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Sharon C. Stewart,* Jaimie T. A. Dick, Peter R. Laming and Almut Gerhardt

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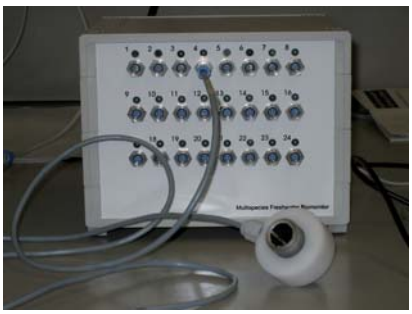

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Assessment of the Multispecies Freshwater Biomonitor™ (MFB) in a marine context: the Green crab (*Carcinus maenas*) as an early warning indicator

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The Multispecies Freshwater Biomonitor™ is an online continuous biomonitor which utilises impedance conversion to quantitatively record behavioural responses of vertebrates and invertebrates to environmental change. Here, we extend the use of the MFB into the marine aquaculture environment using the Green crab (*Carcinus maenas*) as a biological monitor. As a ubiquitous and abundant species, *C. maenas* can be used in applications such as aquaculture and monitoring of diffuse and point source marine pollution. Four experiments were undertaken to establish: (1) if the electrical field generated by the apparatus had any effect on *C. maenas*; (2) if the behaviour of *C. maenas* was altered by the presence of ammonia; (3) if the behaviour of *C. maenas* was affected by the electrical field when ammonia was present and (4) if defined behaviours could be detected by the MFB. There was no significant effect of the current on *C. maenas* in the MFB. There was a significant difference in overall expression of behaviour in response to an increasing gradient of ammonia and activity of the chamber. Five behaviours, 'walking', 'climbing', 'leg stretch', 'cleaning' and 'inactivity' were detected by the MFB. *C. maenas* appears to be a suitable candidate for use in the MFB in a marine context. Further testing of the biomonitor and *C. maenas* is required using other toxicants to establish alarm thresholds that could be used *in situ* for water quality monitoring.

1. Introduction

Manual biomonitoring techniques can be subjective, time consuming and laborious.[1,2] As a result, various methods for automating the process have been developed. For example, the WRc (Water Research centre) has a fish monitor technique[3] that reduces subjectivity and allows long term online biomonitoring using ventilation behaviour of fish as the response variable. Biomonitors have been used based on a variety of methods, including light beam disruption using invertebrates such as *Daphnia* sp. and mussels.[4,5]

A recent development is the Multispecies Freshwater Biomonitor™ (MFB), which is based on the quadropole impedance conversion technique and is a novel biological early warning system (BEWS) for online water quality biomonitoring.[1,2,6,7] The MFB is based on impedance conversion,[6] with organisms placed in flow-through chambers. Online biomonitors register the effects of toxic pollutants on indicator species and therefore allow for fast, continuous and relevant water quality control. BEWS continuously registers sensitive stress responses (behaviour, physiology) of selected indicator species in order to detect pollution pulses. The chambers have two pairs of electrodes, one pair is current carrying (100 kHz) and the other pair senses the changes of impedance caused by the movements of the organism, with the different signals being attributed to different types of behaviours.[7,8] The MFB quantitatively measures changes in the behavioural pattern of both aquatic vertebrates and invertebrates.[1,2,6,9]

Biomonitors rely on rapid and sensitive changes in the behaviour of the indicator species, often recorded as locomotion and/or ventilation. A number of authors have explored the preference or avoidance behaviour of organisms to various toxicants or stimulants.[10,11] Cherry and Cairns[10] in 1982 discovered that two contrasting situations were found: the first appears to be a preference for the organism to enter into a lethal

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Environmental impact

We describe a new marine biomonitoring system using large crabs in the Multispecies Freshwater Biomonitor™ (MFB). *Carcinus maenas*, native in Europe, impacts as invasive marine invertebrate fisheries and maricultures. Both as bait and for human consumption the crab has been fished in Europe, hence being of high ecological and economical importance. The EU Marine Strategy Directive demands a good environmental status for marine environments until 2021. The new biomonitoring system represents an important tool, *e.g.* allowing online surveillance of the crab's fitness as indicator in mariculture systems and along polluted coasts. Extending the MFB to marine biomonitoring, using invasive, widely distributed species, which are also of economic importance represents a new approach in applied ecotoxicological research and risk assessment.

concentration of a toxicant, while the second was where the organism showed neither preference nor avoidance behaviour in the presence of a toxicant. McNicol and Scherer[11] in 1991 carried out a study on lake whitefish, *Coregonus clupeaformis*, and found their reaction to various cadmium concentrations to be bimodal, in that they reacted to low and high concentrations, but showed little reaction to intermediate levels, with the fish showing both preference and avoidance to the cadmium solution. These issues need to be addressed in the development of the MFB in a marine context.

