

Parasite fauna of introduced pumpkinseed fish *Lepomis gibbosus*: first British record of *Onchocleidus dispar* (Monogenea)

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ABSTRACT: A survey of pumpkinseed *Lepomis gibbosus* from a stillwater fishery in southern England revealed infections by the ancyrocephalid monogenean *Onchocleidus dispar* at 100% prevalence. Host specificity of *O. dispar* to North American centrarchid fish suggests that it is a non-native parasite, introduced to Britain with *L. gibbosus*. Mean intensity of *O. dispar* was significantly higher in male (25.5 parasites) compared to female (15) and immature (7) hosts, but was not influenced by host standard length or sampling time. This sex-biased parasitism is likely to be related to both ecological and physiological factors, such as differential exposure to infective stages during nest building, or higher susceptibility to infection due to enhanced host stress levels. *O. dispar* significantly dominated the external surfaces and the outer gill arches. Analysis of the spatial distribution of the parasite between the 9 regions of gill surface determined that as density increased, higher numbers of parasites were found on the anterior and central gill regions compared to posterior (distal) regions. Apparent microhabitat selection is discussed in terms of *O. dispar* lifecycle, maximising oxygen availability, avoiding unstable areas and increasing intraspecific contact. The absence of *O. dispar* in the gills of roach, rudd and gudgeon sampled from the same fishery supports the assumption that this parasite is currently of little threat to native fish populations.

KEY WORDS: *Lepomis gibbosus* · Monogenean · Microhabitat · Exotic species · Sex-biased parasitism

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INTRODUCTION

The pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) is a North American centrarchid fish that has spread throughout Europe with the international fish trade (Copp & Fox 2007, Ozcan 2007). Originally introduced to Britain as an ornamental species, *L. gibbosus* populations have been recorded in the wild since the early twentieth century (Lever 1996, Klaar et al. 2004). However, it is only in the last decade that attention has focussed on their impact and invasiveness in terms of morphology, reproduction, diet, colonisation potential and habitat use in natural environments (Copp et al. 2002, Klaar et al. 2004, Britton et al. 2005,

Villeneuve et al. 2005). In view of the disease risks that can result from the introduction of non-native fish (Copp et al. 2005, Gozlan et al. 2005, 2006) and the influence of parasites on the invasiveness of non-native species (Prenter et al. 2004, Hudson et al. 2006), it is surprising that there are no published accounts of the parasite fauna of *L. gibbosus* in Britain.

Preliminary examinations of *Lepomis gibbosus* conducted by the Environment Agency, as part of routine investigations into non-native fish introductions to freshwater fisheries in England and Wales, revealed infections by the ancyrocephalid monogenean *Onchocleidus dispar*. This parasite naturally occurs in fishes of the family Centrarchidae, natives

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of North America (Hoffman 1999, Collins & Janovy 2003). Due to a general paucity of data on parasites from non-native fish in Britain and limited understanding of this monogenean in *L. gibbosus*, the current study was undertaken to confirm identification and establish the preference of *O. dispar* in terms of host sex, size and gill microhabitat. This study represents the first description of the parasite fauna of *L. gibbosus* in Britain.

MATERIALS AND METHODS

Fish sampling

An initial sample of 39 *Lepomis gibbosus* was obtained for parasitological examination from a fully enclosed stillwater fishery (coarse fishing lake) in southwest England in February 2008. The lake is 1.8 ha, with an average depth of 1.5 m, and has no inflow or outflow. All fish were captured by means of seine netting and transported live to holding facilities at the Environment Agency, Brampton. Following the detection of *Onchocleidus dispar* within these fish, an additional sample of native fish species was obtained in the same month. This sample consisted of 18 roach *Rutilus rutilus*, 10 gudgeon *Gobio gobio* and 11 rudd *Scardinius erythrophthalmus*, which were screened only for the presence of *O. dispar*. A further 85 *L. gibbosus* were netted in October 2008 and February 2009. Fish caught in October were killed on site by anaesthetic overdose using benzocaine solution, and immediately transferred to 90% ethanol fixative. *L. gibbosus* netted in February were transported live to Cardiff University and held in aquaria at 13.5°C under ILFA (Import of Live Fish Act) licence. Directly before dissection, all fish were killed by anaesthetic overdose of tricaine methanesulphonate (MS222) and pithed. The standard length (SL) of all fish was measured. Sex was determined from observation of the gonads, with individuals not sexually mature termed as juvenile.

