths and conch weights of the consecutive collections during 1984 and 1985. The life-span of a venus clam was estimated to be more than nine years for the population.

Using a double logarithmic diagram, metal concentrations against dry soft body weights regressed to linear functions for Fe, Cu and Mn; no critical points are on the slopes. On the other hand, the two critical points for Zn appeared in the ranges of 32–24 and 54–56 mm shell length. The former was equivalent to the range of μ−σ of the second year-class. The latter was situated in the range between μ−σ and μ of the fifth year-class. The regressive patterns for Fe, Cu and Mn in the venus clam differed greatly from those in the wedge clam, but the patterns for zinc in the former was partly similar to that in the latter by having critical points on the slopes.

Studies relating metal concentrations (or contents) to sizes (or weights) of molluscan soft bodies have been developed in the fundamental fields which contribute to monitoring biologically heavy metal pollutions in sea environments. However, growth patterns of specimens have not been taken into consideration except in a few instances for the oysters, the wedge clams, etc. In order to make clear relationships between metal concentrations and individual sizes, it is necessary to clarify the growth patterns of the specimens.

There are several articles1−7 which relate metal concentrations to sizes (or weights) or ages of mollusk species. However, only one instance showing that concentrations of iron, zinc, copper and manganese were related to year-class structure of the natural population of a wedge clam was reported by Ikuta and Nakamura.8 They demonstrated that critical points on slopes of regression lines for metal concentrations or contents against dry soft body weights were divided into the two groups: (1) a critical point on slope for iron, zinc and manganese, and (2) two critical points for copper. They pointed out that critical points of slopes were related to physiological activities for reproduction. In almost all of other papers1−5) relevant to the dependency of metal concentrations or contents on size (or weight), sizes or weights of specimens have been used without regard to their year-class structures.

In this study, venus clams *Meretrix lusoria* which have much longer life-span than wedge clams *Latona cuneata,9*) were used. Concentrations and contents of iron, zinc, copper and manganese in dry soft body weights are discussed in relation to year-class structure correlated with shell length.

Materials and Methods

Environmental and Ecological Aspects of a Venus Clam

Venus clams inhabit tidal flats and sub-tidal zones of the Japanese Islands, Bohai, Korean Bay and Yellow Sea of Korean Peninsula and Chinese Continent, and are found particularly in tidal flats extending from river mouths to foreshores and on shallow water bottoms at 20 meters depth or less.10) They grow in the warm season from spring to autumn, and their growths are suppressed

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in the winter season by low temperature. In the western area of Seto Inland Sea, spawning begins in late June at ca. 23°C and ceases in late August at 26–28°C; that is the maximum temperature in the year. Small and young individuals inhabit the tidal flat near a river mouth, while large and older individuals live in a tidal flat of the foreshore. Young individuals migrate from river mouth to tidal flat in foreshore by drifting on the ebbs of spring tides during the season of warm water temperature. Subsequently, they live and grow near water routes left on the foreshore flat during low water. The segregated inhabitation and migration were observed during the field survey.

Sampling Site and Time, and Collecting Tool

In Japan fishery grounds for venus clams have greatly been reduced by reclaiming land from shallow waters. Wama tidal flat which is left as one of the designated fishery grounds, located in the south-west end of Suou Sound, the western area of Seto Inland Sea, and extends to the left side off the combined mouth of Katsura and Yorimo Rivers which flow towards the north within Bungo-Takada, Ohita Prefecture. The flat is regularly immersed and exposed according to cycles of tide and is composed of a sand bed. The preserves for shellfishes have legally been enacted in a specific area of the flat to protect the venus clam resource. They were selected for the sampling site to ensure a large number of specimens deemed necessary for analyses of year-class structure and metal content. This sampling programme was legally guaranteed with the special permission of Ohita Prefectural Government. Samplings were conducted during 5 days from May 12 to 16, 1984, with “Joren” in Japanese, having facilities of both sieve and trawl. The size of specimens was restricted to over ca. 5 mm in shell breadth by the size of the sieve-like mesh. This tool has been used by fishermen in these areas for commercial collections of venus and little-neck (Ruditapes philippinarum) clams.

