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Olivine avoidance behaviour by marine gastropods (*Littorina littorea* L.) and amphipods (*Gammarus locusta* L.) within the context of ocean alkalinity enhancement

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ABSTRACT

Gigaton scale atmospheric carbon dioxide (CO_2) removal (CDR) is needed to keep global warming below 1.5 °C. Coastal enhanced olivine weathering is a CDR technique that could be implemented in coastal management programmes, but its CO_2 sequestration potential and environmental safety remain uncertain. Large scale olivine spreading would change the surficial sediment characteristics, which could potentially reduce habitat suitability and ultimately result in community composition changes. To test this hypothesis, we investigated the avoidance response of the marine gastropod *Littorina littorea* (Linnaeus, 1758) and marine amphipod *Gammarus locusta* (Linnaeus, 1758) to relatively coarse (83 – 332 μm) olivine and olivine-sediment mixtures during short-term choice experiments. Pure olivine was significantly avoided by both species, while no significant avoidance was observed for sediment with 3% or 30% w/w olivine. For *L. littorea*, aversion of the light green colour of pure olivine (i.e. positive scototaxis) was the main reason for avoidance. Moreover, olivine was not significantly avoided when it was 7.5 cm (45%) closer to a food source/darker microhabitat (*Ulva sp.*) compared to natural sediment. It is inferred that the amphipod *G. locusta* avoided pure olivine to reduce Ni and Cr exposure. Yet, a significant increase in whole body Ni concentrations was observed after 79 h of exposure in the 30% and 100% w/w olivine treatments compared to the sediment control, likely as a result of waterborne Ni uptake. Overall, our results are significant for ecological risk assessment of coastal enhanced olivine weathering as they show that *L. littorea* and *G. locusta* will not avoid sediments with up to 30% w/w relatively coarse olivine added and that the degree of olivine avoidance is dependent on local environmental factors (e.g. food or shelter availability).

1. Introduction

Limiting global warming to 1.5 °C requires fast reductions of anthropogenic greenhouse gas emissions to net zero by 2050 (IPCC, 2022). This primarily requires a shift from fossil fuels to renewable energy sources and improved energy conservation and efficiency (IPCC, 2022; Yousefi et al., 2019). Furthermore, atmospheric carbon dioxide (CO_2) removal (CDR) is needed on a gigaton scale to compensate for carbon budget overshoot and greenhouse gas emissions from sectors that are challenging to decarbonize (e.g. aviation, shipping, industrial processes) (IPCC, 2022; Minx et al., 2018). Ocean alkalinity enhancement (OAE) is a proposed CDR technique that relies on the increase in seawater alkalinity after addition of alkaline substances (e.g. olivine, calcite, lime) to drive atmospheric CO_2 drawdown and enhance the ocean carbon sink (Renforth and Henderson, 2017). Spreading finely ground fast weathering silicate rock in dynamic coastal environments has been put forward as a promising OAE method due the abundance of suitable source rock, technological readiness, and possible co-benefits including promotion of primary production and counteracting ocean acidification (Bach et al., 2019; Caserini et al., 2022; Meysman and Montserrat, 2017; Montserrat et al., 2017). The silicate mineral olivine ($Mg_xFe_{2-x}SiO_4$) has received most attention for use in coastal enhanced silicate weathering (CESW) due to its abundance and relatively fast dissolution kinetics (Meysman and Montserrat, 2017). However, the CO_2 sequestration potential and environmental safety of CESW remain uncertain (Flipkens et al., 2021; Fuhr et al., 2022; Montserrat et al., 2017).

Spreading large amounts of finely ground olivine rich rock in coastal

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environments could alter the local habitat of marine organisms. The impacts could resemble those seen in beach nourishment, encompassing smothering of marine organisms and alterations in both the physical (such as sediment density, grain size, shape, and colour) and chemical (including contaminant and organic carbon content) properties of the surficial sediment (Speybroeck et al., 2006). Freshly ground forsterite olivine sand is typically composed of green, angular grains with sharp edges (Montserrat et al., 2017). The grain size of olivine that would ideally be distributed remains uncertain. Very fine ($\leq$ 10 μm) grains dissolve and sequester CO_2 faster than coarser grains ($\geq$ 100 μm), but cost more energy to produce, are more difficult to transport, and could pose a higher environmental risk (Duan et al., 2022; Flipkens et al., 2021; Foteinis et al., 2023; Hangx and Spiers, 2009). Nickel (Ni) and chromium (Cr) concentrations in olivine are typically significantly higher than in natural marine sediment and could result in toxic effects depending on their release rate during olivine dissolution and subsequent accumulation by marine biota (Flipkens et al., 2021).

The effects of olivine exposure on marine biota have only been subject to limited research, as indicated by a handful of recent studies (Flipkens et al., 2023; Guo et al., 2023; Ren et al., 2021; Hutchins et al., 2023). Ren et al. (2021) observed an increase in the abundance of biofilm forming particle-attached microbes following a 10-day exposure to 1% w/w olivine in natural seawater. In the study by Hutchins et al. (2023), six phytoplankton species exposed to synthetic olivine leachates with Ni(II) and Cr(VI) concentrations up to 0.13 and 0.12 μmol L^{-1} exhibited neutral to positive physiological responses. Guo et al. (2023) reported an increase in the abundance of several small (<20 μm) phytoplankton taxa and the dinoflagellate *Noctiluca scintillans* ((Macartney) Kofoid & Swezy, 1921) after 21 days of olivine exposure in natural seawater. However, ctenopods (*Penilia sp.*) and tunicates (*Oikopleura sp.*) reduced in number, possibly due to impaired feeding in the presence of suspended olivine particles (at a concentration of 1.9 g L^{-1}). Finally, Flipkens et al. (2023) found significant grain size-dependent metal accumulation in the marine amphipod *G. locusta* (Linnaeus, 1758) during acute olivine exposures lasting 24 to 72 h. Additionally, chronic exposure to olivine for 35 days reduced the survival, growth, and reproduction of juvenile *G. locusta* at concentrations of 10% w/w and higher, likely due to metal toxicity. Overall, these findings highlight the critical need for further research on olivine exposure effects on marine biota to ensure that coastal enhanced olivine weathering can be scaled sustainably in the future for climate change mitigation.

Avoidance of olivine-rich sediment patches could possibly occur to reduce Ni and Cr exposure or escape unpreferred substrate types (Araújo et al., 2016). A range of studies have reported dose-dependent avoidance of contaminated sediments by various aquatic invertebrates and fish (Araújo et al., 2016). Additionally, coarse substrates, which often have a lower food content in the form of organic material compared to fine substrates, were significantly avoided by various marine organisms (Boos et al., 2010; Hellou et al., 2005; Moles et al., 1994). Overall, avoidance of coastal olivine application sites could lead to changes in local biodiversity, which could severely impact ecosystem structure and functioning (Araújo et al., 2016; Lopes et al., 2004). Therefore, the behavioural responses of marine organisms to olivine exposure should be considered in ecological risk assessment of CESW.

Amphipods and to a lesser extent gastropods have been frequently used as test organisms in sediment avoidance assays (Araújo et al., 2016). The gastropod *L. littorea* (Linnaeus, 1758) and amphipod *G. locusta* are widespread along shorelines of the Northeast Atlantic. They occur in the intertidal and subtidal zone on a range of substrate types, although *L. littorea* has preferences for rocky substrates and *G. locusta* is mostly found at sheltering structures (e.g. macroalgae and stones) (Carlson et al., 2006; Costa and Costa, 2000). Both species shape intertidal communities by extensive algae grazing and are an important food source for higher trophic levels (e.g. fish and aquatic birds) (Anderson and Underwood, 1997; Andersson et al., 2009; Costa and Costa, 2000).

In this study, we investigated the avoidance response of the periwinkle *L. littorea* and amphipod *G. locusta* to relatively coarse (83 – 332 μm) olivine and olivine-sediment mixtures. These investigations were conducted during several short-term choice experiments aimed at evaluating the potential impact of coastal olivine spreading on the habitat suitability for these two benthic macroinvertebrate species. In addition to the response to equidistant substrates, we investigated whether the degree of pure olivine avoidance in *L. littorea* was dependent on the distance to a food source. Finally, the importance of colour preference on substrate avoidance was assessed for both species. We hypothesized that substrates with a high olivine concentration would be avoided by *G. locusta* and *L. littorea* due to their elevated Ni and Cr content. Additionally, we hypothesized that the distance to a food source could affect the degree of pure olivine avoidance *in L. littorea* since movement is likely driven by food availability in this species (Seuront et al., 2007).

2. Material and methods

2.1. Test organisms

Adult *L. littorea* (shell height: 21.4 ± 1.3 mm, shell width: 18.2 ± 1.1 mm, $N = 632$) originating from Ireland were obtained from a commercial supplier in the Netherlands. All data henceforth are presented as mean ± standard deviation (S.D.), unless stated otherwise. *Littorina littorea* were housed at a temperature of 15 ± 1 °C and photoperiod of 12 h light and 12 h dark (12 L:12D) in a 210 L polyethylene (PE) tank (NORAH Plastics) filled with 200 L of artificial seawater (ASW). The ASW was made by adding 7.15 kg of seasalt (hw-Marinemix®) to ultrapure water (Eurowater DPRO B1 – 1/1) and aerating the solution for at least 24 h before use. *Littorina littorea* were fed *Ulva sp.* ad libitum. A trickling filter was used and 30 to 50% water changes were conducted on a weekly basis to ensure good water quality. Seawater temperature (15 ± 1 °C), pH (8.00 ± 0.08), salinity (33.4 ± 1.1‰), and concentrations of dissolved oxygen (DO) (7.95 ± 0.20 mg L^{-1}) were measured weekly with a HQ30D portable multimeter (Hach). Approximate concentrations of nitrate (NO_3^-), nitrate (NO_2^-) and total ammonia (NH_3/NH_4^+) were determined with commercial test kits (Tetra). The pH and conductivity electrode were calibrated before each with standard pH (pH 4.00, 7.00, and 10.01) and conductivity (12.9 and 50 mS cm^{-1}) solutions (VWR international). The oxygen probe (Intellical LDO101, Hach) was used with factory calibration settings.

