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AGING AND CONTAMINANT ANALYSIS OF MUSSEL SHELLS FROM THE SHUBENACADIE RIVER HEADWATER LAKES IN NOVA SCOTIA

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EXECUTIVE SUMMARY

Frechwater mussels (clams) from the Shubenacadie River Headwater Lakes in Nova Scotia are highly contaminated with arsenic (As) and mercury (Hg). Soft tissue concentrations of these elements vary among species and lakes, and are influenced by body weight. Age is also suspected to be a factor, therefore the shells of three species of mussels from two lakes (n=249) were aged. One of the species (Elliptio complanata) could be aged much more accurately than the other two (Anodonta implicata and Alasmidonta undulata). It also grew more slowly and was longer-lived (17 years or more vs. 8-9 years for the other species). E. complanata from the most contaminated site (Powder Mill Beach) were significantly smaller at a given age than those from the less contaminated Lake Thomas, suggesting the possibility of sublethal toxic effects. The influence of age on differences in the bioaccumulation of As and Hg among the species could not be determined, because age estimates for A. implicata and A. undulata were too inaccurate.

E. complanta is recommended for biomonitoring in the Atlantic region. Because it is long-lived, contaminants should bioaccumulate over time to detectable levels. Also, because it can be accurately aged, different year classes can be analyzed separately. This species is widely distributed in central and eastern Canada, and has been used extensively for biomonitoring in Ontario and Quebec. Therefore, there is an existing data base for comparing environmental contamination in the Atlantic region with other regions in Canada.

Mussels accumulate metals in their shells as well as their soft tissues. Shells retain a sequential record of contamination in the annual growth rings, and therefore have potential for mapping changes in environmental contamination over time. Two techniques for analyzing trace metals in shells, the electron microprobe and secondary ion mass spectrometry (SIMS), were evaluated. Both failed to detect measureable concentrations of As, Hg, Pb or Cd due to a lack of sensitivity and/or various interferences. These methods require further development and refinement before they can be used for this application.

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RESUME EXPLICATIF

Les moules d'eau douce provenant des lacs situés à la source de la rivière de Shubenacadie en Nouvelle-Ecosse sont très contaminées par l'arsenic (As) et le mercure (Hg). Les concentrations de ces éléments dans les tissus mous varient selon les espèces et selon les lacs, et sont influencées par le poids corporel. L'âge serait aussi un facteur à considérer; aussi a-t-on calculé l'âge des coquilles de trois espèces de moules provenant de deux lacs (n=249). Pour l'une des espèces (Elliptic complanata) l'âge pouvait être calculé avec plus de précision pour que les deux autres (Anodonta implicata et Alasmidonta undulata). La première a également grandi plus lentement et vécu plus longtemps (17 ans ou plus par rapport à 8-9 ans pour les deux autres). Les moules de l'espèce E. complanata provenant du site le plus contaminé (Powder Mill Beach) étaient bien plus petites à un âge donné que celles prélevées dans le lac Thomas qui était moins contaminé, ce qui suggère la possibilité d'effets toxiques subléthaux. L'influence de l'âge sur les différences dans la bioaccumulation de As et Hg d'une espèce à l'autre n'a pas pu être déterminée, parce que les estimations au sujet de l'âge des moules des espèces A. implicata et A. undulata étaient trop imprécises.

On recommande l'espèce E. complanata comme bio-indicateur dans la région de l'Atlantique. Étant donné sa longévité, les contaminants devraient s'accumuler avec le temps dans les moules jusqu'à des concentrations décelables. De plus, comme il est possible de calculer leur âge avec précision, on peut analyser séparément les différentes classes d'âge. Cette espèce est largement répartie dans le centre et l'est du Canada et a été énormément utilisée comme bio-indicateur en Ontario et au Québec. Par conséquent, il y a une base de données permettant d'établir des comparaisons en ce qui concerne la contamination de l'environnement entre la région de l'Atlantique et les autres régions du pays.

Les moules accumulent des métaux dans leurs coquilles aussi bien que dans leurs tissus mous. Les coquilles gardent inscrites dans leurs ~~cernes annuels~~ anneaux de croissance les contaminations successives qu'elles subissent et peuvent donc révéler les changements qui se produisent avec le temps dans la contamination de l'environnement. On a évalué deux techniques d'analyse des métaux à l'état de traces dans les coquilles : la microsonde électronique et la spectrométrie de masse (ions secondaires). Les deux techniques n'ont pas réussi à déceler des concentrations mesurables de As, Hg, Pb ou Cd par manque ~~ou~~ de sensibilité ou à cause de diverses interférences. Il faut mettre au point davantage ces méthodes et les perfectionner avant de les utiliser à cette fin.

Ce travail a été exécuté en vertu de contrats octroyés par le ministère des Approvisionnements et Services à l'University of Western Ontario et en partie financé par la Direction générale de la qualité des eaux, région de l'Atlantique.

MANAGEMENT PERSPECTIVE

The freshwater mussel, Elliptio complanta is recommended for biomonitoring in the Atlantic region, based on its high bioaccumulation capacity for environmental contaminants, its longevity, the accuracy with which it can be aged, and its apparent sensitivity to toxicants. In earlier work, we found that E. complanata from the Shubenacadie River headwater lakes bioaccumulated high concentrations of arsenic and mercury in their soft tissues. This species has also been shown to bioconcentrate other heavy metals and organochlorine pesticides. In the present study we found this species to live almost two decades - twice as long as two other species with which it coexists in the Shubenacadie watershed. E. complanata is therefore capable of accumulating persistent contaminants over long periods of time to analytically detectable levels. Because it can be accurately aged, which was not the case for the other two species, relationships between age and contaminant uptake can be confidently evaluated. Furthermore, age classes may be analyzed separately in order to identify trends in environmental contamination. E. complanata from Powder Mill Beach, which is highly contaminated with arsenic and mercury, were significantly smaller at a given age than those from the less contaminated Lake Thomas. This suggests the possibility of sublethal toxic effects.

Mussels accumulate contaminants in their shells as well as their soft tissues. Shells retain a sequential record of contamination in their annual growth rings, and therefore have potential for mapping changes over time. Two techniques for analyzing trace metals in growth rings, the electron microprobe and secondary ion mass spectrometry (SIMS), were evaluated. Both failed due to a lack of sensitivity and/or various interferences. With further development and refinement, however, the SIMS technique shows promise.

PERSPECTIVE-GESTION

La moule d'eau douce Elliptio complanata est recommandée comme bio-indicateur dans la région de l'Atlantique du fait de sa forte capacité d'accumulation de contaminants environnementaux, de sa longévité, de la précision avec laquelle on peut déterminer son âge et de sa sensibilité évidente aux substances toxiques. Au cours de travaux antérieurs, nous avons constaté que les tissus mous de moules du genre E. complanata provenant des lacs situés à la source de la rivière de Shubenacadie contenaient des concentrations élevées d'arsenic et de mercure. On a également montré que cette espèce concentrait d'autres métaux lourds et des pesticides organochlorés. Dans la présente étude, nous avons trouvé que cette espèce avait une durée de vie de près de vingt ans - deux fois plus longtemps que deux autres espèces avec lesquelles elle coexiste dans le bassin versant de la rivière de Shubenacadie. E. complanata peut donc accumuler des contaminants persistants pendant de longues périodes à des teneurs analytiquement décelables. Comme il est possible de calculer son âge avec précision, ce qui n'était pas le cas pour les deux autres espèces, les rapports entre l'âge et l'absorption des contaminants peuvent être évalués avec confiance. De plus, les classes d'âges peuvent être analysées séparément en vue d'identifier les tendances au niveau de la contamination environnementale. Les moules de l'espèce E. complanata provenant de Powder Mill Beach, site fortement contaminé par l'arsenic et le mercure, étaient bien plus petites à un âge donné que celles prélevées dans le lac Thomas qui était moins contaminé, ce qui suggère la possibilité d'effets toxiques sublétaux.

