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Prifysgol Abertawe

Parasitic Diseases of Crabs in Swansea Bay

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by

Grace Crocker

[redacted]

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Dr Charlotte Davies and Professor Andrew Rowley

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Declarations

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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Abstract

The digenean trematode *Microphallus similis* affects *Cancer pagurus* populations, influencing the ecological dynamics of this commercially important species. Therefore, understanding the factors that influence prevalence, and intensity is crucial for understanding crustacean disease dynamics. This study investigated the prevalence, intensity, and identification of trematodes in *C. pagurus* at Mumbles Pier and Oxwich Bay, focusing on temporal variation, host sex, size and environmental conditions. Crabs were sampled from both locations, with analyses of *M. similis* using PCR (polymerase chain reaction). *Microphallus similis* was present at both sites, with greater prevalence in April and August, likely due to favourable temperatures and host availability. Although location was not statistically significant, prevalence was greater at Oxwich Bay, potentially due to favourable conditions. Size was associated with the presence of *M. similis*, but no biometric or environmental variables significantly influenced parasite severity at either site. Additionally, metacercariae size did not differ between locations. The study highlights the potential implications for *C. pagurus* populations and the broader ecosystem, including possible effects on species dynamics and ecological interactions. Future research should focus on the long-term effects of trematodes and their interactions with crustacean hosts, including how these dynamics may influence host health, population dynamics and ecosystem stability.

Lay Summary

The present study investigated the prevalence and intensity of the digenean trematode, *Microphallus similis*, in the edible crab *Cancer pagurus* (Figure 1), across two distinct locations: Mumbles Pier and Oxwich Bay in South Wales. The research aimed to identify whether biometric and environmental factors affected the prevalence and intensity of *M. similis*. Using a molecular approach, the presence and identification of *M. similis* was confirmed at both locations. It was discovered that trematode prevalence was greatest during April and August, likely due to warmer temperatures and greater availability of hosts during these months. Notably, although location was not statistically significant, Oxwich Bay exhibited the greatest prevalence of *M. similis* compared to Mumbles Pier, likely due to favourable conditions. Contrastingly, the heavily modified waterbody surrounding Mumbles Pier may have led to altered parasite transmission. However, no environmental or biometric variables were associated with parasite intensity. Additionally, it was noted that metacercariae size did not differ between locations. This research highlights the presence of *M. similis* in *C. pagurus* populations at both sites. However, there is limited information on how *M. similis* prevalence and intensity affects the population dynamics of this commercially important species.

Figure 1 – Edible crab specimen, measured for biometrics during the present study on trematode prevalence and intensity across two locations

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Chapter 1
Introduction

1.1 A Commercially Important Species – The Edible Crab (*Cancer Pagurus*)

The European edible crab *Cancer pagurus* is a relatively large marine crab species, fulfilling a predatory lifestyle through the consumption of molluscan and crustacean prey, including smaller members of their own species (Bateman *et al*, 2011; Haig *et al*, 2015; Lawton, 1989). *Cancer pagurus* also referred to as the brown crab is most common in UK waters, with a distribution extending from the northwest coast of Norway to south Morocco (Figure 2) (Haig *et al*, 2015). Juvenile crabs occupy the intertidal zone between late summer and early autumn for approximately ~3 years (Bennett, 1995; Regnault, 1994), where they use boulders and holdfasts of kelp as shelter (Moore, 1973; Eriksen & Moen, 1993; Robinson & Tully, 2000; Heraghty, 2013), whilst predating on crustaceans, epifaunic polychaetes and gastropods (Lawton, 1989; Eriksen & Moen, 1993). Once sexually mature, crabs will move increasingly subtidal with subsequent growth depending on age, gender and possibly with ambient depth and location (Bennett, 1979). Additionally, *C. pagurus* is considered a key fisheries resource, with adult crabs supporting an important fishery in European waters. For instance, in 2019, total landings in the EU often exceeded 10,000 tonnes (live weight) with a value of €28 million (*ca*. £23,631,580.00) (NWWAC, NSAC & MAC, 2023). Comparatively, in English waters overall landings of *C. pagurus* were stable between 2016 and 2019 ranging between 13,641 and 14,877 tonnes. However, in 2020 landings declined by 19% to 11,575 tonnes, likely influenced by the COVID-19 pandemic. This decline persisted into 2021, with landings totalling 11,683 tonnes. However, in 2021, the International Council for the Exploration of the Sea (ICES, Figure 3) rectangles off the northeast coast of England saw the highest landing tonnages, specifically in Bridlington accounting for 26% (3,022 tonnes) of total landings. Other significant landings were recorded in ICES rectangles off the southwest coast of England, which saw landings of 952 and 891 tonnes respectively in 2021 (Department for Environment Food & Rural Affairs, 2023). These figures highlight the commercial importance of *C. pagurus*, emphasizing the species economic value across the UK and EU. Monitoring these trends is essential for sustainable management whilst ensuring the long-term viability of this fisheries resource.

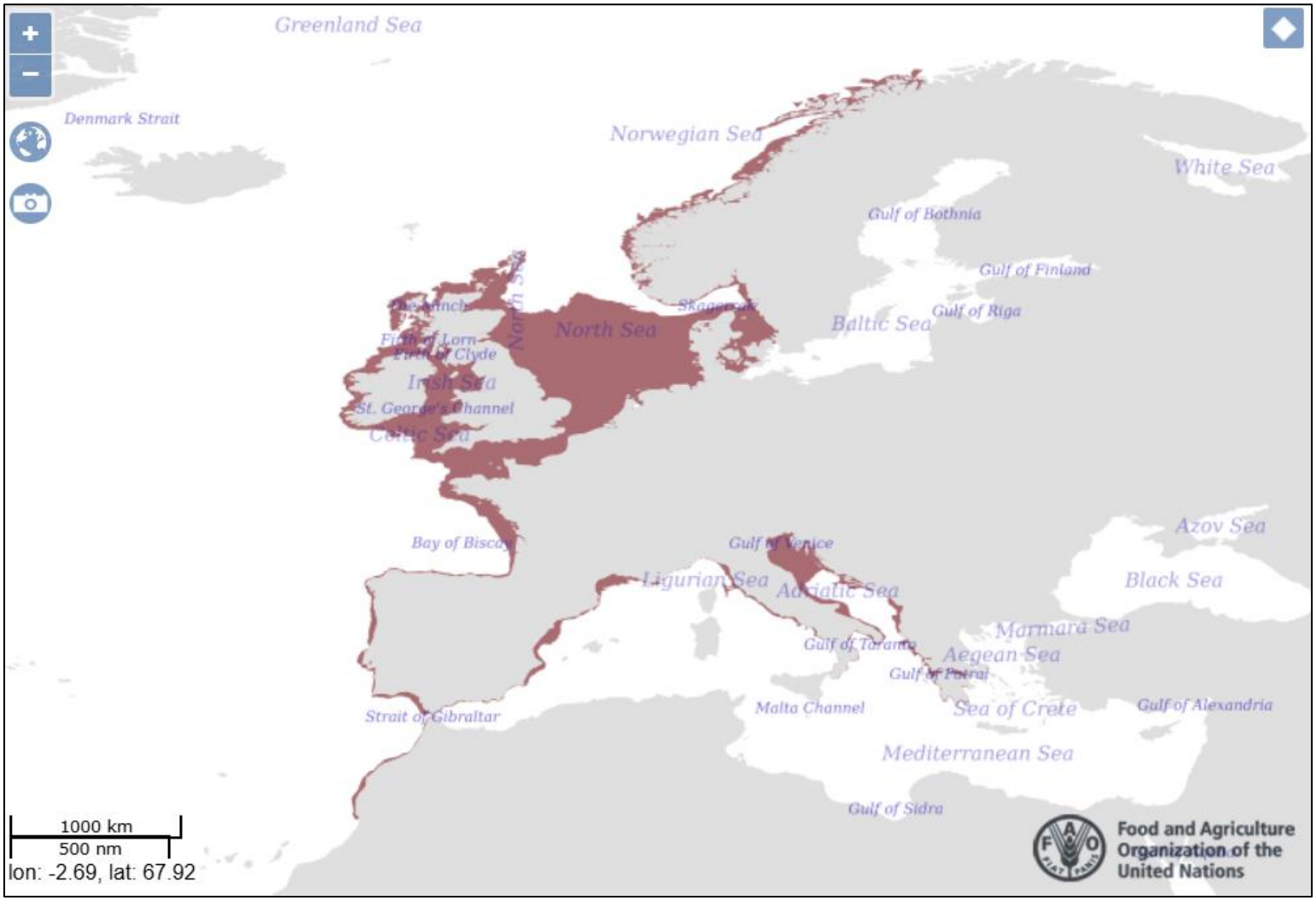

Figure 2 – Spatial distribution of edible crabs, *Cancer pagurus* (red shading). Map created using FAO Aquatic Species Distribution Map Viewer (FAO, 2024)

Figure 3 - International Council for the Exploration of the Sea (ICES) rectangle locations off the northeast and southwest coast of England. Maps created and annotated using QGIS V.3.32.3 3 (Service Layer Credits: Sources: OpenStreetMap, 2024)

1.2 Diseases of *C. pagurus*

Overfishing has been recognized as the foremost environmental and socioeconomic concern in our oceans, leading to depletion of biodiversity, ecosystem modification and concerns for food security (Jackson *et al*, 2001; Worm *et al*, 2006). Infectious diseases have recently inflicted substantial ecosystem and community wide impacts, with increased prevalence and distribution attributed to future climate change predictions (Harvell *et al*, 2004). Stress has often been associated with the development of such diseases, both in aquaculture and natural conditions (Houghton & Matthews, 1986; Ross *et al*, 1996), where the introduction of 'stress cascades' allow latent infections to manifest as a result of generalised host immunosuppression driven by changing environmental conditions (Johnson, 1980). Under such conditions, invertebrate immune systems may become compromised where opportunistic pathogens from both the surrounding environment and those living on or without the host proliferate, causing a multitude of pathological consequences (Stentiford & Feist, 2005). Therefore, monitoring pathogens may be vital in predicting future ecosystem dynamics and assessing impacts on commercially important species. *Cancer pagurus* is susceptible to diseases caused by viruses, bacteria, protists and multicellular organisms (Table 1). However, there is limited knowledge surrounding the prevalence and extent of mortality within populations as a result of these pathogens (Stentiford, 2008). Despite this limited knowledge, a number of studies have provided insight into pathogen profiles of prerecruit (juvenile) and recruit (adult) *C. pagurus*, with examples varying from dinoflagellates (Bateman *et al*, 2011), haplosporidians (Feist *et al*, 2009), and parasitoids (Kuris *et al*, 2002). While these studies have provided insight into pathogen profiles that may be causing direct and indirect losses to *C. pagurus* populations and fisheries, the prevalence of infection is still often poorly estimated (Bateman *et al*, 2011; Stentiford *et al*, 2001; Stentiford & Shields, 2005).

Table 1 – Diseases that have been found to affect the edible crab, *Cancer pagurus*

Disease causing agent	Clinical signs of infection	References
Viral conditions: *Cancer pagurus* bacilliform virus (CpBV), *Cancer pagurus* systematic bunya-like disease (CpSBV)	**CpBV:** Hypertrophic nuclei with eosinophilic nucleoplasm and marginalized chromatin within the hepatopancreatic tubule epithelial cells **CpSBV:** Budding in connective tissue cells and haemocytes	Bateman & Stentiford, 2008, Bateman *et al*. 2011, Corbel *et al*. 2003, Cowley, 2016
Bacterial conditions: Shell disease syndrome, *Vibrio* spp, *Cytophaga-Flavobacter* species, pseudomonads, *Pseudoalteromonas* spp.	Melanised lesions on exoskeleton surface, reduced haemocyte numbers, necrosis of hepatopancreatic tubules, enlarged nephrocytes filled with brown piment	Vogan et al. 2001, Vogan *et al*. 1999, Vogan *et al*. 2002, Powell & Rowley, 2005
Fungal conditions: *Ophiocordyceps* spp.	Encapsulated fungi in hepatopancreas and gills, hemocytic encapsulation, haemocyte necrosis, fungal emergence into hemocoel, septicaemia	Smith *et al*. 2013, Smith & Rowley, 2015
Hematodiniosis: (pink bitter crab disease): *Hematodinium* spp.	Creamy/pink haemolymph, hyperpigmentation of the carapace,	Thrupp *et al*. 2015, Chualain & Robinson, 2011, Stentiford, 2008

	penetration of tissues via haemal sinuses/haemolymph vessels, connective tissue/reserve-inclusion cells displaced, or absent, hepatopancreatic/muscular tissues display changes, lethargy	
Haplosporidiosis: *Paramarteilia canceri*	Lethargy, unresponsive, congested haemal sinuses, influx of haemocytes and fibroblast-like cells resulting in the hepatopancreas resulting in encapsulation, located within the tissues (e.g., gill, heart, skeletal muscle, tegmental gland and gonads)	Collins *et al*. 2022, Feist *et al*. 2009, Ward *et al*. 2016
Microsporidiosis: *Enterospora canceri*	Displacement of the basophilic nucleolus and margination of chromatin, degeneration of hepatopancreatic tubules, encapsulation of affected tubules, necrotic tubules with melanin deposition, degeneration of tubule epithelia, nuclei contains eosinophilic inclusions	Stentiford & Bateman, 2007, Stentiford, 2008, Bateman *et al*. 2011
Paramikrocytosis: *Paramikrocytos canceri*	Hypertrophy of the antennal gland/bladder, layer of yellow gelatinous tissue at the periphery of the antennal gland	Hartikainen *et al*. 2014, Thrupp *et al*. 2015, Bateman *et al*. 2020

Paramoebiasis: Amoebic crab disease (ACD), *Janickina feisti*	Distributed through multiple tissues (e.g., heart, gills. Haemal sinuses, fixed phagocytes, connective tissue cells of hepatopancreas), pronounced haemocytic infiltration, aggregation of phagocytes congests haemal spaces, melanised host reactions in gills and heart	Bateman *et al*. 2022
Digenean trematodes: *Microphallus similis*, *Microphallus primas*, *Cercaria emasculans*	Encysted metacercaria in hepatopancreas, gill and muscle	James, 1967, James, 1968a, Pelseneer, 1906, Bateman *et al*. 2011, Stunkard, 1957
Parasitic barnacle: *Sacculina inflata*	Feminisation of external secondary sexual characters, reduction of primary sexual characters, gonadal degeneration, behavioural changes, inhibition moulting, lower fitness, increased mortality	Waiho *et al*. 2021, Boschma, 1937, Bateman *et al*. 2011
Turbellarians: *Fecampia erythrocephala*	Juvenile *C. pagurus* lighter in colour, single, large turbellarian appears to replace major organ masses within the cephalothorax	Stentiford, 2008, Bateman *et al*. 2011, Kuris *et al*. 2002

1.3 The Lifecycle of Microphallidae

The family Microphallidae (Ward, 1901) are a group within the phylum Platyhelminths (flatworms) containing the class Trematoda subdivided into two subclasses named Aspidogastrea and Digenea (Geraghty, 2018). Digenetic trematodes, the larger of the two subclasses, are among one of the most successful groups of parasitic trematodes (Cribb *et al*, 2003; Olson *et al*, 2003), with the subclass containing 77 families, 18,000 species with ongoing rates of species descriptions still occurring (Poulin, 2014; Kostadinova & Perez-del-Olmo, 2019). Digenean trematodes (Table 2) predominantly act as endoparasites of vertebrates, often characterized by their complex life cycles involving one or two intermediate hosts before reaching the final definitive vertebrate host (Geraghty, 2018). Miracidia are produced via sexual reproduction of adult trematodes within the vertebrate host, eventually being released through host faeces hatching into free-living ciliated infective stages in the water column or upon consumption by the first intermediate host (Sousa, 1994; Esch *et al*, 2001). Within the first intermediate host, typically a mollusc, miracidia infect species-specific tissues developing into asexual reproductive sporocysts. These sporocysts develop into additional sporocysts or a further larval stage characterized by a primitive rediae (gut) and mouth, with the rediae ingesting host tissues and other trematode larval stages (Kuris, 1990; Esch *et al*, 2001). Within the sporocysts and rediae, cercariae, another free-living infective stage develop and are further released into the water column, where they can pursue a second intermediate host to form encysted metacercariae (Haas *et al*, 2008). For example, previous work conducted by Stunkard (1957), and James (1967) revealed that prosobranch molluscs, including *Littorina saxatilis* (rough periwinkle), serve as primary intermediate hosts, through producing motile cercarial stages that penetrate the gills of the second intermediate crustacean host upon ingestion. Subsequently, these cercariae encyst as metacercariae within the tissues of *C. pagurus*, thus completing the lifecycle of *Microphallus similis* (Crothers, 1966).

