Assessing the Isle of Man's brown crab, *Cancer pagurus*, fishery: Does heavy metal contamination have a link with shell disease syndrome?

A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Science (MSc) in Marine Environmental Protection at Bangor University.

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Abstract

75% of European *C. pagurus* landings are in the British Isles; however, *C. pagurus* landings are declining due to several factors, including disease. Shell disease syndrome is prevalent in several *C. pagurus* fisheries in the British Isles, including the Manx fishery; although, very little is known about the causative agent or environmental factors driving shell disease in *C. pagurus*. In 2012, the prevalence of shell disease in the Manx fishery was 24.4% and the average infection severity was 2.1%. Coincidentally, Manx waters are subject to extensive heavy metal contamination, which has been linked to reduced immunocapabilities in crustaceans. Metal contamination in the brown meat of *C. pagurus* is currently unregulated under European legislation, which has led to foreign food standards agencies embargoing *C. pagurus* imports from the British Isles. This study investigated the link between heavy metal contamination in the brown meat of *C. pagurus* and the prevalence and severity of shell disease, along with temporal and spatial variance in shell disease intensities. Furthermore, the biodynamics of metal concentrations in *C. pagurus* brown meat were investigated. The overall prevalence (40.3%) and severity (3.9% ± 0.41) of shell disease found in this study was double that found in 2012. No significant link between metal contamination and shell disease was detected, although there was a trend toward lower metal concentrations in uninfected *C. pagurus*. The concentration of several medically significant metals, specifically arsenic (14.93mg/kg ± 0.75) and cadmium (3.47mg/kg ± 0.48), were found in high concentrations within the brown meat of *C. pagurus*, with cadmium concentrations almost seven times greater than European limits for crustacean white meat. Due to the high concentrations of metals found in the brown meat of *C. pagurus*, it is recommended that food standards agencies advertise dietary recommendations regarding what quantity of brown meat is safe to eat.

Key words

Arsenic · Brown crab · Brown meat · Cadmium · *Cancer pagurus* · Chromium · Contamination · Copper · Heavy metal · Hepatopancreas · Isle of Man · Lead · Nickel · Pollution · Shell disease syndrome · Zinc

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Abbreviations

µg – Microgramme

ANOVA – Analysis of variance

CpBV – *Cancer pagurus* bacilliform virus

CpSBV – *Cancer pagurus* systemic bunya-like virus

DEFA – Department for Environment, Food and Agriculture, Isle of Man

EFSA – European Food Safety Authority

EU – European Union

FAO – Food and Agriculture Organization of the United Nations

FSA – Food Standards Agency

g – Gramme

GDP – Gross domestic profits

ICES – International Council for the Exploration of the Sea

ICP-OES – Inductively coupled plasma – Optical emission spectrophotometry

kg – Kilogramme

mg – Milligrammme

ml – Millilitre

No. – Number

pers. comm. – Personal communication

S.E. – Standard error

t – Tonne

TDI – Tolerable daily intake

TWI – Tolerable weekly intake

UHQ – Ultra high quality

UK – United Kingdom

USB – Universal serial bus

1.0 Introduction

1.1 Context and Background

1.1.1 Global crustacean fisheries

Globally, over $129 billion a year are generated from the extraction of marine species (FAO, 2014), an economic sector that forms an important contribution to the gross domestic profits (GDP) of maritime nations. Over $40 billion of the total worth of the global seafood revenue (FAO, 2014), provided by the exportation and sale of over 12 million metric tonnes of produce (FAO, 2014), is generated from the widespread harvesting of crustaceans from both the natural environment and aquaculture installations. Asia produces the largest quantity of crustaceans, followed by the Americas, Europe, Africa and Oceania (Figure 1) (FAO, 2016). Within Europe, which is the third largest crustacean producing continent (FAO, 2016), brown crab, *Cancer pagurus*, is one of the most economically important crustacean species.

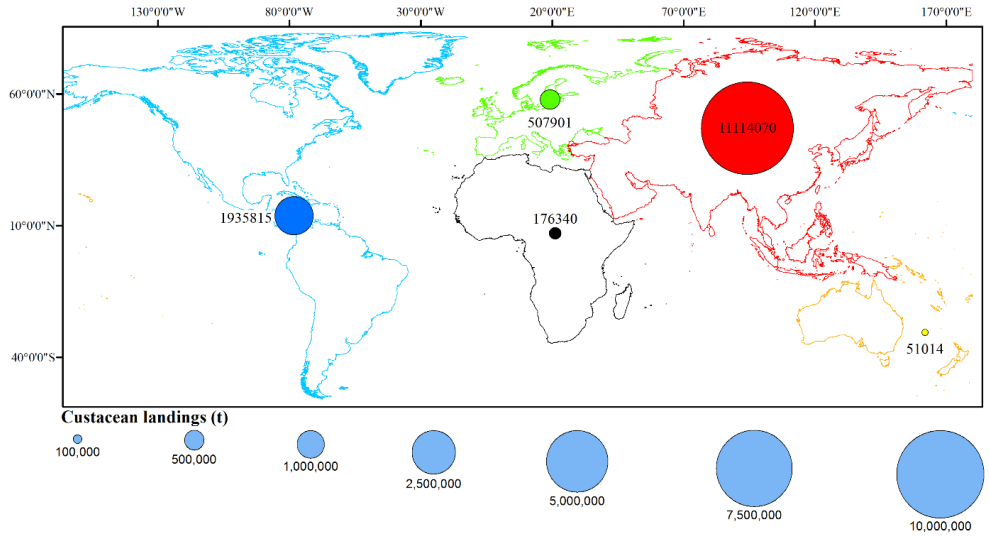


Figure 1: Continental crustacean fisheries production in metric tonnes, according to 2014 data. The Americas have been combined, and Oceania includes Melanesia, Polynesia and Micronesia. Landings data provided by FAO, 2016.

1.1.2 Brown crab, *Cancer pagurus*, fisheries

During 2014, 53,000 tonnes of *C. pagurus* were landed in European waters (FAO, 2016). The British Isles, which includes Ireland, the Isle of Man, the Channel Islands and the United Kingdom, contributed to 75% of overall *C. pagurus* landings within European waters (Appendix 1; Figure 2; FAO, 2016) demonstrating that this region is the most economically important European *C. pagurus* fishery. The *C. pagurus* fishery in Europe is subject to heavy fishing exploitation, as well as several other factors that are detrimental to fisheries, such as accidental bycatch (Jenkins, *et al*., 2001) and disease outbreaks (Stentiford, 2008), which are increasing the risk of *C. pagurus* overexploitation and stock declines.

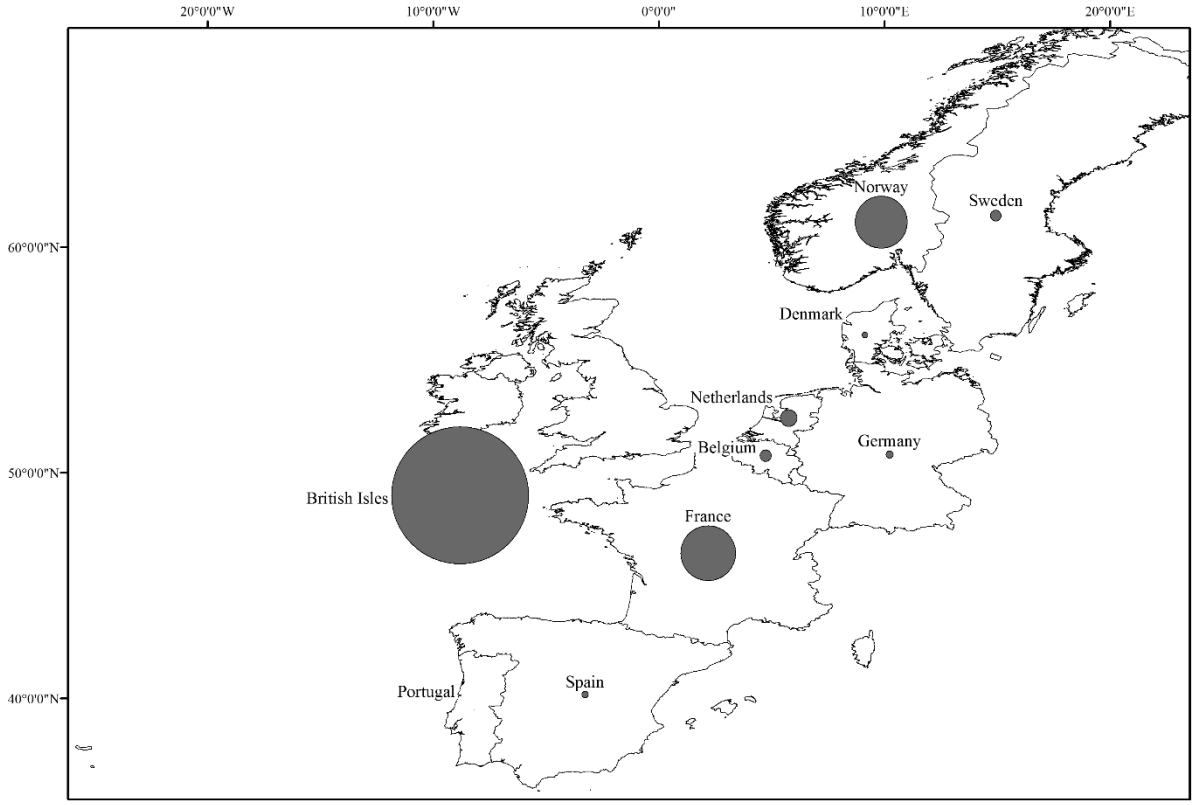


Figure 2: Brown crab, Cancer pagurus, *landings (tonnes, t) in North-West Europe, which are depicted by the graduated symbols during 2014. The British Isles includes Ireland, the Isle of Man, the Channel Islands and the United Kingdom. Landings data supplied by FAO, 2016.*

1.1.3 Diseases of the brown crab, *Cancer pagurus*

A variety of diseases can infect *C. pagurus* during the different phases of the life-cycle (Figure 3) of the species. During the early life stages of *C. pagurus*, relatively few pathogens have been described, which could be due to a lack of economic drivers, including the low aquaculture potential for *C. pagurus* (Stentiford, 2008), that are necessary for funding research into the pathogens affecting the juvenile life phases of *C. pagurus*. Despite the apparent lack of pathogens that infect the early larval stages of *C. pagurus*, several bacterial, viral and fungal pathogens have been described, which infect both the adult and juvenile *C. pagurus* populations.

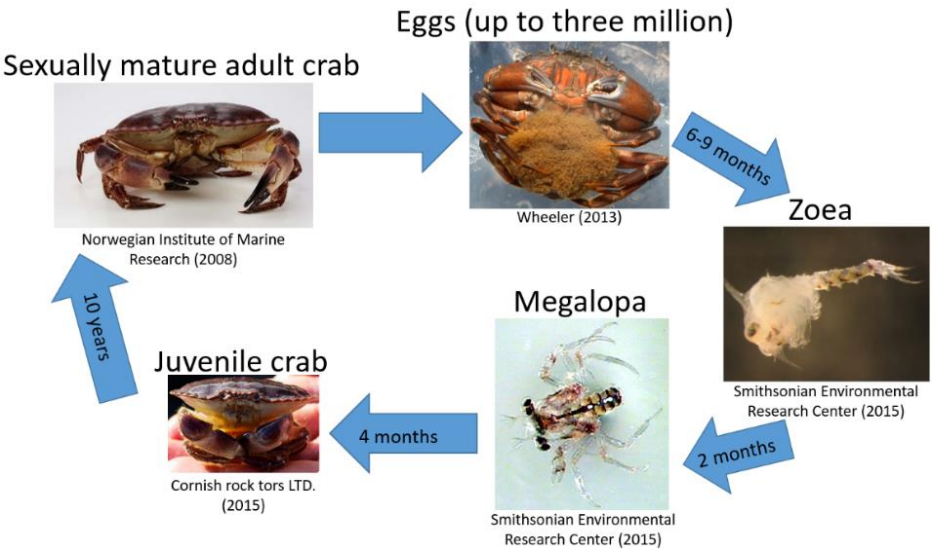


Figure 3: The stages and durations of brown crabs, Cancer pagurus, *lifecycle. The durations of the lifecycle phases were taken from Harms and Seeger (1981), Hartnoll and Mohamedeen (1987), and Eaton* et al. *(2003).*

The number of described viral pathogens that infect *C. pagurus* are limited, especially when compared to other exploited decapod (and *Cancer*) species (Johnson, 1984; Stentiford, 2008), which could be due to an increased effort to identify pathogens in other commercially important species. Of all the described viral pathogens that infect decapod crustaceans, two have been identified as endemic to *C. pagurus*: The *C. pagurus* bacilliform virus (CpBV) (Bateman and Stentiford, 2008) and *C. pagurus* systemic bunya-like virus (CpSBV) (Corbel, *et al*., 2003). Currently, CpSBV prevalence in the natural environment is unknown (Corbel, *et al*., 2003), whereas the natural prevalence of CpBV has been estimated to be around 5% (Bateman & Stentiford, 2008); the aforementioned viral diseases typically leads to mortality within infected individuals, which reduces stock size. Fungal infections described in *C.*

pagurus are also limited to only one yeast-like fungus (Stentiford, 2008), despite the multitude of fungal pathogens described in other marine decapods (Unestam, 1973). The taxonomy of the fungal infection that infects *C. pagurus*, which was identified by Stentiford *et al*. (2003), has yet to be resolved (Stentiford, 2008). Furthermore, there have been no studies conducted on the prevalence of the fungal infection identified by Stentiford *et al*. (2003) within wild populations of *C. pagurus*. While several genera of parasites have been noted in natural *C. pagurus* populations, including trematodes and cestodes (Stentiford, 2008), the data regarding parasite prevalence and severity within wild populations is insufficient (Stentiford, 2008). The lack of data is most likely due to the lack of discernible effects on the host organism's health (Sinderman, 1990). Of all the pathogens that *C. pagurus* are susceptible to, those that are bacterial, particularly those that are associated with the shell of the organism, are the most common. Shell disease syndrome, which is also known as black spot disease, is the most common of all the bacterial diseases affecting decapod crustaceans, including *C. pagurus*, and is prevalent in high concentrations within several economically important European *C. pagurus* fisheries (Vogan & Rowley, 2002a; King, *et al*., 2014).

1.1.4 Shell disease syndrome in the brown crab, *Cancer pagurus*

Figure 4: Black lesions that are associated with shell disease syndrome on the dorsal carapace of a brown crab, Cancer pagurus. *Photograph sources from author.*

Shell disease syndrome infection in *C. pagurus* is characterised by the presence of one or more black lesions on the outer shell (Figure 4). The black lesions that occur on the dorsal carapace of *C. pagurus*, and which are associated with shell disease syndrome, are caused by several undescribed pathogen species, including chitinolytic bacteria and fungi (Sindermann, 1989; Stewart, 1993 Vogan & Rowley, 2002; Vogan, *et al.*, 2008; Stentiford, 2008). Shell disease infection occurs where the waxy cuticle of the chitinous exoskeleton of *C. pagurus* has been damaged, enabling chitinovorous organisms to enter and infect the organism (Sindermann, 1989; Vogan, *et al*., 2001; Vogan & Rowley, 2002b). In addition to entry via damage to the shell of *C. pagurus*, shell disease syndrome associated bacteria can enter a crab through setal pores or hypodermal ducts (Fisher, *et al*., 1978). The black colouration of shell disease lesions is a result

of melanisation of the cuticle, an immunological response of several crustacean species (Nyhlén & Unestam, 1980).

Whilst the physiological effects of shell disease syndrome in *C. pagurus* may be limited (Vogan & Rowley, 2002b), the presence of lesions on the carapace of the crab often prevent the sales of infected crabs (Local fishers, 2016, pers. comm.), which reduced the economic potential of the fishery. Therefore, a high prevalence of shell disease within a *C. pagurus* population reduces the potential economic value and retainable catch per unit effort of the fishery. According to Getchell (1988), the prevalence of shell disease in natural crustacean populations is thought to be less than 10%; however, the overall prevalence in the Langland Bay fishery, which is located in in Swansea, Wales, (60.8%), Isle of Man fishery (24.4%), and Shetland Isles fishery (18%) have been found to be greater than the 10% value suggested by Getchell (1988) (Vogan *et al.*, 1999; King *et al.*, 2014). While several investigations have been made into the causative agent of the unusually high prevalence of shell disease (e.g. Powell & Rowley, 2005; King, 2012), no conclusive evidence has been found.

1.1.5 Environmental factors driving disease outbreaks in crustaceans

Changes in environmental factors have been identified as agents that suppress the immunological responses of several crustacean species (Waterman & Chace, 1960; Le Moullac & Haffner, 2000). The environmental factors that are the most significant contributors to immunosuppression in crustacean species are water temperature (Chen, *et al*., 1995; Hennig & Andreatta, 1998) and environmental stressors (Sindermann, 1979; Smith, *et al*., 1995), such as pollutants. Declines in ambient water temperature are known to reduce immunocapabilities in crustaceans, a phenomenon that has been well researched (e.g. Truscott & White, 1990). Temperature declines lead to a reduction in the kinetic energy possessed by the haemocytes (Truscott & White, 1990), which engulf bacteria (Chisholm & Smith, 1992; Schnapp, *et al*., 1996; Destoumieux, *et al*., 1997), reducing the rate of phagocytosis (Dean & Vernberg, 1966). However, there is evidence in the literature concerning the effects of environmental stress caused by anthropogenic sources of pollution, which may have an equal or more severe immunosuppressing nature (Sindermann, 1979; Truscott & White, 1990; Victor, *et al*., 1990; Smith, *et al*., 1995). A review of the effects of pollution on immunocapabilities by Sindermann (1979) found an increased infection pressure and reduced immunological response time in crustaceans exposed to a polluted environment. Additionally, Smith *et al*. (1995) found that sediments contaminated with polychlorinated biphenyls, polynuclear aromatic hydrocarbons,

and heavy metals could be linked to a reduced total haemocyte count in the common shrimp, *Crangon crangon*. Furthermore, investigations into the effects of heavy metals, which were conducted by Truscott & White (1990), found that exposure to high concentrations of cadmium suppressed the rate of phagocytosis of bacterial pathogens in the common shore crab, *Carcinus maenas*. Moreover, a higher energetic cost to maintain normal health was observed in the Brazilian mangrove crabs, *Ucides cordatus* and *Callinectes danae*, when exposed to environments that were highly contaminated with heavy metals, which was caused by a suppressed hypo-osmotic regulatory ability (Harris & Santos, 2000).

Heavy metal pollution within the coastal sediments surrounding the Isle of Man (Southgate, *et al*., 1983; Daka, *et al*., 2003; Kennington, 2013; Kennington, 2015) and Cornwall (Bryan & Hummerstone, 1977; Bryan & Gibbs, 1983) are a well-studied occurrence. Coincidentally, the prevalence of shell disease syndrome in the Manx and Cornish *C. pagurus* population has been found to be considerably higher than the 10% natural prevalence suggested by Getchell, (1988) (24.4% and 30%, respectively) (Davies, 2007; King, *et al*., 2014). However, no studies have been conducted to investigate whether a link between environmental metal concentration and shell disease syndromes exists.

1.2 Isle of Man's brown crab, *Cancer pagurus*, fishery

1.2.1 Importance of the Isle of Man's brown crab, *Cancer pagurus*, fishery

Despite only covering an area of 572km^2, the Isle of Man lands over 35,000kg of *C. pagurus* each year (Figure 5); however, since 2011, *C. pagurus* landings has slowly declined on an annual basis (Figure 5). There are several theories as to why *C. pagurus* landings are decreasing in the Isle of Man, including the emerging common whelk, *Buccinum undatum*, fishery, which uses *C. pagurus* as bait (Fahy, 2001), a reduced catch effort due to several *C. pagurus* fishers also targeting whelks, increased rate of overfishing as a result of bycatch (Jenkins, *et al*., 2001), and high prevalence of shell disease syndrome (King, *et al*., 2014).

