Infection with cerebral metacercariae of microphallid trematode parasites reduces reproductive output in the gammarid amphipod *Gammarus insensibilis* (Stock 1966) in UK saline lagoons

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ABSTRACT

Saline lagoons are priority habitats in the United Kingdom supporting several protected specialist species. One specialist, the amphipod *Gammarus insensibilis,* is infected with behaviour-altering microphallid trematodes such as *Microphallus papillorobustus*. In saline lagoons around the coast of England (Gilkicker and Lymington-Keyhaven on the Hampshire coast and Moulton Marsh in Lincolnshire) there is variation in the prevalence of this parasite in the gammarid populations (0 at Salterns in the Lymington-Keyhaven lagoon system to 98% at Gilkicker). Infection intensity ranged from 0 to 20 metacercariae in individual amphipods. Higher infection intensity can alter the shape of the amphipod's head. Under experimental conditions respiration rate is significantly reduced in infected animals and reproductive output (expressed as early stage embryos mg g dw^{-1}) is significantly lower in infected females. It is important to consider the role of host-parasite interactions in order to understand the ecology of specialist lagoon species such as *G. insensibilis* and their lagoon habitats.

INTRODUCTION

The amphipod *Gammarus insensibilis* is a priority species, protected under the UK Biodiversity Action Plan. In the UK it is near the northern limit of its range and is restricted to brackish pools and coastal saline lagoons where it can be locally abundant (Gilliland & Sanderson, 2000, Sheader & Sheader, 1985). The ENLag.Veg biotope is utilized by *G. insensibilis* through its close association with the filamentous alga *Chaetomorpha linum* (Bamber et al., 2001), upon which it feeds readily. *G. insensibilis* is not found sympatrically with the morphologically similar but taxonomically distinct (Costa et al., 2004) *G. locusta* (common in fully marine environments). The only recorded fully marine habitats of *G. insensibilis* are in the Mediterranean, where *G. locusta* is absent (Sheader & Sheader, 1987).

Gammarid amphipods demonstrate plasticity of reproductive strategy in which reproductive investment can vary interspecifically but may also vary intraspecifically and can be related to the environmental conditions of their habitat (Johnson et al., 2001, Nelson, 1980, Saintemarie, 1991, Steele & Steele, 1975). In the Mediterranean *G. insensibilis* demonstrates continuous recruitment and a variable (female dominant) sex ratio throughout the year with reproductive output greatest in the winter months (Karakiri & Nicolaidou, 1987). In UK saline lagoons there is an inverse relationship between egg size and temperature (Sheader, 1996), and reproductive investment (clutch volume) is greatest in the summer months (Gates, 2006).

Saline lagoons are listed as priority habitats under the European Union Habitats Directive (Council of the European Communities1992) and are frequently designated as, or lie within, Sites of Special Scientific Interest (SSSI), National Nature Reserves, Ramsar sites and Special Protection Areas (SPAs) (Bamber et al., 2000). In the UK lagoons are of particular conservation importance because they are uncommon habitats (important physiographic features in their own right), and they frequently support plant and animal species and communities absent or rare in other saline habitats (Beer & Joyce, 2013), including “specialist lagoon species” (Bamber et al., 1992, Barnes, 1980, Barnes, 1991). Specialist lagoon species are those that are “distinctly more characteristic of lagoons and lagoon-like habitats than of other habitats” (Bamber et al., 2000). Owing to the rarity of their habitat, specialist lagoon species are uncommon nationally. There are 13 specialist lagoon species of priority concern under the UK biodiversity action plan including *G. insensibilis*. Furthermore, coastal lagoons provide important habitats and feeding grounds for species of charadriiform seabirds such as *Larus melanocephalus* (Mediterranean gull), *Sterna albifrons* (little tern) and *S. sandvicensis* (sandwich tern) (Bamber et al. 2000) and *Recurvirostra avocetta* (avocet) (Hill et al., 1993).

Parasites can have direct (e.g. mortality) and indirect (e.g. behavioural modification) effects on populations and communities (Mouritsen & Poulin, 2002, Thomas et al., 1998a). Differential susceptibility to parasite infection or variation in the symptoms of infection may be as important a factor in determining success and distribution of a species as other factors such as resource availability, physicochemical parameters, and intra/interspecific interactions (Fredensborg et al., 2004). In lagoon systems parasites appear to play a key role (Thomas & Renaud, 2001) and should be considered in the conservation of coastal and wetland habitats (Thomas et al., 1997). *Gammarus insensibilis* can be an intermediate host for a number of trematode parasites including *Maritrema subdolum, Microphallus hoffmanni, Microphallus papillorobustus* and *Levinseniella propinqua* (Kostadinova & Mavrodieva, 2005a) in various localities in continental Europe including Pomorie lagoon in the Black Sea (Kostadinova & Mavrodieva, 2005b) and the Thau lagoon in France (Thomas et al., 1998b).

It has been suggested that the ecology of *G. insensibilis* cannot be fully understood without considering the implications of infection with *M. papillorobustus* (Ponton et al., 2005). *G. insensibilis* is the second intermediate host in a life cycle that begins within a hydrobiid snail. The amphipod is infected by the cercarial stage of the trematode. The cercariae enter through the abdomen of the amphipod and encyst in the head, as cerebral metacercariae. *M. papillorobustus* manipulates the behaviour of *G. insensibilis*, inducing positive phototaxis, negative geotaxis and aberrant evasive behaviour when disturbed, thereby increasing vulnerability to predation by the charadriiform avian definitive host (Arnal et al., 2015, Brown et al., 2003, Helluy, 1984, Thomas et al., 1996b). Behavioural modification only occurs when animals are infected by cerebral metacercariae, not when metacercariae are only located in the abdomen. Behavioural modification is associated with variations in neurological architecture related to levels of the neurotransmitter serotonin (5-hydroxytryptamine) but the mechanism is not fully understood (Tain et al., 2007). Modified behaviour can be induced by injection with serotonin (Helluy & Holmes, 1990, Helluy & Thomas, 2003, Maynard et al., 1996). It is not clear whether serotonin is secreted by the metacercaria itself or whether it is a result of a host response to the infection (Thomas et al., 2005). Documented effects on *G. insensibilis* include assortative pairing; when infected individuals pair with similarly infected animals and vice versa owing to population sub units based on behaviour (Thomas et al., 1996a, Thomas et al., 1995).

