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Are all brachyuran crabs found in the intertidal zone intermediate hosts for digenean parasites?☆

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ABSTRACT

Digenean trematodes with complex life cycles often use marine crabs as intermediate hosts, but their distribution across crab species is not fully understood. Previous reports of *Microphallus similis* in edible crabs (*Cancer pagurus*) relied on morphological identification, leaving potential for misidentification. This study aimed to investigate the prevalence, identity, and host range of digenean parasites in intertidal brachyuran crabs from South Wales, U.K. Over six months, crabs were collected from two rocky shore sites and examined for metacercariae in the hepatopancreas. Parasites were morphologically assessed and identified by sequencing the 28S rDNA region. Metacercariae were found exclusively in juvenile *C. pagurus*, with ~ 30 % prevalence and low individual burdens ranging from 1 to 69 cysts. All sequenced parasites were confirmed as *M. similis*. No infections were detected in *Necora puber*, *Xantho pilipes*, or *X. hydrophilus*. Logistic regression indicated that infection prevalence in *C. pagurus* varied significantly with month and crab size. This study provides the first molecular confirmation of *M. similis* in edible crabs from the U.K. and highlights species-specific susceptibility linked to differences in ecology and feeding behaviour. The absence of infection in co-occurring crabs suggests that *C. pagurus* plays a uniquely important role in the parasite's transmission cycle in intertidal environments.

1. Introduction

Over 30 species of brachyuran (true) crabs are fished worldwide and, in Europe, both edible crabs (sometimes referred to as brown crabs; *Cancer pagurus*) and velvet swimming crabs (*Necora puber*) are commonly fished in these coastal waters. In the case of *C. pagurus*, most commercial fishing takes place in the United Kingdom, Ireland, Norway and France with global landings of 39,446 tonnes live weight in 2022 (Stevens and Miller, 2020; FAO, 2024). Juvenile edible crabs are found in the intertidal zone where they feed on other crustaceans smaller than themselves, bivalve and gastropod molluscs and small fish. After *ca.* 3 years they migrate to the subtidal zone and here they can be found at depths of > 100 m (Shelton and Hall, 1981). These form the basis of the commercial in-shore and offshore fishery with crabs more than 115–160 mm carapace width available to be brought to market (Bennett, 1995). The velvet swimming crab fishery is much smaller than that of edibles. These crabs are often caught as a by-catch during fishing for larger crustaceans including lobsters and edible crabs (Moore et al., 2023) but their decline in France and Spain caused by overfishing and disease (e.g., Wilhelm and Mialhe, 1996) has resulted in increased interest in their capture in Ireland and the UK largely for export for consumption in mainland Europe. Velvet swimming crabs are found in the littoral to sub-littoral zones > 80 m depth. While in the intertidal zone, they reside in rock pools and under crevices and boulders when the tide recedes. They are opportunistic predators eating brown algae, molluscs (including limpets) and crustaceans at high tide (Choy, 1986; Silva et al., 2010).

Disease is a limiting factor in the survival of all aquatic animals but its importance in limiting stocks of capture fish and shellfish in the oceans is less clear (Stentiford et al., 2012; Burge et al., 2014; Rowley et al., 2024). Indeed, although there is information on the range and nature of diseases caused by microbial and macrobial agents of both edible and velvet swimming crabs (e.g. Wilhelm and Mialhe, 1996; Stentiford, 2008; Smith et al., 2013, 2014, 2015; Bateman et al., 2022; Coates and Rowley, 2022; Collins et al., 2022; Martin et al., 2024) their importance in limiting populations is often unclear as mortalities are 'silent' in that they are infrequently observed or recorded in the field, and dead or moribund animals vanish as they are quickly predated upon. Hence, disease levels may be under reported in the wild. Examples of

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diseases that are thought to be important in causing mortality in marine crabs include so-called 'bitter crab' disease caused by the dinoflagellate, *Hematodinium* spp. This condition is endemic in both edible (Smith et al., 2013, 2015) and velvet swimming crabs (Wilhelm and Mialhe, 1996). Indeed, the latter authors considered that this disease was associated with declines in catches of velvet swimmers in France. Other disorders may be less important in affecting the health and survival of crabs. One such condition is caused by various species of digenean parasites that use crabs as their second intermediate host where they encyst in the hepatopancreas and gills (James, 1971; Bateman et al., 2011). Here, they likely make use of their host's nutrients but do not appear to cause major tissue damage (Blakeslee et al., 2015; Davies et al., 2019) and there is usually little evidence of a host response to their presence (Davies et al., 2022).

