The effects of the exposure of green crabs (*Carcinus maenas*) to an anti-sea lice drug: emamectin benzoate in spiked marine sediments

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THE EFFECTS OF THE EXPOSURE OF GREEN CRABS (*CARCINUS MAENAS*) TO AN ANTI-SEA LICE DRUG: EMAMECTIN BENZOATE IN SPIKED MARINE SEDIMENTS

by

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List of Tables

List of Figures

Abstract

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Emamectin benzoate (EMB) is an in-feed antiparasitic used in the finfish aquaculture industry to control sea lice infestations. EMB can accumulate in marine sediments surrounding fish farms due to its hydrophobicity and affinity to sorb onto particulate matter. It is persistent in the sediment with a half-life ranging from 100 to >500 days and potentially bioavailable long-term to non-target benthic organisms. EMB exposure has been documented to affect benthic species like crustaceans through direct exposure to the sediment or by ingestion of wastes through foraging. In this study, we exposed green crabs to EMB-spiked sediment for 130 days and investigated the effects of EMB on growth and survival. There was an effect of EMB on crab weight gain and mortality at the highest EMB sediment concentration evaluated, which is 3 to 12 times higher than the upper range of average environmentally relevant concentrations documented around Canadian aquaculture sites. This study validates the potential for EMB to impact crustaceans and highlights the importance of testing both high concentrations and relevant values to help understand/manage effects on marine fauna.

Keywords: aquaculture; emamectin benzoate; marine; sediment; green crab; toxicity

Résumé

Jonah, L., Kingsbury, M., and Hamoutene, D. 2026. The effects of the exposure of green crabs (*Carcinus maenas*) to an anti-sea lice drug: emamectin benzoate in spiked marine sediments. Can. Tech. Rep. Fish. Aquat. Sci. 3784: vi + 18 p. https://doi.org/10.60825/4aj5-w326

Le benzoate d'émaméctine (EMB) est un antiparasitaire administré dans l'alimentation utilisé dans l'industrie de l'aquaculture des saumons pour contrôler les infestations de poux de mer. L'EMB peut s'accumuler dans les sédiments marins entourant les fermes piscicoles en raison de son hydrophobicité et de son affinité pour se fixer sur les particules en suspension et les sédiments. Il est persistant dans le sédiment, avec une demi-vie variant de 100 à plus de 500 jours et potentiellement biodisponible à long terme avec effets potentiels sur les organismes benthiques. Il a été documenté que l'exposition à l'EMB affecte des espèces benthiques telles que les crustacés, soit par exposition directe au sédiment, soit par ingestion de déchets d'aquaculture. Dans cette étude, nous avons exposé des crabes verts à des sédiments enrichis en EMB pendant 130 jours et étudié les effets de l'EMB sur la croissance et la survie. Un effet de l'EMB sur la prise de poids et la mortalité des crabes a été observé lié à l'exposition a la concentration en EMB la plus élevée testée. Cette concentration est de 3 à 12 fois supérieure à la gamme supérieure des concentrations moyennes documentées dans les sédiments autour des sites aquacoles canadiens. Cette étude valide le potentiel de l'EMB à affecter les crustacés et souligne l'importance de tester à la fois des concentrations élevées et des valeurs pertinentes pour aider à comprendre et gérer les effets sur la faune marine.

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1. Introduction

Emamectin benzoate (EMB) is an in-feed treatment used to control sea-lice infestations in the salmon aquaculture industry (Langford et al. 2014; Chang et al. 2022; Hamoutene et al. 2022; UKTAG 2022; Kingsbury et al. 2023). EMB enters the environment either through the direct deposition of uneaten medicated feed or via excretion from treated fish whether through urine or faeces (Horsberg 2012; Lalonde et al. 2012; Bloodworth et al. 2019). EMB has a low water solubility (Halley et al. 1989; Mushtaq et al. 1996), and due to its hydrophobicity and affinity to sorb onto particulate matter, it does not remain in the water column (Prasse et al. 2009; Strachan and Kennedy 2021; Jacova and Kennedy 2022). As a result, it is not expected to occur in high concentrations in water, and studies suggest that prolonged exposure of pelagic organisms to EMB in solution is unlikely to occur (Davies et al. 1997; Mill et al. 2021). On the other hand, EMB is known to persist in marine sediment with half-lives estimated to range from 100 to >500 days (Benskin et al. 2016; Halley et al. 1989; Hamoutene et al. 2023a; Strachan and Kennedy 2021), which could adversely affect non-target benthic organisms through chronic exposure to the sediment where EMB has been deposited (Burridge et al. 2010; Horsberg 2012).

