Preliminary Histological Assessment of Heavy Metal Accumulation in the Bivalve *Yoldia thraciaeformis* Collected From Alice Arm, Hastings Arm and Satellite Channel, B.C.

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PRELIMINARY HISTOLOGICAL ASSESSMENT OF HEAVY METAL ACCUMULATION IN THE BIVALVE *YOLDIA THRACIAEFORMIS* COLLECTED FROM ALICE ARM, HASTINGS ARM AND SATELLITE CHANNEL, B.C.

by

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Histochemical techniques demonstrated the presence of the trace metals cadmium, copper, iron, lead and zinc within accumulation granules in various tissues of the bivalve Yoldia thraciaeformis. A higher density of granules was found within organisms obtained from Alice Arm, where the AMAX of Canada Ltd. molybdenum mine is located, compared to those from an adjacent inlet, Hastings Arm, and a reference station in Satellite Channel. In addition, granules were present in a greater number of tissue types and stained positively for more metals in Alice Arm Yoldia than in specimens from the other two sampling sites. Yoldia from both Alice Arm and Hastings Arm also appear to have enlarged kidneys compared to specimens from Satellite Channel, however, the small sample size in this study prohibits conclusions concerning a possible cause and effect relationship between metal sequestration and kidney enlargement. Metal granule density and granule distribution in organisms correlated well with sediment trace metal data in Alice Arm, Hastings Arm and Satellite Channel.

RÉSUMÉ

Brand, D.G., M.A. Farrell and B.J. Reid. 1984. Preliminary histological assessment of heavy metal accumulation in the bivalve Yoldia thraciaeformis collected from Alice Arm, Hastings Arm and Satellite Channel, B.C. Can. Ms. Rep. Fish. Aquat. Sci. 1770: vii + 32 p.

L'utilisation de techniques histochimiques a permis de démontrer la présence des métaux traces cadmium, cuivre, fer, plomb et zinc dans les granules d'accumulation se trouvant dans divers tissus du bivalve Yoldia thraciaeformis. Les organismes prélevés dans le bras Alice, où est située la mine de molybdène de l'Amax of Canada Ltd., présentaient des densités plus élevées de granules que ceux provenant d'un inlet voisin, le bras Hastings, ou d'une station témoin située dans le chenal Satellite. De plus, des granules étaient présents dans un plus grand nombre de types de tissus et leur coloration indiquait plus de métaux dans les Yoldias du bras Alice, comparativement aux spécimens des deux autres lieux d'échantillonnage. Les reins des Yoldias des bras Alice et Hastings semblaient aussi plus gros que ceux des spécimens du chenal Satellite, mais la petite taille de l'échantillon interdit de tirer des conclusions quant à une relation de cause à effet entre la séquestration des métaux et l'hypertrophie des reins. La densité et la distribution des granules dans les organismes présentaient une bonne correlation avec les données sur les métaux traces des sédiments des bras Alice et Hastings et du chenal Satellite.

INTRODUCTION

AMAX of Canada Ltd.'s Kitsault mine in Alice Arm began milling molybdenum in April 1981 and remained in operation until November 1982. During the nineteen months of operation more than four million tonnes of tailings were deposited on the bottom of Alice Arm (Burling, et al., 1983).

This tailings discharge has created concern regarding potential accumulation of metals in marine organisms. As a result, considerable biological tissue monitoring has been conducted in the area. Of the four species groups which have been sampled from Alice Arm (algae, bivalves, crustacea and fish), only benthic bivalves have shown increases in tissue trace metal levels (ie. copper, lead, zinc and cadmium) (Burling, et al., 1983). Metal concentrations found within Yoldia thraciaeformis tissue were higher than the concentration in surrounding sediments which suggested that the increase was due to tissue accumulation (Goyette & Christie, 1982).

Most heavy metals may cause morphological and behavioural changes in organisms, the nature of which depends on specific relationships between the organism and metal speciation or biological availability (Eldon, et al., 1980). In a wide variety of phyla the presence of heavy metals leads to the development of granules either intra- or extracellularly which are ultimately associated with the sequestration, detoxification and excretion of metals (Brown, 1982).

The location of metal-containing granules in invertebrates can vary, although organs with digestive, storage and excretory functions appear to be prime sites for metal storage and detoxification. The presence of metal-containing granules has been described in both marine and terrestrial organisms from areas of exceptionally high metal concentrations (Brown, 1982). Several authors (Doyle, et al., 1978; Carmichael, et al., 1979; Icely and Nott, 1980) have also suggested that metal containing granules in certain species may be used as biological indicators of metal contaminated habitats as the form and abundance of granules may be positively correlated with metal levels in the environment. Since bivalves are relatively immobile, are particulate feeders and possess metal-containing granules, they can serve as particularly useful indicators of metal uptake.

In this study, *Yoldia thraciaeformis*, a deposit feeding protobranch clam was histochemically examined for metal containing granules, the presence of cadmium, copper, lead, zinc and iron within these granules and any pathological effects which could be attributed to heavy metal accumulation. *Y. thraciaeformis* were examined from Alice Arm, the site of the Amax/Kitsault mine, Hastings Arm, a reference location for monitoring programs in the area (and the historic site of the Anyox copper smelter); and Satellite Channel, a southern B.C. reference location which has not been subject to major industrial influence.

MATERIALS AND METHODS

SAMPLE COLLECTION

The study areas are presented in Figures 1 and 2.

Specimens of *Yoldia thraciaeformis* were collected from two stations in Alice Arm (near the AMAX/Kitsault Mine outfall and mid-inlet), and from Hastings Arm on October 7-10, 1983. Samples were collected by otter trawl from the survey vessel CSS Vector. The trawl gear consisted of two otter doors attached to a net with a 5.8 m throat and 3.8 cm mesh body. Trawls were conducted at a 3:1 scope over an average distance of 1 kilometer at a speed of 2.5 knots. Additional sampling was also carried out off Boatswain Bank in Satellite Channel on November 8,1983 from the MSSV John Strickland. Samples were collected using an aggassiz dredge which consisted of a 30 x 18 cm throat with a 0.5 cm mesh net. Dredges were towed at a 3:1 scope at a running speed of 1.5 knots. A total of seven otter trawls were carried out in Alice Arm, three at the head of Alice Arm and four from mid inlet. Five otter trawls were carried out in Hastings Arm, while one aggassiz dredge trawl was adequate to collect sufficient numbers of organisms from Satellite Channel. A summary of dates, trawl co-ordinates, sampling depths and numbers of specimens collected is given in Table 1.

