Hitching rides on fish: crustaceans revealed in a pond in south Wales

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ABSTRACT

With the growth of freshwater fishing, the need to monitor fish health in a water system becomes ever more paramount. One of the most prevalent, and arguably the most disastrous, to fish health is parasites that can cause significant damage and mortality in affected fish. With parasite control being of high importance in a fishery or club waters, having no indication of what parasites are present in the fisheries or club waters will limit success of treatment. Therefore, fishery owners need to be made aware of the parasite population residing in their own waters. In this study, 42 freshwater fish from south Wales were examined in response to concern raised by observation of flashing and abnormal behaviour in the fish. Examination of external body surfaces, gastrointestinal tract and abdominal cavity was conducted. Results revealed two parasitic crustacean species infesting the examined fish *Neoergasilus japonicus* (class: Copepoda) and *Argulus foliaceus* (class: Branchiura). The parasitic copepod *N japonicus* was the most abundant species detected with an overall prevalence of 26.2% in all examined fish (n = 42). Rudd recorded the highest prevalence (54.5%) compared to roach (26.6%) and bream, and bream hybrids (6.2%), indicating host specificity. This parasite was found attached to the base of all fins, but predominantly the anal fin (43.3%) and dorsal fin (27.7%). Only one *A foliaceus* was found on 1 out of 15 roach examined. These findings are important because they inform the fishery owner of the residing parasite populations that could potentially threaten their fishery.

The British Isles possess a large number of rivers, streams, ponds and lakes that are either natural or man-made. These aquascapes are home to 57 recognised resident species of fish, of which 28 species are commonly targeted by anglers (Maitland, 2004).

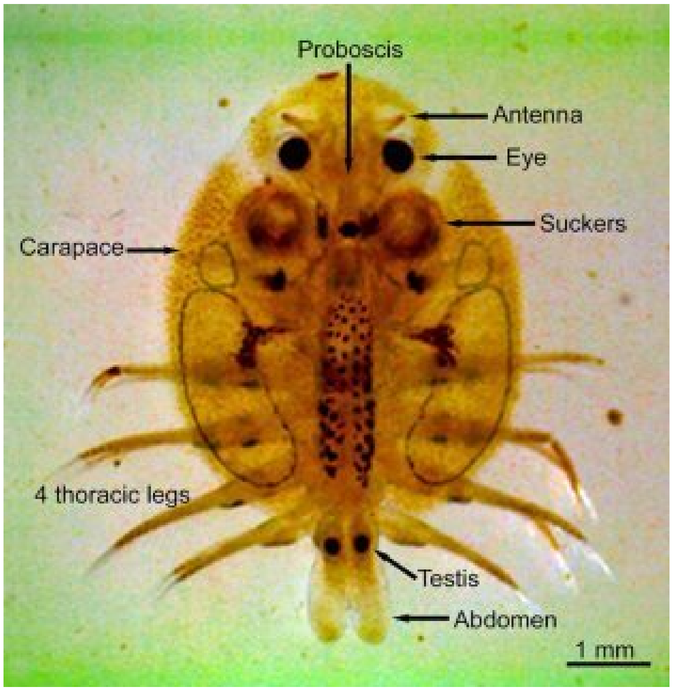

Figure 1. Unstained wet mount preparation of the branchiuran *Argulus foliaceus* isolated from freshwater fish from south Wales.

Interest in fishing or angling has increased in Britain during the past few years. According to the Environment Agency, sales of rod licences increased approximately 31.9% between the year 2000/2001 and year 2010/2011.

With growing popularity of the sport, the risk for disease transmission – particularly parasites – between fisheries is likely to increase due to angling equipment, which can facilitate the dissemination of many disease-causing pathogens to fish. Of these diseases that affect fish, parasites are by far the most abundant and problematic – affecting a wide range of organs, thus affecting the fish's health and aesthetics, which, in turn, can cause significant economic loss to fisheries.

Crustaceans are the most prevalent and arguably the most disastrous threat to fish health because they can cause significant damage and mortality in almost all fish species. However, little is known about the prevalence and pathobiology of these ectoparasites in British waters, and how they interact with the fish host – crucial information in assessing the impact of these parasites.

With the intensification of fish farming and the growth of freshwater fishing, the need to monitor fish health in a water system becomes ever-more paramount. Because parasite control is of high priority in a fishery or club waters, any lack of an indication of what parasites are present will limit the success of any parasite control programme. Therefore, epidemiological surveys should be included in any efforts to control disease transmission and to maintain good health within the fish population.

In this study, the authors investigated the prevalence of ectoparasitic crustaceans in a population of fish in south Wales.

Materials and methods

History and clinical findings

Fish from a private fishery in south Wales were suspected of being infested with ectoparasites – based on the observation of fish jumping out of the water and swimming in an erratic fashion. These are among the early signs that can be observed in fish attempting to rid themselves of ectoparasites.

