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**Understanding the effects of electromagnetic field emissions from Marine Renewable Energy Devices (MREDS) on the commercially important edible crab, *Cancer pagurus* (L.)**

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**Key Words:** *Cancer pagurus*; edible crab; electromagnetic field; environmental stressor; marine renewable energy.

**Conflicts of interest:** none.

**Contributions:** All research was designed and conducted by Kevin Scott and Petra Harsanyi with regular input from Alastair Lyndon. All three authors contributed to the article preparation.

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## **Abstract**

The effects of simulated electromagnetic fields (EMF), emitted from sub-sea power cables, on the commercially important decapod, edible crab (*Cancer pagurus*), were assessed. Stress related parameters were measured (L-Lactate, D-Glucose, Haemocyanin and respiration rate) along with behavioural and response parameters (antennular flicking, activity level, attraction/avoidance, shelter preference and time spent resting/roaming) during 24-hours. Exposure to EMF had no effect on Haemocyanin concentrations, respiration rate, activity level or antennular flicking rate. EMF exposure significantly disrupted haemolymph L-Lactate and D-Glucose natural circadian rhythms. Crabs showed a clear attraction to EMF exposed shelter (69%) compared to control shelter (9%) and significantly reduced their time spent roaming by 21%. Consequently, EMF emitted from Marine Renewable Energy Devices (MREDS) will likely affect edible crabs both behaviourally and physiologically, suggesting that the impact of EMF on crustaceans must be considered when planning MREDS.

## 1 1. Introduction

2 The predicted decline in non-renewable  
3 energy sources in future decades (Pimentel *et al.* 2002) indicates the need for alternative  
4 renewable energy sources. Due to reduced  
5 planning constraints, lack of inexpensive land  
6 near major population centres (Bilgili *et al.*  
7 2011), and perceived aesthetic problems with  
8 many renewable energy structures (Gill 2005),  
9 there is increasing pressure to move potential  
10 locations offshore. Wind speeds tend to be  
11 significantly higher offshore than onshore thus  
12 producing larger amounts of energy per  
13 turbine (Bilgili *et al.* 2011). Vast open spaces  
14 offshore also help avoid wake effects (shading  
15 effect of a turbine on those downwind of it) by  
16 allowing turbines to be placed at greater  
17 distances apart (Chowdhury *et al.* 2012). As  
18 the global energy demand grows, inshore  
19 areas are increasingly being utilised by the  
20 energy sector looking to increase energy  
21 production via wave and tidal energy devices  
22 (Frid *et al.* 2012). Therefore, there is a  
23 requirement for appropriate assessment of the  
24 implications of both offshore and inshore  
25 renewable energy generation with regards to  
26 current ecological status and potential future  
27 consequences (Gill 2005). Currently, the UK is  
28 the largest global producer of electricity from  
29 offshore wind farms and has more projects in  
30 planning or construction than any other  
31 country (Smith *et al.* 1999; Crown Estates  
32 2017). Proposed sites and developments are  
33 based on current knowledge and assessments  
34 of the siting environment, despite relatively  
35 little being known about the ecological effects  
36 of such developments on marine benthic  
37 organisms. Some studies suggest that turbine  
38 arrays could increase biodiversity through new  
39 habitat provision (Landers *et al.* 2001;  
40 Lindeboom *et al.* 2011), whereas detrimental  
41 effects of turbine arrays on birds (Garthe and  
42 Hüppop, 2004) and fish (Westerberg and  
43 Lagenfelt 2008) have also been found.  
44 Furthermore, it is feared that marine mammals  
45 might be sensitive to minor changes in  
46 magnetic fields associated with these

48 developments (Walker *et al.* 2003). There is  
49 currently a gap in our knowledge of the effects  
50 of these arrays on crustaceans.

51 Electromagnetic fields (EMF) are associated  
52 with Marine Renewable Energy Devices  
53 (MREDs). EMFs originate from both  
54 anthropogenic (telecommunication cables,  
55 power cables, marine renewable energy  
56 devices) and natural (Earth's natural  
57 geomagnetic field) sources. It has been shown  
58 that industry-standard AC cables can be  
59 effectively insulated to prevent electric field (E-  
60 field) emissions but not magnetic field (B-field)  
61 emissions (Gill 2005). Standard cable  
62 configurations combined with the existing B-  
63 field creates induced electromagnetic fields  
64 (iEM fields) (Gill 2005). The magnetic field (B-  
65 field) leakage has been shown to be of concern  
66 as it will interact with magnetite-based internal  
67 compasses in marine organisms (Öhman *et al.*  
68 2007). Electric currents between 850 and 1600  
69 A (Amperes) tend to be found in undersea  
70 cables consequently producing an  
71 electromagnetic field of around 3.20 millitesla  
72 (mT) (1,600A) in a perfect wire (Bochert and  
73 Zettler, 2006). As with all electromagnetic  
74 fields this quickly diminishes away from the  
75 source, with values of around 0.32mT and  
76 0.11mT at 1 metre (m) and 4 m respectively  
77 (Bochert and Zettler 2006). In a report by  
78 Normandeau *et al.* (2011) there was shown to  
79 be a great variation in electromagnetic field  
80 strength arising from different structures,  
81 cables and current values. In a recent report  
82 (Thomsen *et al.* 2015) higher EMF emission  
83 values were recorded for export cables  
84 compared to inter turbine cables. It was also  
85 noted in this report that EMF values recorded  
86 were considerably higher around the cables  
87 than around the wind turbine bases. An  
88 assessment of the literature (COWRIE 2003)  
89 highlights that the current state of knowledge  
90 on EMF strengths emitted by undersea power  
91 cables is insufficient to allow an informed  
92 assessment. The European edible crab, *Cancer*  
93 *pagurus* L., is found throughout Western

94 Europe from Norway to northern France. They  
 95 are commonly found from the shoreline to  
 96 offshore depths around 90m. They are a  
 97 heavily exploited commercial species with the  
 98 present UK and Ireland annual catch around  
 99 34,600 tonnes (Bannister 2009). There is a high  
 100 probability that this species will encounter sub-  
 101 sea power cables resulting in increased EMF  
 102 exposures, potentially leading to stress  
 103 responses. In crustaceans, haemolymph  
 104 analysis enables measurement of stress  
 105 through detection of abnormalities in internal  
 106 chemical processes. Previous studies (Taylor *et al.*  
 107 *1997*; Durand *et al.* 2000; Bergmann *et al.*  
 108 2001; Lorenzon *et al.* 2007) show that L-Lactate  
 109 and D-Glucose are useful measures of stress in  
 110 crustaceans, whilst respiration rates in marine  
 111 organisms are also reliable indicators of certain  
 112 environmental stressors (Paterson and  
 113 Spanoghe 1997; Doney *et al.* 2012; Brown *et al.*  
 114 2013). It is also known that behavioural and  
 115 response parameters (attraction/avoidance,  
 116 antennular flicking rate, and activity level) can  
 117 be affected by stress (Stoner 2012). The aim of  
 118 the present paper is to determine the effects  
 119 of EMFs on edible crabs using a combination of  
 120 the above stress indicators.