Parasite screening

The skin and fins of all fish species were screened for ectoparasites, and then the gills were individually removed and placed in sterilised water for further examination. A sub-sample of *Lepomis gibbosus* (n = 49) was also dissected, and the eyes, stomach, intestine, gall bladder, swim bladder, gonads, heart, liver and kidneys visually examined for parasites. Parasite

infection levels are expressed as prevalence (percentage of hosts infected), intensity (number of individual parasites of the species per infected host) and mean intensity (total number of parasite individuals divided by the number of infected hosts) as defined by Margolis et al. (1982). For those fish which had been fixed prior to examination, the fixative was also examined under a dissecting microscope to check for dislodged parasites as standard procedure. To ensure that fixation of the October specimens did not bias the parasite counts obtained (thus confounding any differences between the 2 sample points studied), a subset of the fish collected in February was also fixed and stored for 6 mo prior to their parasite numbers being determined. No significant difference in the number of parasites observed in the fixed and unfixed February specimens was detected ($F = 1.99$, $df = 38$, residual deviance = 368.79, $p = 0.17$) using a generalised linear model (GLM) following the method described below.

Monogenean parasites were removed from the gills under a stereo-microscope. To assess microhabitat specificity, the total number of monogeneans on each attachment site of the gills was recorded. Attachment sites were classified as the right or left side of the fish; the gill arch number 1 to 4 (Fig. 1A) and the arch face (external or internal). Each gill face was further divided along the dorsal-ventral and anterior-posterior axis, giving 9 possible attachment sites on each side of the gill arch (Fig. 1B). Ten specimens were randomly selected and placed on a slide with a drop of sterilised water. A second drop of sodium dodecyl sulphate was added to clear the soft tissues (as described by Wong et al. 2006). A coverslip was added and sealed with nail varnish, and then viewed at 400× magnification using a Leica DMR microscope with differential interference contrast imaging and a Spot digital camera (Diagnostic Instruments). The dorsal and ventral hook length, transverse bar length and width, marginal hook lengths, penis length and accessory piece length were measured using WCIF ImageJ software (www.uhnres.utoronto.ca/facilities/wcif/download.php). Voucher specimens of *Onchocleidus dispar* were deposited in the Helminthological Collection at the Natural History Museum, Oslo (accession number C5266).

Statistical analyses

All analyses were conducted in R version 2.11.1 (R Development Core Team 2005). Differences in parasite intensity with respect to fish length, sex and sam-

