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Manganese handling in the shore crab *Carcinus maenas:* Influence of hypoxia and calcium concentration

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ABSTRACT

Manganese leaching from reduced sediments during oxygen depletion events may expose benthic organisms to elevated manganese concentrations. Various aspects of manganese handling in *Carcinus maenas* were investigated. Exposure to up to 400 mg Mn L^{-1} for 4 d caused no mortality. Crabs accumulated ^{54}Mn from water almost linearly with time, reaching a whole-body concentration factor of 21 after 22 d. After uptake from food or water, major parts of the body burden were eliminated with half-lives of 96 and 44 d, respectively. Most of the manganese entering the haemolymph (by feeding or injection) was removed within 24 h, a substantial part being transferred to the exoskeleton. Exposure to 50 and 300 μg Mn^{++} L^{-1} in the water led to increased manganese concentrations in most tissues. Hypoxic conditions (19 % oxygen saturation) had only moderate effects on manganese accumulation in midgut gland and exoskeleton, and none in gills. Investigations at field locations confirmed that smaller crabs have higher manganese concentrations in their exoskeleton than larger ones. The most important findings in these experiments are: 1) manganese and calcium compete for uptake over the gills into the haemolymph and 2) manganese in the haemolymph may be translocated to the exoskeleton, thus supporting the possibility that the different behavior of smaller and larger crabs during postmoult calcification of the exoskeleton - by uptake of Ca^{++} from the sea water - may explain their different manganese body burdens (of which ≈95 % is found in the exoskeleton).

1. Introduction

Although manganese is an essential metal in living organisms both as a constituent in and an important activator for several enzymes (i.e. Zelko et al., 2002), exposure to elevated concentrations may lead to toxic manifestations in aquatic organisms (e.g. reviewed by Baden and Eriksson, 2006).

Oxygen depletion due to eutrophication in marine waters has been an increasing problem worldwide (reviewed by Diaz and Rosenberg, 2008) and more specifically in some north European coastal areas such as the Swedish west coast (Rosenberg et al., 1990), parts of the Baltic (Kendzierska and Janas, 2024) and the Danish belt area (Hansen, 2021). Porewater concentrations of manganese in anoxic marine sediments of 13 mg Mn^{++} L^{-1} (Thamdrup et al., 1994) and 19 mg Mn^{++} L^{-1} (Magnusson et al., 1996) have been reported. Under hypoxic and anoxic conditions in bottom waters, the flux of reduced manganese (Mn^{++}) from the sediment pore water increases and concentrations in the order of 1 mg Mn^{++} L^{-1} (Kremling, 1983) of dissolved manganese in the water column may be reached; background manganese concentrations in oxic, marine waters generally are in the 0.01–0.05 μg Mn^{++} L^{-1} range (Kremling, 1983; Landing et al., 1995). Manganese liberated as Mn^{++} from the sediment during hypoxic conditions is re-oxidized to MnO_2 fairly slowly (in the order of weeks) after reintroduction of oxygen to the water (Dehairs et al., 1989). Therefore, benthic organisms may encounter concurrent exposure to both increased manganese concentrations and hypoxic conditions.

Crabs are important animal groups in many benthic ecosystems (e.g. Howard et al., 2017) and the shore crab *Carcinus maenas* has been used extensively as model organisms in the investigation of metal handling (e.g. Bjerregaard et al., 2021; Martin and Rainbow, 1998). In crabs, Ca^{++} is

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transported from the ambient medium via the gills – especially during the calcification of the exoskeleton - and other divalent ions, e.g., cadmium, are also taken up during this process in competition with calcium (Bondgaard and Bjerregaard, 2005; Bondgaard et al., 2000; Norum et al., 2005). It is not known if this also happens for manganese in *C. maenas*, although associations between manganese and calcium have been reported from other taxonomic groups (Calhoun and Zou, 2016; Pankau et al., 2022; Ponzoni, 2017; Poteat et al., 2012; Soldati et al., 2016).

Manganese has the potential to accumulate in aquatic organisms from water (Baden et al., 1994, 1995, 1999, 2003; Baden and Eriksson, 2006; Hansen and Bjerregaard, 1995; Rouleau et al., 1995) and food (Hansen and Bjerregaard, 1995) and adverse effects of the accumulated manganese have been observed in several benthic invertebrates (Fedyunin et al., 2019; Hansen and Bjerregaard, 1995; Oweson et al., 2006, 2008, 2010; Skold et al., 2015).

The combined effects of exposure to hypoxia and manganese have only been sparsely investigated (Baden et al., 1995) and because only few investigations have been carried out on the kinetics of manganese in benthic organisms, we wanted to investigate various aspects of manganese kinetics in the shore crab C. *maenas*, including effects of hypoxia and calcium concentrations on manganese uptake (Table 1).

2. Materials and methods

2.1. Experimental animals

Male, intermoult shore crabs, *Carcinus maenas* (L) were collected in seines at the coasts of the Island of Funen, Denmark (Fig. S1), an area in which animals normally experience fluctuating salinities – typically between 12 and 28 ‰. An investigation on the genetics indicates that all of the *C. maenas* around the Island of Funen population belong to the same population (Nissen et al., 2005).

The animals were kept unfed in tanks supplied with aerated, flowing seawater at the Marine Biological Station, Bøgebjerggård, until used in the experiments.

All animals were acclimated in the laboratory for 6–10 days prior to experiments.

2.2. Exposure procedures and general laboratory conditions

The animals were exposed to stable Mn^{++} or ^{54}Mn either in water or food or by injection. The temperature in the laboratory experiments was maintained at 14.5 ± 0.5 °C and 12 h light/dark period was used. Salinities varied between 15 and 22 ‰ (conductivity meter, Struers CDM3). The water was aerated by means of air stones or perforated plastic tubes. No sediment was placed in the aquaria. Unless otherwise stated, crabs were not fed during experiments. Animals were exposed either in groups of 5–10 in 10 L polystyrene aquaria, individually in 1.5 L plastic beakers or larger groups in 40 L glass aquaria.

In feeding experiments, ^{54}Mn or stable manganese were added to food blocks made of homogenized blue mussel soft parts, solidified with commercial gelatin as described by Bjerregaard and Moller (2021).

Table 1
Overview of the experiments.