The typical digenean life cycle has three distinct hosts, a definitive in which sexual reproduction of the parasite occurs, and miracidia hatch from eggs released into the environment that in turn infect the first intermediate host. These infested hosts then release free-living cercariae that penetrate the second intermediate host where they encyst to form metacercaria (James, 1971; Saville and Irwin 2005). The final stage of the life cycle is achieved when these hosts are ingested by the definitive hosts – often birds or fish in the case of microphallid parasites. A well-studied example of a digenean parasite found in the marine environment is the microphallid, *Microphallus similis*. Wading birds and gulls are the definitive hosts (Stunkard 1957; James 1971) and various species of littorinids including *Littorina saxatilis* become infected and form the primary intermediate host (Galaktionov and Bustnes, 1999; Blakeslee et al., 2015; Bojko et al., 2017). Here, the parasites develop in gonadal and digestive tissues often causing damage. They are released from these molluscs as free swimming cercariae that locate and penetrate the tissues of crabs including the European green crab, *Carcinus maenas* (Stentiford and Feist, 2005; Blakeslee et al., 2009, 2015, 2020) and edible crabs (Crothers 1966; Stentiford 2008). When these are predated on by sea birds, the cycle is then completed where the metacercariae emerge from their cysts in the host's digestive system. While there are a few reports of this species parasitising edible crabs (e.g. Stunkard 1957; Crothers 1966; Stentiford 2008) to our knowledge, there are no descriptions of these digeneans inhabiting the tissues of *N. puber*. Furthermore, because these reports that identified *M. similis* in edible crabs only used morphological approaches, including histology, there is the possibility of misidentification with other microphallids including *Microphallus primas*, known to parasitise crabs (Pina et al., 2011) and it is well known that morphological approaches alone are insufficient to identify all such parasites (e.g. Stout et al., 2024).

The main aim of the present study was to determine if commercially important species of crabs (*C. pagurus* and *N. puber*) in the intertidal zone of rocky shores are subject to infection with digenean parasites across two locations in South Wales, UK; assessed both morphologically and via sequencing. The presence, and abundance, of any parasites present were investigated alongside biometric data. These survey sites were chosen as detailed epidemiological studies have previously examined the disease profiles of *C. pagurus* (e.g. Smith et al., 2013, 2014, 2015) and *C. maenas* (e.g. Davies et al., 2019, 2022) crabs in these locations, and histological studies have shown the presence of metacercariae in various tissues of *C. maenas* (Davies et al., 2022) at these sites. A further objective was to assess if juvenile *C. pagurus* crabs have co-infections with both *M. similis* and *M. primas* that has been observed in *C. maenas* at the two survey sites (Bedford, Crocker, Rowley and Davies unpublished observations).

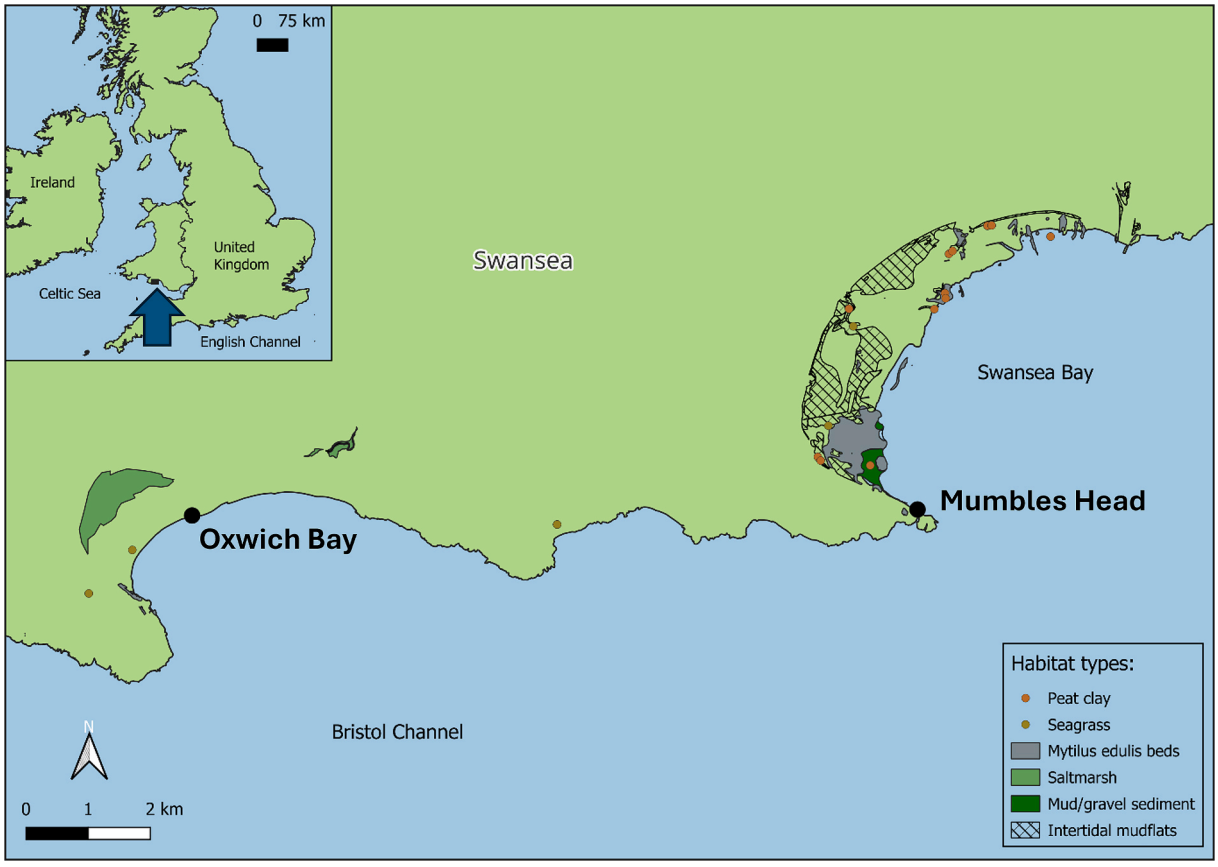

Fig. 1. Collection locations for crabs during this study in South Wales, UK. Maps were created and annotated using QGIS V.3.32.3 (Service Layer Credits: Sources: borders.ukdataservice.ac.uk, osmdata.openstreetmap.de, datamap.gov.wales).