Crustaceans are especially vulnerable to EMB, as it specifically targets nerve cells and nerve impulses in arthropods like all avermectin-classed compounds (e.g., Roberts and Hutson 1999; Burridge et al. 2010). There has been some research completed looking at the effects of EMB exposure in sediment on very different benthic species, including amphipods, polychaetes, and decapods (with lobsters being a dominant study organism) (Mayor et al. 2008; Kuo et al. 2010; Park 2013; Tucca et al. 2014; Daoud et al. 2018; Strachan and Kennedy 2021; Jacova and Kennedy 2022), with the longest exposures ranging from 10 to 40 days. Overall, there is limited research that specifically looks at long-term sediment exposures, which would be a likely exposure scenario due to the persistent properties of EMB.

European green crabs are an invasive species in Canada and may be exposed to aquaculture therapeutants, as their habitats could overlap with some aquaculture locations. They are a widely studied organism and have been used as test animals in ecotoxicology (e.g., Rodrigues and Pardal 2014) as they are indicative of responses of closely related species, such as the rock crab and Jonah crab of eastern Canada, or the northern kelp crab of the Canadian west coast (DFO 2025). In this study, we expose green crabs to EMB-spiked sediment for 130 days and investigate the effects of EMB on growth (weight gain) and survival. We selected one EMB concentration based on Canadian field data around aquaculture sites (from cage edge to ~ 1.5 km away from cages) (Kingsbury et al. 2023; Hamoutene et al. 2025), and a high EMB concentration as a positive control to assess for toxicological effects. This study contributes to addressing the lack of knowledge related to long-term effects of EMB exposure in sediment on benthic species, particularly decapods.

1.1. Materials and Methods

1.2. Standards and reagents

Emamectin Benzoate ((4''R)-4"-Deoxy-4"-(methylamino) avermectin B1 benzoate (CAS# 155569-91-8)) (Batch #BCCG7174) at 97.8% purity with isomer profiles: Emamectin B1a: 82.4%; Emamectin B1b: 4.7% and sodium sulfite were purchased from Sigma Aldrich, and methanol was purchased from Fisher Scientific.

1.3. Experimental setup

Sand was collected from a location near Minister's Island, New Brunswick (NB), Canada (45.103933N; 67.049541W), and refrigerated at 4 °C within 2 hours of collection. Unfiltered seawater from the Saint Andrews Biological Station seawater supply was used at ambient salinity and temperature. Green crabs (*Carcinus maenas*) were collected from Brandy Cove, Saint Andrews, NB (45.085198N; 67.08146W) in October 2024 and were kept at the St. Andrews Biological Station (St. Andrews, NB) in ambient natural seawater and a 16:8 light-dark cycle. During acclimation and exposure, crabs were offered a diet of frozen mixed seafood containing squid, mussels, shrimp, and scallops *ad libitum* every two to three days (less frequently in the colder temperatures).