Given the conservation importance of *G. insensibilis* and the desire to maintain lagoon habitats some consideration of the relationship between *G. insensibilis* and its trematode parasite is necessary in UK lagoons. This study demonstrates the presence of the microphallid trematode parasites in *G. insensibilis* populations in UK saline lagoons of conservation importance. Population structure, physiological rates and estimates of reproductive output are used to consider the effects of the parasite on the amphipod host.

METHODS

Study sites

The Gilkicker lagoon system lies on the shores of The Solent near Gosport on the south coast of England (50.776° N, -1.138° E, Figure 1 c). Its 36,750 m^2 area consists of two connected lagoons and is separated from the sea by a shingle bank and sea wall. The larger (seaward lagoon) is 210 m long and 145 m wide and connected to the sea by a channel of 85 m in length and 10 m wide ending in a metal pipe (0.8 m diameter) that penetrates the barrier. The landward lagoon is 130 m long and 80 m wide and is connected to the seaward lagoon by two pairs of metal pipes of 0.5 m diameter (Al-Suwailem, 1991). The lagoons do not exceed 0.75 m in depth with a low tide mean of 0.46 m. There is a substratum comprised predominantly of mud in the middle and sand on the western and eastern sides with a 1 m belt of shingle around the shores. In much of the lagoon the surface sediments lie above a layer of shingle or silt (Al-Suwailem, 1991).

The Keyhaven-Lymington lagoon system is located approximately 40 km west of Gilkicker (50.742° N, -1.536° E, Figure 1 c). There are a series of lagoons stretching from Normandy Farm lagoon towards Keyhaven at the mouth of the Lymington river estuary. The lagoons are artificial and were created from relict salterns when the sea wall was repaired in the 1980s, in order to maintain the populations of lagoon specialists present in the salterns (Bamber et al., 2000). The lagoons to the west of the system are prone to low salinity resulting from excess freshwater input after high precipitation so specialist lagoon biota is low. The lagoons used in this study were Eight Acre Pong and Salterns. These are located to the east of the system where salinity is usually around 25-35.

The Moulton Marsh lagoon system in Lincolnshire is on the east coast of the UK (52.882° N, -0.010° E, Figure 1 b). There are four lagoons and a series of ‘scrapes’ (small lagoon-like habitats). Specialist lagoon species have previously been recorded in lagoons 1 and 2 of the system. The alga *Chaetomorpha linum* was abundant in all the lagoon systems.

Sample collection

Gammarus insensibilis specimens were collected under the under license of Section 16 (3) (a) of the Wildlife & Countryside Act 1981 (amended by the Environmental Protection Act 1990), licenses were held by Dr. R. N. Bamber (numbers: 2003-2004 no. 20031279; 2004-2005 no. 20041787). At Gilkicker samples were collected monthly (March to July 2004, Table 1) with a hand net from within the mats of *Chaetomorpha linum* near the southern shore of the lagoon, where they were abundant. This varied depending on the location of the mats of *C. linum,* which was affected by the particular wind and wave conditions. The collection method was non-quantitative because of the three dimensional nature of the habitat, and assumed that that all sizes and life history stages were correctly represented in the samples. Samples were collected from the Lymington – Keyhaven system and the Moulton Marsh lagoons using the same method but on fewer occasions (Table 1). Sampling these locations was carried out later in order to find examples of uninfected amphipods. A subset of animals for physiological study were returned to the National Oceanography Centre research aquarium alive and transferred to aquarium water in outdoor holding tanks at ambient temperature. Lagoon water salinity was measured in the field with a t/s probe and on return to the aquarium and diluted to match the water collected in the field. Animals for parasite prevalence and population dynamics study were preserved in 10% buffered formaldehyde solution and transferred to 70% ethanol. In addition a small archive sample of *G. insensibilis* taken from Gilkicker in 1982 was examined.

Population structure and parasite prevalence

Specimens were examined under a dissecting microscope. The body length, dry weight, sex and number of cerebral metacercariae were recorded. The animals were categorised as juvenile (no visible sexual differentiation), immature female (Female with non-setose oöstegites), mature female (setose oöstegites) and mature male (genital papillae and large gnathopods). Mature females were further categorised as ovigerous (carrying embyos in the oöstegites) and empty. The number of embryos carried by ovigerous females and their stage of development were recorded. Embryo classification was based on 6 developmental stages (I, II, III, IV, V, J) in which the early cleavage stages are represented by stage I and newly hatched juveniles are represented by stage J (Sheader & Chia, 1970). In order to increase sample sizes the developmental stages were grouped into early (I-III) and late (IV-V) stage (Ford et al., 2003). Investment in individual embryos as estimated from the mean embryo volume (mm^3) of eight embryos following the methods of Sheader (1996). Brood mass (µg) was determined by removal from the marsupium and weighed in tin foil capsules and dried for 24 hours at 50 °C. Clutch volume (total reproductive investment) was estimated as brood size (number of embyros) x mean embryo volume.

DATA ANALYSIS

Metacercarial presence in the population and the population structure (juvenile, female, male) were not tested statistically. The mean size of juveniles, females and males in the population at Gilkicker were tested independently for significance using one way ANOVA. Reproductive investment data were initially considered on a monthly basis and then pooled to increase sample size. Linear regression was carried out to test the relationship between number of metacercariae and brood size. Following these analyses the monthly data were pooled to increase sample size and one-way ANOVA was used to test for significance of effect on brood size.

Physiological rate experiments

Amphipods used in physiological rate experiments were collected in the field and maintained in aquaria at constant temperature and salinity for a minimum of one week before experiments were conducted.

Respiration rate experiments were carried out to compare infected and uninfected animals. In the first experiment specimens were selected based on their activity. Nine individuals of approximately similar size were taken from within or under their *C. linum* food source (assumed uninfected) and nine animals of similar size demonstrating aberrant escape behaviour were taken from the surface of the aquarium (strongly positively phototactic and negatively geotactic when disturbed, these were assumed infected). In the second experiment animals were selected randomly and dissected after the experiment to determine parasite infection.

In both cases temperature was maintained (± 0.5 °C) in controlled temperature rooms in the aquarium, salinity measured with a t/s probe and experiments were run under a 12 h light, 12 h dark regime. Assays were run for 24 hours and results were standardized to body mass. Before the assays began the animals were acclimatized to the filtered aquarium water and starved for 24 hours. Air-tight control chambers containing filtered seawater and experimental chambers containing an individual amphipod were incubated for 24 hours at the temperature and salinity recorded in the lagoon when the animals were collected. After 24 hours water samples were extracted from the chambers for analysis of oxygen concentrations. Respiration rate was determined as the difference between oxygen concentrations before (control) and after (experiment) the amphipods had been incubated in the chambers, using Winkler titrations.

