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Assessing the history of trace metal (Cu, Zn, Pb) contamination in the North Sea through laser ablation—ICP-MS of horse mussel *Modiolus modiolus* shells

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ABSTRACT: Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to determine the elemental composition of the incremental growth record in the shells of longevous, (~35 yr old) horse mussels *Modiolus modiolus*. Concentrations of the contaminant metals Cu, Pb and Zn were determined from shells collected in 1984 from 2 sites in the southern North Sea, an 'impacted dump' site and a 'control' site distant from known point source inputs. The age of each shell was determined from the pattern of annual growth lines present in thin shell sections, and each growth line was assigned a date, thus allowing annual concentrations of these elements to be determined. Using LA-ICP-MS, concentrations of the metals were determined in 5 replicate laser ablations (size ~100 × 200 µm), from individual summer and winter growth lines along the length of each of 3 shells from the dump site and 3 shells from the control site. A significant effect of age (year) on Cu, Pb and Zn concentrations in the shells from both sites was apparent, indicating changing concentrations with time. Significant seasonal (summer/winter) effects were only obvious in the concentrations of Pb and Zn in the shells; a non-significant difference in seasonal incorporation of Cu was observed. Concentrations of all 3 metals were significantly elevated in the dump site shells compared with the control site shells. Pearson correlation coefficients, determined for pairs of metals in the shells from both sites, indicated a higher number of significant positive correlations in the concentrations amongst the metals in the dump site shells than in the control shells during certain periods of the mussels' life. Levels of Cu, Zn and Pb were significantly higher in the dump site shells between 1968 and 1974 than in the period from 1975 to 1979. In the control site shells, although Pb and Zn significantly declined during the same periods, the concentrations were substantially and significantly lower than in the dump site shells. The implementation of the Dumping at Sea Act (in 1974) and the subsequent decline in dumping in the North Sea may have resulted in a decrease in the concentration of metals in the shells from the impacted dump site.

KEY WORDS: Mussels · *Modiolus modiolus* · Growth lines · Heavy metals · Contamination · North Sea · Laser ablation ICP-MS

INTRODUCTION

Annual variation in seawater temperature is reflected in the shells of bivalve molluscs, both as an annual surface growth ring and an internal growth line (Rosenberg & Runcorn 1975, Rhoads & Lutz 1980, Richardson 1993), and is recorded as a change in the stable oxygen isotope composition $\delta^{18}O$ of the shell carbonate (O'Neil et al. 1969), providing a record of the animals' ontogenetic growth. During the process of biomineralisation of the shell many elements are incorporated, together with the calcium that forms the bulk

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of the skeletal calcium carbonate structure of the animal, at the time of calcification (Wilbur & Saleuddin 1983). If the material within and between the annual growth rings or growth lines could be chemically analysed, then the possibility exists for studying both seasonal and temporal variations in the incorporation of environmentally sensitive elements such as the heavy metals lead, copper, cadmium and zinc found at very low levels in marine environments.

Early studies using the electron microprobe provided an insight into the chemical variation in bivalve shells (Rosenberg 1973, Rosenberg & Jones 1975) and demonstrated tidal fluctuations in sulphur and calcium in the shells of cockles *Cerastoderma* (= *Cardium*) *edule*, and annual patterns of sulphur concentrations in *Spisula* sp. However the electron microprobe can only be used successfully to determine elements which are present at concentrations above the detection limits of the instrument, such as calcium, strontium and magnesium in the case of bivalve shells (Rosenberg & Jones 1975). Using a suite of analytical techniques, proton microprobe (μ-PIXE), instrumental neutron activation analysis (INAA) and α-track autoradiography, Carell et al. (1987) determined the temporal history (~100 yr) of elemental concentrations in the incremental growth record in the shells of the freshwater mussel *Margaritifera margaritifera* from 4 rivers in central Sweden. They found that manganese and sulphur had increased after 1940 in response to the worsening of environmental conditions such as the acidification of rivers.

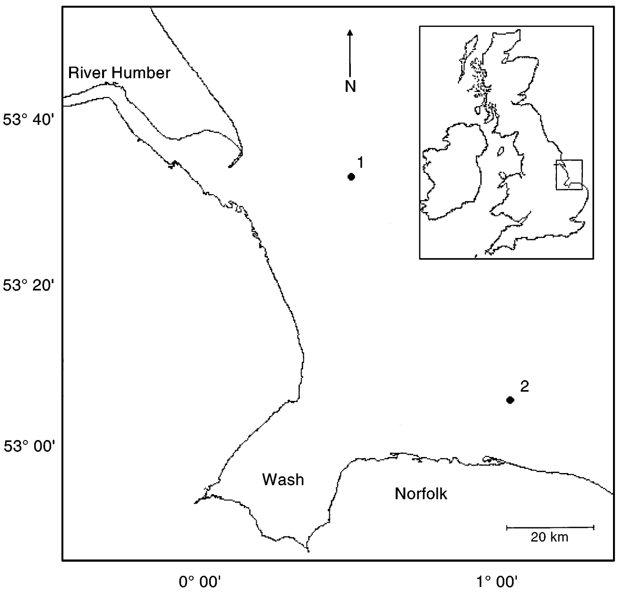

Fig. 1. Location of the sampling sites in the southern North Sea. The dump site (Site 1) off the River Humber and the control (Site 2) off the North Norfolk coast

