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## Metal pollution as a potential threat to shell strength and survival in marine bivalves



Bryce D. Stewart <sup>a,\*</sup>, Stuart R. Jenkins <sup>b</sup>, Charlotte Boig <sup>c</sup>, Catherine Sinfield <sup>d</sup>, Kevin Kennington <sup>e</sup>, Andrew R. Brand <sup>f</sup>, William Lart <sup>g</sup>, Roland Kröger <sup>c,\*</sup>

<sup>a</sup> Department of Environment and Geography, University of York, North Yorkshire, United Kingdom

<sup>b</sup> School of Ocean Sciences, Bangor University, Menai Bridge, United Kingdom

<sup>c</sup> Department of Physics, University of York, North Yorkshire, United Kingdom

<sup>d</sup> Mount Sinai Hospital, New York City, New York, United States

<sup>e</sup> Department of Environment Food and Agriculture, Isle of Man Government, Isle of Man

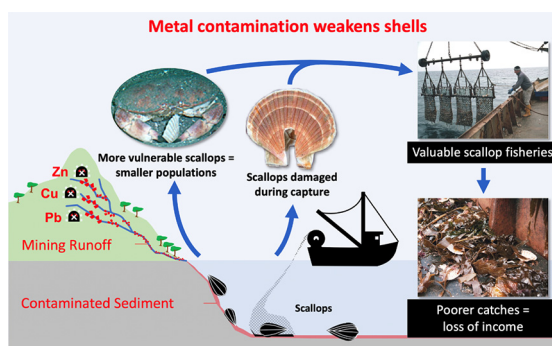
<sup>f</sup> School of Environmental Sciences, University of Liverpool, Liverpool, United Kingdom

<sup>g</sup> Sea Fish Industry Authority, Grimsby, United Kingdom

### HIGHLIGHTS

- Lethal damage to scallops was higher during fishing at a metal contaminated site.
- Higher damage correlated with lower shell strength and disrupted shell structure.
- A wide range of alternative explanations to metal contamination were ruled out.
- Bivalve shell characteristics should be used more in ecotoxicology assessments.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Marine bivalve molluscs, such as scallops, mussels and oysters, are crucial components of coastal ecosystems, providing a range of ecosystem services, including a quarter of the world's seafood. Unfortunately, coastal marine areas often suffer from high levels of metals due to dumping and disturbance of contaminated material. We established that increased levels of metal pollution (zinc, copper and lead) in sediments near the Isle of Man, resulting from historical mining, strongly correlated with significant weakening of shell strength in king scallops, *Pecten maximus*. This weakness increased mortality during fishing and left individuals more exposed to predation. Comparative structural analysis revealed that shells from the contaminated area were thinner and exhibited a pronounced mineralisation disruption parallel to the shell surface within the foliated region of both the top and bottom valves. Our data suggest that these disruptions caused reduced fracture strength and hence increased mortality, even at subcritical contamination levels with respect to current international standards. This hitherto unreported effect is important since such non-apical responses rarely feed into environmental quality assessments, despite potentially significant implications for the survival of organisms exposed to contaminants. Hence our findings highlight the impact of metal pollution on shell mineralisation in bivalves and urge a reappraisal of currently accepted critical contamination levels.

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\* Corresponding authors.

E-mail addresses: [bryce.beukers-stewart@york.ac.uk](mailto:bryce.beukers-stewart@york.ac.uk) (B.D. Stewart), [roland.kroeger@york.ac.uk](mailto:roland.kroeger@york.ac.uk) (R. Kröger).

## 1. Introduction

Marine bivalve molluscs deliver a range of ecosystem services such as water filtration and sediment consolidation and provide a quarter of the world's seafood (Daily, 2003; FAO, 2018; Grabowski et al., 2012). For example, in 2018 scallops supported the third most valuable fishery in the UK (£70 million) (MMO, 2019), and the fourth most valuable in the USA (US\$541 million) (NMFS, 2020).

Bivalve shells provide essential protection from the external environment, particularly against predators (e.g. the crushing claws of crabs and lobsters) and physical disturbance from waves and currents; (Beadman et al., 2003; Telesca et al., 2019). Therefore, shell strength essentially determines a population's resilience. Shells are composed of crystalline calcium carbonate (most common are the calcite and aragonite polymorphs) along with a small percentage of organic matter (Grefsrud et al., 2008). The thickness and microstructure of shells is highly variable between both individuals and areas, which appears to be predominately driven by environmental conditions, as it is not explained by genetic differences (De Noia et al., 2020; Telesca et al., 2019; Vendrami et al., 2017). This variability can affect the mechanical strength of shells, and therefore an organism's survival (Grefsrud et al., 2008; Grefsrud and Strand, 2006; Vendrami et al., 2017). Unfortunately, the estuaries and coastal waters inhabited by marine bivalves are frequently contaminated with a wide range of pollutants, in particular metals, from land-based human activities (Bryan and Langston, 1992; Luoma and Rainbow, 2008). Pollution from metals is an increasing threat to marine ecosystems because of their continued discharge and persistent nature (EEA, 2015; Luoma and Rainbow, 2008), exacerbated by an increased re-mobilisation of contaminated sediments by coastal development and towed fishing gear (Bradshaw et al., 2012; Luoma and Rainbow, 2008). Future increases in ocean acidity and temperature through anthropogenic greenhouse gas emissions (IPCC, 2019) are expected to increase the effects of metal ions and compounds on bivalve molluscs and other marine organisms, by influencing metal speciation and therefore bioavailability, rate of uptake and toxicity in seawater (Millero et al., 2009; Roberts et al., 2013; Sezer et al., 2020; Shi et al., 2016). Moreover, increased storm events due to climate change will increase runoff and further elevate introduction of metals into marine environments (EEA, 2015), even from long disused mining areas.

Bivalves in contaminated areas readily accumulate metals (e.g. Zn, Pb and Cu) into both their soft tissues (predominately during feeding) and shells (Richardson et al., 2001; Sturesson, 1976). In sufficient concentration, such metal contamination is toxic and therefore can significantly elevate mortality and impair growth rates (Beaumont et al., 1987; Calabrese et al., 1977). Incorporation of metals into mollusc shells occurs since elements such as Pb and Zn follow the same intracellular pathway as Ca during the biomineralization process (Jordaens et al., 2006), thereby substituting for Ca when shells develop in contaminated areas. This presence of metals in the shell matrix has been hypothesized to disrupt shell integrity and strength (Jordaens et al., 2006). Alternately, metals may modify the activity of proteins during the crystallisation process and disrupt shell microstructure formation in that way (Sharma et al., 2008).

However, previous studies of the impact of metal pollution on the shell biomineralization have produced variable results, highlighting the necessity for a better understanding (Zuykov et al., 2013). For example, in terrestrial snails, contrasting effects of different metals were found (e.g. negative effects of Pb on shell mass (Beeby et al., 2002), uptake of Zn but no effects on shell strength (Jordaens et al., 2006)), as well as variability between populations and species (Fritsch et al., 2011). In comparison, in freshwater mussels dramatic negative effects of Cu, Cr and Cd on shell morphology have been reported (Faubel et al., 2008; Lopes-Lima et al., 2012). In marine environments, where the chemistry of calcification and the bioavailability of metals is quite different from terrestrial and freshwater systems, shell integrity as indicator of metal pollution has been little studied and is considered unproven (Zuykov

et al., 2013). Given that metal pollution is considered an increasing threat to marine ecosystems (Rogers and Laffoley, 2011), improving predictions of the ecological and economic impact on marine bivalves, including interactions with predicted future changes in ocean chemistry and temperature, should clearly be a priority area for research.

In order to further investigate these effects of metal pollution on marine bivalve shell properties we chose to examine commercially valuable king scallops, *Pecten maximus*, at a site known to be contaminated with metals, and at uncontaminated control sites, around the Isle of Man in the Irish Sea. This design enabled us to explore the long-term effects of metal exposure on susceptibility to damage, fracture strength, thickness and microstructure of scallop shells in a rarely explored real-world setting (Mayer-Pinto et al., 2010). Specifically, a significant loss of scallops due to shell fracturing during dredging had been reported for the Laxey site near the Isle of Man, motivating our interest to investigate the possible causes of this observation.