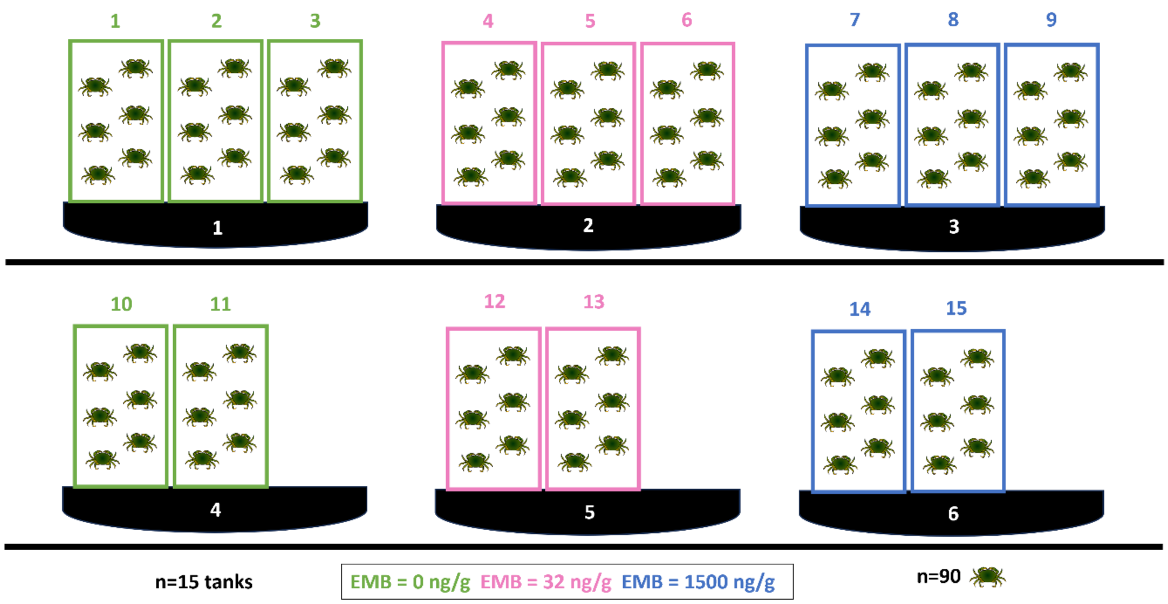

Figure 1. Illustration of the experimental setup as arranged and showing the large tubs containing each of the treatment tanks. They are organised in two rows from no emamectin benzoate (EMB) to the highest concentration tested.

A series of 15 10-gallon tanks containing 7000 g of sediment were set up with separate continuous water flow and sterilized air lines and filters (autoclaved at 132°C for 15 minutes) and arranged into the three treatment groups (Fig. 1). A working solution of EMB was prepared at 250 mg ml^{-1} in methanol for the initial spiking of the sediment. The sediment was then spiked to obtain final concentrations of 1.5 µg g^{-1} (1500 ng g^{-1}) wet weight and 0.032 µg g^{-1} (32 ng g^{-1}) wet weight for the high and low treatments, respectively. After allowing 24 hours for equilibrium to occur, sediment was sampled to determine the initial EMB sediment concentration and the water flow-through system was turned on, six crabs of assorted sizes were placed in each tank after measuring their weight and carapace length. Care was taken to include a consistent variety of sizes in each tank (i.e., a similar number of small, medium, and large crabs). All but six crabs were males in this experiment (6.7%), spread between two high, one low, and three control tanks. Each crab was tagged with a unique colour using nail polish in each tank to track individual crabs.

1.4. Sampling protocol

After the initial sampling (t_0), the crabs were weighed and had their carapace length measured after 30 days (t_1) and then every month until the end of the 130-day incubation period for a total of five sampling timepoints (n=5). Carapace length was recorded in the possibility that the crabs molt over the course of the experiment. Dissolved oxygen and water temperature were monitored weekly along with pH. Sediments were collected at t_0 and t_4 (initial and final sampling), immediately frozen at -80°C, and sent to MB Laboratories Ltd. (Sidney, BC) for chemical analysis (total n=30).

1.5. EMB chemical analysis

A QuEChERS sample preparation approach was performed with a C_{18} clean-up as described in Hamoutene et al. (2023b). Analyses were performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) according to the methodology presented in Hamoutene et al. (2023b). Eight-point matrix-matched calibration curves were prepared for EMB as described in Hamoutene et al. (2023a) (LOQ of 27 pg g^{-1} wet weight). Each extract was spiked with an internal standard to correct for variations in instrument operation. Concentrations were also expressed in dry weight (dry weight) after accounting for moisture content and allow a better comparison to field data (Kingsbury et al. 2023).

1.6. Statistical analysis

First, comparisons of weights and green crab (*Carcinus maenas*) carapace sizes (Kruskal-Wallis test for non-normal distributions) were completed to ensure that all treatments had equivalent-sized individuals. The effect of EMB exposure over time on crab weight was modelled using a Linear Mixed-effects Model (LMM; Schielzeth et al. 2020) in R using the lme4 package (Bates et al. 2015) and the lmerTest package (Kuznetsova et al. 2017). LMM was chosen as it can handle the nested structure of the data (tanks are nested within treatments), as well as accommodate unbalanced data (e.g., missing values due to death) and help correct for potential correlations among repeated measures (Muhammad 2023). Crab weight was the response variable (n=30 crabs per treatment; total n=90). The data was transformed using a Box-Cox transformation to normalize the data. We used treatment (control, low, and high EMB concentration in sediment) and the interaction of treatment with time as fixed effects to understand if the treatments affect weight gain differently over time. Crab ID was included as a random effect to account for the non-independence of measurements of each individual. Tank ID was also evaluated as a random effect; however, it was omitted due to there being no variance associated with it when it was included in the model.