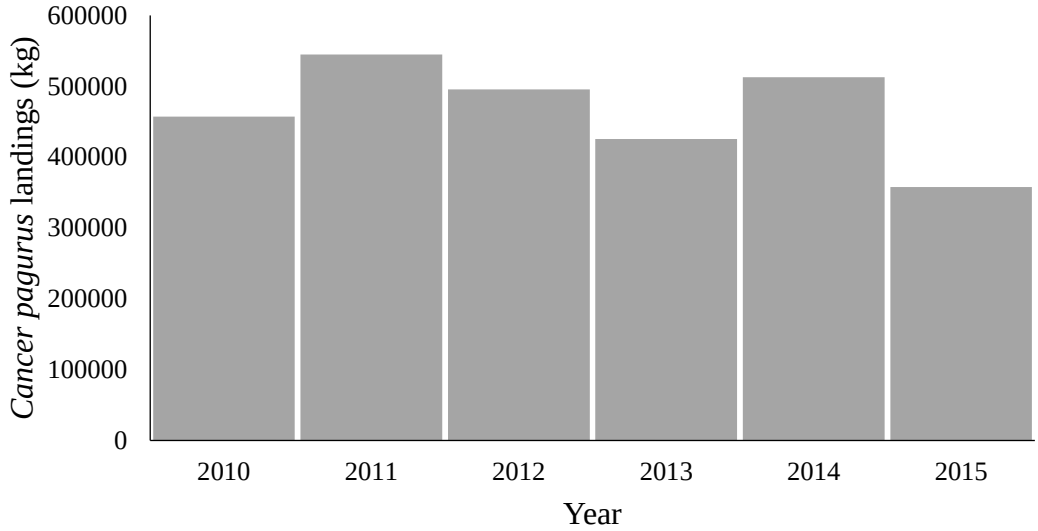


Figure 5: Cancer pagurus*, landings (kg) in the Isle of Man between 2010 and 2015. (DEFA, 2016, unpublished data)*

1.2.2 Shell disease syndrome in the Isle of Man's brown crab, *Cancer pagurus*, fishery

Recent studies by King *et al*. (2014) conducted during 2012 and Haig *et al*. (2016) during 2014/2015 have found a relatively high prevalence of shell disease in the Isle of Man's *C. pagurus* fishery. Moreover, King *et al*. (2014) also examined the infection dynamics of shell disease syndrome in the Isle of Man's *C. pagurus* fishery, in addition to mapping the geographical variation in shell disease's prevalence and severity. During 2012, it was found that 24.4% of the Isle of Man's *C. pagurus* population was infected with shell disease with an average of 2.1% of the carapace covered in black lesions (King *et al*., 2014). In addition, the male *C. pagurus* population was found to have a higher prevalence (30.3%) and severity (2.8%) of shell disease syndrome than the female population (22.2% prevalence and 1.69% severity) (King *et al*., 2014). Furthermore, the fishing areas that surround Castletown, Peel and Ramsey were identified as the three main areas with the highest prevalence of shell disease syndrome in the *C. pagurus* population, with Peel having the highest average severity of infection (Figure 6) (King, *et al*., 2014). However, a more recent study by Haig *et al*. (2016) found the prevalence of shell disease syndrome in the Manx fishery to be 12%, although, the sample size was considerably smaller than King *et al*. (2014), in addition, study was conducted during the winter, whereas King *et al*. (2014) sampled during the summer. Furthermore, King (2012) investigated whether the bacterial species composition around the Isle of Man could be linked to the high prevalence and severity of shell disease, although no such relationship could be

reliably attained. As previously discussed, heavy metal contamination, which is a significant source of pollution in the Isle of Man (Southgate, *et al*., 1983; Daka, *et al*., 2003; Kennington, 2013; Kennington, 2015), has been linked to immunosuppression in several crustacean species (Truscott & White 1990; Smith *et al*., 1995; Harris & Santos, 2000). However, no investigations into the link between heavy metal contamination and shell disease syndrome prevalence and severity have been conducted.

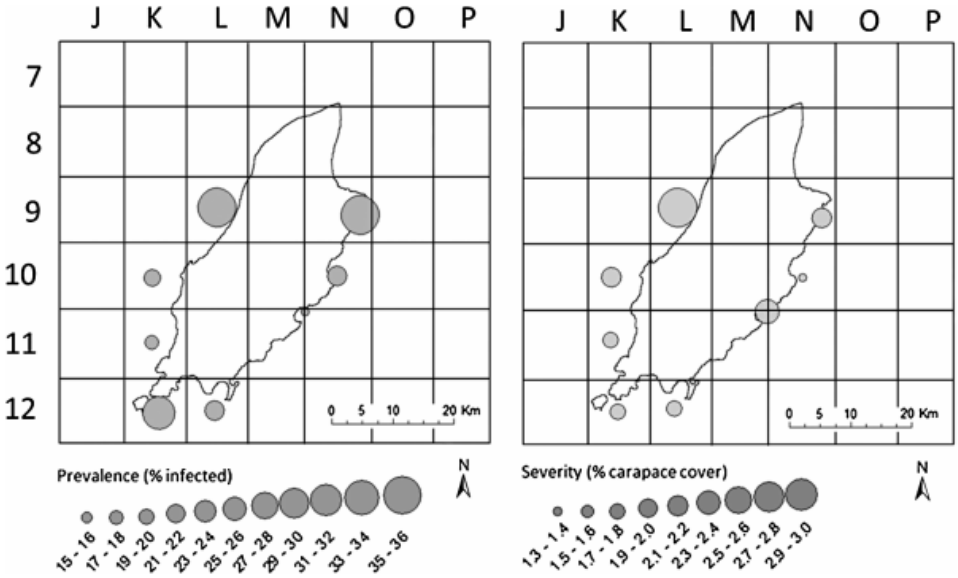


Figure 6: The prevalence and average severity of shell disease syndrome infection in the brown crab, Cancer pagurus, *population within the Isle of Man during 2012. Figure taken from King* et al. *(2014).*

1.2.3 Heavy metal pollution in the Isle of Man

During the Isle of Man's economic development, a significant source of the Island's income was generated through the mining industry (Lamplugh, 1903; Garrod, *et al*., 1972). Today, a number of the mines that are no longer economically viable have been abandoned, which has led to the leaching of metals into several estuaries that run through the island (Southgate *et al., 1983;* Daka *et al*., 2003). Studies by Southgate *et al*. (1983), Daka *et al*. (2003), Kennington (2013) and Kennington (2015) have identified high concentrations of arsenic, cadmium, chromium, copper, lead, nickel and within estuarine and coastal sediments surrounding the Isle of Man, with the highest levels of metals found in Peel (Figure 7).

Additionally, Daka *et al*., 2003 found elevated levels of cadmium, copper, lead and zinc in the blue mussel, *Mytilus edulis*, population surrounding Peel.

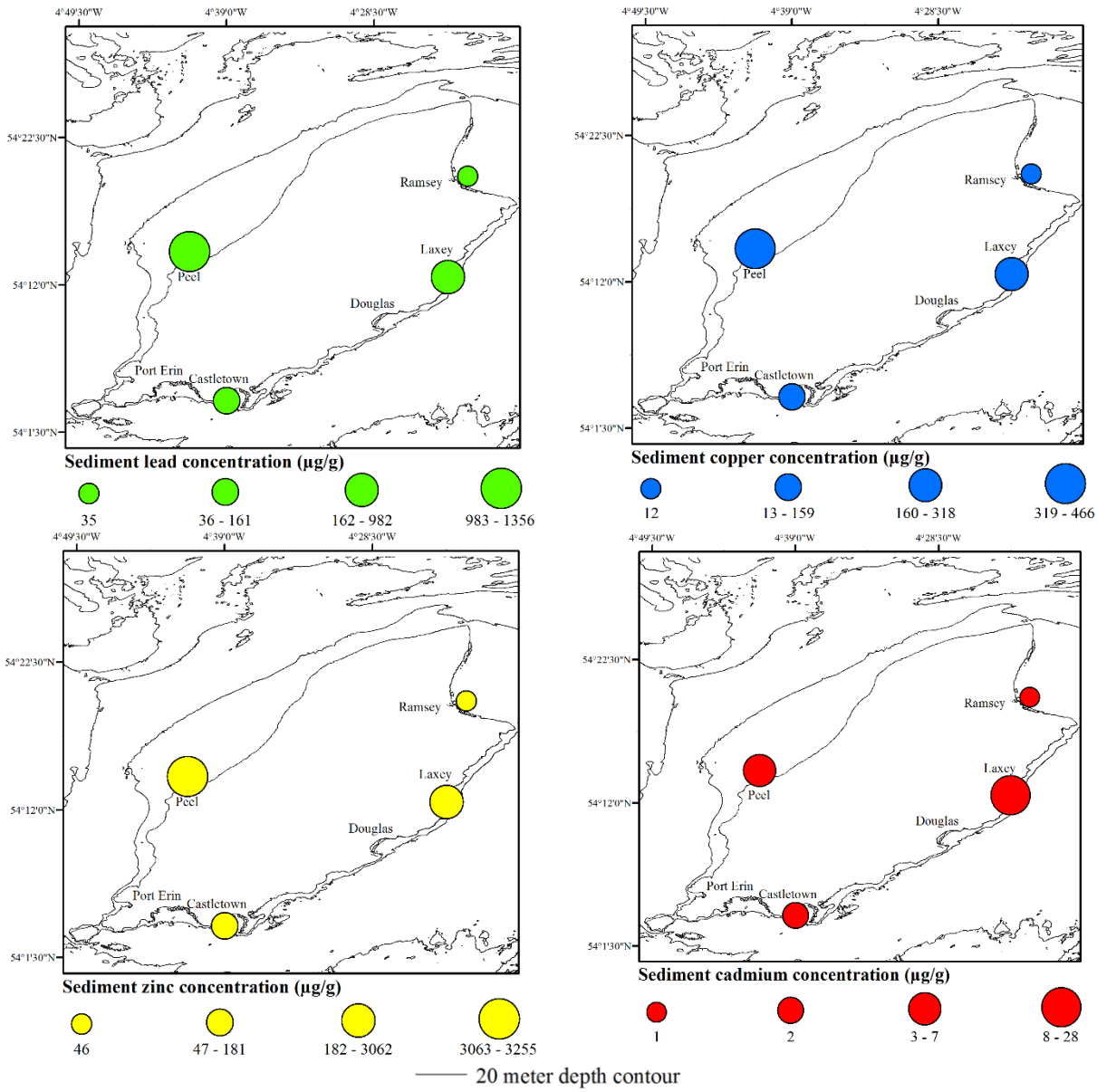


Figure 7: The concentration (µg/g) of cadmium, copper, lead and zinc in the lower estuarine sediments during 1979. Metal concentration data taken from Daka et al. *(2003).*

Heavy metal contamination has also been linked to immunosuppression in several crustacean species. For example, exposure to cadmium, copper, and zinc in the Brazilian mangrove crabs, *Ucides cordatus* and *Callinectes danae*, has been shown to suppress the hypo-osmotic regulatory ability in addition to correlating with an increase concentration of Na^+/K^+ATPase in the gills (Harris & Santos, 2000), which may reduce the energy available for the immune system in crabs. In addition, sediments containing heavy metal contamination *in*

situ have been shown to reduce the total haemocyte count in the common shrimp, *Crangon crangon*, (Smith, *et al*., 1995). Consequently, a reduced rate of phagocytosis was observed in *C. crangon* that were retained in contaminated tanks (Smith, *et al*., 1995). Moreover, there are several human health issues regarding the concentration of heavy metals within food sources.

1.2.4 Implications of heavy metal contamination in food sources

Within Europe, *C. pagurus* is a highly appreciated food source to many consumers. However, consumption of *C. pagurus* can also contribute to the dietary intake of metals that are potentially toxic to consumers (Noël, *et al*., 2011; Bolam & Bersuder, 2013a). Typically, the white meat obtained from the claws is the only portion of the crab that is consumed and therefore regulatory limits have only been set for concentrations of heavy metals in the white meat of crabs (EU Regulation No. 1881/2006 states no more than 0.5mg/kg of cadmium and lead; Appendix 2). However, several coastal European countries, such as the UK, are known to consume the currently unregulated brown meat of *C. pagurus* (Bolam & Bersuder, 2013a). The brown meat of *C. pagurus* often includes the soft meat taken from the body of the crab, the gonads and hepatopancreas, which makes up the largest portion of the brown meat (Davies, *et al*., 1981) and is known to contain high concentrations of trace metals due to the detoxifying nature of the hepatopancreas (Davies, *et al*., 1981; Overnell & Trewhella, 1979; Bolam & Bersuder, 2013a; Bolam & Bersuder, 2013b).

Primarily due to the presence of the hepatopancreas, the brown meat of *C. pagurus* is known to contain several heavy metals, for example, arsenic, cadmium, copper, and lead (Bolam & Bersuder, 2013a; Bolam & Bersuder, 2013b). High concentrations of cadmium in the brown meat of *C. pagurus* has become of particular concern in recent years due to the health implications of high dietary intake of this metal (Benoff, *et al*., 2000; Noël, *et al*., 2011; Bolam & Bersuder, 2013a; Bolam & Bersuder, 2013b). In Europe, concern regarding the concentration of cadmium in the brown meat of *C. pagurus* was first raised during 2007, when Italy refused to import foreign *C. pagurus* following an investigation into the cadmium content of the whole crab, rather than just the white meat, amid concerns of citizens consuming both the white and brown meat (RASFF, 2016). Since then, several nations, including France, Portugal and Spain, have submitted notifications to the Rapid Alert System for Food and Feed (RASFF) regarding the concentration of cadmium in the brown meat of *C. pagurus* (RASFF, 2016). In 2015, the Chinese Centre for Food Safety placed an embargo on *C. pagurus* that were

exported from Anglesey, Wales, due to the cadmium concentration in the brown meat exceeding the maximum acceptable concentrations (BBC, 2015).

Recent testing by the Food Standards Agency (FSA) found the average cadmium concentration within the brown meat of British *C. pagurus* to be 3.9mg/kg of wet weight (FSA, 2013), which is nearly an eight fold increase in the acceptable levels outlined by EU Regulation No. 1881/2006 for the white meat of the crab. In the future, should the acceptable limits of cadmium in the brown meat of *C. pagurus* be set at the same limits as the white meat, a significant proportion of crabs would not be acceptable, which would lead to economic ramifications in the crab fishing industry.

1.3 Aims and hypotheses

The aims of this study are to: 1) quantify the level of heavy metal contamination in the brown meat of the Isle of Man's brown crab, *C. pagurus*, population, making particular note on cadmium and lead concentrations, and compare the observed concentrations to upper limits set by EU Regulation No. 1881/2006; 2) determine if a link between heavy metal contamination and shell disease syndrome severity exists; 3) quantify the prevalence and severity of shell disease syndrome in the various geographic regions of the Isle of Man; 4) determine if a temporal change in shell disease prevalence and severity has occurred since the King *et al.* (2014) study; and 5) determine if heavy metal contamination varies between male and female *C. pagurus* individuals.

This study will investigate the hypothesis that shell disease syndrome infection intensity, as quantified by the total percentage coverage of lesions on the dorsal and ventral carapace, in *C. pagurus* will be significantly greater in organisms with increased concentrations of heavy metals in the brown meat. Investigating the link between shell disease syndrome and heavy metal contamination in the brown meat of *C. pagurus* enabled an additional *a posteriori* investigation into heavy metal contamination in *C. pagurus* to be implemented into this study. In the *a posteriori* component of the current study, the relationship between different heavy metal concentrations and carapace size were investigated. In addition, the varience in heavy metal contamination between male and female *C. pagurus*, was investigated. An *a posteriori* approach was also used to investigate if there was a difference in shell disease syndrome prevalence and severity between 2016 and 2012 (King, *et al*., 2014) and, if there is a difference, why the variation may have occurred.

2.0 Methods

2.1 Field methodology

2.1.1 Catch sampling

All *Cancer pagurus* samples were caught aboard commercial fishing vessels that were launched from the harbour closest to the fishing area; commercial fishers working in the waters surrounding the Isle of Man are typically territorial and therefore different fishing vessels represented separate fishing areas around the island with little to no overlap. As participation in the current study was on an optional basis, fishers were approached for consent prior to surveying. Furthermore, sampling trips were subject to weather conditions as many of the vessels used were susceptible to adverse weather conditions due to their small size (<10 meters).

The Port Erin Marine Laboratory grid squares (Figure 8), which splits the Isle of Man's ICES fishing statistic block (ICES rectangle 37E5) into 45 regions of approximately 75km^2 (Figure 8), were used as a method of assigning the sample regions to an internationally recognised fishing zone. The use of the Port Erin Marine Laboratory squares maintains the small scale resolution that is required to compare the relatively small fishing regions present in the Isle of Man. In addition, a previous investigation into shell disease syndrome in the Isle of Man during 2012, which was conducted by King *et al.* (2014), used the aforementioned grid squares, allowing for direct temporal comparisons to be made between prevalence and severity of shell disease.

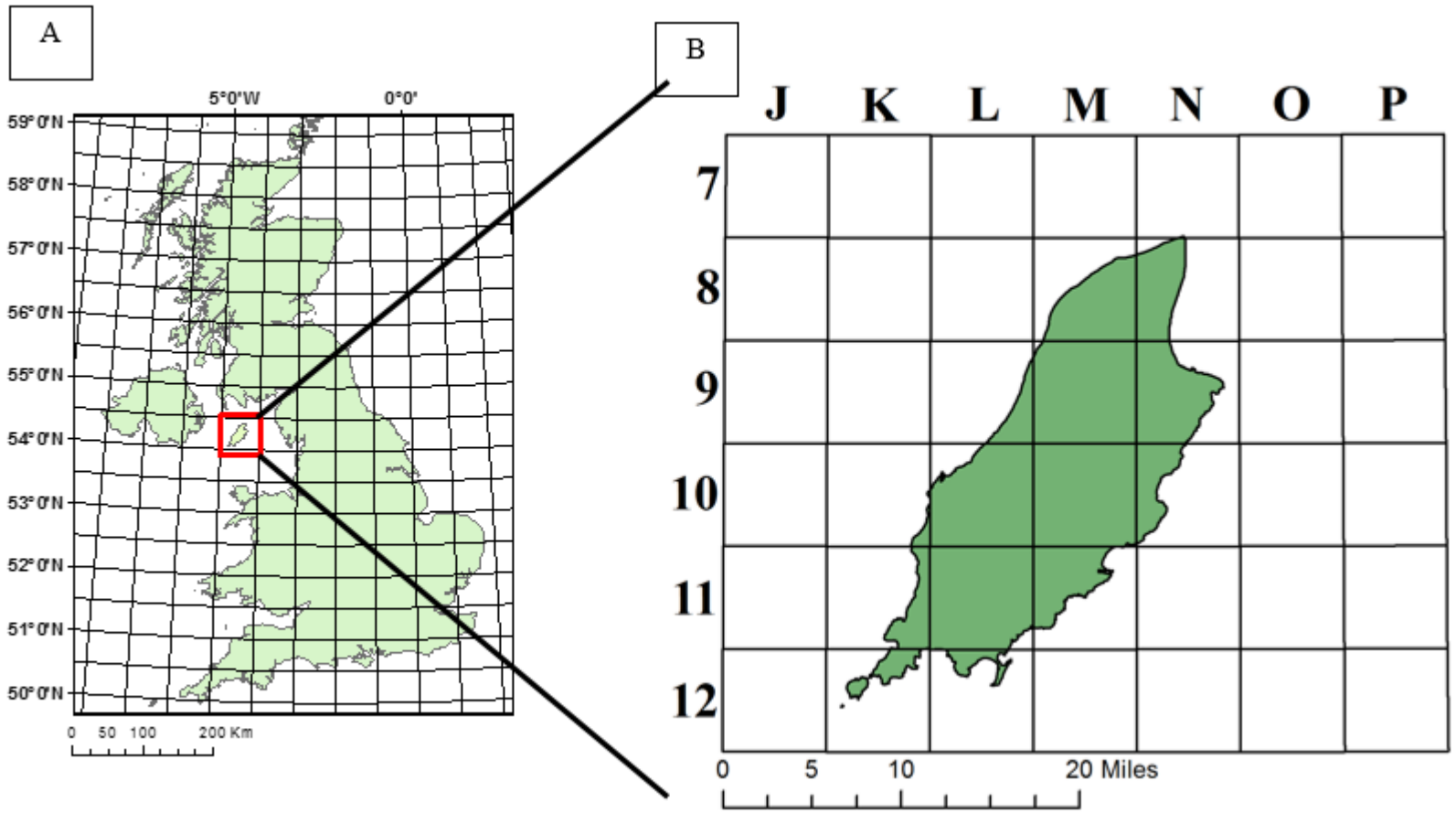


Figure 8: ICES rectangles overlaid on a map of the United Kingdom, with ICES rectangle 37E5 highlighted in yellow (A) alongside the Port Erin Marine Laboratory grid squares (B), which splits ICES rectangle 37E5 into 45 areas of approximately 75km^2.

In accordance with King *et al.* (2014), a minimum sample size of 75 *C. pagurus* was required from each grid square area to determine if the prevalence of shell disease syndrome varied both temporally and spatially. Over 75 *C. pagurus* were sampled from grid squares K10, K11, K12 and L12, enabling temporal and spatial comparisons of shell disease syndrome prevalence and severity to be conducted (Figure 9): grid squares M10, M11, N10 and N11 were pooled into one sample station (Figure 9) as these grid squares represented the Douglas Bay fishing area. In addition to quantifying the prevalence and severity of shell disease within each grid square, a total of 60 *C. pagurus*, consisting of 20 uninfected, 20 moderately infected and 20 severely infected crabs, were retained from a several of the grid squares sampled (Figure 9) for subsequent heavy metal contamination analysis.