Exp.	Purpose of experiment
1 & 2	Establish non-lethal concentrations
3	Investigate whole body uptake kinetics from water and subsequent elimination
4	Establish half-life for Mn in haemolymph
5	Investigate how soon Mn given in food appears in haemolymph
6	Investigate whole body elimination kinetics for Mn injected into the haemolymph and investigate if injected Mn is translocated to the exoskeleton
7	Establish whole body half-life for Mn taken up from food and investigate if ingested Mn is translocated to the exoskeleton
8	Investigate if exposure to low, environmentally realistic Mn concentrations leads to accumulation in tissues
9	Investigate the effect of hypoxia on Mn accumulation
10	Investigate if Mn competes with calcium for uptake over the gills
11	Determination of Mn concentrations in exoskeleton of field collected crabs. Main objective is to confirm that the smallest crabs have highest Mn concentrations. This opens the possibility that these differences may be related to different behaviour of small and large crabs, especially during moult

2.3. Termination of experiments and dissection procedure

The animals were killed by mechanical destruction of the ventral ganglion (Baker, 1962) and tissues were dissected out. The dissected tissues were blotted dry with paper towels, weighed, and frozen at −18 °C. The frozen samples were freeze dried (Hetosicc freeze dryer) for at least 3 days and the dry weight was recorded.

2.4. Manganese analyses

2.4.1. Analysis of stable manganese

Approximately 100 mg freeze dried tissue were transferred to 2 mL concentrated HNO_3 in 20 mL Pyrex glass tubes that were gradually heated to 120 °C. The acid was evaporated, and the samples dissolved in 2 mL 0.2 % nitric acid. Concentrations of manganese were determined by means of a PerkinElmer 2380 atomic absorption spectrometer as described in Bjerregaard et al. (2021). The quality of the determinations was validated by incorporation of certified reference materials from the Canadian National Research Council (TORT-1 and TORT-2 standards; lobster hepatopancreas and Dorm-1; dogfish muscle) with certified values of 23.4 ± 1.0, 13.6 ± 1.2 and 1.32 ± 0.26 μg Mn g^{-1}, respectively. Average recoveries were in the range 90–95 % and the values obtained were not corrected for recovery.

2.4.2. Determination of ^{54}Mn radioactivity

The radioactivity of water samples and dissected tissues was determined in a Wizard 1480 TM3 automatic gamma counter and the radioactivity of the live shore crabs was determined in a well-type Bicron Labtech™ NaI(Tl) crystal gamma counter with a diameter and depth of 7.6 cm. Where needed, results were corrected for different counting efficiencies (CE) for the two counters (CE_{Wizard} ∼ 88 % of CE_{Bicron}). The results were corrected for background counts, but not for self-absorption, which is low because of the relatively high energy (∼ 835 keV) of ^{54}Mn's γ radiation. Results are presented as counts per minute (cpm).

2.5. Hypoxia

In the hypoxia experiment, oxygen saturation was adjusted by means of a GM-602 gas mixer (ADC). N_2 (AGA) and atmospheric air (AGA) from 50 L gas cylinders were mixed and admitted to the aquaria which were covered and closed with lids. The oxygen concentrations were controlled daily by a PHM73 oxygen meter (Radiometer). The nominal oxygen concentration in the hypoxic group was 25 % oxygen saturation versus 100 % in the control.

2.6. Chemicals

Radioactively labelled manganese (^{54}Mn) was obtained from Amersham. Concentrated nitric acid, stable manganese $MnC1_2{\cdot}4H_20$ and chemicals for crab ringer B and artificial sea water were supplied by Merck (all pro analysis).

Artificial seawater (15 ‰ ASW) was made by dissolving NaC1, KC1, $CaC1_2{\bullet}2H_20$, $NaHCO_3$, $MgSO_4{\bullet}7H_20$ and $MgC1_2{\bullet}6H_20$ in deionized water to give a final concentration of: 204 mM Na^+, 238 mM Cl^-, 4.4 mM K^+, 4.5 mM Ca^{2+}, 23.3 mM Mg^{2+}, 12.1 mM SO_4^{2-} and 1 mM HCO_3^-.

Crab ringer B was prepared by dissolving 19.4 g NaCl, 520 mg KCl, 1,23 g $MgSO_4 \bullet 7H_20$, 340 mg $NaHCO_3$, and 590 mg $CaC1_2 \bullet 2H_20$ L^{-1} (pH = 7.85).

2.7. Experiments

2.7.1. Mn toxicity in crabs. Experiment 1

Groups of 10 small crabs (weight not recorded) were exposed to 0, 8, 12, 16, 20, 30, 50, 70, 100 and 200 mg Mn-$MnC1_2 \cdot 4H_20$ L^{-1} and survival was registered daily over 7 d.

2.7.2. Mn toxicity in crabs. Experiment 2

Groups of 5 crabs (28–49 g) were exposed to 0, 25, 50, 100, 200, 400 and 800 mg Mn-$MnC1_2 \cdot 4H_20$ L^{-1} and survival was registered daily over 4 d. After 4 d, haemolymph samples were drawn, the crabs were sacrificed and manganese concentrations in exoskeleton, midgut gland, gills, muscle and haemolymph were determined.

2.7.3. Uptake of ^{54}Mn in crabs from seawater and subsequent elimination. Experiment 3

Twenty-four crabs (10–31 g) were distributed in three 10 L polystyrene aquaria. ^{54}Mn was added to obtain a concentration of 100 cpm mL^{-1}. The concentration of ^{54}Mn in the water was determined daily to maintain the concentration of 100 cpm mL^{-1} by addition. The radioactivity of the crabs was determined daily. Water was changed every second or third day. After 2 and 22 d of uptake, respectively, 5 crabs were sacrificed and ^{54}Mn concentrations in gills, midgut gland, muscle and exoskeleton were determined. After 22 d of exposure, 5 crabs were transferred to uncontaminated water and the radioactivity of the crabs was determined at regular intervals over the next 40 d. After 40 d, the crabs were sacrificed and ^{54}Mn concentrations in gills, midgut gland, muscle and exoskeleton were determined.

2.7.4. Clearance of injected ^{54}Mn from crab haemolymph, experiment 4

Each of four crabs (34–52 g) had 200,000 cpm ^{54}Mn dissolved in 50–60 μL crab ringer B injected through the arthrodial membrane of a walking leg. Over the next 24 h, 50–100 μL haemolymph samples were taken through the arthrodial membrane – at the side opposite injection – for determination of ^{54}Mn concentration at time intervals depicted in Fig. 2A. After 24 h, the crabs were sacrificed and ^{54}Mn concentrations in gills, midgut gland, muscle, haemolymph, exoskeleton and stomach + intestine were determined.

2.7.5. Transfer of manganese from food to haemolymph in crabs. Experiment 5

Four crabs (28–49 g) were individually fed a food cube containing 50 μg Mn-$MnCl_2$ g^{-1}. Haemolymph samples were drawn before the feeding and on each of day 1–6 and day 9 after the feeding. Concentrations of manganese in the haemolymph samples were determined.