In this study, therefore, our aim was to examine the merits and suitability of a marine organism, the Green crab *C. maenas*, as a potential new early warning system species in conjunction with the MFB. *C. maenas* is a common and widely distributed crustacean native to North West Europe.[12] More recently, they have been found on N. American coasts.[12] They are found along all British coasts, making them the most widespread of all British crab species,[13] and they can be found hiding under rocks or buried in the sand during low tide. They are commercially important species that are routinely collected and cultured for Bait.[14] They are also widely collected for human consumption in many parts of mainland Europe and increasingly so in some areas of Britain.[14] As the worldwide demand for seafood increases, aquaculture production has also increased to help meet the demand.[15] Aquaculture facilities are subjected to exogenous and endogenous sources of pollution and these crabs could potentially be used for BEWS in these systems. Ammonia is an important toxicant in these conditions as it can build up in the system from the excretion of the animals and can be brought into the system with the water supply. We thus use ammonia in the present study to examine the use of the MFB for marine aquaculture and water quality monitoring. The MFB has only been used once before with a marine organism in sediment assays with *Corophium volutator*.[2] The aims of this study were to determine: (1) does the Green crab (*C. maenas*) show preference or avoidance behaviour in response to the current in the MFB chambers? (2) Does the presence of ammonia (mg $NH_3N\ l^{-1}$) have an effect on the behaviour of the Green crab in MFB chambers? (3) Does the MFB have an effect on the behaviour of the Green crab due to the current passing through the chambers when ammonia is present? (4) Can individual behaviours exhibited by the Green crab be quantified and qualitatively assessed using the MFB without continual direct observations in a control solution and different concentrations of ammonia?

2. Materials and methods

2.1 Test species

C. maenas were collected from Ballyhenry Island (OS J680055), Strangford Lough, Northern Ireland on several dates spanning two weeks during June 2003. They were manually collected at low tide from under rocks and in rock pools and were of a uniform size (carapace width 3 cm (±0.6 cm)), males and females were collected at a ratio of 50 : 50. Crabs were then transferred to outdoor holding tanks with flow through systems and aeration for 24 hours. The seawater was pumped from Strangford Lough and through a filter before entering the tanks.

2.2 Toxicant

Ammonia can exist in two chemical forms and both are highly soluble in water, with the non-toxic ammonium ions (NH_4^+) predominantly in acidic (low pH) conditions and the highly toxic unionized ammonia (NH_3) (high pH).[16] Together they are referred to as 'total ammonia'. In this study, ammonium chloride (NH_4Cl) solutions were formulated using UV filtered seawater. Average pH and temperature were used to calculate the proportion of unionised ammonia (mg $NH_3N\ l^{-1}$) (Trussell, 1972).

2.3 The Multispecies Freshwater Biomonitor™

The test organisms were placed individually in a cylindrical flow-through clear acrylic plastic test chamber with nylon nets (0.5 cm mesh size) screwed on both ends. The chambers (17 cm in length × 6 cm in diameter) were attached to the MFB, and channel information, noise level (40 mV) and frequency bands (Band 1, 0.5–4.0 Hz for locomotory activity and Band 2, 4.0–8.0 Hz for ventilation and small movements) programmed prior to recording. Recording occurred over 4 minute durations with 6 minute intervals between recording periods (see drawing of MFB in Fig. 1a and b by Kirkpatrick *et al.* in 2006).[1]

2.4 Experimental setup

2.4.1 The quantification and qualification of individual behaviours exhibited by the Green crab (*C. maenas*) using the MFB. The direct observation of individual behaviour with the MFB in a previous experiment enabled the MFB trace to be characterised for each of the specific behaviours; 'walking', 'climbing', 'leg stretch', 'cleaning' and 'inactivity'.

2.4.2 Preference/avoidance response to current passing through the MFB chamber. Two MFB chambers were attached to each other using an intermediate rubber tubing of 5 cm length. The ends of the "dual chamber" were sealed using the mesh caps provided. Each chamber that was part of the dual chamber was then attached to the MFB. One crab was placed into the dual chamber and the mesh end replaced. The chamber was then lowered into a solution ensuring that all air bubbles were removed and the crab was allowed for 15 minutes to acclimatise. The 'dual chamber' was then placed into a 6 L tank which contained 5 L of solution (see below) at pH 7.3 ± 0.3 and 10.8 °C ± 0.2, then one randomly chosen chamber was activated for 7 minutes before the next chamber was activated and the first chamber became inactive. The location of the crab was recorded visually at 2, 6, 10 and 14 minutes. There were five experimental dilution groups: (1) standard seawater; (2) 2.3 mg $NH_3N\ l^{-1}$; (3) 6.9 mg $NH_3N\ l^{-1}$; (4) 9.2 mg $NH_3N\ l^{-1}$ and (5) 11.5 mg $NH_3N\ l^{-1}$. These concentrations were chosen as they were above and below the 96 hour LC_{50} (6.88 mg $NH_3N\ l^{-1}$) for the crab.[16] Forty individuals were used in all the groups, *i.e.* 40 per treatment. The animals used were placed in a chamber for 15 minutes before recording to allow for acclimatising then after 24 hours, the same forty individuals were used in experimental group 2, this was repeated for experimental groups 3–5.