Amphipods were collected by hand in the Eastern Scheldt estuary (51°27'31.6"N 4°04'50.4"E, Krabbendijke, the Netherlands) during low tide and transported to the laboratory in clean HDPE buckets filled with ASW. A subsample of 10 amphipods were identified microscopically as *G. locusta* using the key of Vader and Tandberg (2019). The animals were maintained in a 210 L PE tank filled with ASW and a 1 cm layer of control sediment (Section 2.2) and stones from the collection site. Amphipods were fed *Ulva sp.* ad libitum and Sera Micron Nature (1 mg $individual^{-1}$) three times per week. A trickling filter and protein skimmer were set up and weekly 15 to 30% water changes were conducted. Seawater temperature (18.6 ± 1.1 °C), pH (8.04 ± 0.07), salinity (33.6 ± 0.7‰), DO (7.37 ± 0.25 mg L^{-1}) and nitrogenous waste products were measured before each water change.

2.2. Sediment collection and characterization

Surficial (< 10 cm depth) North Sea sediment was collected from the intertidal zone at the Belgian coast (51°14'28.1"N 2°56'01.0"E, Ostend, Belgium) with a shovel and wet sieved in situ through a 1 mm screen to remove macrofauna. Sediment was brought to the laboratory in clean, sealed HDPE buckets and stored at 4 °C before use in the olivine avoidance assays with *L. littorea*. For the experiments with *G. locusta*, surficial control sediment was collected at the amphipod collection site and wet sieved through a 500 µm screen before storage at 4 °C until experimental use. Norwegian dunite sand was obtained from Sibelco's Aheim site. This dunite sand consists mainly (83 – 86% w/w) of forsterite-rich olivine ($Mg_{1.87}Fe_{0.13}SiO_4$) and will be referred to as olivine throughout the text. The mineral characterization of dunite sand is provided in Supplementary Section SI 1.

Experimental sediments were characterized for their sediment organic matter (SOM) content, grain size distribution and trace metal concentrations. The loss on ignition method according to Heiri et al. (2001) was used to determine SOM content. Grain size distribution was measured with a Mastersizer 2000 particle size analyzer (Malvern) according to standard operating procedures without chemical pre-treatment. The elemental composition was determined via ICP-OES (iCAP 6300 Duo, Thermo Scientific). Sediment samples were acid digested according to a modified chromite ore digestion protocol (CEM Corporation, 1999). Briefly, 1.4 mL of Ultrapure sulphuric acid (≥96%, Merck) and 2.6 mL of orthophosphoric acid (85%, Honeywell Chemicals) were added to 0.1 g of dry sediment and subsequently heated at 240 °C for 30 min in a Discover SP-D 80 microwave digestion system (CEM Corporation). Afterwards, 2 mL of 67–69% nitric acid (Fisher

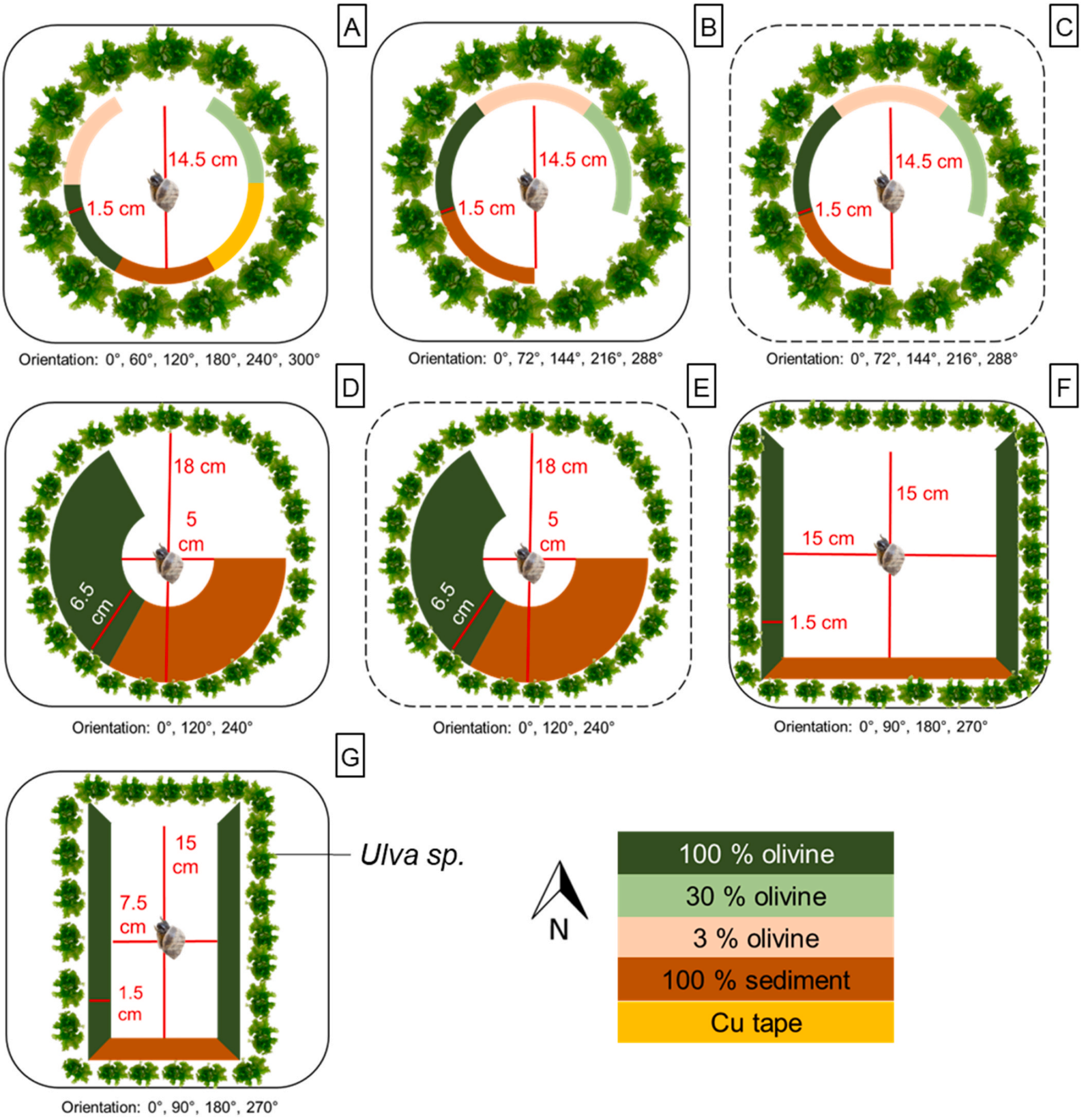

Fig. 1. Schematic representation of the olivine avoidance assays with L. littorea. Square bioassay dishes (A – G) were filled with 200 mL ASW and a circular or rectangular band of various substrates (listed in the figure) surrounded by a band of Ulva sp. (details provided in text). Dotted lines in (C) and (E) indicate that the substrate was put underneath the dish to assess colour preference. Each setup consisted of three to six replicates which were rotated (values below subfigures) to avoid that directional preference would affect the test results.

Scientific) was added and samples were heated again at 240 °C for 15 min. After digestion, samples were diluted 8.3 times with ultrapure Milli-Q (MQ) water. Finally, the supernatant of the samples was collected after centrifugation at 1600 g for 5 min for ICP-OES analysis. For quality control, certified reference material (SARM-6 NIM-D dunite, MINTEK) and procedural blanks were included during analysis. Average elemental recoveries were acceptable for all elements (72 to 93%) except Si due to the insolubility of silicate in HNO_3 (~0.4%).

2.3. *Littorina littorea* olivine avoidance assays

Novel choice experiments were conducted on square Nunc® bioassay dishes (L x W x H = 24.5 x 24.5 x 2.5 cm) to assess which substrate adult *L. littorea* avoided to reach a food source (*Ulva sp.*) and whether this avoidance was distance or substrate colour dependent. All experiments were conducted at controlled temperature (15 ± 1 °C) and light (12 L:12D) conditions and bioassay dishes were cleaned with 2% V/V HCl (VWR International) and ultrapure MQ before use. A similar experimental setup was used for all seven experiments. Namely, dishes were filled with 200 mL of ASW (~6 mm layer) and a ~3 mm thick circular or rectangular band of different substrates (experiment specific) surrounded by a ~2 cm wide band (50.0 ± 0.04 g) of *Ulva sp.* (Fig. 1). The number of dishes was equal to the amount of substrate patches and dishes were rotated by 360/*i* degrees, with *i* equal to the dish number, in order to exclude the possible effect of directional preference on test results. Substrate weights for all experiments are provided in Supplementary Section S2.2.

At the start of an experimental trial, adult *L. littorea* were individually placed in the centre of the dishes with the apex of the shell pointing southeastwards. Subsequently, *L. littorea* could move around freely for 10 min after which the first substrate crossed to reach the *Ulva sp.* was recorded. Next, approximately 95% of the ASW was removed with a syringe and dishes were wiped with paper towel to remove any mucus trails which could affect the path of subsequent *L. littorea* (Erlandsson and Kostylev, 1995). Afterwards, the substrate patches were rearranged if needed and 200 mL of fresh ASW was added to the dishes to start a new trial. A total of 20 trials were conducted for each experiment. A light diffuser (Bresser TR-4) was installed 80 cm above the experimental setup to create soft lighting conditions and experiments were recorded with a digital camera (Fujifilm FinePix XP60).