Table 2 – Digenean trematodes of crabs in Europe

Species	First host	Intermediate host	Final host	Location	References
Accepted name: *Microphallus similis* **Original name:** *Spelotrema excellens*	*Littorina saxatilis*, *Littorina obtusata,* Annelids (e.g., segmented worms)	*Carcinus maenas*, *Cancer pagurus*	*Larus argentatus, Sterna hirundo, Larus schistiagus, Larus marinus, Caldris alpina, Tringa totanus, Charadrius hiaticula, Haematopus ostralegus, Vanellus vanellus*	Western Europe, Belfast, Lough, Ireland, Old Peak, Robin Hood's Bay, Yorkshire, United Kingdom, Sweden, Caernarvonshire, Anglesey, Wales	Ro *et al*. 2022, Galaktionov *et al*. 2012, Bojko *et al*. 2017, Rankin, 1940, Threlfall, 1967, Hansson, 1988, Stentiford, 2008
Accepted name: *Microphallus pygmaeus* **Original name:** *Distomum pygmaeum* (Dixenic lifecycle)	*Littorina saxatilis, Littorina obtusata, Littorina fabalis, Littorina arcana, Littorina compressa, Littorina littorea*		*Somateria mollissima v-nigram, Larus argentatus, Anthus spinoletta*	North Atlantic, Europe, Balsfjord, Norwegian Sea, Norway, Kandalaksha Bay, White Sea, Russia, Grindavik, SW Iceland, Aberystwyth, Wales,	Galaktionov *et al*. 2012, James, 1968a, Bojko *et al*. 2017, Granovitch & Mikhailova, 2004

				Old Peak, Robin Hood's Bay, Yorkshire, Swedish west coast	
Accepted name: *Microphallus primas* **Original name:** *Spelophallus primas*	*Peringia ulvae*	*Carcinus maenas, Cancer pagurus*	*Haematopus ostralegus*, *Somateria mollissima*, *Larus argentatus*, *Larus cachinnans, Haematopus ostralegus*	Sao Jacin-Aveiro estuary, Portugal, Glaicia, north-west Spain, Belfast Lough, Ireland, Alde, Mersey, Tyne, Forth and Clyde estuaries, Southampton	Pina *et al*. 2011a, San-Martin *et al*. 2005, Tkach *et al.* 2003, Stentiford & Feist, 2005, Saville & Irwin, 1991, Bateman *et al*. 2011
Accepted name: *Microphallus piriformes* (Dixenic lifecycle)	*Littorina saxatilis, Nucella lapillus Littorina obtusata, Littorina fabalis, Littorina arcana, Littorina compressa*		*Calidris maritima*, *Larus argentatus*, *Larus marinus*	Kola Peninsula, Russia, North Atlantic, Europe, The Murman Coast of the Barents Sea, Russia, Vaygatch	Galaktionov *et al*. 2012, Kuklin, 2015, Birstein & Mikhailova, 1990, Krupenko & Dobrovolskij, 2018,

				Islands, SE Barents Sea, Russia, Grindavik, SW Iceland, Balsfjord, Norwegian Sea, Norway	Bojko *et al*. 2017, Granovitch & Mikhailova, 2004
Accepted name: *Microphallus pseudopygmaeus*	*Littorina saxatilis*		*Somateria mollissima v-nigram*	Southern Island of Novaya Zemlya, Vaygach Island and Dolgiy Island, Russia	Galaktionov *et al*. 2019, Galaktionov, 1996
Accepted name: *Microphallus triangulatus*	*Littorina saxatilis*, *Littorina obtusata*		*Somateria mollissima*	Kandalaksha Bay, White Sea, Russia, Vaygach Island, SE Barents Sea, Russia	Galaktionov *et al*. 2004, Galaktionov *et al*. 2012
Accepted name: *Microphallus claviformis*	*Peringia ulvae*	*Carcinus maenas, Jarena albifrons*		Sylt Island, Germany, Chupa Bay, White Sea, Russia	Galaktionov & Malkova, 1994, Thieltges *et al*. 2008
Accepted name: *Maritrema portucalense*	*Peringia ulvae*	*Carcinus maenas*	*Larifrom* and *Charadriiform* birds	Aveiro estuary, Portugal	Pina *et al*. 2011b

Original name: *Maritrema portucalensis*					
Accepted name: *Maritrema subdolum*	*Peringia ulvae*	*Carcinus maenas, Cyathura carinata*	Wading birds	Aveiro estuary, Portugal, Mondego estuary, Portugal	Pina *et al*. 2011b, Jensen *et al*. 2004
Accepted name: *Cercaria emasculans*	*Littorina saxatilis, Littorina littorea*	*Carcinus maenas, Cancer pagurus*		United Kingdom, Cardigan Bay, Wales	James, 1967, James, 1968b, Pelseneer, 1906
Accepted name: *Gynaecotyla adunca* **Original name:** *Gynaecotyla longiintestinata*	*Nassarius reticulatus, Tritia reticulata*	*Polybius henslowii, Carcinus maenas*		S. Jacinto channel, Aveiro estuary, Portugal	Costa *et al*. 2017, Pina *et al*. 2007, Russel-Pinto & Bartoli, 2002

1.4 *Cancer pagurus* A Second-Intermediate Host of Microphallidae

Trematodes have been identified as significant parasites affecting marine ecosystems, with potential repercussions for commercially important species such as *C. pagurus*. However, prevalence has been poorly estimated both in recruit and prerecruit populations, as a result of factors including differential catchability of infected animals or through inadvertent preselection of healthy or infected animals by fisheries during sampling (Bateman *et al*, 2011; Stentiford *et al*, 2001; Stentiford & Shields, 2005). Therefore, understanding of pathogen/parasite profiles in commercially important species such as *C. pagurus* is considered crucial. Addressing these challenges, studies including Bateman *et al* (2011), have examined the prevalence and impact of trematodes in recruit and prerecruit populations of *C. pagurus* in the English Channel. Metacercarial stages of *Microphallus primas* (Saville & Irwin, 2005) were observed at high prevalence throughout the study, peaking at 74% in November whilst being present in at least 17% of prerecruit crabs sampled each month (Bateman *et al*, 2011). Notably, high prevalence pathogens including *M. primas* were limited exclusively to prerecruit populations. This observation may be attributed to differences in sampling periods, where sampling efforts may have focused primarily on prerecruit populations or where adult populations were inadvertently under sampled. Despite this, the study highlighted that this was unlikely to significantly impact the disease profiles of the two groups (Bateman *et al*, 2011). However, it was further highlighted that although the presence or absence of particular parasites can be related to differences in host diet and the ecology of the parasite and host (e.g., prerecruit *C. pagurus* are more likely to become infected with *M. primas* through the presence of *L. saxatilis* and the definitive bird host in the littoral zone) (Saville & Irwin, 2005; Stentiford, 2008), the presence or absence of other pathogens are not as easily explained (e.g., shell disease syndrome, white spot disease and *Hematodinium*) (Bateman *et al*, 2011; Castro *et al*, 2006; Millard *et al*, 2021; Stentiford & Shields 2005). Furthermore, additional insights into the prevalence and impact of digenean trematodes has been provided by Crothers (1966), through examining the occurrence of trematodes in marine crustaceans. *Microphallus similis*, a parasite generally found infecting the tissues of the shore crab *Carcinus maenas* was discovered at very low prevalence in *C. pagurus* in British waters. Additionally, James (1967, 1969) reported a second species of encysted metacercaria of *Cercaria emasculans* commonly hosted by intertidal littorinid molluscs, in *C. pagurus* in the UK.

Trematodes exert physiological and behavioural effects by inducing host reactions after the encystment of metacercariae within their second-intermediate hosts (Chubb *et al*, 2010).

These influences can range from minor to extreme, including reproductive impairment, organ dysfunction, tissue damage and behavioural changes (Blakeslee *et al*, 2015). Blakeslee *et al* (2015) highlighted the influence of *M.* similis intensity on *C. maenas* through a series of laboratory experiments, particularly focusing on physiological and behavioural effects. Notably, after a four-week incubation period, physiological and behavioural experiments demonstrated little effect of *M. similis* on *C. maenas* (Blakeslee *et al*, 2015). However, increased immune system activation was observed in experimental crabs after trematode exposure (Blakeslee *et al*, 2015). For instance, significantly fewer haemocytes were observed in the haemolymph of exposed crabs in comparison to control crabs 72 hrs after exposure to cercariae (Blakeslee *et al*, 2015). This decrease in haemocyte number after exposure was likely due to the recruitment of these cells to encapsulate metacercariae, as demonstrated in histological studies of trematode species in *C. maenas* and other crab intermediate hosts (Blakeslee *et al*, 2015). Consequently, trematodes pose significant challenges towards fisheries, impacting both economic viability and ecological sustainability, with parasites potentially leading to reduced productivity and marketability of *C. pagurus* populations. However, due to a basic lack of understanding on a relatively well-studied commercial species such as *C. pagurus* (Bateman *et al*, 2011), further investigation is warranted to elucidate its relationship with digenean trematodes. Despite the existing gaps in our knowledge, exploring this association is essential for comprehensively assessing the factors influencing the dynamics of trematode prevalence and intensity in the edible crab fishing industry.

1.5 Contraction of Disease in Crabs at the Two Selected Survey Sites in South Wales

The coastal regions of Mumbles Pier and Oxwich Bay present stark contrasts in terms of their disease diversity and environmental conditions, most likely due to the hydrology of an open-water pier vs. a sheltered bay. Research by Davies *et al* (2022) provided comprehensive insight into these differences, emphasizing the role of hydrological dynamics in shaping ecological and pathogenic landscapes at Mumbles Pier and the Prince of Wales Dock. Both experimental sites exhibited similar incidences of *Hematodinium* sp. infections in crab populations and demonstrated comparable temporal dynamics of parasite lifecycle (Davies *et al*, 2019a). However, the study highlighted that Mumbles Pier potentially supported a higher diversity of disease-causing agents in terms of eDNA. This included direct transmission of *Hematodinium* sp. from moribund crabs releasing motile zoospores into the water column infecting susceptible crabs, as well as a significantly higher species richness in benthic fauna

contributing to disease transmission (Stentiford & Shields, 2005; Davies *et al*, 2022). Notably, two new species of Haplosporidia including *Haplosporidium carcini* nov. and *Haplosporidium cranc* were further characterised in populations of *C. maenas* sampled from Mumbles Pier (Davies *et al*, 2020). In contrast, Oxwich Bay presents differing ecological and disease profiles, potentially due to the sheltered nature of the bay. For instance, Smith *et al* (2015) explored the parasitisation of juvenile *C. pagurus* by the dinoflagellate *Hematodinium* sp. at Oxwich Bay and Mumbles Head. The study highlighted a significantly higher percentage of *Hematodinium* infections at Mumbles Head, with 29.9% of intermoult crabs found to be infected compared with 21.1% of newly moulted individuals. Comparatively, the percentage of crabs infected with *Hematodinium* at Oxwich Bay was 18.9% compared with 9.6% in newly moulted crabs (Smith & Rowley, 2015). However, at Oxwich Bay the frequency of moulting was greater during the winter months compared with Mumbles Head, with 14% of crabs collected in January being soft bodied. Although the timing of dinospore production and moulting at one site appeared sub-optimal for transmission, the possibility of parasite transfer from other crustacean species (hosts and co-inhabitants), including *C. maenas* and velvet swimming crab *Necora puber* cannot be ruled out (Smith & Rowley, 2015).

Furthermore, other microbes/parasites may differ in their occurrence between the two sites including trematode infestations. A possible explanation of differences in trematode susceptibility at both sites may be due to the presence of reservoirs, physical properties and alternate hosts of disease (Davies *et al*, 2022). For instance, trematodes have multi-host lifecycles with predation of infected crabs by sea birds resulting in this definitive host becoming infected, releasing infective stages via faeces that infect littorinid molluscs (Davies *et al*, 2022). However, due to the sheltered nature of Oxwich Bay and reduced biodiversity, the absence of grazing littorinids may reduce the lifecycle despite the presence of both *C. maenas* and shore birds in comparison to the open-water site of Mumbles Pier. Furthermore, changes in environmental factors as well as pH, xenobiotics and nitrogenous wastes may influence both host and pathogen as well as incidence of disease at Mumbles Pier, potentially due to modification of the water body and limited sewage treatment (Davies *et al*, 2022).

1.6 Aims and objectives

The overall aim of this study is to investigate the presence of digenean trematode parasites in *C. pagurus* at Oxwich Bay and Mumbles Pier. Given the commercial significance of both sites and the increased pressure on global commercial stocks of decapod crustaceans, it is crucial to quantify the prevalence of such parasites within *C. pagurus* communities. The specific objectives of this study are as follows:

1. To identify the species of digenean trematode parasites present in *C. pagurus* across the two locations.
2. To explore if parasite presence, and load (e.g., abundance) correlate with any biometrics taken. This would include size, sex, fouling/presence of epibionts, pigment loss and haemolymph colour across both locations.

These objectives will enable us to draw conclusions as to why and how parasites may alter *C. pagurus* stocks and to alter benthic communities.

Chapter 2

Materials and Methods

2.1 Study Area

The study took place around Mumbles Pier (51° 34’11.00” N, 3° 58’49” W) and Oxwich Bay (51° 33’ 56.92” N, -4° 08’ 48.44” W) off the coast of South Wales (Figure 4). This coastline is often subject to intense hydrodynamic forces as a result of strong winds and tides originating from the Bristol Channel and North Atlantic Swells (Stone *et al*, 2019). Swansea Bay is characterized as an ebb-dominant macro-tidal bay (CEFAS, 2013), with the 12km embayment featuring an intricate hydrodynamic system (i.e. means of 8.5m spring tides; 4.1m neap tides), influenced by its bathymetry and configuration (Collins *et al*, 1979; Smith & Shackley, 2006). Sediments are predominantly fine to medium sand in inner Swansea Bay with increasing proportions of mud occurring inshore to the west, as a result of Mumbles Head providing protection from wave exposure (Smith & Shackley, 2006). Oxwich Bay situated in the outer reaches of the Bristol Channel, on the south shore of the Gower Peninsula (CEFAS, 2013) is renowned for its AONB (Area of Outstanding Natural Beauty) status through the Countryside and Rights of Way Act 2000. The Gower Peninsula is a south facing stretch of open coastline comprising of a series of carboniferous limestone cliffs and embayment’s (CEFAS, 2013). Seabed types comprise mainly of sand with rocky reefs extending into subtidal areas across the eastern half (CEFAS, 2013), with isolated saltmarshes and estuaries to the north. Turbidity within Oxwich Bay primarily arises from inorganic particulates, with sediment distribution predominately regulated by tidal and wave-induced currents (Collins *et al*, 1979). Both locations represent habitats for both commercially important species including *C. pagurus* and *N. puber*, as well as invasive species such as *C. maenas*.

2.2 Sample Collection

Surveys were conducted every two months for a six-month period (April, June, and August 2024) to assess *C. pagurus* populations at both locations. After each low tide, *ca*. 30 *C. pagurus* specimens were randomly selected by hand. Specimens were transferred in seawater and transported to the aquarium where they remained for ≥ 48hr.

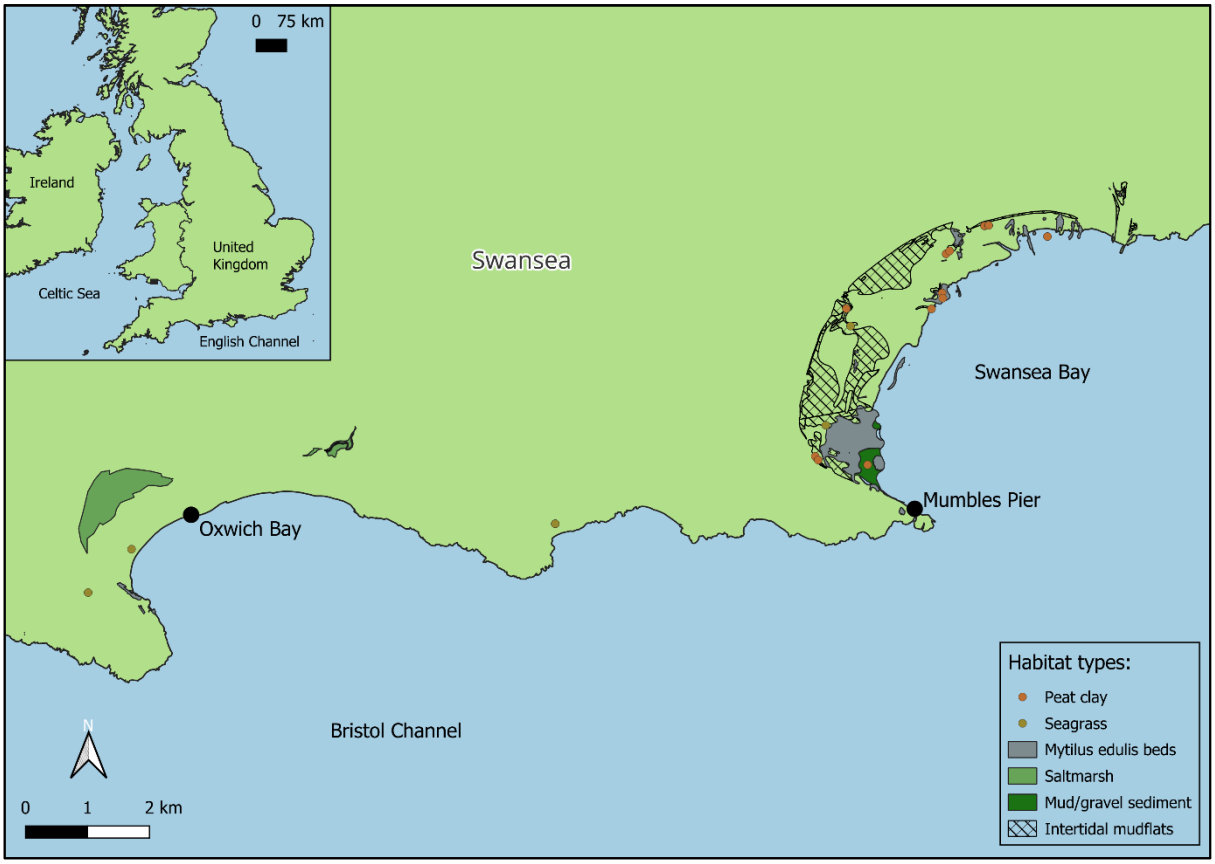

Figure 4 – Collection locations for edible crab (*Cancer pagurus*) during this study, South Wales, UK. Maps created and annotated using QGIS V.3.32.3 (Service Layer Credits: Sources: UK Data Service, 2024; OpenStreetMap, 2024; Welsh Government, 2024)

2.3 Laboratory Regime

All crabs were processed within 48h of collection and placed on ice. The following biometric data were taken for each crab: sex; moult stage (inter-moult [hard] or post-moult [soft]); fouling (presence of epibionts); *Sacculina*, pigment loss, shell disease, encrusting *Spirorbis*; carapace width (CW:mm); and limb loss (see appendix: Table A1). Next, 300 µl of haemolymph was extracted using a 23-gauge hypodermic needle fitted with a sterile 1 ml syringe. Haemolymph appearance was categorised as either normal or milky as an indication of systemic infection. Approximately 25 µl haemolymph was transferred onto a microscope slide, and any samples that deemed positive for *Hematodinium* and fungal infections identified under phase contrast optics of a BX41 microscope (Olympus; Tokyo, Japan) were noted.