Fish samples

From September to November 2013, 42 fish – including 15 roach (*Rutilus rutilus*), 11 rudd (*Scardinius erythrophthalmus*), 16 bream (*Abramis brama*) and bream hybrids – were captured from a private pond in south Wales by using a basic waggler rig on a rod and line, and maggots as bait. Collected fish were placed into a keep net for about two to three hours, and contact between fish and angler was kept to a minimum with the use of a landing net to reduce the risk of removing mucus and any potential parasites. Fish were killed on site via a blow to the head (as stated in Animals [Scientific Procedures] Act 1986, Schedule 1, Table A) and were relocated to containers with the same pond water until processed.

Parasitological examination

For each fish, paper towels and cling film were placed on the workspace – the cling film ensured any protozoan parasites wouldn’t get absorbed into the paper towels. Minimum contact with the fish was achieved by using forceps to handle the areas of the mouth and distal part of the fins to maintain the integrity of the skin and gills during examination.

The entire fish was examined with the naked eye for any parasites, with particular attention to the gills, fins and mouth. Any ectoparasites observed were collected and subjected to microscopical examination. Skin scrapings were also obtained from all body surfaces, fixed in 100% methanol and stained with Giemsa. Stained smears made from scrapes were examined under a microscope at 10× and 40× magnifications. The gill arches were cut at the ventral and dorsal end to allow full removal of the gills, and placed into a Petri dish containing distilled water. The gill arches were held with forceps as a needle was used to separate the gill filaments from the gill arches and then examined under a dissecting microscope. The contents of the abdominal cavity were removed and examined for *Ligula* plerocercoids or other helminths.

Findings

In all examined fish (n = 42), no gill or protozoan parasites were evident and no worms or larval plerocercoids were found. However, 1 *Argulus* species (**Figure 1**) was found on 1 roach (1 out of 15 *R rutilus* fish; 6.6%), with an overall prevalence of 2.38% (1 out of 42 fish). The specific location of this louse on the body of the fish could not be identified because it was found crawling along the cling film. The detected *Argulus* species measures 5.4mm long and possesses rounded lobes of abdomen and other morphological features consistent with those of *A foliaceus*.

The second parasite species detected (**Figure 2**) had morphological attributes consistent with those of the ergasilid *Neoergasilus japonicus*. The overall prevalence of *N japonicus* was 26.2% (11 out of 42) and this parasite seemed to favour fish 7cm to 9.9cm long because no ergasilid was found out of this category of body length. Rudd had the highest prevalence (6 of 11; 54.5%), followed by roach (4 out of 15; 26.6%), whereas bream and bream hybrids recorded the lowest prevalence (1 out of 16; 6.2%).

N japonicus parasites were all isolated from the base of the fins (**Figure 3**) and showed site preference, where disproportionately large numbers of *N japonicus* were found attached to the base of the anal fin (43.3%) and dorsal fin (27.7%), with the caudal fins (6.7%) and pelvic fins (4.4%) being the most scarcely populated – a potential clue in identification of this parasite species.

Remarks on differential diagnosis

Argulus, often coined the fish louse, is a crustacean belonging to the subclass Branchiura and is found worldwide in both freshwater and marine environments (Fryer, 1968; Okland, 1985; Rushton-Mellor, 1992). *Argulus* is easily identified by its oval appearance – due to the U-shaped carapace of the head with a pair of compound eyes, two circular suckers and a proboscis used for attachment and feeding, respectively. The thorax has four carapace-fused segments with a pair of swimming biramous appendages attached to each segment (**Figure 1**). A small bi-lobed abdomen containing the testes in males is present caudally (Hoffman, 1977).

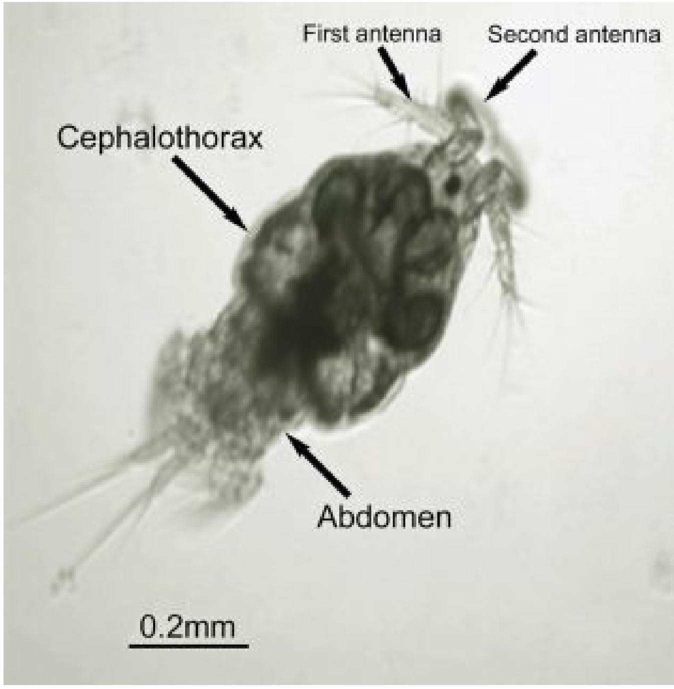

Figure 2. Unstained wet mount preparation of the ergasilid copepod *Neoergasilus japonicus* isolated from freshwater fish in south Wales.