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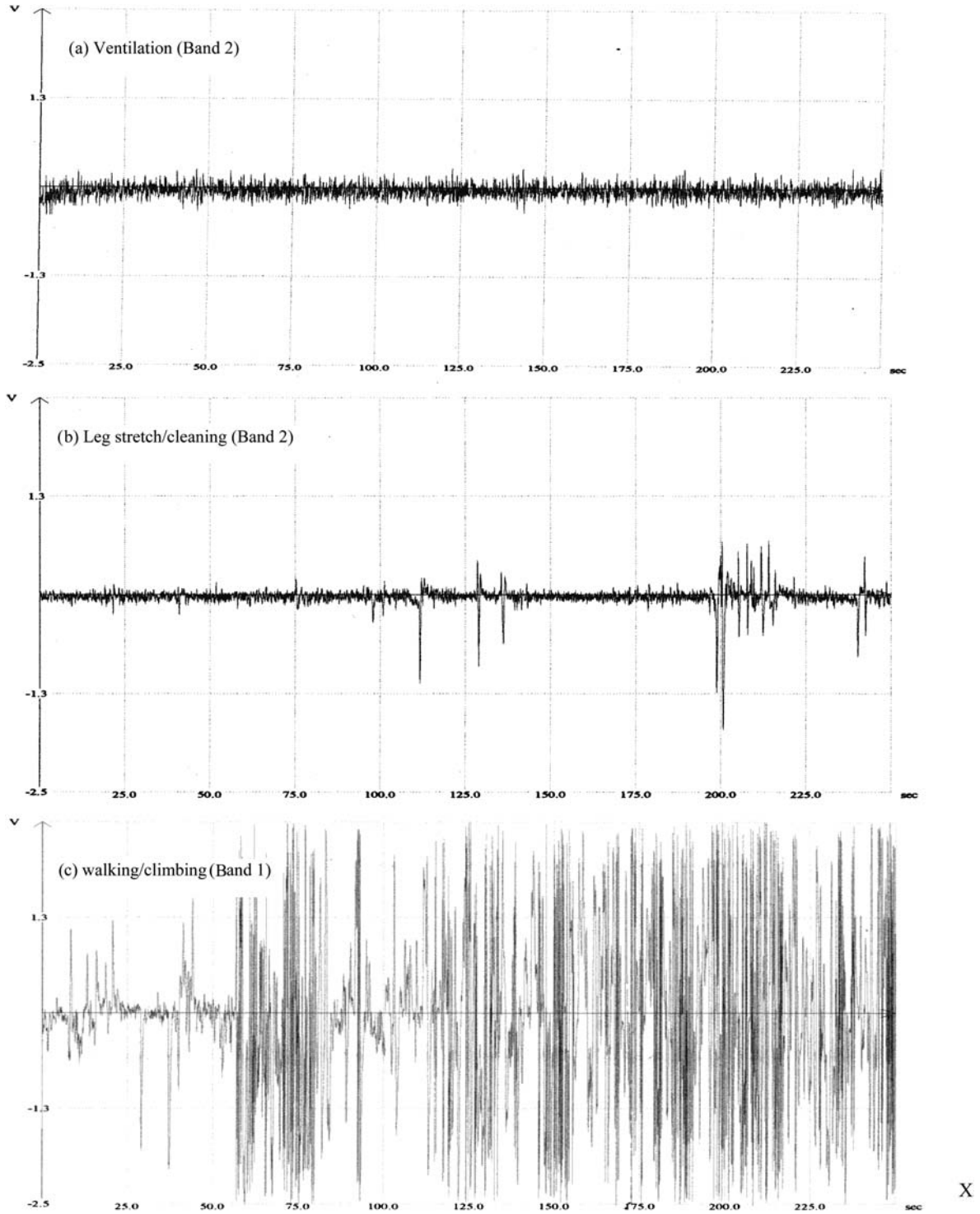

Fig. 1 MFB traces showing characteristic waveforms for *C. maenas* behaviours.

2.4.3 The effect of ammonia (mg NH_3N l^{-1}) on the behaviour of the Green crab (*C. maenas*) in MFB chambers. Individual chambers were used for this test. A crab was placed into each chamber and the chamber was then lowered into 5 L of solution (see above) in a 6 L tank and the crab was allowed for 15 minutes to acclimatise. Crab behaviour was visually recorded using a Psion II hand-held computer (Psion PLC, London, England), programmed using the Observer (4.0) package (Noldus Information Technology, Wageningen, The Netherlands) for five predefined behavioural components: walking, climbing, leg stretch, cleaning and inactivity, obtained from qualitative observations[17] for 15 minutes. This direct observation of the individual behaviour enabled a determination of the effect of the ammonia whilst the crabs were in the chambers, twenty crabs were observed for each experimental group (as above).

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2.4.4 The effect of the current passing through the chambers of the MFB on the behaviour of the Green crab (*C. maenas*) when ammonia is present. An individual crab was placed into each chamber which was then lowered into one of the solutions (see above). Each 6 L tank contained two chambers with a crab in each chamber; the crabs were allowed for 15 minutes to acclimatise. The chambers were attached to the MFB and at random one of the two chambers was activated. The behaviour of the crabs was also recorded using the Psion II hand-held computer (Psion PLC, London, England), programmed using the Observer (4.0) package (Noldus Information Technology, Wageningen, The Netherlands) for five predefined behavioural components, walking, climbing, leg stretch, cleaning and inactivity, obtained from qualitative observations[17] for 15 minutes. Twenty crabs were observed for each experimental group.