Les moules accumulent des métaux dans leurs coquilles aussi bien que dans leurs tissus mous. Les coquilles gardent inscrites dans leurs anneaux de croissance les contaminations successives qu'elles subissent et elles peuvent donc révéler les changements qui se produisent avec le temps. On a évalué deux techniques d'analyse des métaux à l'état de traces dans les coquilles: la microsonde électronique et la spectrométrie de masse d'ions secondaires. Les deux techniques n'ont pas réussi à déceler des concentrations mesurables par manque de sensibilité ou à cause de diverses interférences. Toutefois, avec des perfectionnements la spectrométrie de masse d'ions secondaires est une technique pleine d'avenir.

ABSTRACT

Freshwater mussels from the Shubenacadie River Headwater Lakes in Nova Scotia are highly contaminated with arsenic (As) and mercury (Hg) due to historic gold mining activities in the area. Soft tissue concentrations of these elements vary among lakes, species and size classes, and are suspected to be influenced by age. To evaluate this influence, which is the topic of a later report and to compare longevities and growth rates among species and lakes, the shells of three species of mussels from two lakes were aged. Specimens of *Elliptio complanata* could be aged much more accurately than either *Anodonta implicata* or *Alasmidonta undulata*. They alwo grew more slowly, lived longer, and were significantly smaller at a given age at the most contaminated site, which suggests the possibility of a sublethal toxic response. E. *complanata* is recommended for biomonitoring in the Atlantic region based on its above-described characteristics, as well as its previously demonstrated high bioconcentration capacity for environmental contaminants and its widespread distribution.

Mussels accumulate contaminants in their shells as well as their soft tissues. Shells retain a sequential record of contamination in their annual growth rings, and therefore have potential for mapping changes over time. Two techniques for analyzing trace metals in growth rings, the electron microprobe and secondary ion mass spectrometry, were evaluated. Both failed due to a lack of sensitivity and/or various interferences.

RÉSUMÉ

Les moules d'eau douce provenant des lacs situés à la source de la rivière de Shubenacadie en Nouvelle-Écosse sont très contaminées par l'arsenic (As) et le mercure (Hg) à cause de l'exploitation antérieure de gisements aurifères dans cette région. Les concentrations de ces éléments dans les tissus mous varient selon les lacs, les espèces, la taille des classes et seraient influencées par l'âge. Afin d'évaluer cette influence, ce qui fait l'objet d'un autre rapport, et de comparer la longévité et le taux de croissance des espèces dans différents lacs, on a calculé l'âge des coquilles de trois espèces de moules provenant de deux lacs. Dans le cas de spécimens d'*Elliptio complanata*, l'âge pouvait être calculé avec plus de précision que pour les deux autres (*Anodonta implicata* ou *Alasmidonta undulata*). Les spécimens de la première espèce ont grandi plus lentement et vécu plus longtemps et celles provenant du site le plus contaminé étaient bien plus petites à un âge donné, ce qui suggère la possibilité d'effets toxiques sublétaux. On recommande l'espèce *E. complanata* comme bio-indicateur dans la région de l'Atlantique en s'appuyant sur ses caractères décrits précédemment aussi bien que sur sa grande capacité de concentration de contaminants environnementaux déjà prouvée et de sa vaste répartition.

Les moules accumulent des métaux dans leurs coquilles aussi bien que dans leurs tissus mous. Les coquilles gardent inscrites dans leurs anneaux de croissance les contaminations successives qu'elles subissent et peuvent donc révéler les changements qui se produisent avec le temps. On a évalué deux techniques d'analyse des métaux à l'état de traces dans les coquilles: la microsonde électronique et la spectrométrie de masse d'ions secondaires. Les deux techniques n'ont pas réussi à déceler des concentrations mesurables par manque de sensibilité ou à cause de diverses interférences.

GENERAL INTRODUCTION

Bivalve molluscs have been shown to be excellent organisms for biomonitoring contaminants in aquatic systems. They accumulate and concentrate a wide range of environmentally available pollutants and they are widely distributed both globally and in terms of the habitats they occupy (Green et al, 1985). Marine bivalves have been extensively used to study the occurrence and bioavailability of metals, hydrocarbons and radionuclides in marine environments (Goldberg et al, 1978; Burns and Smith, 1981; Koide et al, 1982). In recent years, the freshwater unionid mussels have received some attention as biomonitors for metals (Smith et al, 1975; Manly and George, 1977; Tessier et al, 1984), chlorinated organic contaminants (Kauss and Hamdy, 1985) and pesticides (Miller et al, 1966; Bedford et al, 1968).

Unionaceans meet many of the criteria for a biomonitoring organism, as summarized by Phillips (1977) and Forester (1980). They accumulate a wide variety of substances without being killed, and are large enough to provide adequate tissue for residue analysis. Because of their low position in the food chain, they provide a direct measure of bioavailability. Finally, they are long-lived. In fact, freshwater mussels probably live the longest among freshwater invertebrates (Imlay, 1982). Their longevity allows for sampling more than one year class, and integrates environmental conditions over long periods of time. A further characteristic of mussels, their amenability to relocation, was considered important by Klumpp and Burdon-Jones (1982).

Resident freshwater mussels from the Shubenacadie River headwater lakes in Nova Scotia were found to have excellent potential as biomonitors for arsenic and mercury (Metcalfe and Mudroch, 1985). Contamination of the lake chain is due to historic gold mining activities in the area. Recent urban and industrial expansion has disturbed the mine tailings and bedrock, and is believed to have aggravated an already existing contamination problem. Mussels may provide a record of past disturbances in the system as well as a means of monitoring the ecological consequences of further development or ameliorative measures.

PART A: AGING OF MUSSEL SHELLS

INTRODUCTION

Metcalfe and Mudroch (1985) found that mussels from the Shubenacadie Lakes were extraordinarily contaminated with arsenic (up to 117 µg/g dry weight) and mercury (up to 9.95 µg/g). Site to site differences were suggested and have since been verified (Metcalfe and Mudroch, unpublished data). Interspecific differences in the bio-accumulation of mercury were apparent, despite the fact that all species are deposit feeders. There was a relationship between the size (dry weight) of the organism and the bioaccumulation of both elements, but particularly mercury. Because weight and age are highly correlated (r = 0.67 - 0.87 for the three resident species), we suspect that age is an important factor influencing the bioaccumulation capacity of mussels.

Mussels can be aged by counting the annual "winter" rings on the surface of their shells which mark the end of each growing season. Because of the occurrence of "pseudoannual" disturbance bands, which could be mistaken for annual rings, age may be verified by preparing thin sections of the shells and counting the internal bands. This technique was used to age mussels which had been collected from two of the most contaminated lakes in the Shubenacadie watershed for analysis of arsenic and mercury residues in their soft tissues.

This report presents the ages of the specimens, and evaluates the accuracy of the aging procedure for each species. The results are used to compare growth rates and longevity among species and between lakes. This information will be used to determine the relationship between age and the accumulation of arsenic and mercury by mussels from the Shubenacadie Lakes.