Feeding rate was determined in a 24 hour experiment carried out at 5°C and salinity of 30 in which individual amphipods were provided with *C. linum* as a food source. The amount consumed was determined as the difference in wet weight of the alga over the course of the experiment. The uninfected animals (n = 17) were from Salterns and the infected animals (n=6) from Gilkicker.

DATA ANALYSIS

Respiration rate experiments were statistically tested using one-way ANOVA. Where data were not normally distributed the non-parametric Kruskall-Wallis ANOVA on ranks test was applied. For the second experiment the individual animals were examined and cerebral metacercariae counted. The effect parasite infection intensity (parasite load) was tested by linear regression for males and females separately and as both sexes combined. Amphipod respiration rate at 5 and 15°C was compared using pooled infection data.

RESULTS

Metacercaria infection in the population

In pooled data from samples collected over four months (April to July 2004) at Gilkicker approximately 85% of the animals were infected by cerebral metacercariae of trematode parasites (Table 1). The greatest proportion of amphipods (45%) was infected by 1 metacercaria with infection intensity decreasing thereafter. The maximum infection intensity was 20 metacercariae in a single amphipod (Figure 2 a). In such cases of high parasite load the shape of the head was distorted. Mean parasite load of infected animals was 2.20 metacercariae per animal (females = 2.16, males = 2.27, juveniles = 2.06). Cerebral metacercariae of trematode parasites were also confirmed from animals from a small archive sample of *Gammarus insensibilis* from Gilkicker lagoon collected in 1982.

In samples from the Keyhaven-Lymington lagoon system parasite prevalence was lower. Infection rate in amphipods from Eight-Acre Pond infection rate was 58.3% but animals from The Salterns were rarely infected (0% and 3.9%). At Moulton Marsh 8% of *G. insensibilis* were infected with cerebral metacercariae, although it must be noted that low numbers were examined (twelve specimens). In uninfected animals there was a male bias to the sex ratio. This is related to the size at which it was possible to differentiate the sexes, 20% of the population was juvenile animals (Figure 2 b). In the infected population there was a decreasing proportion of juveniles with increasing parasite load. Parasite load had no significant effect on mature amphipod size (Figure 2c) but infected juvenile animals were significantly larger than uninfected juveniles (0, 1 and 2+ cerebral infections) (ANOVA; $F_{2, 69} = 21.512$, $P < 0.001$) (Figure 2 c).

Physiological rate experiments

Mean respiration rate of all animals (infected and uninfected) was significantly lower at 5°C than at 15°C (Mann-Whitney U=62.0, n=14, 18, P<0.05). In the first respiration rate experiment (5°C), infected amphipods (selected based on behaviour - positive phototaxis, negative geotaxis) demonstrated significantly lower respiration rate than uninfected individuals (ANOVA; $F_{1\text{-}17} = 6.533$, $P < 0.05$) (Figure 3 a). In animals selected from different lagoons (Salterns and Gilkicker) mean respiration rate of animals from Gilkicker (infected) was significantly lower than those collected at the Salterns (uninfected (Figure 3 b, ANOVA: $F_{1,13} = 8.463$, $P<0.05$). Linear regression showed no significant effect of parasite load (males, females and sexes combined) (Figure 3 c). In feeding experiments there was no significant difference between mean feeding rate of uninfected individuals (11.9 mg g dry wt^{-1} h^{-1}) and infected animals (14.6 mg g dry wt^{-1} h^{-1}).

Reproductive investment

Mean weight-specific early stage brood size of *G. insensibilis* differed significantly with infection by trematode metacercariae when all gravid females from April to July were pooled (Kruskall-Wallis ANOVA on ranks H = 19.808, 2 df; $P < 0.001$, Figure 4 b). Pairwise multiple comparisons (Dunn's method) showed females infected with 3 or more metacercariae to have significantly lower brood size than both uninfected animals, and those infected with 1 or 2 metacercariae. Brood size reduced by 36.6% between uninfected and heavily infected (3+) individuals. There was no significant difference in investment in individual embryos as estimated from embryo volume (mm^3) and embryo mass (µg), changes reproductive investment (clutch volume) were therefore related to the number of embryos.

Mean late-stage brood size was higher in uninfected individuals but there was no significant difference in embryo number in either stage IV or V broods (note, low numbers of uninfected females). Uninfected females showed a rate of loss of embryos of 27.8 % while females infected with three or more metacercariae lost 28.9 % of their embryos during development from early stage (I-III) to late stage (V). Females with intermediate parasite load demonstrated higher rates of embryo loss during development of 44.5 %.

DISCUSSION

The presence of the metacercariae of microphallid trematodes in the cephalic region of *G. insensibilis* became apparent during the examination of animals for a separate study (Gates, 2006). The presence of the metacercariae is consistent with the other studies of the species (Helluy 1983). The size and shape of the metacercariae were consistent with the 270 x 350 µm ovoid cysts of *M. papillorobustus* (Rebecq, 1964) but the parasite species has not been identified in this study. On occasions there were smaller metacercariae present in the abdomen which may represent a different species of trematode or may be immature metacercariae from new infections. The observations reported here are the first from the UK despite other studies of UK populations of *G. insensibilis* (Pearson et al., 2002, Sheader, 1996) but when researchers do not have a “search image” for parasites they can be easily missed (Schwartz & Cameron, 1993). These results have implications for the understanding of the ecology of a priority species under the biodiversity action plan, and more widely the ecology of saline lagoon systems. For example, at an estuarine site in close proximity to Gilkicker high levels of infection by trematode metacercariae had a detrimental effect on the overall population size of *Echinogammarus marinus* (Guler et al., 2015).While our study did not find major detrimental effects on the population there were effects at the individual level.

Mean infected male parasite load at Gilkicker (2.26) was higher than Thau lagoon (1.83) but female parasite load (2.16) was lower than Thau (2.26-3.19) (Ponton et al., 2005). In this study metacercaria infection ranged from 0-20 per host but amphipods with more than five metacercariae were rare. Presumably, increased infections caused an increase in the rate of predation by the definitive host so these animals were removed from the population (Lafferty, 1996). Furthermore, increased mortality of the amphipod *Paracalliope novizealandiae* was reported from wounding and loss of haemolymph following infection with “intermediate” and “high” levels of *Maritrema novaezelandensis* (Microphallidae) infection (Fredensborg et al., 2004). *G. insensibilis* hosting many metacercariae had distorted head shape supporting the idea of maximum volume of infection proposed for metacercariae of microphallids infecting *Cyathura carinata* (Jensen et al., 2004). At low infection intensities a greater proportion of the host animals were juveniles, likely owing to a shorter time period for infection to occur.