2.5 Statistical analyses

2.5.1 Preference/avoidance response to current passing through the MFB chamber. The distribution of crabs between the active/inactive chambers was assessed by Chi-squared analysis (Statview version 5) (c^2). There were four tests (*i.e.* 2, 6, 10 and 14 minutes) undertaken on each dataset, so to avoid type 1 errors (erroneous rejection of a true null hypothesis) we used the Bonferroni adjustment, giving the level of significance as $P < 0.0125$.[18]

2.5.2 The effect of ammonia (mg $NH_3N\ l^{-1}$) on the behaviour of the Green crab (*C. maenas*) in MFB chambers. The file created on the Psion was downloaded onto the observer system (described below). The data recorded on the Observer system were summarised to produce a total duration for each of the behaviours recorded for each crab over the study time (15 minutes). These data were entered into Statview (version 5) and a one-factor MANOVA (Multivariate Analysis of Variance) was undertaken on the dependent variables ($\log_{10}(x + 1)$ data), that is, 'inactivity', 'walking', 'climbing', 'leg stretch' and 'cleaning'. The between subject factor was concentration of ammonia (mg $NH_3N\ l^{-1}$).[1,2,9]

2.5.3 The effect of the current passing through the chambers of the MFB on the behaviour of the Green crab (*C. maenas*) when ammonia is present. The data recorded on the Observer system produced a total duration for each of the behaviours recorded for each crab over the study time (15 minutes). These data were entered into Statview (version 5) and a MANOVA (Multivariate Analysis of Variance) was undertaken on the dependent variables ($\log_{10}(x + 1)$) for 'no movement', 'walking', 'climbing', and 'leg stretch'. The between subject factors were the concentration of ammonia (mg $NH_3N\ l^{-1}$) and whether the chamber was active or inactive. The MANOVA generates a 2-factor ANOVA, with concentration and chamber activity as the between subject factors for each separate behaviour.

3. Results

3.1 The quantification and qualitative assessment of individual behaviours exhibited by the Green crab (*C. maenas*) using the MFB

'Ventilation' has an average frequency of 1.5 Hz (range 1–2 Hz). 'Ventilation' traces have regular peaks and troughs (Fig. 1a). 'Ventilation' is not always so distinctive from direct observation, thus the time for 'ventilation' was considered to be the duration when the organism was 'inactive', *i.e.*, when the organism is inactive the only behaviour that can be registered is ventilation. 'Cleaning' and 'leg stretch' have an average frequency of 3 Hz (range 2.5–3.5 Hz) and have a very similar trace on the MFB, which is characterised by small peaks with additional areas of 'inactivity' (Fig. 1b). Of all the behaviours, 'walking' and 'climbing' (Fig. 1c) have the greatest amplitude. 'Walking' has an average frequency of 6.5 Hz (range 5.5–7.5 Hz) and 'climbing' has an average frequency of 4.5 Hz (range 4–5 Hz).

3.2 Preference/avoidance response to current passing through the MFB chamber

The Chi-squared value for each time interval showed no significant difference (Tables 1 and 2) except for 6.9 mg $NH_3N\ l^{-1}$ at the 14 minute interval ($\chi^2 = 8.1$, df = 1).

Overall, there was no significant effect on the behaviour of *C. maenas* when the MFB is switched on, that is, there was neither preference nor avoidance of a chamber with current passing through it.

3.3 The effect of ammonia (mg $NH_3N\ l^{-1}$) on the behaviour of the Green crab (*C. maenas*) in MFB chambers

There was a significant difference in overall expression of behaviour among the experimental dilution groups ($PF_{20,176} = 3.04$, $P < 0.0001$, Fig. 2). The individual one factor ANOVA results showed that there was a significant difference in time spent 'walking' among groups ($F_{4,45} = 3.8$, $P < 0.01$), although this shows no discernible pattern with increasing concentration (Fig. 2). Fisher's PLSD showed significant differences between the control and 2.3 mg $NH_3N\ l^{-1}$; control and 9.2 mg $NH_3N\ l^{-1}$; 2.3 mg $NH_3N\ l^{-1}$ and 6.9 mg $NH_3N\ l^{-1}$; 2.3 mg $NH_3N\ l^{-1}$ and 11.5 mg $NH_3N\ l^{-1}$; 9.2 mg $NH_3N\ l^{-1}$ and 11.5 mg $NH_3N\ l^{-1}$ (Table 3). 'Cleaning' also showed a significant difference in time spent among groups ($F_{4,45} = 12.2$, $P < 0.0001$) over the range of ammonia concentrations (Fig. 2 and

Table 1 χ^2 results for preference/avoidance experiment

Solution	Time/min	χ^2	df	*P* Value
Control	2	0	1	NS
	6	0.4	1	NS
	10	0.4	1	NS
	14	2.5	1	NS
2.3 mg $NH_3N\ l^{-1}$	2	1.6	1	NS
	6	2.5	1	NS
	10	3.6	1	NS
	14	0.9	1	NS
6.9 mg $NH_3N\ l^{-1}$	2	1.6	1	NS
	6	0.4	1	NS
	10	0.1	1	NS
	14	8.1	1	<0.01
9.2 mg $NH_3N\ l^{-1}$	2	1.6	1	NS
	6	0.9	1	NS
	10	0.4	1	NS
	14	1.6	1	NS
11.5 mg $NH_3N\ l^{-1}$	2	3.6	1	NS
	6	2.5	1	NS
	10	3.6	1	NS
	14	6.4	1	NS

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Table 2 Number of crabs (*C. maenas*, $n = 40$) recorded as located at consecutive time intervals (2, 6, 10 and 14 minutes), in an MFB chamber when the MFB was 'on' compared with when the MFB was 'off' in control (filtered seawater) and several concentrations of ammonia (mg $NH_3N\ l^{-1}$)