SAMPLE PREPARATION

Yoldia samples collected from Alice Arm and Hastings Arm were brushed clean of adhering sediment and placed in a precleaned ten liter glass aquarium (fitted with a raised mesh bottom for depuration purposes) and filled with fresh aerated seawater (30 ppt salinity, 5°c) for 24 hours. Following depuration, the clams were placed in a precleaned polyethylene tray filled with 7.5 percent magnesium chloride seawater solution for three to five hours. Individual clams were then placed in separate 20 ml plastic vials containing either Bouins with mercuric chloride (Atkins, 1937) or 10% buffered formalin (Humason, 1979) and shipped to the University of Victoria. Bouins/Hg Cl_2 was the preferred histological fixative since it decalcifies bivalve shells within 3-5 hours resulting in rapid tissue preservation. In addition, tissues preserved with Bouins do not undergo cell breakdown with storage. Formalin was used as a histochemical fixative as fewer metals leach from tissue during fixing procedures (Pearse, 1972).

Samples collected from Satellite Channel were maintained in coolers filled with seawater for 3-4 hours until they were transported to the University of Victoria where they were placed in a closed cold recirculating seawater system (30 ppt salinity, 11°C, 0.5-1 L min^{-1} flow rate). Samples collected from Satellite Channel also were depurated for 24 hours followed by 3 hours narcotization in 7.5% magnesium chloride seawater solution and then fixed as previously described.

Specimens in buffered formalin fixative were subjected to a 9:1 RDO[1]- buffered formalin solution for 24 hours in order to decalcify the shells (Humason, 1979). Following fixation, samples were dehydrated in ethanol, cleared in xylene and embedded in paraffin for serial sectioning. Shell length and width measurements were taken when samples were in 70 percent ethanol during the dehydration procedure. Prepared six micron thick paraffin sections were subjected to the histochemical procedures indicated in Table 2. All sections were stained then examined microscopically for evidence of metal containing granules within selected organs and photomicrographs were taken of representative slides. Very high granule density within certain tissues produced aggregrates which appeared as large granules and stained very intensely for all metals (Plate 1(a)). This phenomena precluded enumeration of granules, and necessitated a rating system for granule distribution and abundance.

Accordingly the relative stain intensity and granule density in each tissue examined was subjectively rated using a modification

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of the method employed by George et al. (1982). Specifically, rating was accomplished by comparing slides of tissues containing granules which stained positively for the specific metal of concern with; (1) slides of another tissue type in the same organism; (2) the same tissue type stained for different metals in the same organism; and (3) the same tissue type and metal stain, from organisms from another site.

George et al. (1982) noted a positive correlation between stain intensity and concentration (mg/g dry weight) of metals in granules of Mytilus edulis, and accordingly ranked stain intensity (indicative of metal concentration) as +++ (very strong), ++ (moderate), + (detectable), and - (negative) in various tissue granules.

For the purposes of this study the criteria adopted resulted in the following ratings: ++++ (many granules, which stained intensely), +++ (numerous granules which stained moderately), ++ (a limited number of granules which stained positively), and + (very few granules which stained weakly).

RESULTS

PRELIMINARY OBSERVATIONS

All specimens obtained from Alice Arm and Hastings Arm and those specimens fixed in formalin from Satellite Channel had undergone cell lysis to varying degrees thus complicating histochemical and histological analyses. The remaining Yoldia thraciaeformis maintained in Bouins solution did not exhibit the same degree of tissue degradation and were considered suitable for examination. Table 3 presents a summary of the fixatives used, shell measurements and histochemical analysis performed.

HISTOCHEMICAL ANALYSIS OF GRANULES

Intercellular granules, 1-3 microns in size, were found in all samples. They were spherical in shape, with internal laminar structure, and possessed the characteristic orange-brown colour of the lipoprotein lipofuschin (George, et al., 1982) when examined unstained. Table 4 summarizes the results of the various staining methods for heavy metals within the granules. Figure 3 illustrates diagramatically the location of the tissues studied. Micrographs presented in Plates 1 and 2 confirm the presence of stained granules and illustrate the differences in size and location of granules within gill lamellae and stomach epithelial tissues of organisms from each sampling location.

Granules were most abundant in tissues of organisms collected from Alice Arm and least abundant in organisms from Satellite Channel. In addition, granule distribution among tissue types varied considerably in clams from different sampling locations. All specimens examined possessed granules in stomach, digestive diverticular, rectal and gill tissues. Granules were also present in mantle, esophageal, midgut, and kidney tissues in specimens from Alice and Hastings Arm, however, only organisms from Alice Arm contained granules within the foot, labial palp and digestive tubules.

There was no apparent difference in granule abundance versus organism size for specimens collected from the same sampling site. Granules stained positively for all metals examined, with the staining intensity (which reflects concentration of metals within the granules) decreasing for each metal in the order of iron > lead > copper > zinc > cadmium. Granule metal staining intensity was greatest in specimens from Alice Arm, followed by Hastings Arm and Satellite Channel, respectively (Table 4).

PATHOLOGICAL OBSERVATIONS

Due to cellular deterioration in the majority of Alice and Hastings Arm specimens, only preliminary observations were possible. One such observation was that the kidneys in *Yoldia* specimens from Alice Arm and Hastings Arm appeared to be larger than those of Satellite Channel (mean kidney length: 2.36 mm, 2.94 mm and 0.70 mm respectively) (Table 6). Shell length was also greatest in specimens collected from Alice and Hastings Arm and in order to standardize comparisons, the ratio of kidney to organism size was computed and the ratios in organisms from each sampling location were then compared.

While it must be emphasized that the sample size available for this study was very small, the results presented in Table 6 indicate that the kidneys in organisms from Alice Arm and Hastings Arm were 2.1 and 2.7 times larger respectively than in Satellite Channel specimens.

