



# Heterogeneity of chromium in *Mytilus edulis*: Implications for the Cr isotope system as a paleo-redox proxy

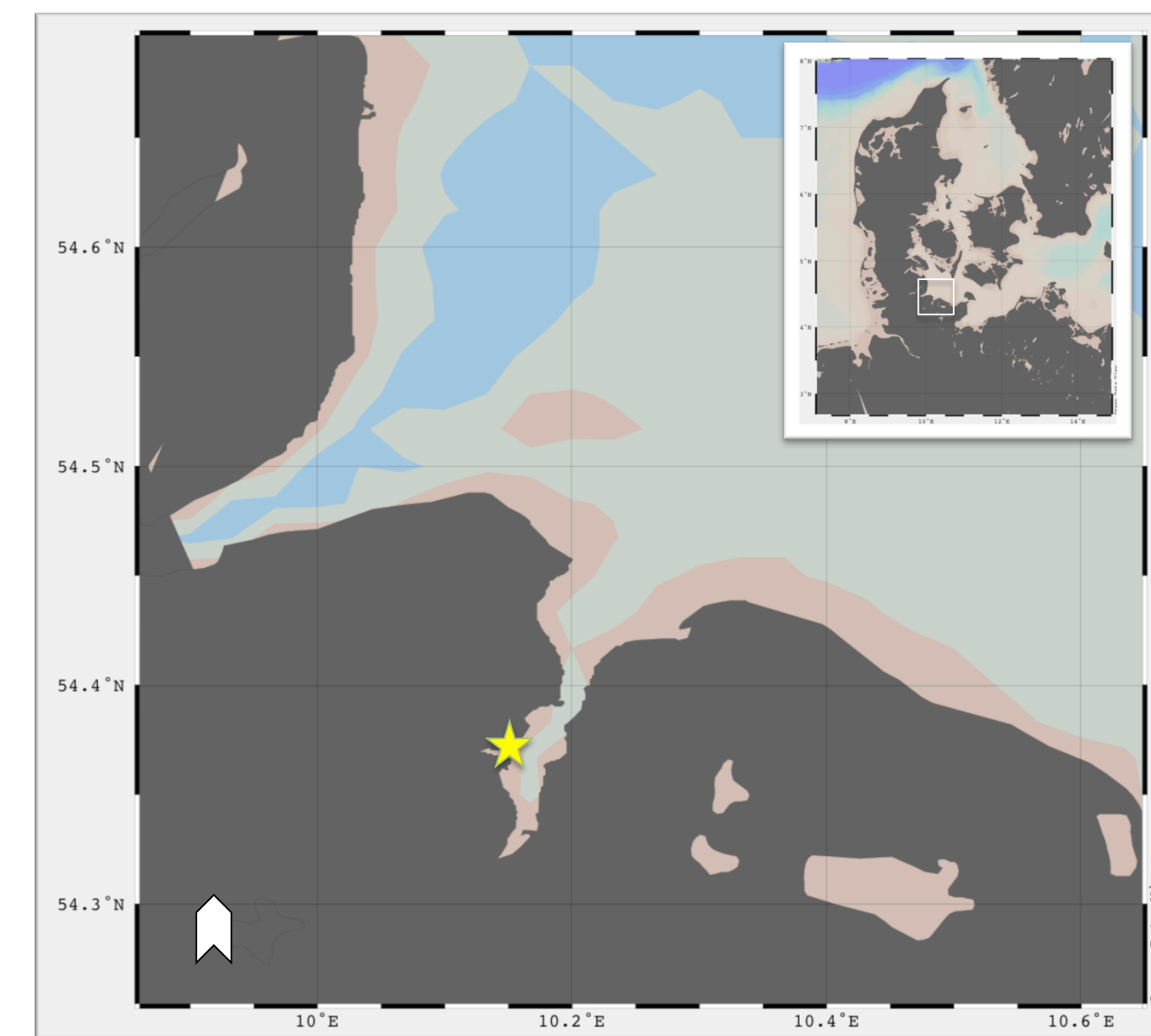
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## Chromium in carbonates