In a first experiment, six substrate types including pure (100% w/w) North Sea sediment, 3%, 30%, and 100% w/w olivine, copper (Cu) tape, and a blank patch were put in a circular band with an inner diameter of 14.5 cm and width of 1.5 cm on the dishes (Fig. 1A). The Cu tape served as a positive control since it is known to be avoided by most gastropod species (Barnes and Hill, 2022), while the blank patch was included in case sandy substrates would be avoided. For the second experiment, the first experimental setup was replicated without Cu tape as a substrate (Fig. 1B), since Cu release could affect periwinkle behaviour (Barnes and Hill, 2022). This experiment was also repeated with the substrate band and 200 mL ASW on a second dish beneath the original dish containing the circular band of 50 g *Ulva sp.* and 200 mL ASW to assess if sediment avoidance was colour based (Fig. 1C).

The width of the circular substrate band was increased from 1.5 to 6.5 cm (decreasing the inner circle diameter to 5 cm) during the fourth experiment to examine whether the size of the sediment patch would affect the choice of *L. littorea* (Fig. 1D). Only pure North Sea sediment, pure olivine and a blank patch were included as substrate types in this experiment. This setup was also replicated with the substrate band in a separate dish below the original dish, similar to the third experiment, to assess whether *L. littorea* exhibited colour preference (Fig. 1E). During the sixth and seventh experiment, substrates were positioned in a rectangular formation of different size to assess whether sediment avoidance was affected by the distance to the food source (Fig. 1F-G). The distance from the periwinkle to the two olivine patches was either half (7.5 cm, Fig. 1F) or equal to (15 cm, Fig. 1E) the distance to a 100% w/w North Sea sediment and blank patch. The width of the sediment patches was equal to 1.5 cm in both experiments (Fig. 1F-G). Pictures of the experimental setups are shown in Supplementary Figure A.2.

Seawater pH (8.18 ± 0.07), temperature (14.8 ± 0.4 °C), salinity (33.2 ± 1.1‰), DO (8.09 ± 0.16 mg L^{-1}), and nitrogenous waste concentrations were measured at the start of each experiment. Furthermore, water samples for dissolved metal analysis were collected with a syringe from all dishes at the end of the first trial and last trial of each experiment. Water samples were filtered through 0.2 μm pore size filters (Chromafil XTRA PES-20/25, Macherey-Nagel) and diluted 20 times with 2% V/V TraceMetalTM Grade nitric acid (Fisher Scientific) before analysis via HR-ICP-MS (Element XR™, Thermo Scientific™). Procedural blanks (2% V/V HNO_3) and certified reference material (1643 f, NIST) were included in the analysis for quality control. Dissolved metal results are provided in Supplementary Section SI 2.3.

2.4. *Gammarus locusta* olivine avoidance assays

A bi-compartmented system according to De Lange et al. (2006) was used with minor modifications to assess olivine avoidance in the marine amphipod *G. locusta* (Fig. 2 and Supplementary Figure A.4). A total of 25 glass aquariums (L x W x H = 30 ×20 x 15 cm) representing 5 treatments were rinsed with 2% V/V HCl, ultrapure MQ and set up in a temperature controlled room (18.6 ± 1.1 °C) with a fixed photoperiod (12 L:12D). For four treatments, aquariums were divided in two equal sections (L x W = 15 ×15 cm) which were filled with a 5 mm layer (175.6 ± 0.007 g, *N* = 20) of wet control sediment on one side, and a 5 mm layer of control sediment, 3% w/w, 30% w/w, or 100% w/w olivine on the other side (Fig. 2). For the remaining treatment a 5 mm layer of control sediment and 5 mm layer of 100% w/w olivine was put underneath the five replicate aquariums to assess if olivine avoidance was colour based (Fig. 2). Aquariums were rotated (0, 90, 180, 180, and 270°) to have the sediments face different directions. Sediment weights are provided in Supplementary Table A.4. Subsequently, 6 L of ASW was added to each aquarium and continuously aerated with aeration lines fitted with plastic tips positioned 8 cm above the sediment surface. The sediment was then left to settle for 2 days and a 50% water changed was conducted 24 h before 10 adult amphipods (70 – 80% males) were added to each aquarium. Survival and position of the amphipods was then recorded three times per day for three consecutive days. Afterwards, surviving amphipods were collected to examine whole body trace metal concentrations.

Amphipods for whole body metal analysis were individually rinsed with ultrapure MQ and dried at 60 °C for 24 h in preweighed 5 mL tubes. Standard reference material (SRM-2976, NIST) and procedural blanks were included during sample preparation and analysis. Sample dry weight was recorded with an ultra-microbalance (Sartorius SE2). Next, samples were digested in 400 μL of TraceMetal™ Grade nitric acid (67 – 69% V/V, Fisher Scientific) at room temperature for at least 12 h. Afterwards, digestion was continued at 105 °C for 15 min in a SC154 HotBlock® (Environmental Express) (Flipkens et al., 2023; Van Ginneken et al., 2015). Subsequently, 50 μL of 30 – 32% w/w H_2O_2 (Fisher Scientific) was added and samples were left to incubate at room temperature for 10 min. Finally, the samples were heated at 105 °C for 15 min and subsequently diluted with ultrapure MQ to a final acid concentration of 2.2% V/V. Elemental concentrations were analysed via HR-ICP-MS (Element XR, Thermo Fischer Scientific). Certified reference material (1643 f, NIST) was measured every 20 to 22 samples for analytical quality control. The recoveries of Ni and Cr for SRM-2976 were 80.1 ± 7.6% (*N* = 8) and 49.3 ± 11.3% (*N* = 8), respectively. The lower Cr recoveries may be attributed to both the relatively low certified value of 0.5 μg g^{-1} for SRM-2976 and the potential small variations during sample preparation and analysis. Measured whole body Cr concentrations for *G. locusta* were not adjusted to account for the lower SRM-2976 Cr recoveries.

Seawater pH (8.12 ± 0.04), temperature (18.4 ± 0.1 °C), and

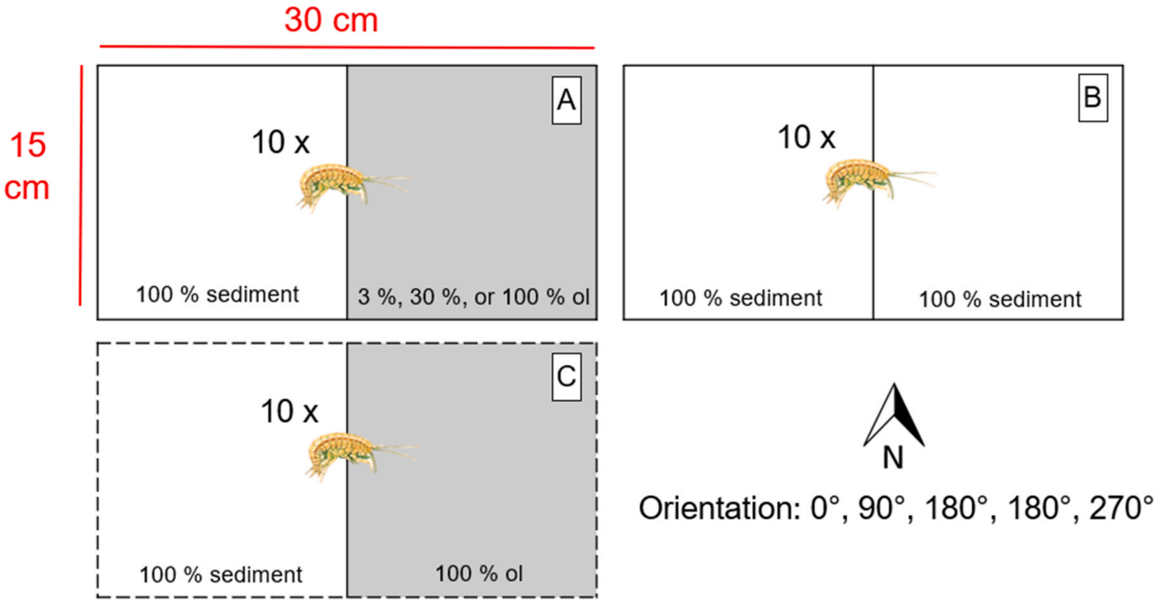

Fig. 2. Schematic representation of the olivine avoidance assays with G. locusta. Glass aquariums (9 L, represented by large rectangles) were filled with 6 L ASW and divided in two equal compartments with a 5 mm layer of 100% w/w sediment on one side (shown in white) and (A) 3%, 30%, or 100% w/w olivine (ol, shown in grey) or (B) 100% w/w sediment on the other side. (C) To assess colour preference, 100% w/w sediment and 100% w/w ol were put underneath the aquarium (indicated by dotted lines). Five aquariums with 10 amphipods were used for each treatment and rotated by 0°, 90°, 180°, 180°, or 270° (orientation) to exclude the effect of possible directional preference on the experimental results.

salinity (32.7 ± 0.3‰) were measured at the start of the experiment and after 48 and 72 h in all aquariums. Simultaneously, DO (7.7 ± 0.1 mg L^{-1}) and nitrogenous waste concentrations ($NO_3^- < 12.5$ mg L^{-1}, $NO_2^- < 0.3$ mg L^{-1}, and $NH_3/NH_4^+ \leq 0.25$ mg L^{-1}) were measured in one replicate of each treatment. A 50% water change was conducted after 48 h of exposure and 10 mL water samples for dissolved metal analysis were collected from all aquariums on each experimental day.