A second observation noted during this study was that gut contents containing zooplankton and sediment were present within all samples examined after a depuration period of 24 hours. Gut sediments stained positively for all metals tested with the exception of cadmium in Satellite Channel specimens.

DISCUSSION

GRANULE FORMATION IN *YOLDIA THRACIAEFORMIS*

Light microscopic examination of serial sections from *Yoldia thraciaeformis* has revealed intracellular accumulation of metals in all of the organisms examined. Granules were more numerous, stained more intensely and were found in a greater number of tissue types in Alice Arm specimens compared to organisms from Hastings Arm and Satellite Channel. It cannot be concluded from this observation however, that granules in Alice Arm specimens have higher concentrations of heavy metals. For example, Reid and Brand (1984) found that granules from the kidneys of the bivalve Spondylus barbatus are more numerous than those of Spondylus nicobaricus and Pteria penquin, but contained lower levels of the metallic elements. In the present study, granule metal concentrations were not determined analytically. However, histochemical techniques revealed that all five metals (eg. cadmium, copper, lead, zinc and iron) were present in granules of specific tissue types in Alice and Hastings Arm specimens, whereas Satellite Channel organisms contained granules which stained positively for only three of these metals. Metal staining intensity, granule abundance and the number of tissue types containing granules were also greater in Yoldia thraciaeformis from these two areas compared to Satellite Channel specimens. Specifically, granules which stained positively for all five metals were found in four tissue types in specimens from Alice Arm, three tissue types in organisms from Hastings Arm and none from Satellite Channel.

The observed distribution of granules among tissues types is probably related to organ function. Yoldia thraciaeformis is a burrowing benthic deposit feeding bivalve which feeds by extending labial palps directly into the substrates. Deposit material adheres to mucous on the surface of the tentacles and is then transported by cilia back to the palps which function as sorting mechanisms. Light particles are carried to the mouth where they are ingested. The process of digestion in these organisms is both intracellular and extracellular (Barnes, 1980). Extracellular digestion occurs in the stomach, where enzymes are secreted by sections of the digestive diverticula and stomach wall. Intracellular digestion occurs within cells in other areas of the diverticula and requires that food be delayed in its passage through the alimentary canal and be evenly distributed over a large area of phagocytic epithelium. The ducts (digestive tubules of the digestive diverticula) are ciliated tracts which carry food particles to the lumen of the small tubules, where they are engulfed by cells of the tubule wall and digested intracellularly. Digestive wastes are then dumped into the lumen, returned to the stomach via excurrent cilia tracts in the digestive tubules, and swept into the intestine.

The greatest accumulation of granules and the highest metal staining intensities noted in this study occurred in the absorptive digestive diverticula of specimens from all areas, and in the stomach epithelia of organisms from Alice and Hastings Arm (Table 4). This finding suggests that metal accumulation in Yoldia is predominantly the result of sediment ingestion. Previous studies with molluscs have also shown that exposure to metals in food results in greater metal accumulation in digestive organs and muscle (Luoma, 1983).

Granules found in gill tissues stained positively for copper, lead and zinc in all specimens examined, with the addition of iron granules in gill tissue of Alice Arm samples. All four of these metals were also present in the mantle tissues of Alice Arm and Hastings Arm clams. Luoma (1983) reports that metal accumulation in external organs such as mantle and gill in molluscs is the result of surface adsorption after exposure to solute metals. Differences in metal content of tissues in bivalves has also been associated with the concentration of specific carrier organic compounds (probably porphyrins) in these tissues (Khistophorova, 1980). This distribution of metals suggests that a second uptake vector for metals in Yoldia may be via interstitial water. Of the metals reported in gill and mantle tissues, lead and zinc have been reported as slightly elevated in the Alice Arm water column (Burling et al, 1983).

In addition to respiratory and absorptive tissues, Yoldia from Alice Arm also had the greatest abundance of metal containing granules in each of the other tissue types examined and were the only specimens which contained granules in the foot, labial palp and digestive tubules. The abundance and presence of granules in organs of Alice Arm Yoldia with such diverse functions as locomotion, feeding, respiration, digestion/adsorption and excretion suggest a higher level of accumulation in these organisms compared to specimens from the other study sites. The differences in tissue granule abundance and staining intensity in Yoldia from Alice Arm, Hastings Arm and Satellite Channel also correlates well with sediment metal data for each of these sampling areas (Table 5). Satellite Channel has not been subject to major industrial influences and is not known to be an area of high mineralization. Accordingly sediments from this area contain comparatively low trace metal concentrations. In contrast, Alice and Hastings Arm are both known to be highly mineralized areas and have been the sites of numerous mining activities. The recently elevated sediment metal levels in areas of Alice Arm are most probably attributable to tailings discharged from the Amax/Kitsault mine, as increases in sediment cadmium, lead and zinc concentrations have been used to delineate tailings deposition patterns in Alice Arm (Burling et al., 1983). Similarly, the relatively high copper, lead, zinc and iron levels reported in Hastings Arm surface sediments reflects the influence of the Anyox copper smelter which operated in Granby Bay until 1935 (Goyette and Christie, 1982).

Coombs and George (1978) propose that heavy-metal containing granules are produced when metals present as particulates are taken up by the process of endocytosis (i.e. engulfment of the metal by the epithelial cell membrane which then pinches off to form a membrane-bound vesicle inside the cell). After endocytosis, the vesicle migrates to the basal end of the cell and is then excreted by the reverse process of exocytosis into the circulating fluid. The metal is then reabsorbed by the circulating amoebocytes for subsequent transfer to the kidney and other tissues such as the midgut and rectum for storage and eventual excretion (George, et al., 1976). Carmichael et al. (1979) demonstrated that radiolabelled manganese, zinc and cadmium were extensively concentrated in mineral concretions which were present in the kidney of the clam Mercenaria mercenaria. Subsequent gel permeation chromatography of the renal cytosol fraction in Carmichael's study also showed that these metals were bound to proteinaceous macromolecules which are typically associated with metal adsorption and detoxification. This process of granule formation and sequestration of metals allows bivalves to tolerate extremely high metal concentrations within membranes and effectively immobilize and detoxify them.