Chromium isotope fractionation is associated with the oxidation state of Cr. Laboratory experiments show that Cr may be directly incorporated into the crystal lattice of inorganic carbonates with no or little isotope fractionation<sup>[1]</sup>. Biogenic carbonates are thought to record changes in the seawater chromium isotope values ( $\delta^{53}\text{Cr}$ ), allowing the reconstruction of changing redox conditions of the Earth's oceans and atmosphere<sup>[2,3]</sup>. We investigate the incorporation of Cr into modern carbonate shells (*Mytilus edulis*) by analyzing Cr in carbonate minerals, organic matter and ambient seawater of *M. edulis* from a cultivation site (Map 1). The observed fractionation effects have implications for the potential of bivalves and other marine calcifiers to preserve  $\delta^{53}\text{Cr}$  compositions of ambient seawater.



name	# samples	length cm	width cm	weight g
S	5	4.7	2.3	1.50
M	37	5.2	2.6	2.23
L	8	6.0	2.8	2.55

**Table 1: Sample specifications**

Characterization of specimens of sizes S, M and L. Lengths may vary max. 0.2 cm, widths max. 0.1cm.

**Map 1: Kiel Fjord**

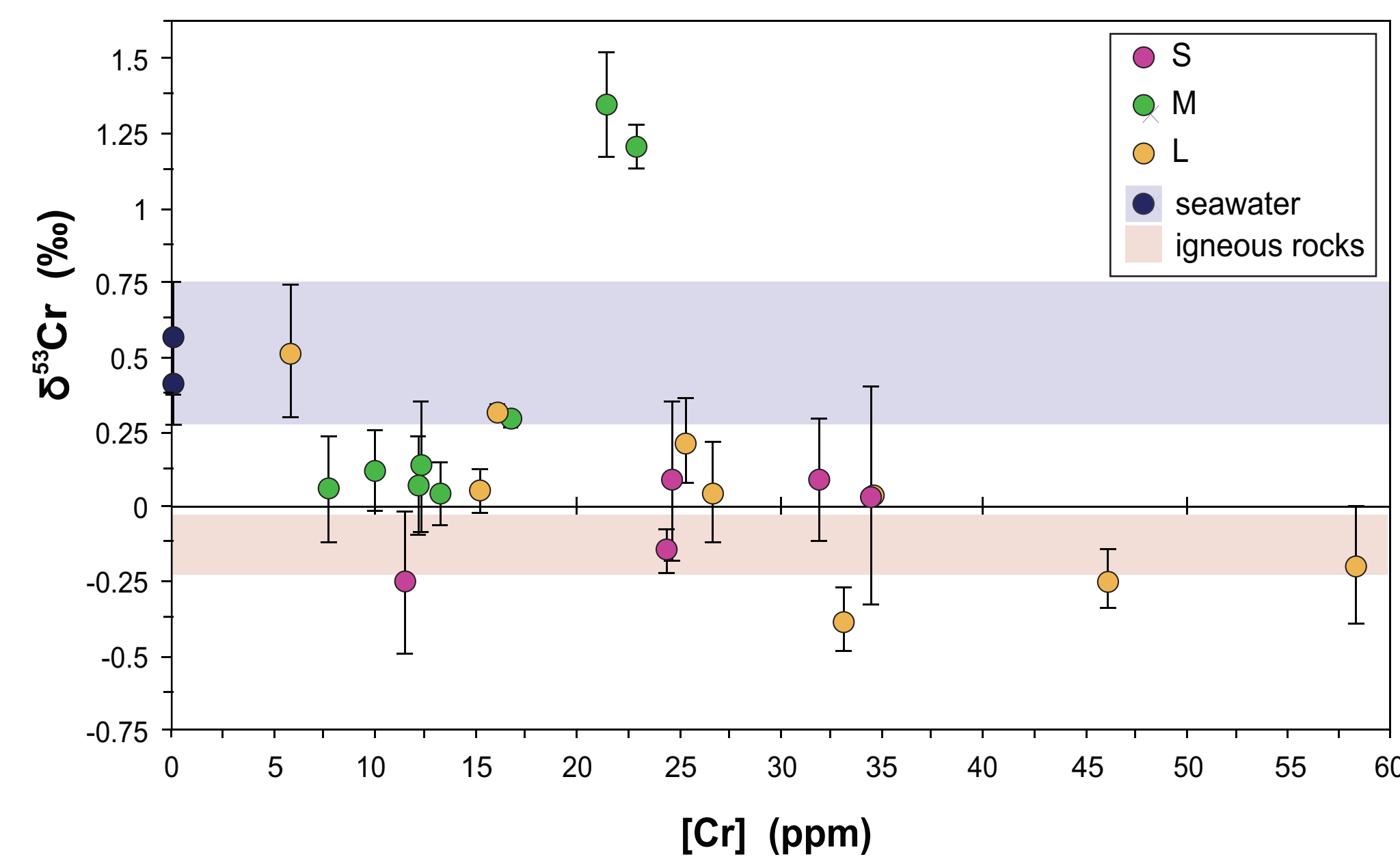
Shell and water samples were collected from a cultivation station (star) in the Kiel Fjord, Germany.