## 121 2. Methods and Materials

122 Intermoult crabs were obtained from local  
 123 fishermen and the St Abbs and Eyemouth  
 124 Voluntary Marine Reserve (St Abbs,  
 125 Berwickshire, UK) for each experiment. Crabs  
 126 were kept in 1000L flow through tanks with  
 127 ambient sea temperature and natural  
 128 photoperiod for a minimum acclimation

129 period of 1 week at densities of no more than  
 130 5 crabs per tank. Each crab was sexed,  
 131 carapace width measured (mm) and a  
 132 condition assigned using a condition index  
 133 (Table 1). Crabs were categorized into size  
 134 classes based on carapace width (10-79mm –  
 135 small, 80-120mm – medium, 121mm+ - large).

### 136 2.1. Physiological Analyses

#### 137 2.1.1. Haemolymph Analysis

138 During experimentation four 70L tanks were  
 139 set up with flow through seawater (UV  
 140 sterilised and filtered) which was temperature  
 141 controlled (TECO TK1000) to ambient  
 142 conditions. Temperature and light intensity  
 143 was constantly measured via data loggers  
 144 (Onset HOBO temperature/light pendant).  
 145 Within each tank a perforated plastic  
 146 enclosure enabled the crab to be held in  
 147 position over the magnets. The EMF was  
 148 produced by placing four electric solenoid  
 149 magnets (24V) connected to variable power  
 150 supplies (QW-MS305D) on ceramic tiles  
 151 underneath the tanks. The magnets were run  
 152 at full power, thus creating an electromagnetic  
 153 field (peak 40mT measured by an AlphaLab, Inc  
 154 Gaussmeter Model GM-2) which covered the  
 155 experimental area. The experiment was  
 156 repeated using a lower strength EMF (peak  
 157 2.8mT) to correspond with the expected,  
 158 although highly variable, levels on the surface  
 159 of a sub-sea power cable and correspond to  
 160 those in previous studies (Bochert and Zettler,  
 161 2006).

162 Haemolymph samples were collected, within  
 163 60 seconds, from the arthroal membrane at

Index	Description
1 – Perfect	Body intact with no damage, black spot or other visible defects.
2 – Good	One or two legs missing no carapace damage.
3 – Ok	More than two legs missing, limited carapace damage or slight blackspot.
4 – Poor	One or both claws missing, damaged carapace and widespread blackspot.
5 - Bad	Legs and claws missing, extensive carapace damage and/or blackspot.

**Table 1** Condition index for *Cancer pagurus*. All crabs used throughout these experiments were grade 1 or 2 (Adapted from Haig *et al.* 2015)

164 the base of the fifth walking leg using 1ml  
165 syringes with 25G needles. Samples of 250µl,  
166 300µl and 700µl were collected from the  
167 different size groups respectively.  
168 Haemolymph was transferred into 1.5ml  
169 cryogenic vials, with 50µl of haemolymph from  
170 each sample stored in a separate vial for  
171 Haemocyanin analysis. Samples were frozen in  
172 liquid Nitrogen and stored in a freezer (-25°C).  
173 To obtain baseline data, haemolymph was  
174 collected before exposure (0h) then again after  
175 4h, 8h and 24h. All haemolymph collection was  
176 staggered with 5 minutes between each  
177 sample to ensure time consistency throughout  
178 the experiment. For all experiments, sample  
179 times were consistent as follows: 0h (09:00),  
180 2h (11:00), 4h (13:00), 6h (15:00), 8h (17:00)  
181 and 24h (09:00).

182 Haemolymph was deproteinated using the  
183 procedure of Paterson and Spanoghe (1997).  
184 Samples were thawed, vortexed and mixed  
185 with an equal volume of chilled 0.6M  
186 perchloric acid (BDH 10175). Inactivated  
187 proteins were separated by centrifugation at  
188 25,000g for 10 minutes (Eppendorf 5417C,  
189 rotor 30 x 1.5-2ml). After neutralizing the  
190 supernatant with 3M potassium hydroxide  
191 (BDH 29628) the precipitated potassium  
192 perchlorate was separated by centrifuging at  
193 25,000g for a further 10 min. The supernatant  
194 was then frozen and stored at -25°C.

#### 195 2.1.1.1. D-Glucose

196 D-Glucose concentration was measured using a  
197 D-Glucose assay kit (Sigma GAGO20-1KT)  
198 according to the procedure in Barrento *et al.*  
199 (2010). Haemolymph samples were incubated  
200 for 30 min at 37°C with an equal part of the  
201 assay reagent. 300µl of 12N sulphuric acid  
202 (BDH) was added to stop the reaction and the  
203 solution added to a 96 well flat-bottomed  
204 microplate (Wheaton 712122). The plates  
205 were then analysed spectrophotometrically at  
206 540nm (Molecular Devices, Spectramax M5)  
207 and D-Glucose concentration calculated using a  
208 calibration curve of standards of known  
209 concentration.

#### 210 2.1.1.2. L-Lactate

211 L-Lactate concentration of deproteinated  
212 haemolymph samples were measured using L-  
213 Lactate reagent (Trinity Biotech, Wicklow,  
214 Ireland no. 735-10), per the procedure  
215 described by Barrento *et al.* (2010). Samples of  
216 haemolymph (2.8µl) were mixed with L-Lactate  
217 reagent (280µl), then incubated for 10 min at  
218 room temperature. These were then added  
219 into the wells of the 96-well flat-bottom  
220 microplate. The plate was then analysed  
221 spectrophotometrically at 540nm and L-  
222 Lactate concentration was calculated from a  
223 calibration curve using standards of known  
224 concentration (Trinity Biotech, Wicklow,  
225 Ireland L-Lactate standards set no. 735-11).

#### 226 2.1.1.3. Haemocyanin

227 Haemocyanin concentrations were  
228 determined spectrophotometrically. 50µl of  
229 haemolymph was diluted with 2ml chilled  
230 distilled water and 280µL added to the wells of  
231 the 96-well flat-bottom microplate and the  
232 absorbance at 335nm was measured twice.  
233 Haemocyanin concentration (mg/ml) was  
234 calculated from the molar extinction  
235 coefficient  $E_{1\text{cm}}^{\text{mM}} = 17.26$  as previously described  
236 by Harris and Andrews (2005).

