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Sediment particles as a cause of nacre staining in the freshwater mussel, *Amblema plicata* (Say) (Bivalvia: Unionidae)

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Abstract

Brown stains sometimes appear in the inner shell layers (nacre) of freshwater mussels. An electron microprobe was used to analyze the stained nacre of the unionid *Amblema plicata* (Say, 1817) from selected localities on the Mississippi River in the vicinity of LaCrosse and Prairie du Chien, Wisconsin.

Several elements such as Na, Mg, Al, Si, P, S, Cl, K, and Fe are more highly concentrated in stained than in unstained nacre. Concentrations of these elements relative to Ca were found to vary significantly among the localities from which the specimens were obtained. Ratios have significantly higher variances downstream of the confluence of the Yellow and Mississippi Rivers, downstream of a barge fleeting area, near the town of Marquette, Iowa, near the site of sewage effluent for Prairie du Chien, Wisconsin and downstream of a scrap metal yard near LaCrosse, Wisconsin in contrast to control localities.

Suspended silt, the result of runoff and river activity (barge traffic, dredging, pleasure boating) may be the stimulus for stain formation. Clay minerals adsorb accessory elements and in turbid water are trapped within the pallial space of *A. plicata*. The mussel secretes an organic-rich, periostracum-like layer over the entrapped sediment, and later reverts to prismatic and finally nacreous shell deposition. Some of the elements found in the stain could directly disturb Ca metabolism by competing with Ca for binding sites in shell aragonite.

Introduction

The purpose of this research is to determine the cause of stained nacre in the shell of the freshwater mussel, *Amblema plicata* (Say, 1817). This bivalved mollusc secretes a thick aragonitic shell with an outer prismatic and inner, lamellar (nacreous) layers. The nacre is generally white, but may be colored various shades of purple. Sometimes brown stains appear within the nacre (Fig. 1). The stains can be distinguished from color variations within the nacre in that they have a distinct boundary with the unstained shell. The stains appear to be products of 'disturbed' calcification, for compositional or structural shell characteristics normally change within the shell according to more gradual, allometric gradients (Rosenberg, 1980).

Stains tend to be circular during early stages of formation, and occasionally have small dark colored pits (nuclei) in the center. A bit of sediment may be trapped at the bottom and overlain by subsequently deposited shell. The stains may be circular due (possibly) to diffusion from the center of sediment. The stains are generally restricted to the area of the shell bounded by the pallial line, and usually in the dorsal half of this area. However, stains can occur anywhere on the shell. Lateral teeth are frequently stained. Stains are also seen on the periphery of the shell beyond the pallial line, especially in the anterior and posterior regions. These peripheral stains, however, are never nucleated or as densely colored as stains dorsal to the pallial line.

There are two practical reasons for studying stained nacre in *Amblema*. First, stained nacre can-