Within the last 15 yr, the development of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (Gray 1985, Jarvis et al. 1992) has opened up the possibility of studying a range of elements in the chemical record of growth. The potential of LA-ICP-MS methodology in such investigations has been demonstrated in the analysis of the limpet *Patella vulgata* and the mussel *Mytilus edulis* from the coastline of Wales (Perkins 1992, Fuge et al. 1993). These studies have indicated the considerable potential of mollusc shells for revealing the past history of chemical contamination, with geographic variations in concentrations of the heavy metals copper (<1 to >5 mg kg^{-1}), lead (<1 to >15 mg kg^{-1}) and zinc (2 to 30 mg kg^{-1}) being demonstrated in limpets and mussels (Perkins 1992). Using LA-ICP-MS, Price & Pearce (1997) demonstrated in spot samples taken across shell profiles of *Cerastoderma edule* from a number of sites in the British Isles that shell growth was punctuated by short-term fluctuations in Zn and Cu, and they speculated that these levels had been caused by contaminated river run-off from mining and mineral processing and local contamination from electroplating effluent.

Horse mussels *Modiolus modiolus*, dominant members of many shallow-water benthic communities, are widely distributed and can attain ages in excess of 35 yr (Anwar et al. 1990). The present study investigates 2 North Sea populations of horse mussels, 1 from an 'impacted waste dump' site to the east of the Humber estuary, which has historically received a mixture of sewage sludge and industrial waste (e.g. 1.1×10^5 and 5×10^3 wet t respectively in 1986), and the other off the Norfolk coast (control), distant from known point-source inputs (Rees & Nicholson 1989) (Fig. 1). Murray et al. (1980) have monitored this dump site and reported the elevation of organic carbon and certain trace metals in sediments at or near the site that could be attributed to both the disposal of sludge at the site and the influence of the industrialised Humber estuary. The Dumping at Sea Act of 1974 (DSA), which subsequently became the Food and Environment Protection Act, was introduced during the period in which the growth of these mussels was being monitored, and thus this legislation may have had an impact on metal accumulation in the shells during their lifetime. Since horse mussels can achieve considerable longevity and their age can be reliably estimated from the alternating patterns of light (summer) and dark (winter) growth lines present in acetate peel replicas of polished and etched shell sections (Anwar et al. 1990), they can potentially be used to study temporal changes in metal contamination at the 2 sites over 30 or 40 yr periods. In the present study we evaluate the use of LA-ICP-MS for analysing the elemental composition of the incremental growth record in horse mussel shells from the impacted and control sites in order to establish a chronology of the temporal changes in environmental levels of trace elements that the organism might have been exposed to during its lifetime.

MATERIALS AND METHODS

Archived samples of air-dried *Modiolus modiolus* shells from 2 sites in the North Sea, an 'impacted waste dump' site to the east of the Humber estuary and a 'control' site off the North Norfolk coast (Fig. 1), collected using an anchor dredge by the Centre for Environment, Fisheries and Aquaculture Research (CEFAS) during February and March 1984, were used in the study. The left shell valve from each of 3 similar-sized (age) shells (length, umbo-rim axis, 54 to 109 mm) from the impacted site (Shells 1 to 3) and 3 similar-sized (age) shells (length 50 to 112 mm) from the control site (Shells 4 to 6) were selected for analysis. Owing to the difficulty of preparing and then accommodating large (>30 mm long), thin shell sections in the laser ablation cell of the ICP-MS, each shell was cut perpendicular to the umbo-rim axis into ~3 pieces of similar size, before mounting in epoxy resin, cutting, and then polishing with abrasives followed by diamond paste to a thickness of 100 ± 10 µm. The age of each mussel was estimated from the alternating pattern of wide (light) and narrow (dark) lines seen in sections examined under a low power microscope (Anwar et al. 1990). Each thin shell section was photographed, and the positions of the winter and summer growth lines marked on photographic enlargements.

Prior to analysis by LA-ICP-MS the elemental concentrations in a *Modiolus modiolus* shell from the dump site were determined to establish the range of concentrations that might be encountered during the study (Chenery & Cook unpubl.). The laser ablation microprobe (LAMP) used in the study consisted of a Spectron frequency quadrupled Nd:YAG UV laser operating in the far-UV spectrum (266 nm), linked to a transmitted light microscope. The shell section under investigation was placed in a perspex cell (30 mm diameter) beneath the microscope, and the specimen illuminated in either transmitted or reflected light. After comparison with photographic enlargements of the section, the appropriate area of shell was selected for investigation, and the laser was fired for 30 s through the microscope while manually rastering the laser beam. The ablated material was transported in a continuous flow of argon to a VG PlasmaQuad 2+ ICP mass spectrometer where vapourisation and ionisation by the hot (6000 °K) argon plasma occurred and concentrations of Cu, Pb and Zn were determined (for further details and the advantages and disadvantages of the techniques see Chenery & Cook 1993, Querol & Chenery 1995a,b).