#### 237 2.1.2. Respiration

238 Thirty juvenile ( $\leq 79\text{mm}$  carapace width)  
239 intermoult crabs were collected from the  
240 intertidal zone around St Abbs and placed into  
241 two 1000L tanks with seawater flow-through.  
242 Crabs with a carapace width of over 80mm  
243 were too large for the respiration chamber so  
244 were discarded. Inside a Helmholtz coil (2.8mT)  
245 a 46L flow through tank was set up as a water  
246 bath, with filtered, UV sterilised seawater  
247 connected to a sump tank and temperature  
248 control unit to ensure temperature stability. A  
249 0.3L respiration chamber was filled with UV  
250 sterilised filtered seawater and placed into the  
251 water bath. The fibre optic probe (Presens  
252 polymer optical fibre POF) was attached to the  
253 chamber. An optical oxygen meter (Presens  
254 Fibox 3) was used to measure oxygen levels  
255 using Presens PSt3 (detection limit 15ppb)

256 sensor spots. This meter was connected to a  
257 computer and a blank was run for a period of  
258 30 minutes. To eliminate bacterial respiration  
259 from water samples, a blank was run prior to  
260 each trial and the information obtained was  
261 considered when calculating oxygen  
262 consumption of the crabs. The system was  
263 calibrated using a conventional two-point  
264 oxygen-free and oxygen-saturated system.  
265 Oxygen-free water was obtained using Sodium  
266 sulphite ( $\text{Na}_2\text{SO}_3$ ) to remove any oxygen, whilst  
267 oxygen saturation was achieved through  
268 bubbling air vigorously into the water sample  
269 for a period of 20 minutes, stirring to ensure  
270 the water was not supersaturated.

271 Crabs were randomly selected, weighed and  
272 carapace width measured. The crabs were  
273 then placed into the respiration chamber and  
274 acclimated for 1 hour with the water flow-  
275 through valve open. After acclimation, the  
276 valve was closed and measurements taken  
277 until a limit of 60% air saturation was reached,  
278 or for a total of 30 minutes. 15 individuals were  
279 ran as control with the Helmholtz coil switched  
280 off and 15 were acclimated with no EMF  
281 present then subjected to an EMF for the  
282 duration of the experiment. The % air  
283 saturation was recorded for each individual  
284 and converted to oxygen consumption  
285 ( $\text{mg/g/h}$ ).

### 286 2.1.3. Helmholtz Coil

287 Two Helmholtz coils were set up with four 12L  
288 glass tanks each, situated in a recirculated  
289 temperature controlled water bath. Tank sides  
290 were covered with netting to reduce visual  
291 stimuli. Tanks were kept at  $10^\circ\text{C}$  and were  
292 constantly aerated with air stones. 10 large  
293 male and 10 large female crabs were randomly  
294 selected (carapace width  $121\text{mm}+$ ), weighed  
295 and carapace width recorded before being  
296 placed into the experimental tanks. After a 1  
297 hour acclimation period, baseline  
298 haemolymph samples were taken from each  
299 crab ( $800\mu\text{L}$ ) and one of the Helmholtz coils  
300 switched on, with the other acting as a control.  
301 Subsequent haemolymph samples were taken

302 at 2, 4 and 6 hours. Haemolymph was sampled  
303 using the previously mentioned protocol. After  
304 6 hours, the Helmholtz coil was switched off  
305 and the crabs were left overnight. 24 hours  
306 after the baseline haemolymph sample was  
307 taken another baseline sample was taken and  
308 the other Helmholtz coil was switched on and  
309 further samples taken at the same times as the  
310 previous day. This allowed all crabs to be  
311 sampled during exposure to EMF and control  
312 conditions and helped to eliminate individual  
313 variances by comparing an individual  
314 throughout both treatments. The EMF created  
315 by the Helmholtz coil was measured and  
316 mapped and gave a field strength of  $2.8\text{mT}$   
317 uniformly distributed throughout the  
318 experimental area. Three additional individual  
319 crabs were sampled over the two-day  
320 experiment with no exposure to EMF to  
321 account for any handling stress. No elevated  
322 stress levels were detected. The aims of these  
323 trials were to detect any changes in  
324 haemolymph parameters over a shorter period  
325 of time. Large crabs were utilised to allow  
326 larger volumes of haemolymph to be sampled  
327 over a short time frame.

## 328 2.2. Behavioural Analysis

### 329 2.2.1. Antennular Flicking Rate

330 A 12L glass tank was set up with a 40L sump  
331 tank containing UV sterilised filtered sea water  
332 that was temperature controlled (TECO  
333 TK1000) to  $12^\circ\text{C}$ . The experimental tank was  
334 placed on top of 4 solenoid electromagnets to  
335 create an EMF of  $2.8\text{mT}$ . The inflow and  
336 outflow were separated from the crab inside  
337 the tank by inserting a perforated plastic sheet  
338 to reduce visual disturbance. Experimental  
339 tanks were placed behind screens to avoid  
340 external stimuli. Crabs were acclimated to the  
341 experimental tanks for 30 minutes prior to  
342 testing after which the camera was set to  
343 record via a remote control. The crab was  
344 recorded for 10 minutes under control  
345 conditions, then a further 10 minutes with an  
346 EMF present. After each trial, the tanks were  
347 sterilised and underwent a full water change to  
348 reduce chemical cues which may affect

349 antennular flicking rates. The system was  
350 monitored for temperature, dissolved oxygen  
351 and salinity during all trials.

352 The video data was post-processed with  
353 flicking rate counted for both antennules by a  
354 minimum of 3 trained people per video file for  
355 accuracy. Trials where the crab was asleep or  
356 did not exhibit any antennular flicking were  
357 discarded.

### 358 2.2.2. Activity Level and Side Selection

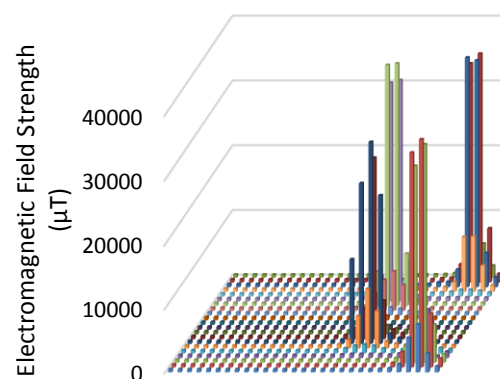
359 Four 70L experimental tanks were set up and  
360 connected to a 1000L temperature-controlled  
361 sump tank which received a constant supply of  
362 UV sterilised filtered sea water. The sides of  
363 the experimental tanks were shaded to reduce  
364 visual disturbances. A wide aperture mesh was  
365 placed over the top of the tanks to prevent the  
366 crabs from escaping. Water was pumped from  
367 the sump tank into the four experimental tanks  
368 at an equal rate for the duration of the  
369 experiment and the temperature was  
370 constantly monitored using data loggers  
371 (Onset HOBO). After each trial the tanks were  
372 drained, sterilised (Virkon aquatic) and refilled.