2.5. Statistical analyses

Statistical analyses were conducted in RStudio (version 2022.12.0 +353) using R version 4.2.1 (R Core Team, 2022) and figures were constructed in GraphPad Prism version 9.5.1 for Windows. For the *L. littorea* olivine avoidance assays, Pearson chi-square tests were performed to test whether observed frequencies deviated significantly from expected frequencies under the null hypothesis that *L. littorea* did not show substrate avoidance. Subsequently, squared standardized residuals were used to determine which substrate types were significantly avoided with the pchisq() function.

For the *G. locusta* olivine avoidance bioassays, differences in amphipod survival between treatments were assessed via one-way ANOVA. A binomial generalized linear mixed model (GLMM) was fit by maximum likelihood (Laplace approximation) to assess the main and interactive effect of time (fixed factor with four levels) and treatment (fixed factor with five levels) on the fraction of amphipods in the control compartment. Aquarium ID and repeated daily observations were included as random factors (25 and three levels, respectively) in the model. Aquarium ID was nested in treatment and observations were nested in time. ANOVA tests were performed to simplify the model by removal of a non-significant (p-value < 0.05) interaction term and explanatory variables. The model was found to not be overdispersed by comparing the sum of squared Pearson residuals to the residual degrees of freedom. Furthermore, normality and homoscedasticity of the model residuals were visually assessed via quantile-quantile (Q-Q) and residuals plots, respectively and found to be acceptable. The conditional and marginal R^2 values of the GLMM were estimated using the “r.squaredGLMM” function of the “MuMIn” package using the delta method (Nakagawa et al., 2017).

Differences in amphipod whole body metal concentrations among treatments were investigated with a GLMM using the R package “nlme”. Treatment was considered a fixed factor and aquarium ID a random factor (25 levels) nested in treatment. Statistical differences in ASW dissolved metal concentrations among experiments with *L. littorea* were analysed similarly. Here, experiment was considered a fixed factor and trial a random factor (two levels). Normality and homoscedasticity of model residuals were assessed as described previously. Data were not transformed after violation of the normality assumption due to the robustness of GLMMs to deviation of model residuals from a normal distribution (Schielzeth et al., 2020). In case of heteroscedasticity, a weights argument was added to the model to differentially weight the data of different treatments. The object containing the weights was created using the “varIdent” function. Post hoc multiple comparisons were performed with the “emmeans” package” to identify significant differences among treatments. Adjustment of p-values was done using Tukey’s method.

3. Results and discussion

3.1. Sediment characteristics

A low average SOM content was observed for the control sediment (0.44% w/w) and pure olivine (0.28% w/w) used in the avoidance assays with *L. littorea* (Table 1). Both olivine and control sediment were sandy substrates, but the olivine (D10 – D90 = 83 – 214 µm) was finer than the control sediment (D10 – D90 = 180 – 385 µm). The average Ni and Cr concentrations of pure olivine (42 and 58 µmol g^{-1} d.w., respectively) were 1150 and 138 times higher those of the control sediment (0.037 and 0.42 µmol g^{-1} d.w., respectively) (Table 1).

For the avoidance assays with *G. locusta*, the average SOM content was also low for both the control sediment (1.0% w/w) and pure olivine (0.29% w/w) (Table 1). The olivine used in these tests was slightly coarser (D10 – D90 = 93 – 332 µm) than the control sediment (71 – 222 µm) and had average Ni and Cr concentrations (38 and 23 µmol g^{-1} d.w., respectively) that were 1078 and 26 times higher than those of the

Table 1
Geochemical properties of the sediments in the olivine avoidance assays with L. littorea and G. locusta. Mean and S.D. (N = 3 – 5) are given for the sediment organic matter content (SOM), volumetric effective grain sizes (D) and sediment nickel (Ni) and chromium (Cr) concentrations (µmol g^{-1} d.w.). The grain size distribution of the 3% and 30% w/w olivine was not measured (N.A.) for the olivine avoidance assays with G. locusta.

***L. littorea* olivine avoidance assays**				
Treatment	100% sediment	3% olivine	30% olivine	100% olivine
SOM (%)	0.44 ± 0.029	0.44[a]	0.39[a]	0.28 ± 0.0066
D10 (µm)	180 ± 2.0	174 ± 1.6	131 ± 2.5	83 ± 0.7
D50 (µm)	263 ± 2.6	261 ± 2.4	236 ± 0.9	135 ± 1.0
D90 (µm)	385 ± 10	391 ± 3.5	404 ± 9.3	214 ± 1.4
Ni (µmol g^{-1} d.w.)	0.037 ± 0.0047	1.3[a]	13[a]	42 ± 0.8
Cr (µmol g^{-1} d.w.)	0.42 ± 0.080	2.2[a]	18[a]	58 ± 3.4
***G. locusta* olivine avoidance assays**				
Treatment	100% sediment	3% olivine	30% olivine	100% olivine
SOM (%)	1.0 ± 0.087	1.0[a]	0.81[a]	0.29 ± 0.0072
D10 (µm)	71 ± 0.80	N.A.	N.A.	93 ± 6.9
D50 (µm)	126 ± 2.8	N.A.	N.A.	189 ± 5.4
D90 (µm)	222 ± 8.1	N.A.	N.A.	332 ± 5.5
Ni (µmol g^{-1} d.w.)	0.036 ± 0.018	1.2[a]	12[a]	38 ± 0.87
Cr (µmol g^{-1} d.w.)	0.90 ± 0.16	1.6[a]	7.8[a]	23 ± 0.85

[a] The SOM content and concentrations of Ni and Cr for the 3% and 30% w/w olivine were not analytically determined, but derived from the measured concentrations in the 100% w/w sediment and 100% w/w olivine treatment.

control sediment (0.036 and 0.90 µmol g^{-1} d.w., respectively). The 60% lower Cr concentration of the different olivine batch used in the avoidance assays with *G. locusta* compared to *L. littorea* can likely be explained by the heterogeneous distribution of chromite ($FeCr_2O_4$) in dunite rock (Santos et al., 2015). The concentrations of Mg, Fe, Al, Mn, Co, and Zn for the different experimental sediments are provided in Supplementary Table A2.

3.2. *Littorina littorea olivine avoidance*

Littorina littorea showed clear avoidance and preference of certain substrate types to reach a food source (Fig. 3). The number of *L. littorea* that crossed the patch without substrate to reach *Ulva sp.* was 20% higher than expected in case of no substrate preference (X^2 (5, N = 104) = 24.6, $p < 0.001$) for the experiment with Cu tape and 26% higher (X^2 (4, N = 93) = 32.0, $p < 0.001$) in the replicated experiment without Cu tape (Fig. 3A-B). Based on the eye anatomy, *L. littorea* are expected to have relatively good vision underwater allowing them to clearly resolve objects of approximately 2 cm in size up to 72 cm away (SEYER, 1992). Hence, *L. littorea* could possibly see that no foreign substrate had to be crossed in order to reach *Ulva sp.* via the blank patch, which could explain the higher choice for this substrate type in the current experimental setup. Conversely, Cu tape was only crossed by 2% of the *L. littorea*, indicating significant avoidance (X^2 (5, N = 104) = 13.6, $p = 0.019$) (Fig. 3A). Previous studies have reported that terrestrial and aquatic gastropods prevent crossing Cu barriers, although the reason for avoidance remains unknown (Barnes and Hill, 2022).

Littorina littorea did not significantly avoid 100% w/w sediment or sediment with 3% w/w or 30% w/w olivine in both experiments (Fig. 3A-B). However, 100% w/w olivine was significantly avoided, with only 1.9% of the individuals crossing pure olivine in the experiment with Cu tape (X^2 (5, N = 104) = 13.6, $p = 0.019$) and 1.1% in the experiment without Cu tape (X^2 (4, N = 93) = 16.7, $p = 0.0023$) (Fig. 3A-B). A number of reasons can possibly explain pure olivine avoidance by *L. littorea* including high trace metal concentrations, low sediment organic matter content (food quantity and quality), and differences in olivine morphology (grain shape, edge sharpness, size, and colour) compared to natural sediment (Araújo et al., 2016). The SOM content of olivine and control sediment was low and not significantly different (Table 1). Hence, olivine avoidance is likely not the result of differences in substrate SOM content. Furthermore, during the experiment most *L. littorea* (initially not located on pure olivine) immediately moved towards a substrate different from 100% w/w olivine, indicating that pure olivine was likely not avoided due to its smaller, less rounded grains compared to natural beach sand (Supplementary Figure A.1A-D). Therefore, olivine avoidance was likely driven by chemical cues or substrate colour.

Significant avoidance of 100% w/w olivine (X^2 (2, N = 58) = 15.5, $p < 0.001$) was also observed when the width of the substrate bands was increased from 1.5 to 6.5 cm (Fig. 3D). In contrast to previous results, significant preference of the 100% w/w sediment patch (X^2 (2, N = 58) = 48.6, $p < 0.001$) and avoidance of the blank patch (X^2 (2, N = 58) = 9.20, $p = 0.010$) was observed (Fig. 3D). These findings are in line with positive scototaxis (attraction to dark shapes) previously observed in *L. littorea* (Moisez and Seuront, 2020), given the relatively dark colour of the natural sediment and light colour of the 100% w/w olivine and blank patch (Supplementary Figure A.2). This behaviour is likely important for *L. littorea* in its natural environment (typically rocky shores) to find dark crevices where they are safe from predation, desiccation, and thermal stress (Moisez and Seuront, 2020; Seuront et al., 2018). The scototactic response is dependent on the dimensions of the dark shape (Moisez and Seuront, 2020), which could explain why no significant preference of the 100% w/w sediment was observed when the patch was only 1.5 cm wide (Fig. 3A-C).