Data collected by the ICP-MS are in raw response units for laser-ablated material. To correct for differences in isotopic abundance, ionisation efficiency and differences in response due to mass, calibration using solutions of known concentration for each isotope was performed. The calibration was applied to the responses from the laser-ablated material, and the data corrected by subtraction of a blank, 1% HNO_3 acid, aspirated into the plasma during laser ablation; several blanks were introduced during the individual analytical runs and applied to the data. The absolute amount of material ablated by the laser varies from ablation to ablation, and thus a correction was made using the naturally occurring internal standard of calcium, the absolute concentration of which was calculated from its stoichiometry in calcium carbonate. Solutions were used as standards, rather than international carbonate reference materials or pure calcium carbonate powders doped with trace element solutions, because of inhomogeneity problems (Perkins & Pearce pers. comm.). However, data produced were checked against reference materials NIST glass (SRM611) and limestone (BGS376). Concentrations of the 3 elements are reported in mg kg^{-1}.

The shell of *Modiolus modiolus* consists of a narrow outer calcite prismatic layer, a wider aragonitic middle nacreous layer (see Fig. 2) and an inner nacreous layer (see Taylor et al. 1969 for further details of mineralogy). The middle nacreous layer was chosen for investigation because: (1) there was a greater amount of material available for ablation compared to the other layers, (2) the outer prismatic layer was more likely to be subject to shell damage (Richardson 1993) and surficial sediment contamination, and (3) the inner nacreous layer in some mytilids is known to undergo seasonal aragonite deposition and dissolution (Lutz & Clark 1984). The laser is capable of routinely achieving high spatial resolution, with crater sizes from 40 µm diameter and 50 µm depth down to <1 µm diameter and <1 µm sample depth, depending on the detection limits required. In this study the relatively large area of shell between and within individual growth lines allowed the laser beam to be rastered manually over an area of ca 200 × 100 µm to obtain both the best detection limits and an integrated 'average' sample from the seasonal growth periods.

The proposed positions of the ablation craters, previously marked on the narrow dark (winter) and wide (summer) growth lines on photomicrographs of each shell section, were compared with the section that was examined through the microscope of the laser ablation system. For consistency, all 3 portions of each shell section were analysed in 1 analytical session. To assess the annual variability in Cu, Pb and Zn between the summer and winter growth lines, 5 replicate ablations were made in each summer and winter growth period along the entire length of each shell from the umbo to the shell margin. The growth periods closest to the shell margin, i.e. the most recently deposited, converge and could not always be analysed. Also the large raster size, relative to the width of the narrow winter growth periods, made it more difficult to analyse the lines accurately. Scanning electron microscopy (SEM) was used to assess the effects of ablation and penetration of the laser beam with respect to the section surface.

To investigate differences in the Cu, Pb and Zn concentrations relating to the age of the mussel, differences between individual mussels from the 2 sites, site differences and seasonal effects (winter or summer), analysis of variance (ANOVA) with 4 factors (age, season, site and individual) using a general linear model (GLM) for each element were undertaken. Normality of data and homogeneity of variance of the data were checked prior to the analyses. The age range over which the metal concentration analyses in the 6 shells were undertaken was restricted to the period between 1968 and 1979. This avoided potential problems associated with the convergence of the growth lines at the shell margin and loss of the outer shell layers in the umbo region during the early period of the life of the horse mussels. The time-scale also covered the period during which the DSA was introduced (i.e. 1974). Possible correlations between the concentrations of the 3 metals in the shells from the dump site and control site were investigated using Pearson correlations during the period prior to the introduction of the DSA (1968 to 1974) and post-DSA (1975 to 1979). A 1-way ANOVA was also used to investigate any differences between the metal concentrations in the dump site shells and in the control site shells during the dumping (1968 to 1974) and post-dumping periods (1975 to 1979).

RESULTS

Sections of horse mussel *Modiolus modiolus* shells show an alternating pattern of narrow, dark, winter lines and wide, white, summer lines (Fig. 2A). A winter line can be traced through the middle shell layer and accurately ablated with the laser (Fig. 2B,C), although at the shell margin of the larger (older) shells the lines are narrow and compressed so that the ablation rasters for each summer and winter region of the shell are stacked progressively closer together and are more difficult to separate. During ablation the laser beam does not usually penetrate the shell section into the underlying resin and glass slide, so there is minimal, if any, contamination from the underlying material. The angular nature of the rastered crater on the shell surface and the rhomboidal appearance of the aragonite crystals parallel to the direction of shell growth at the bottom of the crater indicate that the shell remains undamaged by the laser (Fig. 2D). Instead, during ablation, a significant proportion of crystals are removed by thermo-mechanical shock rather than by melting, vaporisation or decomposition of the shell.

Table 1 shows the results ANOVA/GLM of the Cu, Pb and Zn concentrations in the shells. There is a significant effect of age (year) ($p < 0.001$) on the Cu, Pb and Zn concentrations ($F = 15.55$, 37.97 and 17.86 respectively) in the shells from both sites, indicating changing concentrations with time. Significant ($p < 0.05$) seasonal (summer/winter) effects were only apparent in the concentrations of Pb and Zn in the shells ($F = 20.90$ and 4.23 respectively), although these metals were not consistently elevated or depressed in the winter and summer growth periods. A non-significant ($p > 0.79$) seasonal effect in the concentrations of Cu was observed ($F = 0.07$) (Table 1). Concentrations of all 3 metals were significantly different ($p < 0.001$) between the dump site and control site (site effects) The interactions between age × season and age × site were similarly significant for all 3 metals. However, the interaction term season × site displayed no significant differences ($p > 0.38$) in metal concentrations, thus indicating that the seasonal variability in metal concentrations was similar at both sites (Table 1). The interaction term age × season × site is significant for Pb and Zn ($F = 9.21$ and 4.39 respectively) and just non-significant for Cu ($F = 1.8$, $p > 0.51$). Finally, the analyses demonstrated that there were significant ($p < 0.001$) differences in the concentrations of Cu, Pb and Zn in the shells from the dump site and control site (individual [site]) (Table 1).