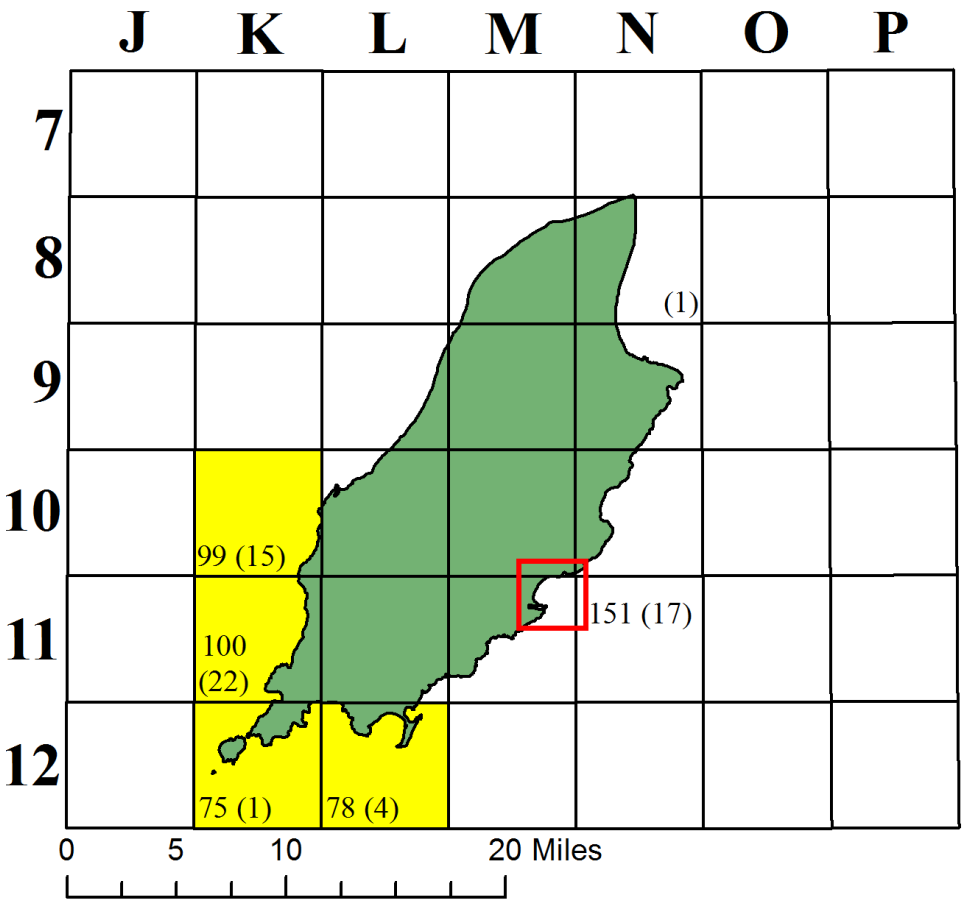


Figure 9: The Port Erin Marine Laboratory squares overlaid on a map of the Isle of Man. Highlighted squares depict sample locations used in this study with the number of sampled brown crab, Cancer pagurus, *provided in the relevant grid square. The bracketed numbers depict the number of crabs retained for heavy metal analysis. The red box represents Douglas Bay, which was pooled as one sample location due to the intersection of four squares and the close proximity of the strings that were sampled.*

2.2 Video camera analysis

A video camera held at a fixed height, as described by Hold *et al.* (2015), was used to determine the size and severity of shell disease infection of the *C. pagurus* surveyed on-board commercial fishing vessels, which minimised the impact on the speed at which the commercial fishermen operated. The video camera equipment consisted of: A *Kodak Playsport* camera (Figure 10), recording at a resolution of 1280x720 at 60 frames per second, a *Newtrent iCarrier* USB portable power pack (Figure 10), a fixed height bracket (Figure 11), a 32GB SD card, allowing for around 8.5 hours of footage to be taken, and a reference ruler.

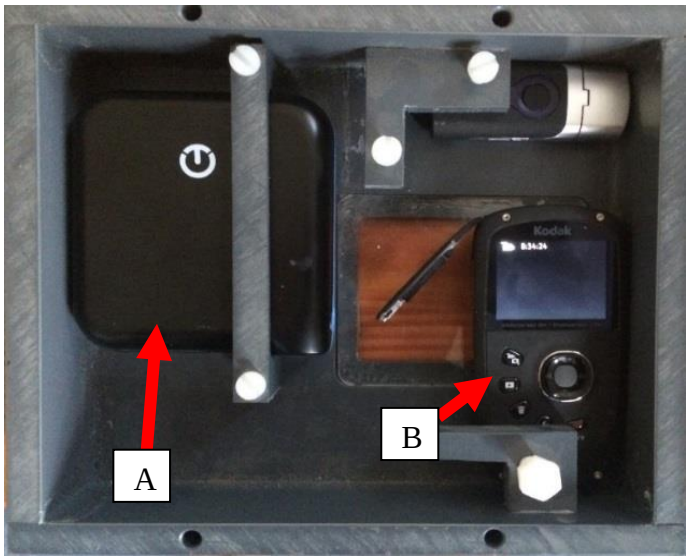


Figure 10: The camera housing of the fixed height camera equipment. (A) Indicates the Newtrent iCarrier *USB portable power pack, (B) indicates the* Kodak Playsport *camera, which housed the SD card. Photograph sourced from the author.*

The dorsal and ventral carapace of *C. pagurus* were passed under the aforementioned video camera set up (Figure 11) for subsequent video analysis. Video footage of captured *C. pagurus* was analysed using *VLC* media player version 2.24 and snapshots of the dorsal and ventral carapace of each crab were taken using the snapshot feature built into *VLC* media player. Furthermore, *C. pagurus* carapace width measurements were determined using the measuring tool within *ImageJ* version 1.50i (Figure 12). However, as described by Hold *et al.* (2015), there is inaccuracy in the carapace width measurements taken from the photographic stills as there is a gap between the reference scale and carapace edge due to the convexly curved structure of the carapace. Therefore, the first 100 *C. pagurus* that were caught, which was considered to be an adequate sample size for calibration by Hold (2016, pers. comm.), were

manually measured using callipers with a precision of 0.01mm prior to being passed under the video camera. The manual carapace width measurements that were taken from the first 100 *C. pagurus* to be caught were pooled with carapace measurements taken in *ImageJ* for later regression analysis, which are described further in section 2.4.

Figure 12: Photographing a brown crab, Cancer pagurus*, under the fixed height camera setup. The claws were bent inwards to ensure that the edge of the carapace was visible to the camera. Photograph sourced from author.*

Figure 11: The two widest points on the carapace of brown crabs, Cancer pagurus*, used for determining carapace width. Photograph sourced from author.*

During observation of the video footage, which was analysed using *VLC* media player, a tally of each crab that was passed under the camera (both with and without shell disease syndrome, which was defined by the presence of at least one black lesion on the carapace of *C. pagurus* (Figure 13)), was made in order to attain an estimate of the total percentage of the Isle of Man's *C. pagurus* population that was infected with shell disease syndrome. Soft shelled crabs were not passed under the video camera as their shells may not have had time to develop a visible manifestation of shell disease (Vogan, *et al*., 1999; Vogan & Rowley, 2002a).

Figure 13: Black legions present on the dorsal carapace of a brown crab, Cancer pagurus. *Photograph sourced from author.*

To determine the average severity of shell disease infection, still images of the dorsal and ventral carapace were taken using the snapshot feature built into *VLC media player*. The carapace width was subsequently measured in *ImageJ*, which was corrected using the linear regression equation generated from calibrating the camera equipment; the scale of the image was subsequently redefined based on this value. Subsequently, the carapace area (mm) was estimated using the freehand draw tool in *ImageJ*. The freehand draw tool was used to determine the total area (mm) of the dorsal and ventral carapace covered in the black lesions along with the total area (mm) of the carapace that was obscured from the camera, which was repeated in the laboratory when dissecting infected *C. pagurus* samples to ensure that severity data was correctly paired with the dissected crab. The following equation was used to determine the total percentage of carapace shell disease syndrome legion coverage:

$$Total\ \%\ carapace\ legion\ coverage = \frac{(Dorsal\ black\ spot\ area + Ventral\ black\ spot\ area)}{(Visible\ dorsal\ carapace\ area + Visible\ ventral\ carapace\ area)} \times 100$$

2.3 Laboratory methodology

2.3.1 Dissection of brown meat

Figure 14: The brown meat of brown crabs, Cancer pagurus*, dissected for heavy metal content analysis. Photograph sourced from author.*

It was decided that all of the brown meat (Figure 14), which includes the hepatopancreas, stomach and gonads, would be dissected and analysed for heavy metal content, rather than just the hepatopancreas, a process in line with similar studies (e.g. Bolam & Bersuder, 2013a; Bolam & Bersuder, 2013b; FSA, 2013). Additionally, analysing all the brown meat enabled the heavy metal content within a portion of *C. pagurus* that is commonly eaten throughout Europe, but not controlled for in regards to the metal content, to be determined.

Brown meat that was dissected from the *C. pagurus* samples, which were retained during the offshore component of the current study, was only conducted on specimens that were frozen for at least 24 hours to ensure the crabs were culled prior to dissection. Dissection was conducted via the removal of the legs, claws and ventral carapace, including all the gills. The brown meat was subsequently removed by hand and placed inside a labelled plastic zip lock bag and was frozen for later analysis.

2.3.2 Analysis of heavy metals

To determine the quantity of arsenic, cadmium, chromium, copper, nickel, lead and zinc in *C. pagurus* brown meat, the brown meat was first homogenised in a blender. Between two and four grammes of the homogenised hepatopancreas was weighed and placed into a Teflon microwave tube, along with 7.5ml of concentrated nitric acid and 2.5ml of concentrated hydrochloric acid. An empty Teflon microwave tube was placed through a *Varian Vista-MPX* inductively coupled plasma-optical emission spectrophotometer (ICP-OES) as a control, whilst the *C. pagurus* brown meat tissues digested for 30 minutes. The microwave tubes containing the digested brown meat sample were subsequently sealed using a pressure-release cap with a

pre-set torque tightening capper, and placed into a *CEM MarsXpress* microwave digester. Once the cycle had completed, the extract was filtered through a hardened ashless filter paper directly into a 100ml volumetric flask, which was filled with ultra-high quality (UHQ) water. The full volumetric flask with UHQ water and the extract was placed into a *Varian Vista-MPX* ICP-OES using yttrium as an internal standard. The output of the ICP-OES was adjusted for the blank tube as calibration and the samples were weighed to give a result in mg/kg.

2.4 Statistics

All statistical analyses were conducted within the *RStudio* Version 0.99.902 statistical software package. Additionally, data were transformed and tested for homogeneity (Bartlett test), normality (Shapiro–Wilk test), and homoscedasticity (Breusch–Pagan test), which required the '*car*' package in *RStudio*, prior to analysis.

The equation that described the relationship between manual and *ImageJ* carapace width measurements was ascertained using linear regression. Investigations into the relationship between manual and video measurements was conducted due to the convexly curved structure of the *C. pagurus* carapace creating a gap between the edge of the carapace and the reference scale, which could lead to an overestimation during the *ImageJ* carapace width measurement (Hold, *et al*., 2015). As was conducted by Hold *et al*., (2015), a linear model was employed to correct for the height of the carapace above the reference scale. As the camera and observer used were the same, additional corrections described by Hold *et al*. (2015) were not required.

χ^2 analysis with contingency tables and standard residuals, with a residual of ±1.96 corresponding to an alpha value of <0.05 (Sheskin, 2004), was conducted to determine if spatial variation between shell disease syndrome prevalence had occurred. Additionally, χ^2 analysis with contingency tables and standard residuals were used to determine if sex ratios varied between the sites sampled.

G-tests with a Yates' continuity correction were conducted to determine if the overall prevalence and severity of shell disease syndrome significantly varied between 2012 and 2016. Likewise, G-tests with a Yates' continuity correction were used to determine if the percentage of males captured in the sites sampled between 2012 and 2016 significantly varied. To conduct G-tests in *RStudio*, the packages '*DescTools*' and '*RVAideMemoire*' were required.

Analysis of variance (ANOVA) tests were conducted to determine if a significant difference between arsenic, cadmium, copper, zinc and total metal content existed between sex and infection category (uninfected, moderately infected and severely infected) existed. A Tukey-HSD *post-hoc* test was conducted to identify where the difference between infection categories lay. Should the data not conform to the assumptions of an ANOVA, Kruskall-Wallis tests, utilising Wilcoxon–Mann–Whitney test *post-hoc*, were performed. Furthermore, as several local fishers had claimed that shell disease syndrome could be fished out of a population and that the months prior to surveying were less prosperous than previous years for fishing (Local fishers, 2016, pers. comm.), an ANOVA test was conducted to determine if the average *C. pagurus* landings between January and May, which were the months prior to the King *et al*., (2014) study and the current survey, significantly varied between 2012 and 2016.

Pearson's product-moment correlation tests were conducted to determine if arsenic, cadmium, copper, zinc and total metal content significantly correlated with shell disease syndromes' infection severity and *C. pagurus* carapace size where the data were normally distributed and homoscedasticity was present, Spearman's rank order correlation tests were conducted if the data were not normally distributed or heteroscedastic. Likewise, the Pearson's product-moment or Spearman's rank order correlation tests were utilised to test for correlation between the content of each heavy metal in the brown meat of crabs respective of normality and homoscedasticity.

3.0 Results

3.1 Sampling data

A total of 7 days of offshore sampling onboard four different vessels were conducted between June and August 2016. In total, 503 individuals were sampled, of which 357 were female (72.24%), and 138 (27.76%) were male.

3.2 Calibration of the camera equipment

Linear regression modelling of actual and video carapace width measurements (Figure 15) found that *C. pagurus* carapace width could be predicted using the equation described in Figure 15. Before applying the correction formula for *C. pagurus* carapace width measurements, the average overestimation of carapace width by *ImageJ* was 3.6%; after the correction formula was applied, the *C. pagurus* carapace width was overestimated by an average of 0.05%.

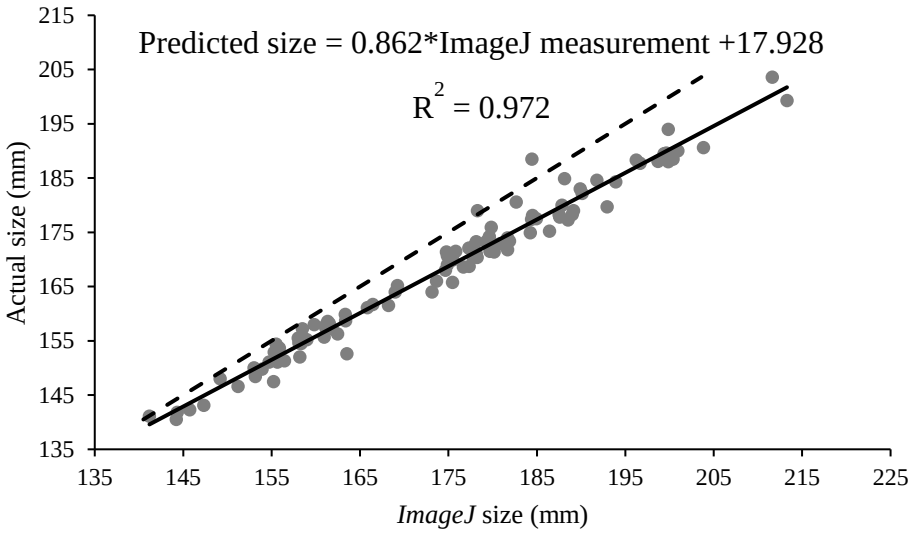


Figure 15: The relationship between actual and ImageJ Cancer pagurus *carapace width measurements (mm). Carapace width can be predicted from the video data using the equation shown on the figure. The solid black line shows the relationship between the actual measurements (mm) and* ImageJ *measurements (mm), the dashed black line shows what the relationship between actual measurements and* ImageJ *measurements would be if* ImageJ *was 100% accurate at measuring* Cancer pagurus *carapace width.*

3.3 Prevalence and severity of shell disease syndrome

3.3.1 Overall prevalence and severity of shell disease syndrome in *Cancer pagurus*

40.3% of the total population surveyed (199 of 503 individuals) exhibited physical manifestation of shell disease syndrome, as described by the presence of at least one black lesion on the carapace. In addition, the overall average infection severity of the sampled *C. pagurus* population was 3.93% (S.E. ± 0.41). The minimum shell disease infection severity exhibited by all the crabs surveyed was 0.04% while the maximum was 53.75%. Within the population sampled, 39.86% of males (55 individuals), exhibited black lesions on the carapace and a minimum infection severity of 0.14%, a maximum severity of 53.76%, and an average infection severity of 5.81% (S.E. ± 1.15) (Figure 16; Figure 17). 40.34% (144 individuals) of the sampled female *C. pagurus* population showed signs of shell disease; the minimum infection severity observed in female *C. pagurus* was 0.04% whilst the maximum severity was 22.68%, and the mean shell disease infection severity was 3.21% (S.E. ± 0.35) (Figure 16; Figure 17). When severity data were log transformed, a significantly greater average shell disease syndrome infection severity was found in male *C. pagurus* (5.81% ± 1.15) when compared to female individuals (3.21% ± 0.35) (ANOVA; $F_{1, 197} = 12.63$, $p < 0.001$). In contrast, no such difference was detected in the prevalence of shell disease syndrome between male and female *C. pagurus*.

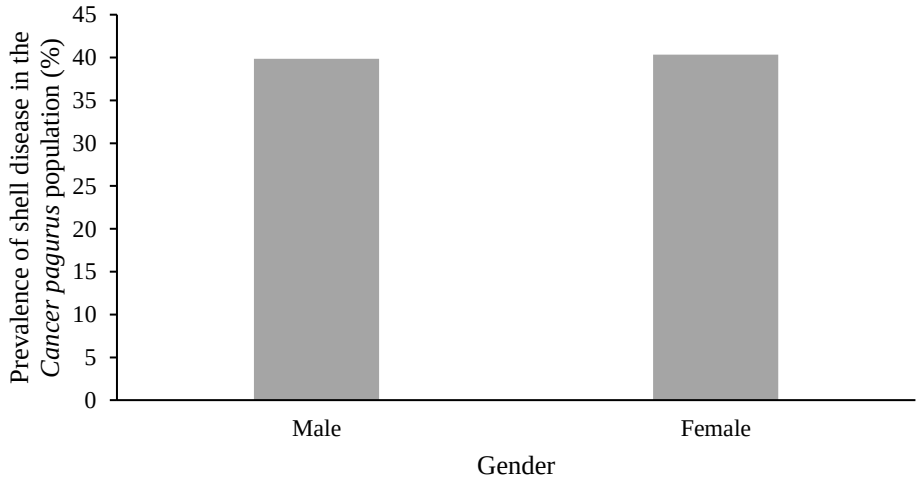


Figure 16: The overall prevalence of shell disease syndrome in male and female Cancer pagurus. *Samples were obtained from the Isle of Man from June to August 2016.*

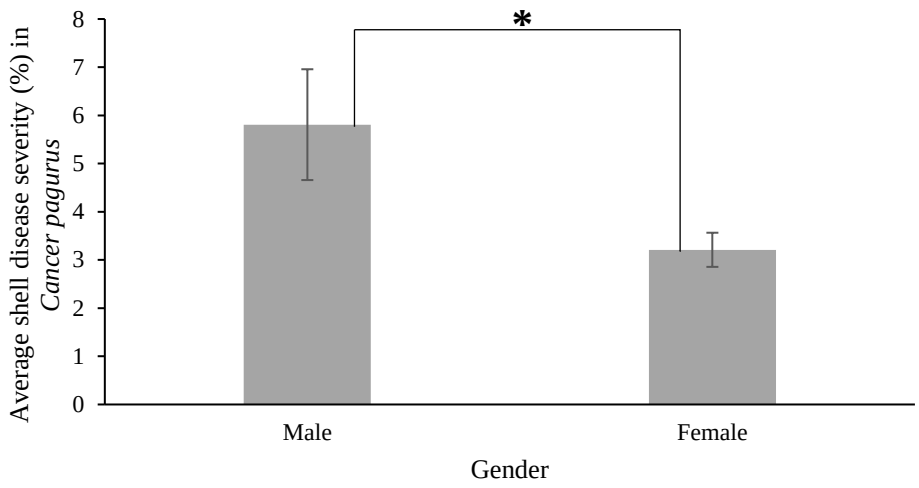


Figure 17: The average severity (± standard error) of shell disease syndrome, as defined by the mean % coverage of black lesions on the dorsal and ventral carapace, of male and female Cancer pagurus. ** Indicates a statistically significant difference. ANOVA was conducted on log transformed data. Samples were obtained from the Isle of Man from June to August 2016.*

3.3.2 Regional prevalence and severity of shell disease syndrome in *Cancer pagurus*

3.3.2.1 Spatial comparisons

A significant difference in the prevalence of shell disease syndrome was detected between the sampled grid squares ($\chi^2_4 = 17.48$, $p = 0.002$) (Figure 18; Table 1). Analysing the standardised residuals, with a residual of ±1.96 equivalent to an alpha value of <0.05 (Sheskin, 2004), indicated that a significantly lower prevalence occurred in Douglas Bay (Residual: -3.08), whereas a significantly greater prevalence was observed within square K11 (Residual: 2.2) (Figure 18). Although the prevalence of shell disease was not a significant increase, the prevalence of shell disease within grid square K10, K12 and L12 was considerably larger than that observed in Douglas Bay. In contrast, a one-way ANOVA with Tukey-HSD *post-hoc* test performed on log transformed data found no significant variation in shell disease syndromes infection in infection severity between the sites sampled (ANOVA; $F_{4, 194} = 1.35$, $p = 0.255$) (Figure 19; Table 1). Indeed, the average severity within grid square L12 was nearly double the severity observed in most other grid squares (Table 1).