373 Four waterproof Infrared cameras were  
374 suspended above the experimental tanks and  
375 set to record during each trial. The trials  
376 consisted of:

- 377 1. Day conditions – (7 hours 30 minutes  
378 (08:30am-16:00pm)
- 379 2. Night conditions – (8 hours (20:00pm-  
380 04:00am)

381 The footage from each tank was post  
382 processed then analysed using Solomon Coder  
383 (version – beta 17.03.22). Each video file was  
384 broken down to still images at 1 minute  
385 intervals for the duration of the trial. The  
386 position of the crab in each image was  
387 analysed and a movement index was created  
388 by assigning a value of 0 to a picture where  
389 there was no movement, when compared to  
390 the previous picture, and a value of 1 where  
391 there was movement. The total movement  
392 index score was recorded for each tank  
393 throughout all the trials and used to indicate  
394 activity levels in the crabs. The individual

395 pictures were analysed to determine the  
396 percentage of time each crab spent on either  
397 side of the tank (magnet or non-magnet). This  
398 was used to indicate an attraction to or  
399 avoidance of the EMF. Trials where there was  
400 no movement for the entire duration or the  
401 crab did not experience both sides of the tank  
402 were omitted. This was deemed necessary as  
403 the individual would not be making a choice  
404 based on treatment preference. It was  
405 concluded during preliminary trials that the  
406 crabs spent a significant amount of time in the  
407 corners of the tanks (approx. 85%), thus  
408 influencing magnet placement.  
409 In the set-up the magnets were evenly spaced  
410 in the middle of one side of the tank in addition  
411 to the two magnets in the corners.  
412 Experiments were conducted under day and  
413 night conditions to fully assess the behaviour  
414 of this crepuscular species. In control  
415 conditions the magnets were present but not  
416 switched on. Magnet placement (left or right)  
417 was randomised to reduce any tank based or



**Fig. 1.** Electromagnetic field strength ( $\mu\text{T}$ ) over the tank floor (square inch) represented by the x axis. Quad magnet set-up with the corner magnets plus an additional two solenoid magnets placed just offset to create an EMF over half of the tank. Magnets were swapped randomly from the left to right sides of the tank during replication.



418 external stimuli that may affect results. The  
419 EMF was mapped for the setup using a 1sq.  
420 inch grid over the base of the tank with each  
421 square being measured by an AlphaLab, Inc  
422 Gaussmeter Model GM-2 (Fig. 1).

### 423 2.2.3. Shelter Selection

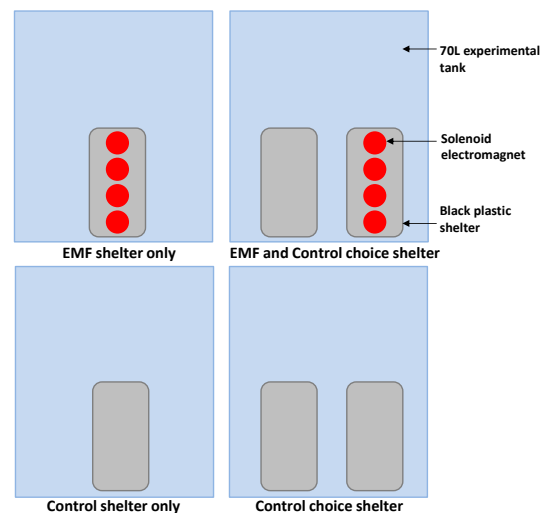
424 To further determine the effects of EMFs on  
425 crab behaviour and potential attraction, four  
426 70L experimental tanks were set up with  
427 temperature controlled (13°C), flow through  
428 UV sterilised seawater (Fig. 2.). Six black ABS  
429 plastic shelters (300mm x 200mm x 100mm)  
430 were constructed and secured to the bottom  
431 of the tanks. In two of the tanks two plastic  
432 shelters were set up, with four solenoid  
433 electromagnets placed under each shelter.  
434 During each trial one of the shelters'  
435 electromagnets would be turned on with the  
436 other remaining off as a control. In the two  
437 remaining tanks a single shelter was set up  
438 with four solenoid electromagnets under each,  
439 one tank having the magnets switched on and  
440 the other remaining off as a control.

441 All magnets were set so that an EMF of 2.8mT  
442 was present under the length of the shelter. An  
443 individual large crab (121+mm carapace width)  
444 was placed in to each tank, using an even split  
445 of male and females. Using the same infrared  
446 camera set-up previously described, the crabs  
447 were recorded from 23:00pm – 06:00am and  
448 the video files post-analysed (Solomon Coder)  
449 to determine the percentage of time spent in  
450 the shelters or free roaming within the tank.  
451 The primary purpose of setting up single  
452 shelter tanks was to determine how the crab  
453 would interact with the shelter under control  
454 conditions, and to determine how the crab  
455 would act if the only shelter available is  
456 subjected to EMF. The dual shelter tanks were  
457 set up to determine if there was an attraction  
458 to EMFs and to discover how crabs would split  
459 their time between seeking shelter and active  
460 roaming.

### 461 2.3 Statistics

462 Results were expressed as mean  $\pm$  standard  
463 error (SEM). When data met ANOVA

464 assumptions (per Shapiro-Wilk test for  
465 normality and Levene's test for equality of  
466 error variances) multiple-comparison tests  
467 (paired t-test, one-way ANOVA, 2-way ANOVA)  
468 were conducted to reveal differences between  
469 groups. If data could not meet ANOVA  
470 assumptions, non-parametrical analysis  
471 (Wilcoxon signed rank test, Mann-Whitney,  
472 Scheirer-Ray-Hare) was performed. Chi-square  
473 test (2 tailed) was utilised for choice  
474 experiments. Post-hoc analysis for  
475 parametrical data (Tukey's test) and non-  
476 parametrical (pairwise Mann-Whitney) were  
477 conducted. All statistics were tested at a  
478 probability of 0.05 (IBM SPSS Statistics v.23  
479 SPSS Corp. Chicago, USA).



**Fig. 2.** The four different shelter experimental tanks. Each tank had 4 solenoid electromagnets underneath each shelter. The shelter with the electromagnets turned on was randomised along with the position of each tank to remove experimental bias and potential external variable factors. (EMF = magnets on, Control = magnets off).

## 480 3. Results

### 481 3.1. Physiological Analysis

#### 482 3.1.1. Haemolymph parameters

483 Exposure to EMF had a significant effect on the  
 484 L-Lactate levels of *Cancer pagurus* (Table 2).  
 485 Throughout the 24-hour high strength (40mT)  
 486 exposure L-Lactate levels followed the same  
 487 circadian rhythm as the control group, with a  
 488 gradual decrease in concentration throughout  
 489 the day before a rise at night (Fig. 3). Despite  
 490 following the same patterns, the EMF exposed  
 491 values were significantly lower at 4h ( $p<0.05$ )  
 492 and 8h ( $p<0.05$ ) when compared to 0h. The  
 493 control group showed a decrease in  
 494 concentration throughout the day, however,  
 495 there were no significant differences between  
 496 the baseline sample and the remaining  
 497 samples taken over the 24 hours. Exposure to  
 498 low strength EMF (2.8mT) disrupted the  
 499 natural circadian rhythm of L-Lactate causing a  
 500 significant decrease throughout the 24-hour  
 501 trial. The typical rise and peak values normally  
 502 obtained at dawn were absent. The different  
 503 EMF strengths had a significant effect on L-  
 504 Lactate level ( $p<0.05$ ). After 4 hours of  
 505 exposure crabs exposed to the high strength  
 506 EMF had significantly lower concentrations of  
 507 L-Lactate compared to those in low strength  
 508 EMF.

