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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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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 (Na_2SO_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 (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°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 2.8mT
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°C . The experimental tank was
334 placed on top of 4 solenoid electromagnets to
335 create an EMF of 2.8mT . 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

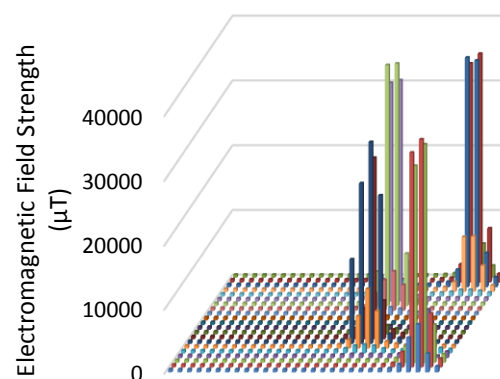


Fig. 1. Electromagnetic field strength (μT) over the tank floor (square inch) represented by the x axis. Quad magnet set-up with the corner magnets plus an additional two solenoid magnets placed just offset to create an EMF over half of the tank. Magnets were swapped randomly from the left to right sides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

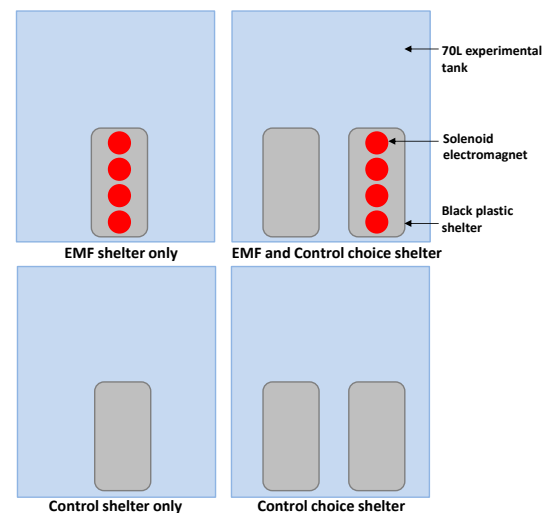


Fig. 2. The four different shelter experimental tanks. Each tank had 4 solenoid electromagnets underneath each shelter. The shelter with the electromagnets turned on was randomised along with the position of each tank to remove experimental bias and potential external variable factors. (EMF = magnets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

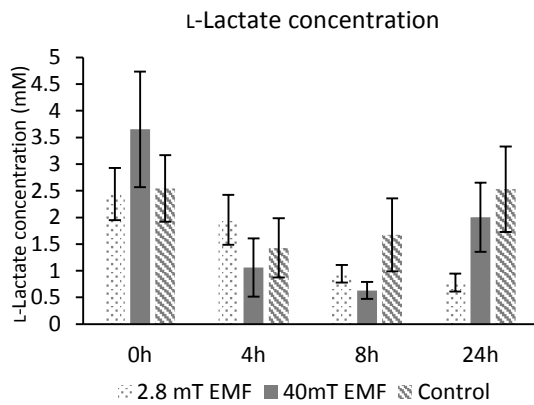


Figure 4.

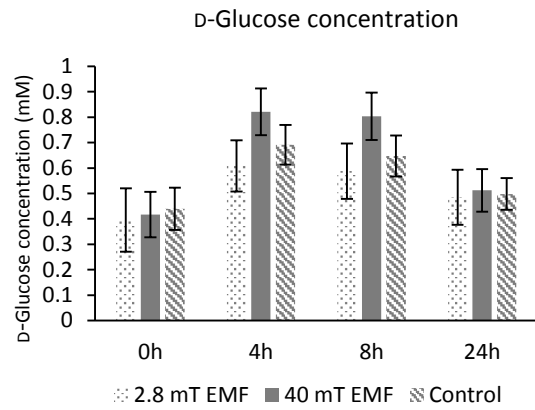


Figure 3., Figure 4. L-Lactate (Fig. 3.) and D-Glucose concentrations (Fig. 4.) (Mean \pm SEM) over a 24-hour period during control conditions and exposure to low strength (2.8mT) and high strength (40mT) EMF. In the control over 24-hours both parameters show a natural circadian rhythm with an increase 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 ± 4 flicks/min remained
 554 unchanged during exposure to EMF (24 ± 4
 555 flicks/min). The mean flicking rate in the first
 556 minute of exposure to EMF (25 ± 4 flicks/min)
 557 remained unchanged from initial
 558 measurements in control conditions (25 ± 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