MATERIALS AND METHODS

Description of Study Area

The Shubenacadie Basin is located north of Halifax, Nova Scotia (Fig. 1). Gold mining, which centred around the village of Waverley at the turn of the century (Trip and Skilton, 1985), is the source of

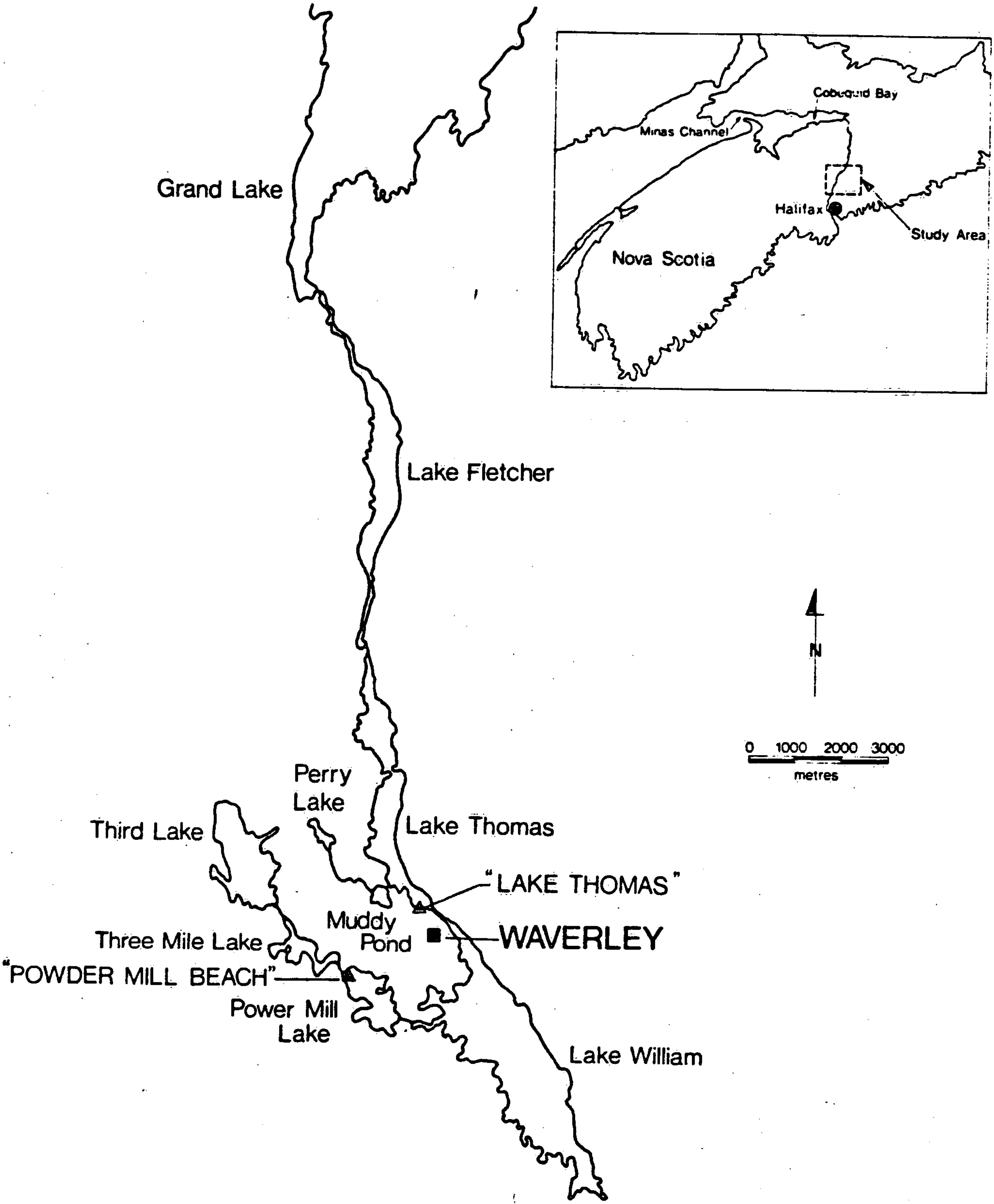


Figure 1. LOCATIONS OF MUSSEL COLLECTION SITES "LAKE THOMAS" AND "POWDER MILL BEACH".

arsenic and mercury contamination in the watershed. The lakes closest to Waverley, especially Powder Mill Lake, Lake Thomas, Muddy Pond and Lake William, are the most contaminated. Large beds of unionid mussels occur in the nearshore areas of the former two lakes at the sites were referred to as "Powder Mill Beach" and "Lake Thomas" (Fig. 1). Relative contamination of the water, suspended sediment and bottom sediment at these two sites are compared in Table I. The data indicate that Powder Mill Beach is more contaminated with both arsenic and mercury. The substrate at Powder Mill Beach was sandy and was easily sampled, whereas at Lake Thomas the substrate was rocky and a representative sediment sample could not be obtained. However, we would expect the sediment at the Lake Thomas site to be less contaminated because this is certainly true for the deposition zone.

Collection of Mussels and Preparation of Shells

Mussels were collected from shallow areas (<1 m depth) at the two sites on July 31, 1984 and July 30, 1985. A total of 249 specimens of the following three species were obtained: *Alasmidonta undulata* (Subf. Anodontinae), *Anodonta implicata* (Subf. Anodontinae) and *Elliptio complanta* (Subf. Ambleminae). Table II presents the numbers of each species collected from each site.

In 1984, mussels were frozen whole on the day they were collected. A week later in the laboratory they were thawed and shucked, and the soft parts were prepared for quantitative determination of arsenic and mercury. In 1985, mussels were shucked

TABLE I. Relative contamination of the Powder Mill Beach and Lake Thomas sites with arsenic and mercury (from Metcalfe and Mudroch, 1985).

Compartment	Powder Mill Beach		Lake Thomas	
	As	Hg	As	Hg
Water (μg/L)[1]	0.2-4.1	ND(<0.02)	2.1-2.6	ND(<0.02)
Suspended Sediment (μg/g)[1]	288-319	2.25-3.30	129-490	.46-.74
Nearshore Bottom Sediment (μg/g; 0-2 cm)	890-3050[2]	5.69[3]	NS	NS
Deposition zone bottom sediment (μg/g; 0-10 cm)	749	20.8	163	10.5

[1]Seasonal ranges
[2]Range of five samples
[3]One sample
NS = not sampled; substrate rocky

TABLE II. Numbers of mussels of each species collected from each site.

Species/Site	Year		Total
	1984	1985	
A. undulata			
Powder Mill Beach	10	20	30
A. implicata			
Powder Mill Beach	20	73	93
Lake Thomas	18	26	44
E. complanata			
Powder Mill Beach	5	10	15
Lake Thomas	13	54	67
			249

fresh on the day of collection. All shells were air-dried and weighed to the nearest milligram. Valve lengths were measured to the nearest 0.05 cm using vernier calipers. The shells were stored in plastic bags until they could be sectioned for aging.

Aging Procedure

For each species at each site, about 12 (10-13) specimens representing the range of size classes present were selected for sectioning. All specimens were taken from the 1985 collection. Following the methods of Clark (1980) and Kennish et al (1980), the right valves of each specimen were coated with epoxy, cut along the axis of maximum growth, mounted on a microscope slide, then further cut to produce a thin section. The internal bands, each of which corresponds to a year's growth, were then counted independently by two "evaluators". External rings were also counted for A. *implicata* only. The reliability of aging methods based on the annular rings of unionids has been well established, as reviewed by McCuaig and Green (1983).

Statistical Analysis

Accuracy of the aging procedure was evaluated by calculating the degree of correlation, as measured by the correlation coefficient "r", between the two independent age estimates for each series of specimens. These independent age estimates were then averaged, and the best regression model predicting valve length as a function of age and site was determined. The "best" model was that in which no significant predictors were omitted but no nonsignificant predictors were included. Site could not be included as a predictor for A. undulata, since all specimens came from Powder Mill Beach. Valve lengths were then used to estimate the ages of the remaining specimens which had not been sectioned. Shell weight was also evaluated as a predictor for age.

RESULTS

Elliptio complanata

The valve lengths, shell weights, and age estimates from internal bands for the 22 specimens which were sectioned are presented in Appendix IA.

Age estimates by the two independent evaluators were found to be highly correlated ($r = .835$), and this relationship was statistically significant ($t = 6.79 > t_{20} = 2.086$; $p \leq .05$). The regression equation was: $y = -0.592 + 1.06\ x_1$, where y = age estimate by evaluator #1 and x_1 = age estimate by evaluator #2. The slope of the line was not significantly different from 1.0 ($t = .38 \ngtr t_{20} = 2.086$), nor was the intercept significantly different from zero ($|t| = .35 \ngtr t_{20} = 2.086$), therefore both evaluators saw the same number of internal bands in a given specimen and the aging procedure can be considered to be very accurate for this species.