## Results

For shell samples, a relatively large range of variability in both [Cr] and  $\delta^{53}\text{Cr}$  values was observed. Bulk valve samples regardless of size range (Tab. 1), have Cr concentrations of 6.8 to 58.3 ppb and  $\delta^{53}\text{Cr}$  values of -0.44 to 1.34‰ (Fig. 1). A subset of shell samples (size M) are distributed along a mixing line between positively fractionated seawater and a negatively fractionated endmember of Cr associated with detrital and organic particles (Fig. 2). Samples (size M) with intact organic outer sheaths (periostraca) show Cr isotopic composition closer to seawater values compared with partial to complete removal of periostraca Fig. 3).

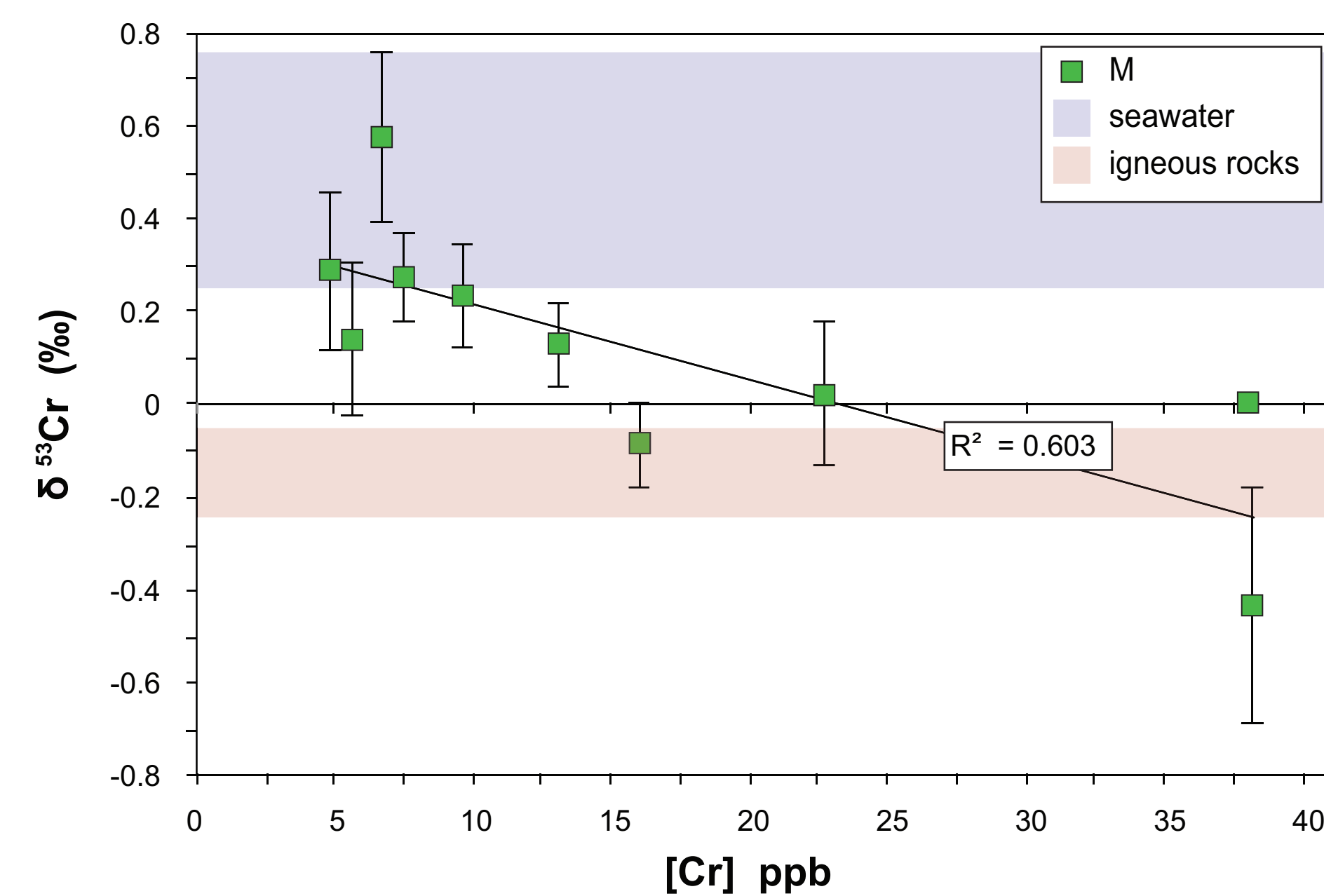
### Offset shell – seawater

Our data reveals a seawater chromium composition (66 ng/kg and  $\delta^{53}\text{Cr} = 0.49 \pm 0.16 \text{‰}$  2SE), which is in agreement with published surface seawater values of the Baltic Sea (0.13 – 0.8 ‰<sup>[4]</sup>). The median  $\delta^{53}\text{Cr}$  value of bulk shells (0.09‰) shows an **offset from local seawater of approximately 0.4‰**. This value is in good agreement with previously published data ranging around 0.5 ‰<sup>[5]</sup>.



**Figure 1: Bulk analyses**

The high variability of both the Cr isotopic composition and concentration in bulk analyses (periostraca, organic matrix & carbonate) of shells of different sizes lead to a variable offset from seawater isotopic values.