The prevalence of cerebral metacercariae in *Gammarus insensibilis* was higher at Gilkicker and Eight-Acre Pond. These lagoons are separated by approximately 40 km but The Salterns, where prevalence was lower, is only about 50 m from Eight Acre Pond. Gilkicker and Eight-Acre Pond are both wide, shallow lagoons with a muddy sediment and gravel in the margins. In contrast, the Salterns and the Moulton Marsh lagoons are narrower, deeper bodies of water. The differential infection rates between the two lagoon types could be explained by the same phenomenon that splits the host population into two discrete subunits based on infection (Ponton et al., 2005, Thomas et al., 1996a, Thomas et al., 1995). Within Gilkicker and Eight-

Acre Pond, the water is shallow (<0.75 m), so such discrete populations are less feasible. The shallower lagoons may also be more suitable for feeding by wading birds, thus increasing the potential for successful completion of the trematode life cycle, and increasing the selective advantage for parasite prevalence at these sites.

At Gilkicker the highest parasite prevalence (98.3%) was recorded in July 2004. The previous month had the highest recorded water temperature (26°C). The release activity of microphallid cercariae from the initial molluscan host is known to increase in warmer water. Amphipods increased their gill ventilation to increase oxygen uptake at higher temperature. In this study this was demonstrated by higher respiration rate at 15°C, as expected for peracarid crustaceans (Dorgelo, 1977, Garnacho et al., 2001). Increased gill ventilation could increase contact with trematode cercariae (Mouritsen & Jensen, 1997). Fredensborg et al (2004) showed that high temperature induced severe *Corophium* sp. mortality as a result of increased risk of injury associated with cercarial penetration in conjunction with temperature stress. Such a situation can be envisaged for lagoonal habitats where the warmest summer months (extremes of temperature and salinity, along with temperature related reduced oxygen levels) in conjunction with high injury rates from cercarial penetration may present a severe physiological challenge.

Respiration rate was significantly lower in *G. insensibilis* infected by metacercariae in both experiments. This does not support the hypothesis that higher respiration rate may be expected in infected animals as a symptom of increased stress. There are conflicting patterns in parasite effects on metabolic rates but similar results have been reported for *G. pulex* infected by the behaviour modifying acanthacephalan *Pomphorhynchus laevis* (Rumpus & Kennedy, 1974). A plausible explanation for reduced respiration rates in animals infected by behaviour modifying parasites is reduced activity in the host. Aberrant escape behaviour occurs in response to disturbance (Ottaviani & Franceschi, 1996). Arnal et al. (2015) reported greatest activity by infected *G. insensibilis* in the presence of fish slime and bird faeces, both associated with potential definitive hosts. In the current study disturbance was minimal in the laboratory assay so activity may be reduced in the infected animals.

In this study there was a reduction in the early-stage brood size with increased parasite load. In any single month there was no difference in the size of individual embryos associated with infection with cerebral metacercariae. In amphipods reproductive plasticity is achieved through the balance of reproductive investment in fewer, larger individual embryos (e.g. in winter) or more smaller individuals (summer) (Kolding & Fenchel, 1981, Sheader, 1996). The same microphallid-gammarid system in the Thau lagoon has demonstrated assortative pairing (Thomas et al., 1996a) influencing the fitness of infected males by pairing with smaller, infected females (Ponton et al., 2005) but there was no evidence of effects infection on the size of reproductive females in this study. Early and late stage brood size was similarly reduced by parasite infection where rates of brood mortality were approximately 29% in infected and uninfected animals. The similar rates of brood mortality suggest that the smaller broods of infected animals was not related to the re-ingestion of the developing embryos at a later stage of development to compensate for energetic imbalances because of parasite infections. Therefore the effects of trematode infection on reproduction were likely initiated prior to the release of eggs into the marsupium, perhaps because of reduced energy to allocate to reproduction. Increased activity by hosts generated by manipulative parasites (Arnal et al., 2015), melanisation and encapsulation responses induced following infection (Kostadinova & Mavrodieva, 2005b) and wounding and haemolymph loss through penetration of the abdomen by cercariae (Fredensborg et al., 2004) are likely energetically costly, reducing resources available for reproduction.

In any consideration of the conservation of lagoon habitats and specialist lagoon species the interaction with parasites must be understood. Microphallid trematodes are frequently associated with lagoons or habitats where temperature can reach high levels, such as the intertidal zone on the seashore. The success of organisms such as *G. insensibilis* in lagoon habitats may be the result of an ability to maintain healthy reproductive rates and population sizes despite stressful environmental conditions, continual parasite infection and occasional parasite-induced reductions in population size.

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REFERENCES

Al-Suwailem A.M. (1991) *The ecology of a saline lagoon in southern England*. PhD, University of Southampton, Southampton.

Arnal A, Droit A, Elguero E, Ducasse H, Sánchez MI, Lefevre T, Misse D, Bédèrina M, Vittecoq M, Daoust S and Thomas F (2015) Activity level and aggregation behaviour in the crustacean gammarid *Gammarus insensibilis* parasitized by the manipulative trematode *Microphallus papillorobustus. Frontiers in Ecology and Evolution*. 3:109.

Bamber R.N., Batten S.D., Sheader M. and Bridgewater N.D. (1992) On the ecology of brackish water lagoons in Great Britain. *Aquatic Conservation: Marine and Freshwater Systems*, **2**, 65-94.

Bamber R.N., Gilliland P.M. and Shardlow E.A. (2000) Saline Lagoons: A guide to their management and creation (interim version). *Saline Lagoon Working Group*.

Bamber R.N., Gilliland P.M. and Shardlow E.A. (2001) Saline Lagoons: A guide to their management and creation *English Nature*, 95.

Barnes R.S.K. (1980) *Coastal Lagoons. The natural history of a neglected habitat*, Cambridge: Cambridge University Press.

Barnes R.S.K. (1991) Dilemmas in the theory and practice of biological conservation as exemplified by British coastal lagoons. *Biological Conservation*, **55**, 315-328.

Beer N.A. and Joyce C.B. (2013) North Atlantic coastal lagoons: conservation, management and research challenges in the twenty-first century. *Hydrobiologia*, **701**(1), 1-11.

Brown S.P., De Lorgeril J., Joly C. and Thomas F. (2003) Field evidence for density-dependent effects in the trematode *Microphallus papillorobustus* in its manipulated host, *Gammarus insensibilis*. *Journal of Parasitology*, **89**(4), 668-672.