The "modelling run" for predicting valve length was initiated with the following model:

$$\hat{y} = a + bx_1 + bx_2 + bx_3 + bx_4.$$

where: $\hat{y}$ = predicted valve length (cm)

a = intercept

b = slope coefficients

x_1 = age (average of two estimates)

x_2 = site (Lake Thomas = 1; Powder Mill Beach = 2)

x_3 = $(age)^2$

x_4 = (age)(site) interaction

The latter two predictors were not significant and the best fitting regression line for predicting valve length for E. complanta was:

$$\hat{y} = 7.9965 + 0.30082x_1 - 1.8096x_2; \qquad r = .88$$

where the effects of both age and site were significant (age: $t = 5.36 > t_{19} = 2.093$; site: $|t| = 4.80 > t_{19} = 2.093$. Thus, mussels from Lake Thomas were larger at a given age than those from Powder Mill Beach. This model was rearranged, as shown below, in order to solve for $\hat{x}$ (estimated age):

$$\hat{x} = y - \frac{7.9965 + 1.8096x_2}{0.30082}$$

The equation was then used to estimate the ages of the remaining 60 specimens which had not been sectioned and for which counts of internal bands had not been made (Appendix IB). The effect of site on mussel size is best illustrated by plotting the regression equations predicting valve length from age for each site individually (Fig. 2). These equations are:

Lake Thomas: $\hat{y} = 6.5435938 + 0.2682652x_1$; $n = 12$; $r = .73$.
Powder Mill Beach: $\hat{y} = 3.8800644 + 0.3534323x_1$; $n = 10$; $r = .85$.

The slopes of these two lines did not differ significantly ($F = .52 < F_{1,18} = 4.41$), therefore the growth rates of mussels five years of age and older do not differ between the sites. Because the intercepts differ, it is apparent that mussels at the Lake Thomas site must grow faster during the early age period (less than five years).

Shell weight was also evaluated as a predictor for age. The best model was:

$$\hat{y} = 17.413 + 2.5059x_1 - 11.711x_2; \quad r = .80.$$

where: $\hat{y}$ = shell weight (g)
x_1 = age (average of two estimates)
x_2 = site (Lake Thomas = 1; Powder Mill Beach = 2).

Length was a better predictor of age ($r = .88$) than weight was ($r = .80$).

Anodonta implicata

The valve lengths, shell weights, age estimates from internal bands, and age estimates from external rings for the 25 specimens which were sectioned are presented in Appendix IIA.

This is the only species for which both internal and external rings were counted. Age estimates by the two independent evaluators were found to be highly correlated for the external counts ($r = .830$), and moderately correlated for the internal counts ($r = .586$). The latter relationship was, nevertheless, statistically significant ($t = 3.47 > t_{23} = 2.069$). Both evaluators saw more external than internal rings. The regressions of external counts (y) on internal counts (x) for each evaluator are as follows:

Evaluator #1: $\hat{y} = 1.4766484 + 0.8770604x$; $r = .71$.

Evaluator #2: $\hat{y} = -0.4575045 + 1.2495479x$; $r = .69$.

The evaluators differed in that #1 saw more external rings in younger specimens (i.e., for $x = 4$, $\hat{y} = 5.0$ and for $x = 10$, $\hat{y} = 10.2$), while #2 saw more external rings in older specimens (i.e., for $x = 4$, $\hat{y} = 4.5$ and for $x = 10$, $\hat{y} = 12.0$).

The model for predicting valve length was based on average internal band counts, despite the fact that there was more agreement between the evaluators in their external ring counts. The "modelling run" was initiated with the same model as for E. <u>complanta</u>. In this case, however, there was no site effect and the best fitting regression line for A. <u>implicata</u> was:

$$\hat{y} \text{ (valve length)} = 1.102 + 1.0710x_1 \text{ (age)}; \quad r = .70.$$

where the effect of age was significant ($t = 4.64 > t_{23} = 2.069$). This model was rearranged, as shown below, in order to solve for $\hat{x}$ (estimated age):

$$\hat{x} = \frac{y - 1.102}{1.0710}$$

The equation was then used to estimate the ages of the remaining 112 specimens which had not been sectioned and for which counts of internal bands had not been made (Appendix IIB). Although site did not influence the length/age relationship, regression equations predicting valve length from age were calculated separately for each site, allowing comparisons with the other two species (Fig. 2). These equations are:

Lake Thomas: $\hat{y} = 2.6571154 + 0.7988462x_1$; $n = 12$; $r = .59$.

Powder Mill Beach: $\hat{y} = -0.8312896 + 1.4211416x_1$; $n = 10$; $r = .81$.

Shell weight was also evaluated as a predictor for age. The best model was:

$\hat{y} = -19.142 + 4.946x_1$; $r = .66$. Where y = shell weight (g) and x_1 = age

Length was a better predictor of age ($r = .70$) than weight was ($r = .66$).

Alasmidonta undulata

The valve lengths, shell weights, and age estimates from internal bands for the 12 specimens which were sectioned are presented in Appendix IIIA.

Age estimates by the two independent evaluators were not strongly correlated ($r = .536$), and in fact the relationship was not statistically significant ($t = 2.01 \ngtr t_{10} = 2.228$). Despite this disagreement, the model for predicting valve length was based on averaged internal band counts. The model could not include site as a predictor because A. *undulata* only occurred at Powder Mill Beach, therefore the "modelling run" excluded the x_2 and x_4 components of the original model (site, and age x site interaction, respectively). The effect of x_3 [$(\text{age})^2$] was not significant, therefore the best fitting regression line was:

$$\hat{y} = 0.8398 + 0.5182x_1; \; r = .80.$$

where the effect of age was significant ($t = 4.24 > t_{10} = 2.228$). This regression line is plotted in Fig. 2 for comparison with the other two species. The model was rearranged, as shown below, in order to solve for x ($\hat{x}$ estimated age):

$$\hat{x} = \frac{y - 0.8398}{0.5182}$$

The equation was then used to estimate the ages of the remaining 18 specimens which had not been sectioned and for which counts of internal bands had not been made (Appendix IIIB).

Shell weight was also evaluated as a predictor for age. The best model was:

$$\hat{y} \text{ (shell weight)} = -5.409 + 1.5627x_1 \text{ (age)}; \; r = .83.$$

This was the only species for which shell weight was a slightly better predictor of age ($r = .83$) than valve length was ($r = .80$).

Interspecific Comparisons

The relationships between valve length and age for each species at each site are compared in Fig. 2. As previously mentioned, *E. complanta* from Lake Thomas were larger at a given age than those from