2.7.6. Clearance of injected ^{54}Mn from whole body crabs. Experiment 6

Each of four crabs (21–33 g) had 5000 cpm ^{54}Mn injected as in Exp. 4. The radioactivity of the crabs was determined at regular intervals over the next 33 d whereafter the crabs were sacrificed and ^{54}Mn concentrations in gills, midgut gland, muscle, haemolymph, exoskeleton and stomach + intestine were determined.

2.7.7. Clearance of ^{54}Mn taken up from food from whole body crabs. Experiment 7

To assess the kinetics of elimination of ^{54}Mn after uptake from the food, 5 crabs (10–32 g) were kept individually in aerated seawater in 1.5-L aquaria. Every second or third day the crabs were transferred to 0.5-L tanks and fed food blocks containing ^{54}Mn. After feeding, the radioactivity of the crabs was determined, and the crabs were transferred to "clean" seawater. After 13 d exposure with ^{54}Mn enriched food blocks, the crabs were fed uncontaminated food blocks every second or third day and the content of ^{54}Mn in the crabs was followed for 48 d. After 48 d, the crabs were sacrificed and samples of gills, midgut gland, muscle, stomach + intestine and exoskeleton were dissected out for analysis of ^{54}Mn concentrations; the muscle samples were lost before analysis.

2.7.8. Accumulation of Mn in crabs from low water concentrations. Experiment 8

Two groups of 25 crabs (22–63 g) were exposed to 50 or 300 μg Mn-$MnC1_2 \cdot 4H_20$ L^{-1} in 40 L glass aquaria. Five crabs were sacrificed from each aquarium after 7 and 14 d exposure Failure of the air supply caused mortality and forced cessation of the 300 μg Mn L^{-1} group after two weeks. Five crabs from the 50 μg Mn exposure group were sacrificed after 21 and 28 d, respectively. Water was shifted twice a week. Manganese concentrations in carapace, midgut gland, gills, muscle, gonad and hypodermis were determined.

2.7.9. Effect of hypoxia uptake on Mn^{++} uptake in C. maenas. Experiment 9

Crabs (20–35 g) were exposed to manganese concentrations control, 0.5, 1.5, 4.0 and 8.0 mg Mn-$MnC1_2 \cdot 4H_20$ L^{-1} for 7½ d – either at full oxygen saturation or a nominal oxygen saturation of 25 %. Each of the 10 groups consisted of 10 crabs. Ten L polystyrene aquaria closed with lids were used. The water remained unchanged during the experimental period. At the end of the experiment the crabs were sacrificed and manganese concentrations in gills, midgut gland and exoskeleton were determined.

2.7.10. Effect of calcium on manganese uptake from water to haemolymph. Experiment 10

Three groups of 8 crabs (41–73 g) were placed in 8 L aquaria with 4.5 L artificially made 15 ‰ seawater with calcium concentrations of 2.0, 4.5 and 10 mM, respectively, in the three aquaria (the calcium concentration in full strength sea water is around 10 mM). 4000 cpm ^{54}Mn L^{-1} were added together with 1 mg 'cold' Mn^{++} L^{-1}; the latter was added to eliminate the possibility that potential trace amounts of manganese in the salts used to produce the artificial sea water might affect the results. Haemolymph samples were drawn from the crabs after 4, 8, 12 and 24 h exposure.

2.7.11. Manganese concentrations in field collected crabs. Experiment 11

Fifty crabs were collected at each of three sites around the Island of Funen (Fig. S1) and manganese concentrations were determined in the exoskeleton of the crabs. Three sediment samples were collected at each of the three locations; 10 cm sediment cores were collected by means of Kajak samplers. At depths of 0–1, 1–4 and 4–10 cm, the sediment cores were split into ½, 1 and 2 cm sections, respectively. Sediment was handled in a glove box supplied with N_2 to avoid oxidation of Mn^{++} in the porewater. Part of the sections were weighed, freeze dried and had their water content and concentration of total manganese determined. Another part of the sections was centrifuged for 10 min at 3000 rpm in N_2 treated, double centrifuge tubes with gas tight lids to obtain the pore water. The supernatant was filtered through a GF/F filter and the filtrate had 50 μL 0.5 M HCl added to avoid bacterial and chemical oxidation of the Mn^{++}. The samples were frozen at −18 °C until determination of pore water manganese. Samples of the dried sediment were heated to 520 °C for 12 h for determination of the organic content (results not shown).

2.8. Data handling and statistical treatment

Concentration factors (CF) for ^{54}Mn during the uptake from water was calculated as cpm g^{-1} crab wet weight/cpm mL^{-1} water. During the uptake phase, data for the concentration factor were fitted to either

linear ($y = a*x + b$) or polynomial ($y = a*x^2+b*x + c$) equations with y = [CF] and x = time. Where retention of ^{54}Mn was followed, the ^{54}Mn content of each animal was set to 100 % at the end of the exposure period and data for concentrations in the crabs over time were fitted to either exponential ($y = P*e^{a*x}$), biexponential ($y = P*e^{a*x} + Q*e^{b*x}$) [with or without residuals] equations according to the multicompartment analysis described by Comar (1955). Half-lives ($T_{½}$) for the elimination of manganese were calculated from the elimination coefficient a ($T_{½} = \ln 2/a$). The computer program FigP was used for curve fitting and production of the figures. One or two-way analysis of variance (ANOVA), repeated measures ANOVA and regression analysis were used where appropriate in statistical evaluation of the data (SYSTAT, version 13). The percentage distribution of manganese among the tissues was calculated from the concentration of manganese in each tissue multiplied by the percentage of each tissue's proportion of the body weight; tissue proportions were taken from Bjerregaard and Depledge (2002). 0.05 was used as level of significance.

3. Results

3.1. Toxicity experiments. Experiment 1 &2

All the crabs - except one crab in the highest exposure group - exposed to up to 200 mg Mn L^{-1} survived for seven days (Exp. 1; not shown). Two crabs exposed to 800 mg Mn L^{-1} in Exp. 2 died at day 4; otherwise, no mortality was registered (not shown). Crabs in Exp. 2 accumulated manganese in all tissues proportionally with exposure concentrations up to 400 mg Mn L^{-1}, while some tissues showed a trend of levelling off at exposure to 800 mg Mn L^{-1} (Fig. S2).

3.2. ^{54}Mn kinetics in shore crabs. Experiment 3-7

3.2.1. Uptake. Experiment 3

Shore crabs accumulated ^{54}Mn from sea water with no trend of levelling off over 3 weeks to whole body concentration factors of 21 after 22 days (Fig. 1A). Over the first week, the concentration factor was described by a polynomic relation ($CF = 0.228 + 1.98*d-0.0972*d^2$; $r^2 = 0.993$). Between 6 and 22 d, the accumulation increased linearly with time ($CF = 0.784*d+4.0$; $r^2 = 0.98$).