Comparisons of Zn-Cu, Pb-Cu and Pb-Zn in the dump site shells and control shells show significant positive Pearson correlations between the concentrations of these elements during the periods 1968 to 1974 and 1975 to 1979 (Table 2). Similar significant positive correlations were apparent only between Zn-Cu and Pb-Zn in the control shells in 1968 to 1974 and between Zn-Cu in 1975 to 1979; Pb-Cu were negatively correlated in 1975 to 1979 (Table 2). In the dump site shells higher metal concentrations were present between 1968 and 1979 compared with those in the control shells (Table 3). Copper, Pb and Zn were significantly elevated between 1968 and 1974 compared with the period 1975 to 1979. However, only Pb and Zn were elevated in the control shells; Cu was not significantly different (Table 3).

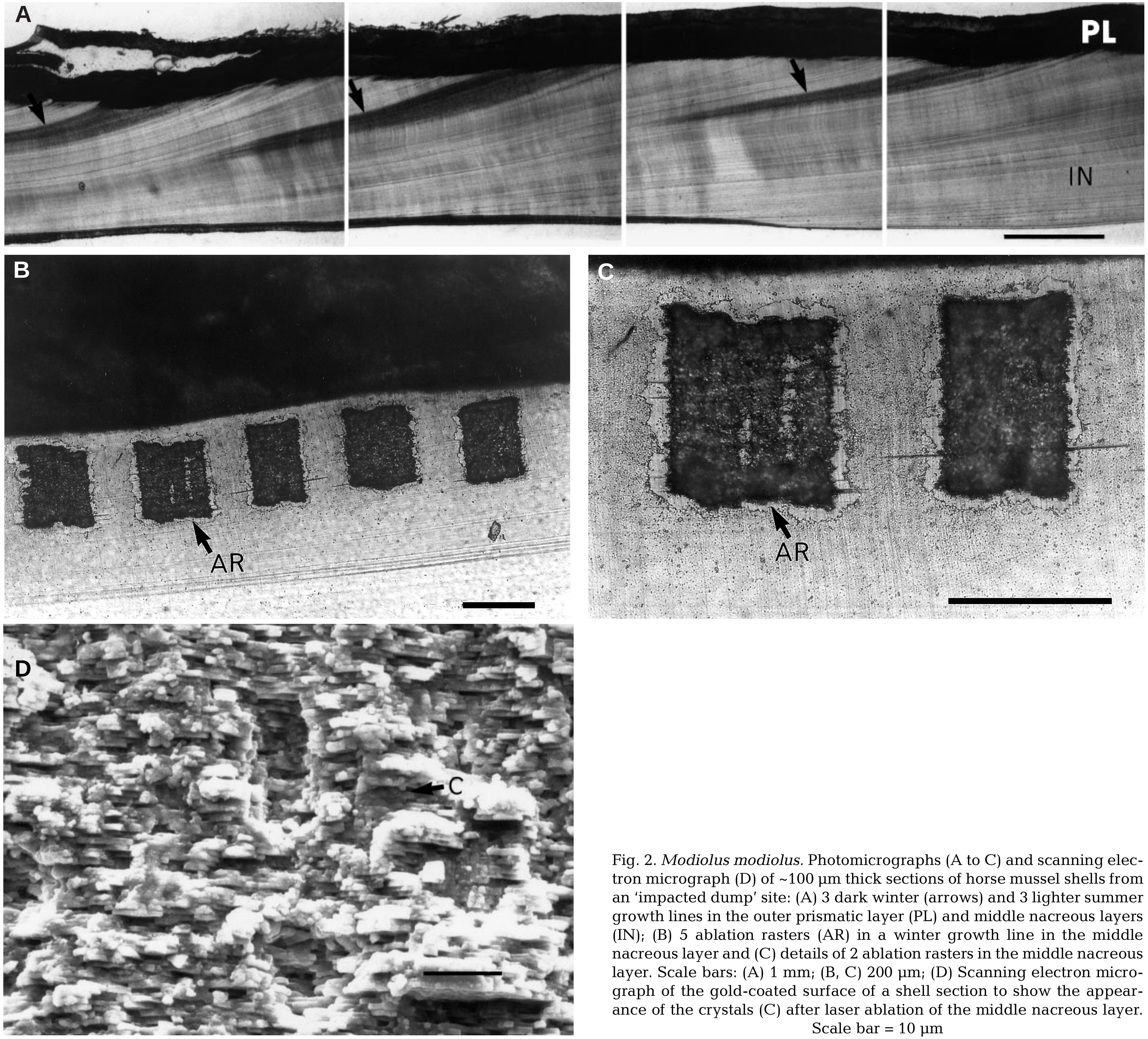

Fig. 2. *Modiolus modiolus.* Photomicrographs (A to C) and scanning electron micrograph (D) of ~100 µm thick sections of horse mussel shells from an 'impacted dump' site: (A) 3 dark winter (arrows) and 3 lighter summer growth lines in the outer prismatic layer (PL) and middle nacreous layers (IN); (B) 5 ablation rasters (AR) in a winter growth line in the middle nacreous layer and (C) details of 2 ablation rasters in the middle nacreous layer. Scale bars: (A) 1 mm; (B, C) 200 µm; (D) Scanning electron micrograph of the gold-coated surface of a shell section to show the appearance of the crystals (C) after laser ablation of the middle nacreous layer. Scale bar = 10 µm