When substrates were put underneath the bioassay dishes, 100% w/w olivine was still significantly avoided in both the experiment with the wider substrate circle (X^2 (2, N = 93) = 15.5, $p < 0.001$) (Fig. 3E) and smaller substrate circle (X^2 (4, N = 83) = 12.84, $p = 0.012$) (Fig. 3C). The relative number of *L. littorea* that crossed 100% w/w olivine was very similar in these experiments (1.6 – 2.4%) compared to the previous experiments (1.1 – 3.4%, Fig. 3A-B and D). Therefore, we can conclude that pure olivine avoidance was mainly a positive scototactic response due to the significantly lighter colour of 100% w/w olivine compared natural sediment (see Supplementary Figure A.2).

Finally, distance towards the food source significantly affected the substrate choice of *L. littorea* (Fig. 3F-G). Similar to previous experiments with 1.5 cm wide substrate patches, olivine was significantly avoided (X^2 (2, N = 78) = 24.6, $p < 0.001$) and the blank patch was preferred (X^2 (2, N = 78) = 38.8, $p < 0.001$) when the distance between the *L. littorea* and the *Ulva sp.* was equal (15 cm) for all substrate types (Fig. 3F). However, no significant avoidance of pure olivine was observed when the distance between the *L. littorea* and the *Ulva sp.* was 45% smaller via the olivine patches (9 cm) compared to the blank or 100% sediment patch (16.5 cm) (X^2 (2, N = 75) = 0.60, $p = 0.74$) (Fig. 3G). Hence, we can infer that distance to a food source or darker microhabitat (*Ulva sp.* in this case) could significantly affect the degree of pure olivine avoidance in *L. littorea* (Moisez and Seuront, 2020; Seuront et al., 2007).

3.3. *Gammarus locusta avoidance assays*

3.3.1. *Avoidance behaviour*

Average amphipod survival ranged between 84 to 90% and was not significantly different among treatments ($F(4, 20) = 0.24$, $p = 0.91$). Overall, control preference was close to 50% throughout the experiment in the 100% w/w sediment control (average values ranging between 31% and 53%, $N = 12$), indicating that amphipods did not exhibit significant directional preference (Fig. 4). No significant effect of exposure day ($X^2 = 2.22$, Df = 3, $p = 0.53$) or the interaction between treatment and exposure day ($X^2 = 8.76$, Df = 12, $p = 0.72$) was observed on amphipod control preference. Therefore, these terms were removed from the binomial GLMM. No significant preference for the control compartment was observed in the 3% w/w ($\beta = 0.060$, $z = 0.14$,

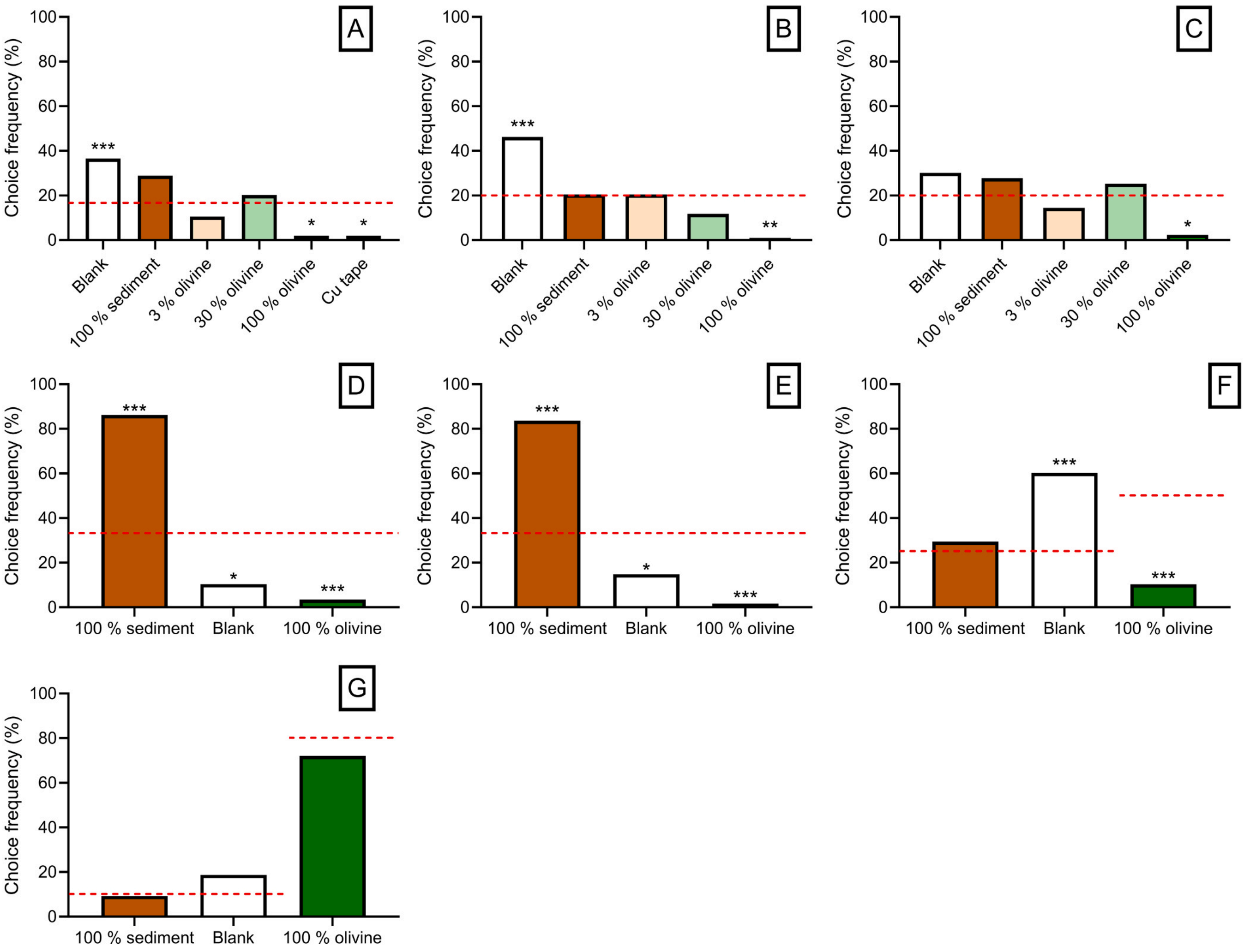

Fig. 3. Substrate preference of L. littorea during acute olivine avoidance assays. Choice frequency (%) of different substrates that were first crossed by L. littorea to reach a food source (Ulva sp.) are shown for the experimental setups displayed in Fig. 1. Subfigure numbers correspond to subfigure numbers in Fig. 1. Significance compared to the expected frequency in case of no avoidance (red dotted line): $* = p < 0.05$, $* * = p < 0.01$, $* ** = p < 0.001$.

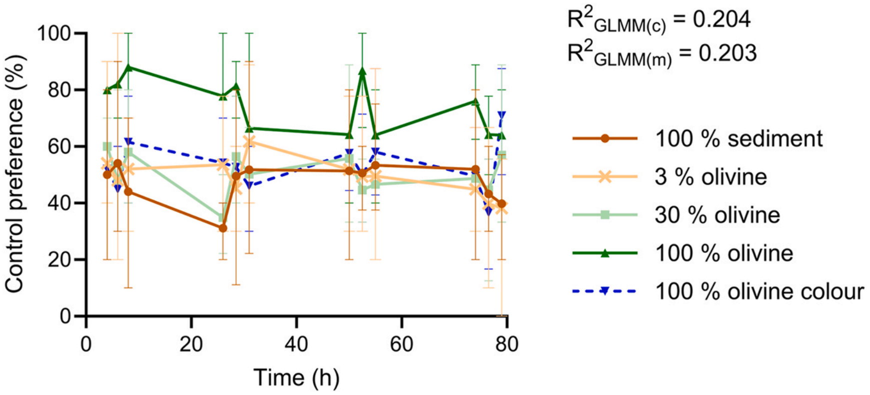

Fig. 4. Control sediment preference (%) of G. locusta as a function of time (h) during the three day bi-compartmented olivine avoidance assays. Mean and range values ($N = 5$) are shown for the 100% w/w sediment control and various olivine treatments (3%, 30%, and 100% w/w olivine) including a pure olivine treatment where the substrate was put underneath the aquariums to investigate colour preference (100% olivine colour). The conditional ($R^2_{GLMM(c)}$) and marginal ($R^2_{GLMM(m)}$) coefficients of determination (R^2) are also given.

$p = 0.89$) and 30% w/w ($\beta = -0.0033$, $z = -0.008$, $p = 0.99$) olivine treatments (Fig. 4). However, pure olivine was significantly avoided ($\beta = 2.67$, $z = 5.07$, $p < 0.001$) by amphipods throughout the experiment (Fig. 4).

Pure olivine avoidance was not significantly driven by colour preference ($\beta = 0.095$, $z = 0.20$, $p = 0.84$, Fig. 4) in contrast to our findings for *L. littorea*. Differences in food availability (sediment organic carbon) can also likely not explain the observed 100% w/w olivine avoidance, since SOM concentrations were low in all treatments (Table 1) and olivine avoidance occurred within hours (Fig. 4) while amphipods were fed prior to the experiment. Furthermore, *G. locusta* inhabits a wide variety of sediment types (from gravel to mud) along its natural distribution (Costa and Costa, 2000), and its survival under laboratory conditions was not significantly affected by the fraction of fine (<63 μm) particles in the sediment during a 10-day exposure (Costa et al., 1996). Therefore, the limited difference in grain size distribution between pure olivine and control sediment (Table 1) also likely does not explain the observed 100% w/w olivine avoidance. However, various studies have shown that amphipods significantly avoid contaminated sediments in a concentration dependent manner (De Lange et al., 2006; Hellou et al., 2005; Szczybelski et al., 2018). Therefore, *G. locusta* possibly avoided pure olivine due to its high Ni (38 μmol g^{-1} d.w.) and Cr (23 μmol g^{-1} d.w.) content (Table 1) in order to reduce trace metal exposure (Araújo et al., 2016).