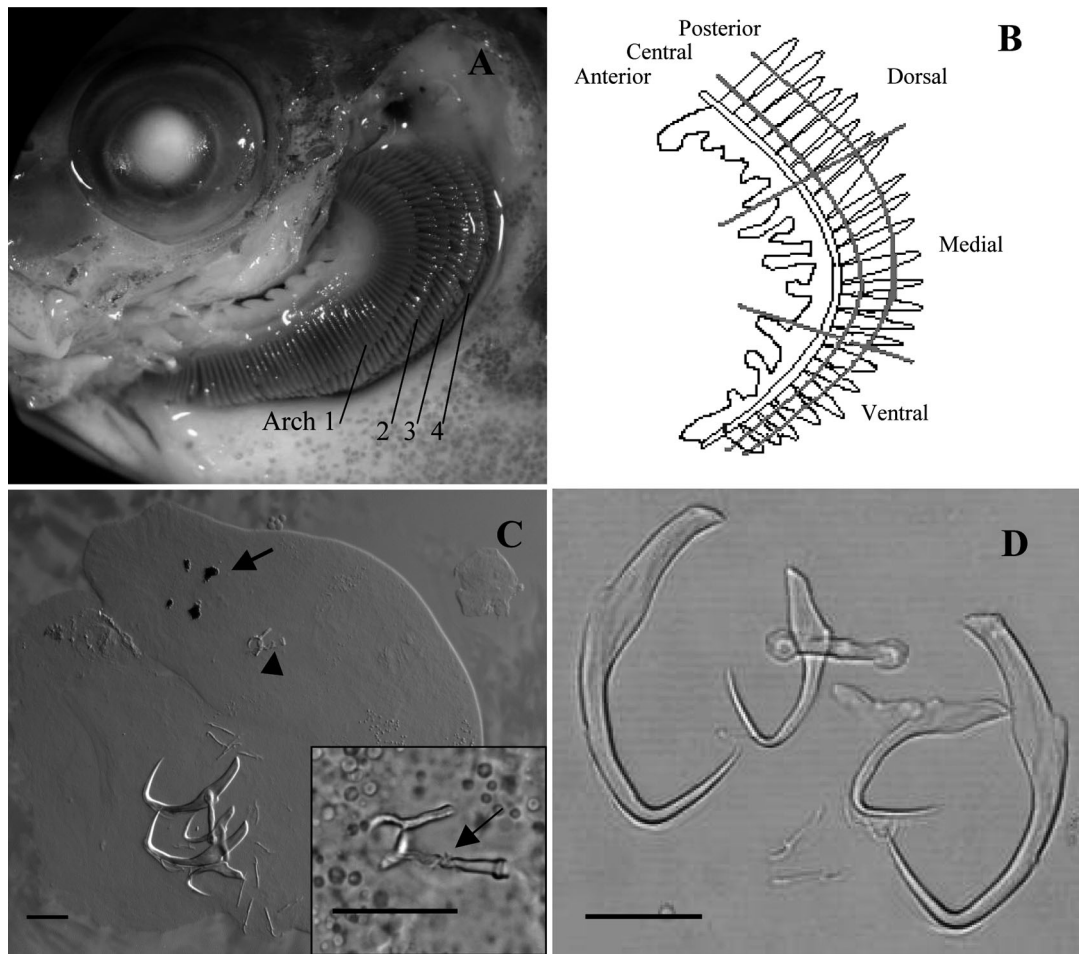


Fig. 1. *Onchocleidus dispar* infecting *Lepomis gibbosus*. (A) Gill arch numbers assigned to the pumpkinseed hosts. (B) Nine regions of each side of the gill arches (not to scale). (C) Whole mount of *O. dispar* showing the eyespots (arrow), opisthaptoral hooks and copulatory complex (arrowhead). Inset: Cirrus of *O. dispar* bright field illumination. The tubular cirrus with an inflated base with 2 spiral filaments (arrow) and an accessory piece which forms a ring through which the cirrus protrudes. (D) Opisthaptoral hooks of *O. dispar*. Scale bars = 25 µm

pling time were analysed by a GLM using a quasi-Poisson (negative binomial) error distribution and log link function to account for over-dispersion observed in the parasite count data. The model was refined by stepwise removal of non-significant terms to produce a final minimal model. Using the same method, parasite intensity on the left and right side of the host, interior and exterior surfaces of each gill arch, and the gill arch number were compared.

Principal component analysis was used to summarise the spatial distribution of the parasites over the 9 gill regions (see Fig. 1B). Relationships between the dominant principal component score, parasite intensity, host sex and host SL were then investigated graphically and by linear models to establish

whether these variables explained variability in the principle component scores obtained, and thus influenced parasite microhabitat selection.