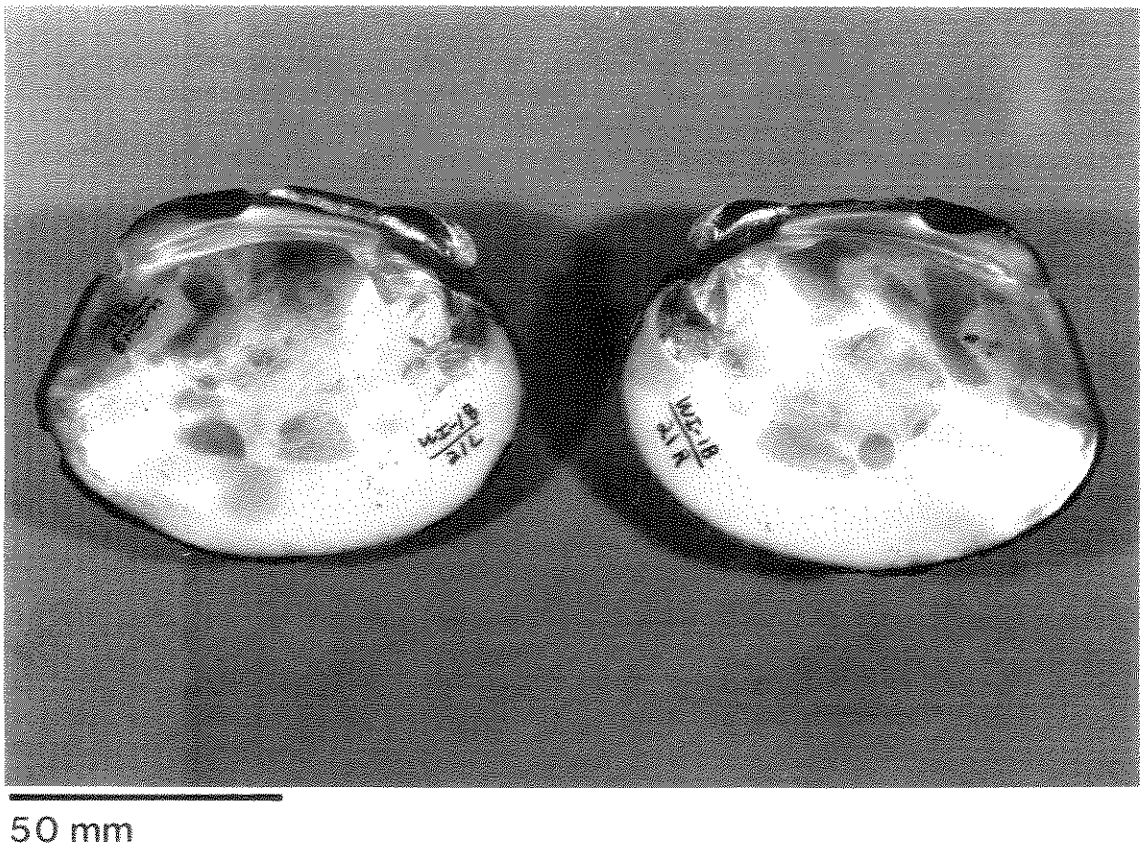

Fig. 1. Stained nacre on interior of both valves of an *Amblema plicata* shell. Stains have well-delimited boundaries. Some end abruptly at the pallial line and do not coalesce with stains ventral to the pallial line.

not be used in the cultured pearl industry. Midwestern mussel shells are commonly thick and white. They are exported to Japan where they are ground into small spheres (seed pearls) which are inserted into oysters. The oysters then secrete lammellae of pearl around the spheres, making a cultured pearl.

If the nacre is stained, discoloration will show through the translucent pearl, rendering it unsuitable for sale. Because suppliers in the Midwestern United States claim that stained nacre is appearing with increasing frequency, it has become important to determine the cause of the discoloration.

Second, stained nacre may be a useful bioindicator of water quality, if stained nacre can be shown to harbor elements not normally concentrated in mussel shells and if the concentrations of these accessory elements can be related to the mussels' proximity to presumed sources of the elements.

Pollution is not the only viable hypothesis, however. Stained nacre is reported frequently in the literature beginning in the early 1900's (e.g. Coker, 1912; Coker *et al.*, 1922) although cases of discolored natural pearls in freshwater mussels were reported much earlier (Woodward, 1868). Either stains are not due to pollution or pollution was a problem in the United States long before awareness of the problem heightened in the 1960s. There is probably truth to both alternatives. Indeed, there are suggestions that some stains may be due to parasites (Parmalee, 1967).

In either case, analysis of stained nacre provides the opportunity to assess the utility of mussel shells as monitors of water quality, or at least to assess the bivalve's responses to variations in water quality. Mussel shell is accretionary and growth incre-

ments preserve a record of the animal's physiological changes and responses to the environment for the duration of the mollusc's growth. Accretionary growth patterns of mollusc shells have been used to determine depth, temperature, salinity, substrate, tidal oscillations and other parameters of habitat (Rhoads & Lutz, 1980; Rosenberg & Runcorn, 1975) but there are relatively few studies on the response of accretionary skeletal growth patterns to pollution (Kennish, 1980; Tevesz & Carter, 1980). This is especially ironic considering the enormous amount of research devoted to establishing cause and effect relationships between pollution and soft tissue composition (Fuller, 1974; Koide *et al.*, 1982; Simkiss & Mason, 1983; Goldberg *et al.*, 1983 are four of the best leads into this vast literature).

This paper reports the results of energy dispersive electron microprobe analyses of 11 elements in the nacre of *Amblema plicata* (Say, 1817) (common name = Three-ridge) from the Upper Mississippi River.

Collecting Sites

Sites from which *Amblema plicata* were collected are listed below and are also shown in Fig. 2. The number of analyzed specimens from each locality is also indicated. Each group has sites selected at varying distances from a suspected polluting source or area of heavy river traffic (barges can stir up the bottom even in 30′ of water). One of the sites within each group is as close as possible to the respective disturbance source. The localities are within three pools (LaCrosse/Prairie du Chien, Wisconsin area) created by a series of locks and dams constructed during the early-mid 1900's.

Group I

Western Bank of the Mississippi River between Harper's Slough, Iowa and Marquette, Iowa.

Locality 8.
Pool 10, Mississippi River mile (MRM) 642.5. Midchannel (noncommercial channel), Harper's Slough, approximately 5.5 miles downstream of Lock and Dam 9 and about 3.5 miles south of Harper's Ferry. Seven specimens.

Locality 9.
Pool 10, MRM 637.5. Just below the mouth of the Yellow River, approximately 200′ from the western shore. Seven specimens.