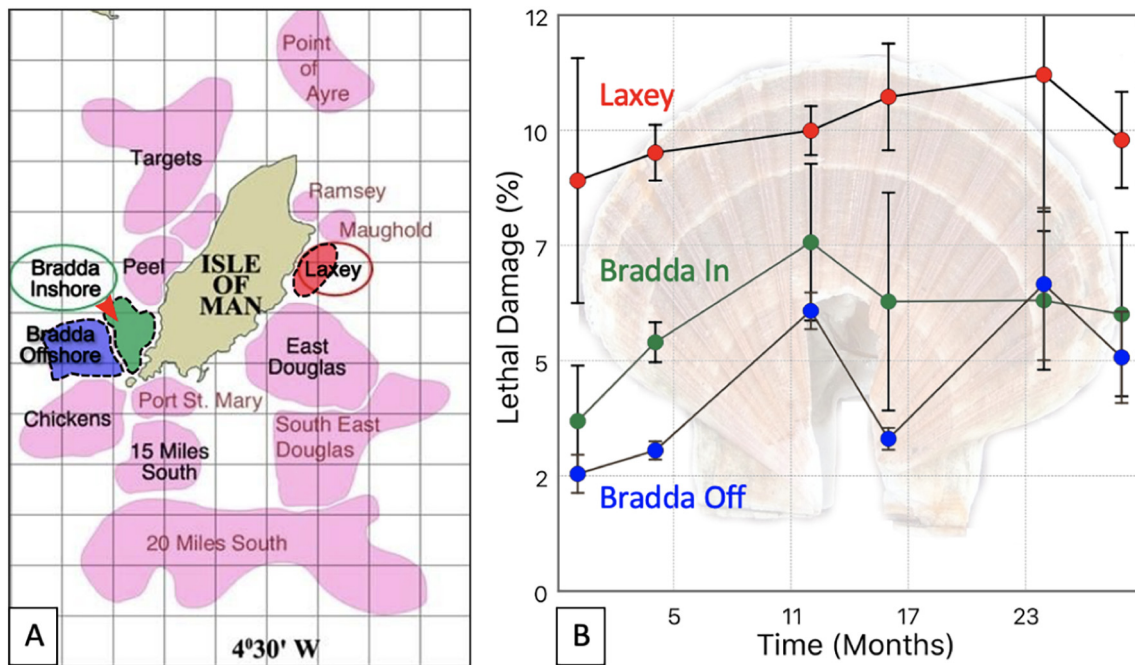
## 2. Methods

### 2.1. Study site and species

Our study was based on samples collected in the coastal waters around the Isle of Man, in the North Irish Sea. The Isle of Man's fisheries are dominated by scallops, particularly the high value king scallop, *Pecten maximus*, and the lower value, but at times highly abundant queen scallop, *Aequipecten opercularis* (Beukers-Stewart et al., 2003; Vause et al., 2007). The king scallop, the focus of this study, can grow to over 20 cm shell length and live for over 20 years (Beukers-Stewart et al., 2005), although most individuals on commercially exploited fishing grounds are less than 12 cm in length and 6 years old (Beukers-Stewart et al., 2003). Due to habitat and environmental preferences, scallops tend to have a patchy distribution around the Isle of Man, occurring in a series of discrete beds ranging from close inshore to the 12 mile boundaries of the territorial sea (Beukers-Stewart et al., 2003), (Fig. 1). Due to the legacy of land-based mining in the 19th and 20th centuries, several rivers, estuaries and therefore nearshore waters and sediments around the Isle of Man experience elevated concentrations of zinc and lead, and to a lesser extent copper and cadmium (Kennington, 2018). At Laxey, off the east coast, such contaminated sediment overlaps with parts of the inshore scallop fishing ground (this study). This situation provided us with the ideal opportunity to directly compare scallop populations at this contaminated site with those at control sites with lower levels of contamination whereby all other known environmental parameters (water temperature, salinity and nutrients) remained similar (Kennington, 2018; Kennington and Hiscott, 2018, see Section 2.3). Sea surface temperatures around the Isle of Man are strongly seasonal, ranging from a low of 5 to 6 °C in February to a high of 15–16 °C in August, while decadal perturbations in the salinity levels reflect variations in the strength of the North Atlantic Oscillation. Nutrient levels are also strongly seasonal, with the nitrogen pool largely exhausted after each spring phytoplankton bloom. However, despite this high level of temporal variability, spatial variability in these parameters appears to be low in the study area (Kennington, 2018). For this reason we selected a number of other locations around the Isle of Man as control sites to study the potential origins of the high rates of damage to the scallops from the Laxey site.

### 2.2. Spatial and temporal variation in scallop damage and mortality

Scallop populations around the Isle of Man have been routinely surveyed (initially bi-annually and then annually) for stock assessment purposes since 1992 (Beukers-Stewart et al., 2003; Murray et al., 2011; Vause et al., 2007). These surveys have typically been conducted using both standard commercial king and queen spring toothed scallop dredges (Lart, 2003) (generally four per side of the survey vessel), during which the densities (catch per unit effort), sizes and ages of both



**Fig. 1.** a) Map of the north Irish Sea showing the major *P. maximus* beds around the Isle of Man in pink. The names of the main fishing grounds are labelled in black, with the ancillary areas labelled with red. The contaminated site, Laxey, is circled in red, while our main control site, Bradda Inshore, is circled in green. Each square of the overlying grid is 5 nautical miles square (adapted from Beukers-Stewart et al., 2003). b) Mean percentage ( $\pm$  SE) of lethally damaged scallops (levels 3 and 4) captured in standard scallop dredges on three fishing grounds between June 2001 and October 2003 ( $n = 3$  dredge tows per site and date). A fatally dredge damaged scallop (grade 4) is shown in the background.

species of scallops are recorded on the main fishing grounds around the island. King scallops (*P. maximus*) are also visually assessed for damage to their shells on a four-point scale (Jenkins et al., 2001). A score of 1 indicates very minor damage, 2 moderate damage, 3 a broken hinge, and 4 major damage (see Fig. S1). Damage scores of 3 or 4 were assumed to be fatal – we tested this assumption using laboratory experiments (see below). We analysed the mean proportion of fatally damaged scallops caught in standard scallop dredges during bi-annual stock surveys at Laxey (contaminated) and five other (uncontaminated) fishing grounds; Bradda Inshore, Bradda Offshore, Chickens, Peel and Targets, from June 2001 to October 2003 (i.e. during a total of six surveys) (Fig. 1). The dredges were towed three times at each site on each date, generally catching between 100 and 200 scallops per tow (minimum 27, maximum 438). During the same surveys in June and July 2001, other characteristics which might be expected to physically damage scallops during the catching process (visual estimates of dredge fullness, and the volume of rocks retained in each dredge) were recorded. Furthermore, we also recorded the density (catch per unit effort / mean number per tow of the scallop gear) of brown crabs, *Cancer pagurus*, the main crustacean predator of king scallops around the Isle of Man (Beukers-Stewart et al., 2006). This was because previous studies (Beadman et al., 2003) had shown that high densities of crabs caused bivalves (in that case mussels, *Mytilus edulis*) to grow thicker and stronger shells, presumably as an anti-predator defence response. If king scallops respond to crabs in similar ways, then thicker and stronger shells could also reduce their susceptibility to damage by scallop dredges. Alternately / in addition, high predation pressure in areas of high crab density might selectively remove weaker shelled scallops, thereby elevating the mean shell strength of the surviving population.

### 2.2.1. Scallop survival experiments

During regular stock surveys (see above), 72 king scallops were collected on the 30th October and 69 on 31st October 2002. On each occasion these scallops were kept alive in 500 l aerated tubs of seawater on board the research vessel for approximately 4 h while they were

transported back to the laboratory. Once there they were transferred to separate 2000 l plastic flow through aquariums (a different one on each day) in the laboratory. Aquariums were maintained at ambient water temperature and on a 12 h day-night lighting cycle. The floor of the aquariums was kept bare. Of the 141 scallops collected, 110 were assessed as damage grade 1, 14 were grade 2 and 17 were grade 3. These scallops were then monitored every 2 to 3 days for 20 days, until no more grade 3 scallops were still alive.

### 2.3. Metal contamination of seabed sediments

Previous sampling in the 1970s and 1990s had demonstrated high levels of metals (particularly Cu, Pb and Zn) in the Laxey estuary / harbour sediments (Kennington, 2018). For this study we wanted to determine the contemporary concentration of metals in sediments at Laxey (the contaminated site), how this varied with distance from shore, and how these levels compared to Bradda Inshore, our main control site. To do this, three single Day Grabs of sediment were taken at each location on the 27th of February 2013. At each site an east to west transect was set up, with the first sample being taken approximately 500 m offshore, the second 1.5 km offshore and the third 3 km offshore. The locations of all of these sediment sample sites overlapped with commercial scallop fishing grounds (Beukers-Stewart et al., 2003).

Sediment samples were processed at the Isle of Man Government Laboratory (certified under ISO 17025). Each sample was dried at 105 degrees C until constant weight achieved and then 100 g of sediment was ground using a pestle and mortar and sieved through 120  $\mu$ m sieve. One gram of material was then microwave digested in reverse aqua-regia (7.5 ml conc HNO<sub>3</sub>, 2.5 ml conc HCl) and after digestion the solute was filtered and made up to 100 ml using deionised water. Analysis for metal content was undertaken using an inductively coupled plasma optical emission mass spectrophotometer (Varian Vista-MPX ICP OES). Multi-element standards used (250, 500, 750 micrograms/l) were obtained from Ultra Scientific (ICP/MS calibration standard #2). Certified reference material LGC 6145 was used for quality control.