Pre-Treatment of Specimen, Separation of Unit Normal Distribution and Constitution of Analytical Lot for Metal Determination

Specimens collected were immersed in an aquarium of running sea water for about 24 hours, during which sand grains entangled in mantle cavities of specimens were discarded through water current for respiration. Subsequently, ligaments of specimens were cut off, with a knife, to let a solution of formalin diluted 10% with sea water to penetrate into soft bodies.

Prior to constituting analytical groups for the detection of elements, shell lengths of all specimens were measured by a calliper to separate year-class groups. Unit normal distributions in the sample population were separated on the basis of a normal probability plot using shell lengths of 3139 individuals collected and Harding’s method.63

Specimens of 28 to 68 mm shell length were divided at 2 mm intervals and an analytical lot of 20 individuals within the ranges was established. In the range of 20 to 22 mm, a lot was prepared, consisting of 100 individuals, and in the ranges from 22 to 28 mm, 50 individuals in each lot were aggregated. In the ranges below 20 mm and beyond 68 mm, 100 and 20 individuals, respectively, were pooled in each lot. According to these treatments of specimens, 26 lots for element analysis were finally prepared.

Metal Determination, Unit Indication and Data Analysis

Soft bodies of specimens were removed from shell valves and weighed and dried at 105°C until constant weights in an electric oven. Dry soft bodies in each lot were ground and homogenized, and about 4 gram’s of tissue were wet-digested with a mixture of nitric and perchloric acids. Iron, zinc, copper and manganese in soft bodies were analyzed by atomic absorption spectrophotometry.

The two units for metals in soft bodies were used: (1) content was indicated as absolute amount (μg on a dry weight basis) in an individual; and (2) concentration (ppmμ) as μg of metal in a gram of dry soft body. Contents and concentrations of metals against dry soft body weights were plotted on a double logarithmic diagram. Regressive relations were calculated according to Boyden’s method.43

Results and Discussion

Year-Class Constitution of Sample Population, Growth Curve, and Estimation of Life-Span

The sample population was divided into seven normal distributions by Harding’s method63 and

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*1 Personal communication from Mr. Y. Kamijou, Ohita Prefectural Shallow Water Fisheries Experimental Station.
Table 1. Mean shell lengths, standard deviations and composition rates for normal distributions
extracted from the sample population of venus clams

<table>
<thead>
<tr>
<th>Normal Distribution</th>
<th>Shell Length (mm)</th>
<th>Composition Rate (%)</th>
<th>Year-Class &amp; Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu - \sigma )</td>
<td>( \mu )</td>
<td>( \mu + \sigma )</td>
</tr>
<tr>
<td>1st</td>
<td>10.0</td>
<td>14.5</td>
<td>19.0</td>
</tr>
<tr>
<td>2nd</td>
<td>23.0</td>
<td>28.1</td>
<td>33.2</td>
</tr>
<tr>
<td>3rd</td>
<td>33.7</td>
<td>37.6</td>
<td>41.5</td>
</tr>
<tr>
<td>4th</td>
<td>45.0</td>
<td>48.0</td>
<td>51.0</td>
</tr>
<tr>
<td>5th</td>
<td>53.5</td>
<td>56.5</td>
<td>59.5</td>
</tr>
<tr>
<td>6th</td>
<td>60.0</td>
<td>62.5</td>
<td>65.0</td>
</tr>
<tr>
<td>7th</td>
<td>67.6</td>
<td>70.0</td>
<td>72.4</td>
</tr>
</tbody>
</table>

Note: The total number of specimens was 3139 individuals. The figures with superscripted plus mark represent age determined by year-class analyses over 7 times collections during 1984 and 1985. Active spawning duration, August is used as a standard month for age determination.