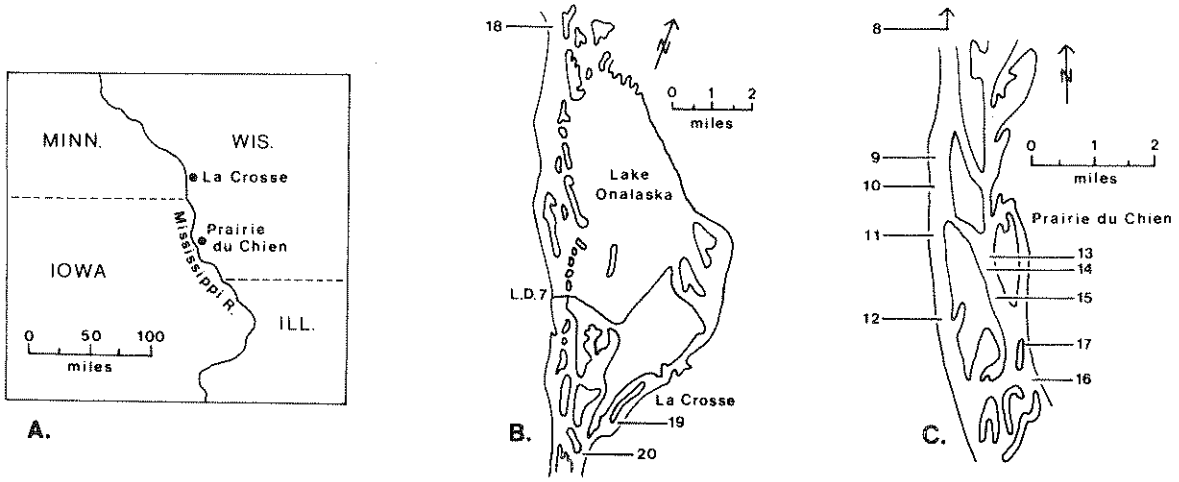

Fig. 2. Index maps to collecting localities.

A. Collecting localities on both shores of Mississippi River between LaCrosse and Prairie du Chien, Wisconsin.

B. Localities 18–20 constitute Group III with pollution increasing to Locality 20, just downstream of a scrap metal yard at the confluence of the Black and Mississippi Rivers. L.D. 7 = Lock and Dam 7.

C. Collecting Localities 9–17. Group I is composed of Localities 9–12 (and Locality 8 which is not shown as it is north of the figured area but also along the west bank). Marquette, Iowa, is at Locality 12. Group II is composed of Localities 13–17. Locality 16 is the site of sewage effluent from the town of Prairie du Chien, Wisconsin. Locality 17 is adjacent to a fertilizer plant.

Locality 10.
Pool 10, MRM 636.7, about 300′ off the west bank, one-half mile north of Marquette Island and 1.0 mile south of the mouth of the Yellow River. Approximately 0.7 mile north of a barge fleeting area. Five specimens.

Locality 11.
Pool 10, MRM 636.0, about 300′ off the west bank, just downstream of the barge fleeting area noted at Locality 10. Five specimens.

Locality 12.
Pool 10, MRM 634.7. Marquette, Iowa, Beneath Marquette Bridge, in the middle of the main channel. A small boat dock is at, and heavy barge traffic passes by this town. Five specimens.

Group II

The Wisconsin side of the River from Lawler Park, Prairie du Chien to Pickerel Slough, Prairie du Chien.

Locality 13.
Pool 10, MRM 635.8, east bank, just upstream of Lawler Park, Prairie du Chien. Five specimens.

Locality 14.
Pool 10, MRM 635.5, east bank, just offshore of Lawler Park, Prairie du Chien. Five specimens.

Locality 15.
Pool 10, MRM 634.8, west side of east channel, just below lengthy wind dam. Five specimens.

Locality 16.
Pool 10, MRM 633.5, 300′ off the east bank, Pickerel Slough, just upstream of a trailer camp and immediately downstream of sewage and fertilizer plants. Five specimens.

Locality 17.
Pool 10, MRM 634.2. Marais de St. Friol Slough. Adjacent to and just downstream of a fertilizer plant and above a sewage treatment plant for Prairie du Chien. Only two mussels were obtained from this locality after three attempts. One was a *Quadrula quadrula,* the other was an *Andonta grandis.* They are analyzed here due to their proximity to a potential polluting site, although they did not show *Amblema*-like stains.

Group III

One locality just north of Lake Onalaska along the western (Minnesota) shore and two localities south of the lake along the eastern (Wisconsin) shore near LaCrosse, Wisconsin.

Locality 18.
Pool 7, MRM 709.0, just north of Dakota, Minnesota, west bank, 200′ from shore. Six specimens.

Locality 19.
Pools 7/8 (near boundary). Black River at Clinton Street Bridge, LaCrosse, Wisconsin. Black River mile 1.7 = MRM 700.5. Five specimens.

Locality 20.
Pool 8, MRM 698.7. Actually within the Black River at approximately mile 0.5 mile (i.e. just upstream of Black River and Mississippi River confluence). Adjacent to and downstream from a scrap metal yard. Five specimens.

Materials and Methods

Specimens were obtained by dragging an eight-foot brail equipped with beaded dovetail hooks. Several species were obtained, but few were as severely stained as *Amblema plicata*, the focus of this report.

The mussels were placed in dark plastic bags and iced down after capture and kept iced until they were sacrificed 48-72 hours later. It should be noted that bivalves respire anaerobically upon removal from water. According to some theories (Lutz & Rhoads, 1977), bivalves buffer the by-products of anaerobiosis by dissolving calcium carbonate from their shells, leaving behind shell enriched in organic matrix ('conchiolin'). We believe that the delay in sacrifice did not affect our results because (1) all specimens were thus treated, (2) none of the stains analyzed were simply surficial – they were up to 0.5 mm thick and thus were deposited over a protracted time interval, (3) cross sections of selected stains were analyzed and accessory elements were found distributed throughout the entire stain (i.e.

were deposited over more than 48-72 hours time).