2. Materials and methods

2.1. Study area and sample collection

The study took place in the intertidal rocky shores at Mumbles Head (51° 34′11.00″ N, 3° 58′49″ W) and rocky outcrops at Oxwich Bay (51° 33′ 56.92″ N, −4° 08′ 48.44″ W) in South Wales, UK (Fig. 1). Both locations represent habitats for both commercially important species including juvenile *C. pagurus* and *N. puber*, as well as other species of crabs such *as C. maenas.* Surveys were conducted every two months for a six-month period (April, June, and August 2024) to assess edible and velvet swimmer populations at both locations. *C. pagurus* and *N. puber* specimens were randomly selected by hand boulder turning. Specimens were transferred to damp seaweed and transported to the aquarium where they remained for up to 48hr. They were fed by the addition of *Mytilus edulis*, blue mussels. Due to their limited on-shore populations, a small number of Risso's crab, *Xantho pilipes* (n = 7) and Montagu's crabs, *Xantho hydrophilus* (n = 8) were also collected from Oxwich Bay only (none were observed at Mumbles Head).

2.2. Laboratory regime

All crabs were processed within 48 h of collection. Crabs were placed on ice and the following biometric data were taken for each crab: sex; moult stage (inter-moult [hard] or post-moult [soft]); fouling (presence of epibionts); externa of *Sacculina*, pigment loss, shell disease, encrusting *Spirorbis*; carapace width (mm); and limb loss (See Supplementary Information Table S1). Next, *ca.* 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 potential systemic infection. Approximately 100 μl haemolymph was transferred onto a microscope slide and examined using phase contrast microscopy for systemic infections including those caused by *Hematodinium* spp. and fungi as previously reported to occur in juvenile edible (Smith et al., 2013, 2015) and velvet swimming crabs (Stentiford et al., 2003).

All crabs were sacrificed by placing at −18 °C for 30–45 min until involuntary motor function was absent. The carapace was detached from the ventral surface and subsequently the whole hepatopancreas was removed, weighed, and stored at −18 °C. This tissue was later thawed at room temperature for *ca.* 30 min before homogenisation in 4–5 ml of 3 % NaCl solution and vacuum filtered through a sterile 150 μm pore size low density polyethylene cell strainer (PluriStrainer, Leipzig, Germany). Filters were examined using a binocular stereomicroscope for the presence of metacercariae, the number of cysts counted, and these were transferred individually or in small groups into 1.5 ml Eppendorf tubes and stored at −18 °C for later DNA extraction. Metacercariae were photographed using an Olympus BX41 microscope equipped with a digital camera. As the edge of the outer cyst walls of metacercariae was indistinct, the diameter of each cyst was measured to the edge of the outer layer of the inner cyst wall (see James 1971 for diagram of encysted metacercariae of *M. similis*).

2.3. DNA extraction, PCR and sequencing conditions

Digenean DNA was extracted directly from multiple thawed metacercariae using a Qiagen Blood and Tissue Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions with an additional extended overnight incubation at 56 °C 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 a DNA concentration of 31.7 ng/μl ± 20.6 ng/μl (mean ± SD).

All PCR reactions were carried out in 25 μl total reaction volumes using 2x BioMix (Bioline, London, UK), 0.5 μl (0.2 mM) oligonucleotide primers synthesised by Eurofins (Ebersberg, Germany), nuclease-free water (Invitrogen™, Leicestershire, UK), 1 μl of genomic DNA (*ca.* 10–60 ng/μl) and performed on a T100 PCR thermal cycler (BioRad Laboratories Inc., Watford, UK). Primers used were refined by Tkach et al. (2003) and Galaktionov et al. (2012) to amplify the 28S rDNA (domains D1-3) gene region; forward primer LSU-5 (5′-TAG GTC GAC CCG CTG AAY TTA AGC A-3′) and reverse primer 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) with an expected product size of ~1400 bps. Cycling conditions were as follows: denaturation at 94 °C for 3 min (1 cycle) followed by 35 cycles of denaturation at 94 °C for 30 *sec*, annealing at 60 °C for 30 *sec* and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. Following cycling, 5 μl of PCR product with 1 μl of 6x DNA Gel Loading Dye (ThermoFisher Scientific, Altrincham, UK) was visualised on a 2 % agarose/TAE gel using GreenSafe Premium nucleic acid stain (NZYTech, Lisbon, Portugal). Gels were electrophoresed for *ca.* 45–60 min at 60 V alongside a 1 Kb 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, purified with ExoSAP-IT/ExoSAP-IT Express (ThermoFisher Scientific, Altrincham, UK) according to manufacturer's instructions. Purified PCR products were identified via Sanger sequencing, using both forward and reverse primers by Eurofins (Ebersberg, Germany).