Associated within the membrane-bound vesicles or granules are pigmented lipoproteins called lipofuschin (Reid et al., 1984). Their abundance has been linked to age of an organism or to the senescence of a cell, however, it has also been found that toxins and other environmental stressors can induce lipofuschin production. Since bivalves are not able to prevent uptake of high concentrations of many metals, the massive accumulation of lipofuschins within the granules may be a metal-induced response (George, et al., 1982).

Many factors such as size, sex and reproductive state may affect the number, appearance and composition of metal-containing granules. For example, Shultz-Baldes (1973) found that the concentration of lead containing granules is significantly higher in small mussels than in large mussels from contaminated environments. In this study however, there was no difference in the number of granules with respect to size when comparing samples from the same site.

There may also be seasonal variability in granule numbers. This variability is associated with reproductive cycles and increases in metabolic rates which differentially affect metal uptake and elimination (Williamson, 1980). Seasonal variability is unlikely to be a factor in this study, however, since sampling at all sites was carried out at the same time of the year (October and November, 1983).

The staining intensity of granules accumulated in different tissue types examined in this study was typically in the decreasing order of iron > lead > copper > zinc > cadmium. While it has been stated that staining intensity reflects metal concentrations within the granules, metals may be selectively removed from the granules during histochemical preparation (Ward, 1982; Brown, 1982). Although the choice of fixatives was intended to minimize incidental leaching of trace metals, the removal of mercury during dehydration and imbedding procedures may have resulted in some removal of cadmium and zinc as all three metals belong to the group IIB metals in the periodic table and have many similar features in their chemical behaviour. Staining intensity therefore can only be considered a relative indication of metal concentration.

While the presence of metal containing granules in invertebrates from areas of elevated metal concentrations has been well documented, the physiological and ecological significance of granule saturation in organisms is less well understood. X-ray microanalysis in Mytilus edulis has shown conclusively that granules contain a variety of metals including iron, zinc, cadmium and lead (George and Pirie, 1979). Light microscope studies have also shown that these granules may contain lysosomal enzymes (Moore, 1977), however, the relationship between metal loading of these lysosomal vesicles and potential labilization of hydrolytic enzymes which could cause autolytic cell damage and subsequent cell death has not been investigated.

While it is possible that metals accumulated in granules may not exert a direct toxic effect, Yoldia are known to be consumed by benthic fish species (J. Westrheim pers. comm.), and it is possible that digestive processes in fish and concurrent hydrolytic enzyme production could result in granule lysis and metal remobilization or liberation. In this manner, granule associated metals may become available for progressive accumulation through successive trophic levels.

PATHOLOGY

Pathologic observations were complicated due to cellular deterioration of all specimens maintained in buffered formalin. Changes in osmality due to the phosphate buffer in the formalin (Baker, 1969) or the length of time between collection and dehydration/imbedding (1 month) may have resulted in tissue breakdown. This has been observed previously for bivalves fixed in formalin (R.G.B. Reid, pers. comm.). Among the organisms fixed in Bouins and $Hg\,Cl_2$, those from Alice and Hastings Arm all exhibited some degree of tissue deterioration, while those from Satellite Channel did not. In contrast to organisms from Satellite Channel, the valves in many of the Alice and Hastings Arm clams remained tightly closed despite narcotization which may have prevented immediate penetration of Bouins into the tissues. Shell valve adduction, followed by total shell closure such as was observed in Alice and Hastings Arm clams, has been reported to be an initial behavioural response in many bivalves exposed to low concentrations of heavy metals (Akberali and Black, 1980; Manley and Davenport, 1980). As transportation and depuration procedures for all collections were similar (in that the organisms were maintained in holding facilities under conditions of salinity and temperature representative of their collection sites), there is the possibility that clams from Alice and Hastings Arm may have been moribund and experienced some tissue lysis prior to collection.

A preliminary histological difference noted in this study was that the kidneys of organisms from Alice Arm and Hastings Arm were approximately twice as large as those in specimens from Satellite Channel. It should be emphasized that this observation is based on a small sample size and while natural variability in populations may be sufficient to account for these differences, these findings correlate well with granule abundance, granule distribution and sediment metal data. Specifically, metal containing granules were absent from the kidneys of all Satellite Channel organisms, while in contrast kidney granules which stained positively for all five metals were present in every organism examined from Alice and Hastings Arm.

A relationship between kidney enlargement and increases in metal loading might be expected as the kidney in most bivalves functions as both a storage and excretory organ (Shultz-Baldes, 1973). Metal sequestration and nephrolith (kidney granule) formation may occur in either of two ways: (1) extension of part of the apical portion of the kidney epithelium, or (2) movement of phagocytic amoebocytes containing metals across the epithelium and coalescence in the renal lumen. The loss of renal columnar epithelium which has been observed to accompany this process in other bivalves, notably Mercenaria mercenaria, has been attributed to renal inflammation caused by prolonged environmental stress (Rheinberger et al., 1979). The inflammation or enlargement of the kidney in Alice and Hastings Arm specimens may therefore be in response to increased demand for granule formation and metal storage or elimination and may cause impaired kidney function. Rheinberger et al (1979) concluded that M. mercenaria is probably unable to expel the granules under these conditions. The paucity of data concerning the relationship between nephrolith density, kidney size and organ function suggests that additional study in this area is warranted and would be necessary to confirm our findings.

A second observation noted during this investigation was that the guts of all Yoldia thraciaeformis examined contained zooplankton, algal debris and sediment that stained positively for all metals. This suggests that a 24 hour depuration period is not long enough to completely purge the organisms. Therefore analytical results reported to date using this depuration period may not represent the true concentrations of heavy metals accumulated in the tissues of Y. thraciaeformis. It has been suggested by Klumpp and Burdon-Jones (1982) that bivalves should be placed in clean seawater for 48 hours rather than the 24 hour depuration period that has been used in the AMAX/Kitsault monitoring programs. Reid and Brand (unpublished) also found that minor disturbances can result in a prolonged cessation of gut activity in another bivalve, Crassostrea gigas, which suggest that handling stress may further decrease the efficacy of a 24 hr. depuration period.