2.3.1 Initial Protocol

Trial experiments were carried out on crabs collected in February 2024. In these, crabs were sacrificed by injection using 1-1.5 ml of 1 M KCl depending on size and left for 10-15 min until involuntary motor functions were absent. Crabs were dissected individually and the hepatopancreas removed. Additionally, four biopsies of the hepatopancreas were weighed. Hepatopancreatic tissue from these was squashed using a 20x50 mm coverslip and examined using a GX stereomicroscope. If samples were deemed positive for metacercariae, the number of cysts were counted and measured. Identified metacercariae were extracted from squashed hepatopancreatic tissue using sterile forceps and stored in a 1.5 ml Eppendorf tube at -18℃ for later DNA extraction. However, this trial proved inaccurate due to the low number of cysts found in *C. pagurus*. This suggested that removing the whole hepatopancreas, rather than biopsies, could be more effective, as cysts in other areas of the hepatopancreas may have been missed when only segments were examined.

2.3.2 Amended Protocol (April – August 2024)

All crabs were sacrificed at -18℃ for 30-45 min until involuntary motor function was absent. Forceps were used to detach the carapace from the ventral surface. Subsequently, the whole hepatopancreas was removed and placed into a pre-weighed 50 ml Falcon tube and stored at – 18℃. Total hepatopancreatic tissue was then removed from the freezer and thawed at room temperature for *ca*. 30 min before homogenisation in 4-5 ml of 3% NaCl and vacuum filtered through a sterile 150 µm (pore size) low density polyethylene (LD-PE) cell strainer (PluriStrainer, Leipzig, Germany). Filters were examined using a GX stereomicroscope. If deemed positive for metacercariae, the number of cysts was counted, and these transferred into 1.5 ml Eppendorf tube and stored at -18℃ for later DNA extraction. This protocol was implemented to enhance the precision of cyst quantity measurements and to accurately identify all metacercarial cyst species, ensuring a reliable survey of digenean trematodes in *C. pagurus* communities at both Mumbles Pier and Oxwich Bay. Metacercariae were photographed using the x4 or x10 objectives of an Olympus BX41 microscope equipped with a digital camera. The maximum diameter of these cysts were determined by reference to a calibration bar.

2.4 DNA Extraction

Digenean trematode DNA was extracted from thawed tissue containing multiple metacercariae using a Qiagen Blood and Tissue Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Samples were, however, incubated overnight at 56℃ with 20 µl of proteinase K to ensure they were completely digested. Extracted DNA was quantified using a Qubit® dsDNA Broad Range Assay Kit and Qubit® Fluorometer (ThermoFisher Scientific; Altrincham, UK), yielding an average DNA concentration of 31.7 ng/µl ± 20.6 ng/µl (range, 50-200 ng/µl) (mean ± SD) (see appendix: Table A9).

2.5 PCR and Sequencing Conditions

All PCR reactions were carried out in 25 µl total reaction volumes using 2x BioMix (Bioline, London, UK), oligonucleotide primers synthesised by Eurofins (Ebersberg, Germany), nuclease-free water (Invitrogen™, Leicestershire, UK), 1 µl of genomic DNA (*ca*. 50-200 ng/µl) and performed on a T100 PCR thermal cycler (BioRad Laboratories Inc., Watford, UK). Primers refined by Tkach *et al* (2003) and Galaktionov *et al* (2012) were used to amplify the 28S rDNA (domains D1-D3) gene region: including forward primer LSU-5 (5'-TAG GTC GAC CCG CTG AAY TTA AGC A-3') with the reverse primer 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3') using 0.5 µl of each primer at a concentration of 0.2 mM with an expected product size of 1400 bps. Cycling conditions were as follows: initial denaturation at 94℃ for 3 min (1 cycle), followed by 35 cycles of denaturation at 94℃ for 30 sec, annealing at 60℃ for 30 sec, and extension at 72℃ for 1 min, with a final extension at 72℃ for 5 min (1 cycle).

Following cycling, 5 µl of post PCR product was mixed with 1 µl of 6x DNA Loading Dye (ThermoFisher Scientific, Altrincham, UK) and visualized on a 2% agarose/TAE gel using GreenSafe Premium nucleic acid stain (NZYTech, Lisboa, Portugal). Gels were electrophoresed for *ca*. 45-60 min at 60 volts alongside a 1Kb Plus Ladder (New England Biolabs, Hitchin, UK). Gel imaging was completed using a Molecular Imager® Gel Doc™ XR System (BioLad Laboratories Inc., Watford, UK). If PCR product deemed positive, samples were re-amplified and purified using ExoSAP-IT/ExoSAP-IT Express (ThermoFisher Scientific, Altrincham, UK) using the manufacturers guidelines in preparation for sequencing. Purified PCR products were identified via DNA Sanger sequencing, using both forward and reverse primers, by Eurofins (Ebersberg, Germany).

2.6 Phylogenetic Analyses

Primers were removed and consensus sequences were constructed using BioEdit sequence alignment editor (Hall, 1999). Sequences were identified using the bioinformatic tool for similarity search BLAST (Altschul, 1990) and submitted to GenBank under the accession numbers PQ314579-PQ314597 (see appendix: Table A6).

Multiple sequence alignments were performed in CLUSTAL X v.2 (Larkin *et al*, 2007). Evolutionary analyses and reconstructions were carried out in MEGA X (Kumar *et al*, 2018) using the maximum likelihood routine based on the Tamura-Nei model. A consensus tree with the highest log likelihood value from 1000 bootstrap re-samplings was annotated using iTOL software (Letunic & Bork, 2019). Maximum likelihood trees are widely used for their efficiency and consistency in providing unbiased parameter estimates, as the most probable tree topology is optimally evaluated by the maximum likelihood estimation method (MLE), this addresses problems such as estimating phylogenetic relationships from molecular data (Dhar & Minin, 2016) (see appendix: 5.6.9 – Multiple Sequence Alignment Viewer). Reference sequences for the corresponding region of *M. similis*, obtained from a variety of hosts, were sourced from GenBank at NCBI (Benson *et al*, 2017): *Larus schistisagus*, *Carcinus maenas*, *Posticobia brazieri*, *Cherax dispar*, *Littorina sitkana*, *Littorina saxatilis*, *Onoba aculeus*, *Somateria mollissima v-nigram*, *Littorina natica*, *Hemigrapsus sexdentatus*, *Zeacumantus subcarinatus*, *Falsicingula kurilensis*, *Buteogallus urubitinga*, *Pleurobrachia* sp., *Xanthocnemis zealandica*, *Oryzomys palustris*, *Eudocimus albus*, *Indochinamon manipurense*, *Austolittorina cincta*, *Juga* sp., *Parabascus lepidotus*, *Longiflagrum nasutus*, *Pleurocera proxima*, *Semisulcospira libertine* (GenBank: HM584122-HM584142; AY220625, AY220628; AB974360; KT355822-KT355823; OQ407755, OQ407760, OQ407761; MG783586-MG783588; OR457720-OR457724; ON036091, ON036092, ON036094; KY62366; KF738451; KJ868216; MW000412-MW000424; MH094413; LC599542). *Microphallus basodactylophallus* was used as an outgroup for the tree (AY220628) (see appendix: Table A5).

2.7 Statistical Analyses

Binomial logistic regression models with Logit link functions were used to assess which predictor variables significantly affected the likelihood of finding crabs testing positive for trematode presence in the sampled crab populations. All logistic models were run in RStudio v.4.3.1. with R v.4.4.1. using the MASS package. Through analysing the residuals, the data was not normally distributed, indicating that the assumptions for logistic regression were met and

the model deemed suitable. Models were initially developed as full models, incorporating all relevant predictor variables with model selection and evaluation based on an information-theoretic approach. Non-significant predictors were systematically eliminated using the drop1 function (stats package) producing final models with improved predictive accuracy, termed the reduced models. The drop1 function was used to compare the initial full model with the same model, whilst removing the least significant predictor variable. If the reduced model differed significantly from the full model (as assessed by a Chi-square test for binomial responses), the excluded variable was permanently removed. This process continued until the final model was achieved. The full models included variables such as month (grouped by April, June and August), sex (male or female), fouling (presence of epibionts, 0 or 1), CW (continuous numbers), pigment loss (0 or 1), fouling (0 or 1), haemolymph appearance (clear or milky, 0 or 1). Initially, location (Mumbles or Oxwich) was also considered in the first model before separating sites. Limb loss was removed from the analysis due to uncertainties surrounding the timing of limb loss, whether it occurred at the site of collection, during transportation, or in the aquarium making it an unreliable predictor.

To assess parasite load (e.g., severity), the dataset was refined to only include crabs harbouring trematodes (n=55). A Poisson regression model was utilized to assess which predictor variables significantly influenced parasite load among infected individuals. This analysis was also conducted in RStudio. The initial model incorporated variables such as month (grouped by April, June and August), sex (male or female), CW (continuous numbers), location (grouped by Mumbles and Oxwich), pigment loss (0 or 1), fouling (0 or 1) and haemolymph opacity (categorized as clear or milky, 0 or 1). Residuals were analysed to ensure the data met the assumptions of the Poisson regression revealing a non-normal distribution. However, the dispersion analysis revealed significant overdispersion (ϕ = 24.91), indicating that a negative binomial was more suitable for modelling trematode severity. A generalized linear model (GLM) with a negative binomial function was utilized using the MASS package. Certain predictor variables including fouling and haemolymph opacity were excluded from the final model due to a low number of individuals displaying trematodes (fouling: n=2, haemolymph opacity: n=2), enhancing the simplicity and interpretability without compromising model performance.

To investigate differences in metacercariae size by location (Mumbles and Oxwich), the normality of the data was tested using GraphPad Prism v.10.3.0. Normality was evaluated using Anderson-Darling, Shapiro-Wilk and Kolmogorov-Smirnov tests. The results of these

tests indicated that the data were not normally distributed ($\alpha = 0.05$). Consequently, a non-parametric Mann-Whitney U (unpaired) test was used to assess the differences in metacercariae size between locations. All graphics were produced using GraphPad Prism v.10.3.0 for Windows.

Chapter 3
Results

3.1 General Population Observations

Overall, 178 crabs were sampled across a six-month period, 87 from Oxwich Bay and 91 from Mumbles Pier (see appendix: Table A1). Of these sampled crabs, 30.9% were trematode-positive, with 34.5% of crabs from Oxwich Bay and 27.5% from Mumbles Pier containing metacercarial cysts in the hepatopancreas. The average number of parasites per individual crab at Oxwich Bay was found to be 14.1 ± 3.8 (mean ± SE), while at Mumbles Pier the average was 10.7 ± 2.9 indicating a potentially higher mean parasite load in crabs sampled at Oxwich Bay. The average carapace width of crabs from Oxwich Bay measured 45 ± 1.3 mm (mean ± SE), with a mean carapace width of 47 ± 1.2 mm at Mumbles Pier. In terms of other diseases observed, 18% of female crabs were *Hematodinium*-positive while 14% of males presented the disease via observations of haemolymph, indicating a possible higher prevalence of *Hematodinium* amongst females. One sample was also found to have an unidentified fungal infection detected via haemolymph observations (Figure 5).

3.1.1 Examining Prescence of Digenean Trematodes in *C. pagurus*

A binomial logistic regression (Model 1, Table 3, see appendix: Table A2) was used to examine the presence of trematodes in response to the following predictor variables: location (Oxwich *vs*. Mumbles), month (April *vs*. June, *vs*. August), sex (male *vs*. female), fouling (presence of epibionts, 0 *vs*. 1), carapace width (cont. in mm), pigment loss (0 *vs*. 1), haemolymph colour (clear *vs*. milky) (see appendix: Table A1). Reduced models revealed month as a significant factor associated with the presence of trematodes. Crabs in June were significantly less likely to present trematodes ($p = 0.00241$) than in April and August (Model 1, Table 3, Figure 6a) (June = 12%, April = 43%, August = 35%).

When separating by location, another binomial logistic regression (Model 2, Table 3, see appendix: Table A2) was used to assess the presence of trematodes, testing the same variables listed above. In Mumbles, month and carapace width were significant factors associated with the presence of trematodes (Model 2, Table 3, Figure 6b, Figure 7b). Crabs collected in June and August exhibited a significantly lower likelihood of displaying trematodes ($p = 0.000441$ (June), $p = 0.000722$ (August)) compared to the baseline month (April) (June = 10%, August = 13%, April = 55%). In terms of carapace width, larger crabs were significantly more likely to harbour trematodes compared with smaller crabs ($\beta = 0.07286$, $p = 0.011856$). This suggests that as carapace width increases, so does the likelihood of trematode presence (Figure 7).

For Oxwich Bay, another binomial logistic regression (Model 3, Table 3, see appendix: Table A2) to analyse the presence of trematodes in the sampled crab populations. This revealed month as a significant factor associated with the presence of trematodes (Model 3, Table 3, Figure 6c). Crabs collected in August were significantly more likely to contain trematodes (p = 0.0398) compared with April and June (August = 57%, April = 30%, June = 15%).

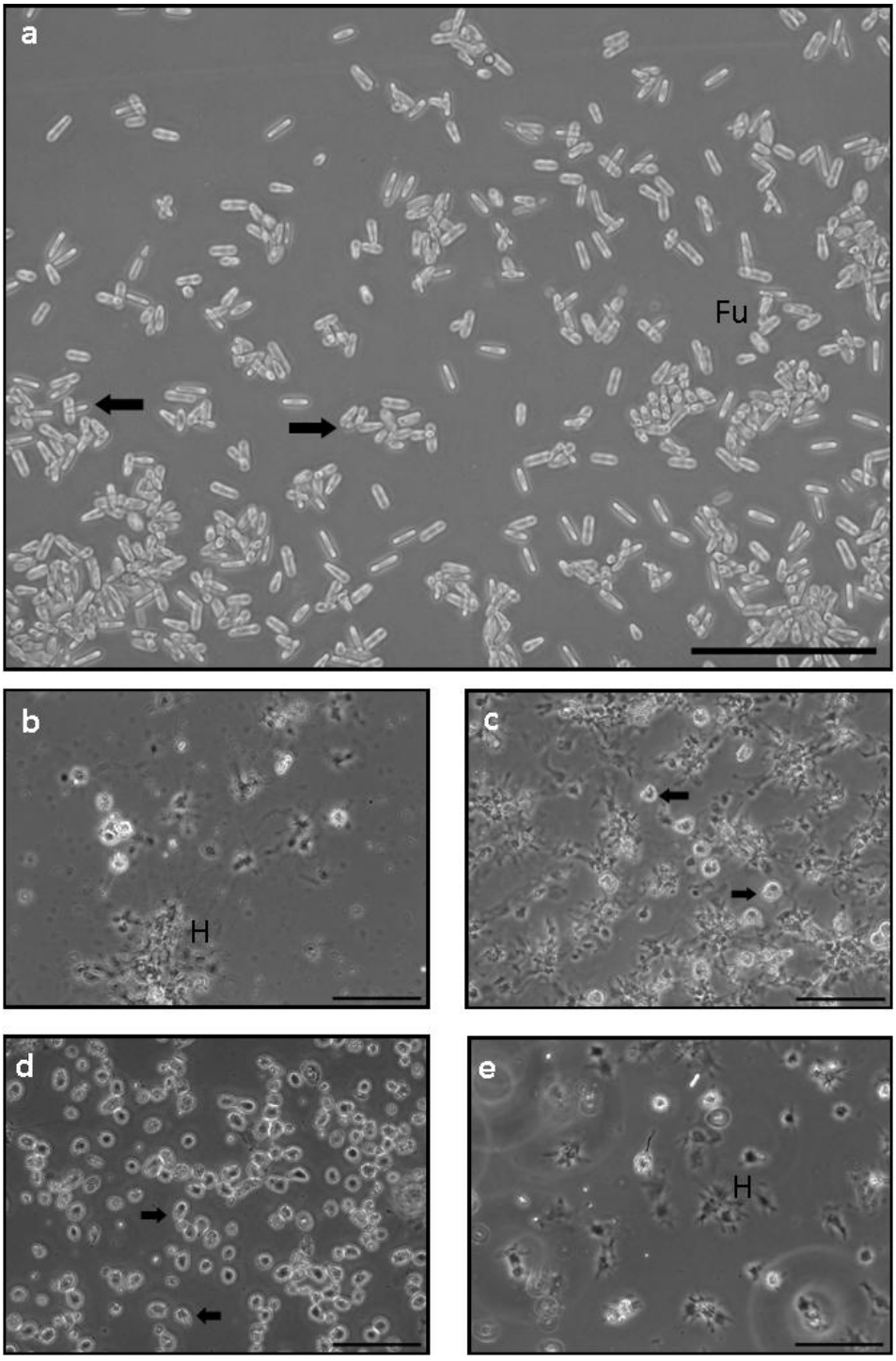

Figure 5 – Identification of *Hematodinium* and unidentified yeast-like fungus in haemolymph preparations viewed under phase contrast microscopy. Parasites were identified by their irregular shape and size. (a) High severity infection with unidentified yeast-like fungus (Fu). (b) Apparent low-grade infection of *Hematodinium*. Haemocytes (H). (c) Low-grade infection with refractile *Hematodinium*. (d) High severity infection (arrows) with numerous refractile *Hematodinium*. (e) Low-grade infection with *Hematodinium*. Haemocytes (H). Oxwich Bay (a, b, c), Mumbles Pier (d and e). Scale-bars: 100 µm

Table 3 – Binomial logistic regression models (reduced from the full models) testing the effects of environmental and biometric predictor variables on the presence of digenean trematodes in the population. Models are separated by location: Model 1, overall population; Model 2, Mumbles Pier; Model 3, Oxwich Bay (see appendix: Table A2)

Model	Predictor variables	Estimate (slope)	SE (standard error)	*P*-value
Model 1				
TremPres ~ Month	Month (August)	-0.1781	0.3836	0.64299
	Month (June)	-1.5271	0.4959	0.00241 **
df = 174				
AIC = 210.58				
Model 2				
TremPres ~ Month + Carapace.Width	Month (August)	-2.66053	0.78684	0.000722 ***
	Month (June)	-2.60586	0.74149	0.000441 ***
	Carapace.Width	0.07286	0.02895	0.011856 *
df = 90				
AIC = 89.82				
Model 3				
TremPres ~ Month	Month (August)	1.1156	0.5427	0.0398 *
	Month (June)	-0.9019	0.6725	0.1799
df = 86				
AIC = 106.35				