## 2.4. Scallop morphometric analysis and strength testing

Based on the observed variation in scallop damage rates between fishing grounds (see results), we decided to investigate scallop shell morphology and strength as a possible explanatory factor. King scallop shells were collected for this purpose on two separate occasions (Table 1). Firstly, during stock surveys in June 2004, 20 scallops were collected from the contaminated site at Laxey, and another 20 scallops from each of five other uncontaminated sites around the island (Fig. 1). Upon return to the laboratory, these scallops (120 in total) were frozen whole (including internal soft tissues) for storage, before being defrosted for later analysis (see below). These scallops ranged in shell length (SL) from 100 mm to 115 mm (mean = 107.06 mm ( $\pm 0.42$ SE) SL). In order to determine the temporal consistency of patterns of shell strength, a second batch of king scallops was collected from just the Laxey (contaminated) and Bradda Inshore (control) grounds (5 from each, 10 in total) during dredge surveys by the Isle of Man government during February 2013 (the same day the sediment samples above were taken) (Table 1). These scallops were slightly larger than previously, ranging in size from 113 to 119 mm SL (mean = 115 mm ( $\pm 0.89$ SE) SL) and were also initially frozen whole, before being defrosted and dissected, with only the shells retained for later analysis. Shells were cleaned using deionised water and dried at room temperature for at least 4 weeks.

### 2.4.1. Shell morphometrics

The morphometrics of all shells was measured to the nearest mm using Vernier callipers. Only the shell length of the 2004 samples was measured (see Beukers-Stewart et al., 2003 for protocol), while for the 2013 samples the height, depth and weight (to the nearest 0.01 g) of the clean shells was also determined. Finally, the thickness of each top (left) and bottom (right) valve of these shells was measured at 1 cm intervals, using shell sections taken along the centre rib, from the umbo to the ventral edge (i.e. along the shell height axis).

### 2.4.2. Shell strength

The shell strength (maximum load force to shell fracture) of the two sets of scallops was assessed in similar, but slightly different ways. The 2004 samples were placed whole into an Instron Universal Testing Machine which had been fitted with the steel tooth from a scallop dredge. These teeth are approximately 11 cm long and 1 cm square, but the ends are cut diagonally to produce a sharp edge. The tooth was placed on the flat valve of each specimen, in between the 1st and 2nd growth ring, before the shells were compressed until they broke. In comparison, the strength of the 2013 samples was assessed using a Tinius Olsen machine fitted with a circular metal rod (diameter 1 cm). This rod was again placed between the 1st and 2nd growth rings on the flat valve of each

specimen. Scallops were held in a customised, cork covered sample holder and again compressed until they fractured.

## 2.5. Structural studies

The observed differences in shell strength between scallops caught at Laxey and Bradda in both 2004 and 2013 (see results) suggested they may also differ with respect to the shell structure. To shed light on this important aspect we employed a set of characterisation techniques of the 2013 samples covering different length scales (light and electron microscopy) and providing information on crystallinity (Raman spectroscopy) and element composition (energy dispersive X-ray spectroscopy).

### 2.5.1. Sample preparation

Cross-sections approximately 80  $\mu$ m thick were prepared, which had been cut in transversal and longitudinal direction of the shell ribs for both top and bottom valves. This preparation was performed using a diamond cutting wheel (Struers) to produce approx. 3 mm  $\times$  3 mm  $\times$  500  $\mu$ m slices which were subsequently ground to approx. 90  $\mu$ m thickness and further polished using diamond lapping pads (in a sequence of grain coarseness from 30  $\mu$ m down to 100 nm) so that specular surfaces were achieved on both sample sides targeting a total slice thickness of approx. 80  $\mu$ m. Selected cross-sections were surface etched for scanning electron microscopy using a 0.1 M acetic acid solution. To prepare a sample for transmission electron microscopy we selected one of the non-etched cross-sections and thinned it down to electron transparency using a tripod polishing holder and applying the H-bar method (Patterson et al., 2002) using a focused ion beam (FIB) system (FEI Nanolab) available at the JEOL York Nanocentre.

### 2.5.2. Optical microscopy

The achieved thickness of approximately 80  $\mu$ m permitted good light transmission and hence observation of structural details down to approximately 1  $\mu$ m extension in transmission optical light microscopy. This was performed using an Olympus BY41 optical microscope equipped with 5 $\times$ , 10 $\times$ , 20 $\times$ , and 50 $\times$  objective lenses and a CCD camera for image recording.

### 2.5.3. Raman microspectroscopy

Micro-Raman spectroscopy was performed using a Horiba Xplora system with a 532 nm semiconductor diode excitation laser providing a lateral spot extension of approximately 1  $\mu$ m. For spectrum recording we used a 2400 T grating to obtain the highest spectral resolution of 1  $\text{cm}^{-1}$  and an acquisition time of typically 1 s and 10 accumulations. This helped to minimize laser induced damaging of the sample and reduced any detrimental interaction with the mineral matrix.

**Table 1**

Dates and details of the field work and laboratory analysis conducted as part of this study. Based on our initial investigations from 2001 to 2004 we decided to conduct further analysis in 2013. This involved repeating the shell strength experiments to ensure consistency over time, testing the sediment for metal contamination and analysing the shell microstructure.

	Scallop damage rate	Dredge fullness & volume	Crab catch per unit effort	Scallop survival rates	Scallop shell strength	Sediment contamination levels	Scallop shell micro-structure
2001	X	X	X				
2002	X			X			
2003	X						
2004					X		
2013					X	X	X

### 2.5.4. Scanning and transmission electron microscopy and energy dispersive X-ray spectroscopy

For the surface topography analysis of cross-sections we used an X30 SEM as part of the FEI Nanolab FIB at 5 kV acceleration voltage as well as an FEI Sirion S-FEG field emission SEM at the York JEOL Nanocentre, which was operated at 5 kV for imaging analysis and 12 kV for energy dispersive X-ray spectroscopy (EDX) analysis. The instrument is coupled with a Noran EDX system which uses an Oxford INCA analysis system and a 30 mm 2 light element Silicon-Lithium (SiLi) detector. The resolution of this microscope under optimum conditions is <2 nm (FEI instrument specifications), however, the sample type and characteristics (e.g. surface morphology and electrical conductivity) limits the resolution to 5 nm.

Transmission electron microscopy (TEM) studies were performed using an image and probe aberration corrected JEOL 2200 FS with a 200 kV acceleration voltage accessible at the York JEOL Nanocentre.

### 2.6. Data analysis

All data were tested for normality and homogeneity of variances (Levene's Test), and if necessary, data were transformed to meet assumptions of parametric tests (proportional data were arcsine square root transformed, ratio data were  $\ln(x + 1)$  transformed). Most data were analysed using Analysis of Variance (ANOVA) followed by post-hoc Student–Newman–Keuls (SNK) tests (as needed). If data did not meet the assumptions of these tests, then non-parametric alternatives (Kruskal–Wallis or Mann Whitney U Tests) were used instead.

## 3. Results

A key motivation of our work was to establish if and how the observed differences in scallop shell-damage were related to the variations of heavy-metal concentrations in the sediments of the fishing grounds around the Isle of Man. For this purpose we conducted a systematic study of the lethal damage of scallops caught at two opposite sites near the Isle of Man, namely Bradda and Laxey, measured the level of metal contamination in the sediments at these sites, determined the mechanical fracture strength of the shells for both sites, as well as a number of additional control sites, and analysed the shell structure for scallop shells from Bradda and Laxey.

### 3.1. Scallop damage and mortality around the Isle of Man

The proportion of scallops that were lethally damaged during capture in stock assessment surveys was determined at six sites around the Isle of

Man over three years (Fig. 1(a), Fig. S3). A four-level visual damage classification system was used, where lethal damage was classified as damage levels 3 and 4, as justified by our laboratory experiment (Fig. S2). The percentage of lethally damaged scallops varied over time at all sites, but was consistently higher at Laxey than at all other fishing grounds (2-way ANOVA: Site  $F_{5,96} = 22.099$ ,  $p < 0.001$ ; Date  $F_{5,96} = 5.485$ ,  $p < 0.001$ ; Site x Date  $F_{21,96} = 1.092$ ,  $p = 0.379$ ) (Fig. S3). Fig. 1b) shows damage rates at the three sites (Laxey and Bradda Inshore and Offshore) for which detailed analyses of scallop shells were subsequently performed. While scallops caught at Bradda Inshore and Offshore experienced lethal damage levels of 2–7%, those caught at Laxey revealed consistently higher mortality rates between 8.5 and 11%. Overall, the average lethal damage rate at Laxey was 9.6%, almost twice the average for the two Bradda sites (5.0%).