2.4. Identification of encysted digeneans and phylogenetic analyses

Primers were removed and consensus sequences were constructed from unclipped sequences using BioEdit sequence alignment editor (Hall, 1999). Sequences were identified using the bioinformatic tool for similarity search BLAST (Altschul, 1990) and were submitted to GenBank.

Multiple sequence alignments were performed in CLUSTAL X v.2 (Larkin et al., 2007). The alignment was analysed for the best fitting model using the IQ-TREE server which resulted in a tree based on the evolutionary model TVM + F + G4, according to Bayesian information criterion (Kalyaanamoorthy et al. 2017; Nguyen et al. 2015; Trifinopoulos et al. 2016). The final tree was constructed using ML process with 1000 bootstrap replicates and annotated in iTOL (Hoang et al., 2018, Letunic and 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 and Minin, 2016). Reference sequences comparing the same genetic region (locus) of *M. similis*, obtained from a variety of hosts, were sourced from GenBank at NCBI (Benson et al., 2017, see Supplementary Information Table S4).

2.5. 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. Assumptions include independence of observations, correct specification of the link function, and linearity of continuous predictors with the logit. Residuals were inspected for evidence of poor fit. 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 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), carapace width (continuous numbers), pigment loss (0 or 1), haemolymph appearance (clear or milky, 0 or 1) and limb loss (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 the mean intensity of infection (i.e., parasite load), the dataset was refined to only include crabs harbouring trematodes (n = 55). A Poisson regression model was first employed to assess which predictor variables significantly influenced the intensity of infection among infected individuals. The initial full model incorporated variables including month (grouped by April, June and August), sex (male or female), carapace width (CW; continuous numbers), location (grouped by Mumbles and Oxwich), pigment loss (0 or 1), fouling (0 or 1) and haemolymph opacity (categorized as clear ['normal'] or milky (Supplementary Information, Table S3). Poisson models assume equidispersion (variance = mean); overdispersion was tested using a dispersion statistic, which indicated strong overdispersion ($\phi = 24.91$). Consequently, a negative binomial GLM (MASS package) was used, which relaxes the equidispersion assumption by introducing a dispersion parameter (Zuur et al. 2009; Bolker et al. 2009). 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 *vs*. Oxwich), the normality of the data was tested using GraphPad Prism v.10.3.0. Normality was evaluated using an Anderson-Darling test, Shapiro-Wilk test and Kolmogorov-Smirnov test. The results of these tests indicated that the data were not normally distributed. Consequently, a non-parametric Mann-Whitney *U* test was used to assess the differences in the size of metacercariae between locations in GraphPad Prism v.10.3.0. All graphics were produced using GraphPad Prism v.10.3.0.

3. Results

3.1. General observations on the prevalence of parasitism

Both survey sites had an abundant population of juvenile edible crabs facilitating a target of *ca*. 30 individuals to be collected on each occasion per site. However, the numbers of resident velvet swimming crabs were much smaller such that only 15 were collected and examined for the presence of encysted digeneans from Oxwich Bay, in April and August only. Oxwich Bay alone also had a small population of other species of crabs, namely Risso's crab (*Xantho pilipes*) and Montagu's crab (*X. hydrophilus*) but the numbers of these were so small that drawing any conclusions from these would be of limited use and therefore they do not feature in the following analysis of digenean prevalence and severity.

Overall, 178 juvenile intermoult edible crabs, *C. pagurus* were examined across a six-month period, 87 from Oxwich Bay and 91 from Mumbles Head (see Supplementary Information Table S1). Across the two survey sites, 31 % of crabs contained one or more metacercariae in the hepatopancreas, with 35 % of crabs from Oxwich Bay and 28 % from Mumbles Head infested. In terms of other diseases observed in the haemolymph samples, 18 % of female edible crabs were clinically *Hematodinium*-positive while 14 % of males presented with this parasite as judged by observations of fresh haemolymph preparations with phase contrast microscopy (Supplementary Information Fig. S1). One edible crab from Oxwich Bay collected in June was also found to have a severe, unidentified systemic fungal infection (Supplementary Information Fig. S2) morphologically like that previously reported in the same location (Smith et al., 2013).

A total of 15 velvet swimming crabs *N. puber* were collected from Oxwich Bay (9 in April and 6 in August, 13 males and 2 females, carapace widths 22–64 mm). Of these, none were found to contain any metacercariae in the hepatopancreas. Similarly, no metacercariae were found in the small number of Risso's (n = 7) and Montagu's (n = 8) crabs examined.

3.2. Variables related to parasite presence or absence in Cancer pagurus

A binomial logistic regression 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 (mm), pigment loss (0 *vs*. 1), haemolymph opacity (clear *vs*. milky) (Supplementary Information, Table S2). Reduced models revealed month as a significant factor associated with the presence of trematodes. Crabs in June were significantly less likely to present with trematodes ($p = 0.00241$) than in April and August (Model 1, Table 2) (June = 12 %, April = 43 %, August = 35 %).