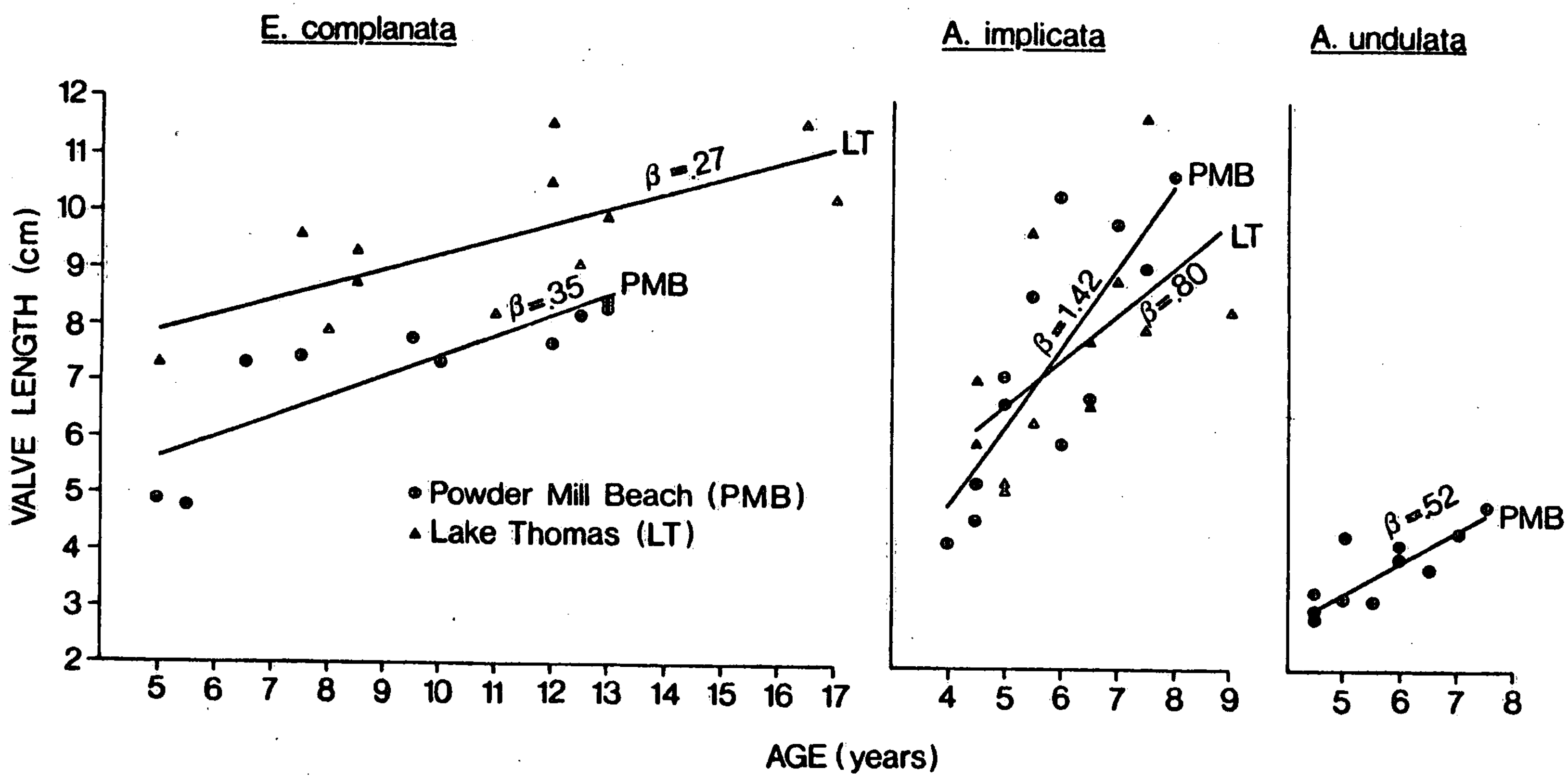


Figure 2. REGRESSION EQUATIONS PREDICTING VALVE LENGTH AS A FUNCTION OF AGE FOR EACH SPECIES OF MUSSEL AT EACH SITE.

Powder Mill Beach. Because the intercepts differ, but the slopes do not, it appears that E. *complanta* grew more rapidly at Lake Thomas than at Powder Mill Beach during the first five years of life. In contrast, there were no significant differences in growth rates of A. *implicata* between the two sites. Although the slopes of the lines appear to differ, this difference is not statistically significant ($F = 1.77 < F_{1,21} = 4.30$). The inaccuracy of our age estimates for this species resulted in a large error term. Therefore, if in fact a difference in growth rates did exist, we would have difficulty detecting it.

A. *implicata* appeared to grow more rapidly than E. *complanta* at both sites (slopes of .80 and 1.42 vs. .27 and .35, respectively), achieving a larger size in a shorter period of time. A. *undulata* was a much smaller organism, with a growth rate at Powder Mill Beach (slope = .52) more similar to E. *complanta* (.35) than A. *implicata* (1.42) from the same site. A. *implicata* and A. *undulata* did not seem to live as long as E. *complanta*. The oldest specimens collected were 17 years for E. *complanta*, 9 years for A. *implicata* and 7-8 years for A. *undulata*.

DISCUSSION

The three species of resident mussels from the Shubenacadie Lakes could be aged with varying degrees of accuracy. Age estimates were based on counts of internal growth bands, and were therefore somewhat subjective. The agreement, or correlation, between counts made by two independent evaluators provided a measure of accuracy. Agreement was very good for E. complanta, but poor for A. implicata and A. undulata. Therefore, we have the most confidence in our age estimates for E. complanta.

It is impractical to section large numbers of shells for internal band counts. Instead, a model may be generated for estimating age from a more easily measured parameter which is a good predictor of age. We found valve length to be the best predictor of age for E. complanta and A. implicata, but shell weight was slightly better for A. undulata. For comparative purposes, valve length was used throughout. For all three species, a linear model for the valve length vs. age relationship was sufficient within the range of the data. This is certainly not true in general, and is due to the limited range of size and age classes sampled. If younger specimens (less than five years) in particular had been included, there may have been a significant curvilinear portion to the model.

Both evaluators counted more external than internal rings in A. implicata, suggesting that disturbance rings were being included in the external counts. This could lead to an overestimate of the true age for this species, therefore external counts should not be relied upon where accurate age estimates are required. In retrospect, internal band/external ring comparisons should have been made for E. complanta instead.

The main reason for aging our specimens was to determine the influence of age on interspecific differences in the bioaccumulation of As and Hg by mussels. Unfortunately, the age estimates for A. implicata and A. undulata were too inaccurate for this purpose.

E. complanata was the best species to work with. Growth rings were the clearest, age estimates were most accurate, and the prediction model had the best fit. As a result, we were able to detect differences in the growth of this species between the two sites. The Powder Mill Beach environment was much more contaminated with As and Hg, and the mussels here were significantly smaller. This suggests the possibility of sublethal toxic effects. We found E. complanta to be a poor regulator of mercury. Soft tissue concentrations increase almost exponentially with increasing body weight and age (Metcalfe and Mudroch, 1985). In contrast, A. implicata of all sizes and ages maintain consistently low concentrations of this metal. It is therefore more likely that body burdens of Hg would accumulate to toxic levels in the tissues of E. complanta.

E. complanta has excellent potential as a biomonitoring species for these and other environmental contaminants in the Atlantic region. Because this mussel is long-lived, any contaminant which is poorly regulated will bioaccumulate over time - probably to detectable levels. Furthermore, a record of input over time can be obtained by analyzing various year classes. This species has been used extensively in environmental monitoring in Ontario and Quebec (Curry, 1977/78; Tessier et al, 1984; Kauss and Hamdy, 1985; A. Hayton, Ontario Ministry of the Environment, personal communication). It occurs in the southern James Bay drainages and the St. Lawrence system (except Lake Huron south of Georgian Bay, Lake Michigan and most of Lake Erie), and the Atlantic drainage (Clarke, 1981). In the U.S.A., it is "... the most widespread of the East Coast mussels occuring in both polluted and unpolluted waters and lotic and lentic habitats from Maine to Florida ..." (Imlay, 1982). A. implicata and A. undulata are more restricted in their distributions, with the former occurring only in the Atlantic coastal drainage from Cape Breton to Maryland, and the latter found in the St. Lawrence River and it tributaries and the Atlantic drainage from Nova Scotia to Florida (Clarke, 1981). Therefore, from ecological, analytical and geographic points of view, E. complanta is the best choice for biomonitoring.

PART B: CONTAMINANT ANALYSIS OF MUSSEL SHELLS

INTRODUCTION

Recent studies suggest that the analysis of bivalve shells may offer several advantages over the analysis of body tissues when monitoring contaminant levels in the environment (Imlay, 1982; Fang and Shen, 1984). Shells can retain a record of contaminant levels over many years, and may provide a more sensitive and more permanent system for monitoring changes in ambient heavy metals (Fang and Shen, 1984). Imlay (1982) noted that for many trace elements the concentration factor is many times greater in the shell than in body tissues. Koide et al (1982) found that metal concentrations in the shells of marine mussels were more strongly correlated than concentrations in the soft tissues with proximity to highly industrialized or populated areas.