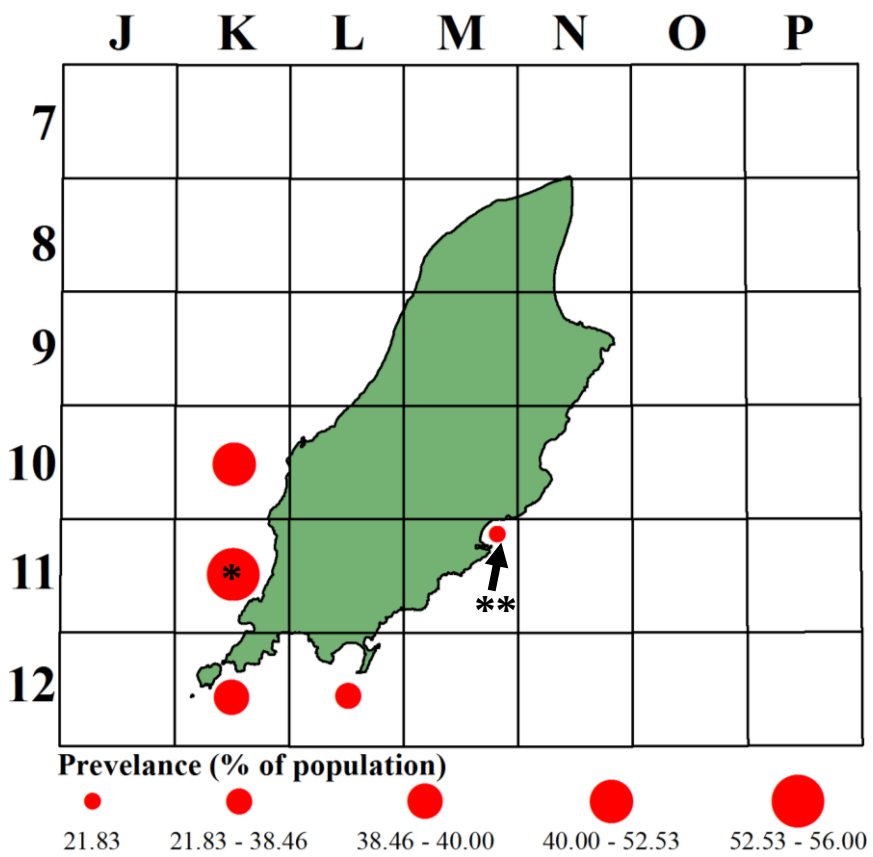


Figure 18: The prevalence of shell disease syndrome in the Isle of Man's brown crab, Cancer pagurus*, population in reference to the Port Erin Marine Laboratory squares sampled, as depicted by the graduated symbols. * indicates a significantly greater prevalence, ** indicates a significantly lower prevalence.*

Table 1: The prevalence and severity, as indicated by the average % coverage of black lesions on the carapace, of shell disease syndrome in the Isle of Man's brown crab, Cancer pagurus*, population. Severity values are given ± standard error.*

Site	Prevalence	Severity
Douglas Bay	21.83	3.33 ± 0.60
K10	52.53	3.37 ± 0.57
K11	56.00	3.26 ± 0.61
K12	40.00	4.24 ± 0.68
L12	38.46	6.44 ± 2.07

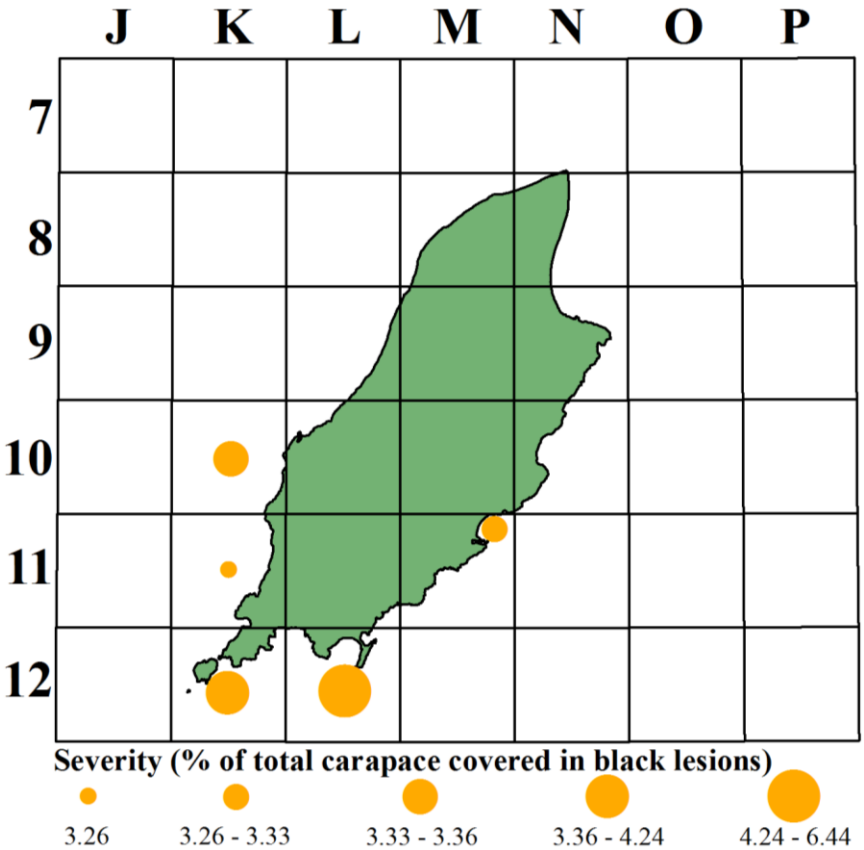


Figure 19: The severity of shell disease syndrome, indicated by the average % coverage of black lesions on the carapace, within the Isle of Man's brown crab, Cancer pagurus, *population. Levels of severity have been grouped according to the Port Erin marine laboratory squares, average severity is depicted by the graduated symbols. An ANOVA with TukeyHSD* post-hoc *performed on log transformed data found no significant difference between sites.*

3.3.2.2 Temporal comparisons

A significant increase in the overall prevalence of shell disease syndrome was detected between 2012 (24.4%) and 2016 (40.34%) (χ^2_1 = 49.30, $p < 0.001$). Indeed, a significant increase in the prevalence of shell disease was found between 2012 and 2016 at sites K10, K11 and L12 (Table 2; Figure 20). A significantly greater severity of shell disease syndrome infection was observed in 2016 when compared with 2012 (Figure 21) (ANOVA; $F_{1, 626}$ = 28.18; $p < 0.001$). A significantly greater severity of shell disease syndrome infection was observed in all sample sites, with the exception of Douglas Bay, in 2016 when compared to 2012 (Figure 21;

Table 3).

Table 2: The results of a G test of independence utilising the Yates' continuity correction. The test was conducted to determine if a significant difference in the percentage of the Cancer pagurus *population was infected with shell disease syndrome within the sites surveyed, which were located in the Isle of Man, existed between 2012 and 2016. The degrees of freedom in all tests conducted was 1. 2012 data taken from King et al. (2014). * indicates a significant difference.*

Site	Prevalence 2012 (%)	Prevalence 2016 (%)	χ^2	p
Douglas Bay	15.3	21.83	1.46	0.228
K10	21.6	52.53	37.02	<0.001*
K11	20.8	56.00	130.89	<0.001*
K12	31.6	40.00	1.42	0.233
L12	23.8	38.46	6.09	0.014*

Table 3: ANOVA outcomes showing the difference in the mean severity of shell disease infection, as indicated by the average percentage coverage of black lesions on the dorsal and ventral carapace, in the Isle of Man's brown crab, Cancer *pagurus, population between 2012 and 2016 within the sample areas surveyed. 2012 data taken from King* et al. *(2014). * indicates a significant difference.*

Site	Average severity in 2012 (%)	Average severity in 2016 (%)	F	d.f.	p
Douglas Bay	2.1 ± 0.62	3.33 ± 0.60	2.01	1, 64	0.161
K10	1.9 ± 0.29	3.37 ± 0.57	6.15	1, 196	0.014*
K11	1.87 ± 0.26	3.26 ± 0.61	5.36	1, 132	0.022*
K12	1.9 ± 0.34	4.24 ± 0.68	11.47	1, 106	0.001*
L12	1.72 ±0.26	6.44 ± 2.07	14.09	1, 120	<0.001*

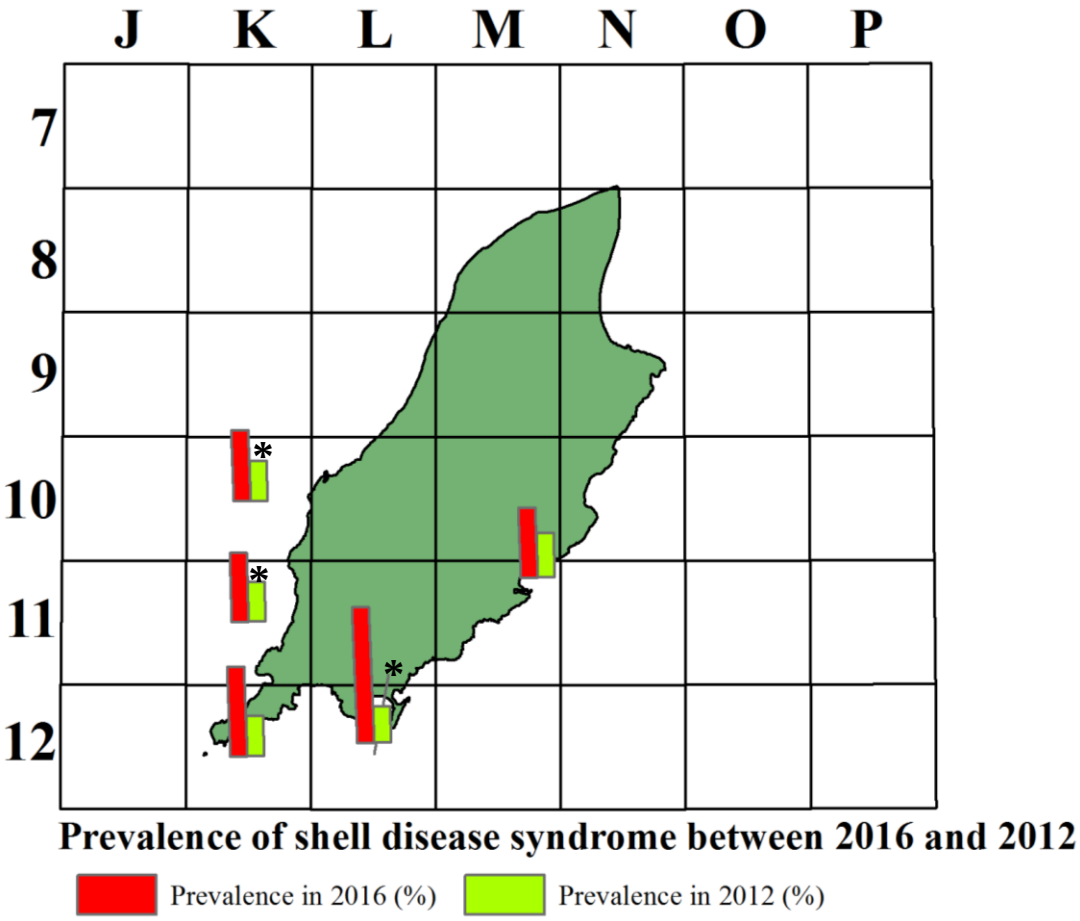


Figure 20: Variation in shell disease syndrome prevalence in the Isle of Man's brown crab, Cancer pagurus, population between 2012 and 2016. Map shows the Port Erin Marine Laboratory grid squares overlaid onto an Isle of Man base map. 2012 data was provided by King et al. *(2014). * indicates a statistically significant difference.*

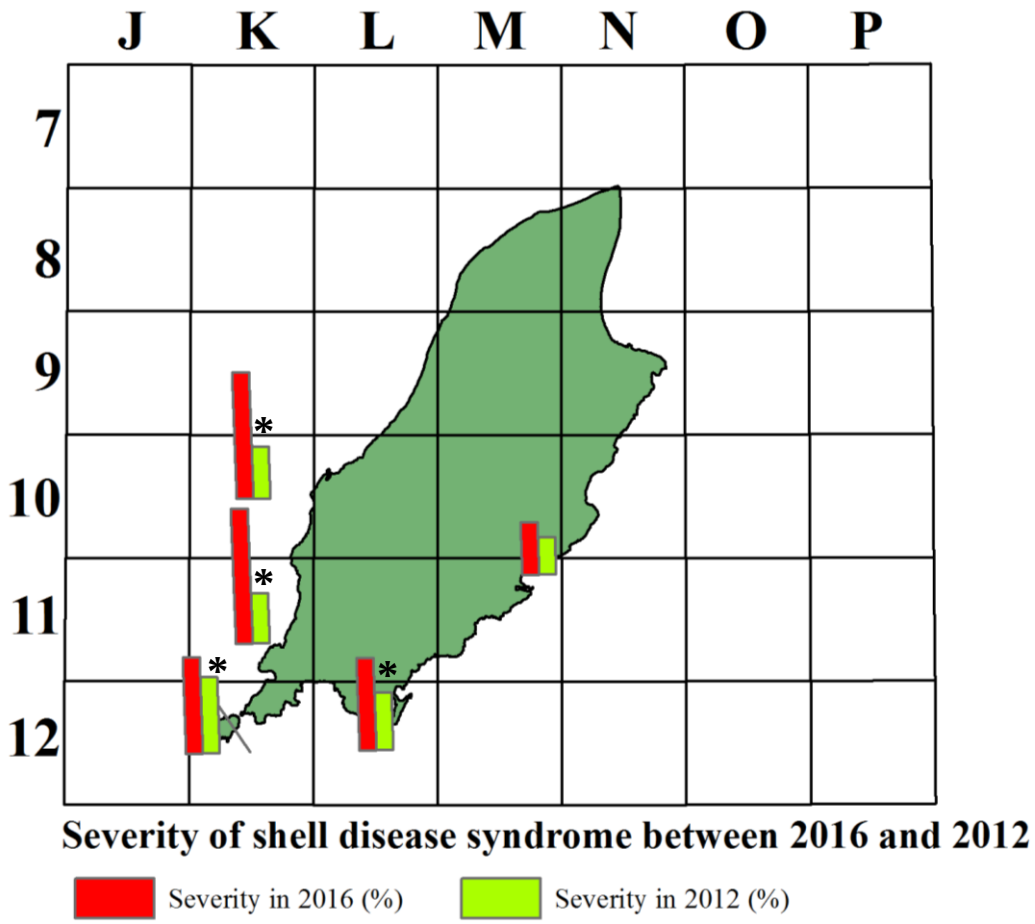


*Figure 21: Variation in shell disease syndrome severity, indicated by the average percentage coverage of black lesions on the dorsal and ventral carapace, in the Isle of Man's brown crab, Cancer pagurus, population between 2012 and 2016. Map incorporates the Port Erin Marine Laboratory grid squares overlaid onto the Isle of Man. * indicates a statistically significant difference in average severity. 2012 data taken from King* et al. *(2014).*

3.4 Sex ratios

3.4.1 Spatial sex ratio comparison

A significant difference in sex ratios was detected between the sites surveyed (χ^2_4 = 89.88, $p < 0.001$), in general, sites had significantly more females than males (Figure 22). Analysis of the standard residuals indicated that Douglas Bay and square L12 had significantly more males (2.00 and 2.42, respectively) than the expected values, whereas grid square K10 and K11 had significantly more females and significantly less males (female residuals: K10 = 2.67, K11 = 2.57; male residuals: K10 = -4.3, K11 = -4.14) than was expected (Figure 22). However, the expected values were significantly less in grid square K12 for females (-2.74) and significantly more males (2.42) (Figure 22).

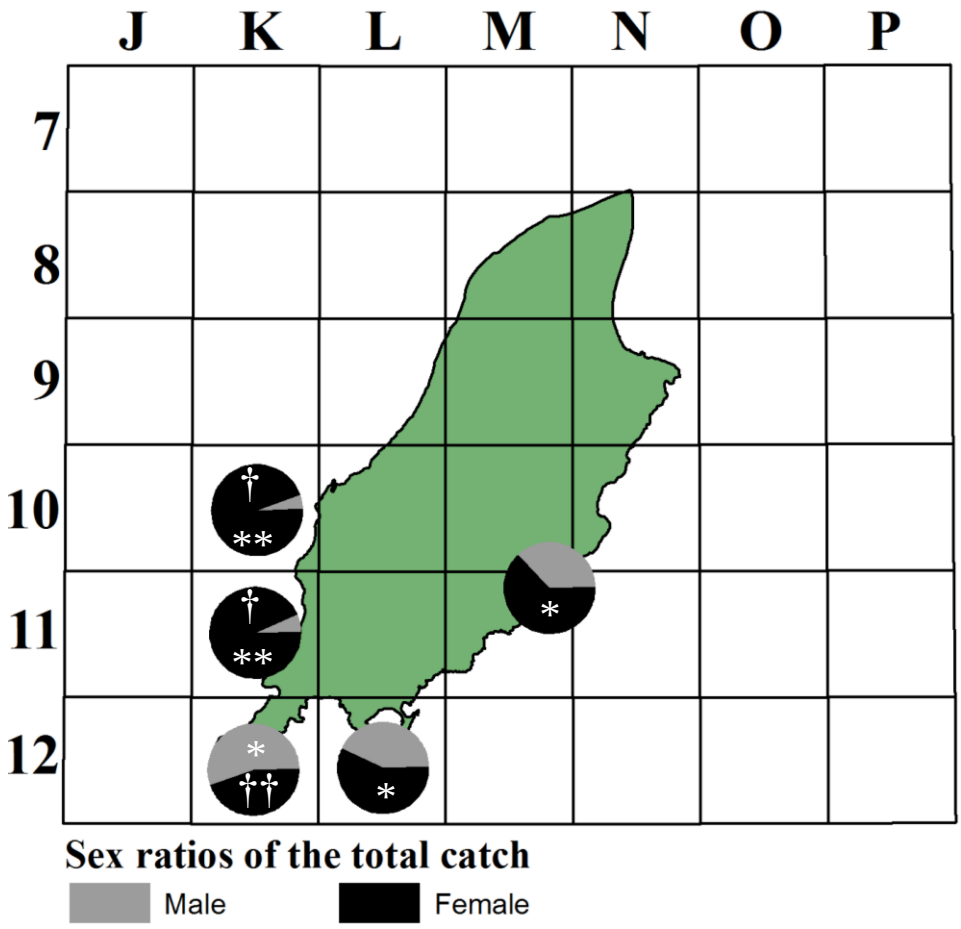


Figure 22: The ratio of male to female Cancer pagurus *in the Port Erin marine laboratory grid squares sampled. * Depicts areas of significantly higher proportion of males than expected, ** depicts areas of significantly lower males than the expected values. † Depicts areas of significantly higher females than was expected, †† depicts areas of significantly lower females.*

3.4.2 Temporal sex ratio comparison

During 2012, significantly more males were surveyed in sites K11 and L12 when compared to the 2016 results (Table 4). However, all other sites displayed insignificant differences (Table 4).

Table 4: The results from a G test of independence utilising the Yates' continuity correction. The test was conducted to determine if a significant difference in the percentage of male Cancer pagurus *were surveyed in the sample squares, which were located in the Isle of Man, existed between 2012 and 2016. 2012 data taken from King et al. (2014). * indicates a significant difference.*

Site	Males captured 2012 (%)	Males captured 2016 (%)	χ^2	d.f.	p
Douglas Bay	32.62	36.42	0.38	1	0.538
K10	10.83	5.05	2.96	1	0.085
K11	94.13	6.00	309.72	1	<0.001*
K12	33.6	45.33	2.88	1	0.090
L12	58.4	42.31	6.15	1	0.013*

3.5 Variation in the total number of *C. pagurus* caught before surveying between 2012 and 2016

Whilst 10% less *C. pagurus* were caught between January and May in 2016 (57013 individuals) than in 2012 (63452 individuals) (Figure 23) (DEFA, 2016, Unpublished data). However, no statistically significant difference was observed between the average number of monthly *C. pagurus* landings that were caught each month between January and May in 2012 and again in 2016 (Figure 24).