Argulus foliaceus, *Argulus coregoni* and *Argulus japonicus* are the three species found in British freshwaters, with *A foliaceus* being the most prevalent. *Argulus* is a large ectoparasite with adults ranging 6mm to 8mm long in *A foliaceus* and *A japonicus* (Shafir and Van As, 1986; Rushton-Mellor and Boxshall, 1994), to 12mm in *A coregoni* (Gurney, 1948). *A foliaceus* and *A japonicus* are very similar morphologically and physiologically, making discrimination between the two species difficult (Rushton-Mellor, 1992). *A japonicus* is known to be an exotic species and was introduced from Asia through the ornamental trade.

A foliaceus and *A coregoni* are regarded as native to the UK. However, they can be discriminated based on size. Also, A foliaceus is more prevalent in shallow, warm lakes with little water movements, compared to *A coregoni*, which favours colder, faster-moving waters (Campbell, 1971). *A foliaceus* has high host specificity for cyprinids, such as carp, perch and roach (Buckley and Morrice, 1976), whereas *A coregoni* has more host specificity for salmonids (Shimura, 1983). *A foliaceus* tends to be located at the head and the basal region of the caudal fin, while *A coregoni* has preference to attach near the pectoral and pelvic fins to protect itself from becoming dislodged from the host from strong water currents where salmonids commonly reside (Shimura, 1983).

The ergasilid *N japonicus* ranged in size from 0.6mm to 0.76mm, but can reach 1mm (Hudson and Bowen, 2002). This parasite has been reported on rudd in Turkey and showed a higher frequency on the anal fins and dorsal fins (Soylu and Soylu, 2012), in agreement with our results. Larvae, males and immature females of *N japonicus* do not live as parasites, but are free-living. Only ovigerous females require a host, while those that are non-ovigerous can detach and reattach to hosts.

In our study, the detected copepod ectoparasite lacked the egg sacs – a similar observation was reported previously (Soylu and Soylu, 2012), where no egg sacs were observed on *N japonicus* in November in Turkey.

Discussion

This investigation was undertaken to delineate the causes of behavioural abnormalities in a freshwater fish pond in south Wales. A high prevalence (26.2%) of the ectoparasitic copepod *N japonicus* was detected in rudd, roach and bream hybrids, with 54.5% prevalence in rudd, indicating host preference. *N japonicus* was not detected on true bream, and was only observed on one bream hybrid – presumably due to the excessive mucus produced, which might act as a mechanical barrier to successful parasite attachment. This high prevalence indicates *N japonicus* could be more prevalent in the UK than thought, but due to its small size and loose attachment to the fish's fins, this parasite can easily be overlooked by routine parasitological examination of infested fish (Tuuha et al, 1992; Hudson and Bowen, 2002).

N japonicus was reported in Europe and the UK 46 and 32 years ago, respectively (Mugridge et al, 1982). This parasite is not native to Britain and is mainly found in eastern Asia, and from where it invaded Europe and North America. The occurrence of *N japonicus* in British waters is probably due to imported pet fish that are then released into the waterways when they either outgrow the tank or are unwanted – and/or due to the increasing rates of fish introductions associated with aquacultural activities (Naylor et al, 2001; Reid and Hudson, 2008; Gozlan et al, 2010).

This species has high infective efficiency (Kim and Choi, 2003), ability to shift hosts (El-Rashidy and Boxshall, 2009) and can parasitise both native and foreign fish species (Suárez-Morales et al, 2010; Nagasawa and Uyeno, 2012) – attributes that can accelerate the spread of this exotic parasite in British waters.

Parasitic crustaceans can cause severe damage to the fish host and substantial economic losses for fish farming and fisheries. *Argulus* infestation can adversely affect the physiology and growth of affected fish – ultimately compromising fish performance and fitness. This fish louse, via attachment and feeding behaviour, can cause damage to the epidermal barrier, which leads to ionoregulatory disturbance and secondary microbial infections.