While analysis of whole shell is useful, more information could be gained by analyzing the annual growth rings separately. In this way changes in environmental contamination could be "mapped" over long periods of time. The methodology for this type of analysis is not currently available, however. Electron microprobe and secondary ion mass spectrometry (SIMS) appear to have good potential for this application. Thin sections prepared for aging may also be used for studying differences in the levels of trace elements between various years' growth. The electron microprobe and SIMS both measure elemental composition of prepared thin sections.

The electron microprobe is an x-ray instrument used to determine quantitatively the chemical composition of thin sections by means of electron bombardment and measurement of the subsequent x-rays produced. A curved crystal spectrometer is used to determine wavelength and intensity of the x-rays being emitted from the specimen. Comparisons are made of the intensities of x-rays from a material of known composition. Sensitivity of the microprobe is about 200 ppm (B. Barnett, Geology Department, University of Western Ontario, pers. comm.).

SIMS, a method similar to the electron microprobe, utilizes an ion beam which is focused onto the surface of a prepared sample. The secondary ions emitted are separated by mass for elemental discrimination and quantification. SIMS is a very sensitive technique, with a detection limit as low as 1 ppb for some elements.

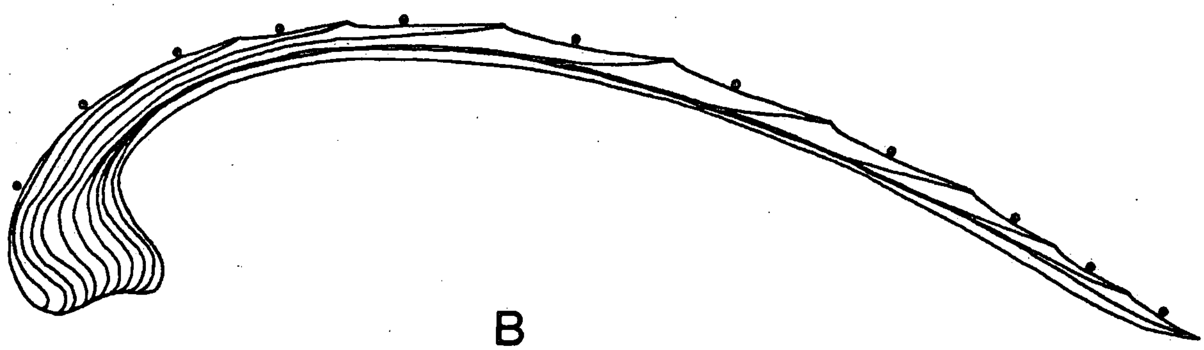
This report evaluates the electron microprobe and SIMS procedures for determining arsenic, mercury and other trace element concentrations in mussel shells from the Shubenacadie Lakes.

MATERIALS AND METHODS

Four mussels were selected for study because of their high soft tissue concentrations of As, Hg and Pb (Table III). Shells were prepared and sectioned in the same manner as for aging, then polished and coated with carbon. Two specimens (LTE43 and PMA61) were chosen for microprobe analysis. Following the methods of Lutz (1981), who used the electron microprobe to measure strontium in *Mytilus edulis* shells, step scan analyses of 10 μm spots were made across the inner nacreous layer and down the surface periostracum as illustrated in Fig. 3. The electron beam was focused onto the thin section, inducing the emission of characteristic x-rays which were then separated in order to discriminate among As, Hg, Pb, Cd, Ca, Sb and Zn.

Specimens PME21 and PMA63 were chosen for the more sensitive SIMS analysis. Step scan analyses of 25 μm spots were made across the nacreous layer and down the periostracum, as described for the

A



B

Figure 3. LOCATION OF SPOTS FOR STEP SCAN ANALYSIS ACROSS THE INNER NACREOUS LAYER (A) AND DOWN THE SURFACE PERIOSTRACUM (B)

TABLE III. Soft tissue concentrations of As, Hg and Pb (ug/g dry weight) in four mussels selected for shell contaminant analysis.

Mussel	Shell Analysis Procedure	Soft Tissue Concentration (ug/g dry weight)		
		As	Hg	Pb
Lake Thomas, E. complanata, 10 years old (LTE43)	Electron microprobe	30.5	4.6	29.0
Powder Mill Beach, A. implicata, 9 years old (PMA61)*	Electron microprobe	45.0	.8	6.5
Powder Mill Beach, E. complanata, 10 years old (PME21)	SIMS	42.0	1.2	NA
Powder Mill Beach, A. implicata, 6 years old (PMA63)*	SIMS	45.0	.8	6.5

NA = not analyzed
* Soft tissues of these specimens were combined for analysis

microprobe method. A survey spectrum was run to measure all elements with masses ranging from 0 to 240 mass units. To examine elements present at low concentrations, extended counting techniques (30s/mass vs. 0.5s/mass) were employed on masses of particular interest (As, Hg, Pb, Cd). The measured ion intensity ratios were converted to concentrations using an ion yield curve determined from a lead silicate glass standard.

RESULTS AND DISCUSSION

Results of the electron microprobe analysis were extremely variable (Table IV), therefore levels of As, Hg, Cd and Zn in the shells could not be quantified. Reliable estimates were not possible due to the instrument's maximum sensitivity of 200 ppm, and its inherent variability at the low concentrations encountered. The electron microprobe is intended for measuring elements in percent concentrations in geological materials. Lutz (1981) reached similar conclusions when he attempted to study seasonal variation in strontium concentration in Mytilus edulis.

Preliminary spectral surveys with SIMS detected only major shell elements in the parts per thousand and percent concentration range (i.e. Ca, C, Sr, Na, Fe, Mn). Subsequent analyses involved counting ion intensity for extended periods on masses of particular interest (i.e. As, Hg, Pb, Cd). Even under these conditions, these elements could not be quantified due to extreme variability (Table V).

TABLE IV. Concentrations of various elements in the shell of mussel LTE43, as determined by the electron microprobe.

Element	Concentration as ug/g dry weight (mean ± s.d.)*
As	190 ± 200
Hg	1100 ± 1600
Pb	ND
Cd	ND
Sb	1600 ± 500
Zn	190 ± 600

* n = 20 spots (14 across the inner nacreous layer and 6 down the surface periostracum).

TABLE V. Concentrations (as ug/g dry weight) of various elements in the shells of mussels PMA63 and PME21, as determined by SIMS.

Element	PMA63	PME21	
		Spot #1	Spot #2
Al	15,800 ± 14,000	2600 ± 1800	7300 ± 3700
V	2000 ± 300	2500 ± 600	3000 ± 1200
Fe	4700 ± 3400	2400 ± 300	6200 ± 800
Cu	90 ± 30	80 ± 60	120 ± 40
As	70 ± 60	ND	30 ± 60
Sr	540 ± 30	570 ± 140	650 ± 200
Pb	90 ± 99	100 ± 170	ND
Cd	ND	ND	ND
Hg	ND	ND	ND

ND = not detectable
* mean ± s.d. for n = 3 readings

A number of modifications could be made to the technique to improve sensitivity. First, a more appropriate calibration standard could be used. Lodding et al (1984) suggested using an apatite crystal when utilizing SIMS in the quantitative analysis of tooth and bone hard tissue. Second, surface charge-up should be controlled by the use of specially adapted metal sample holders or the application of aluminum grids on the sample surfaces (Lefevre, 1980). Finally, Lodding et al (1984) believed that a better understanding of the complicated spectra of hard biological tissues and methods of suppressing intrinisic molecular peaks would greatly aid in the quantitative analysis of hard biological tissues.

Although many authors have suggested that bivalve shells offer several advantages over soft tissues for monitoring pollution events, there remain unsolved problems in shell analysis. Little is known about how contaminants bond within the calcium carbonate matrix. Swann et al (1984) observed changes in Zn levels within the same annual growth increment, and suggested that elemental adsorption onto the shell was occurring. In the same experiment Mg, Al, Si, Sg, Mn and Fe content decreased over time, suggesting that elemental leaching could be occurring. Fang and Shen (1984) also warned that chemicals bound in the old parts of the shell will begin to leach out into the water. They also observed that pollutants tend to accumulate on the outer shell surface where leaching is most likely to occur. Koide et al (1982) believed that microorganisms on the shell surface may account for high surface metal concentrations.