RESULTS

Parasites of *Lepomis gibbosus*

Monogenea recovered from the gills of *L. gibbosus* were identified as *Onchocleidus dispar* (Fig. 1C) based on morphological examination of the copulatory organ (Fig. 1C inset) and sclerotised haptor (Fig. 1D), which conformed to the descriptions by Beverley-Burton & Suriano (1980). *O. dispar* was the

dominant parasite recovered from *L. gibbosus*, and was recorded at a prevalence of 100%, with between 2 and 66 parasites host⁻¹ (mean \pm SD intensity = 20 \pm 16). *O. dispar* was not recorded from the other fish species examined in this study (*Rutilus rutilus*, *Gobio gobio* and *Scardinius erythrophthalmus*).

In addition to *Onchocleidus dispar*, 6 other parasite taxa were detected from *Lepomis gibbosus*. Ciliates belonging to the genera *Apiosoma* and *Trichodina* were observed on the skin and fins, but prevalence was not recorded as these protozoans are opportunistic, have a short generation time and are likely to have transferred between hosts after capture. Glochidia (freshwater mussel larvae) were present in 53% of *L. gibbosus*, with a mean intensity of 1.8 larvae per host (range 1 to 10). They were found mainly on the fins, skin and gills, but only in the February sample. A third-stage larva of the genus *Contracaecum*, possibly *C. rudolphii* (E. Harris pers. comm.), was removed from the stomach of a single male *L. gibbosus*. An unidentified nematode adult was recovered in the dissecting dish from a different *L.*

gibbosus (sex not recorded). Finally, an acanthocephalan was found in the intestinal tract of a single *L. gibbosus* from the February sample, but was too degraded for identification. As many ingested prey were recovered intact within the gut and there was no other evidence of tissue decomposition, the isolated acanthocephalan is assumed to have been a rare occurrence in this population of *L. gibbosus*.

Host and microhabitat preferences of *Onchocleidus dispar*

Host sex had a significant influence on *O. dispar* intensity ($F = 15.26$, $df = 2$, residual deviance = 677.97, $p < 0.01$). There were significant differences in *O. dispar* intensity between female (15 worms) and male hosts (25.5 worms; GLM, $\beta = -0.68$, $t = -2.29$, $p = 0.02$), and between female and immature fish (7 worms; GLM, $\beta = 0.59$, $t = 0.16$, $p < 0.01$; Fig. 2A). However, there were no significant relationships (nor any interaction effects) between parasite intensity