	Time intervals			
	2	6	10	14
Control on	20	22	22	25
Control off	20	18	18	15
2.3 mg on	24	25	14	17
2.3 mg off	16	15	16	23
6.9 mg on	24	22	19	29
6.9 mg off	16	18	21	11
9.2 mg on	24	23	18	16
9.2 mg off	16	17	22	24
11.5 mg on	26	25	14	13
11.5 mg off	14	15	26	27

Table 3 Fisher's PLSD results for the effect of unionised ammonia concentration (mg $NH_3N\ l^{-1}$) on the behaviour of the Green crab (*C. maenas*)

Behaviour	Significant result	Mean difference	*P* Value
Inactive	None, 9.2 mg	−0.155	0.0322
Walking	None, 2.3 mg	0.272	0.0029
	None, 9.2 mg	0.228	0.0115
	2.3 mg, 6.9 mg	−0.202	0.0238
	2.3 mg, 11.5 mg	−0.221	0.0141
	9.2 mg, 11.5 mg	−0.177	0.0468
Climbing	2.3 mg, 11.5 mg	−0.293	0.0350
Cleaning	None, 6.9 mg	0.608	0.0006
	None, 9.2 mg	0.892	<0.0001
	None, 11.5 mg	0.963	<0.0001
	2.3 mg, 9.2 mg	0.606	0.0006
	2.3 mg, 11.5 mg	0.678	0.0002
	6.9 mg, 11.5 mg	0.355	0.0367

Table 3). The amount of time spent on 'cleaning' decreased with increasing ammonia (Fig. 2). Fisher's PLSD showed significant differences between the control and 6.9, 9.2, and 11.5 mg $NH_3N\ l^{-1}$; 2.3 and 9.2 mg $NH_3N\ l^{-1}$; 11.5 mg $NH_3N\ l^{-1}$ and 6.9 and 11.5 mg $NH_3N\ l^{-1}$ (Table 3). The other behaviours showed no overall significant difference over the concentrations of ammonia, although Post hoc tests (Fisher's PLSD) carried out on 'no movement' and 'climbing' showed significant differences between

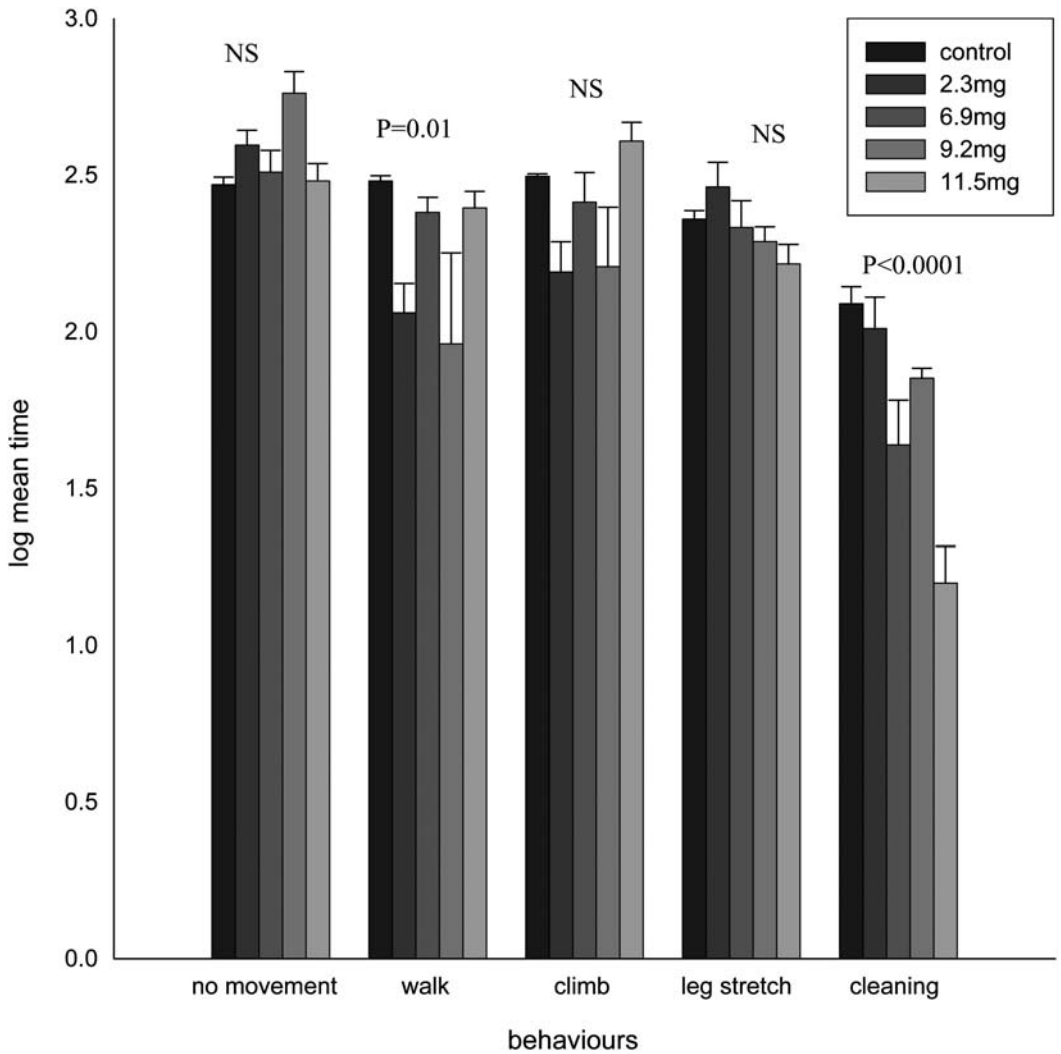

Fig. 2 Log mean time (±SE) spent on each behaviour with increasing concentrations of ammonia (mg $NH_3N\ l^{-1}$).