The Council of the European Communities (1992) Council directive 92/43/EEC of 21 May 1992 on conservation of natural habitats and of wild fauna and flora. 7-50.

Costa F.O., Cunha M.R., Neuparth T., Theodorakis C.W., Costa M.H. and Shugart L.R. (2004) Application of RAPD DNA fingerprinting in taxonomic identification of amphipods: a case-study with *Gammarus* species (Crustacea: Amphipoda). *Journal of the Marine Biological Association of the United Kingdom*, **84**, 171-178.

Dorgelo J. (1977) Comparative ecophysiology of gammarids (Crustacea: Amphipoda) from marine, brackish and fresh-water habitats exposed to the influence of salinity-temperature combinations. III. Oxygen uptake. *Netherlands Journal of Sea Research*, **7**, 253-266.

Ford A.T., Fernandes T.F., Rider S.A., Read P.A., Robinson C.D. and Davies I.M. (2003) Measuring sublethal imapacts of pollution on reproductive output of marine Crustacea. *Marine Ecology - Progress Series*, **265**, 303-309.

Fredensborg B.L., Mouritsen K.N. and Poulin R. (2004) Intensity-dependent mortality of *Paracalliope novizealandiae* (Amphipoda : Crustacea) infected by a trematode: experimental infections and field observations. *Journal of Experimental Marine Biology and Ecology*, **311**(2), 253-265.

Garnacho E., Peck L.S. and Tyler P.A. (2001) Effects of copper exposure on the metabolism of the mysid Praunus flexuosus. *Journal of Experimental Marine Biology and Ecology*, **265**, 181-201.

Gates A.R. (2006) *The physiological ecology of the specialist lagoon amphipod, Gammarus insensibilis*. PhD, University of Southampton, Southampton.

Gilliland P.M. and Sanderson W.G. (2000) Re-evaluation of marine benthic species of nature conservation importance: a new perspective on certain ‘lagoonal specialists’ with particular emphasis on *Alkmaria romijni* Horst (Polychaeta: Ampharetidae). *Aquatic Conservation: Marine and Freshwater Ecosystems*, **10**(1), 1-12.

Guler Y., Short S., Etxabe A.G., Sherhod C.M., Kille P. and Ford A.T. (2015) Impacts of a newly identified behaviour-altering trematode on its host amphipod: from the level of gene expression to population. *Parasitology*, **142**(12), 1469-1480.

Helluy S. (1984) Relations hotes-parasites du trematode *Microphallus papillorobustus* (Rankin, 1940). III Facteurs impliques dans les modifications du comporttement des *Gammarus* hotes intermediaires et tests de predation. *Annales de Parasitologie Humaine et Comparee*, **59**, 41-56.

Helluy S. and Holmes J.C. (1990) Serotonin, octopamine and the clinging behaviour induced by the parasite *Polymorphus paradoxus* (Acanthocephala) in *Gammarus lacustris* (Crustacea). *Canadian Journal of Zoology*, **68**, 1214-1220.

Helluy S. and Thomas F. (2003) Effects of *Microphallus papillorobustus* (Platyhelminthes : Trematoda) on serotonergic immunoreactivity and neuronal architecture in the brain of *Gammarus insensibilis* (Crustacea : Amphipoda). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**(1515), 563-568.

Hill D., Rushton S.P., Clark N., Green P. and Prysjones R. (1993) Shorebird communities on British estuaries - Factors affecting community composition. *Journal of Applied Ecology*, **30**(2), 220-234.

Jensen K.T., Ferreira S.M. and Pardal M.A. (2004) Trematodes in a *Cyathura carinata* population from a temperate intertidal estuary: infection patterns and impact on host. *Journal of the Marine Biological Association of the United Kingdom*, **84**(6), 1151-1158.

Johnson W.S., Stevens M. and Watling L. (2001) Reproduction and development of marine peracaridans. In Southward A.J., Tyler P.A., Young C.M. and Fuiman L.A. (eds) *Advances in Marine Biology*. vol. 39, London: Academic Press.

Karakiri M. and Nicolaidou A. (1987) Population studies on the Amphipoda of Mazoma Lagoon (Greece). *Helgolander Meeresuntersuchungen*, **41**, 453-464.

Kolding S. and Fenchel T.M. (1981) Patterns of reproduction in different populations of five species of the amphipod genus *Gammarus*. *Oikos*, **37**, 167-172.

Kostadinova A. and Mavrodieva A.S. (2005a) Microphallids in *Gammarus insensibilis* Stock, 1966 from a Black Sea lagoon: manipulation hypothesis going East? *Parasitology*, **131**, 337-346.

Kostadinova A. and Mavrodieva R.S. (2005b) Microphallids in *Gammarus insensibilis* Stock, 1966 from a Black Sea lagoon: host response to infection. *Parasitology*, **131**, 347-354.

Lafferty K.D. (1996) Altered behaviour of parasitized killifish increased susceptibility to predation by bird final hosts. *Ecology*, **77**, 1390-1397.

Maynard B.J., DeMartini L. and Wright W.G. (1996) *Gammarus lacustris* harboring *Polymorphus paradoxus* show altered patterns of serotonin-like immunoreativity. *Journal of Parasitology*, **82**, 663-666.

Mouritsen K.N. and Jensen K.T. (1997) Parasite transmission between soft-bottom invertebrates: temperature mediated infection rates and mortality in Corophium volutator. *Marine Ecology Progress Series*, **151**, 123-134.

Mouritsen K.N. and Poulin R. (2002) Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology*, **124**, S101-S117.

Nelson W.G. (1980) Reproductive patterns of gammaridean amphipods. *Sarsia*, **65**(2), 61-71.

Ottaviani E. and Franceschi C. (1996) The neuroimmunology of stress from invertebrates to man. *Progress in Neurobiology*, **48**(4-5), 421-440.

Pearson C.V.M., Rogers A.D. and Sheader M. (2002) The genetic structure of the rare lagoonal sea anemone, *Nematostella vectensis* Stephenson (Cnidaria; Anthozoa) in the United Kingdom based on RAPD analysis. *Molecular Ecology*, **11**(11), 2285-2293.

Ponton F., Biron D.G., Joly C., Helluy S., Duneau D. and Thomas F. (2005) Ecology of parasitically modified populations: a case study from a gammarid-trematode system. *Marine Ecology Progress Series*, **299**, 205-215.

Rebecq J. (1964) *Recherches Systématiques, Biologiques et Écologiques sur les Formes Larvaires de Quelques Trématodes de Camargue.* PhD thesis, University of Marseille, Marseille.