The two techniques, electron microprobe and SIMS, failed to detect quantifiable levels of As, Hg, Pb or Cd in the shells of mussels known to be highly contaminated with As and Hg. The electron microprobe, with its maximum sensitivity of about 200 ppm and high variability near its limits of detection, was not suitable for measuring trace elements at the levels found in these shells. The more sensitive SIMS technique detected only major shell elements. With further method development, including the reduction of interferences and the production of specially prepared standards and appropriately designed sample holders, this time consuming and expensive method may prove useful in the analysis of contaminants within and between annual growth increments. At that time, mussel shells may become invaluable in the study of both past and present pollution events.

SUMMARY

Freshwater mussels from the Shubenacadie River Headwater Lakes in Nova Scotia are highly contaminated with arsenic (As) and mercury (Hg). Soft tissue concentrations of these elements vary among species and lakes, and are influenced by body weight. Age is also suspected to be a factor. To determine the relationship between age and the bioaccumulation of As and Hg, and to compare longevities and growth rates among species and lakes, the shells of three species of mussels from two lakes (n=249) were aged. Fifteen _Elliptio complanata_, _93_

Anodonta implicata and 30 Alasmidonta undulata were collected from the most contaminated site, Powder Mill Beach, while 67 E. complanata and 44 A. implicata were collected from a less contaminated site, Lake Thomas. Ten to 13 representative specimens of each species/site combination were aged directly, that is, shells were thin-sectioned and annual internal growth bands were counted independently by two evaluators. External growth rings were also counted for A. implicata. Ages of the remaining specimens were estimated using a regression model based on valve length as a predictor of age.

E. complanata could be aged with much more accuracy than either A. implicata or A. undulata. Internal growth bands were clearest, agreement between evaluators was closest (r = .835 vs. .586 and .536, respectively), and the regression model had the best fit (r = .88 vs. .70 and .80, respectively). External ring counts overestimated the ages of A. implicata, and should not be used. A. implicata grew more rapidly (.80 - 1.42 cm/yr.) than E. complanata (.27 - .35 cm/yr.), achieving the same maximum valve length (≃11.5 cm) in less time (8.5 - 10.5 vs. 17 - 20 yrs.). A. undulata was smaller, with a growth rate at Powder Mill Beach similar to E. complanata (.52 vs. .35 cm/yr.). E. complanata appeared to live longer (≥17 yrs.) than A. implicata (≥9yrs.) or A. undulata (≥8 yrs.). E. complanata from Powder Mill Beach were significantly smaller at a given age than those from Lake Thomas. Because the growth rates of specimens aged 5-17 yrs. did not differ, E. complanata must have grown more slowly at Powder Mill Beach during the first five years of life. As this site is the most [illegible] and Hg, the possibility of sub[illegible] [illegible]

[illegible] age on interspecific d[illegible] [illegible] and Hg by mussels could no[illegible] because age estimates for both A. implicata and A. undulata were too inaccurate. However, the data for E. complanata suggest that it would be the best choice for biomonitoring purposes in the Atlantic region. Because it is long-lived, contaminants which are [illegible] should accumulate over time to detectable levels. Also, because it can be accurately aged, various year classes can be analyzed separately. This species is widely distributed throughout central and eastern Canada, and has been used extensively for biomonitoring in Ontario and Quebec. Therefore, there is an existing data base for comparing environmental contamination in the Atlantic region with other regions in Canada.

Mussels accumulate metals in their shells as well as their soft tissues. Shells retain a sequential record of contamination in their annual growth rings, and therefore have potential for mapping changes in environmental contamination over time. Several shells were subjected to trace element analysis of their growth rings by two techniques, the electron microprobe and secondary ion mass [illegible] techniques failed to de[illegible] [illegible] Pb or Cd in the shells. [illegible] approximately 200 ppm, was [illegible] SIMS has a theoretical detection limit as low as 1 ppb, but factors such as mass spectral interferences and high counting times may have decreased its sensitivity. This method requires further development and refinement before it can be used for this application.

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APPENDIX IA. Valve lengths, shell weights, and age estimates from internal bands for E. complanata which were sectioned.

Site	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages Evaluator #1	Estimated Ages Evaluator #2
Lake Thomas	1	7.30	13.678	5	5
	3a	7.90	17.173	8	8
	4a	8.20	22.079	14	8
	5a	8.75	23.947	9	8
	6a	9.10	28.367	13	12
	7a	9.30	32.586	8	9
	8b	9.60	29.813	7	8
	9b	9.90	34.941	13	13
	11c	10.20	37.135	20	14
	12b	10.50	64.100	11	13
	14b	11.50	37.300	12	12
	14c	11.55	56.830	17	16
Powder Mill Beach	10	4.80	3.204	5	6
	9	4.90	3.551	4	6
	6	7.30	17.865	6	7
	8	7.35	19.554	9	11
	3	7.45	15.486	7	8
	7	7.70	22.270	11	13
	2	7.80	24.166	10	9
	5	8.20	19.255	12	13
	4	8.30	24.768	12	14
	1	8.40	26.601	13	13

APPENDIX IB. Valve lengths, shell weights, and age estimates from the regression model for E. <u>complanata</u> which were not sectioned.

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Lake Thomas	85	2a	7.55	19.348	4.5
		2b	7.60	20.278	4.5
		3c	7.65	13.092	5.0
		3b	8.10	23.431	6.5
		17b	8.15	22.650	6.5
		4b	8.20	24.385	6.5
		17c	8.20	32.484	6.5
		4c	8.50	25.796	7.5
		17a	8.55	28.239	8.0
		5b	8.75	26.944	8.5
		6c	8.95	22.714	9.0
		16a	9.00	21.462	9.5
		16b	9.00	31.705	9.5
		16c	9.00	23.109	9.5
		6b	9.15	28.115	10.0
		7b	9.25	31.473	10.0
		7c	9.25	36.165	10.0
		18a	9.45	35.564	11.0
		8a	9.50	32.168	11.0
		8e	9.50	27.835	11.0
		8c	9.55	31.122	11.0
		18b	9.55	31.864	11.0
		18c	9.55	39.153	11.0
		9a	9.60	25.073	11.5
		8d	9.65	45.130	11.5
		8f	9.65	34.590	11.5
		9c	9.75	41.590	12.0
		5a	9.80	21.605	12.0
		10a	10.05	33.139	13.0
		10b	10.05	48.660	13.0
		11b	10.15	31.830	13.0
		10c	10.20	53.950	13.5
		11a	10.20	49.610	13.5
		19a	10.20	78.810	13.5
		19b	10.20	43.570	13.5
		19c	10.30	48.120	13.5
		12c	10.50	37.455	14.5
		12a	10.60	67.390	14.5
		13	10.70	41.200	15.0
		14a	11.00	62.210	16.0
		15b	11.15	28.155	16.5
		15a	11.30	43.660	17.0

APPENDIX IB. Valve lengths, shell weights, and age estimates from the regression model for E. complanata which were sectioned. continued

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Lake Thomas	84	21	7.90	27.849	5.5
		22	8.4	20.285	7.5
		24	8.4	21.166	7.5
		23	8.5	18.760	7.5
		25	8.8	31.239	8.5
		34	10.0	49.22	12.5
		32	10.2	43.15	13.5
		44	10.2	83.75	13.5
		31	10.5	43.78	14.5
		33	10.7	50.31	15.0
		43	11.0	61.73	16.0
		42	11.2	51.46	16.5
		41	11.5	61.70	17.5
Powder Mill Beach	84	25	6.7	10.478	7.5
		24	7.2	14.341	9.5
		23	7.4	15.442	10.0
		22	7.6	12.069	10.5
		21	7.6	15.625	10.5

APPENDIX IIA. Valve lengths, shell weights, age estimates from internal bands, and age estimates from external rings for A. <u>implicata</u> which were sectioned.