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the control and 9.2 mg $NH_3N\ l^{-1}$ for 'no movement' and between 2.3 and 11.5 mg $NH_3N\ l^{-1}$ for 'climbing'.

3.4 The effect of the current passing through the chambers of the MFB on the behaviour of the Green crab (*C. maenas*) when ammonia is present

The majority of time during the observations in the ammonia solutions regardless of the MFB state (on/off) was spent 'inactive' or on 'leg stretch' (Fig. 3a and b). There was a significant difference in expression of behaviour among the experimental dilution groups ($PF_{16,760} = 16.8$, $P < 0.001$) and activity of the chambers ($PF_{4,187} = 5.06$, $P < 0.001$). There was also a significant interaction effect between the presence of ammonia and the activity of the chamber ($PF_{16,760} = 7.59$, $P < 0.001$).

The 2-factor ANOVAs showed significantly more time doing nothing when ammonia was present as compared to the control ($F_{4,190} = 289.7$, $P < 0.001$). There was a small difference between

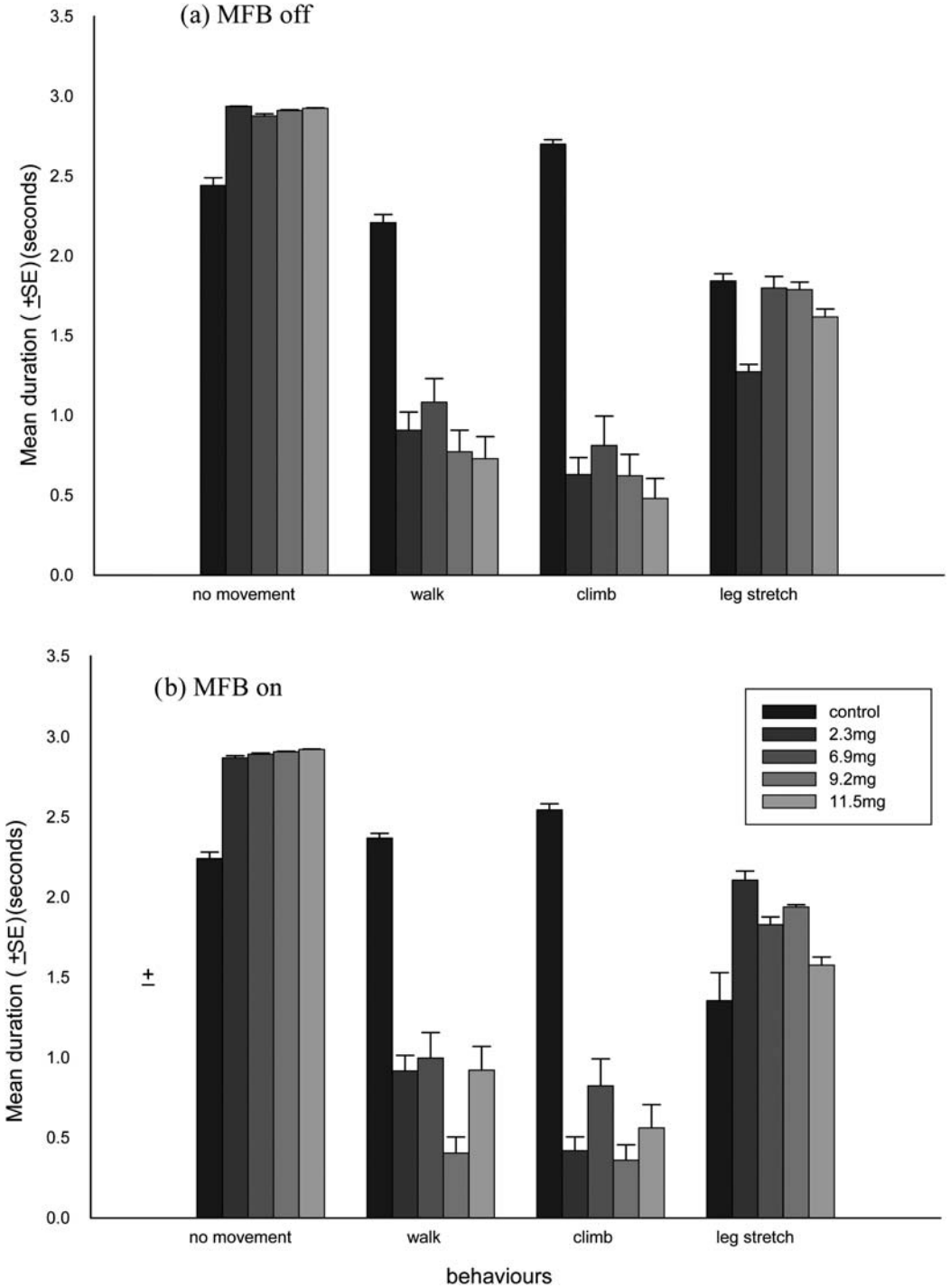

Fig. 3 Duration (+SE) spent per behaviour (in seconds as recorded by the Observer) in filtered seawater and four concentrations of ammonia (mg $NH_3N\ l^{-1}$) ($n = 20$ per solution) when the MFB was switched (a) off and (b) on.