The results of this preliminary histochemical assessment indicate that more experimental work is required in order to accurately assess the effects (if any) of the tailings produced by the AMAX/Kitsault mine on Yoldia thraciaeformis. Since heavy metals were found within granules in specific tissues, these granules could be isolated and subjected to atomic absorption analysis. This would allow quantitative determination of metal concentrations which have bioaccumulated within Yoldia tissues. The drawback to this procedure is that it requires a large sample size. A more widely used (i.e. Reid, et al., 1984; Reid & Brand, 1984; Doyle, et al., 1978; and Mason and Simkiss, 1982) and highly recommended method is electron microscope X-ray microanalysis which would permit examination of the cellular structure of granules and quantitative analyses of metals found within the granules. This method requires a small sample size and would be useful in distinguishing between metal uptake from interstitial water and sediments.

It is apparent that additional work is also required to investigate the effects of granule saturation in marine invertebrates and further our knowledge of the relationship between granule formation, potential lysosomal activation, metal availability and physiological/ecological impacts.

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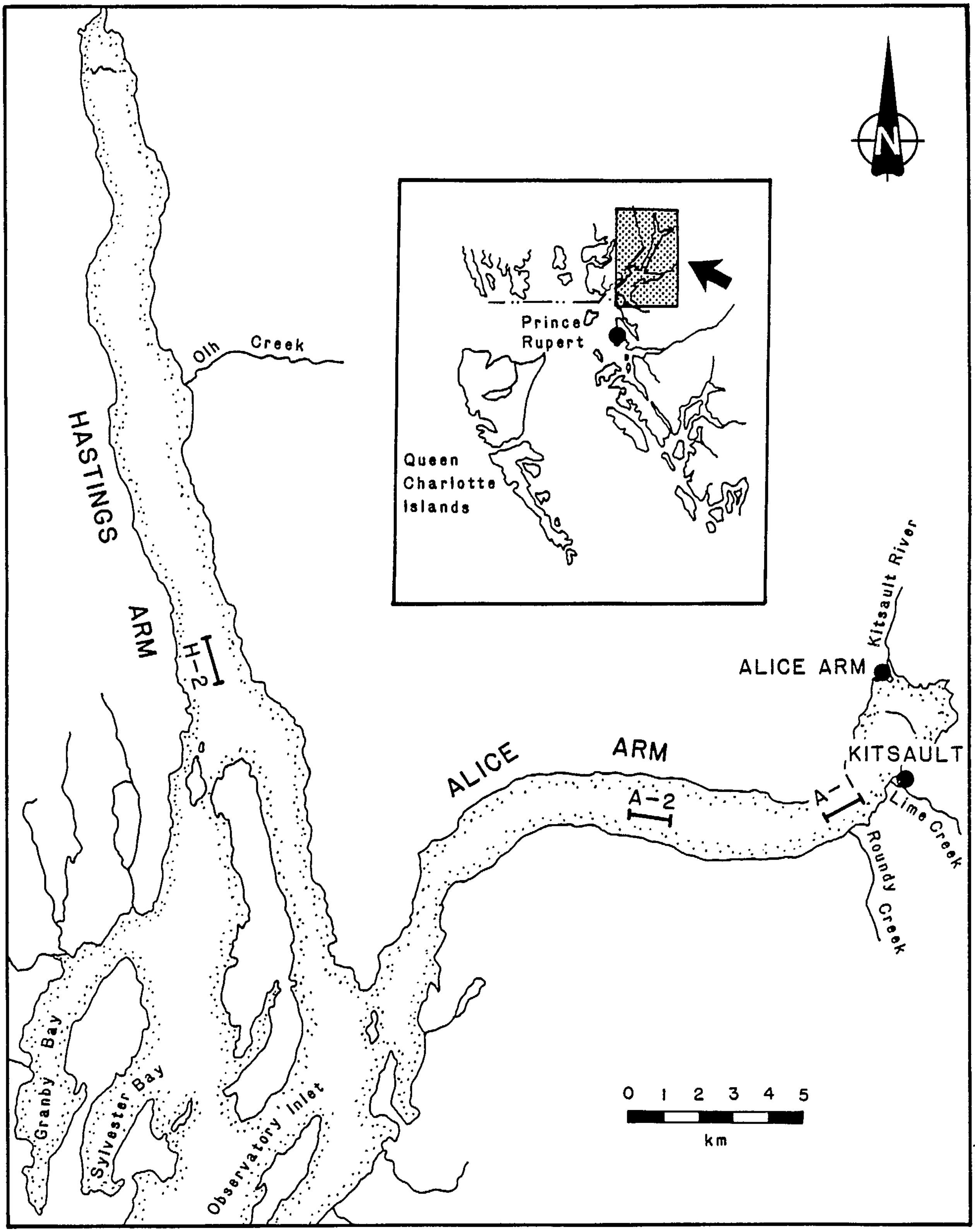


FIGURE 1. Trawl locations in Alice Arm and Hastings Arm, B.C.