Site	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages Internal Bands		External Rings	
				Eval. #1	Eval. #2	Eval. #1	Eval. #2
Lake Thomas	11b	5.20	1.740	5	5	5	5
	11a	5.25	2.101	5	5	5	4
	10a	6.00	2.598	5	4	7	6
	10b	6.35	4.449	6	5	7	5
	9c	6.70	4.713	7	6	6	6
	4	7.10	4.953	5	4	6	5
	5c	7.80	6.479	7	6	7	5
	6	8.00	10.650	9	6	8	7
	7c	8.30	11.772	11	7	10	8
	3	8.90	12.868	7	7	9	8
	2	9.70	12.553	6	5	11	10
	1	11.70	33.330	9	6	11	10
Powder Mill Beach	2b	4.15	0.976	4	4	4	3
	2c	4.60	1.089	5	4	5	3
	3b	5.30	1.819	5	4	5	3
	4b	6.00	2.421	7	5	8	4
	5a	6.70	4.855	6	4	6	5
	6a	6.80	7.381	7	6	7	6
	7c	7.20	5.364	6	4	7	7
	9a	7.80	10.043	7	6	6	7
	10a	8.60	17.184	6	5	6	5
	12b	9.10	22.904	6	9	7	9
	16f	9.90	26.619	8	8	9	10
	17	10.35	24.664	6	6	7	8
	19b	10.70	29.835	8	8	9	10

APPENDIX IIB. Valve lengths, shell weights, and age estimates from the regression model for A. implicata which were not sectioned.

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Lake Thomas	85	11c	4.35	0.803	3.0
		14a	5.70	2.131	4.5
		14b	5.75	2.948	4.5
		14c	5.90	1.916	4.5
		10c	6.30	3.742	5.0
		13	6.40	3.748	5.0
		9a	6.65	5.860	5.0
		8	6.80	4.699	5.5
		9b	6.80	4.244	5.5
		5a	7.30	4.288	6.0
		5b	7.35	5.489	6.0
		12	7.40	5.357	6.0
		7a	8.30	6.154	6.5
		7b	8.45	10.650	7.0
	84	26	4.60	1.369	3.5
		24	5.1	2.031	3.5
		25	5.1	1.975	3.5
		22	5.2	2.276	4.0
		23	5.6	2.759	4.0
		21	5.7	2.484	4.5
		34	6.4	3.685	5.0
		31	6.7	3.962	5.0
		32	6.8	5.007	5.5
		33	7.1	4.003	5.5
		43	7.4	8.236	6.0
		42	7.7	7.259	6.0
		44	8.3	10.594	6.5
		51	8.3	9.508	6.5
		41	8.4	14.263	7.0
		52	8.4	11.601	7.0
		53	9.5	13.719	8.0
		54	9.8	17.738	8.0
Powder Mill Beach	85	2a	4.50	1.241	3.0
		21c	4.80	1.332	3.5
		3a	5.45	2.544	4.0
		21a	5.50	1.874	4.0
		4a	5.80	2.475	4.5
		4c	6.25	3.818	5.0
		5c	6.65	4.508	5.0
		23b	6.70	4.734	5.0
		23a	6.75	6.128	5.5
		23c	6.75	6.404	5.5
		5b	6.80	5.161	5.5
		6c	6.90	6.158	5.5
		6b	7.10	8.873	5.5
		7b	7.25	5.902	5.5
		7a	7.30	9.171	6.0

APPENDIX IIB. Valve lengths, shell weights, and age estimates from the regression model for A. *implicata* which were not sectioned. continued

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Powder Mill Beach	85	24b	7.30	6.984	6.0
		8d	7.40	8.929	6.0
		8a	7.50	7.029	6.0
		8c	7.50	6.302	6.0
		24c	7.55	7.465	6.0
		24a	7.60	5.538	6.0
		8b	7.60	5.941	6.0
		10b	7.70	10.671	6.0
		9d	7.85	6.726	6.5
		9b	7.90	9.205	6.5
		10d	8.20	10.488	6.5
		10c	8.40	15.885	7.0
		11b	8.40	15.432	7.0
		22b	8.70	13.888	7.0
		22c	8.75	12.962	7.0
		11a	8.75	16.387	7.0
		11c	8.80	14.938	7.0
		22a	8.80	13.725	7.0
		9c	8.90	7.786	7.5
		12c	8.90	20.577	7.5
		12a	8.95	20.752	7.5
		13b	9.15	15.322	7.5
		13c	9.25	16.716	7.5
		13a	9.40	17.621	7.5
		14c	9.40	17.456	7.5
		14b	9.45	20.786	8.0
		15b	9.55	24.195	8.0
		14a	9.60	12.582	8.0
		15c	9.70	26.591	8.0
		15a	9.75	18.691	8.0
		25b	9.85	21.460	8.0
		25a	9.90	18.884	8.0
		16b	9.90	20.859	8.0
		16d	10.00	26.550	8.5
		16e	10.00	23.062	8.5
		25c	10.10	22.511	8.5
		16c	10.20	20.810	8.5
		16a	10.35	26.896	8.5
		20a	10.40	27.422	8.5
		18	10.50	28.224	9.0
		19a	10.50	24.783	9.0
		20b	10.75	33.685	9.0
		20c	10.90	30.552	9.0
		3c	NM	1.281	-
		21b	NM	1.367	-

APPENDIX IIB. Valve lengths, shell weights, and age estimates from the regression model for A. *implicata* which were not sectioned. continued

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Powder Mill Beach	84	36	3.9	0.875	2.5
		33	4.3	1.165	3.0
		34	4.3	1.034	3.0
		35	4.3	1.053	3.0
		32	4.4	1.460	3.0
		31	4.7	1.691	3.5
		46	6.3	4.505	5.0
		44	6.6	4.190	5.0
		45	6.7	6.846	5.0
		43	6.8	4.618	5.5
		42	7.0	5.395	5.5
		41	7.2	7.649	5.5
		51	9.2	17.468	7.5
		52	9.2	22.698	7.5
		53	9.7	16.877	8.0
		54	10.0	20.414	8.5
		64	10.2	35.673	8.5
		61	10.3	37.699	8.5
		63	11.2	45.01	-
		62	11.3	41.55	9.5

NM = not measured

APPENDIX IIIA. Valve lengths, shell weights, and age estimates from internal bands for A. <u>undulata</u> which were sectioned.

Site	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages: Evaluator #1	Estimated Ages: Evaluator #2
Powder Mill Beach	20	2.90	1.069	6	3
	19	3.05	1.336	5	4
	18	3.15	1.555	7	4
	17	3.20	1.618	6	4
	16	3.30	1.914	6	4
	14	3.40	2.242	5	4
	12	3.80	3.712	8	5
	7	4.00	3.503	7	5
	6	4.20	4.679	8	4
	4	4.40	4.818	5	5
	2	4.50	5.315	8	6
	1	4.90	7.061	8	7

APPENDIX IIIB. Valve lengths, shell weights, and age estimates from internal bands for A. <u>undulata</u> which were not sectioned.

Site	Year	ID#	Valve Length (cm)	Shell Weight (g)	Estimated Ages
Powder Mill Beach	85	15	3.45	1.674	5.0
		11	3.80	3.554	5.5
		13	3.90	2.845	6.0
		8	4.00	4.031	6.0
		9	4.00	3.677	6.0
		10	4.00	3.362	6.0
		5	4.30	3.995	6.5
		3	4.70	5.517	7.5
	84	25	2.5	0.673	3.0
		23	3.0	1.510	4.0
		21	3.2	1.996	4.5
		24	3.3	1.986	4.5
		22	3.4	2.054	5.0
		34	3.4	2.692	5.0
		35	3.4	2.051	5.0
		33	3.5	2.505	5.0
		32	3.8	3.051	5.5
		31	4.0	3.843	6.0

Vm

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