509 D-Glucose levels showed a significant increase  
 510 between 0h and 4h, 0h and 8h in control crabs  
 511 ( $p<0.05$ ,  $p<0.05$ ) and in crabs exposed to 40mT  
 512 EMF ( $p<0.01$ ,  $p<0.05$ ) (Fig.4). Crabs exposed to  
 513 2.8mT EMF did not show the significant rise in  
 514 D-Glucose level after 8h of exposure.  
 515 Haemolymph D-Glucose levels of low and high  
 516 strength EMF exposed crabs followed very  
 517 similar daily patterns. Although D-Glucose  
 518 concentrations after 4h and 8h were lower in

519 exposed crabs compared to control, the  
 520 difference was not statistically significant.

521 Exposure had no effect on the remaining  
 522 haemolymph parameters. Hemocyanin levels  
 523 remained constant ( $44.08\pm 1.01\text{mg/ml}$ )  
 524 throughout the trials, with no significant  
 525 variation over time or by crab size.

526 To test whether EMF had any effect on the  
 527 measured haemolymph stress parameters  
 528 after exposure, half of the crabs used in the  
 529 Helmholtz trials were sampled the following  
 530 day at 0, 2, 4 and 6 hours. Exposure to EMF has  
 531 no lingering effects on the haemolymph stress  
 532 parameters. The increase in EMF strength from  
 533 2.8mT to 40mT had no effect on the D-Glucose  
 534 or Haemocyanin parameters, but showed an  
 535 overall decrease in mean L-Lactate  
 536 concentration throughout the sample group.  
 537 This change in concentration could potentially  
 538 be explained by high individual variability or  
 539 limits of the assay kit used.

### 540 3.1.2. Respiration

541 The mean respiration rate of juvenile crabs  
 542 exposed to EMF was  $0.05\pm 0.006\text{mg O}_2/\text{g/h}$ ,  
 543 this showed no difference to values obtained  
 544 from individuals under control conditions. EMF  
 545 exposure did not increase  $\text{O}_2$  demand and  
 546 appears to cause no oxidative stress.

## 547 3.2 Behavioural Analysis

### 548 3.2.1. Flicking rate

549 Exposure to EMF caused a slight increase in  
 550 antennular flicking rate in small and medium

	Helmholtz			
	L-Lactate		D-Glucose	
	EMF	Control	EMF	Control
0h	1.21±0.33	2.23±0.59	0.24±0.04	0.31±0.06
2h	1.35±0.25	1.81±0.45	0.47±0.04	0.46±0.06
4h	1.05±0.22	1.47±0.39	0.72±0.7	0.73±0.07
6h	1.03±0.23	1.22±0.47	0.81±0.08	0.71±0.08
N	20	20	20	20

**Table 2** L-Lactate and D-Glucose concentrations (mM) for the Helmholtz (2.8mT) trials (Mean ± SEM).

Figure 3.

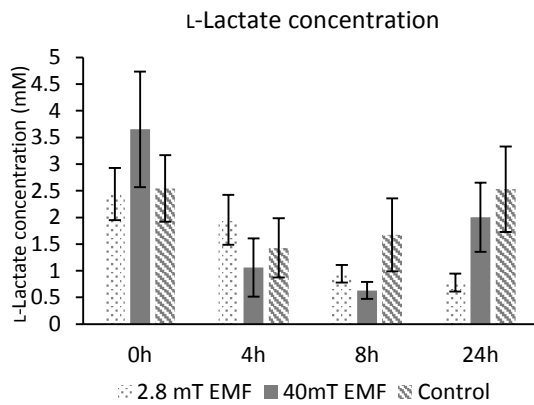
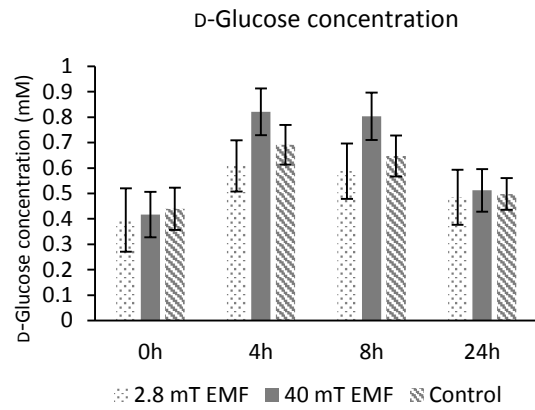


Figure 4.



**Figure 3., Figure 4.** L-Lactate (Fig. 3.) and D-Glucose concentrations (Fig. 4.) (Mean  $\pm$  SEM) over a 24-hour period during control conditions and exposure to low strength (2.8mT) and high strength (40mT) EMF. In the control over 24-hours both parameters show a natural circadian rhythm with an increase leading up to and during the night. The L-Lactate circadian rhythm was disrupted by exposure to 2.8mT EMF and did not follow the usual trend, showing a continuous decrease and significantly lower values after 24 hours. The L-Lactate circadian rhythm was altered during exposure to 40mT EMF resulting in much lower concentrations after 4h and 8h despite following the same trend found in the control results. D- Glucose levels followed similar circadian rhythm in control and 40 mT EMF exposed crabs, with significant increase towards peak locomotor activity, while 2.8 mT exposed crabs were lacking this increase and showed no significant elevation after 8 h.

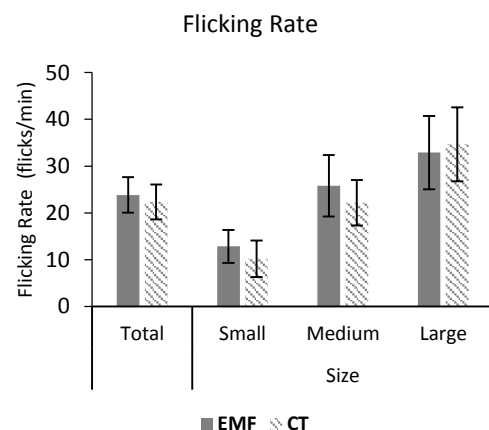
551 crabs although this was not statistically  
 552 significant (Fig. 5). The average pre-EMF  
 553 flicking rate of  $22 \pm 4$  flicks/min remained  
 554 unchanged during exposure to EMF ( $24 \pm 4$   
 555 flicks/min). The mean flicking rate in the first  
 556 minute of exposure to EMF ( $25 \pm 4$  flicks/min)  
 557 remained unchanged from initial  
 558 measurements in control conditions ( $25 \pm 4$   
 559 flicks/min).