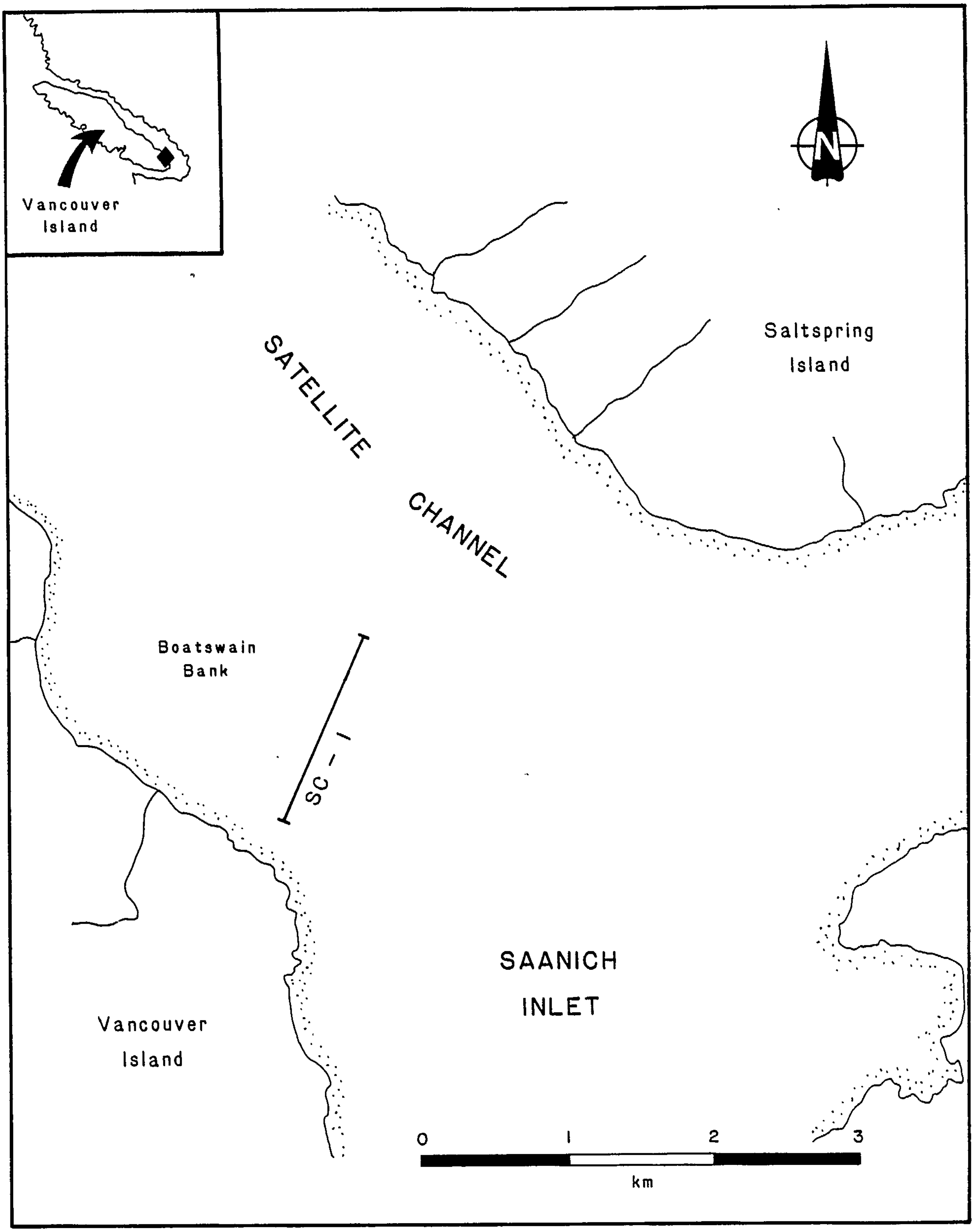


FIGURE 2. Trawl locations in Satellite Channel, B.C.

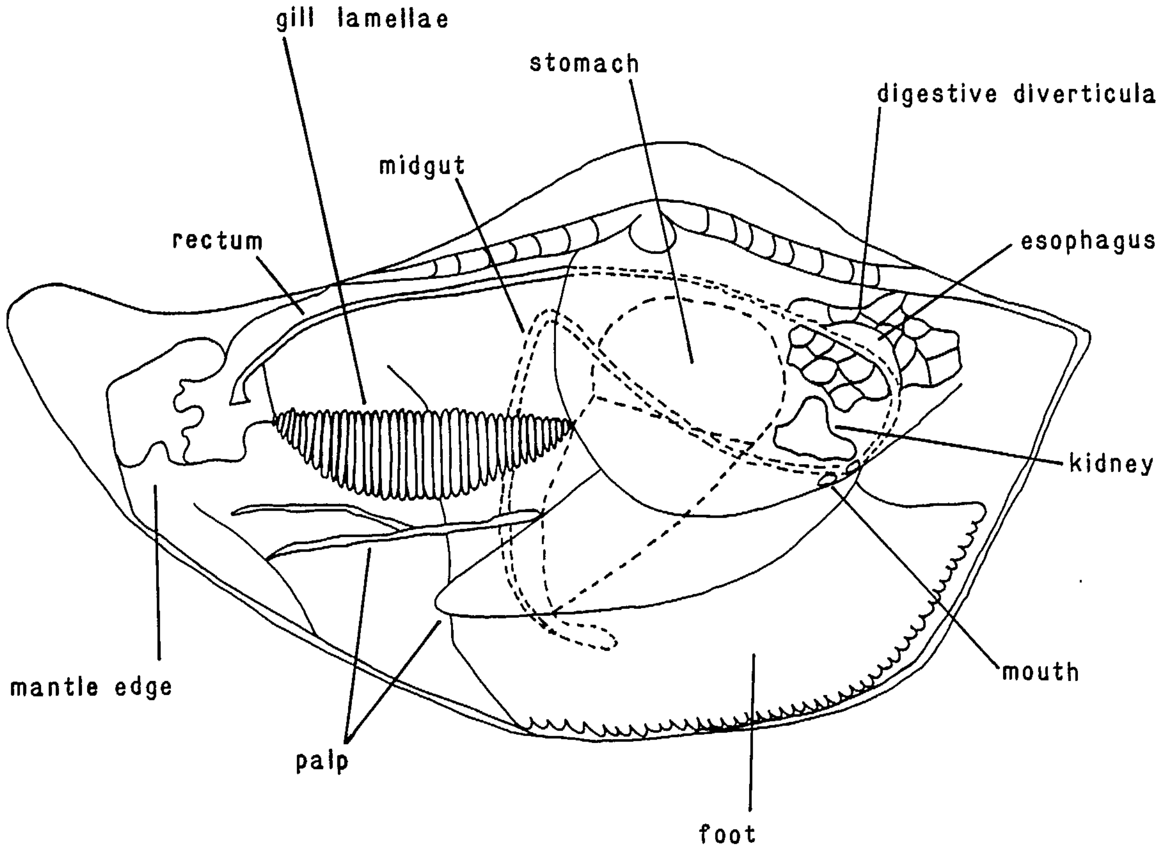
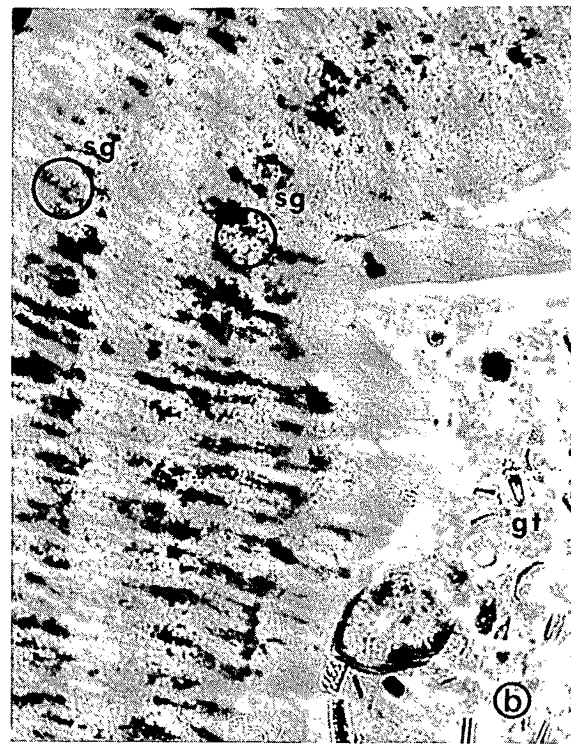
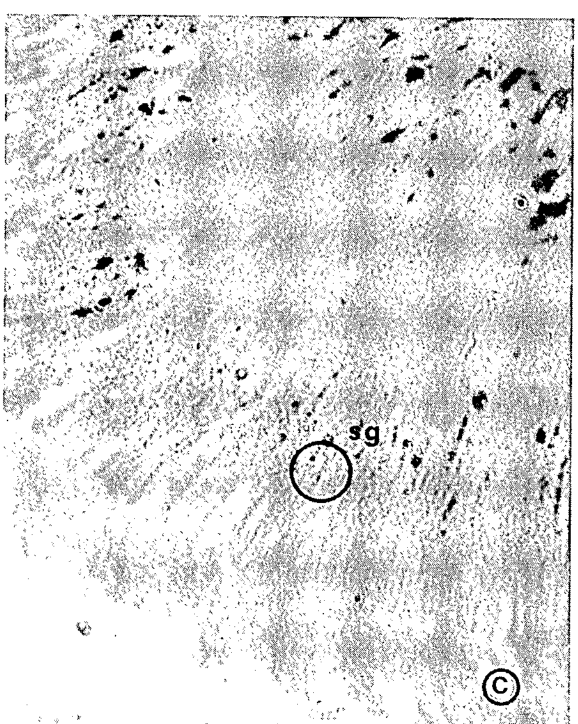