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Table 4 Fisher's PLSD results for the effect of unionised ammonia concentration (mg $NH_3N\ l^{-1}$) on the behaviour of the Green crab (*C. maenas*)

Behaviour	Significant result	Mean difference	*P* Value
Inactive	None, 2.3 mg	−0.562	<0.0001
	None, 6.9 mg	−0.542	<0.0001
	None, 9.2 mg	−0.568	<0.0001
	None, 11.5 mg	−0.581	<0.0001
Walking	None, 2.3 mg	1.376	<0.0001
	None, 6.9 mg	1.248	<0.0001
	None, 9.2 mg	1.7	<0.0001
	None, 11.5 mg	1.463	<0.0001
	2.3 mg, 9.2 mg	0.323	0.0074
	6.9 mg, 9.2 mg	0.451	0.0002
	9.2 mg, 11.5 mg	−0.237	0.0485
Climbing	None, 2.3 mg	2.098	<0.0001
	None, 6.9 mg	1.797	<0.0001
	None, 9.2 mg	2.131	<0.0001
	None, 11.5	2.103	<0.0001
	2.3 mg, 6.9 mg	−0.302	0.0133
	6.9 mg, 9.2 mg	0.334	0.0062
	6.9 mg, 11.5 mg	0.306	0.0121
Leg stretch	None, 6.9 mg	−0.214	0.0036
	None, 9.2 mg	−0.264	0.0003
	2.3 mg, 9.2 mg	−0.174	0.0173
	6.9 mg, 11.5 mg	0.217	0.0032
	9.2 mg, 11.5 mg	0.267	0.0003

the means for 'no movement' when the chamber was active (2.77) compared with when the chamber was inactive (2.82). This may explain the significant difference between the chamber being on and off even though it is not obvious from looking at the figures (Fig. 3a and b). The Post Hoc test, Fisher's PLSD, showed significant differences between the control solution and 2.3, 6.9, 9.2 and 11.5 mg $NH_3N\ l^{-1}$ (Table 4). There was a significant decrease in time spent 'walking' with increasing concentrations of ammonia ($F_{4,190} = 62.5$, $P < 0.001$). However, the presence of the current in the chamber had no significant effect. Post Hoc tests, Fisher's PLSD, showed significant differences between the control solution and all the experimental dilutions, 2.3 and 9.2 mg $NH_3N\ l^{-1}$, 6.9 and 9.2 mg $NH_3N\ l^{-1}$ and 9.2 and 11.5 mg $NH_3N\ l^{-1}$ (Table 4). There was a significant decrease in time spent 'climbing' over the range of experimental dilutions ($F_{4,190} = 115.7$, $P < 0.001$) and the presence of the current had no significant impact on the behaviour. The Post Hoc tests showed significant differences between the same solutions as the results for 'walking' (Table 4). There were significant decreases in time spent on 'leg stretch' with increasing concentrations of ammonia ($F_{4,190} = 65.7$, P 0.0002). Post Hoc tests showed significant differences between the control solution and 6.9 and 9.2 mg $NH_3N\ l^{-1}$, also 2.3 and 9.2 mg $NH_3N\ l^{-1}$, 6.9 and 11.5 mg $NH_3N\ l^{-1}$, and 9.2 and 11.5 mg $NH_3N\ l^{-1}$ (Table 4).

Individual behaviours are affected to different extents, with a general decline in behaviours with increasing ammonia concentration, except for 'no movement'.

4. Discussion

In this study, our aim was to examine the merits and suitability of the Green crab, *C. maenas*, as a potential new 'early warning system' species in conjunction with the MFB in a marine context. The first experiment assessed if the monitoring system imposed any restrictions on the test species' normal behaviour. There was no significant avoidance of, or attraction to, active/inactive test chambers. This indicates that the current passing through the chambers was not affecting the location of crabs in the chamber. This has been observed before for other crustacean species[1] and fish.[9] The addition of ammonia at different concentrations was to determine if there was a behavioural change in response to increasing concentrations of toxicant and the presence or absence of the current. There was no preference for, or avoidance of, a current passing through a chamber when ammonia is present.

The second experiment was designed to assess the effect that the presence of ammonia had on the behaviour of the test crabs. Total ammonia is usually measured in tests, however, the toxicity is primarily attributable to the unionised form (NH_3). Most animals immediately convert the toxic ammonia to a less harmful substance such as urea in fish which continually excrete metabolic ammonia into the surrounding environment *via* cells in the gills.[19] In rivers, lakes and seas this is normally diluted to a safe level, however, in aquaculture systems the levels can rise dangerously.[19] This rise in ammonia is also exacerbated by the decomposition of fish food, fish waste and detritus left in the cages or tanks. With increasing concentration of ammonia, there was a significant difference in some of the behaviours (such as walking and cleaning). In addition, 'inactivity', 'climbing' and 'leg stretch' did not change significantly with increasing ammonia concentration. However, 'inactivity' did increase as the other behaviours decreased, showing a causal relationship. Low levels of ammonia act as an irritant to gills, prolonged exposure to sub-lethal levels can cause skin and gill hyperplasia (this is where the gill lamellae swell and thicken restricting the water flow over the gills[20]). At high levels even short exposure leads to skin, eye and gill damage.[20] It can also suppress the normal excretion of ammonia causing ammonia poisoning which results in organ damage and eventually death.[20]

The third experiment was designed to assess the effect of the current passing through the chambers of the MFB on the behaviour of the Green crab when ammonia was present. Behaviours were significantly affected by the presence of ammonia, but not by the electrical current. When examining the traces formed by the MFB, there was oscillation even when the crab was classified as being 'inactive'. These oscillations are caused by ventilation which is not easily manually observed, but is picked up by the MFB.