During the above surveys, we further estimated fullness and rock volume in each dredge as potential factors affecting damage to scallops. There was no difference in dredge fullness between fishing grounds and significantly fewer stones at Laxey than other grounds - counterintuitive to the higher damage there (Fig. S4a) & b)). We also recorded the density of brown crabs, *Cancer pagurus*, as a potential factor that could affect shell strength (see Methods), but again, there was no relationship between crab density and damage to scallops (Fig. S4c)). Although crab densities were relatively low at Laxey, they were also low at Targets, where damage rates were much lower and shells were significantly stronger than at Laxey (see below).

### 3.2. Metal contamination of seabed sediments

Our analysis of sediment samples at Laxey and Bradda and a previous study (Daka and Hawkins, 2004) revealed that metal contamination (Zn, Cu and Pb) was elevated at the Laxey shoreline and decreased with distance offshore (Fig. 2). Against the internationally recognised Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME, 2002), both Zn and Pb were above the PEL (Probable Effect Level-the probable effect range within which adverse effects frequently occur) at the Laxey shoreline, with Cu being between the TEL (Threshold Effect Level - the possible effect range within which adverse effects occasionally occur) and PEL (CCME, 2002). At 500 m offshore (the approximate distance from shore at which scallops are first found (Beukers-Stewart et al., 2003)) Zn was still above the PEL, but Cu and Pb were between the TEL and PEL. At 1500 and 3000 m from shore both Cu and Zn were below the TEL with Pb between the TEL and PEL. In comparison, levels at Bradda for all metals analysed (Zn, Pb and Cu) were below the TEL level throughout (Fig. 2).

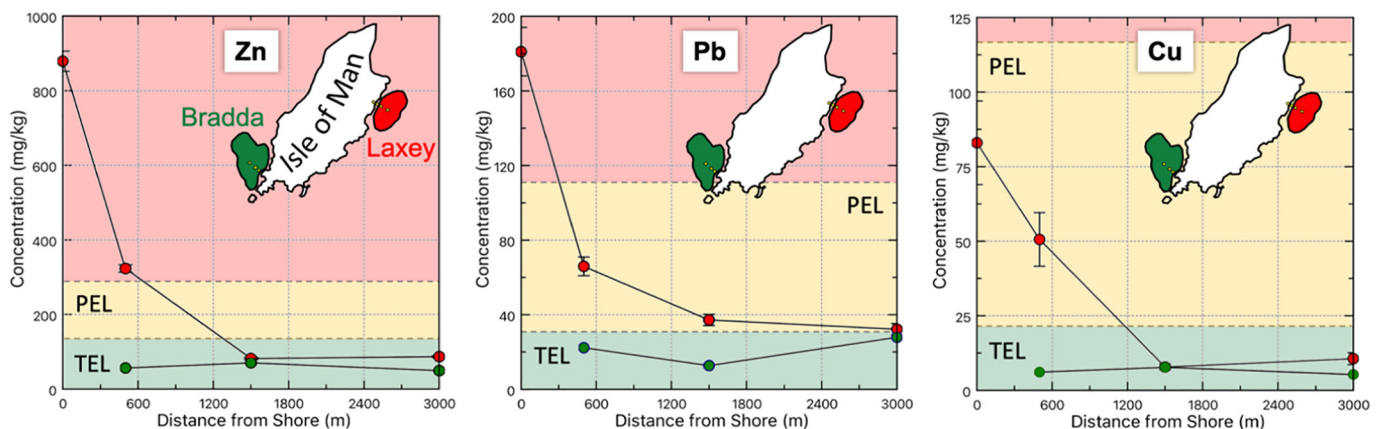
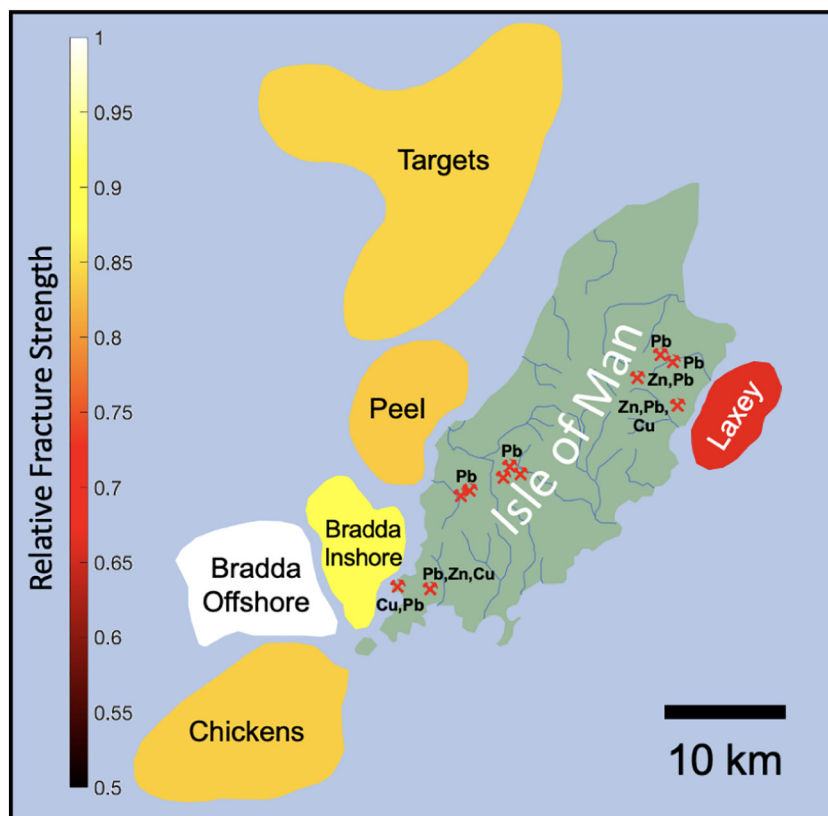


Fig. 2. Metal concentrations as a function of distance from the shore in sediments at the Laxey and Bradda Inshore sites. The shoreline measurement at Laxey (at 0 m) is from Daka and Hawkins (2004) while other measurements are from February 2013. Contamination levels at or above PEL are within the range at which adverse effects to organisms frequently occur, while between the TEL and PEL indicates the possible effect range within which adverse effects occasionally occur. Levels below TEL are considered safe for marine organisms (CCME, 2002). Yellow dots indicate the approximate locations where sediment samples were taken.



**Fig. 3.** Map of the relative mean fracture strength observed for *P. maximus* around the Isle of Man with the value for Bradda Offshore (911.77 N) used to normalise the values for all other sites. Shells were collected from six sites around the Isle of Man in 2004. The colour code indicates the value for the relative fracture strength. Laxey is the contaminated site, all the others are controls ( $n = 20$  per site, 120 in total). The value for the Laxey site is 0.66 whereas the values for the other sites are all above 0.83. Red symbols assign major historical mining sites for zinc, lead and copper ores with the order of importance indicated by their sequence position (Daka and Hawkins, 2004).

### 3.3. Scallop shell strength and morphometrics

Scallops were collected from the Laxey and Bradda Inshore sites for shell strength testing in 2004 and for strength testing and microstructural analysis in 2013 (see Fig. S5). Measurements of the individual weights of shells collected in 2013 allowed us to calculate a weight/length ratio for each scallop which served as an index of shell thickness and/or density. We found that shells at Bradda Inshore had a significantly higher weight/length ratio ( $0.914 \pm 0.021SE$ ) than at Laxey ( $0.763 \pm 0.031SE$ ), (one-way ANOVA:  $F_{1,9} = 16.338$ ,  $p = 0.004$ ). Furthermore, while the thickness of the curved bottom valves was generally similar at both sites, the flat top valves were up to 0.75 mm thinner at Laxey (Fig. S6).

The sample of scallop shells collected from Laxey and the five other fishing grounds in 2004 showed that shell strength varied significantly between fishing grounds (one-way ANOVA:  $F_{5,119} = 5.503$ ,  $p < 0.001$ , Fig. 3). A post-hoc SNK test revealed that shells were significantly weaker at Laxey ( $603.90 N \pm SE 40.67$ ,  $n = 20$ ) compared to all other sites. The second sample of scallops collected in 2013 also found that shells were significantly weaker at Laxey (mean =  $746.32 N$ ,  $\pm SE 85.56$ ,  $n = 5$ ) than Bradda Inshore (mean =  $1174.42 N \pm SE 100.66$ ,  $n = 5$ ), (1-way ANOVA:  $F_{1,9} = 10.50$ ,  $p = 0.012$ ).