FIGURE 3. Diagramatic presentation of Yoldia thraciaeformis identifying locations of organs examined. (Modified after Yonge, 1939)

(a) Stomach epithelia of Alice Arm <u>Yoldia</u>. Note high density and dark staining intensity of large granules (lg) consisting of aggregates of small granules (sg). Gut sediments = (gt).

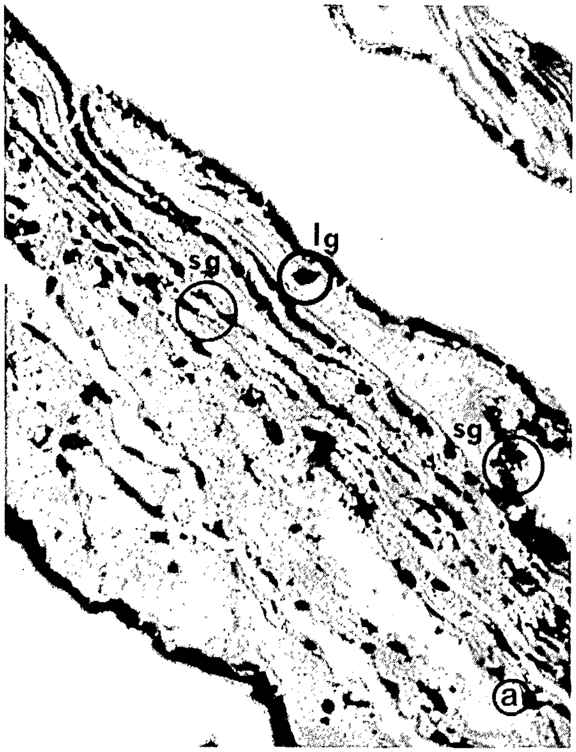
(b) Stomach epithelia of Hastings Arm <u>Yoldia</u>. Note high density of small granules (sg). Also note plankton in gut sediments (gt).





(c) Stomach epithelia of Satellite Channel <u>Yoldia</u>. Note light staining intensity and low density of small granules (sg).

Plate 1: Location, size and structure of metal granules in stomach epithelia of *Yoldia thraciaeformis* from (a) Alice Arm, (b) Hastings Arm, and (c) Satellite Channel (20x mag. All granules stained for lead).



(a) Gill lamellae of Alice Arm *Yoldia*. Note large darkly stained granules (lg) consisting of aggregates of small granules (sg). Also note high density of ◀ small granules (sg).

(b) Gill lamellae of Hastings Arm *Yoldia*. Note low density of large granule aggregates (lg) ▶ and low density of small granules (sg).

(c) Gill lamellae of Satellite Channel *Yoldia*. Note low ◀ density of small granules (sg).

Plate 2: Location, size and structure of metal granules in gill lamellae of *Yoldia thraciaeformis* from (a) Alice Arm, (b) Hastings Arm, and (c) Satellite Channel (40x mag. All granules stained for lead).

TABLE 1: Trawl locations and numbers of Yoldia thraciaeformis obtained from the four sampling sites tested.