3.3.2. *Trace metal accumulation*

Whole body Ni concentrations were significantly elevated in amphipods of the 30% w/w olivine treatment (mean ± S.D. = 0.18 ± 0.13 μmol g^{-1} d.w., range = 0.05 – 0.57 μmol g^{-1} d.w., $N = 20$) and 100% w/w olivine treatment (mean ± S.D. = 0.30 ± 0.27 μmol g^{-1} d.w., range = 0.08 – 1.16 μmol g^{-1} d.w., $N = 18$) compared to the 100% w/w sediment control (mean ± S.D. = 0.063 ± 0.022 μmol g^{-1} d.w, range = 0.04 – 0.13 μmol g^{-1} d.w., $N = 20$) at the end of the experiment (Fig. 5A). This increase in whole body Ni concentrations can be the result of waterborne Ni uptake and dietary Ni uptake via the ingestion of olivine grains (Adams et al., 2011). However, the number of olivine grains that were ingested by experimental amphipods was likely limited, since no significant increase in whole body Cr ($F(4,20) = 2.26$, $p = 0.099$) or Fe ($F(4,20) = 1.03$, $p = 0.42$) concentrations was observed (Fig. 5B and Supplementary Figure A.6 A). Hence, the observed Ni bioaccumulation was mainly the result of waterborne Ni uptake.

Dissolved Ni concentrations in the ASW were elevated in the 100% w/w olivine (112 – 174 nmol L^{-1}, $N = 10$) and 100% olivine colour (107 – 122 nmol L^{-1}, $N = 15$) treatment compared to the sediment control (42 – 64 nmol L^{-1}, $N = 10$) (Fig. 5C). Elevated Ni concentrations in the 100% w/w olivine treatment are the result of olivine weathering (Montserrat et al., 2017), while potential impurities in the sea salt could explain higher concentrations in the 100% olivine colour treatment which was tested separately from the other treatments. Reported waterborne Ni uptake rate constants vary between 0.0052 and 0.24 L g^{-1} d^{-1} for different aquatic invertebrates exposed to dissolved Ni concentrations ranging between 0.26 and 767 nmol L^{-1} (Hédouin et al., 2007; Urien et al., 2017). Given these Ni uptake rate constants and the observed Ni concentrations in the ASW, a whole body Ni concentration between 0.090 and 0.18 μmol g^{-1} d.w. would be expected in amphipods of the 100% w/w olivine treatment (calculations provided in Supplementary Section SI 3.4), which was exceeded in 61% of the exposed amphipods (Fig. 5A). Exposure to elevated Ni concentrations in the porewater compared to the overlying water and interindividual differences in the amount of time spent on the olivine 100% w/w olivine compartment could possibly explain the high and variable whole body Ni

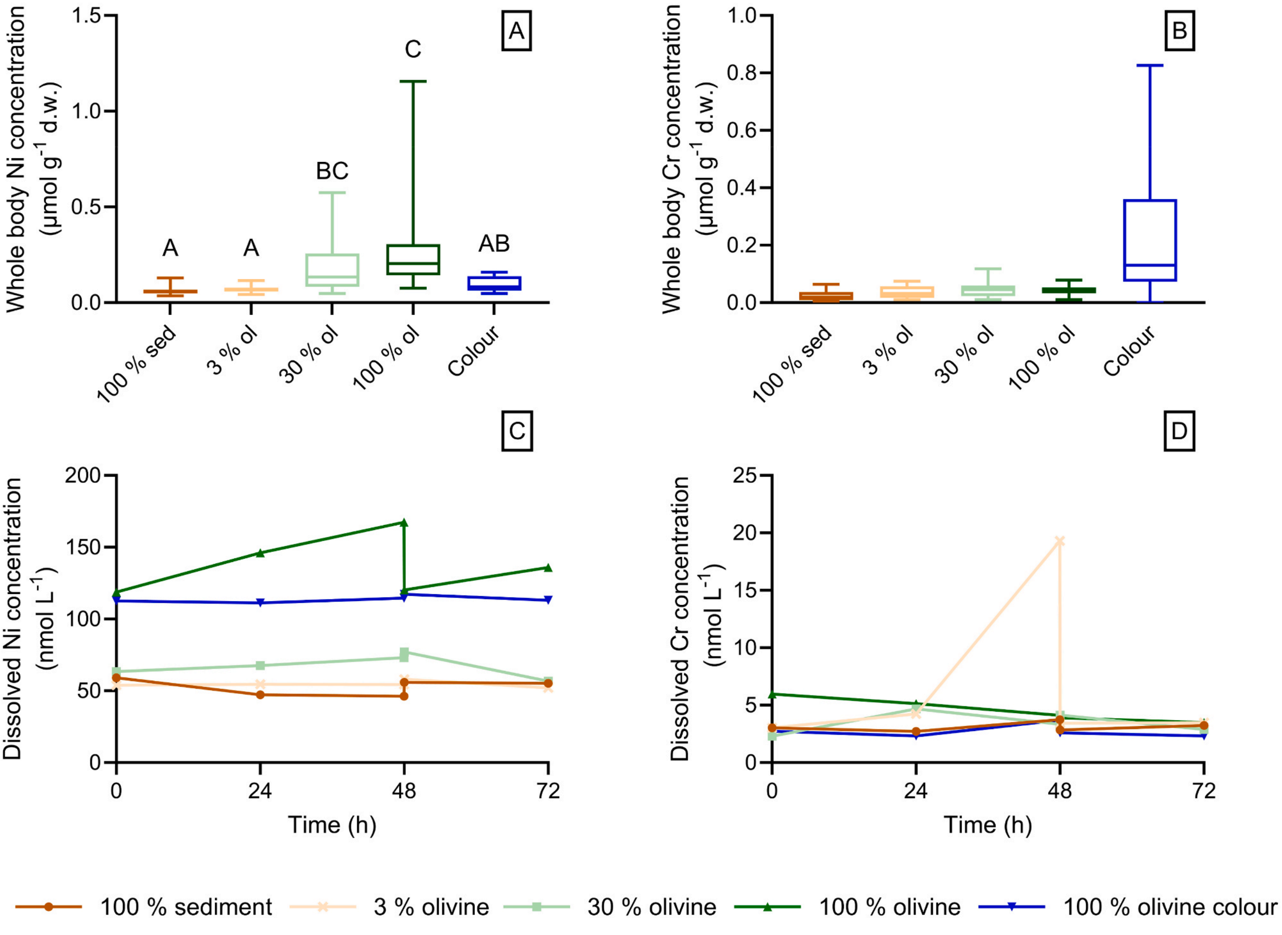

Fig. 5. Metal accumulation in G. locusta and artificial seawater during olivine avoidance assays. Box and whisker plots of the whole body (A) nickel (Ni) and (B) chromium (Cr) concentrations (μmol g^{-1} d.w.) in G. locusta (N = 10 – 20) at the end of the three day olivine avoidance assays. Results are shown for the sediment (sed) control and different olivine (ol) treatment including the 100% w/w olivine treatment to test colour preference (Colour). Temporal evolution of average dissolved (C) Ni and (D) Cr concentrations (nmol L^{-1}) in artificial seawater (N = 2 or 3).

concentrations (Fig. 5A) (Simpson and Batley, 2007).

Whole body Cr concentrations ($<0.34 – 827$ nmol g^{-1} d.w., $N = 84$) were generally lower than whole body Ni concentrations and did not differ significantly between treatments ($F(4,20) = 2.26$, $p = 0.099$) (Fig. 5B). The large variation in whole body Cr concentrations in the 100% olivine colour treatment was unexpected and the cause remains unknown. Dissolved Cr concentrations in ASW were not significantly elevated in the olivine treatments (2.2 – 36 nmol L^{-1}, $N = 40$) compared to the sediment control (2.6 – 4.6 nmol L^{-1}, $N = 10$) (Fig. 5D). The lack of Cr accumulation in the ASW of the olivine treatments can be explained by low water solubility of chromite ($FeCr_2O_4$), which is the dominant Cr-bearing phase in dunite rock (Santos et al., 2015).

3.4. Implications for coastal enhanced olivine weathering

Changes in the physicochemical properties of the surficial sediment (e.g. metal and SOM content, grain size distribution) after large scale coastal olivine spreading for climate change mitigation could possibly alter habitat suitability for marine biota and subsequently lead to avoidance of olivine rich sediment patches (Araújo et al., 2016). Here we found that *L. littorea* and *G. locusta* significantly avoided 100% w/w olivine sand. Under the current experimental designs, olivine was avoided due to its light colour (positive scototaxis) by *L. littorea* and likely its high metal content by *G. locusta*. However, olivine was not significantly avoided when it was mixed with natural marine sediment at concentrations of 3% w/w and 30% w/w olivine. Therefore, these benthic macroinvertebrates will likely not avoid olivine-rich sediment patches after large-scale coastal deployments of relatively coarse olivine. Nevertheless, an olivine deployment method suitable for the local beach conditions should be adopted to avoid short-term disturbance and mortality (e.g. by burial) of marine biota (Speybroeck et al., 2006) in addition to long term adverse effects (e.g. possible trace metal toxicity and changes in community composition) (Bach et al., 2019; Flipkens et al., 2021).