When separating by location, a further binomial logistic regression (Model 2, Table 1) 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 1). 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 % prevalences). 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.

For Oxwich Bay, Model 3 used a binomial logistic regression to analyse the presence of trematodes in the sampled crab populations. This revealed month as a significant factor associated with the presence

Table 1
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 Head; Model 3, Oxwich Bay.

Model	Predictor variables	Estimate (slope)	SE (standard error)	P-value
Model 1 - Overall				
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 - Mumbles Head				
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 - Oxwich Bay				
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.

Table 2
Generalised linear model with a negative binomial function, testing the effects of environmental and biometric predictor variables on parasite intensity in infected crabs.

Model	Predictor variables	Estimate (slope)	SE (standard error)	P-value
Model 4				
Parasite.count ~	Location	0.25415	0.35008	0.4679
Location + Month +	(Oxwich)	−0.07792	0.38853	0.8410
Sex + Pigment loss +	Month	−0.58744	0.55634	0.2910
Carapace width	(August)	0.21572	0.62730	0.7309
	Month	0.22438	0.41549	0.5892
	(June)	−0.00735	0.01620	0.6501
df = 48	Sex (Male)			
AIC = 403.03	Pigment loss			
	Carapace width			

of trematodes (Model 3, Table 1). Crabs collected in August (57 % prevalence) were significantly more likely to contain trematodes ($p = 0.0398$) compared with April (30 %) and June (15 %).

3.3. Variables related to intensity of infection (i.e., parasite loads) in Cancer pagurus

Of the 178 crabs surveyed across the two sites, 55 (31 %) contained trematodes. Male crabs from Oxwich and Mumbles had a mean parasite load of 13.0 ± 2.7 (mean ± SE) while females had 8.9 ± 5.0 (mean ± SE). The mean parasite load in crabs from Mumbles was 10.7 ± 2.9 compared to 14.1 ± 3.8 (mean ± SE) from Oxwich Bay.

Model 4 used this sub-set of 55 crabs (Table 2) to investigate 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). The results of this negative binomial revealed no statistically significant relationship between the intensity of infection (i.e. parasite load) and any of the predictor variables (Model 4, Table 2).

3.4. Morphology of encysted metacercariae in Cancer pagurus

All cysts observed were of similar internal and cyst wall morphology, overall shape and size (Fig. 2A,B) suggesting the presence of only one species of parasite and similar to the morphological descriptions of metacercariae from *M. similis* (e.g., James, 1971). The sizes of metacercariae were determined in crabs from Oxwich Bay and Mumbles Head. The mean cyst diameter from Mumbles Head was 288 ± 23 μm (mean ± SD, n = 20) whereas the average cyst size from Oxwich Bay was 283 ± 25 μm (mean ± SD, n = 24). A Mann-Whitney *U* test of these data indicated that there was no statistically significant relationship ($P = 0.7706$) between cyst size and location.

3.5. Molecular analyses of Microphallus similis

Of the 55 trematode-infected crabs, 13 samples containing multiple metacercariae (mean DNA concentration: 31.7 ng/μl) from Mumbles Head (n = 5) and Oxwich Bay (n = 8) were successfully re-amplified and sequenced using the LSU-5/LSU-1500 oligonucleotides. Following