DATE	ID	LOCATION	CO-ORDINATES START	CO-ORDINATES FINISH	DEPTH RANGE (meters)	# OF CLAMS RETAINED
Oct.7/83	A-1-I	Alice Arm Outfall	55°27'82"N 129°30'57"W	55°27'99"N 129°30'2"W	172-223	4
Oct.9/83	A-2-III	Mid Alice Arm	55°29'N 129°45'48"W	55°28'3"N 129°45'23"W	356-395*	1
Oct.9/83	H2-I	Hastings Arm	55°29'5"N 129°45'8"W	55°29'05"N 129°4'5"W	385-293**	10***
Nov.8/83	SC-I	Satellite Channel	48°42'N 123°23'W	48°43'N 123°31'30"W	60-80	35

* Actual depth not recorded in ships log, taken from trawl A-2-V on October 11, 1983.
** Actual depth not recorded in ships log, taken from Demill, 1983 for same station.
*** Subsample of total collected at this station.

TABLE 2: Histochemical procedures used to detect heavy metals within the tissues of *Yoldia thraciaeformis*.

HEAVY METAL	HISTOCHEMICAL METHOD	REFERENCE
Cadmium, Zinc	Thiazolyazophenol, 2-(2-thiazolyazo)-p-cresol (TAC)	Sumi *et al.* (1979)
Copper, Zinc	Zincon	McNary (1960)
Iron	Perls' method for ferric iron	Pearce (1972)
Lead	Mallory's haematoxylin	Disbrey & Rack (1970)

TABLE 3: Shell measurements (mm) and histochemical tests employed on *Yoldia thraciaeformis*.

STATION	FIXATIVE	SHELL MEASUREMENTS LENGTH	WIDTH	HEAVY METAL TESTED FOR	DEGREE OF TISSUE LYSIS NOTED
Alice Arm-outfall (A-1-I)	Buffered formalin	13.20	3.45		++
	Bouin with $HgCl_2$	19.30	12.50	Iron	+
		20.45	12.70	Lead, Cadmium, Zinc	+
		20.25	13.30	Copper, Zinc	+
Alice Arm-midstation (A-2-III)	Bouin with $HgCl_2$	12.25	6.90		++
Hastings Arm (H2-I)	Buffered formalin	15.40	10.00		++
		14.50	9.80		++
		23.40	14.80		++
		22.20	14.80		++
		21.60	13.80		++
	Bouin with $HgCl_2$	19.50	11.70	Iron	+
		25.40	15.80	Lead	+
		20.30	12.80	Copper, Zinc	+
		21.60	12.40	Cadmium, Zinc	+
		22.45	14.45		++
Satellite Channel (SC-I)	Buffered formalin	12.00	6.70		++
		13.20	7.20		++
		16.60	10.00		++
		11.80	7.30		++
		9.55	5.60		++
	Bouin with $HgCl_2$	12.00	6.95	None	-
		15.00	8.10	Cadmium, Zinc	-
		9.10	5.10	Iron	-
		16.60	10.10	Lead	-
		14.90	9.60	Copper, Zinc	-

\+ Some tissue lysis evident however histochemical analyses was possible.
++ Excessive tissue lysis which precluded histochemical analyses.

TABLE 4: Distribution of heavy metals, relative numbers of granules present and relative staining intensity of granules within the tissues of *Yoldia thraciaeformis*.

STATION	HEAVY METAL	FOOT	MANTLE	GILL LAMELLAE	LABIAL PALP	ESO-PHAGUS	STOMACH	DIGESTIVE TUBLULES	DIGESTIVE DIVERTICULA	MID-GUT	RECTUM	KIDNEY
Alice Arm (A-1-I)	Cadmium					++	+++		++			+
	Copper		++	++		++	+++		+++		+++	+
	Lead		+++	+++	+	+++	++++	+	++++	++	++++	++
	Zinc		++	++		++	+++		+++	+	++	+
	Iron	+	+++	+++	+	+++	++++	+	++++	++	++++	+++
Hastings Arm (H2-I)	Cadmium						++			+	+	+
	Copper		+	+		+	++		++		++	+
	Lead		++	++			+++		*		+++	++
	Zinc		+	+		+	++		++		++	+
	Iron		++			++	+++		+++	+	+++	++
Satellite Channel (SC-I)	Cadmium											
	Copper			+					+		+	
	Lead			++			+		++		++	
	Zinc			+					x			
	Iron						+		++			

++++ Abundant granules which stained very intensely
+++ Numerous granules which stained moderately
++ Fewer granules which stained positively
\+ Granules observed which stained very weakly

* Digestive diverticula too deteriorated to observe granules.

TABLE 5: Trace metal concentrations (ug/g dry weight) in sediments of Alice Arm, Hastings Arm and Satellite Channel.

SAMPLE LOCATION	DATE	CADMIUM	COPPER	LEAD	ZINC	IRON	REFERENCE
UPPER ALICE ARM Station Q-20 (0-2cm) Near trawl Station A-1	Oct/81	14.0	104.0	204.0	521.0	28,200	Goyette and Christie, 1982
HASTINGS ARM Station H-2 (0-2cm) Near trawl Station H-2	Oct/81	<0.60	76.2	25.9	160.0	43,400	Goyette and Christie, 1982
SATELLITE CHANNEL Near Boatswain Bank	Dec/83	<0.50	28.7*	11.0*	84.3*	ND**	McLeay et al, 1984 (IN PREP)

* Mean of three replicate analysis
** ND = Not determined

TABLE 6: Kidney size as a function of shell length in *Yoldia thraciaeformis* from Alice Arm, Hastings Arm and Satellite Channel

LOCATION	SHELL LENGTH (mm)	KIDNEY LENGTH (mm)	RATIO OF SHELL TO KIDNEY LENGTH
Satellite Channel	15.0	0.66	1:23
	9.1	0.69	1:13
	14.9	0.74	1:20
		$\overline{X}$ = 0.70	$\overline{X}$ = 1:19
Hastings Arm	19.5	2.19	1:9
	20.3	2.60	1:8
	21.6	4.03	1:5
		$\overline{X}$ = 2.94	$\overline{X}$ = 1:7
Alice Arm	19.3	2.08	1:9
	25.4	2.58	1:10
	20.3	2.43	1:8
		$\overline{X}$ = 2.36	$\overline{X}$ = 1:9
SC:HA = 1:2.7			
SC:AA = 1:2.1			