### 3.4. Disruption of mineral organisation in scallop shells

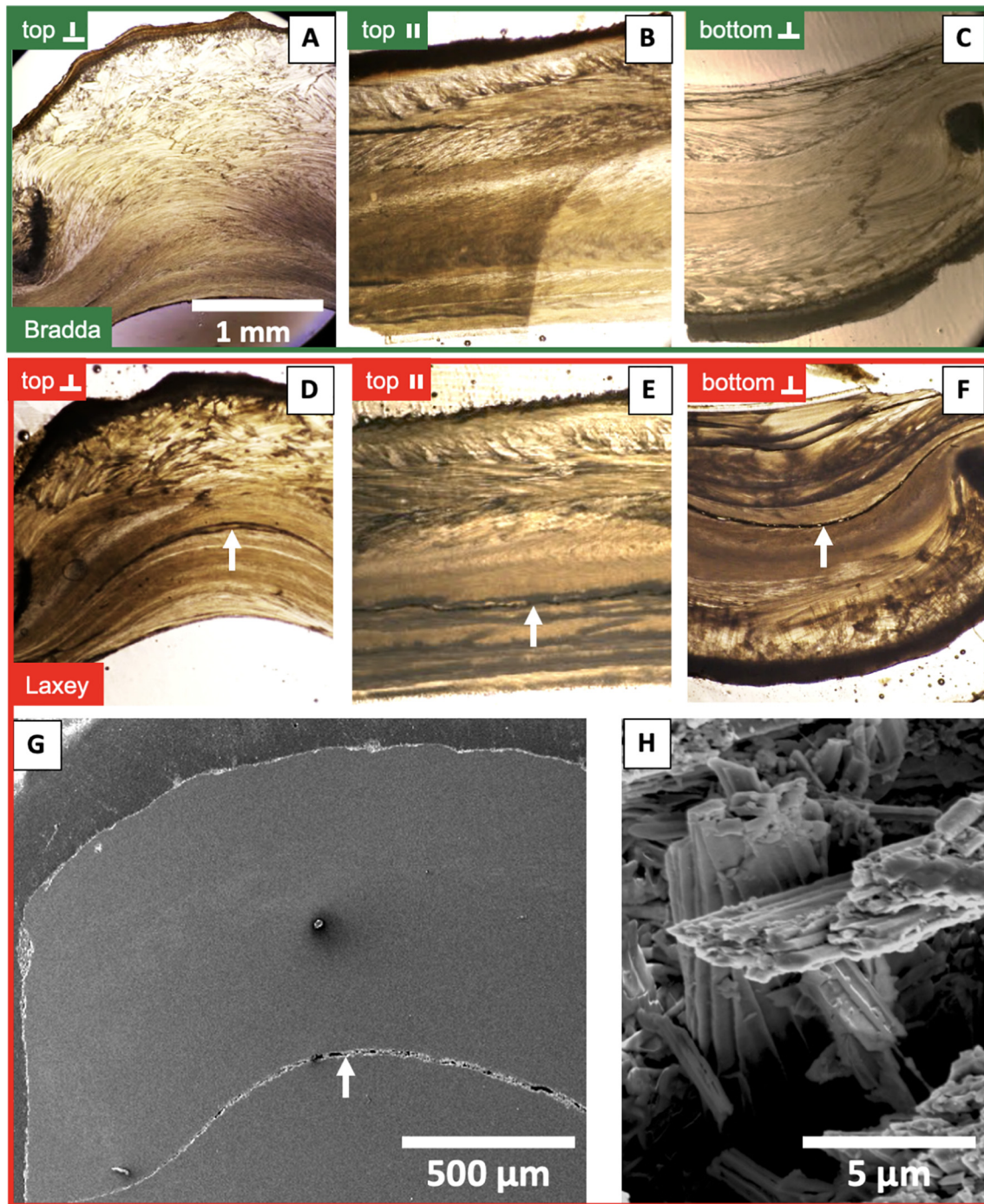
Cross-sections of top and bottom valves from Bradda Inshore and Laxey ( $n = 5$  at each site) were thinned and polished to optical transparency ( $< 80 \mu m$ ) and studied with SEM and TEM to characterize the overall mineral organisation within the shells. Cross-sections were cut

perpendicular and parallel to the rib direction (cut directions are indicated in Fig. S6a). These cross-sections were then polished to specular quality. Optical microscopy (Fig. S7A) shows the distinctive mineral organisation pattern of *P. maximus* where SEM analyses (Fig. S7 B) revealed the intertwined arrangement of the crystals in the foliated region, showing densely packed rod-shaped crystals, perpendicular to the rod direction, with crystal diameters in the range between 300 and 600 nm (Fig. S7 C). These observations are in line with previous studies (Grefsrud et al., 2008) and show that the mineral morphology in the foliated part of the shell is made up of different layers, linked to the shell formation process throughout the scallop's lifetime.

A comparison between the Bradda and Laxey top and bottom valves was performed by preparing perpendicular and parallel cross-sections relative to the rib direction and initially studying these samples with transmission optical light microscopy (Fig. 4). The cross-sections from Bradda Inshore (A, B, C) and Laxey (D, E, F), allow for a direct comparison of the shell structure for both the top and the bottom shells. Three morphological regions can be identified in all samples in agreement with the descriptions given by Bøggild and Kennedy (Bøggild, 1930; Kennedy et al., 1969): i) a thin layer found to consist of aragonite calcium carbonate as identified by Raman spectroscopy, (see Fig. S8) on the interior valve side where the muscle was originally attached, ii) a thick foliated pattern of calcite crystals and iii) an outer calcitic layer. Notably, all Laxey samples showed dark continuous lines positioned at approx. one third of the shell thickness as measured from the inner sides of the valves (Fig. 4 D, E and F, indicated by white arrows). Such lines were not found in any of the Bradda shells.

Investigations of these internal disruption zones by SEM in the perpendicular cross-sections of Laxey valves with high magnification





**Fig. 4.** Comparison of Bradda Inshore (top row) and Laxey (bottom row) shell cross-sections using transmission optical microscopy: (A) Bradda top valve in perpendicular orientation to the rib; (B) Bradda top valve in parallel orientation; (C) Bradda bottom valve, perpendicular; (D) Laxey top valve, perpendicular; (E) Laxey top valve, parallel; (F) Laxey bottom valve, perpendicular. A disruption layer of 10–20  $\mu\text{m}$  thickness is observable in all Laxey cross-sections (indicated by white arrows). Details of the disrupted layer in the Laxey top valve: (G) SEM image of polished cross-section showing disrupted layer (white arrow); (H) Higher magnification SEM image of disrupted region showing a loose arrangement of calcite rod bundles.

revealed these zones to be regions of disorganised mineralisation. Our studies (Fig. 4H) indicate that the disrupted mineralisation regions consist of largely loose bundles of calcite rods, in stark contrast to the well-stacked order of closely packed calcite rods found in the regular shell structure (Fig. S9). EDX analysis in SEM of the disrupted regions did not show the presence of metal elements other than Ca (Supplementary Information 8). Hence, if these metals were incorporated in the disrupted region their concentration would be below the detection limit for EDX ( $< 0.1\%$  for Zn, Pb and Cu).

#### 4. Discussion

Our study demonstrated considerable differences in the morphology, microstructure and strength of shells of commercially valuable king scallops (*Pecten maximus*) at geographically close ( $< 50$  km apart) sites around the Isle of Man in the Irish Sea. The Laxey site, where shells were significantly weaker and thinner compared to the control sites, has a long history of metal contamination due to a legacy of land-based mining (Kennington, 2018). Our surveys showed that



substantial metal contamination (Pb, Cu and Zn) is still present in the sediment on this scallop fishing ground, decreasing exponentially with distance from the shore. The levels of fatal damage to the scallops we caught at Laxey were 1.71 to 3.91 higher than at non-contaminated fishing grounds. Given that approximately 80% of scallops which encounter a dredge are damaged but remain uncaptured on the seafloor (Jenkins et al., 2001), these differences could have major implications for local population size and fisheries yield (Myers et al., 2000). Likewise, weaker shells make young/small scallops more vulnerable to predation by crustaceans (Beadman et al., 2003; Grefsrud et al., 2008), likely reducing the probability of surviving to maturity and commercially viable size.

We believe our results provide a compelling case that metal contamination is playing an important role in the development of thinner and weaker shells at Laxey, and hence the observed high damage rates. Given that the morphology and strength of bivalve shells is well known to be highly variable and affected by environmental conditions (Beadman et al., 2003; Telesca et al., 2019), we do not make this conclusion lightly. However, all of the evidence we investigated points in that direction. Firstly, although Laxey was our only 'treatment' (contaminated) site, it really stood out as an outlier. It was the only site we investigated that is measurably contaminated with metals, and it consistently revealed both the highest damage rates of six sites over a three year period, and the weakest shell strength, at two time periods separated by 9 years (2004 and 2013). We also knew from previous studies that it has been contaminated with metals since at least 1978, and almost certainly much longer (Kennington, 2018). Metals are known to disrupt calcification and therefore shell formation in molluscs, although this phenomenon is poorly understood in marine environments (Zuykov et al., 2013). Secondly, the internal microstructure of the shells at Laxey was markedly different from those at our control site at Bradda. In particular, all of the Laxey shells examined contained a discontinuity or check mark, which could indicate that at a certain stage of growth these scallops lost the ability to form a folded mineral structure within the foliated region of the shell for a period of time. This discontinuity was not found in any of the shells we examined from our control site at Bradda. In all the Laxey shells the affected region was approximately one third of the valve thickness measured from the inner side. This temporary loss of mineral organisation likely began soon after scallops settled onto the sediment as juveniles, but continued throughout the scallop's life, given we found the check mark in sections taken from near the outer edge of shells from adult scallops. Laboratory experiments have shown that exposure of scallops to metal contaminants such as copper during their early life history can dramatically reduce their growth and survival (Beaumont et al., 1987). The particulate pathway (via sediment or food) is also thought to be the main route for accumulation of metals in king scallops (Metian et al., 2009).