After sacrifice and removal of the soft tissue from the exoskeletons, shells were prepared for electron microprobe analyses. Small sections (2 cm in length and width, maximum) were cut using a gem-cutter's diamond saw, and were chosen to include both stained and unstained shell material. Sections were washed and mounted on glass discs with epoxy. For the purposes of this study, unpolished sections were used and specimens were mounted with the inner shell surface up so that it could be analyzed with the probe. Specimens were left unpolished to avoid the possibility of contamination before analyses. We recognized that trace and minor elements in skeletal tissues are normally concentrated in amounts sometimes close to limits of detectability using energy dispersive microprobe techniques. We wanted to be certain that all elements recorded during analyses were actually present in the shell and not introduced during preparation. Present analyses are thus strictly semi-quantitative; polished surfaces are essential to ensure accurate quantitative determinations as topographic variation causes differential absorption of x-rays.

Specimens were analyzed using an ORTEC energy dispersive analyzer (MAC-5 electron microprobe). Spectra were accumulated for 100 seconds to maximize signal to background ratios. The probe was operated at 10-15 kV with a specimen current of 0.01 uA.

Sixty-seven specimens were analyzed from the habitats previously listed. An average of about eight measurements were made on each specimen (on both stained and unstained shell) for a total of about 550 measurements. All samples were taken from relatively flat, inner shell surfaces close to the pallial line to minimize possible variation due to allometric compositional differences (Rosenberg, 1980).

Finally, standard techniques (cf. Rhoads & Lutz, 1980) were used to make acetate peels and thin sections of selected shells. Shell microstructure was examined to determine if the stains disrupted deposition of growth increments and crystal form.

Results: Acetate Peels and Thin Sections

Stains produce a growth reaction similar to the shell damage response that Taylor, Kennedy & Hall (1969) described in *Margaritifera* and Beedham (1965) described in *Anodonta*. This is shown in Figure 3. An organic layer is first produced. A prismatic layer is then secreted, and finally nacreous layers are deposited. Thus, the mantle is stimulated to repeat the shell layers from the outer to inner surface: periostracum, prismatic, and lamellar shell layers.

Results: Compositional Variations

Results of the EDS analyses are graphed in Figures 4-6. Fig. 4 shows the spectrum of elements in the nacre of specimens from Group I (Localities 8-12), Fig. 5 in the nacre of specimens from Group II (Localities 13-17), and Fig. 6 in the nacre of specimens from Group III (Localities 18-20). Except for Ca that is used for normalization, elements detected are listed along the abscissa. Values plotted on the ordinate are mean maximum ratios of the accessory element peak areas to the $Ca_{k\alpha}$ peak area. That is, the maximum ratio of any element relative to $Ca_{k\alpha}$ was determined for each specimen and then averaged for all specimens at a given locality. Probe traverses revealed that accessory elements are not distributed uniformly within the stains, so that use of maximum ratios more faithfully represents presence or absence of accessories.

At first glance, the graphs suggest that ratios increase in downstream direction (towards Marquette, Iowa in Group I; towards the Prairie du Chien sewage effluent in Group II, and toward the scrap metal yard in Group III). The trend in Group III seems especially prominent. However, none of the differences among localities is statistically significant (t-test) because the variances are enormous relative to the mean in almost all cases.

Some appreciation of the large range in accessory element:Ca ratios can be obtained from Table 1. Accessories range from one percent of the Ca peak to several hundred percent times the Ca peak.

The homogeneity of the variance of the ratios was thus tested within each group (F-test, Sokal & Rohlf, 1969:190). The locality having the lowest variance within each group was compared with all of the other localities in the group. In Groups I and III, localities farthest upstream (Localities 8 and 18 respectively) show the least variability. In Group II,