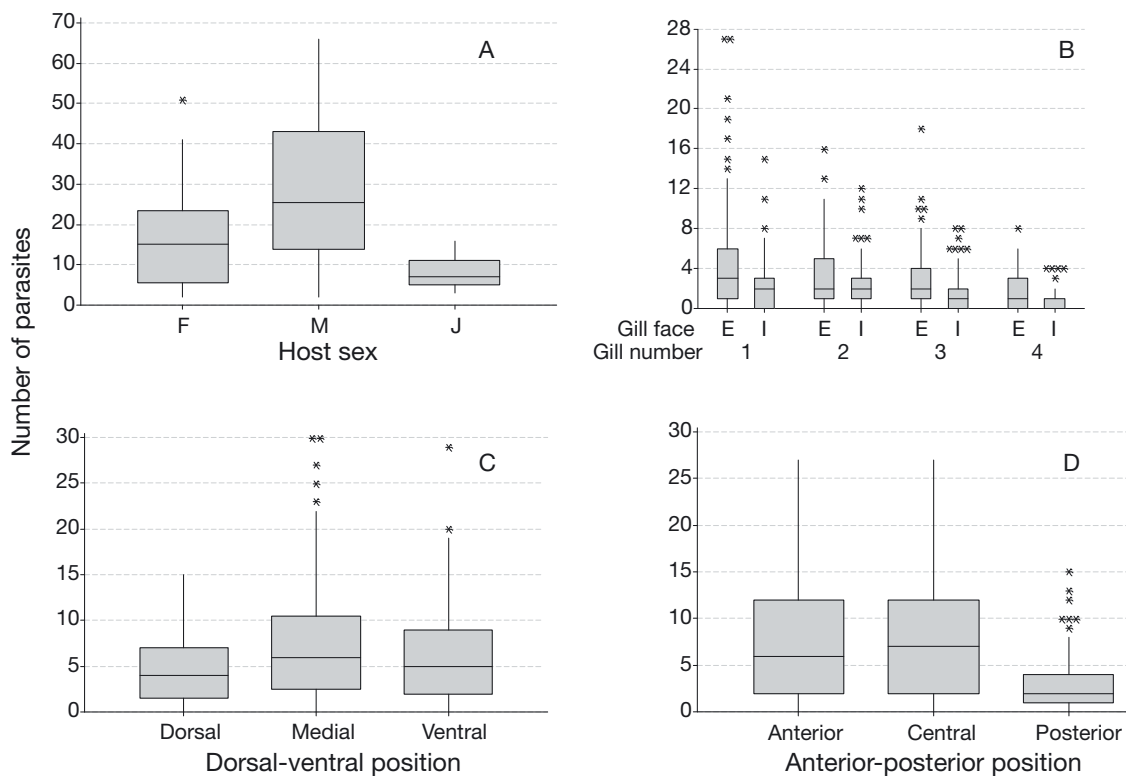
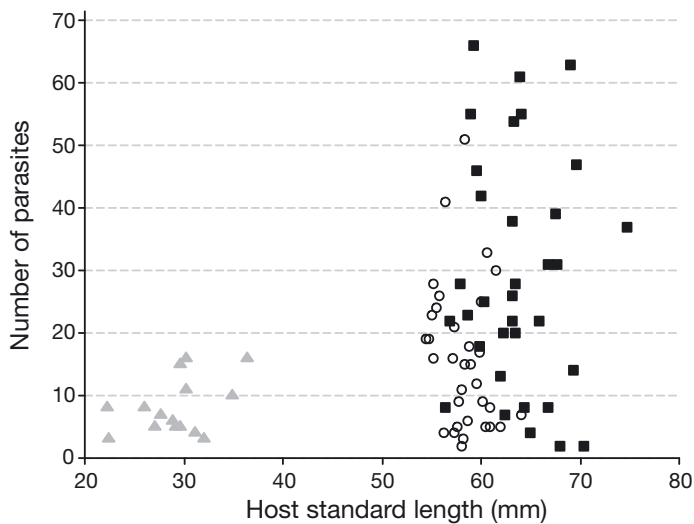


Fig. 2. *Onchocleidus dispar* infecting *Lepomis gibbosus*. Microhabitat preference of *O. dispar* on pumpkinseed. (A) Number of *O. dispar* identified on male (M), female (F) and juvenile (J) *L. gibbosus* hosts. (B) Number of *O. dispar* on the external (E) and internal (I) faces of gill arches 1 to 4. (C) Number of *O. dispar* in the 3 regions along the dorsal-ventral axis of the gill arches. (D) Number of *O. dispar* on the 3 regions along the anterior-posterior axis of the gill arches. Shaded box indicates the interquartile range, centre solid line indicates median number of parasites, whiskers indicate the data range (excluding outliers), and asterisks indicate outliers

Table 1. *Onchocleidus dispar* infecting *Lepomis gibbosus*. Generalised linear modelling results comparing the number of *O. dispar* inhabiting each of the gill arches of pumpkinseed

Factor level	Estimate	Null deviance (df)	Residual deviance (df)	SE	<i>t</i>	<i>p</i>
Intercept	1.54	2266.4 (679)	1880.0 (675)	0.08	19.83	<0.01
Exterior vs. interior	-0.63			0.09	-7.12	<0.01
Gill 1 vs. 2	-0.20			0.11	-1.96	0.05
Gill 1 vs. 3	-0.45			0.11	-3.93	<0.01
Gill 1 vs. 4	-1.08			0.14	-7.65	<0.01

Fig. 3. *Onchocleidus dispar* infecting *Lepomis gibbosus*. Total number of *O. dispar* versus host standard length of male (■), female (○) and juvenile (▲) pumpkinseed (*n* = 85)

and host SL, despite the male hosts being significantly larger than the females (Fig. 3). The time of sampling did not significantly affect parasite intensity.