### 560 3.2.2. Activity level

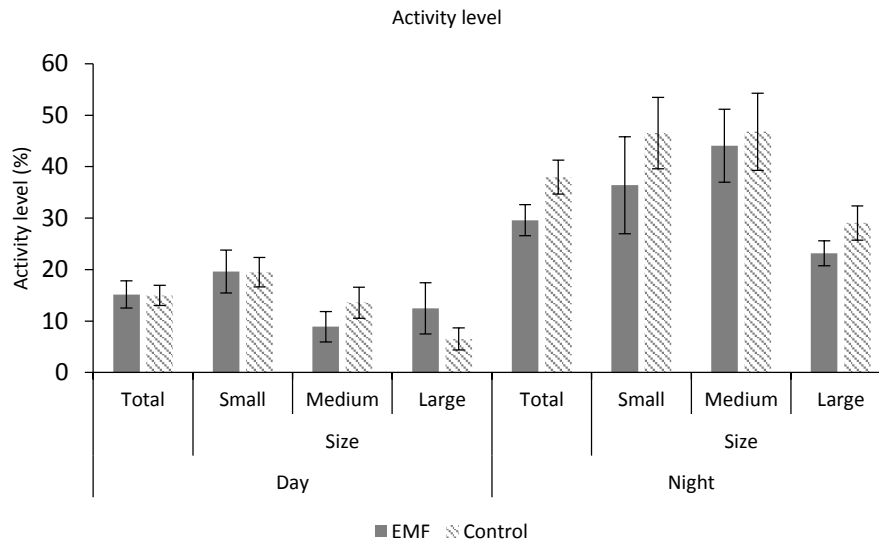
561 During day conditions, there was no significant  
 562 difference in activity levels between EMF  
 563 exposed crabs and control, with size being the  
 564 only significant factor (Fig. 6). The decrease in  
 565 activity level corresponds with an increase in  
 566 crab size, with small crabs ( $19.6 \pm 2.5\%$ ) having  
 567 higher activity levels than large crabs  
 568 ( $10.1 \pm 3.2\%$ ). During night conditions, there  
 569 was a significant increase in activity levels for  
 570 all size groups. Small ( $42.7 \pm 5.6\%$ ) and medium  
 571 crabs ( $45.5 \pm 5.1\%$ ) had significantly higher  
 572 activity levels than large crabs ( $25.9 \pm 2\%$ ).

### 573 3.2.3. Side selection

574 Under control conditions crabs spent  
 575 significantly more time on one side of the tank  
 576 in both day (32-68%) and night (36-64%)  
 577 conditions (N=96). When there was an EMF  
 578 present there were no clear preferences made



**Fig. 5.** Antennular flicking rate (Mean  $\pm$  SEM) of individuals exposed to EMF and control conditions for the three size groups (N=30) and combined.



**Fig. 6.** Activity level (Mean ± SEM) of the different size groups in both treatments for day and night conditions. ( $N_{DAY}=79$ ,  $N_{NIGHT}=117$ ).

579 between sides during both day (44-56%) and  
 580 night (50-50%) conditions ( $N=99$ ). There was a  
 581 significant difference between control and  
 582 EMF exposed crabs' mean side selection for  
 583 both day ( $p<0.05$ ,  $N=77$ ) and night ( $p<0.05$ ,  
 584  $N=118$ ). This shows that in the presence of an  
 585 EMF individual crabs fail to make a clear side  
 586 preference.

### 587 3.2.4. Shelter selection

#### 588 3.2.4.1. Single shelter

589 The mean time spent in the shelter (48%) and  
 590 out (52%) was approximately equal in the  
 591 control trials (Fig. 7B). When there was an EMF  
 592 present in the shelter the proportion of mean  
 593 time spent within the shelter increased to 69%  
 594 (Fig. 7A). These trials also confirmed what was  
 595 discovered in the dual shelter set ups in that  
 596 the percentage time spent roaming the tank  
 597 significantly decreases from 52% in the control  
 598 to 31% when there was an EMF present. The  
 599 overall mean percentage time spent in both  
 600 locations was significantly different between  
 601 control and EMF conditions ( $p<0.001$ ).

#### 602 3.2.4.2. Dual shelter

603 Under control conditions the mean time spent  
 604 in each shelter and out roaming in the tank

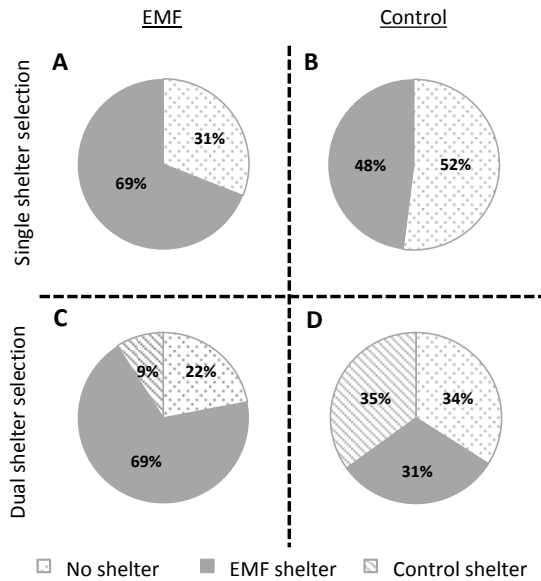
605 were equal (35% EMF shelter, 31% CT shelter  
 606 and 34% No shelter) (Fig. 7D). During EMF  
 607 exposure, there were clear preferences for the  
 608 shelter with the EMF present resulting in 69%  
 609 mean time spent within, with only 9% spent in  
 610 the control shelter and 22% spent roaming the  
 611 tank (Fig. 7C). There was a drop in mean time  
 612 from 34% under control conditions to 22%  
 613 during EMF exposure suggests that once the  
 614 crabs detect EMFs they will begin to seek  
 615 shelter and are drawn to the shelter with the  
 616 EMF emanating from within. The overall mean  
 617 percentage time spent in all three locations  
 618 was significantly different between control and  
 619 EMF conditions ( $p<0.001$ ). Throughout all  
 620 shelter trials an equal number of male and  
 621 female crabs were used. There were no  
 622 significant differences in behaviour between  
 623 the sexes.

## 624 4. Discussion

### 625 4.1. Physiological Analysis

#### 626 4.1.1. Haemolymph parameters

627 L-Lactate and D-Glucose both followed a  
 628 natural circadian rhythm with a rise in D-  
 629 Glucose, and a subsequent fall in L-Lactate  
 630 concentrations throughout the day. L-Lactate



**Fig. 7. Effect of EMF on shelter selection.** Time spent in single shelter and outside of the shelter, if exposed to EMF (A) and when the magnets are switched off (B). EMF (N=11) and control (N=11). Time spent in each shelter and outside the shelters, when one of the shelter is exposed to EMF (C) and if none are exposed (D). EMF (N=15) and control (N=15). Shown as a percentage of total trial time (%).