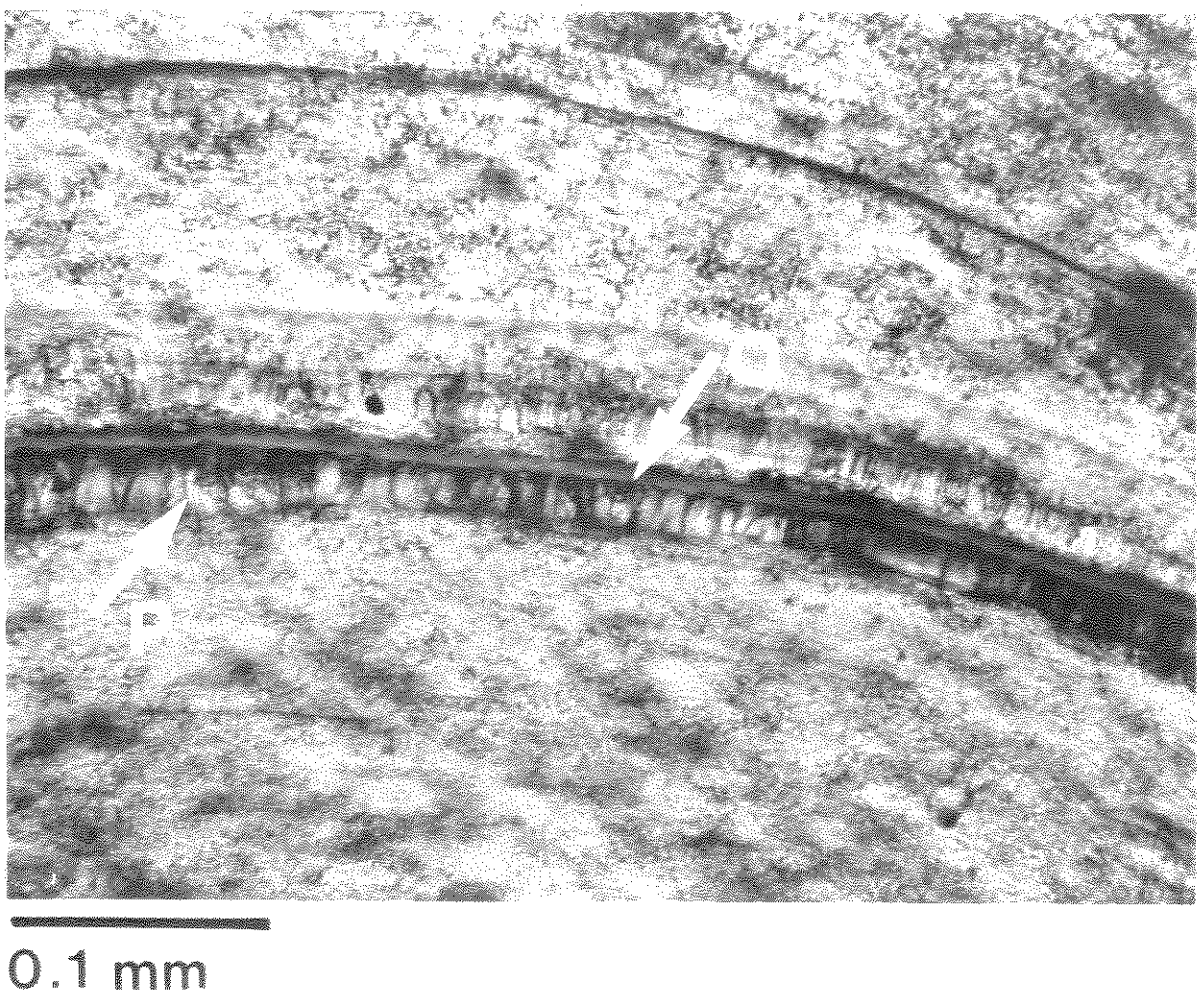

Fig. 3. Thin section of stained nacreous layer in *Amblema plicata* shell. Organic-rich lamellae (o) are initial disturbance response. Prismatic crystals (p) are deposited next, followed by return to nacreous shell secretion.

Locality 14 has the lowest variance for all elements, and it is upstream of all but Locality 13. The ratios of the variances are not recorded in the table, but the significance level at which they are greater (one tailed test) is given by * ($p<0.05$) and ** ($p<0.01$). In many cases the level of significance is considerably less than (0.01).

Table 1 shows that the variances increase just below the confluence of the Yellow and Mississippi Rivers (Locality 9), where the variances of all accessory ratios (except Cl and K) are significantly greater than those of their respective ratios within Locality 8. Except for Al and Si, the variances at Locality 10 are not significantly greater than those at Locality 8. The variances increase at Locality 11 and are maximal (not shown except in terms of level of significance) at Locality 12. Locality 10 is upstream of a barge fleeting area, Locality 11 is downstream of the same fleeting area, and Locality 12 is at Marquette, Iowa.

The trends are similar in Groups II and III on the eastern side of the river; variances for most elements increase downstream. They are greatest near Prairie du Chien's sewage treatment plant and a fertilizer plant (Localities 16 and 17, respectively within Group II) and just downstream of a scrap metal yard (Locality 20 within Group III).

Finally, it is possible to give 'order of magnitude'

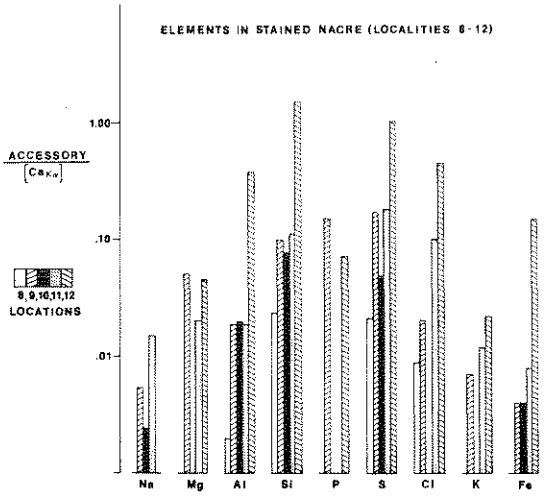

Fig. 4. Spectrum of accessory elements found within nacre of *Amblema plicata* from Group I. Elements detected are listed along abscissa. For each element there are 5 bars. Each bar refers to elemental concentration within mussels from one locality (respectively, Localities 8–12 from left to right). Mean-maximum concentration of each element relative to Ca (peak area ratios) is graphed on the ordinate. There are no significant differences ($p = 0.05$) in ratios within any locality, due to the large variances (Table 1).