The analysis of environmental variables such as dredge fullness and stone volume did not indicate any other obvious explanation for the high damage rates and weaker scallop shells at the Laxey fishing ground. In fact, stone volume was lowest at Laxey, where damage was highest. This particular difference is more explained by local geology rather than differences in hydrographic conditions around the Isle of Man (Kennington and Hiscott, 2018). All of the above findings indicate that damage likely occurs when scallops first encounter the dredges on the seabed, rather than once they are in the dredge (Lart, 2003), and that damage levels are therefore predominantly related to shell strength. Given the close proximity of our study sites, oceanographic conditions such as water temperature, salinity and food availability are known to be relatively similar across all areas (Kennington, 2018; Kennington and Hiscott, 2018). Therefore these factors do not explain the spatial variations in shell strength that we observed. Likewise, our data give no indication that those patterns were driven by either a phenotypic anti-predator response (Beadman et al., 2003) or selective predation on individuals with weaker shells (Grefsrud et al., 2008), possibly because predator numbers are temporally variable in the

study area (Öndes et al., 2019). Finally, although genetic variation is not thought to strongly influence plasticity in bivalve shells (Vendrami et al., 2017), there is little genetic diversity in populations of *Pecten maximus* around the UK (Morvezen et al., 2016) excluding this factor as a potential cause for shell strength variability.

Biological responses to metal contamination are generally explored in short-term laboratory experiments (Mayer-Pinto et al., 2010), whereas we examined the long-term effects of exposure under field conditions. Our findings imply a non-apical response (i.e. a response not directly linked to enhanced mortality) to metal contamination leading to shell weakening in a marine bivalve mollusc. To our knowledge this is the best evidence to date for such an effect in a real-world setting. Such non-apical responses are rarely considered in toxicity assessments (Marty et al., 2017). However, our study indicates that such an effect needs to be considered as a cofactor, which strongly reduces survival rates. In our study this effect was apparent during both fishing operations and from the increased vulnerability of the scallops to predators. For example, Jenkins et al. (2004) show enhanced scavenger and predator aggregation to even lightly damaged scallops. This finding is of particular significance since the metal contamination we recorded was generally below the regulatory limits thought likely to harm marine life (CCME, 2002), and the Laxey scallops are considered safe for human consumption (Kennington, 2018). Given the similarity of shell formation and calcification in scallops and other bivalves (Zuykov et al., 2013), our findings have therefore broader implications for a group of species of considerable ecological and economic importance.

Naturally, a key question here is, what is the mechanism by which metal bearing sediments affect the shell formation process? Some evidence exists that contaminant metals can affect the mineralisation process in freshwater molluscs (Faubel et al., 2008) and there are in principle two mechanisms or a combination of these mechanisms conceivable:

(i) Contaminant metal ions could directly substitute  $\text{Ca}^{2+}$  ions during shell formation (Jordaens et al., 2006) and thereby disrupt the organisation of the shell matrix leading to poisoning of certain crystal facets and thereby changing growth rates and morphological evolution. This process would require a direct transport of the metal ions towards the active mineralisation sites. In this case the mineralising organism would act as a transit stage with possibly minor effects on the organism itself. Although it is well established that heavy metal pollution can significantly affect shell formation in bivalves, the precise impact pathways of metal ions such as  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Pb}^{2+}$  on the formation of crystalline calcium carbonate in mollusc shells is still to be unravelled. Systematic studies by Kurmaç (Kurmaç, 2009) have found that microbial calcium carbonate precipitation is strongly diminished by the presence of comparably low concentrations of these ions where Cu(II) shows the strongest impact followed by Pb(II) and Zn(II). This indicates a notable sensitivity of the biomineralization process to these bivalent ions. It is likely that such sensitivity is related to the strong affinity of these heavy metal ions for incorporation into calcium carbonate substrates such as limestone (Sdiri et al., 2012) where certain crystallographic orientations become inhibited by these impurities and thereby leading to a suppression of the overall crystal growth via adsorption to specific growth sites (Meyer, 1984).

(ii) Contaminant metal ions interact with the organism and, by means of affecting the production of mineralisation related proteins, lead to a deterioration of mineral morphology. The early stages of mineral formation (where ions assemble into stable amorphous clusters), are regulated by proteins transporting  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$  ions from the sea water possibly via an intermediary amorphous precursor phase into the final crystals (Freeman et al., 2010; Rao et al., 2014). The metal ions may bind with proteins involved in the mineralisation process and be carried into the shells (Freitas et al., 2016), or they might lead to protein folding and thus modifying their activity and thereby the crystallisation dynamics of microstructure formation (Sharma et al., 2008).

Our EDX analysis did not detect any of the metals found in the sediments at the disruption layer of the Laxey shells. However, the detectable concentration is relatively high (approx. 0.5%) and hence does not preclude a possible direct role of metal ions in the formation of the disruption layer, since only minor levels of contaminants can substantially disrupt the crystal formation process.

Given that metal pollution is considered an increasing threat to marine ecosystems (Rogers and Laffoley, 2011), improving our predictions of the ecological and economic impact on bivalves, including interactions with predicted future changes in ocean chemistry (pH) and temperature, clearly needs to form a priority area for research (Sezer et al., 2020; Shi et al., 2016). The fact that comparably low levels of heavy metal contaminations can affect the shell structure and strength in such a potent way represents a challenge to marine species management and conservation strategies, particularly when considering the potential and already established enhancements of these effects by ocean acidification and global warming in the present and the future.

## 5. Conclusion

The potential long-term impact of anthropogenic metal pollution on marine organisms, as shown in our work, is remarkable since the last major mine on the Isle of Man closed in 1908 (Government, n.d.). Given both the persistent nature of such threats, and the likelihood that they will be amplified in the future by ongoing human activities and climate change (Rogers and Laffoley, 2011; Sezer et al., 2020; Shi et al., 2016), we urge a renewed research focus on the effects of metals on calcification in marine organisms. Our study indicates that bivalve shell structure and strength may be a more sensitive, but very ecologically relevant, end-point or bio-indicator than those currently used in ecotoxicology assessments. Given the joint prevalence of both bivalve molluscs and metal contamination in inshore coastal waters around the world, it is quite possible that significant bivalve population losses and reductions in associated biodiversity and ecosystem services have been occurring unseen because of these effects on calcification. Patterns of spatial variation in bivalve shell strength should therefore be used to help identify potentially affected sites. Through subsequent field sampling and modelling of effects (Dong et al., 2016), it would then be possible to confirm the marine and coastal areas where marine life and fisheries are most threatened from the geochemical time bombs generated by metal pollution. Such an approach could potentially result in a paradigm shift for how risks of metal contamination are assessed and managed in the marine environment.

## CRedit authorship contribution statement

BDS, RK and SRJ came up with the key concepts behind this work. BDS and RK led the writing of the paper. CB, CS and KK conducted much of the laboratory analysis, while BDS and SRJ led the scallop survey field work. ARB, WL and RK secured funding. All authors contributed to drafts of the manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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## References