In the group sacrificed after 2 d, the majority of the accumulated ^{54}Mn was associated with the exoskeleton and some in muscle (Table 2, A1); this trend was accentuated in the groups sacrificed after 22 d uptake (Table 2, A2) and further after 40 d elimination (Table 2, A3).

3.2.2. Elimination. Experiment 3-7

^{54}Mn taken up from the sea water was eliminated according to 1. order kinetics with a half-life of 44 days (% ^{54}Mn retained = $98.9*e^{-0.0167*d}$; $r^2 = 0.962$; Fig. 1B).

Elimination of ^{54}Mn taken up from food could be described by biexponential kinetics (% ^{54}Mn retained = $7.1*e^{-1.75*d} + 92.6*e^{-0.0060*d}$; $r^2 = 0.737$; Fig. 2B); the majority of the ^{54}Mn was eliminated with a half-life of 96 days. After 21 d, the majority of the ^{54}Mn was located in the exoskeleton Table 2, B). Elimination from the whole crab of ^{54}Mn injected into the haemolymph could also be described by biexponential kinetics (% ^{54}Mn retained = $23.3*e^{-0.289*d} + 75.4*e^{-0.0053*d}$; $r^2 = 0.972$; Fig. 2B); three quarters of the ^{54}Mn was lost with a half-life of 131 days. One day after the injection, the majority of the ^{54}Mn was in the midgut gland (Table 2, C1), whereas the exoskeleton contained more than half of the ^{54}Mn remaining after 33 d (Table 2, C2).

Elimination from the haemolymph of injected ^{54}Mn was relatively quick and it could be described by biexponential kinetics (% ^{54}Mn retained = $74.0*e^{-0.512*h} + 26.9*e^{-0.0634*h}$; $r^2 = 0.998$; Fig. 2A); approximately 95 % of the injected ^{54}Mn was removed from the haemolymph after 24 h. Stable manganese fed to the crabs appeared in the haemolymph within 24 h and the majority was lost from the haemolymph within 2 d (Fig. 2B).

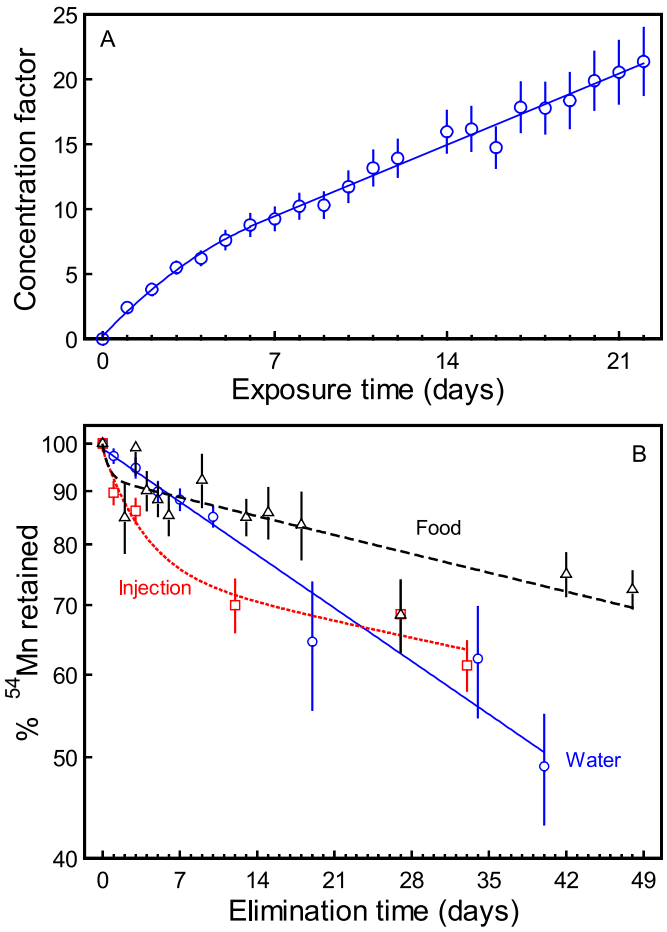

Fig. 1. *Carcinus maenas*. A: Accumulation of ^{54}Mn in crabs from sea water. N = 24 → 5. B: Retention of ^{54}Mn acquired from water (○; n = 5) or food (Δ; n = 5) or by injection in crabs (□; n = 4). Mean ± SEM shown.

3.3. Accumulation of stable manganese in crabs from water at μg L^{-1} quantities. Experiment 8

Crabs exposed to 50 μg Mn L^{-1} accumulated manganese linearly with time in carapace ($p = 0.006$; $r^2 = 0.26$), midgut gland ($p < 0.001$; $r^2 = 0.40$), gills ($p = 0.015$; $r^2 = 0.24$), and hypodermis ($p < 0.001$; $r^2 = 0.54$) (Fig. 3). Manganese concentrations in gonads and muscle did not show statistically significant changes. Crabs exposed to 300 μg Mn L^{-1} over 14 days accumulated manganese linearly with time in midgut gland ($p = 0.017$; $r^2 = 0.31$), gills ($p = 0.009$; $r^2 = 0.38$), muscle ($p = 0.046$; $r^2 = 0.23$), hypodermis ($p = 0.003$; $r^2 = 0.44$) and gonad ($p = 0.006$; $r^2 = 0.39$). Manganese concentrations in the exoskeleton did not show statistically significant changes. In some of the tissues, single, high values resulted in a fairly high variability in the manganese concentrations.

3.4. Manganese accumulation in hypoxic water, experiment 9

The respiration of the crabs lowered the oxygen saturation in the hypoxic aquaria from the nominal 25 % to 19.0 ± 3.0 %.

Exposure to 0.5 and 1.5 mg Mn L^{-1} for 7.5 days at 100 % oxygen saturation led to an almost linear increase in manganese concentrations in the tissues analyzed (gills, midgut gland and carapace) whereas exposure to 4 and 8 mg Mn L^{-1} caused no or little further accumulation (Fig. 4).