*Statistically significant *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001

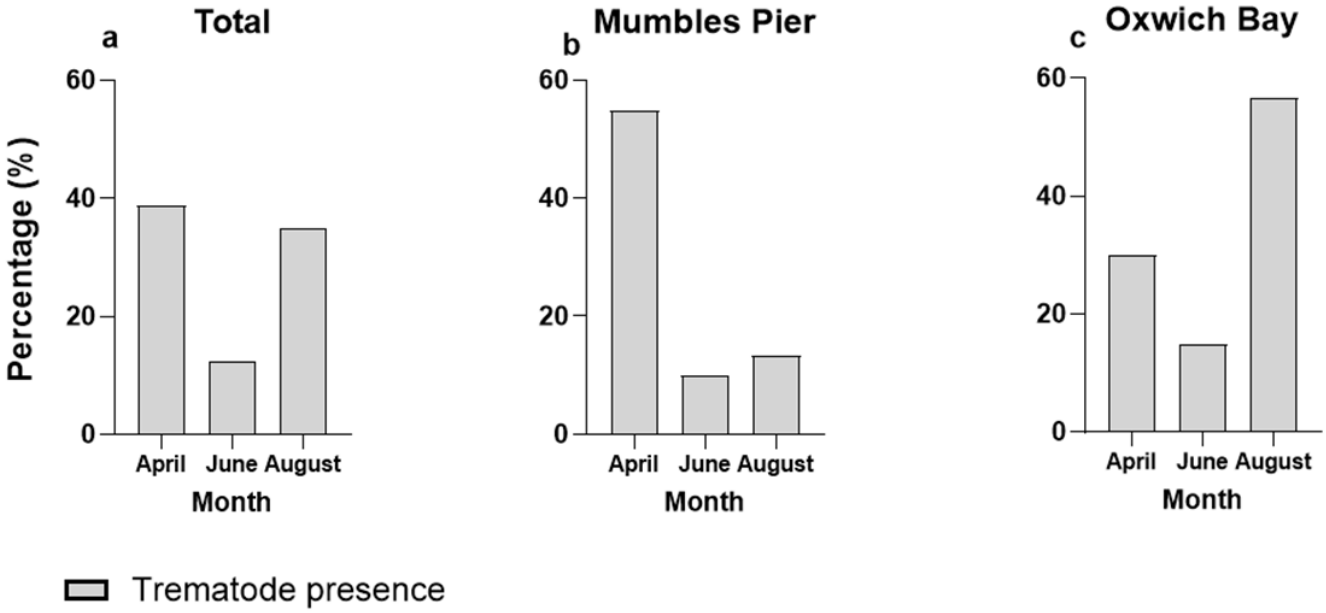

Figure 6 – Percentage of crabs where digenean trematodes were present, per location: total population, Mumbles Pier and Oxwich Bay, in relation to predictor variables month (a-c)

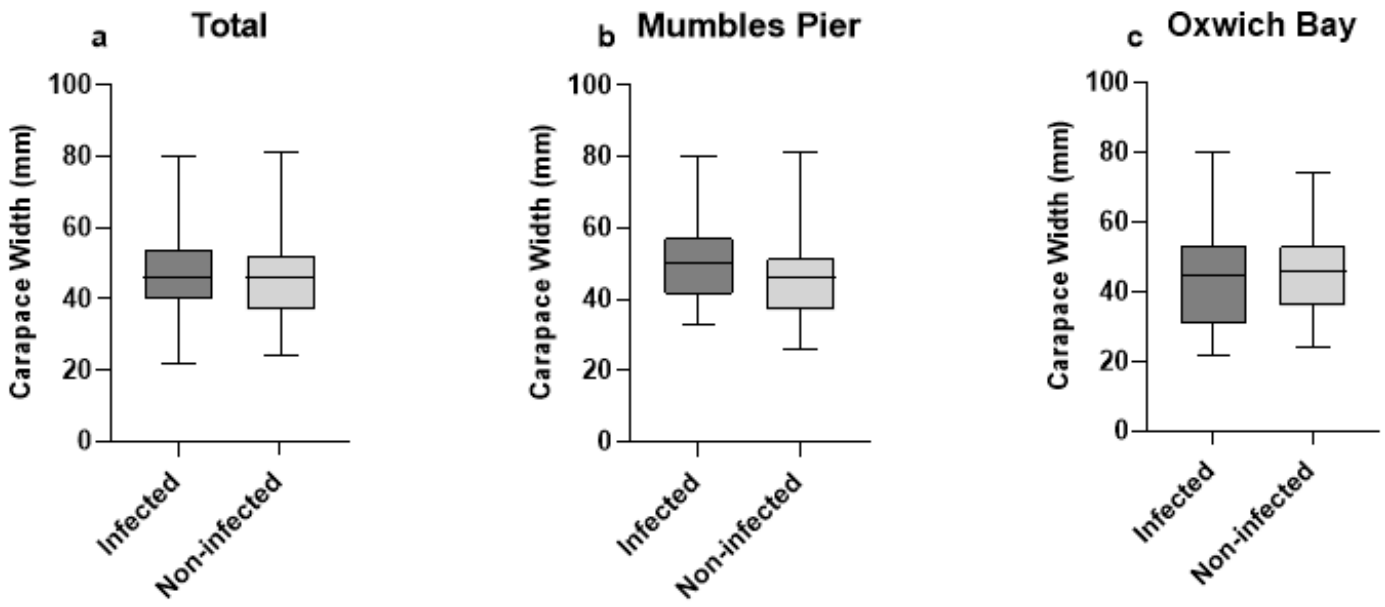

Figure 7 – Carapace width (mm) of trematode-infected and non-infected *C. pagurus* per location: total population (a), Mumbles Pier (b) and Oxwich Bay (c). For the total population (a): infected (SE = 1.7, median = 46, IQR = 14, min = 22, max = 80); non-infected (SE = 1.1, median = 46, IQR = 15, min = 24, max = 81). For Mumbles Pier (b): infected (SE = 2.2, median = 50, IQR = 15.5, min = 33, max = 80); non-infected (SE = 1.4, median = 46, IQR = 14.3, min = 26, max = 81). For Oxwich Bay (c): infected (SE = 2.6, median = 44.5, IQR = 22.3, min = 22, max = 80); non-infected (SE = 1.6, median = 46, IQR = 17, min = 24, max = 74)

3.1.2 Parasite load in *C. pagurus*

Of the 178 crabs, 55 (30.9%) contained trematodes. A generalised linear model with a negative binomial function (Model 4, see appendix: Table A3) was used to investigate the total parasite load per crab in response to location (Oxwich *vs*. Mumbles), month (April *vs*. June, *vs*. August), sex (male *vs*. female), carapace width (cont. in mm) and pigment loss (no pigment loss *vs*. pigment loss). Across the two sites, male crabs exhibited an apparently greater mean parasite load (mean ± SE) (13 ± 2.7) than females (8.9 ± 5). Crabs collected from Oxwich Bay displayed a higher parasite load (14.1 ± 3.8), in comparison to Mumbles Pier (10.7 ± 2.9). In terms of month, those crabs collected in June displayed a lower mean parasite load (7.2 ± 3.3) than those in April (12.5 ± 3.1) and August (14.2 ± 4.8). Individuals displaying pigment loss exhibited a greater parasite load (13.3 ± 5.2) than crabs without pigment loss (12.3 ± 2.8). Crabs with larger carapace widths exhibited a lower parasite load (β = -0.00735, p = 0.6501),

suggesting that as carapace width increases, trematode intensity decreases. However, the results of the negative binomial revealed no statistically significant relationship between parasite severity and any of the predictor variables, including location (Oxwich: $p = 0.4679$), month (August: $p = 0.8410$, June: $p = 0.2910$), sex (male: $p = 0.7309$), pigment loss ($p = 0.5892$) and carapace width (Model 4, see appendix: Table A3).

3.2 Metacercariae Size and Morphology

All metacercariae observed had similar morphology with round shapes, prominent cyst walls and of similar size (Figures 8 and 9). Metacercariae sizes were analysed in crabs from Oxwich Bay and Mumbles Pier (see appendix: Table A4). The median cyst size from Mumbles measured 277 µm (n=20) whereas the average cyst size from Oxwich measured 283 µm (n=24). To determine whether the observed difference in cyst size was statistically significant a Mann-Whitney U test was used to compare both locations. The results indicated that there was no statistically significant relationship (Mann-Whitney U = 227, $p = 0.7706$) between cyst size and location (Figures 8-9).

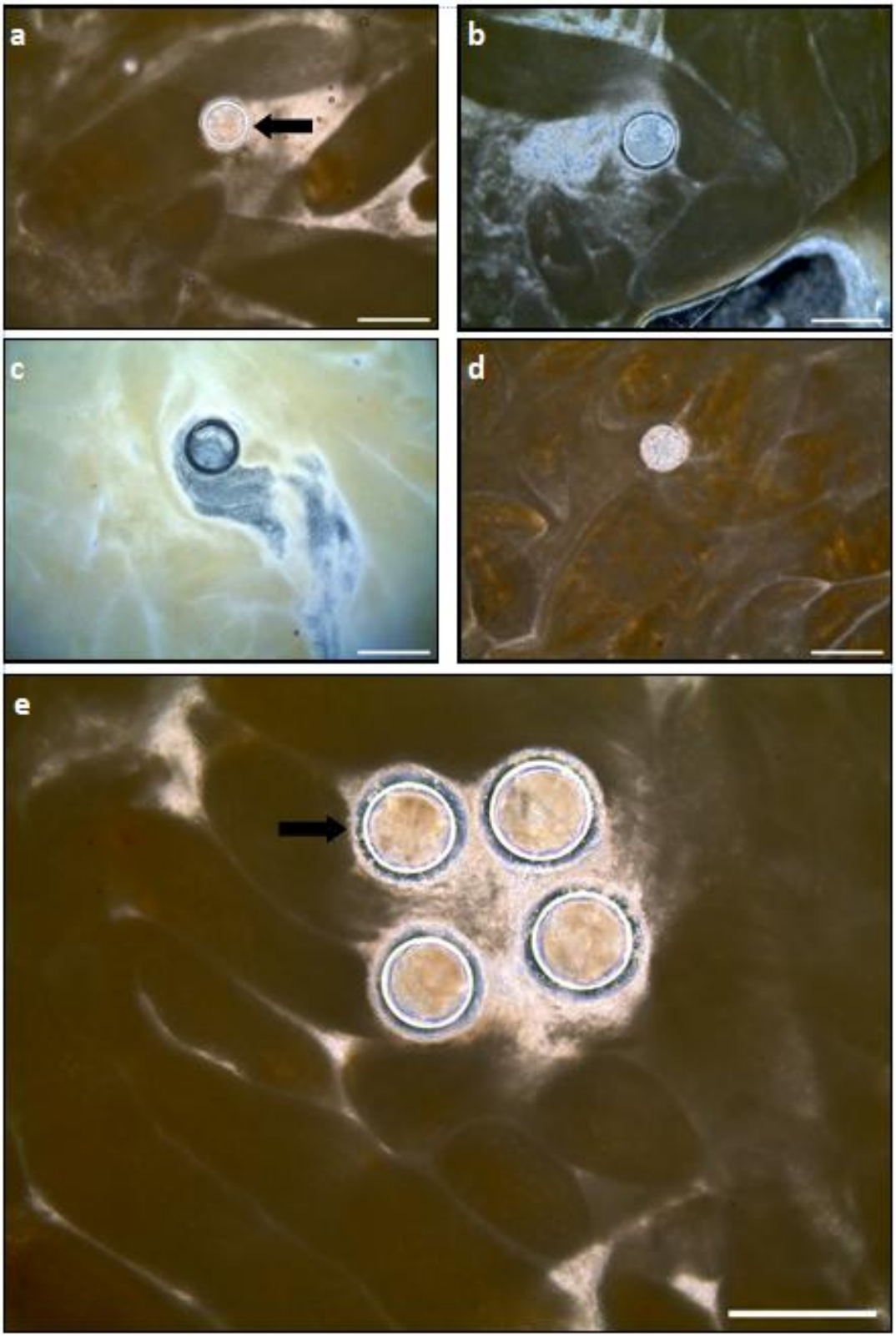

Figure 8 – Metacercariae of *M. similis* in hepatopancreas samples identified from Mumbles Pier. Metacercariae were identified as spherical with a thick cyst wall. (a, b (dark field) c (bright field), d, e (dark field)). Scale-bars: 500 µm

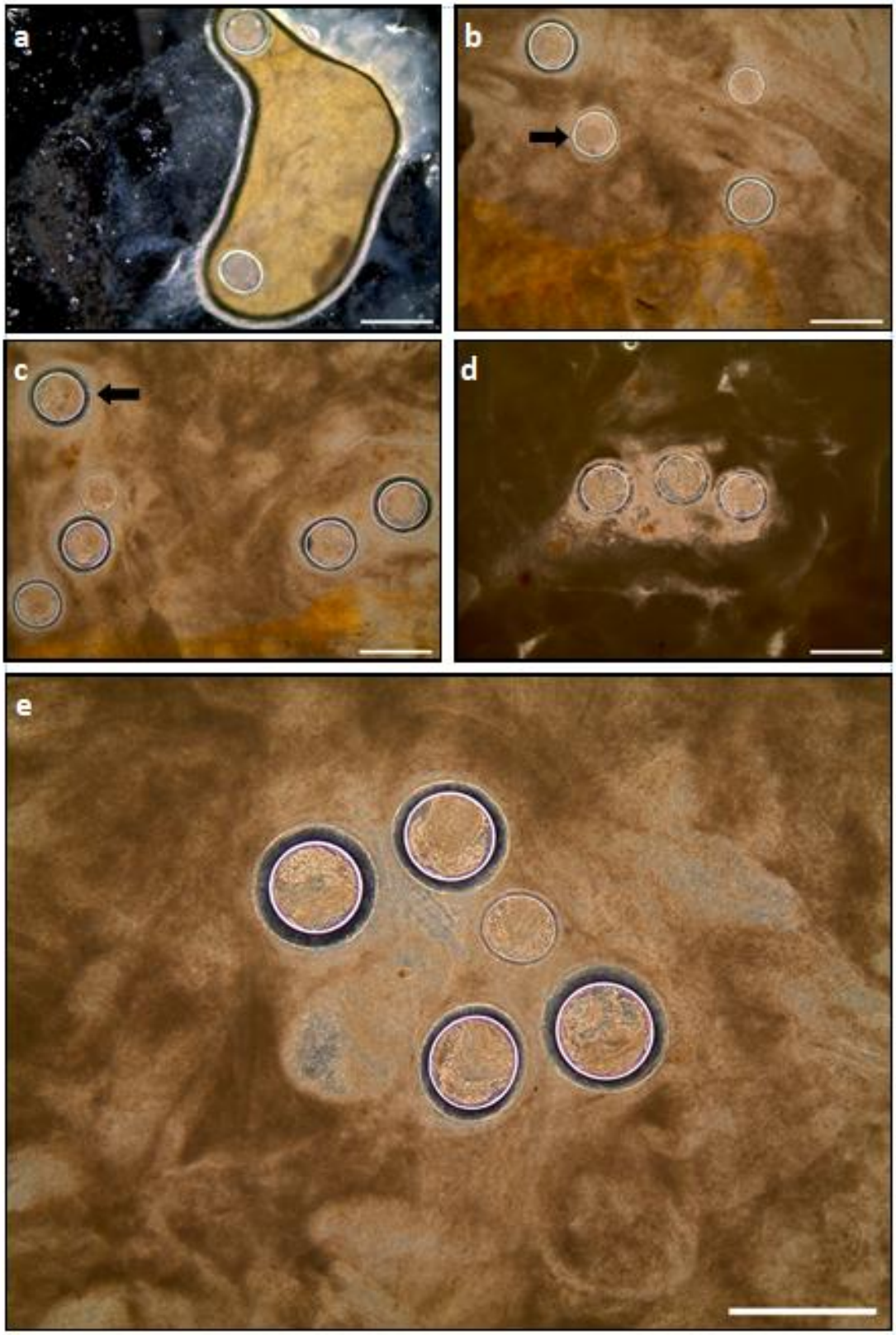

Figure 9 – Metacercariae of *M. similis* from Oxwich Bay. Metacercariae were identified as spherical with a thick wall. (a, b, c, d, e). Scale-bars: 500 µm

3.3 Phylogenetic Analyses

Of the 55 trematode-infected crabs, a total of 13 samples containing multiple metacercariae (average DNA concentration of samples sent for sequencing: 31.7 ng/µl ± 20.6 ng/µl) (mean ± SD) (see appendix: Table A9) from Mumbles Pier (n=5) and Oxwich Bay (n=8) were successfully re-amplified and sequenced using the LSU-5/LSU-1500 oligonucleotides. Of these sequences, 61.5% shared considerable similarity (1257 bp, 100% coverage, 100% identity) with *M. similis* from the shore crab (*C. maenas*) (GenBank: AY220625, see appendix: Table A5) reported by Tkach *et al* (2003), with one sequence showing a slightly lower identity of 99.62% from the same host species. All sequences shared high similarity (>95% coverage and identity) with *M. similis* retrieved from the salty-backed gull (*L. schistisagus*) (GenBank: HM584136-HM584138, see appendix: Table A5). The constructed phylogram revealed a clear separation of *M. similis* from other microphallid species (Figure 10), with all *M. similis* sequences forming a robust clade, suggesting little genetic variation between *M. similis* and other microphallid species.