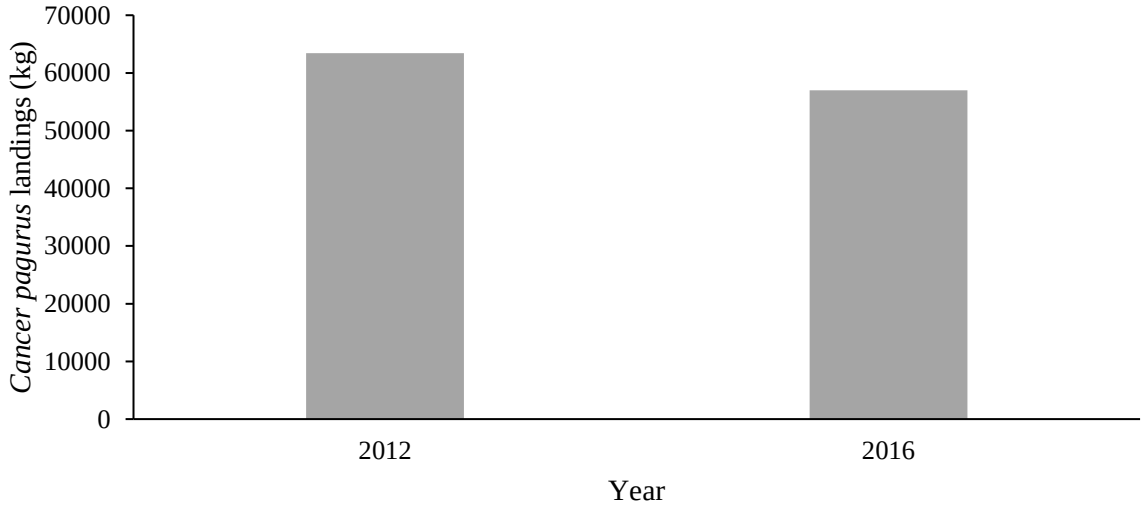


Figure 23: The Cancer pagurus *landings (kg) by the Manx fleet between January and May in 2012 and again in 2016. Landings data supplied by DEFA, 2016, Unpublished data.*

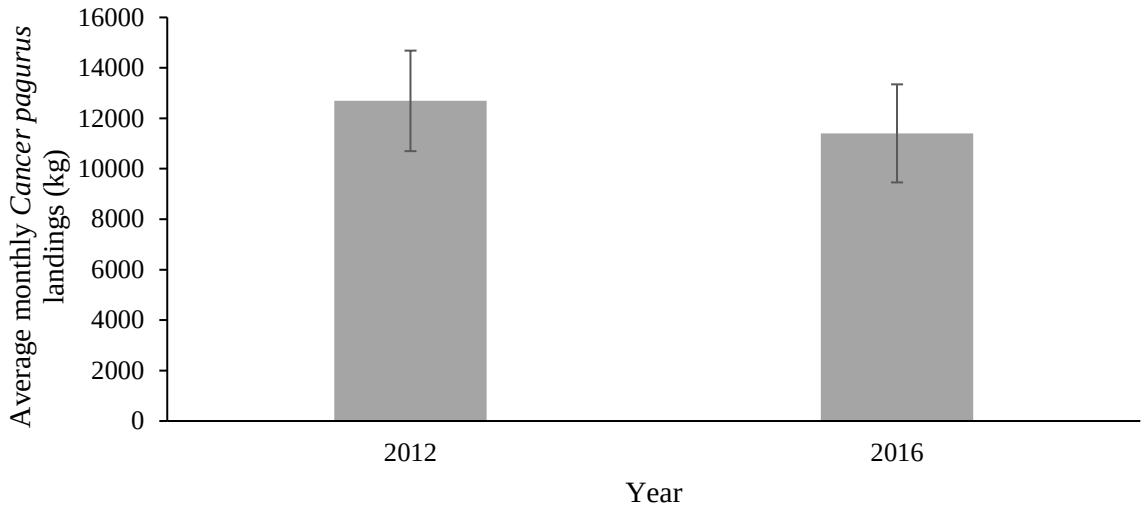


Figure 24: The average monthly Cancer pagurus *landings (kg) by the Manx fishing fleet between January and May in 2012 and 2016. Error bards depict the standard error of the mean. Landings data supplied by DEFA, 2016, Unpublished data.*

3.6 Heavy metals

A total of 60 *C. pagurus* brown meat samples were analysed for heavy metal contamination using ICP-OES. Of the 60 samples analysed, 27 were female (45%) and 33 were male (55%). Within the 60 *C. pagurus* samples retained and dissected there were three subcategories of infection severity that were: uninfected (0% of carapace covered in black lesions), moderately infected (5% - 10% of carapace covered in black lesions), and severely infected (10%+ of carapace covered in black lesions); all three infection subcategories were represented by 20 *C. pagurus* individuals.

3.6.1 Average metal concentrations in *Cancer pagurus* samples

Concentrations of arsenic, cadmium, copper and zinc were found in the brown meat of the *C. pagurus* samples analysed, which were above the detection limit in all *C. pagurus* brown meat samples analysed (Table 5). Conversely, chromium, nickel and lead were not above the detection limit in several samples and were only present in relatively low concentrations (Table 5). Cadmium concentrations within 85% of the *C. pagurus* brown meat samples exceeded the upper limit of 0.5mg/kg set for the white meat of crustaceans, which was issued by EU Regulation No. 1881/2006. However, lead concentrations in all samples did not exceed the upper limit of 0.5mg/kg for crustacean white meat issued by EU Regulation No. 1881/2006 (Table 5). Copper concentrations exhibited the widest range of observed concentrations while lead concentrations showed the lowest range of concentrations (Table 5).

Table 5: The average (± standard error), minimum, and maximum concentration (mg/kg of wet weight) of heavy metal concentrations, including the average concentration of total heavy metals (arsenic, cadmium, chromium, copper, lead, nickel and zinc), which were detected within the brown meat of 60 Cancer pagurus *samples. Heavy metal concentrations were determined using inductively coupled plasma optical emission spectrophotometry. EU Regulation No. 1881/2006 sets the maximum legal concentration of lead and cadmium within the white meat of crustaceans at 0.5mg/kg.*

Metal	Average (mg/kg)	Minimum concentration (mg/kg)	Maximum concentration (mg/kg)	% of population observed with metal in the brown meat	% of population exceeding maximum permissible limits for white meat set by EU Regulation No. 1881/2006
Arsenic	14.93 ± 0.75	5.21	33.13	100.00	N/A
Cadmium	3.47 ± 0.48	0.10	17.48	100.00	85.00
Chromium	0.06 ± 0.01	0.00	0.80	96.67	N/A
Copper	51.57 ± 5.13	8.97	205.84	100	N/A
Lead	0.11 ± 0.01	0.00	0.36	95.00	0.00
Nickel	0.06 ± 0.01	0.00	0.38	68.33	N/A
Zinc	26.27 ± 1.12	11.60	54.50	100.00	N/A
Total metal content	96.47± 6.48	30.61	290.94	-	N/A

3.6.2 Relationships between heavy metal concentrations and shell disease syndrome

3.6.2.1 *Cancer pagurus* brown meat metal concentrations correlation with shell disease syndromes infection severity

No significant correlations between the severity of shell disease syndrome infection, as determined by the total percentage coverage of black lesions on the dorsal and ventral carapace, and the concentration of heavy metals within the brown meat of *C. pagurus* was detected (Figure 25; Table 6). For the correlation analysis to be conducted, no uninfected crabs were included to ensure that the analysis was not dominated by uninfected crabs. Furthermore, one outlier, which was considerably greater in chromium concentration than the rest of the samples, was removed in the chromium concentration analysis.

Table 6: Spearman's rank correlation test results showing the correlations between different metal concentrations (mg/kg of wet weight) in the brown meat of 40 shell disease syndrome infected Cancer pagurus *and the severity of shell disease infection. One outlier was removed from the chromium analysis.*

Metal	ρ	d.f.	p
Arsenic	0.160	38	0.325
Cadmium	0.032	38	0.845
Chromium	-0.255	37	0.118
Copper	0.142	38	0.383
Lead	-0.012	38	0.940
Nickel	0.085	38	0.603
Zinc	-0.214	38	0.185
Total metal content	0.099	38	0.542

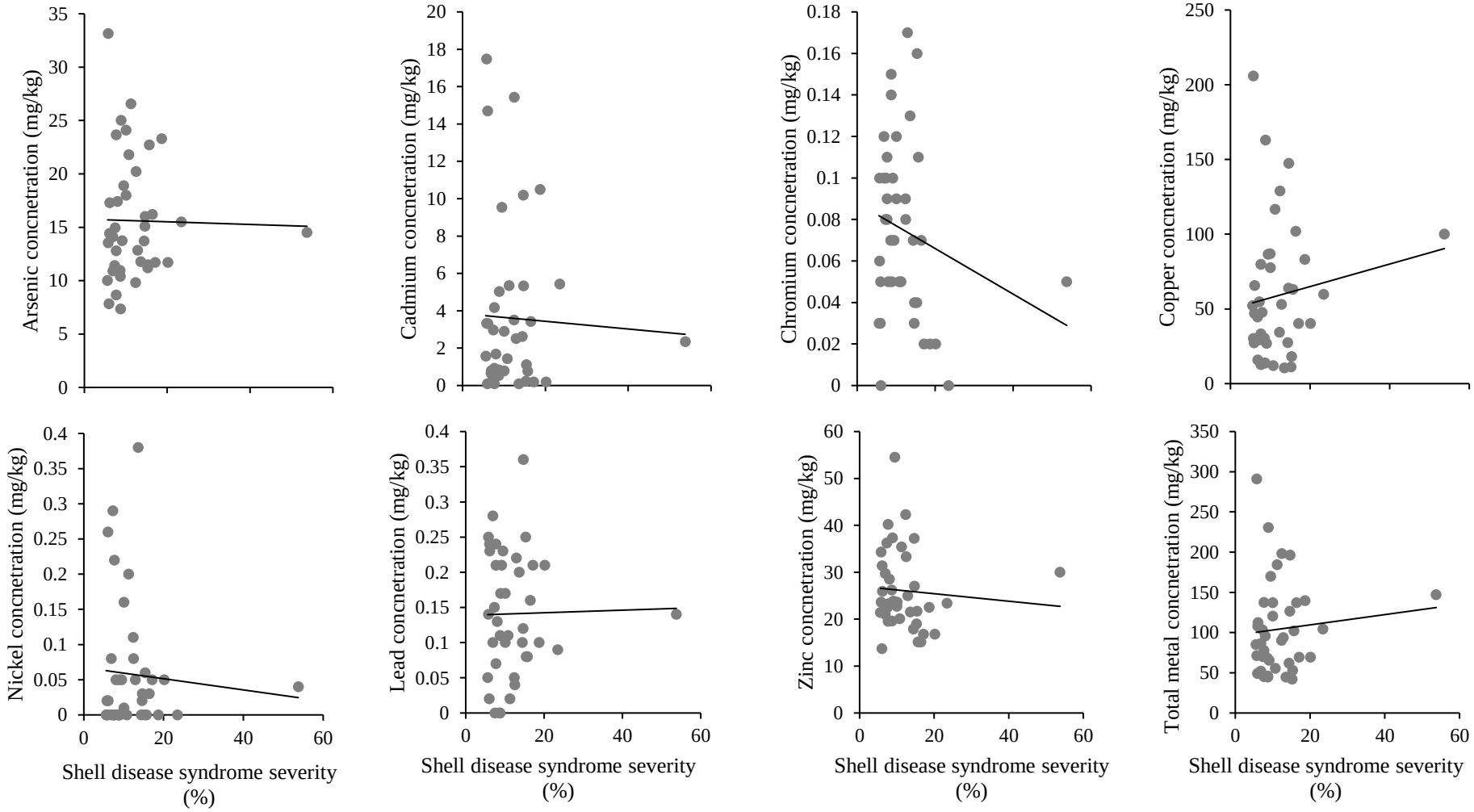


Figure 25: The correlations between all heavy metal concentrations, including total metal concentration, within the brown meat of Cancer pagurus *and shell disease syndrome infection severity within the Isle of Man. Spearman's ranked correlation tests found that all correlations were insignificant. One outlier was removed from the chromium correlation analysis.*

3.6.2.2 Difference in *Cancer pagurus* brown meat metal concentrations between infection category

A statistically significant difference in nickel concentrations was observed between infection categories (Figure 26; Table 7), *post-hoc* tests found a significant increase in nickel concentration within uninfected C. pagurus compared to moderately infected C. pagurus ($p = 0.016$). However, the average concentration of nickel within the brown meat of uninfected C. pagurus was only 0.07mg/kg greater than the average concentration within moderately infected crabs. Although concentrations of arsenic, cadmium copper, and total metals was lower in uninfected C. pagurus, no significant differences were detected (Figure 26; Table 7).

Table 7: ANOVA and Kruskall-Wallis test results, which were conducted to determine if the average (± standard error) concentration (mg/kg of wet weight) of metals within the brown meat of the 60 Cancer pagurus *samples analysed significantly varied between shell disease syndrome infection categories. Total metals were calculated through the sum of arsenic, cadmium, copper and zinc concentrations. Data transformations: † = natural log.*

Metal	Infection category	Average	F/χ^2	d.f.	p
Arsenic†	Uninfected	13.57 ± 1.30			
	Moderate	14.82 ± 1.43	1.56	2, 57	0.22
	Severe	16.41 ± 1.13			
Cadmium	Uninfected	3.20 ± 0.39			
	Moderate	3.48 ± 1.09	1.97	2	0.374
	Severe	3.72 ± 0.91			
Chromium	Uninfected	0.09 ± 0.02			
	Moderate	0.07 ± 0.04	2.02	2	0.364
	Severe	0.03 ± 0.01			

Metal	Infection category	Average	F/χ^2	d.f.	p
Copper†	Uninfected	36.83 ± 4.35			
	Moderate	54.02 ± 11.12	1.82	2, 57	0.172
	Severe	63.85 ± 9.13			
Lead	Uninfected	0.16 ± 0.02			
	Moderate	0.08 ± 0.02	0.41	2, 57	0.668
	Severe	0.08 ± 0.02			
Nickel	Uninfected	0.12 ± 0.03			
	Moderate	0.05 ± 0.02	8.03	2	0.018*
	Severe	0.02 ± 0.01			
Zinc†	Uninfected	26.68 ± 2.02			
	Moderate	27.81 ± 2.06	0.85	2, 57	0.432
	Severe	24.32 ± 1.72			
Total metals†	Uninfected	80.28 ± 6.32			
	Moderate	100.14 ± 14.40	1.51	2, 57	0.231
	Severe	108.30 ± 11.04			

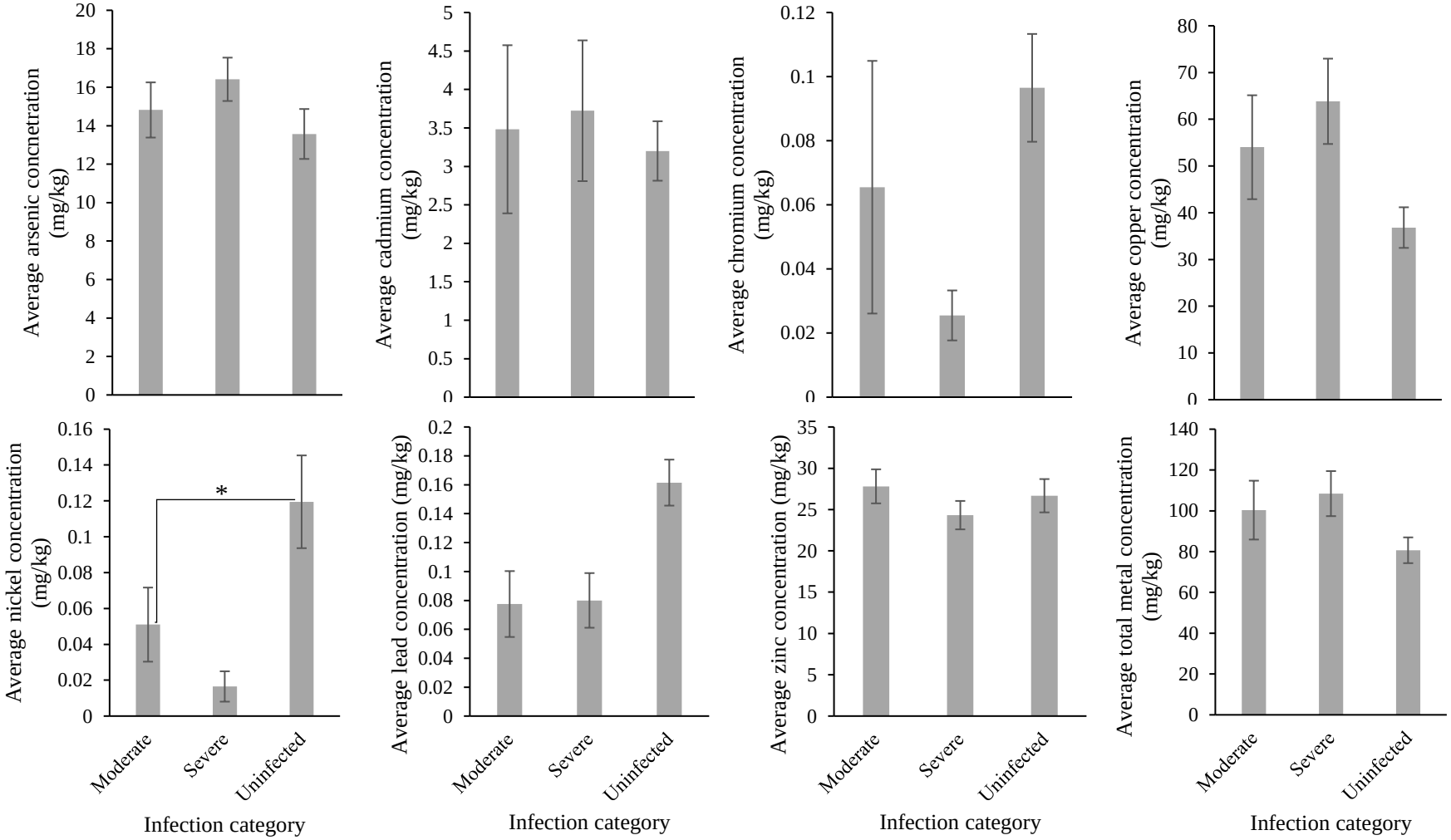


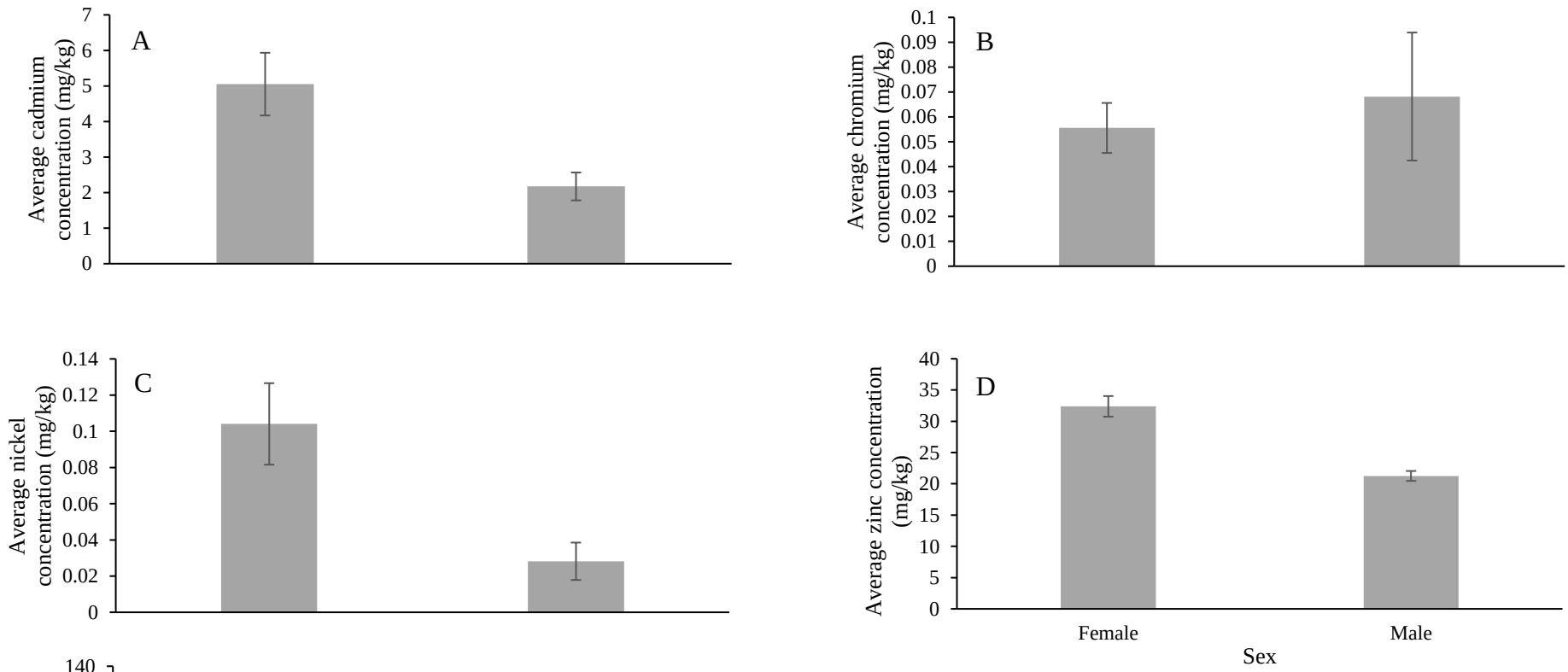
Figure 26: The average concentration (± Standard error) of heavy metals in the brown meat of Cancer pagurus *that were uninfected (0% of the carapace covered in black lesions), moderately infected (5-10% of the carapace covered in black lesions) or severely infected (over 10% of the carapace covered in black lesions) with shell disease syndrome. The number of individuals within each infection category was 20. * indicates a statistically significant difference.*

3.6.3 Brown meat heavy metal concentrations within male and female *Cancer pagurus*

The total concentration of heavy metals in female *C. pagurus* were significantly greater than in males, with females displaying a 41% greater mean total heavy metal concentration than males (Figure 27; Table 8). There was also a significant difference in cadmium concentrations, with female *C. pagurus* containing over double the concentration than was found in males (Figure 27; Table 8). Although a nearly significant increase concentration of chromium was observed in male *C. pagurus*, an average of only 0.01mg/kg more were detected in male crabs (Figure 27; Table 8). While the average concentration of copper was 43% more in female *C. pagurus* than in males, no significant difference was observed (Table 8). Nickel concentrations were also significantly higher in female *C. pagurus*¸ although, the average concentration was only 0.07mg/kg greater in females (Figure 27; Table 8). There was a minimal increase of 0.06mg/kg of lead in the brown meat of female *C. pagurus* (Figure 27; Table 8). However, the 52% increase in female *C. pagurus* zinc concentrations was significantly different (Figure 27; Table 8). In addition, the average total metal concentration in the brown meat of female *C. pagurus* were significantly greater than males, exhibiting a 40% increase (Figure 27; Table 8).