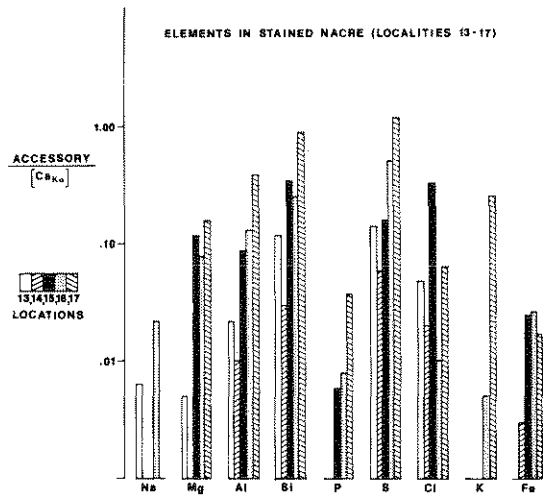

Fig. 5. Spectrum of accessory elements found within the nacre of *Amblema plicata* from Group II. Format of listing elements and their relative concentrations is the same as in Figure 4. There are 5 bars for each element respectively representing mean-maximum accessory to Ca ratios for Localities 13–17. There are no significant differences ($p = 0.05$ in ratios within any locality due to the large variances (Table 1).

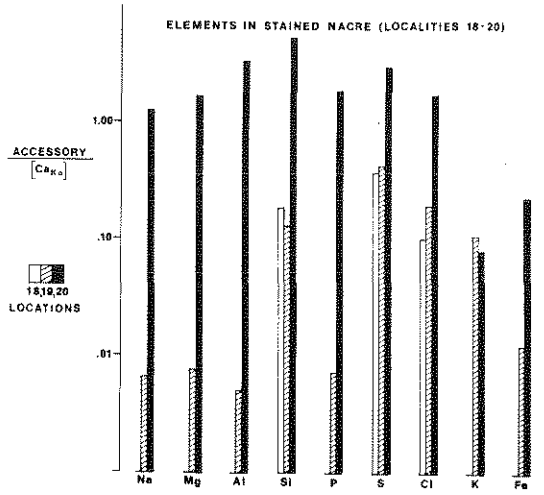

Fig. 6. Spectrum of accessory elements found within the nacre of *Amblema plicata* from Group III. Format of listing elements and their relative concentrations is the same as in Figure 4. There are 3 bar graphs for each element, respectively representing values for Localities 18–20. There are no significant differences ($p = 0.05$) in ratios within any locality due to the large variances (Table 1).

estimates of the maximum weight percentages of the accessory elements. Even though we used unpolished sections, it is possible to find flat areas on each specimen, analyses of which could be compared with standard analyses. Estimated maximum weight percentages of accessory elements are: 3% Si, 3% Al, 10% S, 6% P, 2% Fe and 1% for each of Mg, Cl, K, and Na. Unstained, white shell typically has less than 0.10% of any of these elements. Also, in extreme cases, Ca can be as low as 24% by weight in contrast to 40% Ca in stoicheometric $CaCO_3$ shell.

Discussion

Although there may be several causes of stained nacre (see Introduction), our data strongly suggest that turbidity is the most important cause. The high concentrations of Si and Al relative to Ca in some shells is clear evidence of clay minerals and finely particulate quartz trapped within the nacre. In some cases, we could rub stains off the nacre with a thumb, which indicates that these stains are adventitious and impermanent until subsequently co-

Table I. Maximum accessory element: Ca ratios in *A. plicata* shell.

	Locality	*Na*	*Mg*	*Al*	*Si*	*P*	*S*	*Cl*	*K*	*Fe*
	8	0.01	0.02	0.03	0.08	0.08	0.06	0.05	0.03	0.01
	9	0.03*	0.25**	0.07*	0.20*	1.03**	0.76**	0.05	0.05	0.03*
Group I	10	0.02	0.01	0.08*	0.17*	0.05	0.08	0.03	0.03	0.02
	11	0.07**	0.05**	0.10**	0.31**	0.08	0.63**	0.22**	0.09**	0.04**
	12	0.02	0.27**	1.50**	7.54**	3.49**	4.65**	1.91**	0.17**	7.45**
	13	0.05**	0.04*	0.12	0.34*	0.07	0.33	0.14	0	0.01
	14	0.01	0.01	0.06	0.10	0.08	0.21	0.10	0.01	0.01
Group II	15	0.01	0.60**	0.40**	1.54**	0.16	0.39	1.65**	0.03*	0.12**
	16	0.15**	0.43**	0.60**	1.16**	0.29*	2.31**	0.06	0.07**	0.14**
	17	0.03**	0.33**	0.74**	1.72**	0.83**	2.28**	0.08	0.48**	0.04**
	18	0.01	0.02	0.03	1.02	0.05	2.04	0.64	0.02	0.01
Group III	19	0.05**	0.34**	0.19**	0.56	0.14**	1.89	0.85	0.57**	0.06**
	20	2.76**	6.41**	10.47**	11.50**	4.58**	6.03*	3.34	0.44**	5.83**

* Significantly greater variance ($p < 0.05$) than control locality within respective group (Locality 8, 14, or 18).
** Significantly greater variance ($p < 0.01$) than control locality within respective group (Locality 8, 14, 18).

vered by additional shell material. Subsequent deposition of 'clean' shell material over stains also helps to explain why accessory elements are not always distributed uniformly over stained surfaces. If periods of high turbidity are not of long duration or are not severe, the presence of the accessory substances will be obscured with continued secretion of the shell.