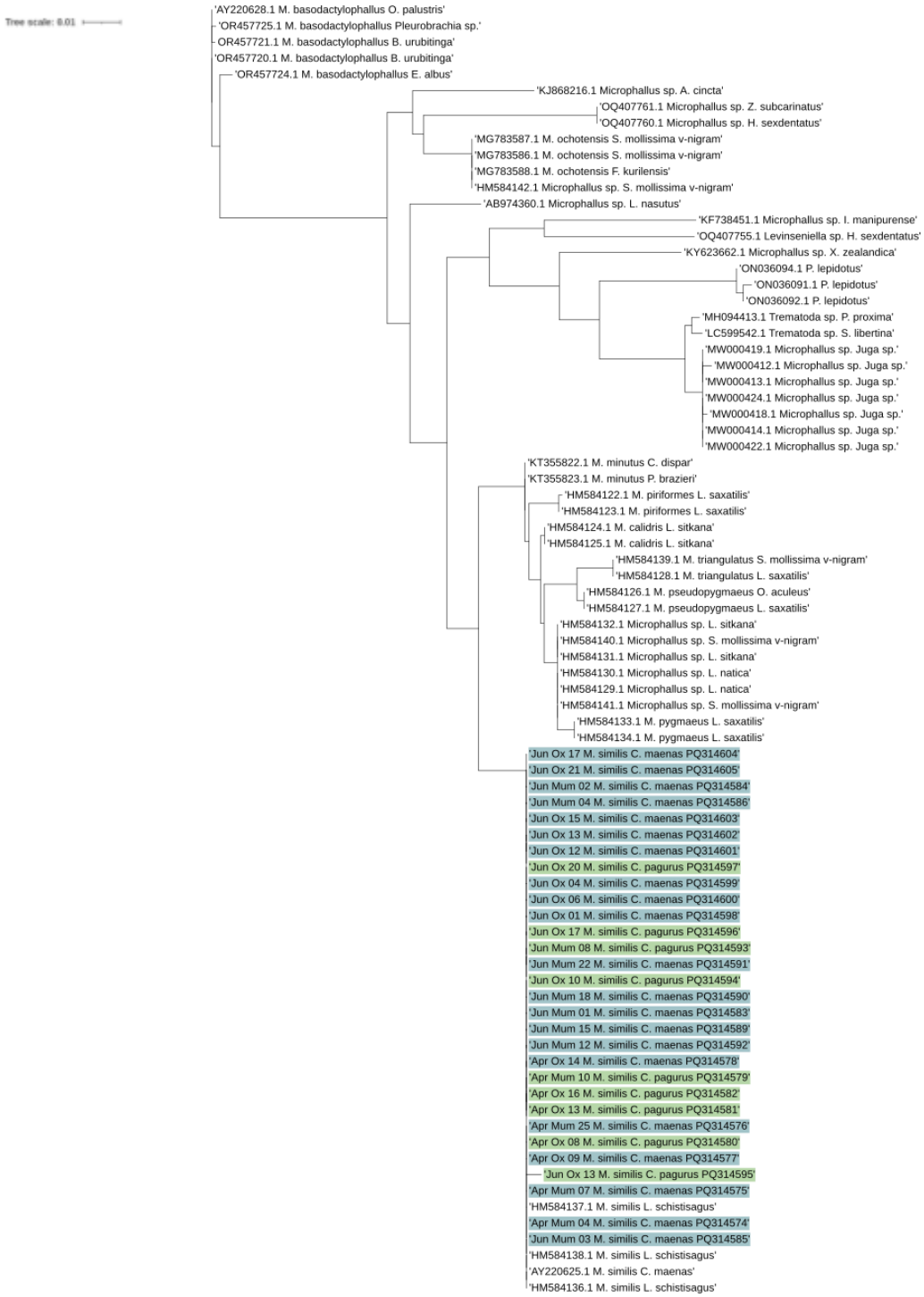

Figure 10 – Phylogram of the partial 28S rRNA gene region from trematode-infected crabs (Maximum Likelihood estimation, with highest log likelihood value (-5446.52) from 1000 bootstrap replicates). Genomic DNA was isolated from the hepatopancreatic tissue of trematode-infected edible crabs across two locations (prefix Mum, Mumbles Pier; prefix Ox, Oxwich Bay, *C. maenas* (blue), *C. pagurus* (green)) in Swansea Bay, UK (GenBank: PQ314579-PQ314597, see appendix: Table A6 for individual numbers), and probed *via* PCR for microphallid diversity. Reference nucleotide sequences for microphallid species from

various hosts (see full methods list) were retrieved from GenBank. The tree was rooted using the corresponding region from *M. basodactylophallus* (GenBank: AY220628)

Chapter 4
Discussion

The digenean trematode *M. similis* is present in *C. pagurus* populations at both Mumbles Pier and Oxwich Bay. Both locations sampled displayed monthly difference in terms of trematode presence, with crabs collected in April and August exhibiting more parasites. In June, the number of crabs found to harbour *M. similis* was significantly lower with these individuals displaying low prevalence. Carapace width was also associated with the presence of *M. similis*, with larger crabs being more likely to harbour trematodes than smaller individuals, at Mumbles Pier only. However, no biometric or environmental variables were associated with trematode intensity at either location. In terms of phylogeny all parasites were identified both morphologically and genetically as *M. similis*.

4.1 Seasonal Effects on Trematode Presence

The role of season in relation to trematode presence has been documented in a range of host species. Seasonal prevalence of trematodes also seems to be host specific, mostly relating to temperature, salinity and light. In the present study, *M. similis* prevalence was higher in both April and August compared to June but with no clear evidence of a seasonal effect probably due to the short timescale of these surveys concentrating on late spring to summer only. A number of studies have used wider sampling ranges over the year to look for seasonal effects. For instance, Studer and Poulin (2012) reported seasonal patterns in *Maritrema novaezealandensis* an intertidal trematode, particularly focusing on the lifecycle and interactions with various hosts. Warmer months were generally found to support higher transmission rates with the timing of emergence of cercariae being optimised to enhance the probability of successful transmission, whereas colder months were found to slow or halt the trematodes lifecycle. (Studer & Poulin, 2012). Additionally, higher densities of the second intermediate *Paracalliope novizealandiae* and definitive bird hosts during the summer resulted in more cercariae being ingested by a larger number of suitable hosts, leading to an increase in metacercariae loads (Studer & Poulin, 2012). Prokofiev *et al* (2023) documented similar patterns in daily cercarial emission during experiments with ten littoral trematode species from the White Sea and two freshwater species from Lake Chudskoe. Temperature was identified as a key factor regulating cercarial emergence from molluscan hosts, with higher temperatures increasing cercarial activity and release (Koprivnikar *et al*, 2010; Prokofiev *et al*, 2023). In some species, transmission was entirely temperature-dependent, more so than light, in controlling the release of cercariae as seen in *Himasthla* spp (Prokofiev *et al*, 2023). Furthermore, it was noted that maintaining molluscs at higher temperatures (20˚ and 25℃)

during the experiment led to a significant increase in the intensity of cercariae development in parthenitae (Prokofiev *et al*, 2023).

The movements of the first molluscan, intermediate crustacean and definitive hosts, may influence the prevalence and intensity of infections by *M. similis*. For example, gulls, including *L. argentatus*, often migrate to coastal breeding grounds in the summer, potentially increasing parasite transmission to the first molluscan hosts. The intermediate host, *C. pagurus* exhibit vertical diurnal movements, foraging on rocky shores during the summer and early autumn, with relatively young post-moult crabs feeding at night on mussels (*Mytilus edulis*) (Dannevig and Gunderson, 1982). Karlsson and Christiansen (1996) documented similar diurnal movements in *C. pagurus* in an exposed rocky inlet on the Norwegian Skagerrak coast, noting that temperature influenced feeding activity, with warmer conditions driving crabs to shallow waters at night. These feeding forays of crabs increase their chance of encountering cercariae released from the molluscan host. Additionally, Stafford and Davies (2004) reported that *L. saxatilis* (first intermediate host) often aggregate in crevices or pits, reducing the risk of predation and encouraging mating, which could enhance *M. similis* transmission when in similar habitats as *C. pagurus* during spring and late summer. The low prevalence of *M. similis* in June remains unexplained and it is clear that a longer-term study could unearth a better understanding of the dynamics of parasitisation. Future studies should extend the observation period to better understand how environmental and biological factors affect trematode dynamics throughout the year. Employing temperature loggers on site would also provide valuable data on this key parameter that controls cercarial movement.

4.2 Effect of Crab Size on Parasite Presence

In the present study, carapace width influenced trematode prevalence, with larger crabs being more likely to have trematodes compared to smaller crabs at Mumbles Pier only. Briones-Fourzan *et al* (2016) and Davies *et al* (2019b) found that the abundance of *Cymatocarpus solearis* in *Panulirus argus* was not influenced by sex but increased with size. This could indicate that larger, older *C. pagurus* have likely been exposed to infective cercariae for a longer period of time, potentially allowing them to accumulate greater parasite burdens before succumbing to mortality (Overstreet, 1983). However, as the crabs in the present study were all juveniles, this hypothesis is difficult to test, as juveniles may exhibit different behavioural patterns that influence their susceptibility to trematodes. In contrast, it has been reported that

juvenile *C. pagurus* in Weymouth Bay, UK, were more susceptible to *M. primas* than adult crabs (Bateman *et al*, 2011). This was likely due to juvenile edible crabs residing in the intertidal zone, where both first intermediate and definitive hosts of *M. primas* are abundant, whereas adult crabs are found in deeper waters (Bateman *et al*, 2011). Despite the fact that larger crabs were more likely to harbour trematodes, this was not the case for the total number of parasites. No significant correlation was found between crab size and parasite intensity in the present study, indicating that other factors other than size may be more critical in determining trematode intensity. Future studies should aim to analyse how different life stages of trematodes interact with hosts of various sizes.

4.3 Location

Although not statistically significant, trematode prevalence was apparently higher at Oxwich Bay (34.5%) than at Mumbles Pier (27.5%) during the sampling period, perhaps suggesting that Oxwich may offer more favourable conditions for trematode transmission including hosting larger numbers of first intermediate hosts such as periwinkles and/or the definitive sea bird hosts. However, no statistical relationship was found between trematode prevalence and intensity at either location. The original aim of this study was to determine parasite dynamics in crabs only and no attempt was made to examine the distribution of these other hosts in the lifecycle of *M. similis*. Mumbles Pier is located in Swansea Bay's designated 'Heavily Modified Waterbody', where the ecological status, according to the WFD Infaunal Quality Index (IQI), is rated as poor or bad due to the close proximity to diffuse sources of historical pollution (Callaway, 2016). Oxwich Bay is situated further away from the industrial embayment that is Swansea Bay that is headed at Mumbles point. In particular, Callaway (2016) reported that the previous sewage outfalls in Swansea Bay have significantly impacted benthic community composition. Additionally, samples from the outer bay indicated poor ecological status, likely linked to the nearby dredge spoil ground used for discarding material from dredging the bay's shipping channels (Callaway, 2016; Callaway *et al*, 2020). This spoil disposal may directly affect the benthic community by altering sediment composition, increasing turbidity and mobilizing toxic materials causing a severe localised negative effect in Swansea Bay (Callaway *et al*, 2020; Callaway, 2016). While these anthropogenic changes may not have affected community patterns over the past 30 years, they have likely caused localised shifts in Swansea Bay's ecological status (Callaway, 2016). Such declines in biodiversity and habitat quality could correlate with reduced trematode prevalence and

intensity. At Mumbles Pier, the degraded ecological conditions and reduced species richness could limit the diversity and abundance of potential hosts for *M. similis*, restricting their lifecycle and transmission leading to reduced prevalence and intensity of parasitism.

4.4 Phylogeny

Phylogenetic reconstructions demonstrated little ecotype diversity of *M. similis* from edible crabs between locations (Mumbles Pier and Oxwich Bay) or sampling months, as the majority of sequences clustered within one robust clade. However, a single *M. similis* sequence from a shore crab appeared as an outlier, suggesting higher genetic variation. This indicates that the parasite infecting *C. pagurus* at both locations is *M. similis*. Galaktionov *et al* (2012) reviewed transmission patterns and diversification with respect to historical events, host switching and host-parasite co-evolution of *pygmaeus* microphallids in the Northern Hemisphere. Tkach *et al* (2003) reported the phylogenetic interrelationships of 32 species belonging to the superfamily Microphallidae. Blakeslee *et al* (2020) demonstrated genetic overlap of microphallid lineages between crab species, with *C. maenas* acting as a competent host in Placentia Bay. Clade B was the most common, with one haplotype (identified as *M. similis*) displaying the highest frequency across crab species, *Littorina* spp. and regions (Blakeslee *et al*, 2020). *Microphallus similis*, a prevalent parasite in *C. maenas* and cosmopolitan across the Atlantic, may have experienced trans-Atlantic gene flow via definitive bird hosts or through the introduction of *C. maenas* to North America (Miura *et al*, 2006; Blakeslee *et al*, 2020). Although, it was noted that native hosts in eastern North America could also harbour European genetic variants of *M. similis* (Blakeslee *et al*, 2020). By combining sequence data with 50 references from GenBank, this provided strong evidence of *M. similis* presence across *C. pagurus* populations at both locations. However, research on the diversity of *M. similis*, particularly in *C. pagurus*, remains limited.

4.5 Conclusions

The study highlights the presence and variation of *M. similis* in *C. pagurus* populations across Mumbles Pier and Oxwich Bay, showing that prevalence was influenced by seasonality and carapace width. Additionally, no environmental or biometric variables were associated with *M. similis* intensity at either site. Trematode prevalence was highest during April and August, likely reflecting the lifecycle of *M. similis*, driven by temperature and host availability.

Although no clear associations between host sex or size and trematode intensity were found, male-biased susceptibility at Oxwich Bay and the association between crab size and trematode prevalence may suggest that host biology could still influence parasitism, as noted in other studies. Encountering *M. similis* at relatively high percentages in *C. pagurus* populations at Oxwich Bay provides insight into reservoirs of crustacean disease, where protected habitats may support a more diverse range of hosts and ecological niches compared to degraded aquatic environments. This could have significant implications for commercially important species like *C. pagurus*, as increased parasitism may alter population dynamics, potentially affecting overall fishery yields. Future research should focus on monitoring the long-term effects of trematode prevalence and intensity on *C. pagurus* populations, including changes in parasitism over time. Additionally, studies should aim to investigate the interactions between environmental variables, parasite lifecycle and host biology to gain insights into mitigating the impacts of parasitism on commercially important species.

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Chapter 5
Appendix

5.1 Abbreviations

CW: carapace width

PCR: polymerase chain reaction

ICES: the International Council for the Exploration of the Sea

eDNA: environmental DNA

rRNA: ribosomal ribonucleic acid

AONB: Area of Outstanding Natural Beauty

KCl: potassium chloride

BLAST: basic local alignment search tool

NCBI: National Centre for Biotechnology Information

SE: standard error

IQI: WFD Infaunal Quality Index

SD: standard deviation

MLE: Maximum Likelihood Estimator

GLM: Generalized Linear Model

IQR: Interquartile range

5.2 List of Tables and Figures

5.3 Supplementary Methods

5.3.1 (Table A1) - Biometric data taken from *C. pagurus* populations from Mumbles Pier and Oxwich Bay

Site(s)	Month	No. of crabs surveyed	Mean CW & range (mm)	Sex ratio (M:F)	Epibionts (%)	Limb loss (%)	Pigment loss (%)	Shell disease (%)	Spirorbis (%)
Mumbles Pier	April	31	44 mm, 33 mm	30:1	0	45	13	13	3
	June	30	45 mm, 38 mm	24:6	23	57	47	3	0
	August	30	52 mm, 55 mm	26:4	13	53	10	3	3
Oxwich Bay	April	30	53 mm, 56 mm	19:11	3	37	23	10	13
	June	27	45 mm, 40 mm	27:0	5	30	48	19	0
	August	30	38 mm, 36 mm	30:0	3	40	10	0	0

5.3.2 (Table A2) - Binomial logistic regression **(**full model) used in order to predict response variable of trematode presence before reduction. Asterix denotes significance ($P \leq 0.05$)

Model	Predictor variable	Estimate (slope)	SE (standard error)	*P*-value
Model 1				
TremPres ~	Location(Oxwich)	0.32666	0.36174	0.36781
Location +	Month (August)	-0.14348	0.39775	0.71876
Month + Sex +	Month (June)	-1.70107	0.53773	0.00185 **
Fouling +	Sex (Male)	0.89906	0.66294	0.17685
Carapace.Width	Fouling	-0.62416	0.83908	0.45800
+ Pigment.Loss	Carapace.Width	0.02005	0.01686	0.23593
+ Hemo.col	Pigment.Loss	0.55423	0.46646	0.23644
	Hemo.col (Milky)	-1.06752	0.84114	0.20614
df = 169				
AIC = 215.30				
Model 2				
TremPres ~	Month (August)	-2.41923	0.83728	0.00492 **
Month + Sex +	Month (June)	-2.38512	0.95674	0.01466 *
Fouling +	Sex (Male)	0.04766	1.02581	0.96306
Carapace.Width	Fouling	-0.79740	1.33541	0.55205
+ Pigment.Loss	Carapace.Width	0.06648	0.03443	0.05691.
+ Hemo.col	Pigment.Loss	0.71017	0.87421	0.41891
	Hemo.col (Milky)	-0.95302	1.26568	0.45360
df =83				
AIC =96.51				
Model 3				
TremPres ~	Month (August)	1.45800	0.68566	0.0335 *
Month + Sex +	Month (June)	-1.10241	0.79082	0.1633
Fouling +	Sex (Male)	1.00458	0.98402	0.3073
Carapace.Width	Fouling	0.13159	1.3512	0.9224
+ Pigment.Loss	Carapace.Width	0.03718	0.02571	0.1482
+ Hemo.col	Pigment.Loss	0.45495	0.64655	0.4816
	Hemo.col (Milky)	-1.31439	1.33889	0.3262
df = 86				
AIC = 112.65				

*Statistically significant *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

Abbreviations: SE, standard error

5.3.3 (Table A3) – Generalised linear model with negative binomial function used in order to predict response variable of trematode intensity.