To assess survivorship of crabs across EMB treatments, a Kaplan-Meier survival analysis was conducted in R after the 130-day exposure (i.e., at the final time-point, t_4). A pairwise comparison was conducted to assess differences in survival distributions between EMB treatment groups (i.e., control, low and high) using the Log-Rank test with the Benjamin-Hochberg (BH) correction for multiple comparisons. The analysis was conducted using the "survival" package (Therneau et al. 2024) and the "survminer" package (Kassambara et al. 2024) for visualizations and pairwise comparisons.

2. Results and discussion

Concentrations of EMB around aquaculture cages have been observed to vary on average between EMB values not reliably detectable (< LOQ; different LOQ values depending on the method) to over 100 ng g^{-1} dry weight (Bloodworth et al. 2019; Hamoutene et al. 2018; Hamoutene et al. 2025; Kingsbury et al. 2023; Langford et al. 2014; Tucca et al. 2017). The latest sediment sampling (2021-2023) around Canadian sites (from cage edge to ~ 1.5 km away) confirm values varying on average from 0.149 ng g^{-1} to 91.804 ng g^{-1} dry weight (Hamoutene et al. 2025) with concentrations varying depending on sampling areas, and sites.

In this study, average initial concentrations of EMB were measured in each treatment at the beginning (t_0) and end (t_4) of the experiment (Table 1). The control was also evaluated to confirm that no EMB was present in the sediment. An average moisture content of 23.9% was measured in sediment samples (N=29) allowing us to calculate concentrations per dry weight (Table 1). The EMB values measured in the low treatment are at the lower range of the averages observed in the field, while concentrations in the high treatment are 3 to 12 times the highest averages measured around Canadian sites (Hamoutene et al. 2025).

Table 4. Summary of the initial and final emamectin benzoate sediment concentrations [EMB] in each of the treatments. Concentrations are expressed in wet weight with values between brackets and in bold in dry weight using a moisture content of 23.9 % for calculations (concentration in dry weight = concentration in wet weight /(1-percent moisture/100)).

Treatments	Nominal concentrations (in wet weight only)	Initial [EMB] (ng g^{-1})	Final [EMB] (ng g^{-1})
Control (N=5)	0	0.0 ± 0.0	0.0 ± 0.0
Low EMB (N=5)	32	0.56 ± 0.40 **(0.74 ± 0.52)**	0.10 ± 0.02 **(0.13 ± 0.03)**
High EMB (N=5)	1500	855.57 ± 202.60 **(1124.30 ± 266.23)**	228.60 ± 49.46 **(300.39 ± 65.00)**

Overall, sediment EMB concentrations measured after spiking were lower than the target nominal concentration values with coefficients of variation (standard deviation divided by the mean) in EMB concentrations ranging from 21 to 71% (Table 1). Spiking bulk-sediments for ecotoxicological studies remains challenging especially for non-soluble compounds. Piccone et al. (2022) recommend that spiked sediment be stored at 4 °C during equilibration to minimise possible biological degradation of the toxicants, to use the minimum volume solvent carrier, and that actual concentrations be measured, preferably in aliquots, to assess spiking efficiency, and ensure conclusions on effects are accurately related to the measured amount of toxicant. These points were carefully considered in this study but as for any large volumes of sediments, there is a potential for chemical patchiness, as efficient mixing of large volumes of sediment is trying (Picone et al. 2022). Additionally, there was a decline in EMB sediment concentrations as observed in other studies with EMB-spiked sediment (e.g., Hamoutene et al. 2023a,b; Asnicar et al. 2024). This decline could be due to the equilibrium processes (water/sediment) (Strachan and Kennedy 2021), photodegradation (Tariq et al. 2014), and/or a potential minimal adsorption of EMB to the walls of the tanks (Kuo et al. 2010; OECD 2019).

Each treatment had a similar combination of various-sized crabs, which was confirmed by the initial analyses of crab weights (Kruskal-Wallis test, $H2 = 0.118$, $P = 0.974$), and carapace widths (Kruskal-Wallis test, $H2 = 0.052$, $P = 0.975$). Individual crab weights ranged from 18.6 g to 132.3 g with an average difference of 81 g in each tank. There were no changes in individual carapace width between treatments over time as no crab moulted over the course of the experiment. As mentioned, sex was not considered as a factor in this experiment, nevertheless, four of the six females produced eggs over the course of the exposure; therefore, once a female had eggs present, they were no longer weighed as part of the analysis at that point due to the extraneous weight. We also noted a change of colour in most crabs over time; however, colour coding was not fully standardized and therefore remains an anecdotal observation at this stage. Tracking colour change in future studies could provide information on intermolt duration (Lee and Vespoli 2015) and used to determine if EMB may affect time to molt.