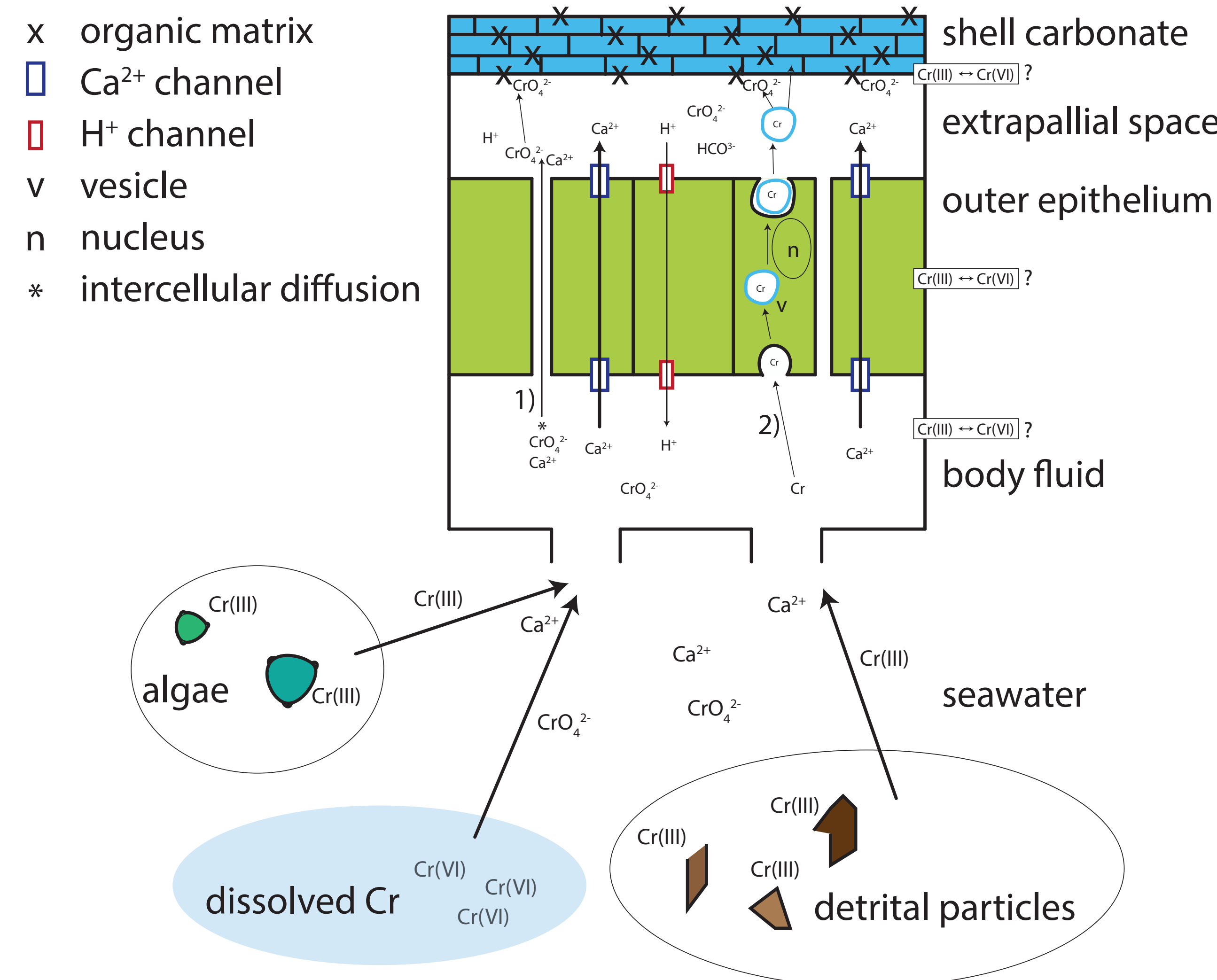


**Figure 2: Cr associated with organic matrix**

After removal of the organic outer sheaths, samples of size M were incinerated and dissolved in 2 N HCl to analyse Cr associated with the organic matrix and to a very low extent, with the carbonate minerals and show a wide range of isotopic compositions and concentrations.

## Chromium incorporation into mollusk shell carbonate

Analyses of Cr associated with carbonate minerals result in [Cr] of < 4 ppb for size M shells and are below the limit of detection to analyze the Cr isotopic composition. The lack of Cr in the carbonate minerals strongly suggests that carbonate shells as *M. edulis* are, in contrast to other biogenic carbonates (e.g. corals<sup>[6]</sup>), unlikely to incorporate Cr(VI) directly into the crystal lattice from seawater. Instead we propose an alternative mechanism, where Cr is associated with the organic matrix of the shell (Fig. 4).