are listed in Table 1 together with their shell lengths (\( \mu - \sigma \), \( \mu \) (mean shell length) and \( \mu + \sigma \)) and composition rates of individual numbers. It was estimated that the seven normal distributions separated by shell length (SL) were equivalent to respective year-classes in the order determined on the basis of the consecutive samplings during 1984 and 1985. Year-class groups are also indicated in Table 1 with a figure superscripted by a plus symbol (+). Using \( \mu \) values of each year-class, a maximum shell length was estimated by Walford’s plot as follows:

\[
SL_{(n+1)} = 29.80 + 0.60SL_{(n)},
\]

and a maximum size was calculated as 75.17 mm from a solution of simultaneous equations (I) and a standard line, \( Y = X \).

Subsequently, the growth curve by Bertalanffy’s equation was computed out as follows:

\[
SL = 75.17(1 - e^{-0.100(1 - e^{-0.073})})\]

The same calculations as year-class analysis by Harding’s method, Walford’s plot and Bertalanffy’s equation for shell length were conducted for conch weights (CHW). The life span over 9 years was estimated from the corresponding relations between both parameters obtained allometrically and from Gompertz equations obtained using sample populations mixed with small individuals collected at the river mouth flat. The life span of a venus clam was about two or three times longer than that of a wedge clam Latona cuneata.17

Allometry of Dry Soft Body Weight against Shell Length

As shown in Fig. 1, a critical point in the arrangement of dots relating dry soft body weight (DSBW) on \( Y \)-axis against shell length (SL) on \( X \)-axis was visually recognized. Therefore, three regression lines were calculated as shown in Fig. 1: (a) for dots below 42 mm in shell length; (b) for dots beyond 42 mm; and (c) for all dots. Value conversion from shell length to dry soft body weight was made from the equation (b). The converted values were used for correlations to concentrations and contents of metals. Arrow marks are explained in the text in relation to the critical points on the slope of zinc concentration.

Fig. 1. Allometories between dry soft body weights and shell lengths. Three regression lines were calculated as explained in the text, i.e. (a) for dots below 42 mm in shell length; (b) for dots beyond 42 mm; and (c) for all dots. Value conversion from shell length to dry soft body weight was made from the equation (b). The converted values were used for correlations to concentrations and contents of metals. Arrow marks are explained in the text in relation to the critical points on the slope of zinc concentration.
Table 2. Mean concentrations (ppm*) of metals for each year-class and all specimens

<table>
<thead>
<tr>
<th>Year-class &amp; Age</th>
<th>Iron</th>
<th>Zinc</th>
<th>Copper</th>
<th>Manganese</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st (1*)</td>
<td>259</td>
<td>155</td>
<td>10.6</td>
<td>6.47</td>
</tr>
<tr>
<td>2nd (2*)</td>
<td>287±53</td>
<td>88.5±24.9</td>
<td>10.9±0.3</td>
<td>6.38±0.77</td>
</tr>
<tr>
<td>3rd (3*)</td>
<td>250±50</td>
<td>92.7±19.1</td>
<td>12.6±1.2</td>
<td>6.53±0.46</td>
</tr>
<tr>
<td>4th (4*)</td>
<td>191±12</td>
<td>98.0±9.10</td>
<td>15.0±1.4</td>
<td>6.35±0.38</td>
</tr>
<tr>
<td>5th (5*)</td>
<td>280±22</td>
<td>81.2±0.20</td>
<td>15.6±0.7</td>
<td>7.07±0.13</td>
</tr>
<tr>
<td>6th (6*)</td>
<td>252±4.0</td>
<td>86.3±15.2</td>
<td>16.3±0.4</td>
<td>7.29±0.57</td>
</tr>
<tr>
<td>7th (7*)</td>
<td>237</td>
<td>112</td>
<td>15.0</td>
<td>5.98</td>
</tr>
</tbody>
</table>

Total 251±47 97.5±23.8 13.6±2.40 6.62±0.70

Note: Mean concentrations of metals were calculated from data ranged in μ−σ to μ+σ for respective year-classes. Data of the first and seventh year-class groups were presented as only a datum, since data in the range (from μ−σ to μ+σ) were only one, respectively. In the row of “Total” data of all size ranges were used for calculations.