Even though turbidity is a compelling cause of staining, the presence of stains dorsal to the pallial line (where the mantle becomes closely appressed to the shell) is something of a mystery. It is not clear how even fine clay particles can be transferred across the mantle in this area of the shell. Beedham (1965) did suggest that some silt could enter the mantle cavity through a crack in the ligament.

It is reasonable to suppose that high levels of other accessories in the nacre are due to their adsorption to the entrapped clay. Much pollution-related research has suggested that both inorganic and organic pollutants are concentrated on finely particulate clay minerals suspended in water and by organic compounds dissolved in water (see Fuller, 1974, for a discussion). This conclusion is not surprising because geologists have long known that heavy metals are often concentrated in organic rich shales and coals.

The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) notes that more than 150 agricultural pesticides are in use in this country and that 2/3 are potentially adsorbed onto sediment rendering them more biologically available. Phillips (1977) found that uptake of trace metals in bivalves is greater when metals are bound to food and to particulate inorganic matter. Pentreath (1973) determined that direct uptake of Zn, Mn, Co, and Fe by *Mytilus edulis* is minimal compared with that from food and particulate sources. Kapkov (1971) claimed that Cu bound with organic complexes is more toxic than the ionic form. Chaisemartin (1977) also found that Cu was more highly concentrated in the soft tissues of the freshwater bivalve *Margaritifera margaritifera* when the Cu was complex with organic matter. Imlay (1982) reported that freshwater mussel shells concentrate metals to a greater extent when metals are combined with suspended matter. Wiener *et al.* (1984) reported that toxic contaminants in the Upper Mississippi River occur largely in association with suspended particulate material and deposited sediments. One of the few sources that seem to contradict this relationship is that of Winger *et al.* (1984) who report that suspended sediment has no effect on the toxicity of copper-diquat to apple snails in Florida. However, it could be that apple snails have a low tolerance to copper-diquat to begin with.

Compositional analyses of molluscan soft tissue are typically highly variable, so it is not surprising that mean maximum accessory ratios in the shell of

A. plicata also have large variances.

Manly & George (1977) determined that Zn, Ni, Pb, and Cd concentrations were elevated in *Anodonta* (mantle, ctenidia, and kidneys) subjected to urban sewage. However, they found a considerable difference in concentrations within individuals from any given locality, with the greatest variation in juveniles. Fischer's (1983) study of Cd concentration in marine molluscs revealed that Cd content of soft tissues could be used as an index of pollution, but only in the most polluted habitats, because of the variability within individuals. Bryan (1973) described seasonal variations in (primarily heavy) metal content of *Pecten maximus* and *Chlamys opercularis* digestive gland and kidney. The data were highly variable in different individuals, but repeatable at the population level. Some of the seasonal variability was accounted for by seasonal oscillations in food supply. Concentration of the metals in tissues tended to rise in the autumn and winter months when phytoplankton productivity was lowest, and concentrations fell when productivity increased in the spring. Bryan (1973) noted that the reasons for the variability were probably very complicated, but one interesting hypothesis suggested that metal pollutants were distributed over a higher biomass during peak phytoplankton productivity periods. This effectively diluted the concentration of the metals in the scallop's food and consequently diluted the concentrations in the tissues. Carriker *et al.* (1982) analyzed 16 elements in the shell of living *Crassostrea virginica* with the proton microprobe. These researchers measured a range of concentrations of many of the elements (such as Na, Mg, Al, Si, S, Cl, Mn, Fe, Cu, Zn, Br, and Sr) in consecutive analyses of single as well as in different valves of *Crassostrea.* Carriker *et al.* (1982) accounted for the variations with several hypotheses. These included contamination of the shells by adsorption directly from seawater, ontogenetic changes in concentration, and passive concentration of metals adsorbed to inorganic detritus incorporated in the shell. The latter idea was originally developed by Immega (1976).