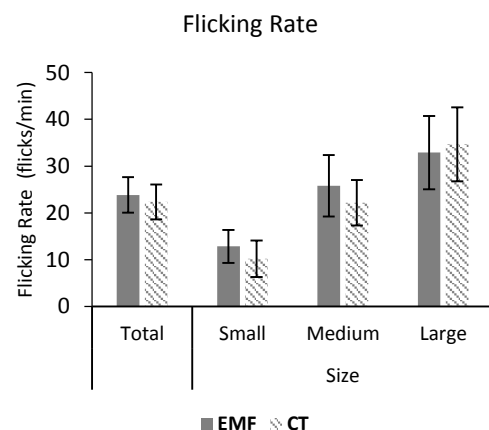


Fig. 5. Antennular flicking rate (Mean \pm SEM) of individuals exposed to EMF and control conditions for the three size groups (N=30) and combined.

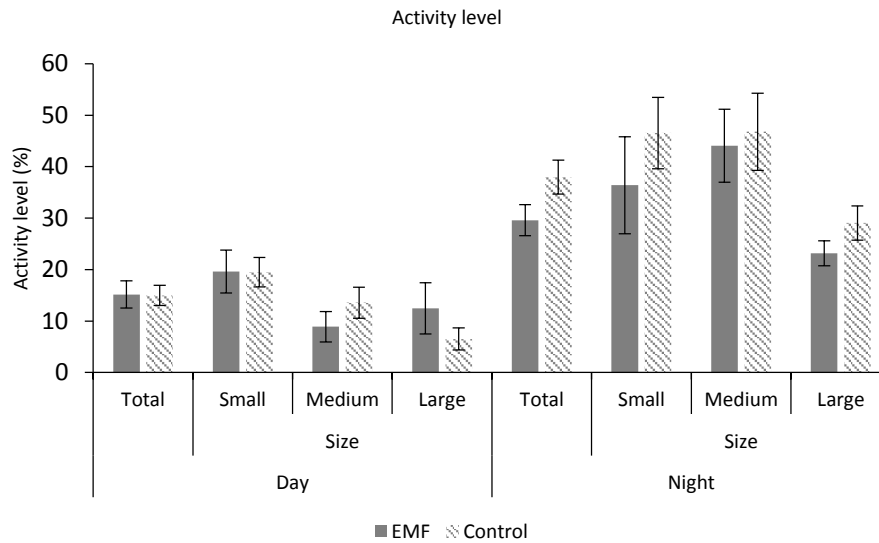


Fig. 6. Activity level (Mean ± SEM) of the different size groups in both treatments for day and night conditions. ($N_{\text{DAY}}=79$, $N_{\text{NIGHT}}=117$).

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

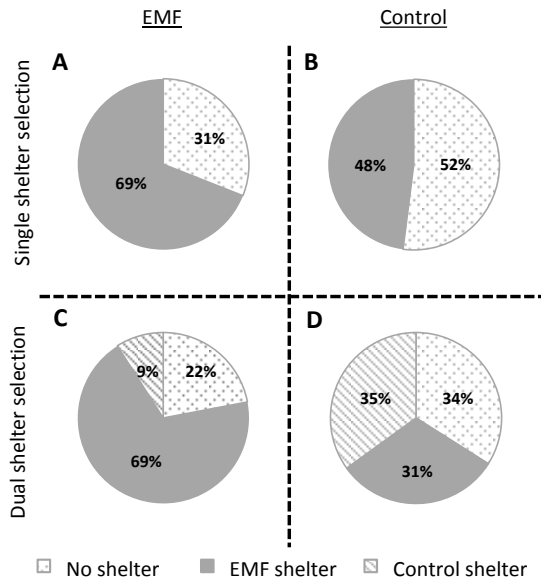


Fig. 7. Effect of EMF on shelter selection. Time spent in single shelter and outside of the shelter, if exposed to EMF (A) and when the magnets are switched off (B). EMF (N=11) and control (N=11). Time spent in each shelter and outside the shelters, when one of the shelter is exposed to EMF (C) and if 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 O₂ 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 O₂/g/h (Small *et al.* 2010)); spider crab, *Hyas*
740 *araneus*, (0.025 mg O₂/g/h (Camus *et al.*
741 2002)); Dungeness crab, *Cancer magister*,
742 (0.044 mg O₂/g/h (Johansen *et al.* 1970)) and
743 shore crab, *Carcinus maenas*, (0.036 - 0.126 mg
744 O₂/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 O₂/g/h during pre-pause and 4.42 mg
748 O₂/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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