Model	Predictor variable	Estimate (slope)	SE (standard error)	*P*-value
Model 4				
Parasite.count ~ Location	Location (Oxwich)	0.25415	0.35008	0.4679
+ Month + Sex +	Month (August)	-0.07792	0.38853	0.8410
Pigment.Loss +	Month (June)	-0.58744	0.55634	0.2910
Carapace.Width	Sex (Male)	0.21572	0.62730	0.7309
	Pigment.Loss	0.22438	0.41549	0.5892
	Carapace.Width	-0.00735	0.01620	0.6501
df = 55				
AIC = 403.03				

5.3.4 (Table A4) – Mann-Whitney U test testing the effects of environmental predictor variables such as location on metacercariae size (µm)

Group A	**Group B**	**U statistic**	**Sum of Ranks (A)**	**Sum of Ranks (B)**	**Median Rank (A)**	**Median Rank (B)**	***P*-value**
Mumbles Pier	Oxwich Bay	227	463	527	15.5	16.7	0.7706

5.3.5 (Table A5) – Accession numbers, from reference sequences deposited in GenBank, and used in phylogenetic tree (Figure 13)

GenBankID	Host species	Target	Location	Sample type	Reference
KF738451	*Indochinamon manipurense*	*Microphallus* sp.	Manipur, Motbung, India	Genomic DNA	Athokpam & Tandon, 2015
KY623662	*Xanthocnemis zealandica*	*Microphallus* sp.	New Zealand	Genomic DNA	Filion *et al*. 2017
MW000414	*Juga* sp.	*Microphallidae* sp.	Oregon, USA	Genomic DNA	Preston *et al*. 2021
MW000412	*Juga* sp.	*Microphallidae* sp.	Oregon, USA	Genomic DNA	Preston *et al*. 2021
MW000422	*Juga* sp.	*Microphallidae* sp.	Oregon, USA	Genomic DNA	Preston *et al*. 2021
MW000419	*Juga* sp.	*Microphallidae* sp.	Oregon, USA	Genomic DNA	Preston *et al*. 2021
MW000413	*Juga* sp.	*Microphallidae* sp.	Oregon, USA	Genomic DNA	Preston *et al*. 2021
MW000424	*Juga* sp.	*Microphallidae* sp.	Oregon, USA	Genomic DNA	Preston *et al*. 2021
MW000418	*Juga* sp.	*Microphallidae* sp.	Oregon, USA	Genomic DNA	Preston *et al*. 2021
MH094413	*Pleurocera proxima*	*Trematoda* sp.	Alleghany County, North Carolina, USA	Genomic DNA	Zemmer *et al*. 2020
LC599542	*Semisulcospira libertina*	*Trematoda* sp.	Hokkaido, Asahikawa, Japan	Genomic DNA	Nakao & Sasaki, 2021
OR457724	*Eudocimus albus*	*Microphallus basodactylophallus*	Tlacotalpan, Veracruz, Mexico	Genomic DNA	Aldama-Prieto *et al*. 2024

OR457721	*Buteogallus urubitinga*	*Microphallus basodactylophallus*	Playa Paraiso, Tabasco, Mexico	Genomic DNA	Aldama-Prieto *et al*. 2024
OR457720	*Buteogallus urubitinga*	*Microphallus basodactylophallus*	Tupilco, Tabasco, Mexico	Genomic DNA	Aldama-Prieto *et al*. 2024
OR457725	*Pleurobrachia* sp.	*Microphallus basodactylophallus*	Tampamachoco, Veracruz, Mexico	Genomic DNA	Aldama-Prieto *et al*. 2024
ON036091	No information	*Parabascus lepidotus*	Republic of Mordovia, Russia	Genomic DNA	Kirillova *et al*. 2022
ON036092	No information	*Parabascus lepidotus*	Republic of Mordovia, Russia	Genomic DNA	Kirillova *et al*. 2022
ON036094	No information	*Parabascus lepidotus*	Republic of Mordovia, Russia	Genomic DNA	Kirillova *et al*. 2022
AY220628	*Oryzomys palustris*	*Microphallus basodactylophallus*	USA	Genomic DNA	Tkach *et al*. 2003
AY220625	*Carcinus maenas*	*Microphallus similis*	United Kingdom	Genomic DNA	Tkach *et al*. 2003
AB974360	*Longiflagrum nasutus*	*Microphallidae* sp.	Okinawa, Japan	Genomic DNA	Kakui, 2014
KJ868216	*Austrolittorina cincta*	*Microphallus* sp.	Lower Portobello Bay, New Zealand	Genomic DNA	O'Dwyer *et al*. 2014

OQ407755	*Hemigrapsus sexdentatus*	*Levinseniella* sp.	Otago, New Zealand	Genomic DNA	Bennett *et al*. 2023
OQ407761	*Zeacumantus subcarinatus*	*Microphallus* sp.	Otago, New Zealand	Genomic DNA	Bennett *et al*. 2023
OQ407760	*Hemigrapsus sexdentatus*	*Microphallus* sp.	Otago, New Zealand	Genomic DNA	Bennett *et al*. 2023
MG783587	*Somateria mollissima v-nigram*	*Microphallus ochotensis*	Skipper Creek, Sea of Okhotsk, Russia	Genomic DNA	Galaktionov *et al*. 2018
MG783586	*Somateria mollissima v-nigram*	*Microphallus ochotensis*	Skipper Creek, Sea of Okhotsk, Russia	Genomic DNA	Galaktionov *et al*. 2018
MG783588	*Falsicingula kurilensis*	*Microphallus ochotensis*	Skipper Creek, Sea of Okhotsk, Russia	Genomic DNA	Galaktionov *et al*. 2018
KT355822	*Cherax dispar*	*Microphallus minutus*	Moggil Creek, Queensland, Australia	Genomic DNA	Kudlai *et al*. 2015
KT355823	*Posticobia brazieri*	*Microphallus minutus*	Churchbank Weir, Queensland, Australia	Genomic DNA	Kudlai *et al*. 2015
HM584142	*Somateria mollissima*	*Microphallus* sp.	Cape Taygonos, N Sea of Okhotsk, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584122	*Littorina saxatilis*	*Microphallus piriformes*	SW Grindavik, Iceland	Genomic DNA	Galaktionov *et al*. 2012
HM584123	*Littorina saxatilis*	*Microphallus piriformes*	Vaygach Island, SE Barents Sea, Russia	Genomic DNA	Galaktionov *et al*. 2012

HM584124	*Littorina sitkana*	*Microphallus calidris*	Sakhalin, Sea of Okhotsk, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584125	*Littorina sitkana*	*Microphallus calidris*	Kunashir, Kuril Islands, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584139	*Somateria mollissima*	*Microphallus triangulatus*	Yamskaya Bay, Sea of Okhotsk, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584128	*Littorina saxatilis*	*Microphallus triangulatus*	Kandalaksha Bay, White Sea, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584126	*Onoba aculeus*	*Microphallus pseudopygmaeus*	Kandalaksha Bay, White Sea, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584127	*Littorina saxatilis*	*Microphallus pseudopygmaeus*	Kandalaksha Bay, White Sea, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584133	*Littorina saxatilis*	*Microphallus pygmaeus*	SW Grindavik, Iceland	Genomic DNA	Galaktionov *et al*. 2012
HM584134	*Littorina saxatilis*	*Microphallus pygmaeus*	Kandalaksha Bay, White Sea, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584129	*Littorina natica*	*Microphallus* sp.	Egvekinot Inlet, Bering Sea, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584131	*Littorina sitkana*	*Microphallus* sp.	Kunashir, Kuril Islands, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584130	*Littorina natica*	*Microphallus* sp.	Egvekinot Inlet, Bering Sea, Russia	Genomic DNA	Galaktionov *et al*. 2012

HM584140	*Somateria mollissima*	*Microphallus* sp.	Yamskaya Bay, N Sea of Okhotsk, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584141	*Somateria mollissima*	*Microphallus* sp.	Yamskaya Bay, N Sea of Okhotsk, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584132	*Littorina sitkana*	*Microphallus* sp.	Kunashir, Kuril Islands, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584136	*Larus schistisagus*	*Microphallus similis*	Impoveem, Sea of Okhotsk, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584138	*Larus schistisagus*	*Microphallus similis*	Impoveem, Sea of Okhotsk, Russia	Genomic DNA	Galaktionov *et al*. 2012
HM584137	*Larus schistisagus*	*Microphallus similis*	Impoveem, Sea of Okhotsk, Russia	Genomic DNA	Galaktionov *et al*. 2012

5.3.6 (Table A6) – Accession numbers, deposited in GenBank, and corresponding sampling numbers for all trematode-positive crabs successfully sequenced from study, and used in phylogenetic tree (Figure 13)

GenBankID	Sample	Target	Primers	Sample type
PQ314580	AOCP08	*Microphallus similis*	LSU-5/LSU-1500	*Cancer pagurus* Metacercariae DNA
PQ314579	AMCP10	*Microphallus similis*	LSU-5/LSU-1500	*Cancer pagurus* Metacercariae DNA
PQ314581	AOCP13	*Microphallus similis*	LSU-5/LSU-1500	*Cancer pagurus* Metacercariae DNA
PQ314582	ACOP16	*Microphallus similis*	LSU-5/LSU-1500	*Cancer pagurus* Metacercariae DNA
PQ314593	JMCP08	*Microphallus similis*	LSU-5/LSU-1500	*Cancer pagurus* Metacercariae DNA
PQ314594	JOCP10	*Microphallus similis*	LSU-5/LSU-1500	*Cancer pagurus* Metacercariae DNA
PQ314595	JOCP13	*Microphallus similis*	LSU-5/LSU-1500	*Cancer pagurus* Metacercariae DNA
PQ314596	JOCP17	*Microphallus similis*	LSU-5/LSU-1500	*Cancer pagurus* Metacercariae DNA
PQ314597	JOCP20	*Microphallus similis*	LSU-5/LSU-1500	*Cancer pagurus* Metacercariae DNA

5.3.7 (Table A7) – MRes Biosciences Statement of Expenditure

Student name: Grace Olivia Nancy Crocker

Student number: [redacted]

Project title: Parasitic Diseases of Crabs in Swansea Bay

Category	Item	Description	Cost
Travel	Driving	Sampling at Mumbles Pier	£3.00
Travel	Driving	Travelling from Mumbles Pier to aquarium (Swansea University)	£2.50
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£4.50
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£2.50
Travel	Driving	Sampling at Mumbles Pier	£3.00
Travel	Driving	Travelling from Mumbles Pier to aquarium (Swansea University)	£5.50

Travel	Driving	Travelling from Oxwich Bay to aquarium (Swansea University)	£4.50
Travel	Driving	Travelling from Mumbles Pier to aquarium (Swansea University)	£2.50
Travel	Driving	Sampling at Mumbles Pier	£5.50
Travel	Driving	Travelling from Oxwich Bay to aquarium (Swansea University)	£9.00
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£9.00
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£9.00
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£9.00
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£9.00
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£9.00
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£9.00
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£6.50

Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£9.00
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£9.00
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£9.00
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£9.00
Travel	Driving	Travelling from Carmarthen to benthos/molecular lab (Swansea University)	£9.00
Travel	Driving	Cleaning aquarium/benthos lab (Swansea University)	£9.00
Travel	Driving	GenBank accession uploads (Swansea University)	£9.00
Travel	Driving	Sampling at Oxwich Bay	£5.00
Travel	Driving	Sampling at Oxwich Bay	£6.00

Travel	Driving	Meeting	£6.50
Travel	Driving	Meeting	£9.00
Total cost:			**£192.50**

*Including VAT and delivery where applicable

I hereby certify that the above information is true and correct to the best of my knowledge.

Signature (Supervisor)

Signature (Student)

5.3.8 (Table A8) - Statement of Contributions

Contributor Role	Role Definition
Conceptualization	AFR, CED
Data Curation	ATB, GONC, AFR, CED
Formal Analysis	ATB, GONC
Funding Acquisition	AFR, CED
Investigation	ATB, GONC, AFR, CED
Methodology	ATB, GONC, AFR, CED
Project Administration	AFR, CED
Resources	AFR, CED
Software	CED, ATB, GONC
Supervision	CED, AFR
Validation	CED, AFR
Visualisation	CED, AFR, ATB, GONC
Writing – Original Draft Preparation	GONC, CED, AFR
Writing – Review & Editing	GONC, CED, AFR

5.3.9 (Table A9) – Qubit DNA concentrations

Sample	DNA concentrations (ug/µl)
AOCP08	13.0
AOCP13	19.0
AOCP16	10.8
AMCP09	Out of range
AMCP10	19.5
AMCP26	Out of range
AMCP28	3.16
JMCP08	40.6
JOCP10	63.2
JOCP13	58.2
JOCP17	47.2
JOCP18	18.4
JOCP20	55.6

5.4 R Script for Binomial Logistic Regression

```
rm(list=ls(all=TRUE)) # Removes everything from R (all objects) - a good thing to do if you are starting fresh work

graphics.off()        # Removes any graphics or graphic windows that may exist - again a good thing to do if you are starting to work fresh


setwd()


install.packages("quantreg")

install.packages("SparseM")

install.packages("Rearrangement")

install.packages("reshape")

install.packages("MuMIn") #only needed if we want to use DREDGE

library(MASS)

library(Rearrangement)

library(reshape)

library(MuMIn)


BenthosCPresultsnew1<-read.csv(file.choose())

names(BenthosCPresultsnew1)

head(BenthosCPresultsnew1)

str(BenthosCPresultsnew1) # gives full structure of dataset

attach(BenthosCPresultsnew1)

detach(BenthosCPresultsnew1)


Carapace.Width <- factor(Carapace.Width)

Location<- factor(Location)

Pigment.Loss<- factor(Pigment.Loss)

Fouling<- factor(Fouling)

Hemo.col<- factor(Hemo.col)
```

```
Gender<- factor(Gender)

TremPres<- factor(TremPres)

Month<- factor(Month)


## note: categorical variables are automatically taken as factors

# when written into models - however continuous variables need to be specified as factors, e.g. CW


BenthosCPresultsnew1.Mumbles<-subset(BenthosCPresultsnew1,Location=='Mumbles') #subsetting for location for later models

BenthosCPresultsnew1.Oxwich<-subset(BenthosCPresultsnew1,Location=='Oxwich')


#options(na.action = "na.fail")


#FULL MODEL 1 INC Pigment.Loss


Trem_ALL <- glm(TremPres ~ Location + Month + Gender + Fouling + Carapace.Width + Pigment.Loss + Hemo.col , data=BenthosCPresultsnew1, family=binomial(link = "logit"), na.action=na.exclude)

summary.lm (Trem_ALL)

extractAIC (Trem_ALL)


stres<- (Trem_ALL$residuals - mean(Trem_ALL$residuals))/sd(Trem_ALL$residuals)

hist(stres)

plot(stres ~ Trem_ALL$fitted.values)

plot(Trem_ALL)



# capture.output(summary(Trem_ALL),file="Trem_ALL.doc") # exporting model result to a word doc.


drop1(Trem_ALL, test="Chisq") #remove fouling
```

```
Trem_reduced1 <- glm(TremPres ~ Location + Month + Gender + Carapace.Width +
Pigment.Loss + Hemo.col , data=BenthosCPresultsnew1, family=binomial(link = "logit"),
na.action=na.exclude)

summary.lm (Trem_reduced1)

extractAIC (Trem_reduced1)


drop1(Trem_reduced1, test="Chisq") # remove location


Trem_reduced2 <- glm(TremPres ~ Month + Gender + Carapace.Width + Pigment.Loss +
Hemo.col , data=BenthosCPresultsnew1, family=binomial(link = "logit"),
na.action=na.exclude)

summary.lm (Trem_reduced2)

extractAIC (Trem_reduced2)


drop1(Trem_reduced2, test="Chisq") # remove cw


Trem_reduced3 <- glm(TremPres ~ Month + Gender + Pigment.Loss + Hemo.col,
data=BenthosCPresultsnew1, family=binomial(link = "logit"), na.action=na.exclude)

summary.lm (Trem_reduced3)

extractAIC (Trem_reduced3)


drop1(Trem_reduced3, test="Chisq") # remove gender


Trem_reduced4 <- glm(TremPres ~ Month + Pigment.Loss + Hemo.col ,
data=BenthosCPresultsnew1, family=binomial(link = "logit"), na.action=na.exclude)

summary.lm (Trem_reduced4)

extractAIC (Trem_reduced4)


drop1(Trem_reduced4, test="Chisq") # remove pigment loss


Trem_reduced5 <- glm(TremPres ~ Month + Hemo.col, data = BenthosCPresultsnew1,
family=binomial(link = "logit"), na.action=na.exclude)
```

```
summary.lm(Trem_reduced5)
extractAIC(Trem_reduced5)



#  Month is significant overall

###SEPARATING BY LOCATION####

#FULL MUMBLES MODEL 1

Trem_Mum <- glm(TremPres ~ Month + Gender + Fouling + Carapace.Width +
Pigment.Loss +Hemo.col, data=BenthosCPresultsnew1.Mumbles, family=binomial(link =
"logit"), na.action=na.exclude)
summary.lm (Trem_Mum)
extractAIC (Trem_Mum)

stres<- (Trem_Mum$residuals - mean(Trem_Mum$residuals))/sd(Trem_Mum$residuals)
hist(stres)
plot(stres ~ Trem_Mum$fitted.values)
plot(Trem_Mum)



drop1(Trem_Mum, test="Chisq") #remove gender

Trem_Mum_reduced1 <- glm(TremPres ~ Month + Fouling + Carapace.Width +
Pigment.Loss + Hemo.col, data=BenthosCPresultsnew1.Mumbles, family=binomial(link =
"logit"), na.action=na.exclude)
summary (Trem_Mum_reduced1)

drop1(Trem_Mum_reduced1, test="Chisq") # remove fouling
```

```
Trem_Mum_reduced2 <- glm(TremPres ~ Month + Carapace.Width + Pigment.Loss +
Hemo.col, data=BenthosCPresultsnew1.Mumbles, family=binomial(link = "logit"),
na.action=na.exclude)

summary (Trem_Mum_reduced2)


drop1(Trem_Mum_reduced2, test="Chisq") #remove pigment loss


Trem_Mum_reduced3 <- glm(TremPres ~  Month + Carapace.Width + Hemo.col,
data=BenthosCPresultsnew1.Mumbles, family=binomial(link = "logit"),
na.action=na.exclude)

summary (Trem_Mum_reduced3)


drop1(Trem_Mum_reduced3, test="Chisq") #remove hemo col


Trem_Mum_reduced4 <- glm(TremPres ~  Month + Carapace.Width,
data=BenthosCPresultsnew1.Mumbles, family=binomial(link = "logit"),
na.action=na.exclude)

summary (Trem_Mum_reduced4)

extractAIC(Trem_Mum_reduced4)


#therefore, in the Mumbles, CW (e.g. size) and month (August/June) are significant in
explaining the presence of Hematodinium. Let's see what happens when we compare the
external factors.


#FULL Oxwich MODEL 1


Trem_Ox <- glm(TremPres ~ Month + Gender + Fouling + Carapace.Width + Pigment.Loss
+ Hemo.col, data=BenthosCPresultsnew1.Oxwich, family=binomial(link = "logit"),
na.action=na.exclude)

summary (Trem_Ox)

extractAIC(Trem_Ox)


stres<- (Trem_Ox$residuals - mean(Trem_Ox$residuals))/sd(Trem_Ox$residuals)

hist(stres)
```

```
plot(stres ~ Trem_Ox$fitted.values)

plot(Trem_Ox)


drop1(Trem_Ox, test="Chisq") #remove fouling


Trem_Ox_reduced1 <- glm(TremPres ~ Month + Gender + Carapace.Width + Pigment.Loss
+ Hemo.col, data=BenthosCPresultsnew1.Oxwich, family=binomial(link = "logit"),
na.action=na.exclude)

summary (Trem_Ox_reduced1)

drop1(Trem_Ox_reduced1, test="Chisq") #remove pigment loss


Trem_Ox_reduced2 <- glm(TremPres ~ Month + Gender + Carapace.Width + Hemo.col ,
data=BenthosCPresultsnew1.Oxwich, family=binomial(link = "logit"), na.action=na.exclude)

summary (Trem_Ox_reduced2)

drop1(Trem_Ox_reduced2, test="Chisq") #remove gender


Trem_Ox_reduced3 <- glm(TremPres ~ Month + Carapace.Width + Hemo.col,
data=BenthosCPresultsnew1.Oxwich, family=binomial(link = "logit"), na.action=na.exclude)

summary (Trem_Ox_reduced3)

drop1(Trem_Ox_reduced3, test="Chisq") #remove hemo col


Trem_Ox_reduced4 <- glm(TremPres ~ Month + Carapace.Width,
data=BenthosCPresultsnew1.Oxwich, family=binomial(link = "logit"), na.action=na.exclude)

summary (Trem_Ox_reduced4)

drop1(Trem_Ox_reduced4, test="Chisq") #remove cw


Trem_Ox_reduced5 <- glm(TremPres ~ Month, data=BenthosCPresultsnew1.Oxwich,
family=binomial(link = "logit"), na.action=na.exclude)

summary(Trem_Ox_reduced5)

extractAIC(Trem_Ox_reduced5)

#Therefore, in Oxwich, month is significant and could explain overall significance in whole
population model.
```

