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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-h periods. 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. Introduction

The predicted decline in non-renewable energy sources in future decades (Pimentel et al., 2002) indicates the need for alternative renewable energy sources. Due to reduced planning constraints, lack of inexpensive land near major population centres (Bilgili et al., 2011), and perceived aesthetic problems with many renewable energy structures (Gill, 2005), there is increasing pressure to move potential locations offshore. Wind speeds tend to be significantly higher offshore than onshore thus producing larger amounts of energy per turbine (Bilgili et al., 2011). Vast open spaces offshore also help avoid wake effects (shading effect of a turbine on those downwind of it) by allowing turbines to be placed at greater distances apart (Chowdhury et al., 2012). As the global energy demand grows, inshore areas are increasingly being utilised by the energy sector looking to increase energy production via wave and tidal energy devices (Frid et al., 2012). Therefore, there is a requirement for appropriate assessment of the implications of both offshore and inshore renewable energy generation with regards to current ecological status and potential future consequences (Gill, 2005). Currently, the UK is the largest global producer of electricity from offshore wind farms and has more projects in planning or construction than any other country (Smith et al., 1999; The Crown Estates, 2016). Proposed sites and developments are based on current knowledge and assessments of the local environment, despite relatively little being known about the ecological effects of such developments on marine benthic organisms. Some studies suggest that turbine arrays could increase biodiversity through new habitat provision (Landers Jr et al., 2001; Lindeboom et al., 2011), whereas detrimental effects of turbine arrays on birds (Garthe and Hüppop, 2004) and fish (Westerberg and Lagenfelt, 2008) have also been found. Furthermore, it is feared that marine mammals might be sensitive to minor changes in magnetic fields associated with these developments (Walker et al., 2003). There is currently a gap in our knowledge of the effects of these arrays on crustaceans.

Electromagnetic fields (EMF) are associated with Marine Renewable Energy Devices (MREDs). EMFs originate from both anthropogenic (telecommunication cables, power cables, marine renewable energy devices) and natural (Earth's natural geomagnetic field) sources. It has been shown that industry-standard AC cables can be effectively insulated to prevent electric field (*E*-field) emissions but not magnetic field (B-field) emissions (Gill, 2005). Standard cable configurations combined with the existing B-field creates induced electromagnetic fields (iEM fields) (Gill, 2005). The magnetic field (B-field) leakage has been shown to be of concern as it will interact with magnetite-based

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internal compasses in marine organisms (Öhman et al., 2007). Electric currents between 850 and 1600 Amperes (A) tend to be found in undersea cables consequently producing an electromagnetic field of around 3.20 millitesla (mT) (1,600 A) in a perfect wire (Bochert and Zettler, 2006). As with all electromagnetic fields this quickly diminishes away from the source, with values of around 0.32 mT and 0.11 mT at 1 m and 4 m respectively (Bochert and Zettler, 2006). In a report by Normandeau et al. (2011) there was shown to be a great variation in electromagnetic field strength arising from different structures, cables and current values. In a recent report (Thomsen et al., 2015) higher EMF emission values were recorded for export cables compared to inter turbine cables. It was also noted in this report that EMF values recorded were considerably higher around the cables than around the wind turbine bases. An assessment of the literature (CMACS, 2003) highlights that the current state of knowledge on EMF strengths emitted by undersea power cables is insufficient to allow an informed assessment. The European edible crab, *Cancer pagurus* L., is found throughout Western Europe from Norway to northern France. They are commonly found from the shoreline to offshore depths around 90 m. They are a heavily exploited commercial species with the present UK and Ireland annual catch around 34,600 t (Bannister, 2009). There is a high probability that this species will encounter sub-sea power cables resulting in increased EMF exposures, potentially leading to stress responses. In crustaceans, haemolymph analysis enables measurement of stress through detection of abnormalities in internal chemical processes. Previous studies (Taylor et al., 1997; Durand et al., 2000; Bergmann et al., 2001; Lorenzon et al., 2007) show that L-Lactate and D-Glucose are useful measures of stress in crustaceans, whilst respiration rates in marine organisms are also reliable indicators of certain environmental stressors (Paterson and Spanoghe, 1997; Doney et al., 2012; Brown et al., 2013). It is also known that behavioural and response parameters (attraction/avoidance, antennular flicking rate, and activity level) can be affected by stress (Stoner, 2012). The aim of the present paper is to determine the effects of EMFs on edible crabs using a combination of the above stress indicators.