We used a linear mixed effects model to determine if EMB exposure influenced crab weight over time. Random effects initially included individual crabs nested in tanks to account for potential tank differences and variability in initial crab weights. Tank number ultimately had no variance associated with it; therefore, it was removed from the model. The results of the final model (Table 2) revealed that neither the high nor low EMB treatment groups differed significantly in weight from the control group at time zero (LMM, High: $\beta = 0.0035$, $p = 0.95$; Low: $\beta = -0.015$, $P = 0.80$). Over time, crabs in the control group exhibited a small but significant increase in weight (LMM, $\beta = 0.0000604$, $p = 0.0023$), however, the high EMB treatment group showed a significant reduced rate of change relative to the control group (LMM, $\beta = -0.0000787$, $P = 0.0074$), indicating that the crabs in this treatment lost weight over time (Table 2). In contrast, the low EMB treatment group did not differ from the control group in their growth rate (LMM, $\beta = 0.00000825$, $P = 0.76$). As previously described, there were some individual differences existing within the groups (crab to crab (intercept) variance = 0.05), however, the residual variance was minimal (0.00011).

Table 5. Results of the linear mixed model of crab weight with time, treatment (control, low EMB, high EMB), as well as the interaction of time and treatment. Individual variability was classified as a random effect due to the varied sizes of crabs. Significant results (** if P < 0.001, *** if P < 0.0001) are indicated by asterisks.

Fixed Effects	Estimate (β)	Std. Error	Df	t value	P (>|t|)	Sig.
Control (Intercept)	2.68	0.041	87.13	65.335	$< 2e^{-16}$	***
Treatment High	0.0035	0.058	87.13	0.06	0.95213	
Treatment Low	-0.015	0.058	87.13	-0.255	0.79969	
Time	0.0000604	0.0000197	321.00	3.071	0.00232	**
Treatment High: Time	-0.0000787	0.0000292	321.00	-2.695	0.00740	**
Treatment Low: Time	0.00000825	0.0000275	321.00	0.299	0.76484	

Random Effects	Variance	Std. Dev.
Crab ID (Intercept)	0.0504525	0.22462
Residual	0.0001122	0.01059

Sig.: significance, Std. Error: Standard error, Df: degrees of freedom, Std. Dev.: Standard deviation

After the 130-day exposure, survivorship was 93.3% in the control group, 96.7% in the low EMB group and 70% in the high EMB group (Fig. 2). There was a statistically significant difference in survival between the three treatment groups (Chi-square test, $\chi^2_2 = 10.90$, P = 0.0043, Fig.2) with the high EMB group showing significantly lower survival probability compared to the low (Log-Rank test with Benjamin-Hochberg (BH) correction for multiple comparisons, P = 0.020, Table 3), and control EMB treatments (Log-Rank test with Benjamin-Hochberg (BH) correction for multiple comparisons, P = 0.033, Table 3).