Table 3. Components of regressive relations of metal concentrations against dry soft body weights

<table>
<thead>
<tr>
<th>Metal</th>
<th>Range (SL, mm)</th>
<th>log a</th>
<th>a</th>
<th>b</th>
<th>r</th>
<th>Significance of b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>whole</td>
<td>2.3855</td>
<td>242.9</td>
<td>-0.02</td>
<td>-0.1250</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Zn</td>
<td>&lt;32</td>
<td>1.4915</td>
<td>31.0</td>
<td>-0.51</td>
<td>-0.9004</td>
<td>p&lt;0.0005</td>
</tr>
<tr>
<td></td>
<td>32-54</td>
<td>1.9677</td>
<td>92.8</td>
<td>-0.01</td>
<td>-0.0455</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>54&lt;</td>
<td>1.6470</td>
<td>44.4</td>
<td>1.33</td>
<td>0.7411</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>whole</td>
<td>1.9631</td>
<td>91.9</td>
<td>-0.04</td>
<td>-0.1919</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Cu</td>
<td>whole</td>
<td>1.1692</td>
<td>14.8</td>
<td>0.14</td>
<td>0.8991</td>
<td>p&lt;0.0005</td>
</tr>
<tr>
<td>Mn</td>
<td>whole</td>
<td>0.8302</td>
<td>6.76</td>
<td>0.04</td>
<td>0.4236</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

Note: Components are picked up from equations for metals, log Y = log a + b log X. Values of "r" are correlation coefficient.

Recognized only between the equations of (a) and (b). It was suggested, therefore, that the three equations were suitable for establishing a relationship between two parameters (DSBW and SL). In the range of larger than 42 mm in shell length, it is seen that the increment of dry soft body weight was somewhat suppressed.

Mean Metal Concentrations of Sample Population and Respective Year-Classes

Mean concentrations of metals calculated for sample population were as follows: iron (251 ppm*), zinc (97.5 ppm*), copper (13.6 ppm*), manganese (6.62 ppm*) as seen in Table 2. Coefficients of variation in percentage were 18.7 for iron, 24.4 for zinc, 17.6 for copper, and 10.6% for manganese. Cadmium, chromium, lead and nickel in soft bodies were below their respective detection limits.

Mean concentrations of metals in each year-class were calculated from data arranged from μ−σ to μ+σ for respective year-classes and listed in Table 2. Data of the first and seventh year-class groups were presented with only a single data point, since there was only one value each in the range from μ−σ to μ+σ, respectively.

Regressive Relations of Metal Concentrations and Contents in Soft Bodies against Dry Soft Body Weights

The values of SL’s were converted to those of DSBW’s from the allometry equation (c) given in Fig. 1. When a metal content differed suddenly between plotted dots adjacent to each other, according to increase of dry soft body weight, this was temporarily decided as a critical point. The regressive relations for plotted dots in the range decided temporarily were tested over 95% of significance level. Dividing for dots before and after a temporarily critical point and tests of significant level for regression were repeatedly counted until an appropriate value for significant level could be obtained. Finally, the best fit regressive relation for each metal was decided by the repetition. Critical points finally obtained by these repetitions for contents were directly applied to concentrations, for which regression lines were calculated. Components for these regressive relations are listed in Table 3.

Except zinc (Fig. 3), iron, copper and manganese concentrations and contents regressed to single linear equations on a double logarithmic diagram, as shown in Figs. 2, 4 and 5. Iron and manganese
Fig. 2. Correlations between iron contents (left scale, solid circle) or concentrations (right scale, open circle) and dry soft body weights, and the same scales and symbols are used in Figs. 3-5. In upper area of diagram, sizes of year-classes were drawn horizontally with μ's and three σ's ranges. The regression line for concentration was log Y = log 2.3855 - 0.02 log X.