**Figure 4: Model for Cr incorporation into mollusk shells**

In seawater, the thermodynamically stable species of Cr is Cr(VI), which occurs as  $\text{CrO}_4^{2-}$ . Cr(III) is highly particle reactive and sorbs onto (in)organic particles. As *M. edulis* are filter feeders, seawater (including major and trace elements) is ingested. From body fluids, our model suggests that Cr may reach the extrapallial space (EPS) over two different pathways: 1) intercellular diffusion, 2) endocytosis of the fluid containing Cr, which is transported to vesicles, where the precursor of the carbonate is precipitated (amorphous calcium carbonate ACC). Together with ACC, Cr is excluded from the epithelial cell into the EPS, where  $\text{CrO}_4^{2-}$  is proposed to be bound to the organic matrix responsible for crystal shape and growth. The pathway of Cr from seawater into the shell might include several redox-cycles, e.g. from Cr(VI) to Cr(III) as detoxification mechanism in vesicles. Model adapted after <sup>[6,7,8]</sup>.

## Chromium heterogeneity

Due to the chromium heterogeneity in the studied samples, we suggest a mixing model with at least three Cr endmembers:

- **detrital particles** with negatively fractionated Cr compositions in the range of igneous rock values ( $-0.124 \pm 0.101\text{‰}$ <sup>[9]</sup>).
- **algae**, which are suggested to be negatively fractionated due to bioreduction of Cr(VI)<sup>[10]</sup>
- **direct uptake of dissolved Cr(VI) from seawater** with the isotopic composition of the ambient water body. We propose that dissolved Cr(VI) is mainly accumulated in the organic outer sheath (Fig. 3)<sup>[11]</sup>.

However, as all shell samples lived at the same cultivation site, external factors such as changing ocean currents or food availability cannot account for the Cr variability. Instead, we propose that **metabolic efficiency**, possibly related to **growth rates**, as well as the **storage of elements in ACC** may be the reasons for the high Cr variability in modern bivalves, in analogy to other elements such as Sr.<sup>[7,12,13]</sup>

## Implications for $\delta^{53}\text{Cr}$ as paleo-redox proxy

In contrast to other biogenic carbonates (e.g. corals) we found very little Cr directly incorporated in the carbonate minerals of modern *M. edulis* from seawater. Instead, we observed that chromium is most likely hosted in their organic matrix. The Cr isotopic composition and concentration may depend in part on external factors (detrital particles, algae, dissolved Cr(VI)), but are proposed to be mostly influenced by vital effects.

Valves with intact periostraca were least affected by changing admixtures of Cr from endmembers other than seawater, but are unlikely to preserve the information through time due to the low preservation potential of organic matter in the oxygenated marine water column.

Investigation of the preservation potential of Cr associated with organic matter in the fossil record will help to assess the utility of chromium isotopes in carbonate shells as a paleo-redox proxy.

## Methods

The tissue of shell samples was removed from the shell valves, which were then washed in 0.1 N HCl using an ultrasonic bath. Valves were ashed in a furnace at 750°C for 16h to break-down organic compounds within and around the valves and release Cr. Step digestions were performed using 2N HCl and 0.5N HCl to attack calcite and aragonite minerals respectively, and 7% NaOCl to leach the organic outer sheath of each valve.