Two-way ANOVA analysis demonstrated that hypoxic conditions augmented ($p = 0.001$) accumulation of manganese in midgut gland

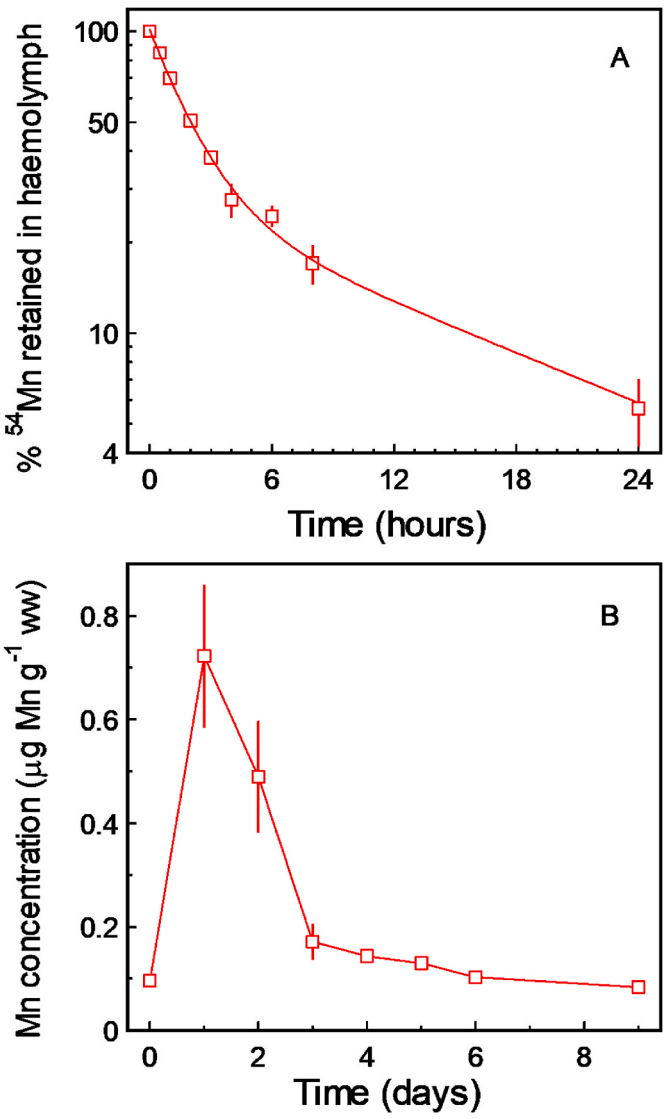

Fig. 2. *Carcinus maenas*. A: Retention of ^{54}Mn in the haemolymph of crabs after injection of ^{54}Mn into the haemolymph. (n = 4). B: Concentration of manganese in haemolymph of crabs fed a food block with 50 µg Mn-$MnCl_2$ g^{-1} at day 0. Mean ± SEM for 4 crabs.

(Fig. 4B) and lowered (p = 0.039) accumulation in carapace (Fig. 4C) whereas the accumulation in gills (Fig. 4A) was not affected by the oxygen tension.

3.5. Effect of calcium on Mn uptake. Experiment 10

Uptake of manganese from the ambient water into the haemolymph of the crabs increased with decreasing concentrations of calcium in the water ($p < 0.001$; repeated measures ANOVA) and manganese concentrations in the haemolymph tended to reach a steady state within 24 h (Fig. 5) with concentration factors of approximately 0.1, 0.15 and 0.2 at 10-, 4.5- and 2-mM Ca, respectively.

3.6. Manganese in field collected crabs and sediments. Experiment 11

Manganese concentrations in the exoskeleton of the crabs decreased significantly with size of the crabs at all three stations – more pronounced in Kertinge Nor and Gamborg Fjord (Fig. 6A–C) than in Kerteminde Fjord (Fig. 6B). When divided into size groups with10 g intervals (Fig. 6 D), crabs ≥40 g showed no statistically significant differences in manganese concentrations, whereas crabs ≤30 generally showed lower concentrations at Kerteminde Fjord than at the two other stations.

Total manganese concentrations in the sediment reached 150–250 mg Mn L^{-1} in the upper 1 cm at all 3 stations with no difference between the three stations (P = 0.27; Fig. 6E). Below 1 cm, concentrations decreased to stable levels of 20–70 mg Mn L^{-1} at Kertinge Nor and Kerteminde Fjord and significantly ($P < 0.001$) higher – but also more variable - levels of 110–125 mg Mn L^{-1} at Gamborg Fjord.

Porewater manganese concentrations in the upper 1 cm - peaking at 550–700 µg Mn L^{-1} at app. 1 cm's depth - did not differ between the three stations (P = 0.43; Fig. 6F). Below 2.5 cm average pore water concentrations at Kerteminde Fjord and Gamborg Fjord (55–130 µg Mn L^{-1}) were not different, whereas at Kertinge Nor they stabilized at a statistically significant (P = 0.004) higher (135–165 µg Mn L^{-1}) level.

4. Discussion

Whole-body concentration factors for *Carcinus maenas* exposed to ^{54}Mn in the water reached 22 after 3 weeks' exposure, which is higher than in flounder *Pleuronectes platessa* (CF approximately 1 after 30 days; Pentreath, 1973) but in the same order of magnitude as for the sea star *Asterias rubens* (CF 19 after 23 d; Hansen and Bjerregaard, 1995) and brown trout *Salmo trutta* (CF 15 after 3 weeks; Rouleau et al., 1995). After 3 d exposure to ^{54}Mn in the water, Norway lobster *Nephrops norvegicus* (Baden et al., 1995), lobster *Homarus vulgaris* (Bryan and Ward, 1965) obtained similar CF's (5.4 and 3.8–4.8, respectively) as the shore crab (5.3) in the present investigation.

The half-life of ^{54}Mn assimilated from the water in *C. maenas* of 44 days is in the same order of magnitude as described for another decapod crustaceans the Norway lobster *N. norvegicus* (69 d; Baden et al., 1995). Half-lives for stable manganese accumulated from 5 to 10 mg Mn L^{-1} in the internal tissues of *N. norvegicus* were somewhat shorter – in the order of 1–4 d (Baden et al., 1999). Half-life for accumulated ^{54}Mn in another benthic invertebrate, the sea star *A. rubens* was similar to that of *C. maenas* (36 d; Hansen and Bjerregaard, 1995).

With a half-life of 96 d for ^{54}Mn assimilated from the food, manganese is eliminated more slowly than several other metals - 5–7 d for plutonium (Fowler and Guary, 1977; Guary and Fowler, 1990), 7 d for zinc (Chan and Rainbow, 1993), 5–10 d for americium (Guary and Fowler, 1990), 4–27 d for neptunium (Guary and Fowler, 1990),10 d for vanadium (Miramand et al., 1981), 55 d for inorganic mercury (Larsen

Table 2
Distribution of ^{54}Mn in the tissues in % of the total amount of ^{54}Mn determined.