Table 1. ANOVA with 4 factors (age, season, site and individual) for copper, lead and zinc concentrations in the shells of *Modiolus modiolus* (NS: not significant)

Source of variation	df	MS	*F*-value	p
Copper concentrations				
Age (year)	11	131.54	15.55	0.001
Season	1	0.59	0.07	0.792^{NS}
Site	1	1063.20	125.67	0.001
Individual (site)	4	769.42	90.95	0.001
Age × Season	11	17.46	2.06	0.021
Age × Site	11	144.02	17.02	0.001
Season × Site	1	6.47	0.77	0.382^{NS}
Age × Season × Site	11	15.21	1.80	0.051^{NS}
Error	610	8.46		
Total	661			
Lead concentrations				
Age (year)	11	30.41	37.97	0.001
Season	1	16.74	20.90	0.001
Site	1	325.25	406.12	0.001
Individual (site)	4	6.46	8.07	0.001
Age × Season	11	5.97	7.46	0.001
Age × Site	11	14.2	17.72	0.001
Season × Site	1	0.08	0.10	0.746^{NS}
Age × Season × Site	11	7.38	9.21	0.001
Error	610	0.80		
Total	661			
Zinc concentrations				
Age (year)	11	202.27	17.86	0.001
Season	1	47.87	4.23	0.040
Site	1	2395.85	211.53	0.001
Individual (site)	4	1270.95	112.21	0.001
Age × Season	11	49.56	4.38	0.001
Age × Site	11	279.05	24.64	0.001
Season × Site	1	5.50	0.49	0.486^{NS}
Age × Season × Site	11	49.75	4.39	0.001
Error	610	11.33		
Total	661			

Table 2. Summary of Pearson correlations between copper, lead and zinc concentrations in shells of *Modiolus modiolus* from the 'impacted dump' site and 'control' site during the periods 1968 to 1974 and 1975 to 1979. (All correlations significant at $p < 0.001$, except * where $p < 0.05$; NS: not significant)

Site, period	Metal	1968–1974		1975–1979	
		Copper	Zinc	Copper	Zinc
Dump site					
1968–1974	Zinc	0.290	–		
	Lead	0.191*	0.457		
1975–1979	Zinc			0.620	–
	Lead			0.339	0.339
Control site					
1968–1974	Zinc	0.230	–		
	Lead	-0.103^{NS}	0.292		
1975–1979	Zinc			0.413	–
	Lead			−0.256*	0.361^{NS}

Temporal variations in Cu, Pb and Zn in the 6 shells are shown in Fig. 3. The strongest observable Cu event was in Shell 3 from the dump site and lasted from 1968 to 1973, although the magnitude of the event was not seen in the other shells from either site during this time (Fig. 3A,B). In this individual there was a small amount of damage to the outer surface of the shell where high copper levels were recorded. A smaller event occurred in an undamaged region of the same shell (Shell 3), and of Shell 2 from the impacted site during 1975, but not in Shell 1. Concentrations of Cu in the 3 control shells, however, remained around 1.5 mg kg^{-1} during the same period (Fig. 3B). Fig. 3C,D shows similar temporal variations in Zn concentrations. Overall, Shell 3 from the impacted site contained higher concentrations of Zn than the other 2 shells. In this mussel, 2 events can be seen, 1 in 1972 and 1 in 1975. Whilst the event in 1972 is only based on 1 point, 2 points comprise the rise in 1975, and a similar event occurred in this latter year in Shell 2, albeit with a smaller increase in concentration. These 2 events coincide with generally elevated zinc levels in Shell 1 between 1970 and 1975 (Fig. 3C). Zinc concentrations remained around 4 mg kg^{-1} in the 3 control shells, with a suggestion that levels have been gradually declining since 1968 (Fig. 3D). Of the 3 metals analysed, lead shows the most significant number of similar events (Fig. 3E,F). At the dump site, concentrations of Pb were elevated in all the shells in 1970, 1972 and 1974, whereas from 1969 to 1975 levels showed a decrease in Shells 1 and 3. Additionally there is an underlying trend in all the shells, indicating that Pb was at an elevated level between 1970 and 1975. Lead concentrations were lower in the control shells (~1 mg kg^{-1}). Amongst the 3 control site shells there were correlated events in 1968, 1969 and 1972 (Fig. 3F), although the magnitude of the events was considerably less than those observed in the dump site shells (~2 to 5 mg kg^{-1}). Since 1969, Pb levels in the control shells have declined.

The elemental analyses have demonstrated that there were events during the life of the mussels from the impacted site when elevated concentrations of the heavy metals Cu, Zn and Pb were incorporated into the shells and that a correlation in events existed in certain years between shells from the same site. Concentrations of Cu, Zn and Pb display large fluctuations both within and between years, with the highest levels recorded between 1968 and 1975. Concentrations of metals, particularly Cu and Zn, in the control site shells, however, remained at a much lower level and showed little variation (Fig. 3B,D). Pb concentrations, though, were more variable (Fig. 3F). These temporal variations in Cu, Pb and Zn are summarised in Fig. 4 to illustrate the general trend in the metal concentrations in the dump site and control site shells. The DSA was introduced in 1974, and the figure allows a visual comparison of metal concentrations in the pre-DSA versus the post-DSA periods.