The mean parasite intensity was similar in the left and right gill chambers; however, significantly higher numbers of parasites were observed on the exterior as opposed to interior surfaces of the gills (Table 1, Fig. 2B). Additionally, the number of parasites declined with respect to the gill arch number, with the outer arches holding the most parasites (Fig. 2B). In total, 35% of all *Onchocleidus dispar* individuals were attached to gill arch 1, significantly more than observed on gill arches 2, 3 and 4, which held 31%, 23% and 11% of the parasites, respectively (Table 1).

Principal components 1 and 2 summarised 61% of the variability in parasite microhabitat selection over the 9 regions of the gill arch (Table 2). Axis 1 explained 49% of the variability in distribution of parasites between hosts (Table 2). Linear regression models confirmed a significant positive association between the axis 1 score and total parasite intensity

Table 2. *Onchocleidus dispar* infecting *Lepomis gibbosus*. Principal component analysis scores summarising microhabitat distribution of *O. dispar* on different regions of pumpkinseed gills

Variable loading	Axis (variability summarised)	
	1 (49%)	2 (12%)
Dorsal anterior	0.37	-0.19
Dorsal central	0.37	0.03
Dorsal posterior	0.24	0.62
Media anterior	0.36	-0.19
Medial central	0.39	-0.06
Medial posterior	0.28	0.56
Ventral anterior	0.35	-0.11
Ventral central	0.39	-0.12
Ventral posterior	0.16	-0.45

($F = 1376.78$, $df = 1$, $p < 0.01$), suggesting that as total parasite intensity increased, the number of parasites in each region also increased. However, the variable loadings for each axis (Table 2) suggested that the rate of increase is faster in some regions than others, with the slowest increase occurring in all 3 posterior regions. Differences in host sex also explained a significant amount of variability observed in axis 1 scores ($F = 240.27$, $df = 2$, $p < 0.01$), with males and females having significantly higher scores (and therefore more parasites in each region) than juveniles. A significant interaction effect between sex and host length was also present ($F = 14.62$, $df = 2$, $p < 0.01$), suggesting the rate at which axis 1 scores increased with parasite abundance was lower in males and females than in juveniles. After accounting for the variability in axis 1 scores explained by parasite abundance and host sex, no significant relationship with host length was detected. Axis 2 explained a further 12% of the variability in microhabitat selection by the parasite, and suggested that for the 12% of fish that fell into this category, some negative associations occurred between the gill regions in which parasites were found, i.e. if parasites were found in a particular region they would avoid another (Table 2). Variable loadings for this

axis suggest that high parasite numbers on the dorsal- or medial-posterior regions were associated with low parasite numbers on the ventral-posterior and dorsal- and medial-anterior regions, and vice versa. Relationships between the other regions were considerably weaker. No significant relationships were found between axis 2 scores, host sex, length or parasite intensity.