	Midgut	Gills	Muscle	Exoskeleton	Stomach + intestine	Haemolymph
A1: After 2 d water exposure	0.6	0.8	10.8	87.8		
A2: After 22 d water exposure	0.69	0.54	2.1	96.3	0.06	0.28
A3: 22 d water exposure+40 d elimination	0.86		0.60	92.0	0.17	0.32
B: 21 d after feeding	5.8	1.4	#	90.4	2.5	
C1: 24 h after injection	54	3.3	11.0	20.6	2.1	8.9
C2: 33 d after injection	10.6	1.0	30.1	53.6	1.8	2.8

#: Sample lost

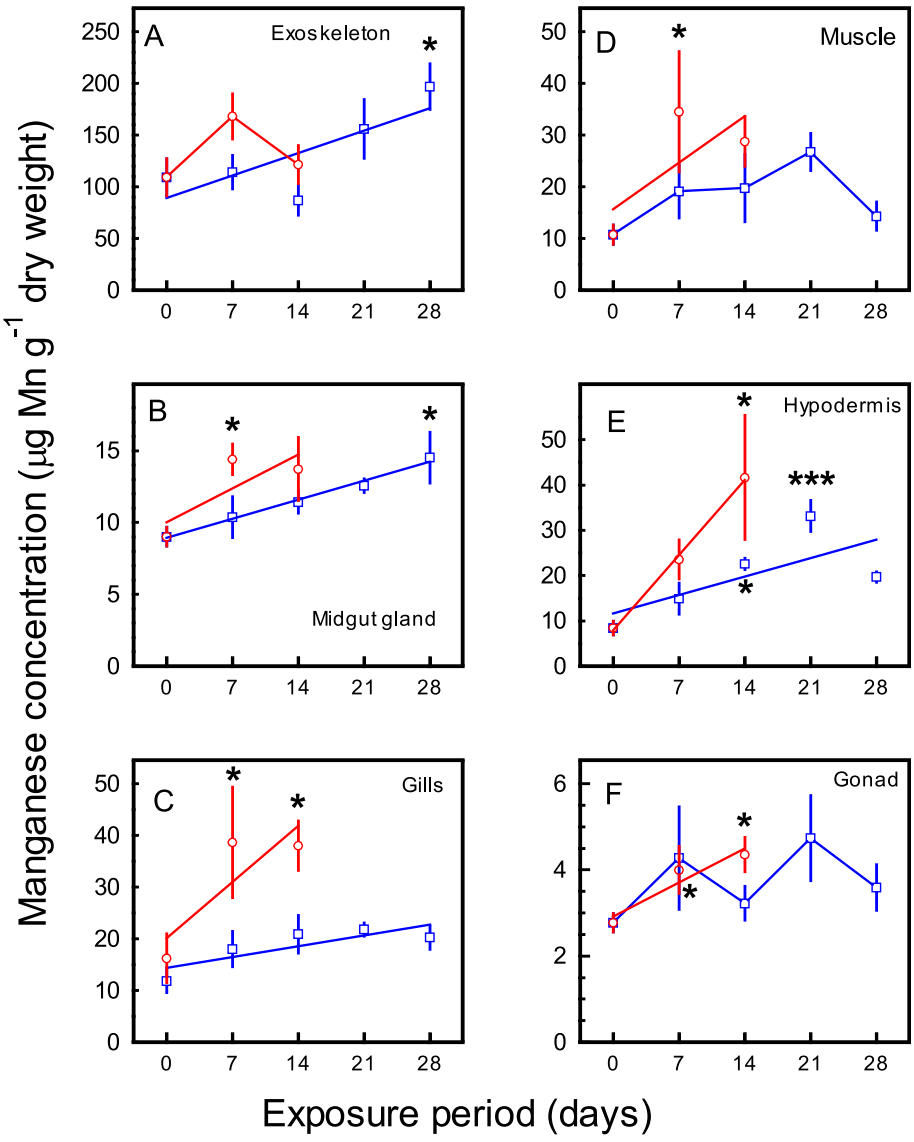

Fig. 3. *Carcinus maenas*. Concentrations of stable manganese in tissues of crabs exposed to 50 (□) or 300 (○) µg Mn L^{-1}. Mean ± SEM for 5–7 crabs shown (except n = 2 for gonad at 28 d). * and *** indicate statistically significant difference from day 0 at 0.05 and 0.001. Straight lines indicate statistically significant regressions.

and Bjerregaard, 1995) - but still faster than organic mercury (more than 700 d (Larsen and Bjerregaard, 1995) and cadmium 626 d (Bjerregaard et al., 2005).

Whereas shore crabs accumulated manganese in the tissues at exposure to 50 µg Mn L^{-1} and higher, exposure of the Norway lobster to 555 µg Mn L^{-1} for two weeks did not lead to increased manganese concentrations in any of the tissues investigated; exposure to 1755 µg Mn L^{-1} led to increased concentrations in haemolymph, gills and muscle but not in the midgut gland where increases were only seen at exposure to 5555 µg Mn L^{-1} (Baden et al., 1995). Manganese concentration increased in all soft tissues after exposure to 5 and 10 mg Mn L^{-1} for 20 d (Baden et al., 1999). Manganese concentrations in the exoskeleton of *N. norvegicus* were not augmented in any of these exposures (Baden et al., 1995, 1999). Exposure of the lobster *H. vulgaris* to 50, 100 and 1000 µg Mn L^{-1} for up to 3 weeks led to increased concentrations in the haemolymph and gills at the highest exposure concentration but had no effect on manganese concentrations in carapace, midgut gland and muscle (Bryan and Ward, 1965). Reasons for these differences in accumulation patterns between the 3 decapods remain unknown.

Norway lobsters collected in the field under hypoxic conditions had 2 to 4 times higher manganese concentrations in the internal tissues than animals living under normoxic conditions (Baden et al., 1994). However, in a laboratory experiment, hypoxic conditions (12 % oxygen saturation) did not affect manganese accumulation from 555, 1755 or 5555 µg Mn L^{-1} in the tissues of the Norway lobster (Baden et al., 1995; Eriksson and Baden, 1998). The present investigation revealed a statistically significant, albeit moderate effect of hypoxia on the accumulation of manganese in midgut gland and exoskeleton. *C. maenas* increases ventilation during hypoxia and the ventilation rate is approximately 4 times higher at 19 % oxygen saturation than at 100 % (Taylor, 1976). This might result in increased uptake into the organism - including midgut gland. It cannot be excluded that some of the manganese accumulated in the exoskeleton under normoxic conditions might be MnO_2 – oxidized from Mn^{++}. If this were true, the same phenomenon does apparently not take place in the sea star *Asterias rubens*, in which accumulation of manganese in both external and internal tissues from 1 mg Mn^{++} L^{-1} over 4 weeks were identical at normoxia and hypoxia (Bjerregaard and Hansen, 2025).