Table 3. 1-way ANOVA of copper, lead and zinc concentrations in shells of *Modiolus modiolus* from the 'impacted dump' site and 'control' site during the periods 1968 to 1974 and 1975 to 1979. (NS: not significant)

Site	Metal	Mean ± SD (mg kg^{-1}) 1968–1974	1975–1979	*F*-value	p
Dump site	Copper	5.04 ± 7.42	2.13 ± 1.90	21.67	<0.001
	Lead	3.65 ± 1.55	1.76 ± 1.46	130.57	<0.001
	Zinc	9.52 ± 7.29	6.59 ± 6.13	15.27	<0.001
Control site	Copper	1.51 ± 0.88	1.38 ± 0.73	1.68	NS
	Lead	1.67 ± 0.60	0.87 ± 0.47	157.18	<0.001
	Zinc	4.04 ± 2.69	2.15 ± 0.69	56.48	<0.001

DISCUSSION

Horse mussels *Modiolus modiolus* have a wide distribution from the low intertidal where they occur occasionally, to fjords and deeper offshore locations where they can form extensive mussel beds which may be at risk from anthropogenic activities such as dredging and pollution. Unlike their smaller, common and more widely distributed relative *Mytilus edulis*, the large size, greater longevity and clear pattern of annual growth lines in horse mussel shells make them suitable organisms for the chronological study of metal contaminants in the marine environment. Mussels are generally sedentary in habit, tolerant of a wide range of environmental conditions and can filter large volumes (2 to 3 l h^{-1}) of seawater across their gills, concentrating many chemicals in their tissues by factors of 10 to 10^5 relative to the concentration in seawater. They are also integrators of chemical contamination in a given area, making them useful organisms for monitoring environmental contamination (Widdows & Donkin 1992). Aspects of the mechanisms of uptake and deposition of metals in bivalves are reviewed in Viarengo (1989), Livingston & Pipe (1992) and Simkiss et al. (1992).

The accumulation of metals in molluscs has been mainly studied from the standpoint of their accumulation in the soft tissues. However, elements can accumulate in the shell, which acts as a receptor for some metals (Lin & Liao 1999). Koide et al. (1982) found that Zn, Pb and Cd levels were higher in the tissues of *Mytilus edulis* and *M. californianus* than in their shells; although Cu concentrations were greater in the shell than in the tissues. Bourgoin (1990) demonstrated that Pb levels in the nacre of *M. edulis*, collected from near a lead smelter off the coast of Dalhousie, Canada, were only $\frac{1}{10}$ of the levels in the tissues. Lead levels in whole shells of the New Zealand cockle *Chione* (*Austrovenus*) *stutchburyi* were similar to those in the soft tissues (Purchase & Ferguson 1986). Highest lead levels were found in the umbo region (oldest part), and lowest in the posterior younger regions of the shell. Lead and cadmium enrichment of *M. edulis* shells has been demonstrated experimentally by Sturesson (1976 and 1978 respectively).

Bivalve shells could prove useful in determining historical records of metal contamination. Bertine & Goldberg (1972) analysed the shells of museum specimens of *Mytilus edulis* and razor clams *Ensis siliqua* and *E. directus* collected from the Belgian coast during the last 100 yr, but could