For beach nourishment practices, olivine characteristics (e.g. grain size, metal content) in addition to the scale, timing, and method of deployment likely play an important role to minimize ecological impacts (Speybroeck et al., 2006). A number of smaller olivine deployments are generally preferred over a single large deployment, although this would reduce the net CO_2 sequestration efficiency of CESW as a result of higher transport associated CO_2 emissions (Hangx and Spiers, 2009; Speybroeck et al., 2006). Furthermore, olivine spreading is preferably done in periods of low biological activity and the type of deployment (classic, foreshore, or backshore nourishment) should be chosen based on local biodiversity taking into account social and political acceptance (Low et al., 2022; Speybroeck et al., 2006). Ideally, olivine with a similar grain size distribution as that of the local sediment would be dispersed to minimize ecological impacts, although finer grain sizes are possibly preferred from a climate change mitigation perspective due to their faster dissolution and CO_2 sequestration rate (Foteinis et al., 2023; Hangx and Spiers, 2009; Speybroeck et al., 2006). The distribution of fine-grained olivine (<63 μm) could affect the particle-attached microbial community composition (Ren et al., 2021) and sediment siltation could have adverse impacts on various marine biota including shellfish, mangroves, and corals (Erftemeijer et al., 2012; Noor et al., 2015; Poirier et al., 2021). Finally, the positive (e.g. improved calcification and phytoplankton growth) and negative effects (e.g. Ni and Cr toxicity) of olivine dissolution products on marine biota and contribution to climate change mitigation should be considered to assess the scale at which olivine could be safely deployed in coastal waters (Bach et al., 2019; Flipkens et al., 2021; Hutchins et al., 2023). Hence, additional studies on olivine exposure effects for various marine organisms are needed to accurately determine the most suitable, location specific, olivine deployment strategy for atmospheric CO_2 removal.

4. Conclusions

Coastal enhanced olivine weathering has been proposed as a large scale atmospheric CO_2 removal technique for climate change mitigation. However, changes in the surficial sediment physicochemistry after olivine addition could possibly alter the habitat suitability and result in avoidance of olivine rich substrates by some marine species. To test this hypothesis, we conducted several short-term choice experiments to assess the avoidance response of the marine gastropod *L. littorea* and the marine amphipod *G. locusta* to both pure olivine and olivine mixed with natural sediment. Pure olivine was significantly avoided by both organisms, while 3% w/w and 30% w/w olivine mixtures with natural sediment were not. In contrast to our hypothesis, *L. littorea* avoided pure olivine due to its light colour rather than its high trace metal content as a result of an innate attraction to dark shapes (i.e. positive scototaxis). As hypothesized, the degree of avoidance was dependent on the distance to a food source, since pure olivine was not significantly avoided when it was 7.5 cm (45%) closer to *Ulva sp.* than natural sediment. The amphipod *G. locusta* did not avoid pure olivine due to its colour, but likely to reduce Ni and Cr exposure. Nevertheless, a significant increase in whole body Ni concentrations was observed in *G. locusta* of the 30% and 100% olivine treatments compared to the sediment control after 79 h of exposure. Overall, our results indicate that *G. locusta* and *L. Littorea* would not significantly avoid coastal zones with relatively coarse olivine mixed in the surficial sediment up to concentrations of 30% w/w olivine. However, additional research on the effects of long-term olivine exposure on various marine biota is needed to accurately determine the scale and suitable deployment strategy of coastal olivine spreading to maximize the benefits and minimize the ecological risks.

CRediT authorship contribution statement

Gunter Flipkens: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – Original draft, Writing – Review & Editing, Visualization **Vincent Dujardin:** Investigation **Jordy Salden:** Investigation, Writing – Review & Editing **Kyle T'Jollyn:** Investigation, Writing – Review & Editing **Raewyn M. Town:** Conceptualization, Methodology, Writing – Review & Editing, Supervision **Ronny Blust:** Conceptualization, Methodology, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Co-author is a member of the International Editorial Board of Ecotoxicology and Environmental Safety - R.M.T.

Data Availability

Our research data is shared in a supplementary Excel file.

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Appendix A. Supplementary material

Pictures of the experimental setups, substrate weights, and measured and expected metal accumulation are given in the supplementary Word file.

Appendix B. Research data

Substrate preferences and dissolved metal concentrations in artificial seawater of the olivine avoidance assays with *G. locusta* and *L. littorea* are given in the supplementary Excel file. Additionally, temporal seawater pH, temperature, conductivity, and dissolved oxygen concentrations during the olivine avoidance assays with *G. locusta* are shown. Finally, whole body metal concentrations in *G. locusta* at the end of the experiment are available.

Appendix C. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115840.

References

Adams, W.J., Blust, R., Borgmann, U., Brix, K.V., DeForest, D.K., Green, A.S., Meyer, J.S., McGeer, J.C., Paquin, P.R., Rainbow, P.S., Wood, C.M., 2011. Utility of tissue residues for predicting effects of metals on aquatic organisms. Integr. Environ. Assess. Manag. 7 (1), 75–98. https://doi.org/10.1002/ieam.108.

Anderson, M., Underwood, A., 1997. Effects of gastropod grazers on recruitment and succession of an estuarine assemblage: a multivariate and univariate approach. Oecologia 109, 442–453. https://doi.org/10.1007/s004420050104.

Andersson, S., Persson, M., Moksnes, P.-O., Baden, S., 2009. The role of the amphipod Gammarus locusta as a grazer on macroalgae in Swedish seagrass meadows. Mar. Biol. 156, 969–981. https://doi.org/10.1007/s00227-009-1141-1.

Araújo, C.V., Moreira-Santos, M., Ribeiro, R., 2016. Active and passive spatial avoidance by aquatic organisms from environmental stressors: A complementary perspective and a critical review. Environ. Int. 92, 405–415. https://doi.org/10.1016/j.envint.2016.04.031.

Bach, L.T., Gill, S.J., Rickaby, R.E., Gore, S., Renforth, P., 2019. CO_2 removal with enhanced weathering and ocean alkalinity enhancement: Potential risks and co-benefits for marine pelagic ecosystems. Front. Clim. 1, 7 https://doi.org/10.3389/fclim.2019.00007.

Barnes, A.J., Hill, J.M., 2022. Copper barriers can cause behavioral artifacts in experiments with marine snails. Mar. Ecol. Prog. Ser. 685, 127–136. https://doi.org/10.3354/meps13959.

Boos, K., Gutow, L., Mundry, R., Franke, H.-D., 2010. Sediment preference and burrowing behaviour in the sympatric brittlestars Ophiura albida Forbes, 1839 and Ophiura ophiura (Linnaeus, 1758)(Ophiuroidea, Echinodermata). J. Exp. Mar. Biol. 393 (1-2), 176–181. https://doi.org/10.1016/j.jembe.2010.07.021.

Carlson, R.L., Shulman, M.J., Ellis, J.C., 2006. Factors contributing to spatial heterogeneity in the abundance of the common periwinkle Littorina littorea (L.). J. Mollusca Stud. 72 (2), 149–156. https://doi.org/10.1093/mollus/eyi059.

Caserini, S., Storni, N., Grosso, M., 2022. The availability of limestone and other raw materials for ocean alkalinity enhancement. Glob. Biogeochem. Cycles 36 (5), e2021GB007246. https://doi.org/10.1029/2021GB007246.

CEM Corporation, 1999. Chromite Ore Microwave Sample Preparation Note: 5OS-47. 〈http://www.uwm.edu.pl/kchemsr/MARS/OXIDSUL/Chromit.pdf〉 (accessed 04/07/2022).

Costa, F., Costa, M., 2000. Review of the ecology of Gammarus locusta [L.]. Pol. Arch. Hydrobiol. 47 (3-4), 541–559.

Costa, F.O., Correia, A.D., Costa, M.H., 1996. Sensitivity of a marine amphipod to non-contaminant variables and to copper in the sediment. Ecol. Brunoy 27 (4), 269–276.

De Lange, H.J., Sperber, V., Peeters, E.T., 2006. Avoidance of polycyclic aromatic hydrocarbon–contaminated sediments by the freshwater invertebrates Gammarus pulex and Asellus aquaticus. Environ. Toxicol. Chem. 25 (2), 452–457. https://doi.org/10.1897/05-413.1.

Duan, L., Song, J., Li, X., Yuan, H., Zhuang, W., 2022. Potential risks of CO_2 removal project based on carbonate pump to marine ecosystem. Sci. Total Environ. 862, 160728 https://doi.org/10.1016/j.scitotenv.2022.160728.

Erftemeijer, P.L., Riegl, B., Hoeksema, B.W., Todd, P.A., 2012. Environmental impacts of dredging and other sediment disturbances on corals: a review. Mar. Pollut. Bull. 64 (9), 1737–1765. https://doi.org/10.1016/j.marpolbul.2012.05.008.

Erlandsson, J., Kostylev, V., 1995. Trail following, speed and fractal dimension of movement in a marine prosobranch, Littorina littorea, during a mating and a non-mating season. Mar. Biol. 122, 87–94. https://doi.org/10.1007/BF00349281.

Flipkens, G., Blust, R., Town, R.M., 2021. Deriving nickel (Ni (II)) and chromium (Cr (III)) based environmentally safe olivine guidelines for coastal enhanced silicate weathering. Environ. Sci. Technol. 55 (18), 12362–12371. https://doi.org/10.1021/acs.est.1c02974.

Flipkens, G., Horoba, K., Bostyn, K., Geerts, L.J., Town, R.M., Blust, R., 2023. Acute bioaccumulation and chronic toxicity of olivine in the marine amphipod Gammarus locusta. Aquat. Toxicol. 262, 106662 https://doi.org/10.1016/j.aquatox.2023.106662.

Foteinis, S., Campbell, J.S., Renforth, P., 2023. Life cycle assessment of coastal enhanced weathering for carbon dioxide removal from air. Environ. Sci. Technol. 57 (15), 6169–6178. https://doi.org/10.1021/acs.est.2c08633.