Manganese concentrations were higher in the exoskeleton, midgut gland and gills in the control crabs of the hypoxia experiment (Exp. 9) than in the exposure to 50 and 300 µg Mn L^{-1} (Exp. 8). Crabs in the two experiments were approximately equally sized and collected at the same

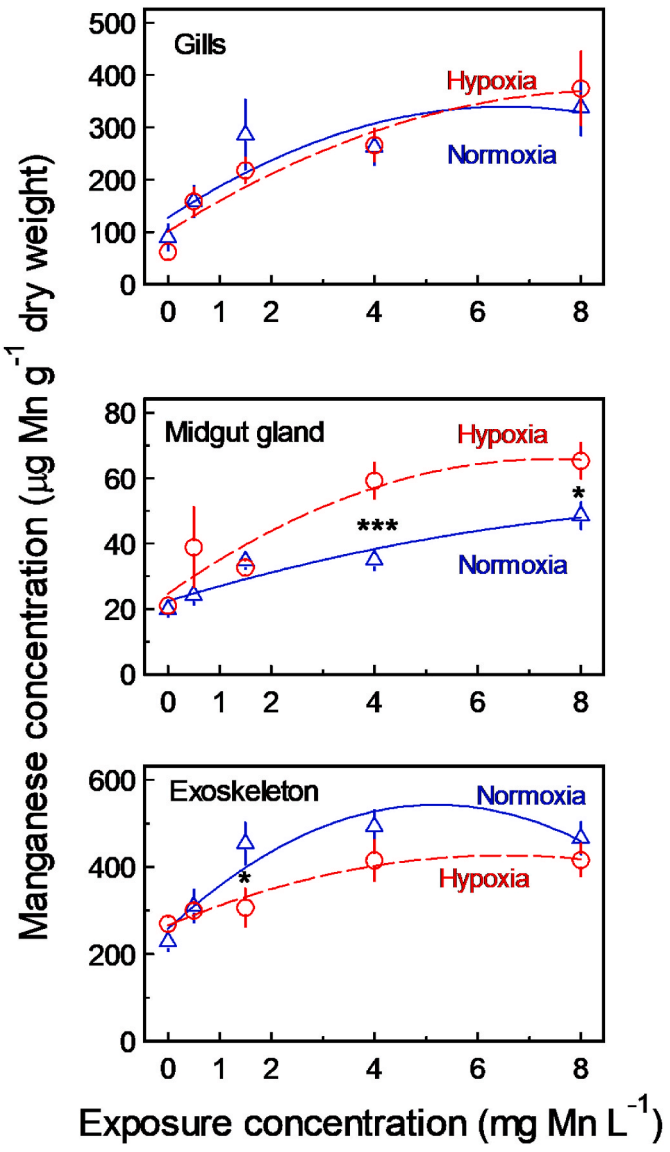

Fig. 4. *Carcinus maenas*. Concentrations of stable manganese in the tissues of crabs exposed to manganese for 7½ d under full (△) or 19 ± 3 % (○) oxygen saturation. Mean ± SEM for 10 crabs shown. * and *** indicate statistically significant difference between normoxia and hypoxia at 0.05 and 0.001.

location (Odense Fjord). Crabs in Exp. 9 were freshly caught in April and crabs in Exp. 8 were kept in running sea water tanks for the winter from November until used in experiments in February early March. This might potentially explain the difference, although, in Norway lobster, no clear seasonal variation in manganese content was demonstrated (Eriksson, 2000).

The present results confirm earlier reports (Bjerregaard and Depledge, 2002) that the major part (approximately 95 %) of the body burden of manganese is located in the exoskeleton of *C. maenas* and that the concentration shows a clear size dependence. Manganese in the exoskeleton may be acquired by two mechanisms: 1) either by adsorption of manganese directly on the surface from the sea water or 2) manganese taken up via gills or from food and transported in the haemolymph and further across the hypodermis to end up being incorporated into the exoskeleton. Two of the findings in the present investigation support the plausibility of the latter mechanism: 1) A substantial amount of ^{54}Mn injected into the haemolymph ends up in the exoskeleton and 2) Mn^{++} competes with Ca^{++} in *C. maenas*. Shore crabs normally maintain higher calcium concentrations in their haemolymph

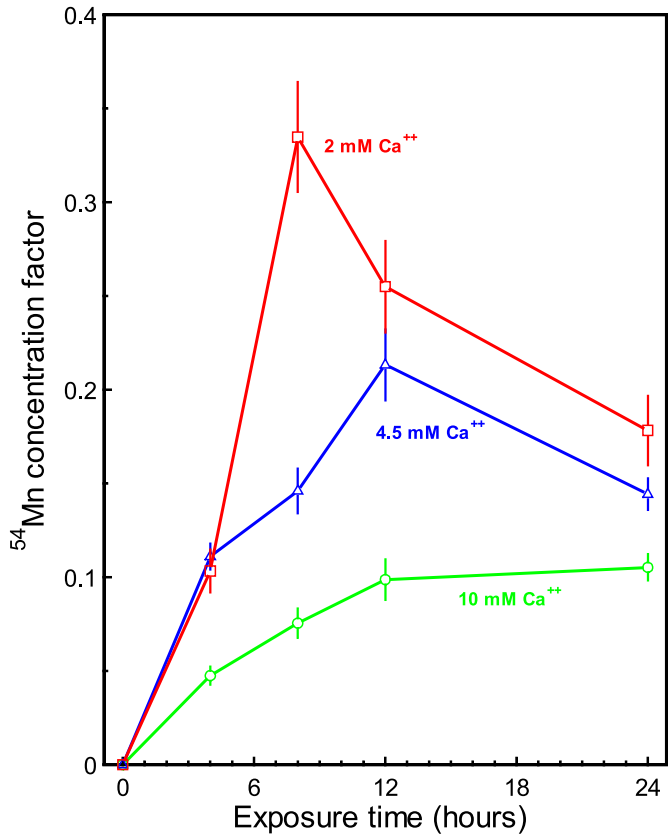

Fig. 5. *Carcinus maenas*. Uptake of ^{54}Mn from ambient, artificial seawater (15 ‰) into the haemolymph at calcium concentrations of 2.0 (○), 4.5(△) or 10 (□) mM Ca^{++}. Mean ± SEM for 8 crabs.

than in the ambient sea water by active uptake over the gills (e.g. Bjerregaard and Vislie, 1985; Greenaway, 1985). Active transport of calcium from the haemolymph over the hypodermis to the exoskeleton takes place by means of a Ca-ATPase and a Na/Ca exchange mechanism in the hypodermis (Roer, 1980). During postmoult calcification of the exoskeleton, the influx of calcium over the gills is an order of magnitude higher than in the intermoult stages (Norum et al., 2005) and influx over the gills from sea water of divalent metal ions competing with Ca^{++} - e.g. cadmium - may increase one hundred fold (Norum et al., 2005) - leading to accumulation in the tissues (Bondgaard and Bjerregaard, 2005; Bondgaard et al., 2000; Norum et al., 2005).