Our data suggest that the variance of the accessory ratios may prove to be a sensitive indicator of water quality. The increases and decreases in the variance are consistent with apparent changes in habitat. For example, within Group I the increase in variance of the ratios just south of the mouth of the Yellow River indicates that the Yellow is a significant source of turbidity. (The Yellow River drains the western bluffs which are extensively farmed). The decline in variance less than one mile downstream suggests that the effect is local. And the increase downstream of the barge fleeting area (Locality 11) suggests that stationary barges act like wing dams which are known to trap sediment and increase turbidity (Thiel, 1981). This is consistent with the increase in the variance within Group II at Locality 15, immediately downstream of a wing dam. The large variance of the ratios near Prairie du Chien's sewage effluent (Locality 16) testify to an effect on treated sludge on the biota. Finally, the highest maximum ratios and highest variances are found in Group III at Locality 20. This may be due to considerable river traffic within the Black River north of the confluence with the Mississippi, but it may also suggest that metals from the scrap metal yard nearby are being leached into the River. The fact that the largest ratios and highest variances are seen on the eastern shore of the Mississippi River at Locality 20 is consistent with Dawson *et al.*'s (1984) determination that pollution of the Mississippi near LaCrosse, Wisconsin is more severe on the eastern, than western shore. This is reasonable considering that farming activity (and consequent runoff) as well as industrial activity and population density are greater along the Wisconsin side of the River.

Although trends in the variance of the accessory ratios are consistent with apparent changes in habitat, we acknowledge that direct measurements of water parameters such as turbidity, metal ion content, and organic compound levels are needed to firmly establish the precision with which accessory ratios are bioindicators of water quality.

Finally, we wish to approach the biological significance of the accessory elements in *A. plicata* nacre. The question is whether any of the accessories are harmful in the maximum amounts detected or whether they are simply sequestered passively in the shell. Two lines of evidence suggest the former. First, the mussels were scarce at the localities where the maximum accessory ratios were highest and most variable: Localities 16, 17 and 20. Second, it is known that pollution induces bivalves to secrete extensive mucous (Ellis, 1936) or specific metal-binding proteins (in some cases sulfated metallothioneins) (Viarengo *et al.*, 1982) in an attempt to

protect themselves against the toxic effects of heavy metals and particulate material in the environment. The high levels of P, S, and Mg in some of the shells suggest to us an adverse response to particulate material and adsorbed metals in the water.

S is normally a component of the protein fraction of the shell (although in the maximum amounts recorded, some S could also occur within the crystal lattice – it is not readily possible to distinguish the different phases with the electron microprobe). It is not clear in which phase the P occurs within the *A. plicata* nacre. Bevelander & Benzer (1948) and Biedermann (1902) long ago reported $Ca_5(PO_4)_3$ within the mollusc shell. These researchers proposed that the shell was initially secreted as $Ca_5(PO_4)_3$ which was subsequently converted to $CaCO_3$. Other researchers who tried to repeat their work could not detect $Ca_5(PO_4)_3$ and so the suggestion was rejected by most malacologists (Wilbur, 1964). However, recent reports of P as well as many other accessory elements in molluscan mantle spherulites (Davis *et al.*, 1982; Petit *et al.*, 1981) and even in the inner shell lamellae (Marsh & Sass, 1983, 1984) have reopened the matter. In any event, the large maximum amounts of S and P detected in *A. plicata* (see Results) does suggest a disturbance in the mussel's calcium physiology. Moreover, Mg is a crystal poison, and there is growing consensus that Mg normally present within the extrapallial fluid acts to inhibit crystallization of mollusc shell (Wilbur & Saleuddin, 1983). The high Mg content, high S and P content (as indices of high organic content) and low Ca content in the shell seem to present a consistent picture of inhibition of normal Ca metabolism. Accessory metals probably retard mineralization by competing with Ca for binding sites (Wilbur & Bernhardt, 1984). As Simkiss & Mason (1983) write, the molluscan body load of metals reflects that of the environment; the mollusc seems to be a non-specific accumulator of toxic substances. It may be that the mollusc's use of its shell to sequester toxic substances has diminishing returns if the pollution is continuous and severe. Then pollution inhibits the normal metabolism of Ca, ultimately depressing the amoung of Ca in the shell.

Alternatively, it must be noted that Ca content is depressed only in stained areas and these are not always extensive. This may be a small price to pay for safely sequestering toxic ions and crystal poisons. We see no apparent growth deformities in stained vs. unstained *A. plicata*. But Murphy, Paparo & Sparks (1980) found shell deformities in fingernail clams (*Musculium transversum*) that were kept in well water with elevated levels of elements such as K, Al, Cd, Zn, Mg, and Fe. The researchers concluded that deformities were directly due to substitution of Si for Ca in the shell.

Of course, *M. transversum* has a paper-thin shell and is at most 12 mm in length. *A. plicata* and other thick-shelled species can easily deal with these substances without suffering shell deformities which afflict thin-shelled species. This may help to explain why *A. plicata* can survive at all in turbid waters.

If further research supports the role of silt and dissolved organic matter in exasperating the deleterious effects of metal pollutants on calcification, then increased agricultural activity and urban growth within the Midwestern United States have important implications for mussel diversity. Increased tillage of the soil promotes increased siltation in, and eutrophication of, lakes and streams. Increased population promotes increased sewage and industrial effluent. Those who have suggested that diminishing mussel diversity along the Mississippi River since the 1900's is due to increased siltation (Havlik & Stansberry, 1977; Thiel, 1981; Duncan & Thiel, 1983) may wish to consider the synergistic effects of combined particulate and metallic pollution on aquatic biota.

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