- Beadman, H.A., Caldwell, R.W.G., Kaiser, M.J., Willows, R.I., 2003. How to toughen up your mussels: using mussel shell morphological plasticity to reduce predation losses. *Mar. Biol.* 142, 487–494. <https://doi.org/10.1007/s00227-002-0977-4>.
- Beaumont, A.R., Tserpes, G., Budd, M.D., 1987. Some effects of copper on the veliger larvae of the mussel *Mytilus edulis* and the scallop *Pecten maximus* (Mollusca, Bivalvia). *Mar. Environ. Res.* 21, 299–309. [https://doi.org/10.1016/0141-1136\(87\)90052-3](https://doi.org/10.1016/0141-1136(87)90052-3).
- Beeby, A., Richmond, L., Herpe, F., 2002. Lead reduces shell mass in juvenile garden snails, (*Felix aspersa*). *Environ. Pollut.* 120, 283–288. [https://doi.org/10.1016/S0269-7491\(02\)00151-3](https://doi.org/10.1016/S0269-7491(02)00151-3).
- Beukers-Stewart, B.D., Mosley, M.W.J., Brand, A.R., 2003. Population dynamics and predictions in the Isle of Man fishery for the great scallop, *Pecten maximus* (L.). *ICES J. Mar. Sci.* 60, 223–241. [https://doi.org/10.1016/S1054-3139\(03\)00005-5](https://doi.org/10.1016/S1054-3139(03)00005-5).
- Beukers-Stewart, B.D., Vause, B.J., Mosley, M.W.J., Rossetti, H.L., Brand, A.R., 2005. Benefits of closed area protection for a population of scallops. *Mar. Ecol. Prog. Ser.* 298, 189–204. <https://doi.org/10.3354/meps298189>.
- Beukers-Stewart, B.D., Vause, B.J., Mosley, M.W.J., Brand, A.R., 2006. Closed areas and stock enhancement of scallops - What's the catch? *J. Shellfish Res.* 25, 267–268.
- Boggild, O.B., 1930. The shell structure of the mollusks. *Det Kongelige Danske Videnskabernes Selskabs Skrifter. Naturvidenskabelig og Matematisk Afdeling. Raekke.* 9, 231–326.
- Bradshaw, C., Tjensvoll, I., Skold, M., Allan, I.J., Molvaer, J., Magnusson, J., Naes, K., Nilsson, H.C., 2012. Bottom trawling resuspends sediment and releases bioavailable contaminants in a polluted fjord. *Environ. Pollut.* 170, 232–241. <https://doi.org/10.1016/j.envpol.2012.06.019>.
- Bryan, G.W., Langston, W.J., 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. *Environ. Pollut.* 76, 89–131. [https://doi.org/10.1016/0269-7491\(92\)90099-V](https://doi.org/10.1016/0269-7491(92)90099-V).
- Calabrese, A., MacInnes, J.R., Nelson, D.A., Miller, J.E., 1977. Survival and growth of bivalve larvae under heavy-metal stress. *Mar. Biol.* 41, 179–184. <https://doi.org/10.1007/BF00394024>.
- CCME, 2002. Canadian sediment quality guidelines for the protection of aquatic life: summary tables. Updated. In: Canadian Council of Ministers of the Environment, Canadian environmental quality guidelines, Winnipeg. [https://www.ccme.ca/en/resources/canadian\\_environmental\\_quality\\_guidelines](https://www.ccme.ca/en/resources/canadian_environmental_quality_guidelines).
- Daily, G., 2003. What are ecosystem services? In: Lorey, D.E. (Ed.), *Global Environmental Challenges for the Twenty-First Century: Resources, Consumption and Sustainable Solutions*. Rowman & Littlefield Publishers, Lanham, pp. 227–231.
- Daka, E.R., Hawkins, S.J., 2004. Tolerance to heavy metals in *Littorina saxatilis* from a metal contaminated estuary in the Isle of Man. *J. Mar. Biol. Assoc. U. K.* 84, 393–400. <https://doi.org/10.1017/S0025315404009336h>.
- De Noia, M., Telesca, L., Vendrami, D.L., Gokalp, H.K., Charrier, G., Harper, E.M., Hoffman, J.J., 2020. Population genetic structure is unrelated to shell shape, thickness and organic content in European populations of the soft-shell clam *Mya arenaria*. *Genes* 11, 298. <https://doi.org/10.3390/genes11030298>.
- Dong, Y., Rosenbaum, R.K., Hauschild, M.Z., 2016. Assessment of metal toxicity in marine ecosystems: comparative toxicity potentials for nine cationic metals in coastal seawater. *Environmental Science & Technology* 50, 269–278. <https://doi.org/10.1021/acs.est.5b01625>.
- EEA, 2015. State of Europe's seas. EEA Report. 2, pp. 220. <https://www.eea.europa.eu/publications/state-of-europes-seas> (Accessed 9 July 2020).
- FAO, 2018. The State of World Fisheries and Aquaculture 2018: Meeting the Sustainable Development Goals. FAO, Rome <http://www.fao.org/documents/card/en/c/19540EN/> (Accessed 9 July 2020).
- Faubel, D., Lopes-Lima, M., Freitas, S., Pereira, L., Andrade, J., Checa, A., Frank, H., Matsuda, T., Machado, J., 2008. Effects of Cd<sup>2+</sup> on the calcium metabolism and shell mineralization of bivalve *Anodonta cygnea*. *Mar. Freshw. Behav. Physiol.* 41, 131–146. <https://doi.org/10.1080/10236240802194123>.
- Freeman, C.L., Harding, J.H., Quigley, D., Rodger, P.M., 2010. Structural control of crystal nuclei by an eggshell protein. *Angew. Chem.* 49, 5135–5137. <https://doi.org/10.1002/anie.201000679>.
- Freitas, P.S., Clarke, L.J., Kennedy, H., Richardson, C.A., 2016. Manganese in the shell of the bivalve *Mytilus edulis*: Seawater Mn or physiological control? *Geochimica et Cosmochimica Acta.* 194, 266–278. doi:<https://doi.org/10.1016/j.gca.2016.09.006>.
- Fritsch, C., Coeurdassier, M., Gimbert, F., Crini, N., Scheifler, R. and De Vaulfleury, A., 2011. Investigations of responses to metal pollution in land snail populations (*Cantareus*