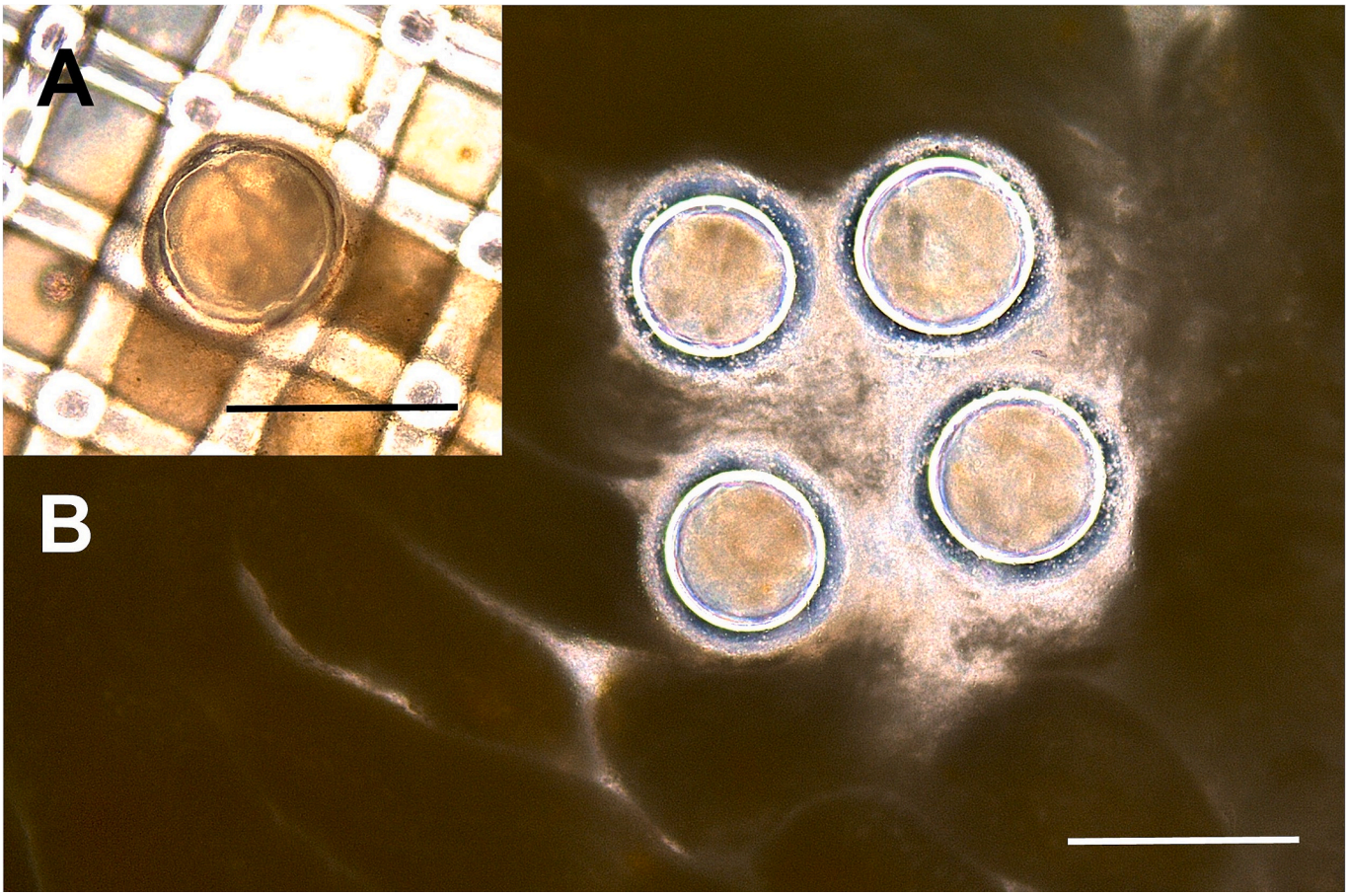

Fig. 2. (A). Photograph of encysted cercaria *ex situ* on the 150 μm pore size cell strainer. (B). Group of metacercariae photographed using darkfield optics. Because the margins of the outer cyst wall were indistinct, all diameter measurements were made using the edge to edge of the distinct inner cyst wall (arrow). Scale bars = 500 μm.

quality control, 9 of these sequences (of the partial 28S rRNA gene region of digenean trematodes) were deposited in GenBank under accession numbers PQ314579 – PQ314582 and PQ314593 – PQ314597 (See Supplementary Information Table S5). 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) reported by Tkach et al. (2003), with one sequence showing a slightly lower identity of 99.6 % 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). The constructed phylogram revealed a clear separation of *M. similis* from other microphallid species (Fig. 3), with all *M. similis* sequences forming a robust clade.

4. Discussion

The current study has provided evidence using molecular approaches for the presence of only *Microphallus similis* in juvenile edible crabs collected from two intertidal sites in South Wales, U.K. Previous studies using only histological approaches have also reported the presence of *M. similis* in edible crabs from other intertidal locations in the U.K. (e.g. Crothers, 1966; James, 1971; Stentiford, 2008) suggesting that this digenean is widely present in this species of crab across several locations. While the morphology of metacercariae can be useful in determining their identity, utilising the size and shape of these digeneans in histological sections, however, can result in errors in the interpretation resulting in potential misidentification. Histological wax-embedded sections are typically 4–10 μm in diameter, much less than the maximum diameters of encysted metacercariae (*ca.* 200 μm), and unless