631 levels rise throughout the night due to  
 632 increased activity and subsequent increase in  
 633 glucose metabolism. In crustaceans,  
 634 haemolymph glucose and lactate levels are  
 635 affected by various environmental conditions  
 636 and stressors (Kallen *et al.* 1990, Reddy *et al.*  
 637 1996, Chang *et al.* 1998,) and should cycle  
 638 together under normal, unstressed conditions.  
 639 L-Lactate is an indicator of anaerobic  
 640 respiration, typically due to impaired gill  
 641 function or hypoxic conditions (Durand *et al.*  
 642 2000). D-Glucose, the primary fuel for ATP  
 643 production in crustaceans is crucial in  
 644 maintaining metabolic processes (Barrento *et al.*  
 645 2010). D-Glucose levels show a negative  
 646 correlation with vigour where healthy  
 647 individuals have lower levels and moribund  
 648 crabs become hyperglycaemic (Barrento *et al.*  
 649 2010). Activity levels in crabs should partially  
 650 be reflected in D-Glucose concentrations  
 651 (Briffa and Elwood 2001). D-Glucose levels  
 652 were found to correlate well with current  
 653 literature in that there was a continual rise in  
 654 concentration in relation to locomotor activity  
 655 (Hamann 1974; Reddy *et al.* 1981; Kallen *et al.*  
 656 1988; Kallen *et al.* 1990; Tilden *et al.* 2001).  
 657 This suggests that D-Glucose levels continually  
 658 rise throughout the night until peak locomotor  
 659 activity has been reached during which the

660 levels will begin to decrease back to original  
 661 values. EMF exposure did not significantly  
 662 influence activity level which is consistent with  
 663 minor changes in D-Glucose levels. Several  
 664 studies have shown that EMF can alter the  
 665 circadian rhythm of animals through altering  
 666 melatonin levels (Reiter 1993; Schneider *et al.*  
 667 1994; Levine *et al.* 1995; Wood *et al.* 1998).  
 668 Melatonin, a neuropeptide, present in  
 669 crustaceans, can cause shifts in L-Lactate and D-  
 670 Glucose cycles (Tilden *et al.* 2001). This  
 671 suggests that exposure to a field of 2.8mT  
 672 could affect melatonin secretion, which  
 673 consequently alters L-Lactate and D-Glucose  
 674 circadian rhythms. At 2.8mT, the L-Lactate  
 675 concentration follows the circadian rhythm  
 676 and decreases throughout the day when  
 677 activity levels are generally lower. During the  
 678 night there were no differences in activity  
 679 levels between 2.8mT and control crabs,  
 680 however there were no increases in L-Lactate  
 681 levels.

682 The suppression of the rise in L-Lactate  
 683 prevents the increase in O<sub>2</sub> affinity of  
 684 Haemocyanin that would naturally occur  
 685 (Sanders and Childress 1992). An increase in L-  
 686 Lactate has been shown to occur in *Carcinus*  
 687 *maenas* during emersion when the crabs  
 688 would be relying on anaerobic respiration.

689 During re-immersion L-Lactate levels remained  
690 elevated after 1 hour suggesting that the crabs  
691 have to repay an oxygen debt (Simonik and  
692 Henry 2014). Exposure to EMF suppresses the  
693 rise in L-Lactate which enables the crabs to  
694 repay the oxygen debt accrued during periods  
695 of higher activity. During long exposures to  
696 EMF, crabs may be unable to repay this oxygen  
697 debt, potentially leading to increased  
698 mortality. Both D-Glucose and L-Lactate  
699 concentrations show high individual variability  
700 with D-Glucose levels influenced by individual  
701 status and reactions to external stimulus  
702 (Matsumasa and Murai 2005). The values  
703 observed for L-Lactate and D-Glucose  
704 corresponded with those found in previous  
705 literature (Watt *et al.* 1999; Lorenzon *et al.*  
706 2008; Barrento *et al.* 2010; Barrento *et al.*  
707 2011). Haemocyanin, as the primary oxygen  
708 carrying protein in invertebrates, has been  
709 shown to increase in concentration during  
710 periods of hypoxia (Hagerman *et al.* 1990). The  
711 lack of deviation in concentrations observed  
712 suggests that EMF exposure does not elicit  
713 similar physiological responses as hypoxic  
714 conditions. The overall lack of change on these  
715 parameters suggests this species can maintain  
716 homeostasis during exposure to high strength  
717 EMFs.

#### 718 4.1.2. Respiration

719 Although increased oxygen demand and high  
720 gill ventilation rates typically occur in  
721 crustaceans subjected to different stressors  
722 (Jouve-Duhamel and Truchot 1985; Paterson  
723 and Spanoghe 1997), EMF (2.8mT) did not  
724 significantly alter the respiration rate of  
725 juvenile crabs. Respiration rates in *Cancer*  
726 *pagurus* are highly variable due to the  
727 alternating periods of apnoea and bradycardia  
728 that have been observed in pausing behaviour  
729 (Bottoms, 1977; Burnett and Bridges, 1981).  
730 This pausing behaviour will alternate but can  
731 be present for significant periods of time. This  
732 was concluded by Burnett and Bridges (1981)

733 when individuals were found to be exhibiting  
734 pausing behaviour for 40-50% of the time.  
735 These results show that juvenile *Cancer*  
736 *pagurus* respiration levels correlate well with  
737 other species of crabs of similar size: velvet  
738 swimming crab, *Necora puber*, (0.21 ±0.119 mg  
739 O<sub>2</sub>/g/h (Small *et al.* 2010)); spider crab, *Hyas*  
740 *araneus*, (0.025 mg O<sub>2</sub>/g/h (Camus *et al.*  
741 2002)); Dungeness crab, *Cancer magister*,  
742 (0.044 mg O<sub>2</sub>/g/h (Johansen *et al.* 1970)) and  
743 shore crab, *Carcinus maenas*, (0.036 - 0.126 mg  
744 O<sub>2</sub>/g/h (Newell *et al.* 1972; Taylor and Wheatly  
745 1979). Current respiration values for adult  
746 *Cancer pagurus* found in the literature are  
747 28.03mg O<sub>2</sub>/g/h during pre-pause and 4.42 mg  
748 O<sub>2</sub>/g/h post pause (Bradford and Taylor 1982).

#### 749 4.2. Behavioural Analysis

##### 750 4.2.1. Flicking rate

751 The lack of deviation in the number of  
752 antennular flicks during initial exposure and  
753 throughout the trials suggest that the  
754 antennules may not be utilized in the detection  
755 of EMF in this species, or as a reliable indicator  
756 of detection. Similar results were reported by  
757 Woodruff *et al.* (2013) after exposing  
758 Dungeness crab, *Metacarcinus magister*, to a  
759 3mT EMF.

##### 760 4.2.2. Activity level

761 Exposure to EMF did not have any effect on the  
762 overall activity level in *Cancer pagurus*. This  
763 suggests that if there is a behavioural change  
764 during exposure to EMF it may be more subtle  
765 than basic movement levels. The side selection  
766 results confirm that there is a decreased ability  
767 to find a suitable resting spot, however the  
768 crabs did not have higher activity levels within  
769 the EMF treatment. Under control conditions  
770 the crabs alternated their time between  
771 resting and roaming, subsequently spending  
772 larger amounts of time resting in the same  
773 spot. EMF exposure did not affect the resting  
774 and roaming behaviour but appeared to inhibit

775 the crabs from spending large amounts of time  
776 in the same location. Overall activity levels  
777 were not affected by EMF exposure, but the  
778 distribution of time spent in specific locations  
779 (see 4.2.3.) within the tank and between  
780 resting and roaming behaviours were. The low  
781 activity levels during the day could be a result  
782 of behaviour consisting largely of shelter  
783 seeking (Chapman and Rice 1971; Hockett and  
784 Kritzler 1972; Hazlett and Rittschof 1975; Hill  
785 1976). The discrepancies between size groups  
786 could be explained by smaller crabs typically  
787 inhabiting the sub-littoral zone where there  
788 will be higher risks of predation and higher  
789 competition for food and shelter, whereas  
790 larger crabs which tend to be found in deeper  
791 waters may not be subjected to the same  
792 pressures as the juveniles given their larger  
793 size (Paine 1976). The increase in activity levels  
794 during the night corresponds with this species'  
795 nocturnal behaviour and will be due to  
796 foraging or potential mate seeking (Seed 1969;  
797 Skajaa *et al.* 1998). The increase in antennular  
798 flicking rate of larger crabs combined with the  
799 decreased activity levels suggest that adult  
800 crabs rely more on chemical sensing than  
801 physical exploration to survey the  
802 environment.