Fuhr, M., Geilert, S., Schmidt, M., Liebetrau, V., Vogt, C., Ledwig, B., Wallmann, K., 2022. Kinetics of olivine weathering in seawater: an experimental study. Front. Clim. 4, 831587 https://doi.org/10.3389/fclim.2022.831587.

Guo, J.A., Strzepek, R.F., Swadling, K.M., Townsend, A.T., Bach, L.T., 2023. Influence of ocean alkalinity enhancement with olivine or steel slag on a coastal plankton community in tasmania. EGUsphere 2023, 1–27. https://doi.org/10.5194/egusphere-2023-2120.

Hangx, S.J., Spiers, C.J., 2009. Coastal spreading of olivine to control atmospheric CO_2 concentrations: a critical analysis of viability. Int. J. Greenh. Gas. Control. 3 (6), 757–767. https://doi.org/10.1016/j.ijggc.2009.07.001.

Hédouin, L., Pringault, O., Metian, M., Bustamante, P., Warnau, M., 2007. Nickel bioaccumulation in bivalves from the New Caledonia lagoon: seawater and food exposure. Chemosphere 66 (8), 1449–1457. https://doi.org/10.1016/j.chemosphere.2006.09.015.

Heiri, O., Lotter, A.F., Lemcke, G., 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. J. Paleolimnol. 25 (1), 101–110. https://doi.org/10.1023/A:1008119611481.

Hellou, J., Cheeseman, K., Jouvenelle, M.L., Robertson, S., 2005. Behavioral response of Corophium volutator relative to experimental conditions, physical and chemical disturbances. Environ. Toxicol. Chem. 24 (12), 3061–3068. https://doi.org/10.1897/05-100R.1.

Hutchins, D.A., Fu, F.-X., Yang, S.-C., John, S.G., Romaniello, S.J., Andrews, M.G., Walworth, N.G., 2023. Responses of globally important phytoplankton species to olivine dissolution products and implications for carbon dioxide removal via ocean alkalinity enhancement. Biogeosciences 20 (22), 4669–4682. https://doi.org/10.5194/bg-20-4669-2023.

IPCC. (2022). Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [P.R. J. Shukla, Skea, R., Slade, A., Al Khourdajie, R., van Diemen, D., McCollum, M., Pathak, S., Some, P., Vyas, R., Fradera, M., Belkacemi, A., Hasija, G., Lisboa, S., Luz, J. Malley, (eds.)]. C. U. Press. http://doi.org/10.1017/9781009157926.

Lopes, I., Baird, D.J., Ribeiro, R., 2004. Avoidance of copper contamination by field populations of Daphnia longispina. Environ. Toxicol. Chem. 23 (7), 1702–1708. https://doi.org/10.1897/03-231.

Low, S., Baum, C.M., Sovacool, B.K., 2022. Taking it outside: exploring social opposition to 21 early-stage experiments in radical climate interventions. Energy Res. Soc. Sci. 90, 102594 https://doi.org/10.1016/j.erss.2022.102594.

Meysman, F.J.R., Montserrat, F., 2017. Negative CO_2 emissions via enhanced silicate weathering in coastal environments. Biol. Lett. 13 (4), 20160905. https://doi.org/10.1098/rsbl.2016.0905.

Minx, J.C., Lamb, W.F., Callaghan, M.W., Fuss, S., Hilaire, J., Creutzig, F., Amann, T., Beringer, T., de Oliveira Garcia, W., Hartmann, J., 2018. Negative emissions—Part 1: Research landscape and synthesis. Environ. Res. Lett. 13 (6), 063001 https://doi.org/10.1088/1748-9326/aabf9b.

Moisez, E., Seuront, L., 2020. Deciphering the known unknowns in the behavioural ecology of the intertidal gastropod Littorina littorea. J. Exp. Mar. Biol. Ecol. 524, 151313 https://doi.org/10.1016/j.jembe.2020.151313.

Moles, A., Rice, S., Norcross, B.L., 1994. Non-avoidance of hydrocarbon laden sediments by juvenile flatfishes. Neth. J. Sea Res 32 (3-4), 361–367. https://doi.org/10.1016/0077-7579(94)90013-2.

Montserrat, F., Renforth, P., Hartmann, J., Leermakers, M., Knops, P., Meysman, F.J., 2017. Olivine dissolution in seawater: implications for CO_2 sequestration through enhanced weathering in coastal environments. Environ. Sci. Technol. 51 (7), 3960–3972. https://doi.org/10.1021/acs.est.6b05942.

Nakagawa, S., Johnson, P.C., Schielzeth, H., 2017. The coefficient of determination R 2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. J. R. Soc. Interface 14 (134), 20170213. https://doi.org/10.1098/rsif.2017.0213.

Noor, T., Batool, N., Mazhar, R., Ilyas, N., 2015. Effects of siltation, temperature and salinity on mangrove plants. Eur. Acad. Res. 2 (11), 14172–14179.

Poirier, L.A., Clements, J.C., Coffin, M.R., Craig, T., Davidson, J., Miron, G., Davidson, J.D., Hill, J., Comeau, L.A., 2021. Siltation negatively affects settlement and gaping behaviour in eastern oysters. Mar. Environ. Res. 170, 105432 https://doi.org/10.1016/j.marenvres.2021.105432.

R Core Team, 2022. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. URL 〈http://www.R-project.org/〉.

Ren, H., Hu, Y., Liu, J., Zhang, Z., Mou, L., Pan, Y., Zheng, Q., Li, G., Jiao, N., 2021. Response of a coastal microbial community to olivine addition in the muping marine Ranch, Yantai. Front. Microbiol. 12, 805361 https://doi.org/10.3389/fmicb.2021.805361.

Renforth, P., Henderson, G., 2017. Assessing ocean alkalinity for carbon sequestration. Rev. Geophys. 55 (3), 636–674. https://doi.org/10.1002/2016RG000533.

Santos, R.M., Van Audenaerde, A., Chiang, Y.W., Iacobescu, R.I., Knops, P., Van Gerven, T., 2015. Nickel extraction from olivine: effect of carbonation pre-treatment. Metals 5 (3), 1620–1644. https://doi.org/10.3390/met5031620.

Schielzeth, H., Dingemanse, N.J., Nakagawa, S., Westneat, D.F., Allegue, H., Teplitsky, C., Réale, D., Dochtermann, N.A., Garamszegi, L.Z., Araya-Ajoy, Y.G., 2020. Robustness of linear mixed-effects models to violations of distributional assumptions. Methods Ecol. Evol. 11 (9), 1141–1152. https://doi.org/10.1111/2041-210X.13434.

Seuront, L., Duponchel, A.-C., Chapperon, C., 2007. Heavy-tailed distributions in the intermittent motion behaviour of the intertidal gastropod Littorina littorea. Phys. A: Stat. Mech. Appl. 385 (2), 573–582. https://doi.org/10.1016/j.physa.2007.07.029.

Seuront, L., Ng, T.P., Lathlean, J.A., 2018. A review of the thermal biology and ecology of molluscs, and of the use of infrared thermography in molluscan research. J. Mollusca Stud. 84 (3), 203–232. https://doi.org/10.1093/mollus/eyy023.

SEYER, J.-O., 1992. Resolution and sensitivity in the eye of the winkle Littorina littorea. J. Exp. Biol. 170 (1), 57–69. https://doi.org/10.1242/jeb.170.1.57.

Simpson, S.L., Batley, G.E., 2007. Predicting metal toxicity in sediments: a critique of current approaches. Integr. Environ. Assess. Manag. 3 (1), 18–31. https://doi.org/10.1002/ieam.5630030103.

Speybroeck, J., Bonte, D., Courtens, W., Gheskiere, T., Grootaert, P., Maelfait, J.P., Mathys, M., Provoost, S., Sabbe, K., Stienen, E.W., 2006. Beach nourishment: an ecologically sound coastal defence alternative? A review. Aquat. Conserv.: Mar. Freshw. Ecosyst. 16 (4), 419–435. https://doi.org/10.1002/aqc.733.

Szczybelski, A.S., Kampen, T., Vromans, J., Peeters, E.T., van den Heuvel-Greve, M.J., van den Brink, N.W., Koelmans, A.A., 2018. Avoidance tests as a tool to detect sublethal effects of oil-impacted sediments. Environ. Toxicol. Chem. 37 (6), 1757–1766. https://doi.org/10.1002/etc.4129.

Urien, N., Farfarana, A., Uher, E., Fechner, L., Chaumot, A., Geffard, O., Lebrun, J., 2017. Comparison in waterborne Cu, Ni and Pb bioaccumulation kinetics between different gammarid species and populations: Natural variability and influence of metal exposure history. Aquat. Toxicol. 193, 245–255. https://doi.org/10.1016/j.aquatox.2017.10.016.

Vader, W., Tandberg, A.H.S., 2019. Gammarid amphipods (Crustacea) in Norway, with a key to the species. Fauna Norv 39, 12–25. https://doi.org/10.5324/fn.v39i0.2873.

Van Ginneken, M., De Jonge, M., Bervoets, L., Blust, R., 2015. Uptake and toxicity of Cd, Cu and Pb mixtures in the isopod Asellus aquaticus from waterborne exposure. Sci. Total Environ. 537, 170–179. https://doi.org/10.1016/j.scitotenv.2015.07.153.

Yousefi, S.R., Masjedi-Arani, M., Morassaei, M.S., Salavati-Niasari, M., Moayedi, H., 2019. Hydrothermal synthesis of DyMn2O5/Ba3Mn2O8 nanocomposite as a potential hydrogen storage material. Int. J. Hydrog. Energy 44 (43), 24005–24016. https://doi.org/10.1016/j.ijhydene.2019.07.113.