Table 8: The results of ANOVA and Kruskall-Wallis tests, which were conducted to determine if there was a significant variation in average Cancer pagurus *brown meat metal concentrations (mg/kg of wet weight) between gender. Average values given ± standard error. * indicates a statistically significant difference, ** indicates a nearly significant difference. Total metals were calculated through the sum of arsenic, cadmium, chromium, copper, nickel, lead and zinc concentrations. Data transformations: † = natural log, †† = 1/4th root. 33 male and 27 female* Cancer pagurus *were sampled in the Isle of Man between June and August 2016.*

Metal	Sex	Average ± SE (mg/kg)	F/χ^2	d.f.	p
Arsenic †	Female	15.25 ± 1.17	0.19	1, 58	0.665
	Male	14.67 ± 0.98			
Cadmium ††	Female	5.05 ± 0.88	11.91	1, 58	0.001*
	Male	2.17 ± 0.39			
Chromium	Female	0.06 ± 0.01	3.78	1	0.052**
	Male	0.07 ± 0.03			
Copper †	Female	61.83 ± 9.20	3.25	1, 58	0.076
	Male	43.17 ± 5.22			
Lead	Female	0.14 ± 0.02	2.13	1, 58	0.15
	Male	0.08 ± 0.02			
Nickel	Female	0.10 ± 0.02	8.51	1	0.004*
	Male	0.03 ± 0.01			
Zinc †	Female	32.39 ± 1.65	39.76	1, 58	<0.001*
	Male	21.26 ± 0.79			
Total metals †	Female	114.82 ± 11.16	7.80	1, 58	0.007*
	Male	81.46 ± 6.50			



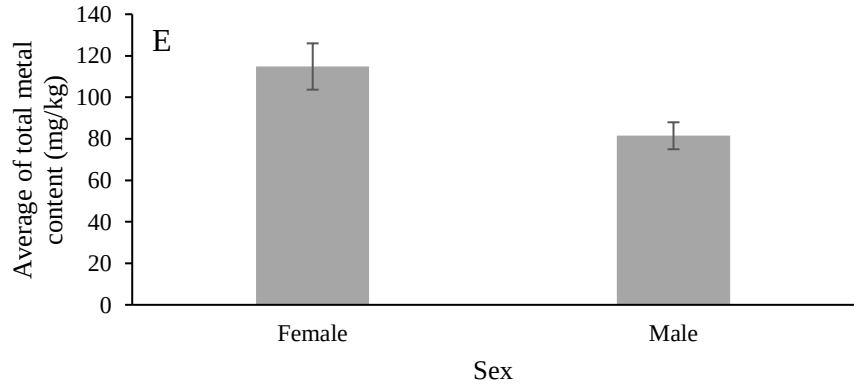


Figure 27: The average (± standard error) concentration (mg/kg of wet weight) of cadmium (A), chromium (B), nickel (C), zinc (D) and total metals (E) in male and female Cancer pagurus *where statistically significant or nearly statistically significant differences occurred. Total metals were calculated through the sum of arsenic, cadmium, copper and zinc concentrations. Note the different scale on the y axis between metals. Statistical tests were conducted on transformed data. 33 male and 27 female* Cancer pagurus *were sampled in the Isle of Man between June and August 2016.*

3.6.3 Correlations between *Cancer pagurus* brown meat heavy metal concentrations and size

During the current study, it was found that as the size of *C. pagurus* increases, so does the concentration of cadmium, nickel and zinc (Figure 28; Table 9). However, chromium concentrations were found to significantly decrease as the crab got larger (Figure 28; Table 9). In addition, arsenic, copper, lead and the total metal content did not significantly correlate with carapace size (Table 9).

Table 9: Results from the Spearman's rank correlation tests, which investigated the relationship between heavy metal concentrations (mg/kg of wet weight) in the brown meat of 60 Cancer pagurus *samples and the carapace width (mm) of the crab. The degrees of freedom in all tests were 58, although chromium had 57, due to the removal of one outlier. Total metals were calculated through the sum of arsenic, cadmium, copper and zinc concentrations.*

Metal	ρ	p
Arsenic	0.071	0.590
Cadmium	0.441	<0.001*
Chromium	-0.447	<0.001*
Copper	0.130	0.323
Lead	0.138	0.294
Nickel	0.296	0.021*
Zinc	0.409	0.001*
Total metals	0.228	0.080

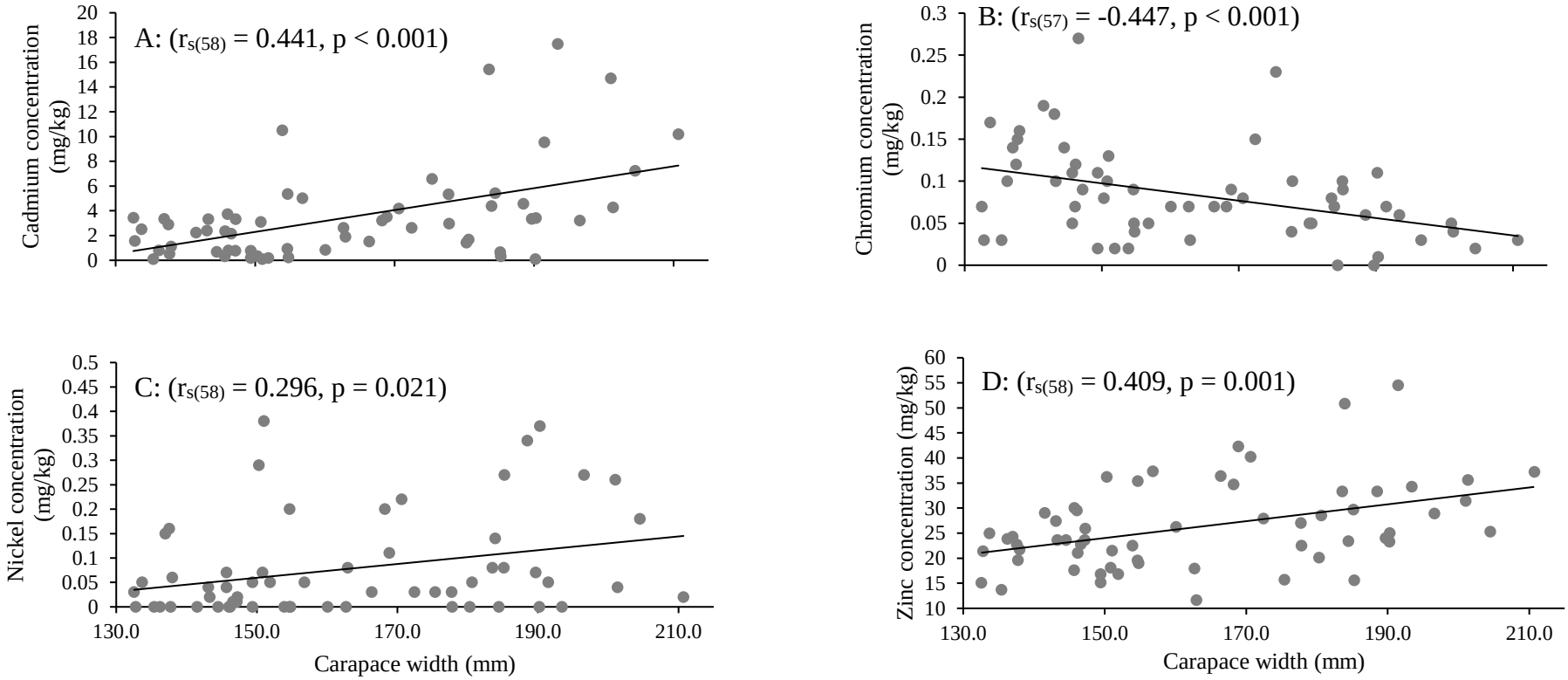


Figure 28: Significant Spearman's ranked correlations showing the relationship between brown meat cadmium (A), chromium (B), nickel (C) and zinc (D) concentrations in Cancer pagurus *(mg/kg of wet weight) and carapace width (mm). Text on each graph depicts the ranked Spearman's correlation and significance values along with the degrees of freedom in each test. Note the different scales on the y axis between the different metals. One outlier from the chromium analysis was removed*

3.6.4 Heavy metal concentration correlations

Pearson's product-moment tests conducted on data, with different transformations applied (Table 10), determined that the concentration of arsenic, cadmium, copper and zinc in the brown meat of *C. pagurus* all significantly positively correlated with one another (Figure 29; Table 10). However, spearman's rank order correlation tests found that concentrations of chromium displayed a significant negative correlation with the concentration of cadmium and copper in the brown meat of *C. pagurus* (Figure 30; Table 11). Additionally, the concentration of lead or nickel did not significantly correlate with the concentration of any other metal in the brown meat of *C. pagurus* (Table 10; Table 11).

Table 10: Results of Pearson's product-moment correlation tests conducted to determine whether the concentrations of the metals that were isolated in the brown meat of 60 Cancer pagurus *sampled, taken from the Isle of Man, significantly correlated. Data transformations: † = natural log, †† = 1/4th root.*

Correlation test		t	Correlation	p
Arsenic†	Zinc†	1.89	0.241	0.047*
	Copper†	6.12	0.626	<0.001*
	Cadmium††	4.75	0.525	<0.001*
	Lead	-0.21	-0.027	0.835
Cadmium††	Zinc†	4.50	0.509	<0.001*
	Copper†	6.27	0.635	<0.001*
Copper†	Zinc†	3.36	0.404	0.001*
	Lead	0.64	0.084	0.524
Zinc†	Lead	0.62	0.081	0.540

Table 11: Results of Spearman's correlation tests conducted to determine whether the concentrations of metals isolated from the brown meat of 60 Cancer pagurus *sampled from the Isle of Man significantly correlated. The degrees of freedom in all tests were 58 apart from chromium, which was 57, due to the presence of one outlier.*

Correlation test		ρ	p
Arsenic	Chromium	-0.150	0.256
	Nickel	-0.081	0.537
Cadmium	Chromium	-0.275	0.034*
	Nickel	0.204	0.119
	Lead	0.017	0.897
Copper	Chromium	-0.390	0.002*
	Nickel	0.210	0.108
Chromium	Nickel	-0.097	0.466
	Lead	-0.071	0.591
	Zinc	-0.101	0.448
Nickel	Lead	0.191	0.143

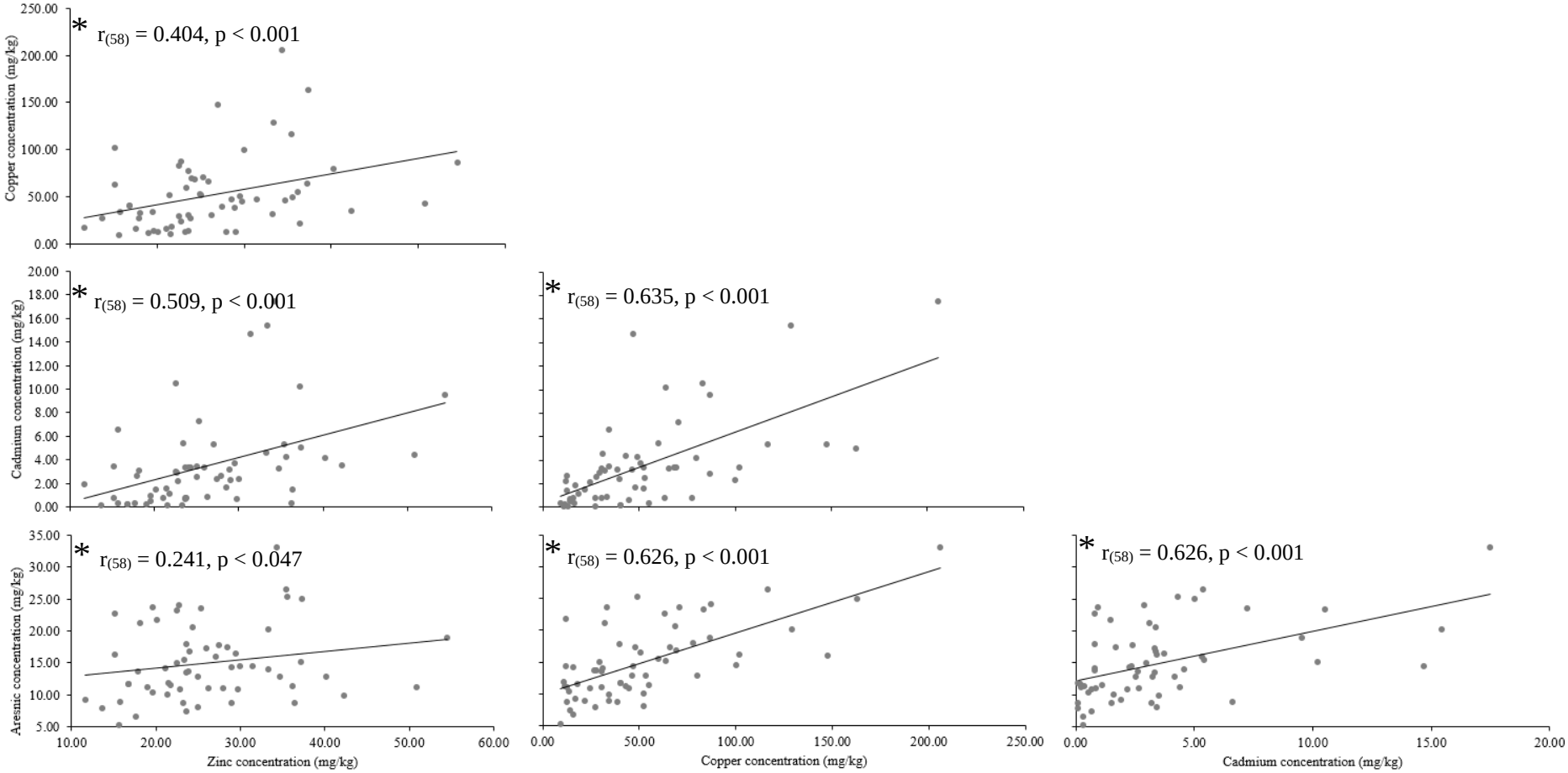


Figure 29: The relationship between the concentrations (mg/kg of wet weight) of arsenic, cadmium, copper, and zinc, which were isolated from the brown meat of 60 Cancer pagurus *samples. * indicates a significant correlation, as determined by Pearson's product-moment correlation tests. Pearson product-moment correlation tests were conducted on transformed data. Text on each graph represents the correlation, degrees of freedom and significance value.*

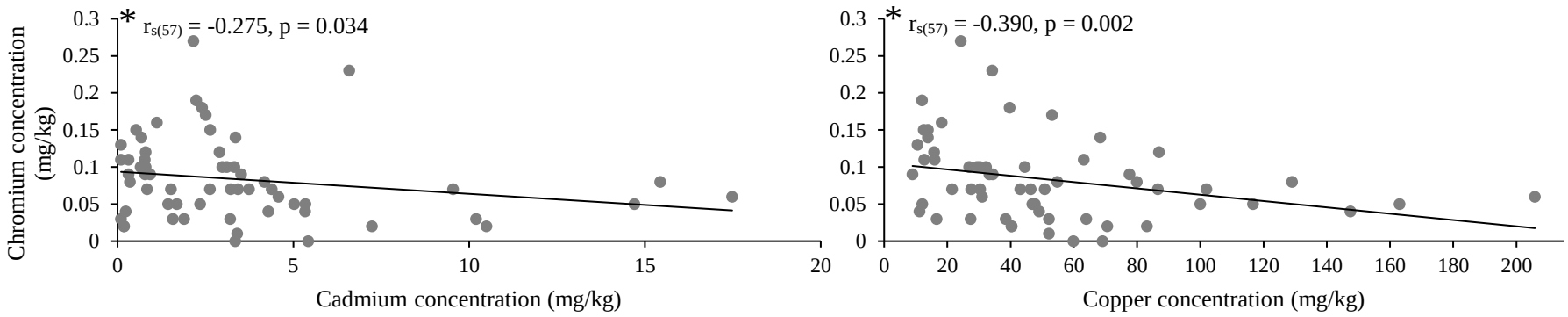


Figure 30: The concentration (mg/kg of wet weight) of chromium's correlation with cadmium and copper in the brown meat of 60 Cancer pagurus *samples taken from the Isle of Man. * indicates a significant correlation, as determined by Spearman's rank correlation tests. Text on each graph represents the correlation, degrees of freedom and significance value.*

4.0 Discussion

4.1 Prevalence and severity of shell disease syndrome in *Cancer pagurus*

4.1.1 Overall prevalence and severity of shell disease syndrome in the Isle of Man's *Cancer pagurus* fishery

This study found that the overall prevalence of shell disease syndrome in the Manx *C. pagurus* population was 40.28%, a value that is considerably greater than the natural prevalence of 10% within crustaceans that was defined by Getchell (1988). Additionally, within other Brachyuran crab species fisheries, the prevalence of shell disease syndrome often exhibits interspecific variability. For example, Rosen (1970) found a 3% prevalence of shell disease in the blue crab, *Callinectes sapidus*, population in Chesapeake Bay. Also, Benhalima *et al*. (1998) observed the prevalence of shell disease within the population of snow crabs, *Chionoecetes opilio*, in southern Gulf of St Lawrence, Canada, which was 2%. Both the snow and blue crab populations that were studied have a much lower prevalence of shell disease than what was found in the Manx *C. pagurus* population. Conversely, populations of red crabs, *Geryon quinquedens*, that were caught at depths of over 100m within continental shelf seas off the east coast of New York, displayed a much higher shell disease syndrome infection prevalence than was observed within the current study, with the prevalence within the red crab population reaching 90% (Bullis, *et al*., 1988).

In the current study, the observed prevalence (40.3%) and severity (3.9%) of shell disease in the Isle of Man's *C. pagurus* fishery was nearly double that of the 24.4% incidence rate and average severity (2.1%) detected in 2012 by King *et al.* (2014). The prevalence of shell disease within the current study was also three times greater than prevalence (12%) detected in 2015 by Haig *et al*. (2016). However, it is worth noting that the sample size used in King *et al*., (2014) was considerably larger for the prevalence (2361 in 2012 compared to 503 in 2016, respectively) and severity (429 in 2012 compared to 199 in 2016, respectively) analysis. In addition, King *et al*. (2014) sampled a larger proportion of the *C. pagurus* fishery. The sample size used by Haig *et al*. (2016) (274 individuals) was smaller than the sample size used in the current study. The variance in the number of *C. pagurus* individuals sampled between this study, King *et al*. (2014) and Haig *et al*. (2016) may have influenced the observed prevalence of shell disease. The reduced sampling effort in this study when compared to King *et al*. (2014) was due to adverse weather conditions restricting the number of sampling voyages and vessels

that participated in the study. Moreover, as the prevalence of shell disease varied between localities, the larger number of grid squares sampled by King *et al*. (2014) may have accounted for the reduced the average prevalence that was observed. A delayed arrival of *C. pagurus* to the Isle of Man in 2016 may have affected shell disease intensities and incidence rates as there may have been a reducing fishing effort prior to conducting the current study, which may have decreased the rate at which infected *C. pagurus* were harvested from the population through commercial exploitation (Local fishers, 2016, pers. comm.). However, there is no evidence for commercial harvesting lowering shell disease syndrome incidence rates in the literature. Indeed, a 10% decrease in total landings between January and May, which were the months prior to the 2012 and 2016 study, was observed in 2016 when compared to 2012 (DEFA, 2016, Unpublished data). Despite the decrease in total *C. pagurus* landings during 2016, no significant decrease in average monthly *C. pagurus* landings occurred prior to surveying between 2012 and 2016 (DEFA, 2016, Unpublished data). Variation in shell disease intensities between this study and Haig *et al*. (2016) could also be attributed to differences in sampling dates, as Haig *et al*. (2016) sampled during the winter whereas the current study sampled during the summer. Additionally, the regions that were sampled around the Isle of Man by Haig *et al*. (2016) were not clearly defined and therefore the incidence rate of shell disease may not be representative of the entire fishery. Furthermore, the operator variability in the identification of smaller black lesions on the carapace of *C. pagurus* may have accounted for the increased prevalence of shell disease observed since the King *et al*. (2014) and Haig *et al.* (2016) studies. However, the effect of operator variability was minimised between this study and King *et al*. (2014) as the methods were identical.