The extent of the damage caused is dependent on the parasite load on the fish. A low overall prevalence of *Argulus* (2.38%) does not guarantee a low infestation in the waters, as one of the signs fish louse can cause is decrease in appetite, hence using rod and reel might not be an accurate collection method to determine the prevalence of *Argulus* in a fish population (Taylor et al, 2006).

Ergasilid copepods can serve as intermediate hosts for other fish parasites, act as vectors of diseases or be fish parasites themselves (Piasecki et al, 2004). Heavy infestation can lead to bleeding, skin ulcers and hypovolaemia (Menezes et al, 1990), leading to loss of physical condition, loss of appetite, higher susceptibility to secondary bacterial and fungal infections, and aesthetic problems (Jafri and Ahmed, 1994; Rahman, 1996; Taylor et al, 2006). Both trout and coarse fisheries can incidentally exacerbate the problem: the former removes healthy individuals from the lake and so artificially selects fish susceptible to *Argulus* to pass on to progenies, and the latter because of the legalities of removing fish any infected fish caught are placed back into the waters to reinfect other hosts (Taylor et al, 2006).

High temperature and, in turn, seasonality, affect *Argulus* development and longevity by increasing *Argulus* infestations during the summer months, coinciding with the peak angling season, further amplifying the infestation (Taylor et al, 2006). Other factors related to an increase in *Argulus* infestation are slow stock turnover rates, low water visibility and dense population of fish (Taylor et al, 2006). Careful stock management, maximising stock turnover, trickle stocking and either stopping or minimising catch and release, are among the main measures used by trout farmers to tackle *Argulus*.

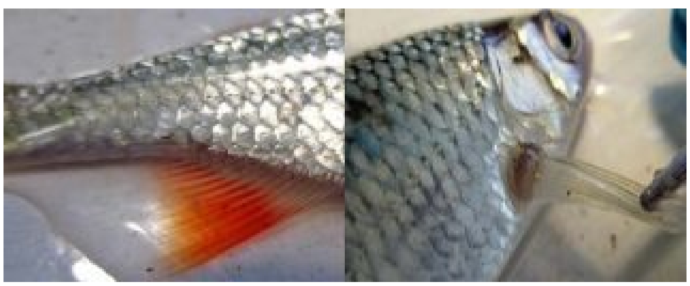

Figure 3. The anatomical location, and in situ appearance, of the ergasilid *Neoergasilus japonicus*. Left: adult *N japonicus* spreading evenly along the base of the fish anal fin. Right: pectoral fin with approximately three *N japonicus* at the base of the fin.

In fish crustacean infestation the two things that matter most to fishery owners are the availability of appropriate treatments and ability to prevent or control infestation. Established treatments are not able to address the problems caused by these parasites and the pipeline of new drugs for treating them is dry. It is clear we need to explore new opportunities to discover new therapeutics. This is a high-risk, high-return scenario and increasing applications of the emerging interdisciplinary technologies in understanding host-parasite interaction is a promising pathway for the development of new therapeutic interventions.

With regard to prevention, it is important to be aware of, and able to, tackle risk factors for the occurrence and spread of these parasites. Both crustacean parasites have broad host range and complex (multi-stages) life cycles; thus, particular attention should be paid to understanding the drivers of cross-fish species parasite sharing, and the identification of the high-risk situations in which new host-shift events are likely to occur. It is also important to recognise factors that can limit our ability to understand the mechanism of diseases caused by these parasites. Many crustacean species and even strains with different virulence capacities exist, thus affected fish can be asymptomatic or exhibit overt clinical signs of the disease. Also, the pattern of infestation can be influenced by varying environmental factors that can impact the parasite’s transmission dynamics.

Comprehensive understanding of the epidemiology of these parasites and how they interact with, and affect, fish health is necessary for the development of vaccine and other control measures, and can be incorporated into a surveillance framework to monitor the range of fish species these parasites can affect.

Taken together, our findings highlight the importance of parasitic crustaceans as pests with potentially adverse effects on the health of native fish species. Further and larger surveys are advisable due to the need for defining the geographical distribution, host range, transmission dynamics, economic impact and pathologies caused by these parasitic crustaceans – crucial information in guiding future fish parasite surveillance and control programmes.

Key points

- Fishery owners need to be aware of the type and frequency of the parasite populations residing in their own waters.
- A high prevalence of non-native parasitic copepod *N japonicus* and one *A foliaceus* was found in a population of 42 fish in south Wales.
- Not only has ergasilid shown host-specificity for rudd, but also site preference to the anal fins and dorsal fins of fish.
- The findings reported herein may serve to alert fish vet practitioners to the occurrence of ectoparasitic crustaceans in freshwater fish in the UK.
- The pathology and distribution of *N japonicus* in the UK is unknown and should be investigated in future studies.

Competing interests

The authors declare no competing interests.

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Because of space limitation, the authors note they were unable to comprehensively cite many worthy contributions to the field.

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