#### 803 4.2.3. Side selection

804 Exposure to EMF does not affect the activity  
805 levels of the crabs but affects their ability to  
806 select a site to rest. This may be explained by  
807 crabs seeking shelter (see 4.2.4 below) when  
808 they detect EMF as opposed to natural  
809 movement patterns (Skajaa *et al.* 1998)  
810 observed in those within the control group.  
811 The crabs under control conditions spent a  
812 higher percentage of their time on one side of  
813 the tank interspersed with active roaming.  
814 EMF exposure inhibited a clear side preference  
815 within the tank which resulted in an  
816 approximately 50-50% split across the tank,  
817 potentially reflecting shelter seeking  
818 behaviour. *Cancer pagurus* has been shown to

819 inhabit pits when inactive (Hall *et al.* 1991) and  
820 were observed spending large amounts of time  
821 resting during the day in acclimation tanks with  
822 minimal movement. This behaviour appears to  
823 have been altered by exposure to EMF.

#### 824 4.2.4. Shelter selection

825 During the single shelter trials when crabs  
826 were exposed to control conditions there was  
827 an equal amount of time spent inside and  
828 outside the shelters. The same pattern was  
829 recorded during the dual shelter trials, with an  
830 equal amount of time spent in each of the  
831 shelters and roaming the tank. This suggests  
832 that when there are no environmental  
833 stressors present the crabs will spend a portion  
834 of their time resting in shelter and an equal  
835 portion of their time surveying their  
836 environment exhibiting roaming behaviour.  
837 When there was an EMF present the amount  
838 of time spent exhibiting roaming behaviour  
839 significantly decreased in both single and dual  
840 shelter trials. This has clear implications on the  
841 *Cancer pagurus* population in the areas  
842 surrounding MREDs. If there is an EMF present  
843 then crabs will be drawn to the source of the  
844 emission and spend significant amounts of  
845 time within the affected area. This will come at  
846 the cost of time spent foraging for food,  
847 seeking mates and finding shelter, potentially  
848 leading to higher predation rates, increased  
849 death due to starvation and/or decreased  
850 number of successful matings. Many offshore  
851 sites have introduced no-take zones around  
852 turbine arrays, with speculation that the  
853 decrease in fishing pressure, combined with  
854 the addition of artificial reefs in the form of  
855 scour protection blocks, could enhance the  
856 overall crustacean population (Langhamer and  
857 Wilhelmsson 2009) by providing refuge and  
858 breeding areas. However, this experiment  
859 highlights the potential lack of spill-over effect  
860 from these areas due to a high attraction to the  
861 emitted EMF. This suggests that fishing zones  
862 in close proximity to subsea power cables

863 could potentially see an overall decrease in  
864 crab numbers. Scour protection zones are  
865 estimated to create 2.5 times more habitat  
866 than is lost by array installation (Linley *et al.*  
867 2009) and with the inclusion of drilled holes  
868 have an estimated carrying capacity of  
869 65,000kg of fish per year per turbine (Linley *et*  
870 *al.* 2009). If specific habitat requirements are  
871 considered for individual target species around  
872 MREDS during the construction of these  
873 artificial habitats, then abundance and  
874 diversity of associated species, including  
875 commercially important species, could be  
876 enhanced (Bortone *et al.* 1994; Kawasaki *et al.*  
877 2003) subject to EMF emission mitigation.

## 878 5. Conclusion

879 Several decapod crustaceans are known to be  
880 magneto sensitive, yet information available  
881 on the effects of electromagnetic fields  
882 emitted from MREDS is scarce. The aim of this  
883 study was to fill some of these knowledge gaps.  
884 Exposure to electromagnetic fields, of the  
885 strength predicted around sub-sea cables, had  
886 significant physiological effects on *Cancer*  
887 *pagurus* and changed their behaviour. EMF  
888 disrupted the circadian rhythm of  
889 haemolymph L-Lactate and D-Glucose levels.  
890 Melatonin levels in several species have been  
891 found to be affected by EMF exposure. This  
892 suggests that EMF exposure could affect  
893 crustaceans on a hormonal level. Further  
894 studies are needed to understand the  
895 underlying mechanism responsible for  
896 disrupted glucose and lactate cycles.

897 In this study it was shown that EMF exposure  
898 altered behaviour, with crabs spending less  
899 time roaming around the tank and more time  
900 in a shelter in direct contact with the EMF. This  
901 suggests that the natural roaming behaviour,  
902 where individuals will actively seek food

903 and/or mates has been overridden by an  
904 attraction to the source of the EMF. When  
905 given the choice between a shelter exposed to  
906 EMF and one without exposure, the crabs were  
907 always drawn to the EMF. These results predict  
908 that in benthic areas surrounding MREDS,  
909 where there is increased EMFs, there will be an  
910 increase in the abundance of *Cancer pagurus*  
911 present. This potential aggregation of crabs  
912 around benthic cables and the subsequent  
913 physiological changes in L-Lactate and D-  
914 Glucose levels, brought about by EMF  
915 exposure, is a cause for concern.

916 Berried female Edible crabs move offshore and  
917 spend 6-9 months, buried with minimal  
918 movement and lower feeding rates  
919 (Williamson, 1900; Edwards, 1979; Howard,  
920 1982; Naylor *et al.*, 1997). Given this species'  
921 proven attraction to EMF sources, incubation  
922 of the eggs may take place around areas with  
923 increased EMF emissions. Long term studies  
924 are needed to investigate the effects of chronic  
925 EMF exposure. The effects of EMF on egg  
926 development, hatching success and larval  
927 fitness are unknown and need to be addressed.  
928 As larval stages are critical population  
929 bottlenecks, any negative effect of EMF on  
930 crab larvae will have a drastic effect on the  
931 edible crab fishery.

932 With the recent large scale renewable energy  
933 developments, it is clear more research is  
934 needed to reduce uncertainty of the  
935 environmental effects of these activities on  
936 benthic marine species, particularly on other  
937 commercially and ecologically important  
938 decapod crustaceans. This is important to  
939 develop an understanding of population level  
940 consequences and cumulative impacts of  
941 MREDS' stressors. These knowledge gaps need  
942 to be addressed before the implementation of  
943 the many approved wind farm sites around the  
944 UK to help mitigate an ever growing problem.



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