5.5 R Script for Generalised Linear Model with Negative Binomial Function

```
rm(list=ls(all=TRUE)) # Removes everything from R (all objects) - a good thing to do if you are starting fresh work

graphics.off()        # Removes any graphics or graphic windows that may exist - again a good thing to do if you are starting to work fresh


setwd()


install.packages("quantreg")

install.packages("SparseM")

install.packages("Rearrangement")

install.packages("reshape")

install.packages("MuMIn") #only needed if we want to use DREDGE

install.packages("MASS")

library(MASS)

library(Rearrangement)

library(reshape)

library(MuMIn)

install.packages("pscl")

install.packages("MASS")

require(ggplot2)

require(boot)

require(pscl)

install.packages("car")

library(car)


NewdataCP2025<-read.csv(file.choose())

names(NewdataCP2025)

head(NewdataCP2025)

str(NewdataCP2025) # gives full structure of dataset
```

```
attach(NewdataCP2025)

detach(NewdataCP2025)

Carapace.Width <- factor(Carapace.Width)

Location<- factor(Location)

Pigment.Loss<- factor(Pigment.Loss)

Gender<- factor(Gender)

Month<- factor(Month)

Parasite.count <- factor(Parasite.count)

glm_load <- glm(Parasite.count ~ Location + Month + Gender + Pigment.Loss +
Carapace.Width, family=poisson, data=NewdataCP2025)

summary(NewdataCP2025)

summary(glm_load)

stres<- (glm_load$residuals - mean(glm_load$residuals))/sd(glm_load$residuals)

hist(stres)

plot(glm_load$fitted.values)

plot(glm_load)

# Residuals suggest a non-normal distribution for poisson model

dispersion_ratio <- sum(residuals(glm_load, type = "pearson")^2)/glm_load$df.residual

print(dispersion_ratio)

# Checked for dispersion, dispersion = 24.91422. This suggests that we use a negative
binomial instead of a poisson model due to large overdispersion

neg_load <- glm.nb(Parasite.count ~ Location + Month + Gender + Pigment.Loss +
Carapace.Width, data=NewdataCP2025)

summary(neg_load)
```

5.6 Health and Safety Risk Assessments and Ethics

5.6.1 – Risk Assessment for Teaching, Administration and Research Activities Aquarium

Risk Assessment for Teaching, Administration and Research Activities

Swansea University; College of Science/Medicine

Name: Grace Crocker **Signature**: [redacted] **date: 01/02/24**

Supervisor*: CE Davies/AF Rowley **Signature**: [redacted] **date**: 01/02/24

Activity title: Parasites of crabs **Base location (room no.)**
Aquarium

(* the supervisor for all HEFCW funded academic and non-academic staff is the HOC)

University Activity Serial # (enter Employee No. or STUREC No. .....
Start date of activity (cannot predate signature dates) 05/01/24
End date of activity (or 'on going') 30/06/24

Level of worker (delete as applicable) PG

UG, PG, research assistant, technician, administration, academic staff, other (state)

Approval obtained for Gene Manipulation Safety Assessment by SU? Yes/not applicable
Licence(s) obtained under "Animals (Scientific Procedures) Act (1986)"? Yes/not applicable
Approval obtained for use of radioisotopes by COS/COM? Yes/not applicable

Record of specialist training undertaken

Course	date
Aquarium	01/02/24

Summary of protocols used; protocol sheets to be appended plus COSHH details for chemicals of category A or B with high or medium exposure

Protocol Details						Protocol Details					
#	Assessment					#	Assessment				
	1st date	Frequency of re-assessment	Hazard category	Secondary containment level	Exposure potential		1st date	Frequency of re- assessment	Hazard category	Secondary containment level	Exposure potential
1						11					
2						12					
3						13					
4						14					
5						15					
6						16					
7						17					
8						18					
9						19					
10						20					

See notes in handbook for help in filling in form (Continue on another sheet if necessary)

(Dept.) **Protocol Risk Assessment Form**

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol # **1**	**Title:** **Crab storing**
Associated Protocols **#**.....	**Description:**

Location:

Highlight which (Dept.) Local Rules apply –

Boat (Field) Genetic-Manipulation (Laboratory) Office/Facility Radioisotope

Identify here risks and control measures for work in this environment, additional to Local Rules

Chemicals	**Quantity**	**Hazards**	**Category (A,B,C,D)***	**Exp. Score**
N/A	N/A	N/A	N/A	N/A

Hazard Category (known or potential)

A (e.g. carcinogen/teratogen/mutagen)
B (e.g. v.toxic/toxic/explosive/pyrophoric)
C (e.g. harmful/irritant/corrosive/high flammable/oxidising)
D (e.g. non classified)

Exposure Potential Highlight the **highest Exposure Score** above. Use this to calculate the exposure potential for the entire protocol (see handbook). Indicate this value below.

Low Medium High

Primary containment (of product) sealed flask/bottle/glass/plastic/other (highlight/state) :-

Storage conditions and maximum duration :- N/A

Secondary containment (of protocol) open bench/fume hood/special (highlight/state) :- N/A

Working Practice – Good Laboratory Practice under (dept.) local rules PLUS the following (highlight/state)

(latex/nitrile/heavy gloves) screens full face mask dust mask protective shoes spillage tray ear-defenders other (state)

Other risks & control measures e.g. pressure, temperature, electrical, mechanical, autoclave, field, boat. N/A

Disposal e.g. autoclaving of biohazard, SU chemical disposal

N/A

Identify other control measures (highlight or delete) ~~latex/nitrile/heavy gloves~~; screens; full face mask; dust mask; protective shoes; spillage tray; ear-defenders; ~~other~~ (state)

Justification and controls for any work outside normal hours

No outside hours

Emergency procedures (e.g. spillage clearance; communication methods) Yes

Supervision/training for worker (highlight) None required Already trained Training required Supervised always	
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures. *Name & signature of worker: Grace Crocker* [redacted] *Name & counter-signature of supervisor: Andrew Rowley* [redacted] *Date: 05/02/24*	
Date of first reassessment	Frequency of reassessments

5.6.2 - Risk Assessment for Teaching and Research Activities Benthos Laboratory

V1.1 2023

Risk Assessment for Teaching and Research Activities*

Swansea University; FSE: Biosciences

Name: Grace Crocker Signature: [redacted] **date: 14/02/24**

Supervisor*: CE Davies/AF Rowley **Signature:** [redacted] **date: 14/02/24**

Activity title Parasites of crabs **Base location (room no.)** W043

(* the supervisor for all HEFCW funded academic and non-academic staff is the HOD or their nominee)

University Activity Serial # (enter Employee No. or Student No.). [redacted]
Start date of activity (cannot predate signature dates) 14/02/24
End date of activity (or 'on going') 30/06/24

Level of worker (choose from the list below) Postgraduate

UG, MSc, M.Res, M.Phil/PhD, RA/Postdoc, technician, administration, academic staff, visitor, other (state)

Ethics approval number: 2/2024/84117714

Approval obtained for Biological Hazards and/or GMO Safety Assessment by SU? Yes/not applicable

Is your project: (circle the appropriate choice A-D)

A. Laboratory-based only (i.e. you **never** work in the field)
B. Field **AND** laboratory-based
C. Field-only based (i.e. you do not have an allocated laboratory space and **never** work in a laboratory)
D. Desk based (i.e. no field or laboratory base. i.e. you are only allocated office space [if you are a PhD or research member of staff])

For **category A** complete this Risk Assessment template and associated laboratory protocols, and a Training Record form.
For **category B** complete this Risk Assessment template and associated laboratory protocols, a Training Record form, AND either complete the FSE on-line Field Risk Assessment (**for UG, MSc**) or the relevant University-template form (i.e. Red Form- Off Campus Activities & Risk Assessment Form) (**for MRes, PhD, all staff, visitors**)
For **category C** complete this Risk Assessment template (but not the protocol sheets) and the relevant on-line FSE field risk assessment or University-template forms (see B above for details) and complete a Training Record
For **category D** complete the Training Record template **and** this front page.

Summary of laboratory and/or field protocols used; protocol sheets to be appended and updated as necessary

#	Title	1st Assessment Date	Frequency of re-assessment
1	Protocol 1	14/02/24	N/A
2			
3			
4			
5			
6			
7			
8			
9			
10			

Reassessment - the first reassessment must be undertaken as soon as possible after the first time the protocol has been undertaken in order to identify any unforeseen hazards. After this first reassessment, the protocol should be reassessed every 6-12m. The protocol must be reassessed immediately if new knowledge on the chemical hazards becomes available.

Protocol Risk Assessment Form (Laboratory-only)

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol # **1**	**Title:** **Crab data and bleeding**
Associated Protocols #.....	**Location and local rules** *In addition to Good Laboratory Practice, identify any local rules that apply (specific risks and control measures for work in this environment).* W043

Description of the protocol:

- Crab bleeding dissection, recording of data, sacrifice crab(s) with 1ml KCL

Additional risks and control measures specific to this protocol:

In addition to the local rules, identify the risks associated with use of equipment (e.g. autoclaves, centrifuges), other mechanical and electrical hazards AND control measures.
**Note chemical hazards are summarised below and any biological hazards should be identified in a separate Biological Risk Assessment form.*

Needle puncture/KCL exposure resulting, crab pincers

Who or what may be harmed?	
✱ Staff/ PG student carrying out the activity ☐ Contractors ☐ Visitors ☐ Cleaners ☐ Maintenance staff ☐ UG student carrying out activity ☐ Other staff/ students in the vicinity	**Vulnerable groups present:** ☐ U18/ U16 ☐ New or expectant mother ☐ Other: N/A ☐ **Environment** (via release to air/water/ground, or incorrect disposal) N/A

PROTOCOL RISK MANAGEMENT

Secondary Containment (of protocol): e.g. open bench/fume hood/special

N/A

Measures taken to eliminate or substitute/reduce: e.g. using less hazardous, less volume of chemicals

N/A

Personal Protective Equipment and all specific control measures Include a full description e.g. latex/nitrile/heavy gloves; safety glasses, screens; full face mask; dust mask; protective shoes; spillage tray; ear-defenders; other (state)

Emergency procedures (include first aid, fire, spillage, communication methods) N.B. full emergency plans for each chemical are detailed in individual Chemical data Sheets

N/A

Is exposure monitoring required? Yes (give details) or No	**Is health surveillance required?** Yes (give details) or No

Justification and controls for any work outside normal hours (N.B. UG project students cannot work outside normal hours in a laboratory)

No outside hours

Supervision/training for worker (highlight) **N.B. All relevant training forms (e.g. for specific laboratories) should be completed**

None required | Already trained | Training required | Supervised always

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker: Grace Crocker [redacted]

Name & counter-signature of supervisor: Andrew Rowley [redacted] *Date: 14/02/24*

Date of first reassessment | Frequency of reassessments

5.6.3 - Risk Assessment for Teaching and Research Activities Molecular Laboratory

V1.1 2023

Risk Assessment for Teaching and Research Activities*

Swansea University; FSE: Biosciences

Name: Grace Crocker **Signature**: [redacted] **date: 17/04/24**

Supervisor*: Charlotte Davies/Andrew Rowley [redacted] **Signature**: **date**: 17/04/24

Activity title: PCR amplification and DNA visualisation via gel electrophoresis **Base location (room no.)**: 131a

(* the supervisor for all HEFCW funded academic and non-academic staff is the HOD or their nominee)

University Activity Serial # (enter Employee No. or Student No.): [redacted]
Start date of activity (cannot predate signature dates): 17/04/24
End date of activity (or 'on going'): 30/08/24

Level of worker (choose from the list below): MRes

UG, MSc, M.Res, M.Phil/PhD, RA/Postdoc, technician, administration, academic staff, visitor, other (state)

Ethics approval number: 2/2024/84117714

Approval obtained for Biological Hazards and/or GMO Safety Assessment by SU? Yes/not applicable

Is your project: (circle the appropriate choice A-D)

A. Laboratory-based only (i.e. you **never** work in the field)
(B) Field **AND** laboratory-based
C. Field-only based (i.e. you do not have an allocated laboratory space and **never** work in a laboratory)
D. Desk based (i.e. no field or laboratory base. i.e. you are only allocated office space [if you are a PhD or research member of staff])

For **category A** complete this Risk Assessment template and associated laboratory protocols, and a Training Record form.
For **category B** complete this Risk Assessment template and associated laboratory protocols, a Training Record form, AND either complete the FSE on-line Field Risk Assessment (**for UG, MSc**) or the relevant University-template form (i.e. Red Form- Off Campus Activities & Risk Assessment Form) (**for MRes, PhD, all staff, visitors**)
For **category C** complete this Risk Assessment template (but not the protocol sheets) and the relevant on-line FSE field risk assessment or University-template forms (see B above for details) and complete a Training Record
For **category D** complete the Training Record template **and** this front page.

Summary of laboratory and/or field protocols used; protocol sheets to be appended and updated as necessary

#	Title	1st Assessment Date	Frequency of re-assessment
1	PCR (amplification of DNA) and DNA visualisation via gel electrophoresis	17/04/24	
2			
3			
4			
5			
6			
7			
8			
9			
10			

Reassessment - the first reassessment must be undertaken as soon as possible after the first time the protocol has been undertaken in order to identify any unforeseen hazards. After this first reassessment, the protocol should be reassessed every 6-12m. The protocol must be reassessed immediately if new knowledge on the chemical hazards becomes available.

Protocol Risk Assessment Form (Laboratory-only)

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol # 2	**Title: PCR (amplification of DNA) and DNA visualisation via gel electrophoresis**
Associated Protocols #.....	**Location and local rules** *In addition to Good Laboratory Practice, identify any local rules that apply (specific risks and control measures for work in this environment).* Shared molecular facility Wallace 131a Benthos lab Wallace 043

Description of the protocol:

PCR (amplification of DNA) and DNA visualization via gel electrophoresis

Additional risks and control measures specific to this protocol:

In addition to the local rules, identify the risks associated with use of equipment (e.g. autoclaves, centrifuges), other mechanical and electrical hazards AND control measures.
**Note chemical hazards are summarised below and any biological hazards should be identified in a separate Biological Risk Assessment form.*

Use of centrifuges, vortex, class II hood, UV light sterilization, bleach for cleaning, PCR machine, gel tanks

Who or what may be harmed?	
☒ Staff/ PG student carrying out the activity ☐ Contractors ☐ Visitors ☐ Cleaners ☐ Maintenance staff ☐ UG student carrying out activity ☐ Other staff/ students in the vicinity	**Vulnerable groups present:** ☐ U18/ U16 ☐ New or expectant mother ☐ Other: ☐ **Environment** (via release to air/water/ground, or incorrect disposal)

CHEMICAL RISK – Summary sheet

A copy of each Chemical COSSH form should be readily available in the lab for use (e.g. in an emergency)

Chemical Name (& Conc.) for chemicals to be used and generated	**GHS symbols** (SH, AT, H, C, Ex, F, O, Env, CG) All that are applicable.	**Skin/Eyes Group** (SA, SB, SC, SD, SE)	**Inhalation Group** (A,B,C,D,E)	**Quantity**	***In use* dustiness or volatility**	**Disposal**	**Primary containment & storage**	**Other comments:** **In use factors affecting exposure and special control measures** *(e.g. <15 mins duration/ frequency/ splash protection only/ hand immersion/ spraying)* **Safety/ environmental hazards (H2XX/H4XX)**
Master Mix	N/A	SA	A	1 - Small	Low volatility	*SU chemical disposal*	*General chemical storage*	
Primers	N/A	SA	A	1 - Small	Low volatility	*SU chemical disposal*	*General chemical storage*	
TAE buffer (1x and 10x)	Env, H	SA	A	1 - Small	Low volatility	*SU chemical disposal*	*General chemical storage*	
Agarose	N/A	SA	A	1 - Small	Low volatility	*SU chemical disposal*	*General chemical storage*	
Bleach	C, Env	SB	B	1 - Small	Choose an item.	*SU chemical disposal*	*General chemical storage*	
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			

GHS symbols– SH (serious health hazard), AT (acute toxicity), H (health hazard), C (corrosive), Ex (explosive), F (flammable), O (oxidiser), Env (environment), CG (compressed gas). These should be obtained from chemical SDS documentation. See Appendix (hazard symbols).