Comparisons made with other studies conducted that investigated the incidence of shell disease syndrome in *C. pagurus* fisheries that are located elsewhere in the British Isles indicate that the Isle of Man fishery encompasses one of the largest populations of shell disease infected crabs in the British Isles. The observed prevalence of 40.28% of shell disease syndrome in the current study is considerably greater than the 30% and 18% reported in the Cornish and Shetland Isles, Scotland, fisheries, respectively (Tallack, 2002; Davies, 2007). However, shell disease prevalence is greater in the Langland Bay, Wales, *C.* pagurus population (60.8%) (Vogan, *et al*., 1999). Again, the variation in shell disease prevalence between studies may have been due to operator variability in the detection of low severities of infection, the effect of which may have been further exacerbated between the investigations discussed above and this study due to a variation in sampling regime. Operator variability between studies may have

contributed to the increased incidence of shell disease in the Manx *C. pagurus* fishery observed as this study utilised photograph analysis. Re-analysing still images enabled the *C. pagurus* specimens to be double checked for the manifestation of shell disease, which reduced the likelihood of organisms displaying lower severities of infection being not being counted, which could have increased incidence rates within the current study. To reduce the effect of operator variability between studies characterising shell disease syndrome within a *C. pagurus* fishery, a standard protocol during the assessment methodology should be implemented, which takes into account the species size distribution, sampling effort, timing and methodological process. Should a standardised methodology be employed when assessing shell disease within *C. pagurus* fisheries, valid statistical comparisons between fisheries could be conducted. Furthermore, a significant variation in the prevalence of shell disease syndrome between male and female *C. pagurus* has been observed in several studies (Vogan, *et al*., 1999; Tallack, 2002; King, *et al*., 2014). Therefore, a variation in the sex ratio of observed *C. pagurus* between studies may also account for the change in the observed shell disease incidence. Additionally, due to differences in the seasonal migration of male and female *C. pagurus*, which can influence catch sex ratios within a population (Brown & Bennett, 1980), a difference in sampling dates chosen between studies may also account for the variation in shell disease prevalence observed. However, abiotic factors and anthropogenic sources of pollution could also account for the increased prevalence and severity of shell disease syndrome in the Isle of Man's *C. pagurus* population.

The severity of shell disease syndrome in the *C. pagurus* population surveyed in this study was considerably higher than that observed in other studies around the British Isles. For example, the average gender severities within two sites located on the Gower Peninsula in South Wales, UK, were substantially lower (Langland Bay: Male = 1.0% ± 0.22, Female = 0.2% ± 0.02; Rhossili Bay: Male = 0.4% ± 0.09, Female = 0.1% ± 0.03) (Vogan & Rowley, 2002a). However, the techniques that were used to quantify infection severity by Vogan and Rowley, (2002a) were considerably different than the methods used in this study. Therefore, the severity data from Vogan and Rowley, (2002a) and this study cannot be compared. Should a shell disease severity study, which uses the same methods as the current study, be conducted to assess the *C. pagurus* population around the Gower Peninsula, factors that have been shown to influence shell disease severities, such as regional sediment characteristics and land use, should be taken into account to enable later studies to compare such factors and to determine if a cause-effect relationship exists.

The increased intensity of shell disease in the Manx *C. pagurus* population could be due to regional sediment characteristics. For instance, coarse sediments are thought to have a significant impact on incidence and severities of shell disease as coarser sediments will have an abrasive action of the sediment grains on the shell of crabs (Young, 1991; Vogan, *et al*., 1999; Vogan, *et al*., 2008), which can remove the outer cuticle and enable chitinovorous organisms to access to the chitinous shell (Young, 1991; Vogan, *et al*., 1999). In addition, coarser sediments in the sample region could contain higher abundances of faecal bacteria (Howell, *et al*., 1996). The presence of finer sediments within the sample regions may have also influenced shell disease intensities as finer sediments are known to contain more contaminants, such as heavy metals (Caeiro, *et al*., 2005; Liu, *et al*., 2006). Habitat maps of the Manx benthos conducted at the same time as this study, as well as a previous studies by Hinz, *et al*. (2010) and White (2010), found both coarse and fine sediments in all of the areas sampled (Allison, 2016, pers. comm.; Dempster, 2016, pers. comm.), which can create the abrasive effect described by Young (1991) and damage the outer cuticle of *C. pagurus* in addition to containing more bacteria (Howell, *et al*., 1996) and pollutants (Caeiro, *et al*., 2005; Liu, *et al*., 2006), which leads to an increase in shell disease infection intensity (Young, 1991; Vogan, *et al*., 1999). Furthermore, during 2013, 4,000 tonnes of highly contaminated materials were dredged from Peel Marina and disposed offshore (Kennington, 2015), which would have significantly contaminated local habitats and species. The disposal of the contaminated materials offshore will have contaminated species that reside on the benthos, including *C. pagurus*, which may have increased their susceptibility to disease.

Land use around the Isle of Man, in particular, the amount of land utilised for agricultural purposes, could be a causative agent that contributes to the high shell disease intensities observed in the local *C. pagurus* population. The percentage of land used for agriculture may increase shell disease intensities due to use of chemicals commonly used in farming, such as insecticides and the growth regulator methoprene, which have been found to inhibit the development of the outer cuticle of the shell and the synthesis of chitin in blue crabs, *C. sapidus*, (Horst & Walker, 1999). Walker *et al*. (2005a; 2005b) found that, in adult American lobsters, *Homarus americanus*, methoprene inhibited chitin synthesis as the hormone analogue was found to accumulate in the epidermal cells. Additionally, studies into the effects of exposure to the insecticides resmethrin and malathion have shown that American lobsters, *Homarus americanus*, exhibit a reduced phagocytic ability for three weeks after exposure, which reduces their immunocapability (De Guise, *et al*., 2004; De Guise, *et al*., 2005). Whilst

it is unknown to the author whether the use of methoprene, resmethrin and malathion is standard practice in the Isle of Man's agricultural industry, it is highly likely that insecticides and growth hormones in some form are used, which may interfere with crustacean chitin and cuticle synthesis and therefore increase shell disease incidence within the Isle of Man's *C. pagurus* population.

Individual *C. pagurus* that are infected with shell disease syndrome are often unmarketable as they fail to meet consumer expectations, this results in a financial loss to the fishery as profitable catch yields are reduced. With incidence rates of shell disease nearly doubling in the past three years, the fishery is indisputably suffering increasing financial losses from shell disease syndrome. However, shell disease syndrome infection of *C. pagurus* does not always equate to rejection and failure to sell. Consequently, a financial loss is not always incurred. Personal observations during this study, and by King (2012), noted that *C. pagurus* that exhibited signs of shell disease syndrome severity less than 5% were still accepted by the fishers and successfully sold to the processors. However, the estimation of 5% of infection severity as an upper limit of organism acceptance was only conducted *in situ* and the discard threshold was not quantified. Therefore, it is recommended that a more detailed study be conducted to determine what the actual shell disease infection severity displayed by *C. pagurus* must be before rejection occurs. Calculations of the acceptable severity of shell disease syndrome would enable a more comprehensive investigation into the financial impact of shell disease syndrome within the Manx *C. pagurus* fishery to be made. Moreover, due to the recent development of the common whelk, *B. undatum*, fishery around the Isle of Man (Fahy, 2001), a high severity of shell disease syndrome in *C. pagurus* is not a total financial loss to fishers as infected crabs can be retain or sell the infected *C. pagurus* at a reduced rate for use as whelk bait (Local fishers, 2016, pers. comm.).

4.1.2 Sample location shell disease syndrome prevalence and severity

Sample locations that displayed greater intensities of shell disease syndrome were observed during the present study, both spatially within this study sites and temporally between the current study and the King *et al*. (2014) study. The spatial and temporal differences in shell disease intensity may have been driven my several factors. Variation in shell disease prevalence and severity was noted between male and female *C. pagurus*; if different sex ratios between sample sites within the current study and 2012 were observed, shell disease intensities may have varied. Regional sediment characteristics and pollution from nearby sewage outfalls may

have influenced the incidence rate and severity shell disease syndrome. There are well-documented cases both within the sample area (King, *et al*., 2014) and in other fisheries (Tallack, 2002; Vogan & Rowley, 2002a), of a greater prevalence and severity of shell disease in male *C. pagurus* to be greater than females. Therefore, the greater proportion of males surveyed within grid squares K12 and L12 may have accounted for the increased prevalence and severity of shell disease observed. However, an increased intensity was also observed within grid square K11, a site which had significantly more sampled females than males. Also, no significant variation in the prevalence of shell disease syndrome was observed in grid square K12, where significantly more males than expected were sampled. Furthermore, there was no significant increase in shell disease prevalence between male and female *C. pagurus* observed in the current study. A significant temporal increase in shell disease prevalence and severity was observed in almost all sample squares even though more males were surveyed during the 2012 study conducted by King *et al*. (2014), which could indicate that the increase in shell disease intensity during 2016 was more severe than was calculated during the current study. A 10% decrease in *C. pagurus* landings in the months prior to surveying during this study when compared to the 2012 study may have accounted towards the temporal increase in shell disease intensities within the sample sites. However, there are no data to quantify the total landings within the sample squares. Spatial differences between sample site sex ratios may have occurred due to the habitat structure of sampling areas, because females migrate to sandy offshore habitats post-coitus (Hartnoll, 1969). According to biotope maps created by White (2010) and Hinz *et al*. (2010), the benthic sediment of the offshore locations sampled in grid squares K10 and K11 are predominantly composed of sandy substrate, which may have influenced the sex ratio observed (Hartnoll, 1969), which could have led to in a lower prevalence and severity of shell disease. While more males will have been present in sample squares K10 and K11 than were observed in this study, males were more likely to be located within the rockier substrates found further inshore (Pawson, 1995; White, 2010; Hinz, *et al*., 2010). While a study that samples shell disease in both rocky inshore and sandy offshore sites would enable a more accurate assessment of shell disease intensity in the Manx *C. pagurus* population. Accordingly, if only small scale local fishers are fishing the inshore regions, studying shell disease within such areas would be of little importance to the greater fishery and would result in prevalence and severity data that was unrepresentative of the majority of the fisher fishery. In addition, the increased prevalence of shell disease in sample squares K10 and K11 could be due to the close proximity of a sewage effluent pipe to the sample squares (Figure 31).

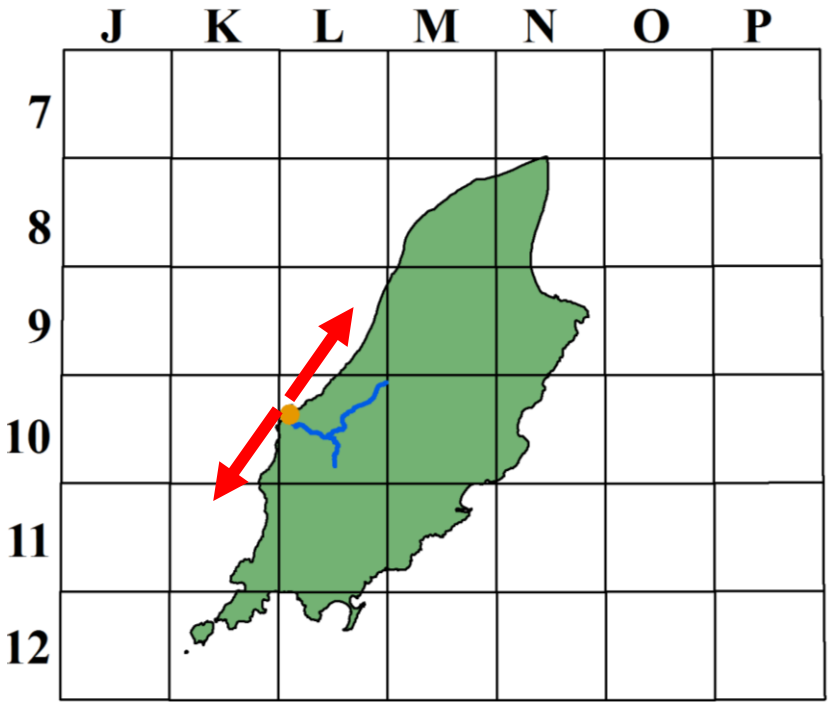


Figure 31: The location of a raw sewage effluent pipe located near grid squares K10 and K11, along with the extent and direction of the ebb tides, as depicted by the red arrows.

Marine habitats located near sewage outfalls have been shown to encompass crustacean populations with high incidences of shell disease syndrome. For example, American lobsters, *H. americanus*, exposed to sediment taken from a sewage dumping ground around New York were found to have an increased prevalence of shell disease lesions (Young & Pearce, 1975). However, the Young & Pearce (1975) study was conducted within aquarium conditions and therefore may not be representative of the processes that occur in the natural environment. Studies on the effects of sewage effluent pipes discharge on the prevalence of shell disease syndrome in *C. pagurus* caught off Langland Bay in South Wales, into which a wastewater effluent pipe used to drain, also displayed an increased prevalence and severity of shell disease (Vogan, *et al*., 1999). However, amid concerns over the effects the sewage pipe's discharge on the local environment and as an effort to increase the quality of the water to achieve bathing beach status, the effluent pipe was decommissioned in 1999, which allowed a comparative shell disease prevalence study to be conducted in 2003 (Powell & Rowley, 2005). Powell & Rowley (2005) found no significant decrease in the prevalence and severity of shell disease syndrome after the closure of the sewage outfall pipe in the local *C. pagurus* population, indicating that the link between sewage effluent and shell disease may not be as clear as initially thought.

Despite the tenuous link between sewage and shell disease in Langland Bay (Powell & Rowley, 2005), the potential for an increased intensity of shell disease due to habitat degradation via sewage in the Isle of Man's *C. pagurus* population should not be disregarded.

It has been well documented that global water temperatures are rising due to global warming; while rising temperatures may not directly increase the prevalence and severity of shell disease syndrome, there is potential for a cumulative impact. Increased temperatures may increase disease susceptibility in all crustacea as temperature is known to affect rates of metabolism, oxygen consumption, survival, moulting, growth, and immune system (Dean & Vernberg, 1966; Chen, *et al*., 1995; Hennig & Andreatta, 1998). Increased temperatures are also known to increase the rate at which bacteria and fungi reproduce, which are causative agents of shell disease syndrome (Sindermann, 1989; Stewart, 1993; Vogan & Rowley, 2002; Vogan, *et al*., 2008; Stentiford, 2008). Moreover, increased temperatures are known to increase enzyme activity (Cornish-Bowden, 2012), furthering the rate at which chitinase, the enzyme responsible for breaking down chitin within chitinovorous organisms (Sindermann, 1989; Vogan & Rowley, 2002; Vogan, *et al*., 2008; Stentiford, 2008), can break down chitin and therefore increase shell disease intensity in decapod crustaceans. A recent review of the potential impact of climate change on diseases of shellfish by Rowley *et al*. (2014) in the Irish Sea also predicted that the increased temperatures associated with global warming could increase disease susceptibility. However, the current understanding of shell disease associated pathogens relationships with temperature is too limited to confidently predict the extent of climate changes effect on shell disease diseases in crustaceans (Rowley, *et al*., 2014).

4.2 Heavy metal pollution inducing shell disease syndrome in Manx *Cancer pagurus* population

The environmental factors that can influence the susceptibility of shell disease infection within marine crustaceans are not well researched and often extrapolation from studies that found lower haemocyte counts, and therefore reduced immunocapabilities, within crustaceans under different conditions have to be made. Environmental factors, such as temperature (Truscott & White, 1990) and sediment pollution (Sindermann, 1979; Truscott & White, 1990; Harris & Santos, 2000), including sediments contaminated with polychlorinated biphenyls, polynuclear aromatic hydrocarbons (Sindermann, 1979), and heavy metal contamination (Sindermann, 1979; Truscott & White, 1990; Sindermann, 1979; Truscott & White, 1990; Harris & Santos, 2000; Le Moullac & Haffner, 2000; Lorenzon, *et al*., 2001), have been shown

to influence the immunological response time in crustaceans, which may also be linked to the increase in the susceptibility of *C. pagurus* to shell disease syndrome.

It was expected that heavy metal contamination in the brown meat of *C. pagurus* would be higher in individuals that had an increased incidence and severity of shell disease syndrome because crustaceans contaminated with heavy metals exhibit reduced immunocapabilities (Sindermann, 1979; Truscott & White, 1990; Le Moullac & Haffner, 2000; Lorenzon, *et al*., 2001). However, the findings of the current study indicate that the concentration of heavy metals within the brown meat of *C. pagurus* had no effect on the severity or susceptibility of *C. pagurus* to shell disease. However, although statistically insignificant, the average concentration of several heavy metals, including total metal content, isolated from the brown meat of *C. pagurus* were between 16% and 73% lower in uninfected crabs. The reduced average metal concentrations in uninfected *C. pagurus* could indicate that this study required a larger sample size for a significant difference to be detected. Also, due to the difficulty in ascertaining how long the individual *C. pagurus* had spent in the sample area, this study did not take into account sample location. Disregarding sample position may have concealed the influence of metal concentrations on shell disease infection as coastal sediments are known to have highly variable metal concentrations throughout the Isle of Man (Southgate, *et al*., 1983; Daka, *et al*., 2003; Kennington, 2013; Kennington, 2015); therefore, the uptake of metals in crabs caught in more polluted habitats could have been greater (Harris & Santos, 2000). Furthermore, mercury and zinc have been identified as agents that reduce the rate at which chitinovorous organisms, which are the primary cause of shell disease syndrome (Sindermann, 1989; Stewart, 1993; Vogan & Rowley, 2002; Vogan, *et al.*, 2008; Stentiford, 2008), can digest chitin (Vogan, unpublished observations). Incidentally, mercury and zinc, which may reduce the rate at which chitinase can digest chitin, are also known to bind to chitin (Muzzarelli, 1977), which may influence shell disease severities. Therefore, analysis of metals bound to the shell of *C. pagurus* may be more appropriate for determining the effects of metals on shell disease severity.

Despite the present study finding no apparent link between metal pollution and shell disease, a study investigating metal contents with blue crabs, *C. sapidus*, found that higher quantities of heavy metals, particularly manganese, within the organs of shell disease infected organisms (Weinstein, et al., 1992). However, Weinstein *et al*. (1992) concluded that metals only played a minor role in shell disease infection within the Albemarle-Pamlico estuarine system, North Carolina, USA. The findings of Weinstein *et al*. (1992) suggested that the metals

that were most responsible for increased incidence of shell disease were not quantified in this study. Additionally, several other factors that can influence shell disease intensities within a population have been identified in the literature, which may have concealed the impact of heavy metal contamination on shell disease syndrome in the Manx *C. pagurus* population.

4.3 Heavy metals in the brown meat of *Cancer pagurus*

The current study found concentrations of all the heavy metals that were surveyed to be above the detection limit in the brown meat of *C. pagurus* in over 68% of the population surveyed, and the concentrations of arsenic, cadmium, copper and zinc were above the detection limit in every sample analysed.

Arsenic is a highly toxic (Mayer, *et al*., 1993) and carcinogenic (Kaur, *et al*., 2011) metal found in many food sources. However, relatively little is done in Europe to monitor and control consumer's dietary intake of arsenic in non-rice products due to the difficulty of quantifying arsenic in several food sources. This study found arsenic to range between 5.21-33.13mg/kg, with an average concentration of 14.93mg/kg ± 0.75. Conversely, a study by CEFAS along the British coastline during 2013 found that the concentration of arsenic in *C. pagurus* brown meat varied between 0.35-29mg/kg, with an average concentration of 9.81mg/kg (Bolam & Bersuder, 2013a), which is 34% lower than what was found within this study. The increased concentration of arsenic in the brown meat of Manx *C. pagurus* observed in this study when compared to Bolam & Bersuder (2013a) may indicate that the Isle of Man's crab fishery could be under threat if maximum arsenic concentrations within crab brown meat are set in the future. Moreover, the increased concentration of arsenic in the brown meat of *C. pagurus* caught to the Isle of Man could indicate that anthropogenic sources of arsenic pollution, such as pesticides used in agriculture (Azcue & Nriagu, 1994; Garelick, *et al*., 2008) or the Isle of Man's mining history (Lamplugh, 1903; Garrod, *et al*., 1972; Garelick, *et al*., 2008), are causing contamination of the Isle of Man's marine environment, especially as many marine invertebrates are bioindicators of heavy metal pollution (Chiarelli & Roccheri, 2014). However, the usefulness of *C. pagurus* as a bioindicator of metal contamination is uncertain due to the migratory nature of the species.