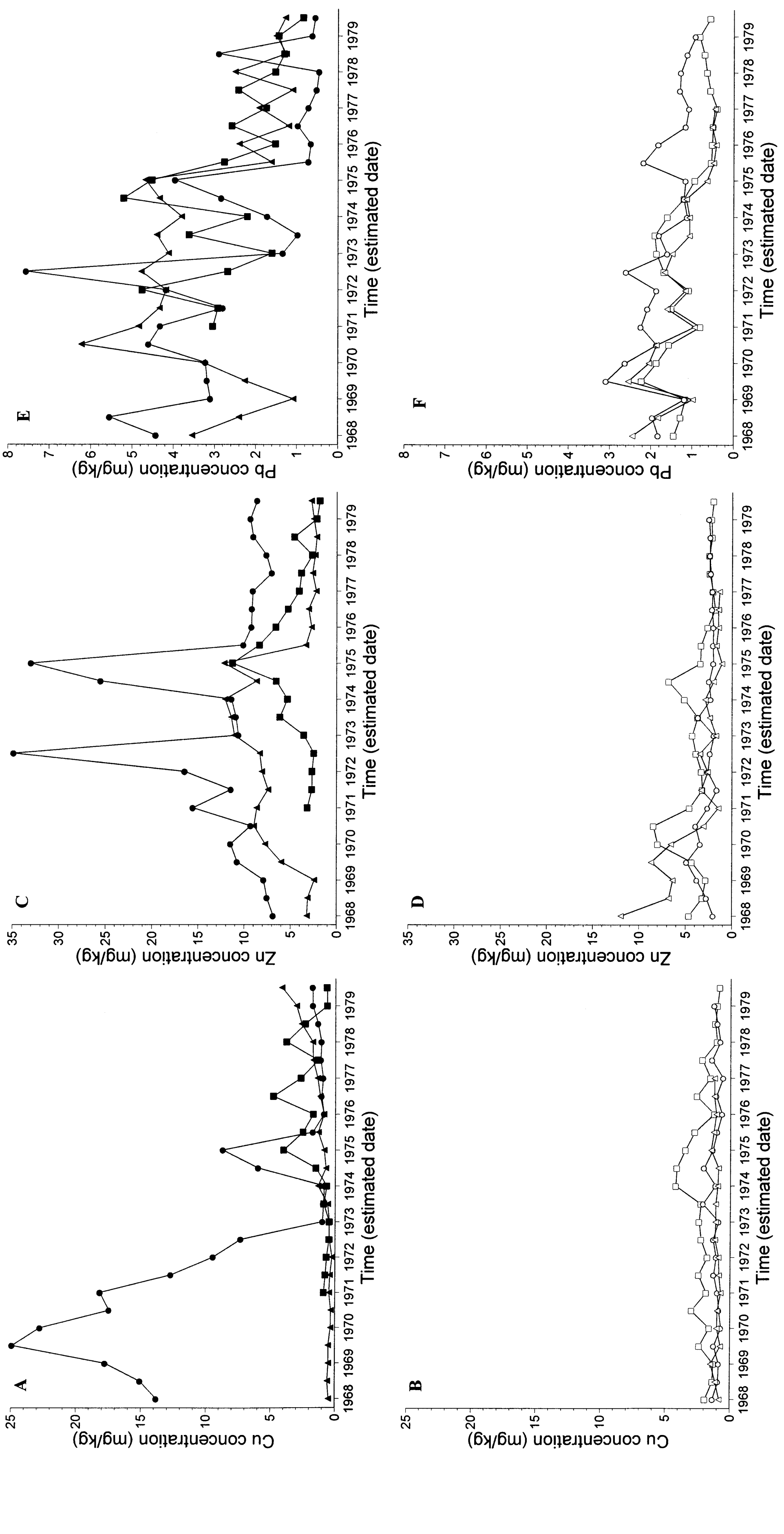

Fig. 3. Variations in contaminant element concentrations in 3 *Modiolus modiolus* shells from an 'impacted dump' site (▲: Shell 1, ■: Shell 2, ●: Shell 3) and 3 from the 'control' site (□: Shell 4, ○: Shell 5, △: Shell 6) between 1968 and 1979: (A, B) copper levels, (C, D) zinc levels and (E, F) lead levels