The explanation as to why smaller crabs have higher concentrations of manganese in their exoskeleton than larger ones remains speculative. If the majority of the manganese in the exoskeleton – and thereby a major part of the body burden – is acquired from the ambient medium during the postmoult calcification of the exoskeleton, behavioural differences between the small and large crabs may play a role. Manganese concentrations in coastal, intertidal areas have been shown to be variable over time (Kowalski et al., 2012) and space (Laes et al., 2007; Statham et al., 2005). Thus, different distributional preferences in the coastal zone for crabs of various sizes and physiological forms (Crothers, 1967; McGaw and Naylor, 1992) could explain larger uptake in smaller individuals. Other explanations may relate to larger surface:volume ratios and gill:body volume and higher respiration rate favouring uptake from water in small crabs. Also, small crabs moult more often, and more animals may moult during periods with hypoxia and small crabs live more submerged in the sediment than larger to avoid predation. A potential size-dependency in feeding behaviour and preferred food items can also not be excluded.

It has been suggested that the manganese content in the Norway lobster can be used as an efficient marker for oxygen depletion in marine

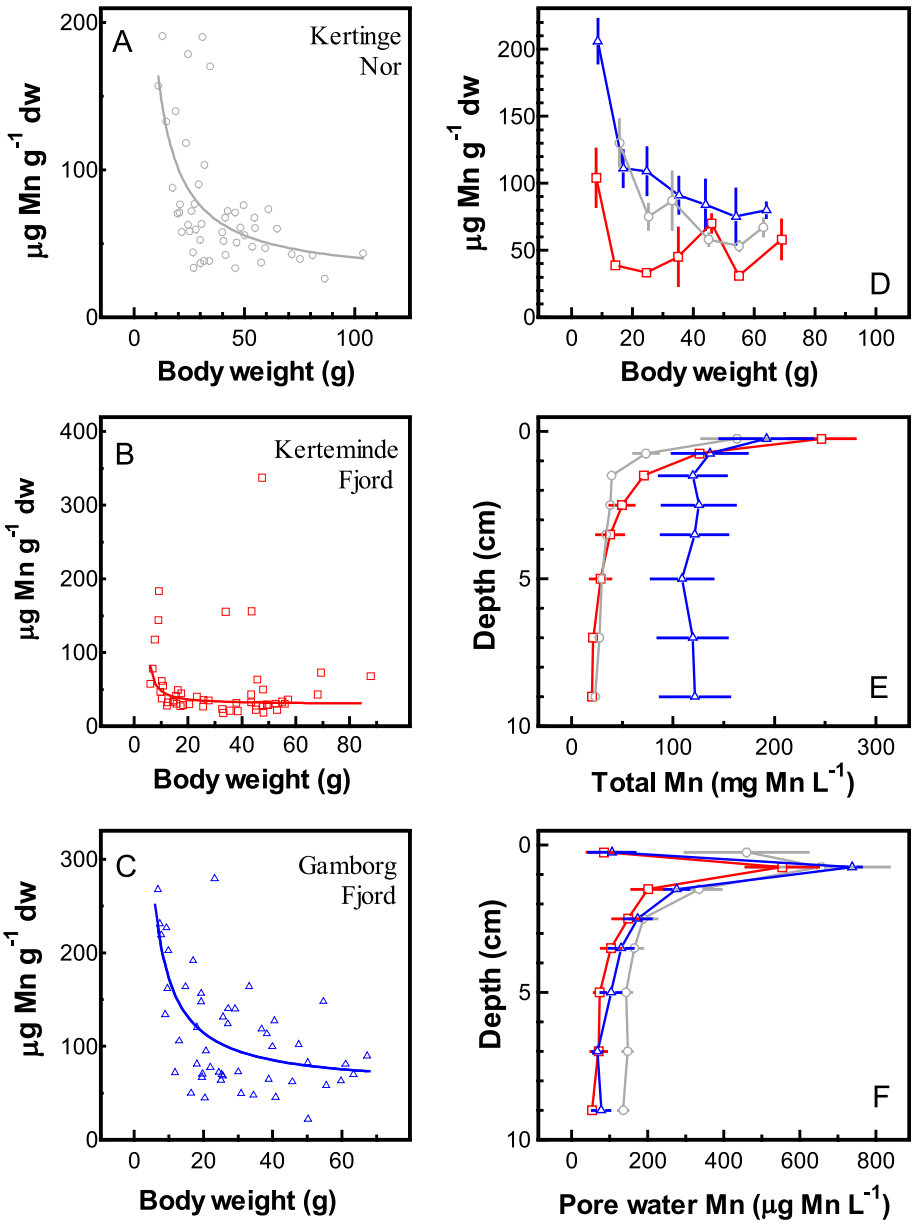

Fig. 6. *Carcinus maenas*. A–C: Concentrations of manganese in exoskeleton versus size of crabs from three different locations. Individual values for 50 animals from each location and regression lines shown. D: Data broken up per 10 g from the three locations. E: Concentration of total manganese in sediments from Kertinge Nor (○), Kerteminde Fjord (□) and Gamborg Fjord (△). Mean ± SEM for 3 stations from each location shown. F: As E, only for pore water.

waters (Baden et al., 1994; Eriksson and Baden, 1998). In the lobster *H. vulgaris* the majority of the manganese body burden is also found in the exoskeleton (Bryan and Ward, 1965) and to avoid potential errors in the conclusions from investigations on *N. norvegicus* it would be useful to know if manganese concentrations in this species are correlated with size – such as they are in *C. maenas*.

Gamborg Fjord had higher concentrations of total manganese in the sediment than the two other field locations, but this was not directly reflected in the manganese concentrations in the crabs. This corresponds to the findings for mercury (Bjerregaard et al., 2020) that a simple and straight forward relationship between the concentrations of mercury in sediment and benthic invertebrates does not necessarily exist.

CRediT authorship contribution statement

Poul Bjerregaard: Writing – original draft, Formal analysis, Data curation. **Michael Hastrup:** Writing – review & editing, Investigation, Data curation. **Kasper Nowack:** Investigation, Data curation. **Jens Malmkvist:** Writing – review & editing, Investigation, Data curation.

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2025.107246.

Data availability

Data will be made available on request.

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