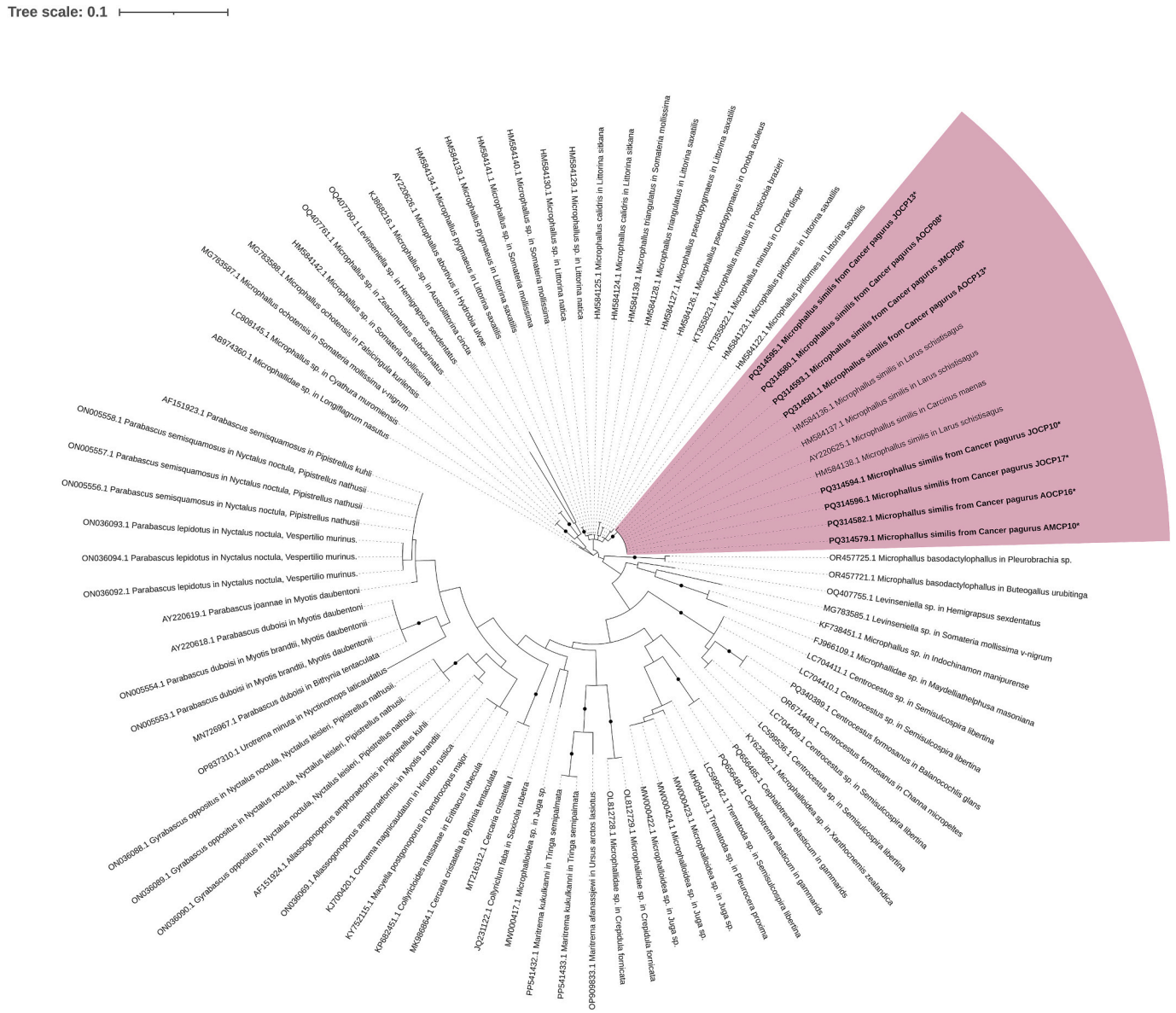

Fig. 3. Phylogram of the partial 28S rRNA gene region from trematodes infecting crabs in this study, and reference nucleotide sequences for trematode species from various hosts retrieved from GenBank. Sequences from this study are in bold font annotated with (*) and are clearly in a robust clade (highlighted pink) with other Microphallus similis. All clades with 100 % bootstrap support are annotated with (●) at the node. Tree based on the evolutionary model TVM + F + G4, according to Bayesian information criterion from the IQ-TREE server (Nguyen et al. 2015; Trifinopoulos et al. 2016). The final tree was constructed using ML process with 1000 bootstrap replicates and annotated in iTOL (Letunic and Bork, 2019). Log-likelihood of the tree: −2046.5814 (s.e. 120.6477). Akaike information criterion (AIC) score: 4395.1629. Bayesian information criterion (BIC) score: 4936.8374. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

serial sections are examined it can result in difficulties in accurate measurement of cyst sizes and interpretation of their internal morphology. For example, Bateman et al. (2011) employed a histological approach to quantify the prevalence of juvenile and adult edible crabs infested with metacercariae collected from Weymouth Bay, U.K. Based on their morphology, they considered these to belong to a related species, *Microphallus primas*, but the cyst shape and sizes shown in their micrographs is conceivably more indicative of *M. similis* than *M. primas*. Interestingly, Bateman et al. (2011) also found that while onshore, juvenile edible crabs were heavily infected by these parasites, but adult crabs caught close by in coastal waters were free from these parasites. The explanation of these findings is problematic as once crabs are infested with such parasites, they would be expected to retain these through to adulthood and it would seem unlikely that the presence of digeneans in juveniles (pre-recruits) alone would cause their death resulting in their failure to enter the fishery.