Rumpus A.E. and Kennedy C.R. (1974) The effect of the acanthocephalan *Pomphorhynchus laevis* upon the respiration of its intermediate host, Gammarus pulex. *Parasitology,* **68**(02), 271-284.

Saintemarie B. (1991) A review of the reproductive bionomics of aquatic gammaridean amphipods - Variation of life-history traits with latitude, depth, salinity and superfamily. *Hydrobiologia*, **223**, 189-227.

Schwartz S.S. and Cameron G.N. (1993) How do parasites cost their hosts? Preliminary answers from trematodes and *Daphnia obtusa*. *Limnology and Oceanography*, **38**(3), 602-612.

Sheader M. (1996) Factors influencing egg size in the gammarid amphipod *Gammarus insensibilis*. *Marine Biology,* **124**(4), 519-526.

Sheader M. and Chia F.-S. (1970) Development, fecundity and brooding behaviour of the amphipod, *Marinogammarus obtusatus*. *Journal of the Marine Biological Association of the United Kingdom*, **50**, 1079-1099.

Sheader M. and Sheader A.L. (1985) New distribution records for *Gammarus insensibilis* Stock, 1966, in Britain. *Crustaceana*, **49**(JUL), 101-105.

Sheader M. and Sheader A.L. (1987) The distribution of the lagoonal amphipod *Gammarus insensibilis* Stock in England. *Porcupine Newsletter*, **3**, 220-223.

Steele D.H. and Steele V.J. (1975) The bioloigy of *Gammarus* (Crustacea, Amphipoda) in the northwestern Atlantic. XI. Comparison and discussion. *Canadian Journal of Zoology*, **53**, 1116-1126.

Tain L., Perrot-Minnot M.-J. and Cézilly F. (2007) Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on brain serotonergic activity in two congeneric host species. *Biology Letters*, **3**(1), 69-72.

Thomas F., Adamo S. and Moore J. (2005) Parasitic manipulation: where are we and where should we go? *Behavioural Processes,* **68**(3), 185-199.

Thomas F., Crivelli A., Cezilly F., Renaud F. and deMeeus T. (1997) Parasitism and ecology of wetlands: A review. *Estuaries,* **20**(3), 646-654.

Thomas F. and Renaud F. (2001) *Microphallus papillorobustus* (Trematoda): A review of its effects in lagoon ecosystems. *Revue D Ecologie-La Terre Et La Vie*, **56**(2), 147-156.

Thomas F., Renaud F. and Cezilly F. (1996a) Assortative pairing by parasitic prevalence in *Gammarus insensibilis* (Amphipoda): Patterns and processes. *Animal Behaviour*, **52**, 683-690.

Thomas F., Renaud F., de Meeus T. and Poulin R. (1998a) Manipulation of host behaviour by parasites: ecosystem engineering in the intertidal zone? *Proceedings of the Royal Society of London Series B-Biological Sciences*, **265**(1401), 1091-1096.

Thomas F., Renaud F., Derothe J.M., Lambert A., Meeüs T. and Cézilly F. (1995) Assortative pairing in *Gammarus insensibilis* (Amphipoda) infected by a trematode parasite. *Oecologia*, **104**, 259-264.

Thomas F., Verneau O., Santalla F., Cezilly F. and Renaud F. (1996b) The influence of intensity of infection by a trematode parasite on the reproductive biology of Gammarus insensibilis (Amphipoda). *International Journal for Parasitology*, **26**(11), 1205-1209.

Thomas F., Villa M., Montoliu I., Santalla F., Cezilly F. and Renaud F. (1998b) Analyses of a debilitating parasite (*Microphallus papillorobustus*, Trematoda) and its "hitchhiker" parasite (*Maritrema subdolum*, Trematoda) on survival of their intermediate host (*Gammarus insensibilis*, Amphipoda). *Journal of the Helminthological Society of Washington*, **65**(1), 1-5.

Tables

Table 1. The prevalence of cerebral metacercariae of microphallid trematodes in *G. insensibilis* from saline lagoons on the UK coast.

Site	**Sampling date**	**Temperature**	**Salinity**	**Number of amphipods**	**% prevalence**
Salterns	04/10/2004	16.8	31.4	51	0
Salterns	09/02/2005	7.9	18.3	69	3.9
8-Acre Pond	09/02/2005	7.3	25.7	24	58.3
Moulton Marsh 1	12/10/2004	10.7	29.1	12	8.0
Gilkicker	08/03/2004	7.6	33.1	68	76.5
Gilkicker	19/04/2004	11.0	32.4	150	92.7
Gilkicker	20/05/2004	19.1	36.9	110	60.9
Gilkicker	14/06/2004	26.2	39.5	167	80.2
Gilkicker	19/07/2004	21.3	37.7	181	98.9
Gilkicker Total	March – July 2004	-	-	676	84.3