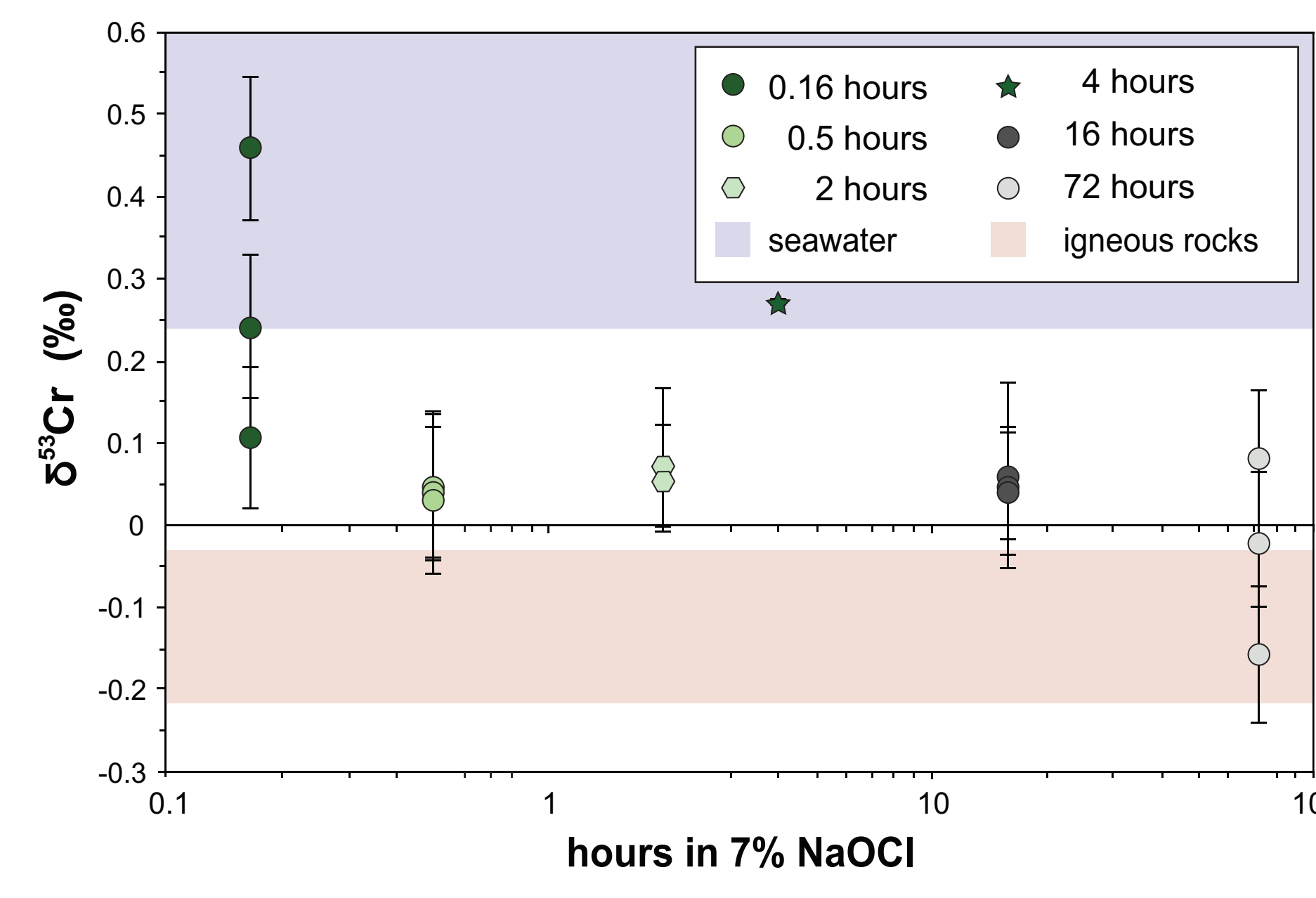
All samples were doped with a  $^{50}\text{Cr}$ - $^{54}\text{Cr}$  double-spike before elemental Cr was isolated from samples using chromatographic column separation. The Cr isotopic composition ( $\delta^{53}\text{Cr}$ ) and Cr concentration ([Cr]) of each sample was measured using thermal ionization mass spectrometry on a GV IsoProbe T.  $\delta^{53}\text{Cr}$  values were normalized to the SRM979 international standard using the following equation:

$\delta^{53}\text{Cr} (\text{‰}) = \left( \frac{{}^{53}\text{Cr}_{\text{sample}}/{}^{52}\text{Cr}_{\text{sample}}}{({}^{53}\text{Cr}_{\text{SRM979}}/{}^{52}\text{Cr}_{\text{SRM979}})} - 1 \right) \cdot 1000$

The external shell standard (KIESS-1) was repeatedly processed through the chromatographic column separation and reveals an external reproducibility of 0.24 ‰ (2SE, n = 3). The total amount of chromium of procedural blanks is <3 ng Cr.

## Literature

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**Figure 3: Valves leached in NaOCl for variable time periods**

Samples (size M) subjected to NaOCl for only very short time periods (10 minutes) are isotopically similar to seawater.