A key finding of our study is that not all species of crabs present in the intertidal zone at Oxwich Bay were subject to parasitism by digeneans (Table 3). Although we were only able to collect relatively small numbers of velvet swimmers, Montagu's and Risso's crabs, none of these contained any encysted metacercariae in the hepatopancreas. In contrast, shore crabs (*C. maenas*) also collected in the same surveys, were heavily parasitised with *ca*. 76 % prevalence across the two sites and higher parasite loads than found in *C. pagurus* (Bedford, Crocker, Rowley and Davies, unpublished observations; Table 3). There are several potential explanations for our findings. Firstly, it could be that some species of crabs are resistant to the penetration and survival once in the host of the invasive cercariae of *M. similis* and other microphallids into their tissues. Indeed, James (1971) conducted a series of experiments exposing various species of crabs (*C. pagurus*, *C. maenas*) and fish (*Lipophrys pholis* and *Ciliata mustella*) commonly found in the intertidal zone to cercariae of *M. similis*. He reported that only shore crabs contained encysted metacercariae, in what was undoubtedly a preliminary experiment with no mention of replication, or the number of potential hosts exposed. A further explanation of the variability of parasitism across crab species summarised in Table 3 may be due to differences in the movement and feeding behaviour of these five species of crabs in relation to the location of the first intermediate hosts for *M. similis*. Various species of periwinkles, including *Littorina saxatilis,* are the hosts for this microphallid (James, 1971: Blakeslee et al., 2015) and they are abundant in the intertidal region at both survey sites. Periwinkles, in general, are often found on the mid-upper shore (James, 1971). The infective cercariae of *M. similis* are motile and laboratory-based observations have revealed that they swim horizontally away from light and survive > 24 hr in these conditions (McCarthy et al., 2002). Therefore, the chance of these cercariae encountering potential crab hosts will depend on the excursions of these animals at high tide. At low tide, when we sampled at Oxwich, all crabs were found in a similar range of tide heights and so this could suggest an equal chance of all crabs encountering free swimming cercariae of *M. similis*. However, contact with cercariae is much more likely to occur at high tide when the intertidal zone is under water, and it is known that littorinid snails release their cercariae at such times usually in the night (Combes et al., 1994; Haas, 1994) when crabs are actively feeding. These different species of highly mobile crabs are known to show dissimilarities in their movement between the subtidal and intertidal zones of rocky shores associated with their feeding and reproductive behaviours. For instance, Silva et al. (2014) carried out a detailed mark-recapture study of the foraging excursions of *C. maenas*, *C. pagurus* and *N. puber* on rocky shores in southwest England. They concluded that *N. puber* and *C. pagurus* are predominantly subtidal animals that feed in the intertidal zone at high tide but mainly in the lower shore. Shore crabs, however, are more capable of wider feeding excursions where they can move further up the shore (Hunter and Naylor, 1993; Silva et al., 2014) resulting in them more readily encountering the cercariae of *M. similis* emerging from littorinid snails. Hence, these differences in crab feeding excursions at high tide could be a further explanation for the elevated levels of parasitism in shore crabs in comparison to edibles but may not on their own fully explain the apparent lack of digenean metacercariae in *N. puber*, *X. hydrophilus* and *X. pilipes* (Table 3).

We sought to determine if various biometric data had any association with parasitism. Of interest was the finding that larger (i.e. older) crabs were more likely to be parasitised by *M. similis* but that these crabs did not appear to harbour more parasites per crab despite the greater size of the hepatopancreas. Several studies have revealed that large, older crabs are more likely to host greater numbers of parasites (Koga, 2008; Koehler and Poulin, 2010; Blakeslee et al., 2015) simply because with aging, the number of potential encounters with such parasites increases and presumably, they do not disappear once they become established in the host's tissue. The lack of a significant relationship in terms of parasite load, however, could be because the size range of crabs examined was perhaps too small, or not varied enough, to analyse such an association.

Table 3
Summary table showing the prevalence and severity of infection (parasite load) of various species of brachyuran crabs from Mumbles Head and Oxwich Bay with *Microphallus similis* during the collection period April – August 2024.

Crab (genus and species)	Prevalence (%)		Mean parasite load (total per infected crab)	
	Mumbles Head	Oxwich Bay	Mumbles Head	Oxwich Bay
Shore crab (*Carcinus maenas*)*	85	67	41	25
Edible crab (*Cancer pagurus*)	28	35	11	13
Velvet swimming crab (*Necora puber*)	0	0	0	0
Risso's crab (*Xantho pilipes*)	N.F.	0	N.F.	0
Montagu's crab (*Xantho hydrophilus*)	N.F.	0	N.F.	0

*Data from Bedford, Crocker, Rowley and Davies unpublished observations (2025).
N.F. = crabs not found at this site.

5. Conclusions

Edible crabs are subject to modest levels of parasitism in terms of prevalence and severity by the microphallid *M. similis* at two locations in South Wales, U.K. All crabs found were proven to be parasitised by *M. similis* alone. Other species of crabs including *N. puber*, *X. pilipes* and *X. hydrophilus* did not host any microphallid parasites in their hepatopancreas. Whether this condition causes any adverse effects on their hosts is unknown, but similar studies with another crab host, *C. maenas*, again with *M. similis*, have shown only modest effects on their behaviour (Blakeslee et al., 2015; Ro et al., 2022) unlikely to cause any significant alteration in either their health or susceptibility to predation by other animals while in the intertidal zone. Since edible crabs are part of a commercial fishery, it is important to determine any adverse effects of parasitism by *M. similis* and other digenean parasites.

Ethics

Research was approved by Swansea University Research Ethics Approval Number: 2 2024 8411 7714. Sampling of *Cancer pagurus* was approved by Welsh Government Marine and Fisheries Division under dispensation Ref: DISP_2024_001.

CRediT authorship contribution statement

Grace N. Crocker: Writing – review & editing, Investigation. **Alexander T. Bedford:** Investigation. **Andrew F. Rowley:** Writing – review

& editing, Supervision, Investigation, Conceptualization. **Charlotte E. Davies:** Writing – review & editing, Supervision, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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