Cadmium concentrations within the brown meat of *C. pagurus* are relatively well researched by the food standards agency (FSA, 2013), CEFAS (Bolam & Bersuder, 2013a; Bolam & Bersuder, 2013b) and other European food safety authorities (Garrett, *et al*., 2015; VKM, 2015). Furthermore, the Italian and Chinese food authorities both rejected *C. pagurus*

due to the cadmium content within the brown meat (BBC, 2015; RASFF, 2016). Even in low concentrations, cadmium is toxic and carcinogenic to humans (Koizumi & Li, 1992) and is known to bioaccumulate in animals (Croteau, *et al*., 2005). Overexposure to cadmium can induce nausea, diarrhoea and vomiting when consuming highly contaminated food items (EFSA, 2009; Thévenod & Lee, 2013). Long term chronic exposure to cadmium can result in infertility (Benoff, *et al*., 2000), itai-itai ('ouch-ouch') disease (Åkesson, *et al*., 2014), deterioration of renal functions (EFSA, 2009) and impaired DNA repair (Åkesson, *et al*., 2014). Therefore, monitoring dietary intake of cadmium from food sources, such as the brown meat of *C. pagurus*, should be paramount. However, a maximum cadmium concentration of 0.5 mg/kg has only been set for the white meat of crabs, not the brown meat. This study found that cadmium concentrations ranged between 0.1mg/kg and 17.48mg/kg and averaged at 3.47mg/kg ± 0.48, which is almost identical to the mean of 3.9mg/kg (FSA, 2013) and 3.36mg/kg (Bolam & Bersuder, 2013a) found by other studies conducted in the British Isles. All studies that quantified cadmium in *C. pagurus* that were caught in the British Isles have shown concentrations to be between 7 and 9 times greater than the maximum concentration of 0.5mg/kg of cadmium set for crustacean white meat by EU Regulation No. 1881/2006. Additionally, a study of the concentrations of cadmium in the brown meat of *C. pagurus* consumed in France found the average concentration to be 14.3mg/kg (Noël, *et al*., 2011), which is over 28 times the maximum permissible concentration in crustacean white meat in Europe (EU Regulation No. 1881/2006) and over 4 times the concentration observed in this study. In all of the studies discussed, the majority *C. pagurus* surveyed contained a considerably greater concentration of cadmium in the brown meat than the maximum limit set by European guidelines for the white meat indicating a need to inform consumers about how much brown meat they should consume based on the tolerable weekly intake (TWI) of cadmium of 2.5µg/kg of body weight, as was done by the Norwegian food safety authority (VKM, 2015). VKM (2015) calculated that only 0.48 of a filled shell crab should be consumed by an adult, and 0.13 for adolescents, as not to exceed the TWI of cadmium. However, setting a maximum amount of brown meat that should be eaten by consumers may be difficult to attain due the high variation observed in brown meat cadmium concentrations. Furthermore, if maximum cadmium concentrations in crustaceans sold were set uniformly at 0.5mg/kg, *C. pagurus* fisheries would incur a severe economic decline. Similar levels of cadmium observed in this study and by Bolam & Bersuder (2013a) indicates that that cadmium contamination is uniform throughout the British Isles, because crabs can be used as bioindicators of heavy metal pollution (Chiarelli & Roccheri, 2014). However, as previously discussed, *C. pagurus* may not

be the most accurate indicator of metal contamination. Samples collected from France displayed considerably greater levels of cadmium pollution than have been observed in the British Isles (Noël, *et al*., 2011; Bolam & Bersuder, 2013b), signifying that cadmium contamination in other European fisheries may be much greater.

Although the dietary intake of chromium is currently not controlled by current European legislation, hexavalent chromium can be carcinogenic to humans (Costa & Klein, 2006; Smith & Steinmaus, 2009). Even so, there is little evidence of hexavalent chromium's presence within crustaceans. This study found chromium concentrations to be very low (average: 0.06mg/kg ± 0.01; range: 0.00-0.80mg/kg), which is very similar to the findings of Bolam & Bersuder (2013a) in other fisheries located in the British Isles (average: 0.11mg/kg; range: 0.01-0.96mg/kg). The low concentration of chromium, along with the lack of evidence regarding the presence of the carcinogenic hexavalent chromium in crustaceans, found in the brown meat of *C. pagurus* suggests that additional monitoring is not required.

The average concentration of copper within the brown meat of *C. pagurus*, that was in this study was more than double the concentration found across the rest of the United Kingdom (51.57mg/kg and 23.7mg/kg respectively) (Bolam & Bersuder, 2013a). However, no major health problems were observed when exposed to increased copper concentrations (Turnlund, *et al*., 1990), due to the body's ability to regulate the rate of copper absorption (Turnlund, *et al*., 1989). Despite the ability of the human body to regulate copper intake, copper can be toxic to humans during periods of chronic exposure, which can lead to nervous system dysfunctions (Murata & Araki, 1991). Therefore, high concentrations of copper in *C. pagurus* should be monitored.

Despite the absence of a maximum limit for nickel in food sources, the European Food Safety Authority (EFSA) has set a tolerable daily intake (TDI) of nickel at 0.28mg per kilogramme of body weight (EFSA, 2015). This study found the average concentration of nickel in the brown meat of *C. pagurus* to be 0.06mg/kg, thus, a 60kg individual would have to consume 280kg of *C. pagurus* brown meat before the TDI of nickel was exceeded. Therefore, the concentration of nickel within the brown meat of *C. pagurus* caught in the Manx fishery is not of significant concern. Furthermore, the average concentration of nickel in the brown meat of *C. pagurus* that were captured in the Isle of Man is similar, if not slightly less, than the mean concentration identified in the UK population by Bolam & Bersuder (2013a) (Manx: 0.06mg/kg; U.K.: 0.17mg/kg).

The EU has set a maximum permissible level of 0.5mg/kg for lead concentrations in the white meat of crustaceans due to the ability of the substance to sterilise males (Benoff, *et al*., 2000), inhibit DNA repair and synergistically interact with other carcinogenic mutagens (Steenland & Boffetta, 2000). The maximum permissible concentration of lead was not exceeded within the brown meat of any *C. pagurus* sampled from the Isle of Man (maximum concentration: 0.38mg/kg). Average lead concentrations within the brown meat taken from the Manx *C. pagurus* fishery were similar to samples collected around the UK and the rest of Europe (Europe: 0.08mg/kg; Manx: 0.11mg/kg; UK: 0.06mg/kg) (Noël, *et al*., 2011; Bolam & Bersuder, 2013a). Although, the brown meat taken from some *C. pagurus* samples within the UK fisheries exceeded the limit of 0.5mg/kg set for crustacean white meat by EU Regulation No. 1881/2006 by 6 times (maximum concentration: 3.00mg/kg) (Bolam & Bersuder, 2013a), no crab sampled in this study or by Noel *et al* (2011) exceeded this limit (Isle of Man maximum concentration: 0.38mg/kg; European maximum concentration: 0.22mg/kg). As brown meat lead concentrations exceeded the maximum limit for crustacean white meat in *C. pagurus* samples collected in the UK (Bolam & Bersuder, 2013a), it is important to regularly monitor the Manx fishery to ensure that consumers are not at risk of chronic dietary lead exposure. Furthermore, as there is the potential for *C. pagurus* brown meat to exceed the upper limits of lead concentration set by EU Regulation No. 1881/2006, it is imperative to start monitoring and regulating for lead concentrations within crab brown meat products.

Even though zinc is considered an essential mineral for immunocompetence, neurological function, wound repair, and infant growth and development (EFSA, 2006), regularly exceeding the TDI of 50mg per day of zinc (EFSA, 2006) can impair several physiological functions, including a reduced intestinal copper absorption (Fischer, *et al*., 1981) and impaired immune function (Sandstead, 1994). However, possibly owing to the high TDI of zinc, there are currently no upper limits of zinc concentrations for food products in Europe. Average concentrations of zinc within the brown meat of *C. pagurus* that were sampled in the current study (26.27mg/kg ± 1.12) were only 16% less tahn those found in *C. pagurus* fisheries around the UK (32.0mg/kg) (Bolam & Bersuder, 2013a). While both this study and Bolam & Bersuder (2013a) found examples of *C. pagurus* that had a higher zinc content in a kilogram of meat than the TDI, it is unlikely that consumers will eat a kilogram of brown meat in one day. The average quantity of brown meat required in recipes found online was around 100g, this demonstarates that that an average of only 2.63mg of zinc would be consumed if the crab was caught in the Manx fishery. Thus, there are few concerns regarding the concentration of

zinc in the brown meat portion of *C. pagurus*. However, it is not uncommon for consumers to be taking additional zinc supplements or to have allergic reactions to zinc; as a result, it is important to openly advertise the content of zinc within brown crab meat products.

Several other studies have quantified the concentration of arsenic within the brown meat of *C. pagurus* (Topping, 1972; Barrento, *et al*., 2009a; Barrento, *et al*., 2009b) and other crab species (Al-Mohanna & Subrahmanyam, 2001). However, the methods used in other studies to quantify the concentrations of arsenic are not directly comparable with this study either due to the following reasons: (i) the separation of the hepatopancreas and gonads from the rest of the *C. pagurus* brown meat (Barrento, *et al*., 2009a), (ii) homogenising all edible tissues of the *C. pagurus* (Topping, 1972), (iii) having a small sample size (Topping, 1972), (iV) or quantifying metal concentrations in the dry weight of the product (Al-Mohanna & Subrahmanyam, 2001), whereas this study used the wet weight. Although a convertion ratio of 16.5% can be applied to convert dry weight to wet weight in marine decapods (Ricciardi & Bourget, 1998), the convertion ratio was found to be highly variable througout different decapod populations (Ricciardi & Bourget, 1998). Nevertheless, although Topping (1972) only surveyed 5 crabs at each sample location, similar concentrations of cadmium, lead and zinc in the edible tissues of *C. pagurus* taken from the Arbroath fishery, Scotland (cadmium: 3.6mg/kg; lead: <0.4mg/kg; zinc: 21.3mg/kg) (Topping, 1972) when compared to the Manx fishery. Conversely, the average concentration of copper within the brown meat of *C. pagurus* in the Arbroath fishery (21.7mg/kg) was over half that of the Isle of Man's fishery (51.27mg/kg). While zinc and copper concentrations in the edible tissues of *C. pagurus* were similar to the concentrations found in the brown meat of the Isle of Man's fishery (26.27mg/kg) when compared to the Buckie, Scotland, fishery (21.8mg/kg) (Topping, 1972), the average concentration of cadmium in Buckie (6.89mg/kg) was almost double that of the concentrations found in the Isle of Man (3.47mg/kg) (Topping, 1972). The brown meat taken from *C. pagurus* that were sampled from Orkney, Scotland, by Topping (1972) displayed reduced concentrations of cadmium (1.38mg/kg) and copper (14.34mg/kg), an increased average zinc concentration (48.5mg/kg) and a similar lead concentration (0.22mg/kg) when compared to this study. However, Topping (1972) only analysed the brown meat metal content of 5 *C. pagurus*. Therefore, the average concentration of heavy metals may not be representative of the population. Topping (1972) found that the Scottish *C. pagurus* fishery exhibited a similar level of metal contamination to the Manx fishery, however, a more recent study by Barrento *et al*. (2009a) along the Scottish Coast and the English Channel found greater arsenic and

cadmium concentrations when compared to the current study. A study on the blue swimming crab, *Portunus pelagicus*, in Kuwait found that the average concentrations of arsenic, chromium, copper, lead and zinc in the dry weight of the hepatopancreas to be between 2 and 3 orders of magnitude lower than have been isolated in the brown meat and hepatopancreas of *C. pagurus* (Al-Mohanna & Subrahmanyam, 2001), representing either interspecific variation in metal uptakes or a lower degree of metal contamination within Kuwaiti waters.

In the current study, a significant difference in the concentrations of cadmium, chromium, nickel zinc and total metals, which were isolated from the brown meat of male and female crabs. Increased concentrations of metals within the hepatopancreas on female crabs was also observed by Barrento *et al*. (2009a; 2009b). Reasons for the increased heavy metal concentrations within female *C. pagurus* could stem from behaviour as female crabs typically bury themselves in the sediment after mating (Ondes, 2015), potentially increasing exposure to metals in the sediment; however, a comparison of metal uptake between buried and non-buried crabs would be required to prove this hypothesis. Barrento *et al*. (2009a; 2009b) also noted an increased concentration of metals in the gonads of females, which may have also accounted towards the increase of metal concentrations observed in females during this study. Seasonal variability in *C. pagurus* gonadal, and hepatopancreas metal concentrations were observed by Barrento *et al*. (2009b), with the hepatopancreas exhibiting larger concentrations of metals in the Autumn and the gonads displaying higher concentrations in the summer, which corresponds to when the gonads are ripe and possibly indicates a depuration of metals. Seasonal variability in metal concentrations of *C. pagurus* organs may have caused concentrations of metals observed during this study to not be representative of the yearly average, thus, should the concentrations of metals in brown meat become monitored in the future, monitoring should be conducted during all four seasons to attain a yearly average metal concentration.

Cadmium, nickel and zinc concentrations on the brown meat of *C. pagurus* were all found to psoitively correlate with the carapace size, and therefore the age, of the crab, suggesting bioaccumulation of metals, which may occur as crab shells are a known bioabsorbent of several metals (An, *et al*., 2001). Nickel concentrations were uniformly low within the *C. pagurus* population sampled and high dietary zinc intake in humans is not of primary medical concern to the majority of consumers. Hence, bioaccumulation of nickel and zinc is not of concern. However, due to the medical implications of high dietary intakes of cadmium (Koizumi & Li, 1992; Benoff, *et al*., 2000), establishing an upper limit for crab landing size may be necessary to reduce the average cadmium content in the *C. pagurus*

population landed should upper limits of cadmium found in the brown meat be set in the future. Concentrations of chromium were found to negatively correlate with carapacem size in this study; however, the concentration of chromium found in all samples was less than 1mg/kg. Thus, the reduction in chromium with size was not of biological significance.

The concentrations of the four most abundant metals in the brown meat of *C. pagurus*, notably arsenic, cadmium, copper and zinc, were all positively correlated with each other. The positive correlation between metal concentrations observed is likely due to similar carrier proteins required by the metals for entry into the organisms (Rainbow, 1997). Additionally, positive correlations between metal concentrations could be indicative of a similar level of bioavailability of metal ions within the environment.

4.4 Conclusions

The average prevalence of shell disease syndrome during the summer months within the sample squares of the Isle of Man's *C. pagurus* fishery was one of the highest in the British Isles and is over four times the national historic levels suggested by Getchell (1988); although, variation in sampling methodology and season may have accounted for some of the variation in shell disease incidence between studies. Additionally, the incidence of shell disease had almost tripled since 2015 (Haig *et al*., 2016), although there was a smaller sample size and seasonal variation between sampling dates between the present study and the work of Haig *et al*. (2016). Furthermore, the average prevalence of shell disease has nearly doubled since the 2012 study by King *et al*. (2014), although, there was a lager sample size and a larger proportion of the fishery was sampled in the 2012 study. There was little evidence as to why the increase in shell disease incidence had occurred either by the variables measured in this survey or the literature. Any future studies that investigate the increase in shell disease prevalence should be multifactorial and examine all factors that have been identified in the literature that may increase shell disease susceptibility, for example, sediment characteristics, quantities of agricultural pesticides and growth hormones in the water, metal contamination, water temperature and the number of chitinolytic bacteria in the area.

Although metal contamination within the brown meat of *C. pagurus* was not found to be significantly different between crabs that were infected and not infected with shell disease, metal content was notably lower in uninfected crabs, which may indicate that other factors may have disguised metals influence. Future studies that examine the susceptibility of shell disease

within a fishery should be multifactorial and should not disregard the influence of water chemistry and sampling location.

The concentration of two metals that are detrimental to human health, arsenic and cadmium, within the brown meat of *C. pagurus* were substantial. The concentration of cadmium in the brown meat of the *C. pagurus* population averaged at nearly seven times the upper limit set for white meat by EU Regulation No. 1881/2006. Additionally, the concentration of cadmium in the brown meat of female crabs was over ten times that set for white meat, which was established by EU Regulation No. 1881/2006 and were found to bioaccumulate within *C. pagurus*. The current study recommends that current European legislation should be amended to incorporate an upper limit for arsenic, cadmium and lead in the brown meat of *C. pagurus*, which will limit the potential for chronic dietary exposure to metals that are detrimental to human health. It is advised that fishery managers start testing *C. pagurus* brown meat for heavy metal contamination in local waters in a standardised manner, as is the standard practice for metal contamination testing in scallops, which takes into account the season of catch and sex of the crab. Furthermore, food safety authorities and *C. pagurus* brown meat distributors should clearly advertise the metal contents of crab brown meat products, depending on the location of the catch, and make dietary recommendations on how much brown meat should be eaten by the consumer in order to not exceed the TWI of metals.

5.0 References

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Appendices

Appendix 1 – European *Cancer pagurus* landings

Appendix Table 1: The total number of brown crab, Cancer pagurus*, landed by European nations during 2013. Data supplied by FAO, 2016.*

Country	Landings/t
Belgium	271
British Isles	36782
Denmark	69
France	5925
Germany	115
Netherlands	554
Norway	5241
Portugal	1
Spain	82
Sweden	223

Appendix 2 – The upper limits of metals in foodstuffs set by EU Regulation No. 1881/2006

Appendix Table 2: The maximum level (mg/kg of wet weight) of cadmium and lead in foodstuffs, as defined by EU Regulation No. 1881/2006. Table adapted from EU Regulation No. 1881/2006.

Metal	Foodstuffs	Maximum levels (mg/kg of wet weight)
Lead	Raw milk, heat-treated milk and milk for the manufacture of milk-based products	0.020
	Infant formulae and follow-on formulae	0.020
	Meat (excluding offal) of bovine animals, sheep, pig and poultry	0.10
	Offal of bovine animals, sheep, pig and poultry	0.50
	Muscle meat of fish	0.30
	Crustaceans, excluding brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (*Nephropidae* and *Palinuridae*)	0.50
	Bivalve molluscs	1.5
	Cephalopods (without viscera)	1.0
	Cereals, legumes and pulses	0.20
	Vegetables, excluding brassica vegetables, leaf vegetables, fresh herbs and fungi. For potatoes the maximum level applies to peeled potatoes	0.10
	Brassica vegetables, leaf vegetables and cultivated fungi	0.30
	Fruit, excluding berries and small fruit	0.10
	Berries and small fruit	0.20
	Fats and oils, including milk fat	0.10
	Fruit juices, concentrated fruit juices as reconstituted and fruit nectars	0.050
	Wine (including sparkling wine, excluding liqueur wine), cider, perry and fruit wine	0.20
	Aromatized wine, aromatized wine-based drinks and aromatized wine-product cocktails	0.20

Cadmium	Meat (excluding offal) of bovine animals, sheep, pig and poultry	0.050
	Horsemeat, excluding offal	0.20
	Liver of bovine animals, sheep, pig, poultry and horse	0.50
	Kidney of bovine animals, sheep, pig, poultry and horse	1.0
	Muscle meat of fish, excluding species listed below	0.050
	Muscle meat of the following fish:	
	anchovy (*Engraulis species*)	
	bonito (*Sarda sarda*)	
	common two-banded seabream (*Diplodus vulgaris*)	
	eel (*Anguilla anguilla*)	
	grey mullet (*Mugil labrosus labrosus*)	
	horse mackerel or scad (*Trachurus species*)	0.10
	louvar or luvar (*Luvarus imperialis*)	
	sardine (*Sardina pilchardus*)	
	sardinops (*Sardinops species*)	
	tuna (*Thunnus species, Euthynnus species, Katsuwonus pelamis*)	
	wedge sole (*Dicologoglossa cuneata*)	
	Muscle meat of swordfish (*Xiphias gladius*)	0.30
	Crustaceans, excluding brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (*Nephropidae* and*Palinuridae*)	0.50
	Bivalve molluscs	1.0
	Cephalopods (without viscera)	1.0
	Cereals excluding bran, germ, wheat and rice	0.10
	Bran, germ, wheat and rice	0.20
	Soybeans	0.20
	Vegetables and fruit, excluding leaf vegetables, fresh herbs, fungi, stem vegetables, pine nuts, root vegetables and potatoes	0.050
	Leaf vegetables, fresh herbs, cultivated fungi and celeriac	0.20
	Stem vegetables, root vegetables and potatoes, excluding celeriac. For potatoes the maximum level applies to peeled potatoes	0.10