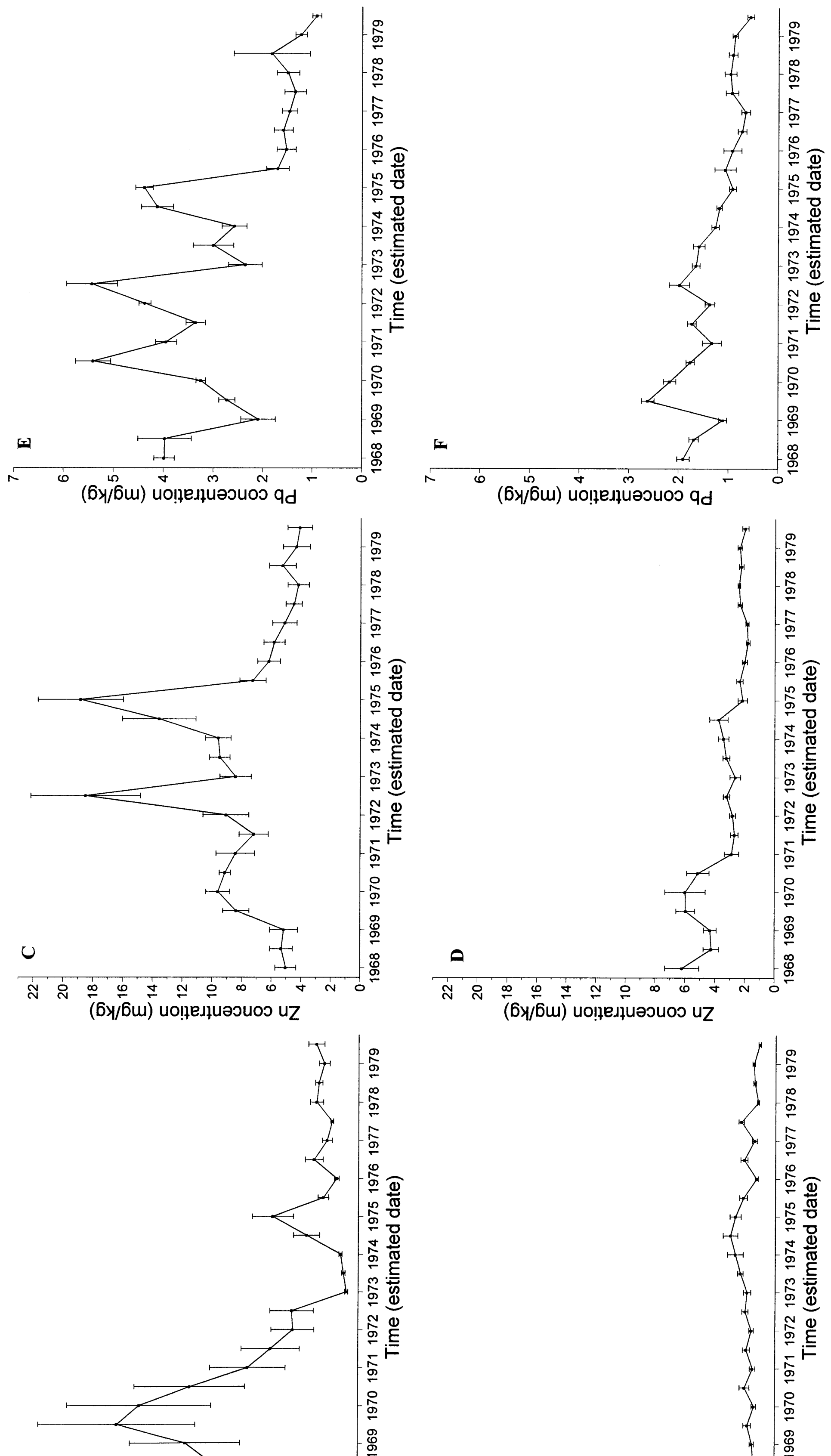

Fig. 4. Temporal variations in the mean (±SD) contaminant elements in *Modiolus modiolus* shells from an 'impacted dump' site (upper panels) and 'control' site (lower panels) between 1968 and 1979: (A, B) copper levels, (C, D) zinc levels and (E, F) lead levels

find no evidence of recent increases in metal contamination as a consequence of Man's activities. Koide et al. (1982), however, found markedly higher concentrations of Cd, Cu, Zn and Pb in the shells of *M. edulis* and *M. californianus* from industrialised and populated areas, compared to those areas devoid of human habitation. Geographical variations in the Cu, Zn and Pb content of the shells of the limpet *Patella* sp. from along the rocky intertidal coastline of Wales were accounted for by differences in their exposure to drainage water from Pb, Zn and Cu mines discharging into Cardigan Bay, Wales (Perkins 1992).

The use of shell growth patterns for identifying the physical and biological factors affecting shell growth is widely established (Rosenberg & Runcorn 1975, Rhoads & Lutz 1980, Richardson 1993 and references therein). A combination of growth increment analysis with elemental investigations provides an opportunity for interpreting the high-resolution-detailed record preserved in marine mollusc shells. The development of LA-ICP-MS, together with a detailed understanding of the molluscan shell growth record, has opened up new possibilities for linking contamination to particular years in the shell, thus offering the potential for investigating historical records of contamination of coastal waters in a fashion not previously possible with soft tissue analysis. The growth record in horse mussel shells is best preserved in the middle nacreous layer of the shell (Anwar et al. 1990), where there is sufficient material for elemental analysis.

The approach using detailed sampling of the summer/winter lines has demonstrated the potential for investigating seasonal and annual variations in metal contamination in horse mussel shells. The concentrations of Cu, Zn and Pb correlated in the shells from the contaminated dump site, and Zn and Pb displayed significant, but not consistent, differences between summer and winter growth. There were also significant differences in the metal concentrations between the impacted and control site shells. In the impacted shells there were periods during the life of the mussels, between 1968 and 1976, when Cu, Zn and particularly Pb levels were elevated in the shells. During the same period, concentrations of these elements in the control shells generally remained at around background levels. The elemental concentrations observed in this study are similar in magnitude to those recorded in other bivalve shells from the coastal waters of the British Isles. Perkins (1992) and Fuge et al. (1993) typically recorded concentrations of Cu, Zn and Pb of from 4 to 14, 20 to 330 and 3 to 17 mg kg^{-1} respectively in *Mytilus edulis* shells and Cu and Pb levels of 0.5 to 3 and 2 to 50 mg kg^{-1} respectively in *Arctica islandica* shells from Cardigan Bay, Wales. These concentrations, in bivalves from an area known to be contaminated from mining activities, are within the range of values recorded in horse mussels from the impacted dump site in the southern North Sea.

It is known that historically there have been changes in the contaminants emanating from the Humber estuary, although it has been difficult to find quantitative evidence in this regard. Gardiner (1982) reported the results of trace metal monitoring in this estuary from 1961 to 1981, and concluded there was some evidence that concentrations of Cu and Pb in waters entering the Humber estuary had decreased between the mid-1970s and the 1980s. Murray et al. (1980) compared various sources of inputs into the coastal waters off the Humber in the area where the dump site horse mussels were collected, and concluded that dumping was a significant source of Cu and Hg, although they did not investigate whether there were any temporal variations in inputs. Taken together, both these studies suggest that there has been a change in contamination inputs during the period of the horse mussels' growth, between the early 1960s and 1970s, and 1984, when the horse mussels were collected.

The introduction of the DSA in 1974, during the monitored period of the mussel growth, is likely to have had an impact on heavy metal concentrations in the shells between 1964 and 1984. Significant differences in Cu, Pb and Zn were observed in the dump site shells during the period prior to and after introduction of the DSA. Thus, there is indication that implementation of the legislation in 1974 may have contributed to the decrease in metal concentrations in the shells from this site (Fig. 3). However, both Pb and Zn concentrations in the control shells also showed a significant decline during the 2 periods, although the levels were substantially lower than in the dump site shells, possibly suggesting a general improvement in metal contamination levels of the Humber estuary and the surrounding coastal waters. Given the small number of shells analysed it is perhaps premature to draw any significant conclusions about the actual implementation of the legislation on the Cu, Zn and Pb concentrations in the shells. Nevertheless, the feasibility of using ontogenetic records in bivalve shells for investigating past metal contamination in the marine environment has been demonstrated. Such information, together with experimental evidence of metal incorporation, should allow the shells to be used as historical archives of the concentration of heavy metals in the marine environment.

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