Fig. 3. Correlations between zinc contents or concentrations and dry soft body weights. Indications for sizes of year-classes are the same as Fig. 2. The regression lines were (1) log Y = log 1.4915 - 0.51 log X for the smaller range than 32 mm in shell length, (2) log Y = log 1.9677 - 0.01 log X for the range from 32 to 54 mm, and (3) log Y = log 1.6470 + 1.33 log X for the larger range than 54 mm.

Fig. 4. Correlations between copper contents or concentrations and dry soft body weights. Indications for sizes of year-classes are the same as Fig. 2. The regression line for concentrations was log Y = log 1.1692 + 0.14 log X.

Fig. 5. Correlations between manganese contents or concentrations and dry soft body weights. Indications for sizes of year-classes are the same as Fig. 2. The regression line for concentrations was log Y = 0.8302 + 0.04 log X.

Concentrations were relatively stable irrespective of weight parameter. Namely, iron concentrations decreased very gradually according to increase of weight (Fig. 2), while manganese concentrations increased very slowly (Fig. 5). Copper concentrations increased depending on weight.
increase with a relatively steep slope for the regression line (Fig. 4).

On the other hand, as shown in Fig. 3, two critical points were in common in the regressive relations for zinc concentrations and contents. The first critical point was located at near \( \mu + \sigma \) of the second year-class that was situated in the range from \( \mu - 2\sigma \) to \( \mu - \sigma \) of the third year-class. This point and range were equivalent to those of 32 mm and from 17 to 32 mm shell length, respectively. The second critical point was located at near \( \mu \) of the fifth year-class and was equivalent to 54 mm in shell length. Before the first point, the slope of the regression line was negative. Between the first and the second points the slope was almost parallel to X axis, with a weakly negative trend. This range was equivalent to that from 32 to 54 mm in shell length. After the second point the slope was strongly positive. This range was equivalent to the one larger than 54 mm in shell length. Variation trends for the three regression lines were nearly equal to those for the three obtained against shell lengths. Moreover, these critical points were almost similar to those (arrows marked in Fig. 1) recognized in the allometry of dry soft body weight against shell length.

Dependence of Metal Concentrations and Contents on Weight or Linear Parameter

Zinc concentrations and contents are indicated by the two critical points and the three regression lines. These critical points and regressive relations may be related to various physiological activities in relation to growth. On the other hand, the other three metals, iron, copper and manganese concentrations and contents regressed to single linear equations on a double logarithmic diagram. Ikuta and Nakamura\(^7\) pointed out that in *Latona cuneata*, the critical points of regression slopes were found and fell into two groups: the slopes and one critical point for iron, zinc and manganese that were situated at the range of \( \mu + \sigma \) of the first year-class; and three slopes and two critical points for copper situated at the range of \( \mu - \sigma \) of the first year-class and around \( \mu \) of the second year-class. They suggested that the critical points appearing in the soft compartment could be related to reproductive activity. In this study, the sample population consisted of individuals beyond about 20 mm in shell length on account of the sampling tools used and the segregation by inhabitation for small and large individuals. If the sample population included individuals smaller than 20 mm in shell length, the regressive relations may have varied from the results obtained here. This was also suggested from the results for the burrowing bivalve, *Screbiculata plana* as reported by Bryan and Uysal.\(^5\)

Utility of a Venus Clam as Biological Indicator for Heavy Metal Pollutions in Coastal Waters

It is recommended from the results of regressive relations of heavy metals in soft bodies against dry soft body weights of venus clams that medium sizes in the population must be used for specimens to biologically monitor sea environments for metal pollutions. This monitoring programme will be applied to the coastal waters of Japanese Islands along the Pacific, Bohai, Korea Bay and Yellow Sea along Korea Peninsula and Chinese Continent where venus clams are inhabiting.

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References

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