2. Methods and materials

Intermoult crabs were obtained from local fishermen and the St Abbs and Eyemouth Voluntary Marine Reserve (St Abbs, Berwickshire, UK) for each experiment. Crabs were kept in 1000 L flow through tanks with ambient sea temperature and natural photoperiod for a minimum acclimation period of 1 week at densities of no > 5 crabs per tank. Each crab was sexed, carapace width measured (mm) and a condition assigned using a condition index (Table 1). Crabs were categorized into size classes based on carapace width (10–79 mm – small, 80–120 mm – medium, 121 mm + - large).

2.1. Physiological analyses

2.1.1. Haemolymph analysis

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

Haemolymph samples were collected, within 60 s, from the arthrodial membrane at the base of the fifth walking leg using 1 ml syringes with 25 G needles. Samples of 250 µl, 300 µl and 700 µl were collected from the different size groups respectively. Haemolymph was transferred into 1.5 ml cryogenic vials, with 50 µl of haemolymph from each sample stored in a separate vial for Haemocyanin analysis. Samples were frozen in liquid Nitrogen and stored in a freezer (−25 °C). To obtain baseline data, haemolymph was collected before exposure (0 h) then again after 4 h, 8 h and 24 h. All haemolymph collection was staggered with 5 min between each sample to ensure time consistency throughout the experiment. For all experiments, sample times were as follows: 0 h (09:00), 2 h (11:00), 4 h (13:00), 6 h (15:00), 8 h (17:00) and 24 h (09:00).

Haemolymph was deproteinated using the procedure of Paterson and Spanoghe (1997). Samples were thawed, vortexed and mixed with an equal volume of chilled 0.6 M perchloric acid (BDH 10175). Inactivated proteins were separated by centrifugation at 25,000*g* for 10 min (Eppendorf 5417C, rotor 30 × 1.5–2 ml). After neutralizing the supernatant with 3 M potassium hydroxide (BDH 29628) the precipitated potassium perchlorate was separated by centrifuging at 25,000*g* for a further 10 min. The supernatant was then frozen and stored at −25 °C.

2.1.1.1. D-Glucose. D-Glucose concentration was measured using a D-Glucose assay kit (Sigma GAGO20-1KT) according to the procedure in Barrento et al. (2010). Haemolymph samples were incubated for 30 min at 37 °C with an equal part of the assay reagent. 300 µl of 12 N sulphuric acid (BDH) was added to stop the reaction and the solution added to a 96 well flat-bottomed microplate (Wheaton 712,122). The plates were then analysed spectrophotometrically at 540 nm (Molecular Devices, Spectramax M5) and D-Glucose concentration calculated using a calibration curve of standards with a known concentration.

2.1.1.2. L-Lactate. L-Lactate concentration of deproteinated haemolymph samples were measured using L-Lactate reagent (Trinity Biotech, Wicklow, Ireland no. 735–10), per the procedure described by Barrento et al. (2010). Samples of haemolymph (2.8 µl) were mixed with L-Lactate reagent (280 µl), then incubated for 10 min at room temperature. These were then added into the wells of a 96-well flat-bottom microplate. The plate was then analysed spectrophotometrically at 540 nm and L-Lactate concentration was calculated from a calibration curve using standards of known concentration (Trinity Biotech, Wicklow, Ireland L-Lactate standards set no. 735–11).

2.1.1.3. Haemocyanin. Haemocyanin concentrations were determined spectrophotometrically. 50 µl of haemolymph was diluted with 2 ml chilled distilled water, 280 µl was added to the wells of the 96-well flat-bottom microplate and the absorbance at 335 nm was measured twice. Haemocyanin concentration (mg/ml) was calculated from the molar extinction coefficient $E^{mM}_{1\,cm} = 17.26$, as previously described by Harris and Andrews (2005).

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

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.