- asperus and *Cepaea nemoralis*) from a smelter-impacted area. *Ecotoxicology*. 20, 739–759. doi:<https://doi.org/10.1007/s10646-011-0619-z>.
- Grabowski, J.H., Brumbaugh, R.D., Conrad, R.F., Keeler, A.G., Opaluch, J.J., Peterson, C.H., Piehler, M.F., Powers, S.P., Smyth, A.R., 2012. Economic valuation of ecosystem services provided by oyster reefs. *BioScience* 62, 900–909. <https://doi.org/10.1525/bio.2012.62.10.10>.
- Grefsrud, E.S., Strand, Ø., 2006. Comparison of shell strength in wild and cultured scallops (*Pecten maximus*). *Aquaculture* 251, 306–313. <https://doi.org/10.1016/j.aquaculture.2005.06.004>.
- Grefsrud, E.S., Dauphin, Y., Cuif, J.P., Denis, A., Strand, Ø., 2008. Modifications of microstructure of cultured and wild scallop shells (*Pecten maximus*). *J. Shellfish Res.* 27, 633–641. [https://doi.org/10.2983/0730-8000\(2008\)27\[633:MIMOCA\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2008)27[633:MIMOCA]2.0.CO;2).
- IPCC. IPCC Special Report on the Ocean and Cryosphere in a Changing Climate, 2019. In: Pörtner, H.-O., Roberts, D.C., Masson-Delmotte, V., Zhai, P., Tignor, M., Poloczanska, E., et al., (Eds.). <https://www.ipcc.ch/srdoc/> (Accessed 10 July 2020)10/07/20.
- Isle of Man Government, Mines and Minerals, n.d. <https://www.gov.im/about-the-government/departments/environment-food-and-agriculture/ecosystem-policy-and-energy/mines-and-minerals> (Accessed 9 July 2020).
- Jenkins, S.R., Beukers-Stewart, B.D., Brand, A.R., 2001. The impact of scallop dredging on benthic megafauna: a comparison of damage levels in captured and non-captured organisms. *Mar. Ecol. Prog. Ser.* 215, 297–301. <https://doi.org/10.3354/meps215297>.
- Jenkins, S.R., Mullen, C., Brand, A.R., 2004. Predator and scavenger aggregation to discarded by-catch from dredge fisheries: importance of damage level. *J. Sea Res.* 51, 69–76. <https://doi.org/10.1016/j.seares.2003.05.002>.
- Jordaens, K., De Wolf, H., Vandecasteele, B., Blust, R., Backeljau, T., 2006. Associations between shell strength, shell morphology and heavy metals in the land snail *Cepaea nemoralis* (Gastropoda, Helicidae). *Sci. Total Environ.* 363, 285–293. <https://doi.org/10.1016/j.scitotenv.2005.12.002>.
- Kennedy, W.J., Taylor, J.D., Hall, A., 1969. Environmental and biological controls on bivalve shell mineralogy. *Biol. Rev.* 44, 499–530. <https://doi.org/10.1111/j.1469-185X.1969.tb00610.x>.
- Kennington, K., Marine Pollution. In: Hanley, L., et al., 2018. (Eds.) Manx Marine Environmental Assessment. Isle of Man Government, Douglas 42 pp. <https://www.gov.im/about-the-government/departments/infrastructure/harbours-information/territorial-seas/manx-marine-environmental-assessment/#:~:text=The%20Manx%20Marine%20Environmental%20Assessment,from%20the%20Departments%20of%20Infrastructure%20> (Accessed 10 July 2020).
- Kennington, K., Hiscott, A., 2018. Hydrology, weather and climate, climatology. In: Hanley, L., et al., (Eds.) Manx Marine Environmental Assessment Isle of Man Government, Douglas, 45 pp. <https://www.gov.im/about-the-government/departments/infrastructure/harbours-information/territorial-seas/manx-marine-environmental-assessment/#:~:text=The%20Manx%20Marine%20Environmental%20Assessment,from%20the%20Departments%20of%20Infrastructure%20> (Accessed 10 July 2020).
- Kurmac, Y., 2009. The impact of toxicity of metals on the activity of ureolytic mixed culture during the precipitation of calcium. *J. Hazard. Mater.* 163, 1063–1067. <https://doi.org/10.1016/j.jhazmat.2008.07.061>.
- Lart, W., et al., 2003. Evaluation and improvement of shellfish dredge design and fishing effort in relation to technical conservation measures and environmental impact: [ECODREDGE FAIR CT98-4465]. Sea Fish Industry Authority, Hull.
- Lopes-Lima, M., Freitas, S., Pereira, L., Gouveia, E., Hinzmann, M., Checa, A., Machado, J., 2012. Ionic regulation and shell mineralization in the bivalve *Anodonta cygnea* (swan mussel) following heavy-metal exposure. *Can. J. Zool.* 90, 267–283. <https://doi.org/10.1139/z11-129>.
- Luoma, S.N., Rainbow, P.S., 2008. *Metal contamination in aquatic environments: science and lateral management*. Cambridge University Press, Cambridge.
- Marty, M.S., Blankinship, A., Chambers, J., Constantine, L., Kloas, W., Kumar, A., Lagadic, L., Meador, J., Pickford, D., Schwarz, T., Verslycke, T., 2017. Population-relevant endpoints in the evaluation of endocrine-active substances (EAS) for ecotoxicological hazard and risk assessment. *Integr. Environ. Assess. Manag.* 13, 317–330. <https://doi.org/10.1002/ieam.1887>.
- Mayer-Pinto, M., Underwood, A.J., Tolhurst, T., Coleman, R.A., 2010. Effects of metals on aquatic assemblages: what do we really know? *J. Exp. Mar. Biol. Ecol.* 391, 1–9. <https://doi.org/10.1016/j.jembe.2010.06.013>.
- Metian, M., Warnau, M., Hédouin, L., Bustamante, P., 2009. Bioaccumulation of essential metals (Co, Mn and Zn) in the king scallop *Pecten maximus*: seawater, food and sediment exposures. *Mar. Biol.* 156, 2063–2075. <https://doi.org/10.1007/s00227-009-1237-7>.
- Meyer, H.J., 1984. The influence of impurities on the growth rate of calcite. *J. Cryst. Growth* 66, 639–646. [https://doi.org/10.1016/0022-0248\(84\)90164-7](https://doi.org/10.1016/0022-0248(84)90164-7).
- Millero, F.J., Woosley, R., Ditrolio, B., Waters, J., 2009. Effect of ocean acidification on the speciation of metals in seawater. *Oceanography* 22, 72–85. <https://doi.org/10.5670/oceanog.2009.98>.
- MMO, 2019. UK Sea Fisheries Statistics 2018. Office for National Statistics, London <https://www.gov.uk/government/statistics/uk-sea-fisheries-annual-statistics-report-2018> Accessed 10 July 2020.
- Morvezen, R., Charrier, G., Boudry, P., Chauvaud, L., Breton, F., Strand, Ø., Laroche, J., 2016. Genetic structure of a commercially exploited bivalve, the great scallop *Pecten maximus*, along the European coasts. *Conserv. Genet.* 17, 57–67. <https://doi.org/10.1007/s10592-015-0760-y>.
- Murray, L.G., Hinz, H., Kaiser, M.J., 2011. Functional response of fishers in the Isle of Man scallop fishery. *Marine Ecology-Progress Series* 430, 157–169. <https://doi.org/10.3354/meps09067>.
- Myers, R.A., Fuller, S.D., Kehler, D.G., 2000. A fisheries management strategy robust to ignorance: rotational harvest in the presence of indirect fishing mortality. *Can. J. Fish. Aquat. Sci.* 57, 2357–2362. <https://doi.org/10.1139/f00-250>.
- NMFS, 2020. Fisheries of the United States, 2018. <https://www.fisheries.noaa.gov/national/sustainable-fisheries/fisheries-united-states> Accessed 10 July 2020.
- Öndes, F., Emmerson, J.A., Kaiser, M.J., Murray, L.G., Kennington, K., 2019. The catch characteristics and population structure of the brown crab (*Cancer pagurus*) fishery in the Isle of Man. Irish Sea. Marine Biological Association of the United Kingdom. *Journal of the Marine Biological Association of the United Kingdom*. 99, 119–133. <https://doi.org/10.1017/S0025315417001849>.
- Patterson, R.J., Mayer, D., Weaver, L., Phaneuf, M.W., 2002. “H-Bar lift-out” and “plan-view lift-out”: robust, re-thinnable FIB-TEM preparation for ex-situ cross-sectional and plan-view FIB specimen preparation. *Microsc. Microanal.* 8, 566–567. <https://doi.org/10.1017/S1431927602105502>.
- Rao, A., Berg, J.K., Kellermeier, M., Gebauer, D., 2014. Sweet on biomineralization: effects of carbohydrates on the early stages of calcium carbonate crystallization. *Eur. J. Mineral.* 26, 537–552. <https://doi.org/10.1127/0935-1221/2014/0026-2379>.
- Richardson, A.J., Chenery, S.R.N., Cook, J.M., 2001. History of trace metal (Cu, Pb, Zn) contamination in the North Sea through laser ablation - ICP-MS analysis of horse mussel *Modiolus modiolus* shells. *Marine Ecology-Progress Series*. 211, 157–167. <https://doi.org/10.3354/meps211157>.
- Roberts, D.A., Birchenough, S.N., Lewis, C., Sanders, M.B., Bolam, T., Sheahan, D., 2013. Ocean acidification increases the toxicity of contaminated sediments. *Glob. Chang. Biol.* 19, 340–351. <https://doi.org/10.1111/gcb.12048>.
- Rogers, S.I., Laffoley, Dd.A., 2011. International Earth system expert workshop on ocean stresses and impacts. Summary report. IPSO, pp. 18. <https://portals.iucn.org/library/node/12801> (Accessed 10 July 2020).
- Sdiri, A., Higashi, T., Jamoussi, F., Bouaziz, S., 2012. Effects of impurities on the removal of heavy metals by natural limestones in aqueous systems. *J. Environ. Manag.* 93, 245–253. <https://doi.org/10.1016/j.jenvman.2011.08.002>.
- Sezer, N., Kılıç, Ö., Sıkkokur, E., Çayır, A., Belivermiş, M., 2020. Impacts of elevated pCO<sub>2</sub> on Mediterranean mussel (*Mytilus galloprovincialis*): metal bioaccumulation, physiological and cellular parameters. *Mar. Environ. Res.*, 104987 <https://doi.org/10.1016/j.marenvres.2020.104987>.
- Sharma, S.K., Goloubinoff, P., Christen, P., 2008. Heavy metal ions are potent inhibitors of protein folding. *Biochem. Biophys. Res. Commun.* 372, 341–345. <https://doi.org/10.1016/j.bbrc.2008.05.052>.
- Shi, W., Zhao, X., Han, Y., Che, Z., Chai, X., Liu, G., 2016. Ocean acidification increases cadmium accumulation in marine bivalves: a potential threat to seafood safety. *Sci. Rep.* 6, 20197. <https://doi.org/10.1038/srep20197>.
- Sturesson, U., 1976. Lead enrichment in shells of *Mytilus edulis*. *Ambio* 5, 253–256. <https://www.jstor.org/stable/4312228>.
- Telesca, L., Peck, L.S., Sanders, T., Thyrring, J., Sejr, M.K., Harper, E.M., 2019. Biomineralization plasticity and environmental heterogeneity predict geographical resilience patterns of foundation species to future change. *Glob. Chang. Biol.* 25, 4179–4193. <https://doi.org/10.1111/gcb.14758>.
- Vause, B.J., Beukers-Stewart, B.D., Brand, A.R., 2007. Fluctuations and forecasts on the fishery for queen scallops (*Aequipecten opercularis*) around the Isle of Man. *ICES J. Mar. Sci.* 64, 1124–1135. <https://doi.org/10.1093/icesjms/fsm089>.
- Vendrami, D.L., Telesca, L., Weigand, H., Weiss, M., Fawcett, K., Lehman, K., Clark, M.S., Leese, F., McMinin, C., Moore, H., Hoffman, J.L., 2017. RAD sequencing resolves fine-scale population structure in a benthic invertebrate: implications for understanding phenotypic plasticity. *R. Soc. Open Sci.* 4, 160548. <https://doi.org/10.1098/rsos.160548>.
- Zuykov, M., Pelletier, E., Harper, D.A., 2013. Bivalve mollusks in metal pollution studies: from bioaccumulation to biomonitoring. *Chemosphere* 93, 201–208. <https://doi.org/10.1016/j.chemosphere.2013.05.001>.