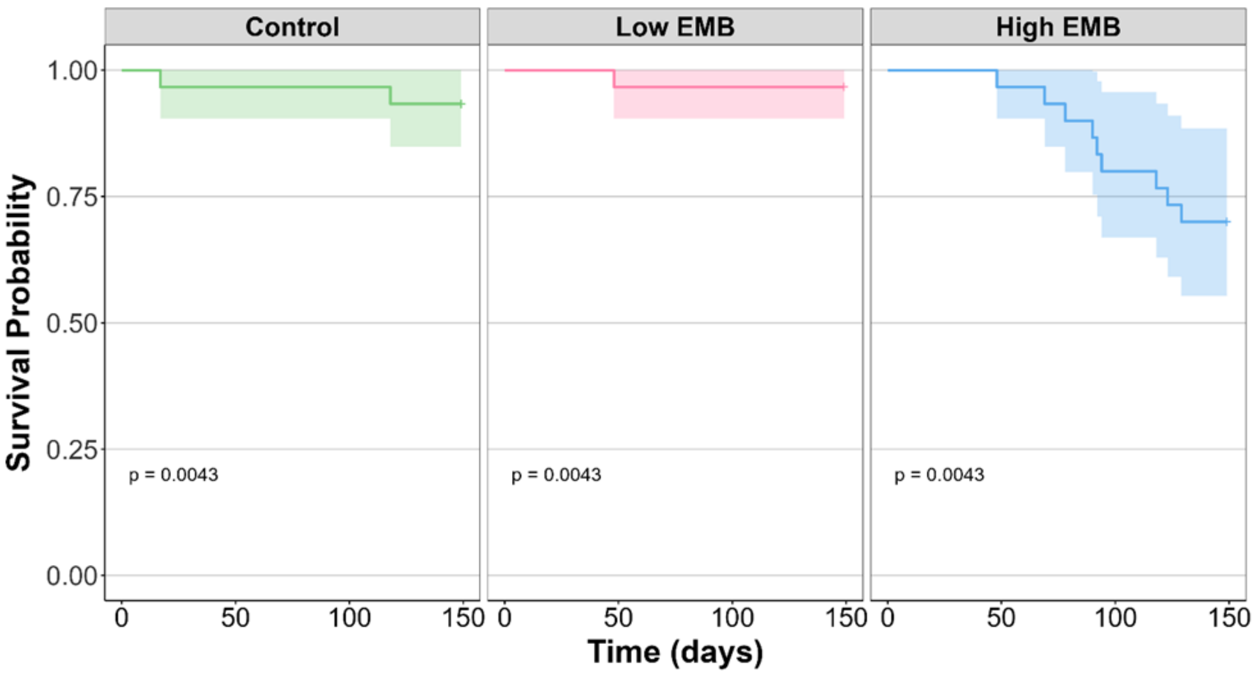

Figure 2. Survival probability of green crabs over time in the 130-day exposure based on emamectin benzoate (EMB) exposure with Control in green, Low EMB in pink, and High EMB in blue. The shaded areas represent the 95% confidence bands of the Kaplan-Meier curve.

Table 6. Results of pairwise comparisons testing for differences in survival between EMB treatment groups; fit to Kaplan-Meier survival curves. Significant results (P < 0.05) are indicated by an asterisk

Treatment Comparison	Adj. P value (BH)	Significance
Control – High EMB	0.033	*
Control – Low EMB	0.557	
Low - High EMB	0.020	*

Adj.: adjusted

As we did not measure tissue or stomach contents of the crabs to assess if EMB was ingested, it is not clear if the observed mortality is due to exposure through direct contact with contaminated sediments, ingestion of EMB through contamination of the mixed seafood diet in contact with spiked sediments, or a combination of the two. However, this would be similar to what would be potentially experienced around aquaculture sites, where both exposures would be likely to occur. Opportunistic feeding by decapods on aquaculture-derived wastes and associated fauna/flora has been documented by several researchers. The opportunistic feeding by crabs (brown crabs or rock crabs) on aquaculture-derived wastes and/or their presence at finfish sites have been documented by several authors (Woodcock et al. 2018; Sardenne et al. 2020; Lees et al. 2023). Brown crabs (*Cancer pagurus*) have been documented through fatty acid analysis to consume waste feed released from salmon aquaculture directly beneath the farms and, in the case of rock crabs (*Cancer irroratus*), up to 1 km away (Woodcock et al. 2018). In addition, at mussel sites, scavenging crabs have been found to remain within the mussel farms, exploiting fallen mussels and associated organisms (Lees et al. 2023). Related, the mussel *Mytilus edulis* is considered a fouling organism of salmon aquaculture facilities (Callier et al. 2018 and references within), and their presence could encourage foraging by crabs similar to what has been observed at mussel sites.

Our study demonstrates the potential for continuous EMB exposure (130 days) to have a toxic effect on crabs (both on growth and mortality) at concentrations exceeding the highest average values measured around aquaculture sites (Langford et al. 2014; Tucca et al. 2017; Hamoutene et al. 2018, 2025; Bloodworth et al. 2019; Kingsbury et al. 2023; Hamoutene et al. 2025). This finding does not infer a direct risk of toxicity within the average environmentally relevant concentrations recorded recently in the field (Hamoutene et al. 2025), however, it does confirm the toxicity of EMB through continuous exposure. The present trial confirms the importance of testing environmentally relevant exposure pathways and discussing results in relation to field relevant datas. It would also be important to conduct experiments that can capture intermittent exposure scenarios of mobile species like crabs and/or other decapods.

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