Kirkpatrick (2006b)[2] showed that the MFB could be used in sediment toxicity assays with *C. volutator* as well as the freshwater *Crangonyx pseudogracilis*.[1] The MFB has been used successfully with a variety of freshwater crustacean and fish species (*e.g. Gammarus pulex*,[8,21] *Macrobrachium nipponense*,[23] and fish:[9] *Daphnia magna* and *Gambusia holbrooki*[23]). During *in situ* MFB tests in Sweden using *G. pulex* exposed to a copper pulse, it was found that there was an overall increase in activity which correlated with the changing copper concentration.[8] Overall activity in this case involved significant increases in leg movements, body stretches and ventilation, with response times between 30 and 60 minutes occurring at sub-lethal concentrations. During the current investigation, there were significant increases in leg movement, and periods of inactivity (ventilation) with increasing concentrations of ammonia. Gerhardt (1996)[22]

carried out another study using *G. pulex* and the MFB, and found that it again reacted within 1 hour to a combination of heavy metals and organic xenobiotics from a factory effluent. The initially high levels of activity decreased as the duration of the exposure increased. The response variable in that study was overall activity. In the same study, *Hydropsyche angustipennis* showed increased locomotion, but only at night. It also showed a decrease in ventilation, which has been suggested to be a mechanism to try to delay exposure to toxicants until they had passed downstream, a technique which has been suggested by other authors for semi-sessile organisms.[21] Ventilation has been used as a response variable by many researchers.[3] In fish, the responses include gill purging (coughing), ventilatory frequency and amplitude.[24] Some authors only used ventilatory frequency in their biomonitoring systems[26,27] as frequency is easily monitored and was the most sensitive indicator of fish health.

There are a number of advantages of the MFB over other biomonitors, for example, it can record in sediment, cloudy, turbid or coloured water samples or using pale coloured organisms.[8] Problems have been found when using pale coloured organisms, especially with detection systems that utilise video recordings. This was overcome by feeding organisms such as *Daphnia* with a dark coloured food and by only using large *Daphnia* in the investigation[28] or even using fluorescent food. The colour of the organisms, sample colour or time of day are irrelevant in the MFB as this system does not depend on visual detection, but rather measures the change in impedance due to changes in the electrical field.[8] The MFB can also be utilised without affecting the photoperiod to which the organism is acclimitatised.[28]

Overall, BEWS can complement but not replace existing water quality monitoring.[29] The use of organisms enables the detection of anything toxic or detrimental as the organisms react automatically, whereas water quality monitors and toxicity tests can only examine for the toxicant for which they are designed or investigating. Field applied BEWS have two applications: (1) to protect drinking water supplies by detecting toxicants within it and (2) to protect the aquatic environment from the toxicants.[29] The protection of aquatic ecosystem health is more reliable than determining the effects to human health. Some studies have shown that BEWS only detected levels of some chemicals, such as cadmium, ammonia and zinc that were too high to protect human health.[29] For example, Morgan *et al.*[30,31] carried out a study on the guppy, *Poecilia reticulate*, and found that the detection of chronically toxic levels of chemicals was poor.

Individual behaviours were characterised using the MFB and this was fundamental in the investigations to assess the impact of toxicants on specific behavioural components. For the Green crab (*C. maenas*) there was a reduction in the number of behavioural components recorded when compared with the manual observations undertaken. This arose from the subjectivity of the direct observations with the human eye able to distinguish factors such as 'cleaning', and the objective measurements taken by the automated technique. The MFB was investigated as a relatively new biomonitor that is little used with marine species. From an operational point of view, the system was easy to set up, run and obtain information from. This equipment would take little specialist knowledge to run once installed as an online biomonitor system. Whilst the system was only used with one test species in the present study, it would be possible to use it with a variety of other marine species simultaneously, *e.g.* with a crab and benthic *Corophium* sp. The MFB can be used in saltwater. It is a portable system and could be installed on site for continuous monitoring. The MFB at present requires further work, such as the remote access, *e.g.* in offshore situations, before it is ready for sea cages and widespread marine application, but could now be used to monitor water quality in crustacean aquaculture and holding facilities.

5. Conclusions

The Green crab (*C. maenas*) appears to be a suitable candidate for biological monitoring using the MFB in marine aquaculture facilities.

Their behaviour is not adversely affected by the passage of the current through the chambers.

Future widespread studies are required to assess sensitivity to other toxicants and using multiple species to increase the sensitivity of the MFB.

The MFB can be used in a marine setting but requires further research on remote access operation so that it could be used in sea cages, *e.g.* in offshore mariculture.

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