Figures

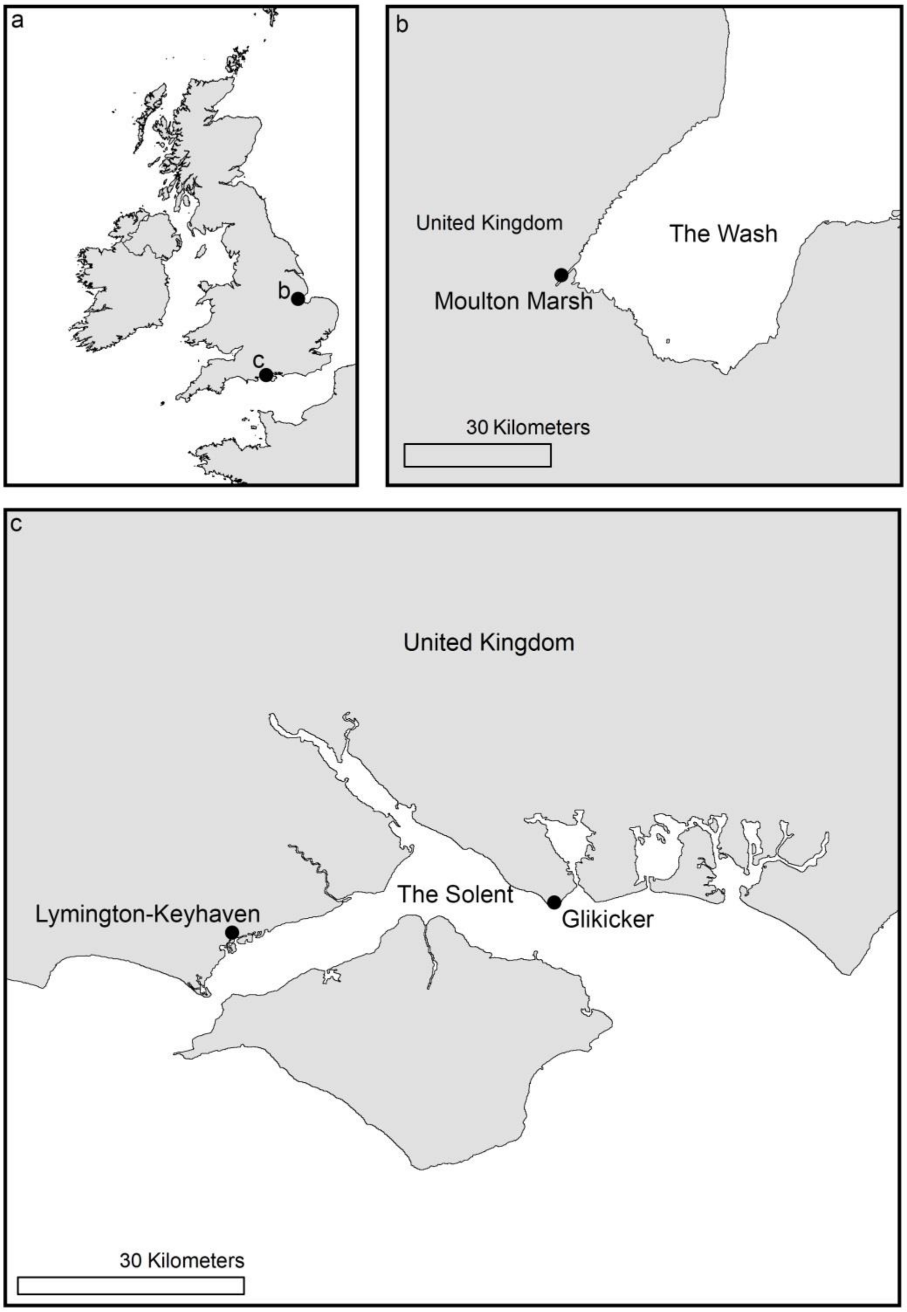


Fig 1. a) Location of the study sites in the United Kingdom, b) Location of Moulton Marsh on the east coast and c) location of the Gilkicker and Lymington-Keyhaven lagoon systems on the south coast.

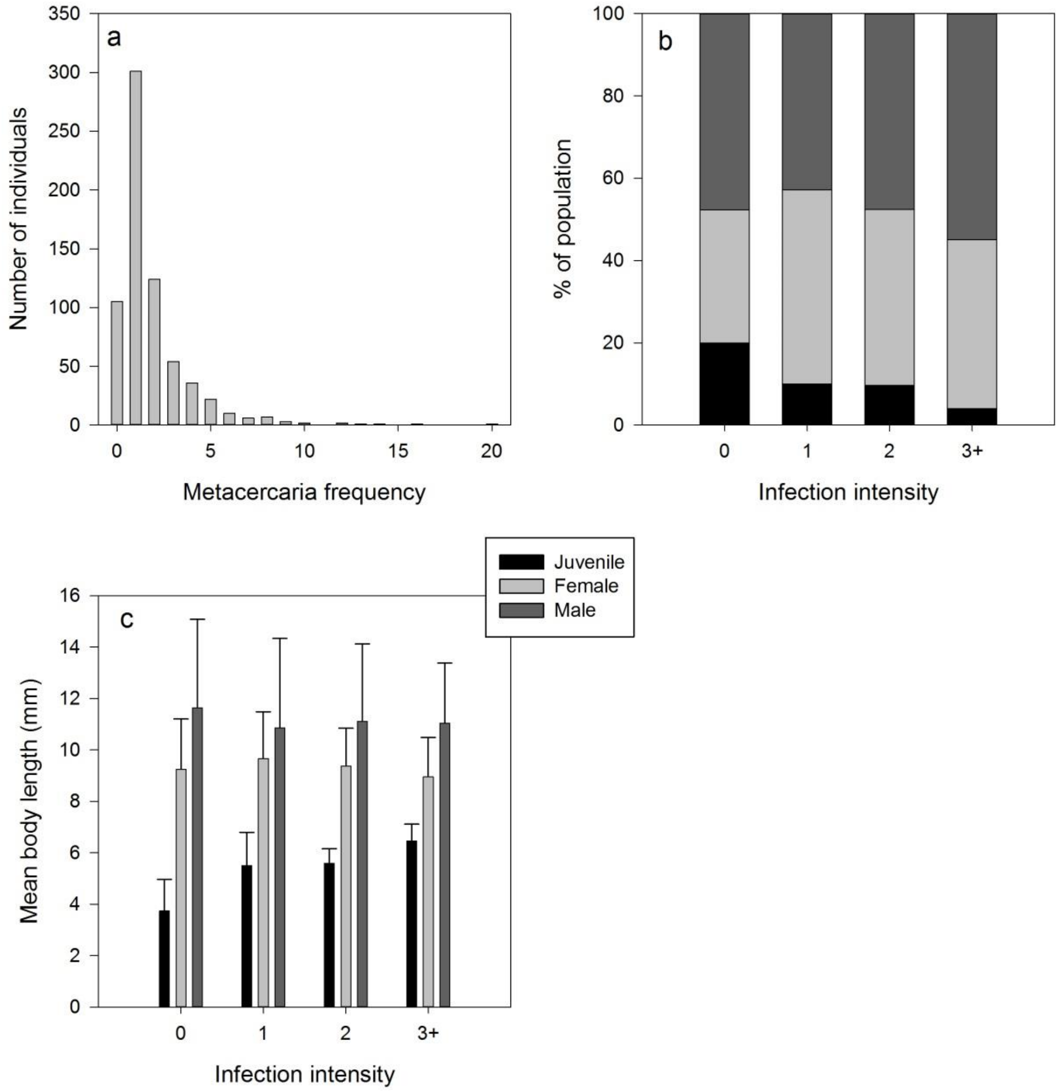


Fig 2. Trematode metacercaria infection in the *Gammarus insensibilis* population from Gilkicker between March and July 2004. a) Metacercaia infection intensity in the *G. insensibilis* population, b) proportion of the population at increasing infection intensity, c) The size of amphipods (± 1 sd) from three life history categories with increasing infection intensities (legend refers to b and c).