Inhalation Group and Skin/Eyes Group- Hazard groups are classified as A/SA (least hazardous) to E/SE (most hazardous). See Appendix for hazard phrases associated with each group. Hazard phrases can be found on chemical SDS documentation.

Dustiness. Low (Pellet- does not break up), Medium (granular or crystalline), High (fine solid or light powder/dust)

Volatility. Low, medium, high, gas. Consider boiling point of liquid and operating temperature.

Disposal e.g. autoclaving of biohazard, SU chemical disposal

Primary containment: e.g. sealed flask, supplied vessel. **Storage:** e.g. secure chemical storage, fridge, freezer, general chemical storage

Note: A **specific DSEAR risk assessment** must be carried out if:

- The work activity involves the use or storage of **flammable, oxidising or corrosive gas cylinders.**
- The work activity is likely to create an **explosive atmosphere** even after the application of controls stated in the chemical risk assessment.
- The work activity involves the **use of explosives.**

PROTOCOL RISK MANAGEMENT

Secondary Containment (of protocol): e.g. open bench/fume hood/special Open bench and fume hood for larger volumes	
Measures taken to eliminate or substitute/~~reduce~~: e.g. using less hazardous, less volume of chemicals Using less hazardous, less volume of chemicals	
Personal Protective Equipment and all specific control measures Include a full description e.g. latex/nitrile/heavy gloves; safety glasses, screens; full face mask; dust mask; protective shoes; spillage tray; ear-defenders; other (state) Nitrile gloves, lab coats, safety goggles where necessary	
Emergency procedures (include first aid, fire, spillage, communication methods) N.B. full emergency plans for each chemical are detailed in individual Chemical data Sheets	
Is exposure monitoring required? Yes (give details) or No N/A	**Is health surveillance required?** Yes (give details) or No N/A
Justification and controls for any work outside normal hours (N.B. UG project students cannot work outside normal hours in a laboratory) N/A	
Supervision/training for worker (highlight) **N.B. All relevant training forms (e.g. for specific laboratories) should be completed** None required Already trained Training required Supervised always	
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures. *Name & signature of worker: Grace Crocker* [redacted]	

Name & countersignature of supervisor: Andrew Rowley [redacted]	*Date 14/02/24*
Date of first reassessment	Frequency of reassessments

5.6.4 - Risk Assessment for Teaching and Research Activities Molecular Laboratory

V1.1 2023

Risk Assessment for Teaching and Research Activities*

Swansea University; FSE: Biosciences

Name: Grace Crocker **Signature:** [redacted] **date**: 17/04/24
Supervisor*: Andrew Rowley [redacted] **Signature**: **date**: 17/04/24

Activity title: DNA Extraction **Base location (room no.)** 131a
(* the supervisor for all HEFCW funded academic and non-academic staff is the HOD or their nominee)

University Activity Serial # (enter Employee No. or Student No.): [redacted]
Start date of activity (cannot predate signature dates): 17/04/24
End date of activity (or 'on going'): 31/08/24

Level of worker (choose from the list below) MRes

UG, MSc, M.Res, M.Phil/PhD, RA/Postdoc, technician, administration, academic staff, visitor, other (state)

Ethics approval number: **2/2024/84117714**

Approval obtained for Biological Hazards and/or GMO Safety Assessment by SU? Yes/not applicable

Is your project: (circle the appropriate choice A-D)

A. Laboratory-based only (i.e. you **never** work in the field)
B. Field **AND** laboratory-based
C. Field-only based (i.e. you do not have an allocated laboratory space and **never** work in a laboratory)
D. Desk based (i.e. no field or laboratory base. i.e. you are only allocated office space [if you are a PhD or research member of staff])

For **category A** complete this Risk Assessment template and associated laboratory protocols, and a Training Record form.
For **category B** complete this Risk Assessment template and associated laboratory protocols, a Training Record form, AND either complete the FSE on-line Field Risk Assessment (**for UG, MSc**) or the relevant University-template form (i.e. Red Form- Off Campus Activities & Risk Assessment Form) (**for MRes, PhD, all staff, visitors**)
For **category C** complete this Risk Assessment template (but not the protocol sheets) and the relevant on-line FSE field risk assessment or University-template forms (see B above for details) and complete a Training Record
For **category D** complete the Training Record template **and** this front page.

Summary of laboratory and/or field protocols used; protocol sheets to be appended and updated as necessary

#	Title	1st Assessment Date	Frequency of re-assessment
1	DNA extraction from crab tissue, haemolymph and parasite cysts	17/04/24	
2			
3			
4			
5			
6			
7			
8			
9			
10			

Reassessment - the first reassessment must be undertaken as soon as possible after the first time the protocol has been undertaken in order to identify any unforeseen hazards. After this first reassessment, the protocol should be reassessed every 6-12m. The protocol must be reassessed immediately if new knowledge on the chemical hazards becomes available.

Protocol Risk Assessment Form (Laboratory-only)

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol # 1	**Title: DNA extraction from crab tissue, haemolymph and parasite cysts**
Associated Protocols **#.....**	**Location and local rules** *In addition to Good Laboratory Practice, identify any local rules that apply (specific risks and control measures for work in this environment).* Shared molecular facility Wallace 131a Benthos lab Wallace 043

Description of the protocol:

DNA extraction from crab tissue, haemolymph and parasite cysts using Qiagen Blood and Tissue Kits

Additional risks and control measures specific to this protocol:

In addition to the local rules, identify the risks associated with use of equipment (e.g. autoclaves, centrifuges), other mechanical and electrical hazards AND control measures.
**Note chemical hazards are summarised below and any biological hazards should be identified in a separate Biological Risk Assessment form.*

Use of centrifuges, incubator, vortex, class II hood, flow hood, UV light sterilization, bleach for cleaning

Who or what may be harmed?	
☒ Staff/ PG student carrying out the activity ☐ Contractors ☐ Visitors ☐ Cleaners ☐ Maintenance staff ☐ UG student carrying out activity ☐ Other staff/ students in the vicinity	**Vulnerable groups present:** ☐ U18/ U16 ☐ New or expectant mother ☐ Other: ☐ **Environment** (via release to air/water/ground, or incorrect disposal)

CHEMICAL RISK – Summary sheet

A copy of each Chemical COSSH form should be readily available in the lab for use (e.g. in an emergency)

Chemical Name (& Conc.) for chemicals to be used and generated	**GHS symbols** (SH, AT, H, C, Ex, F, O, Env, CG) All that are applicable.	**Skin/Eyes Group** (SA, SB, SC, SD, SE)	**Inhalation Group** (A,B,C,D,E)	**Quantity**	***In use* dustiness or volatility**	**Disposal**	**Primary containment & storage**	**Other comments:** **In use factors affecting exposure and special control measures** *(e.g. <15 mins duration/ frequency/ splash protection only/ hand immersion/ spraying)* **Safety/ environmental hazards (H2XX/H4XX)**
Buffer AW2	N/A	SA	A	1 - Small	Low volatility	*SU chemical disposal*	*General chemical storage*	
Proteinase K	SH	SE	E	1 - Small	Choose an item.	*SU chemical disposal*	*General chemical storage*	
Buffer ATL	N/A	SA	A	1 - Small	Low volatility	*SU chemical disposal*	*General chemical storage*	
Buffer AE	N/A	SA	A	1 - Small	Low volatility	*SU chemical disposal*	*General chemical storage*	
Buffer AW1	H	SB	B	1 - Small	Low volatility	*SU chemical disposal*	*General chemical storage*	
Buffer AL-T/M	H	SE	E	1 - Small	Low volatility	*SU chemical disposal*	*General chemical storage*	
Bleach	C, Env	SB	B	1 - Small	Choose an item.	*SU chemical disposal*	*General chemical storage*	
Ethanol (100% HPLC)	F	SB	A	1 - Small	High volatility	*SU chemical disposal*	*General chemical storage*	
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			

GHS symbols– SH (serious health hazard), AT (acute toxicity), H (health hazard), C (corrosive), Ex (explosive), F (flammable), O (oxidiser), Env (environment), CG (compressed gas). These should be obtained from chemical SDS documentation. See Appendix (hazard symbols).

Inhalation Group and Skin/Eyes Group- Hazard groups are classified as A/SA (least hazardous) to E/SE (most hazardous). See Appendix for hazard phrases associated with each group. Hazard phrases can be found on chemical SDS documentation.

Dustiness. Low (Pellet- does not break up), Medium (granular or crystalline), High (fine solid or light powder/dust)

Volatility. Low, medium, high, gas. Consider boiling point of liquid and operating temperature.
Disposal e.g. autoclaving of biohazard, SU chemical disposal
Primary containment: e.g. sealed flask, supplied vessel. **Storage:** e.g. secure chemical storage, fridge, freezer, general chemical storage

Note: A **specific DSEAR risk assessment** must be carried out if:

- The work activity involves the use or storage of **flammable, oxidising or corrosive gas cylinders.**
- The work activity is likely to create an **explosive atmosphere** even after the application of controls stated in the chemical risk assessment.
- The work activity involves the **use of explosives.**

PROTOCOL RISK MANAGEMENT

Secondary Containment (of protocol): e.g. open bench/fume hood/special Open bench and fume hood for larger volumes	
Measures taken to eliminate or substitute/~~reduce:~~ e.g. using less hazardous, less volume of chemicals Using less hazardous, less volume of chemicals	
Personal Protective Equipment and all specific control measures Include a full description e.g. latex/nitrile/heavy gloves; safety glasses, screens; full face mask; dust mask; protective shoes; spillage tray; ear-defenders; other (state) Nitrile gloves, lab coats, safety goggles where necessary	
Emergency procedures (include first aid, fire, spillage, communication methods) N.B. full emergency plans for each chemical are detailed in individual Chemical data Sheets	
Is exposure monitoring required? Yes (give details) or No N/A	**Is health surveillance required?** Yes (give details) or No N/A
Justification and controls for any work outside normal hours (N.B. UG project students cannot work outside normal hours in a laboratory) N/A	
Supervision/training for worker (highlight) **N.B. All relevant training forms (e.g. for specific laboratories) should be completed** None required Already trained Training required Supervised always	
Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.	

Name & signature of worker: Grace Crocker [redacted]	
Name & countersignature of supervisor: Andrew Rowley [redacted]	*Date 17/04/24*
Date of first reassessment	Frequency of reassessments

5.6.5 - Aquarium Risk Assessment

Risk Assessment			
College/PSU	Science and Engineering	Assessment Date	01/02/24
Location	Aquarium	Assessor	
Activity	Crab storing	Review Date (if applicable)	
Associated documents	•	•	

Part 1: Risk Assessment

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?	Action by whom?	Action by when?	Done Yes/No
Electrical	Myself/ others	Burns, electric shock	Ensure drip loops are used, regular inspections	Ensure aquarium lab door is propped open incase help is needed	Myself	Everyday	Yes
Chemcial exposure	Myself/others	Burns, rashes, irritarion	Ensure checmicals are used and stored safely, wear PPE	Ensure aquarium lab door is propped open incase help is needed	Myself	Everyday	Yes
Slips/trips/ falls	Myself/others	Sprains/cuts/bruises/broken bones	Leave belongings in a safe place out of the way, ensure work area is clear	Ensure aquarium lab door is propped open incase help is needed	Myself	Everyday	Yes
Open wounds	Myself/others	Infection	Ensure all cuts are covered, wear PPE	Ensure aquarium lab door is propped open incase help is needed	Myself	Everyday	Yes
Spillages	Myself/others	Injuries/falling over	Ensure to clear any spillages	Ensure aquarium lab door is propped open incase help is needed	Myself	Everyday	Yes

5.6.6 - Biosciences Training Proforma

BIOSCIENCES TRAINING PROFORMA (v1.1 AFR2023)

NAME OF TRAINEE	NAME OF TRAINER(S)	DATE(S)
Grace Crocker	Jess Minett Charlotte Davies/Andrew Rowley	13/02/24

It is the responsibility of the PI or supervisor to determine the local training needs for each trainee and to ensure the trainee has suitable access to this training.

This form must be used to record the health and safety training **and** training in specific procedures.

This document should be updated over time (i.e. level of competency and specific procedures).

The trainer must ensure the competence of the trainee in each area before signing the form. This may be done by any or a combination of the following:

- Written test
- Oral test
- Practical demonstration by the trainee
- Reference to completed on-line training provided by ~~University~~

Level of attainment competency of the trainee (use the codes A-D below and place these in the right-hand boxes within the tables)

A: The task must be directly supervised.

B: The supervisor's advice and approval must be sought before the procedure is started.

C: The work entails risks that require careful attention to safety. The trainee has been trained in the task and demonstrated competence.

D: The risks are insignificant and carry no special supervisory considerations.

BASIC LABORATORY PROCEDURES (**if field or desk-based only** then place N/A in boxes. Amend & extend table as necessary)

PROCEDURE	TRAINING ~~ACHIEVED, METHOD~~ OF ASSESSMENT & LEVEL OF ATTAINMENT (A-D codes)	TRAINER & DATE
Laboratory induction (add details of individual laboratories on new lines)	General induction 131A 043 D	J Minett 13/02/24
Waste disposal	Yes D	J Minett 13/02/24
Use of analytical balances		
Use of fume hoods		
Use of biological safety cabinets		
Use of pipettes		
Use, storage & disposal of toxic chemicals (poisons)		
Use, storage & disposal of systemic health hazards (e.g. carcinogens/mutagens)		
Use, storage & disposal of biological hazards and GMO		
Handling & disposal of sharps (e.g. needles, blades)	Disposal - D	J Minett 13/02/24

OFFICE-BASED ('Dry') PROCEDURES (extend table as necessary)

PROCEDURE	TRAINING ACHIEVED, METHOD OF ASSESSMENT & LEVEL OF ATTAINMENT
Awareness of posture, monitor level, etc. (complete Document from central H&S entitled *DSE Self-Assessment Checklist* & HSE *Working with display screen equipment* leaflet). Participation of training course 695 on DSE is mandatory for staff. See https://staff.swansea.ac.uk/healthsafety/training/	

SIGNATURES:

Trainee: [redacted] Date: 13/02/24

Trainers/Supervisor(s)/Approvers (add as appropriate)

Name: [redacted] Date: 13/02/24

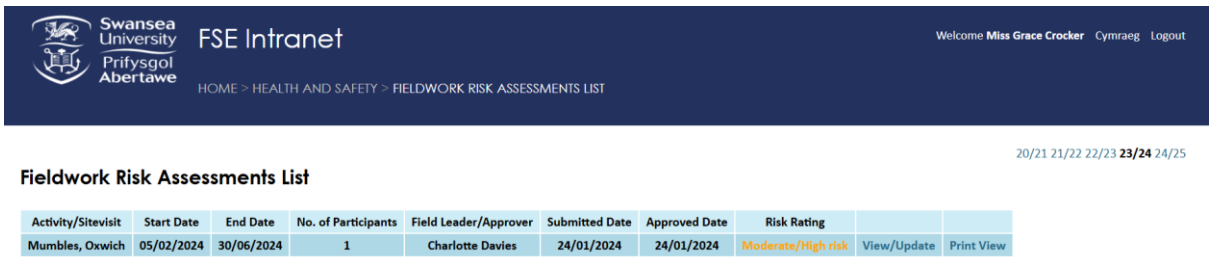
Name: [redacted] Date 13/02/24

Name: ..... Date.....

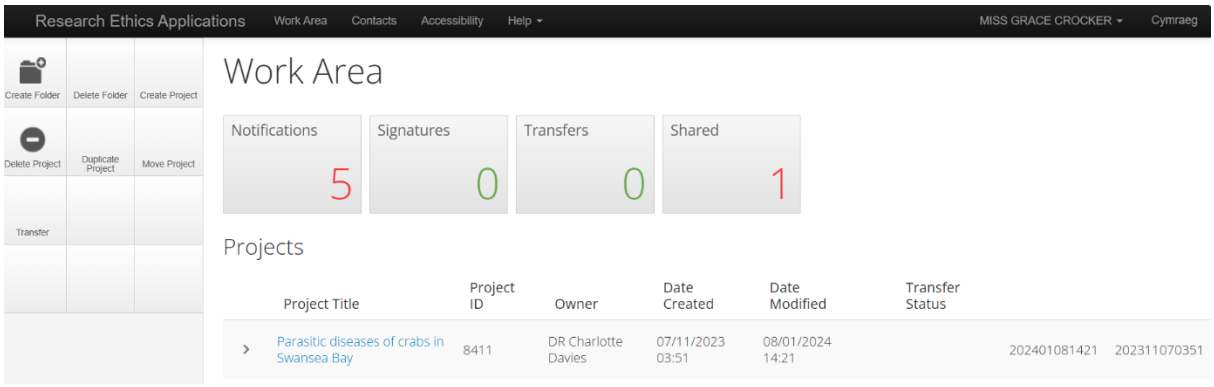
(Reassessment due N/A)

COMPLETED FORM, ONCE SIGNED OFF, SHOULD BE KEPT WITH RISK ASSESSMENTS AND PROTOCOLS (i.e. in a shared TEAMS folder). IT IS THE TRAINEE'S RESPONSIBILITY TO STORE THIS FORM AND TO KEEP IT UPDATED.

5.6.7 - Fieldwork Risk Assessment



5.6.8 - Research Ethics Application Approval



5.6.9 – Multiple Sequence Alignment Viewer