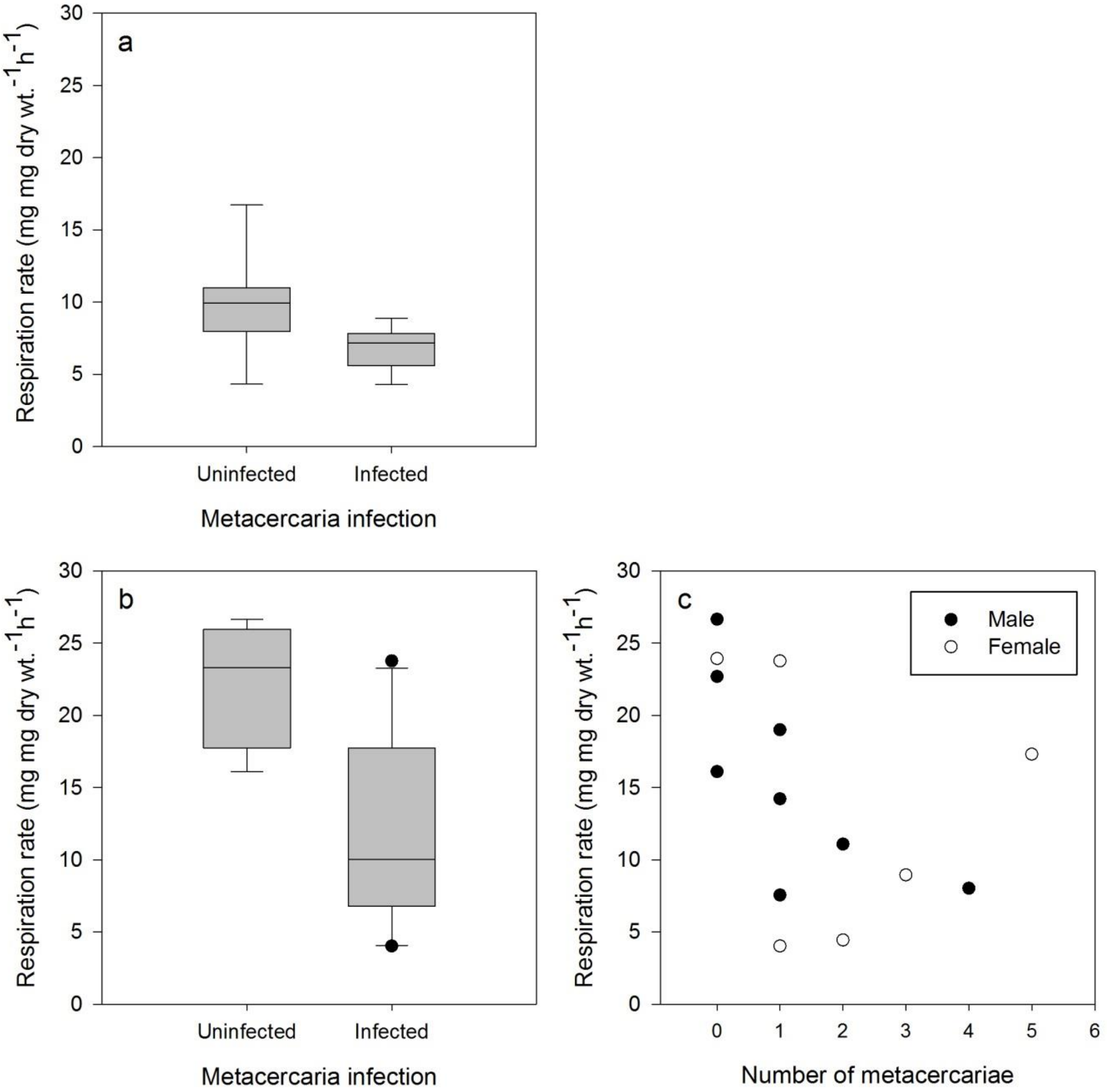
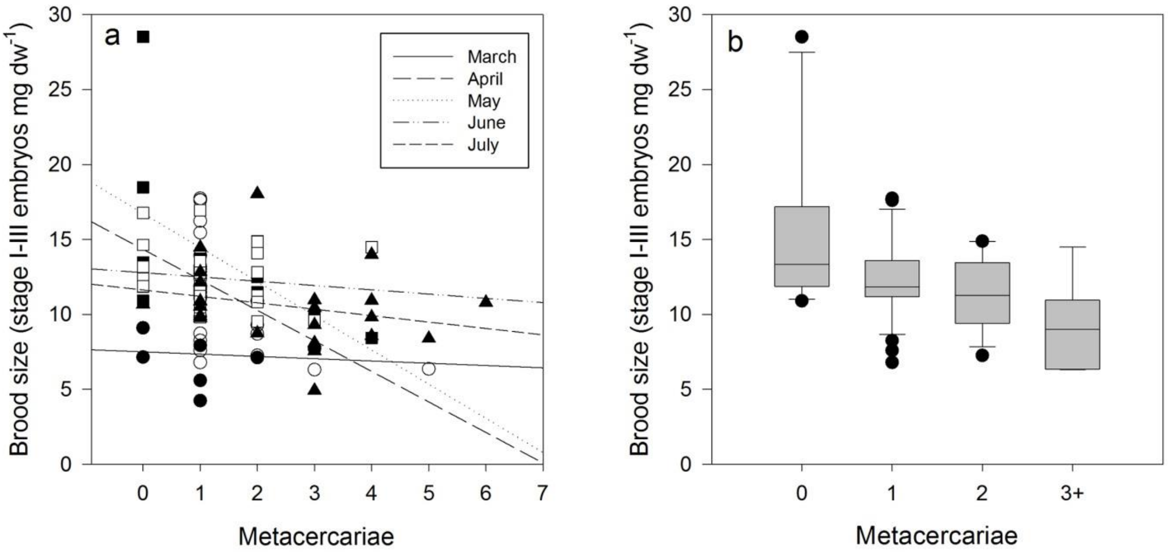


Fig. 3. The effect of metacercarial infection on respiration rate of *Gammarus insensibilis*. a) Experiment 1, Respiration rate (± 1 sd) of uninfected and infected *G. insensibilis* selected based on behaviour (n=9). b) Respiration Experiment 2, Respiration rate of infected (n=4) and uninfected (n=10) *G. insensibilis* randomly selected and dissected after the experiment to determine metacercaria presence. c) Scatter plot of data from b) separated by sex.



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584 **Fig 4.** The effect of intensity of cerebral metacercaria infection on the early stage brood size

585 (embryos mg dry wt.$^{-1}$) of *G. insensibilis*. a) Monthly data b) all monthly data pooled.

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