DISCUSSION

Following the introduction and spread of *Lepomis gibbosus* throughout Europe, *Onchocleidus dispar* has been recorded from Romania, Italy, the Czech Republic, Slovakia, Norway and France (Roman-Chiriac 1960 cited by Hoffman 1999, Lambert 1977, Hoffman 1999, Galli et al. 2003, Sterud & Jorgensen 2006). The current discovery of *O. dispar* in southern England represents the first record of this parasite in Britain (cf. Kirk 2000). This is the latest of a number of recent additions to the parasite fauna of freshwater fish in Britain, which includes other monogenean species, such as *Gyrodactylus sommervilleae* (Turgut et al. 1999) and *Pellucidhaptor pricei* (Harris 2003) in bream. More recently, infections of the ancyrocephalid monogenean *Thaparocleidus vistulensis* were detected in the European catfish *Siluris glani* in southern England, which was introduced with the movement of catfish stocks (Environment Agency unpublished data). Such examples highlight how the parasite fauna in British freshwaters continues to change as a result of fish translocations, but also questions how well monogenean species of freshwater fish are documented.

The detection of *Onchocleidus dispar* on *Lepomis gibbosus* shares similarities with the introduction of *Urocleidus principalis* with largemouth bass *Micropterus salmoides* to southern England (Maitland & Price 1969). Both parasites are specialists of North American fish which were introduced to England during the 19th century, for either sporting or ornamental purposes (Davies et al. 2004). However, unlike *L. gibbosus*, largemouth bass failed to establish in Britain due to the higher temperature requirements for reproduction. Because of the strict host specificity of *U. principalis*, the parasite is no longer considered established in Britain as a result of the extirpation of its host (Davies et al. 2004). In contrast, *L. gibbosus* has successfully colonised a number of freshwater habitats in England. At present, this species is believed to be limited to lakes and reservoirs in southern England, and has failed to estab-

lish breeding populations in riverine environments (Copp et al. 2004, Klaar et al. 2004, Villeneuve et al. 2005, Copp & Fox 2007). However, in other parts of Europe where *L. gibbosus* have been introduced, their invasive potential has been realised (Fox et al. 2007, van Kleef et al. 2008), and this may increase further in England as a consequence of climate change (Britton et al. 2005, Dembski et al. 2006, Copp et al. 2009). Records of *O. dispar* have so far only been on centrarchid hosts, namely *Lepomis* spp., *Micropterus* spp. and *Pomoxis nigromaculatus* (e.g. Beverley-Burton & Suriano 1980, Hoffman 1999, Collins & Janovy 2003, Sterud & Jorgensen 2006). It therefore appears likely that *O. dispar* will remain established in Britain, but its range will be limited by the distribution of its host. The apparent host specificity of *O. dispar* suggests that the parasite poses no threat to native fish populations; however, experimental studies (e.g. King & Cable 2007) are essential before host-switching to non-centrarchid fish can be ruled out.

In their native geographical range in North America, 129 parasite taxa, including 27 monogenean species, have been recorded from *Lepomis gibbosus* in several regions (Hoffman 1999). As only one location in England was examined in the current study, direct comparisons cannot be made with Hoffman's (1999) summary. However, the enemy release hypothesis (Tourchin et al. 2002, Vignon et al. 2009) may explain the successful establishment of *Onchocleidus dispar* in southern England. The hypothesis predicts that newly established species are released from natural pressures, such as predation and parasitism, and are therefore likely to carry only a small subset of their native parasites. Introduced parasites with direct life cycles such as monogeneans are more likely to co-establish with their hosts as they are not reliant on the presence of other host species in the new environment. The establishment and successful colonisation of introduced parasites also depends on factors such as host specificity, with generalists more likely to establish, reproduce and proliferate in new environments (Kennedy 1994, Tompkins & Poulin 2006). The direct life cycle and apparent capability of *O. dispar* to adapt to changes in the environment between the native and introduced habitats has allowed this species to successfully establish with its fish hosts.

Intensity of *Onchocleidus dispar* was significantly higher in sexually mature males compared to female *Lepomis gibbosus*. Sex-biased parasitism is commonly observed, particularly in small mammals, but has also been recorded in several fish species (Zuk & McKean 1996 and references therein). For example,

Reimchen & Nosil (2001) considered the ecological basis for sex-biased parasitism in three-spined sticklebacks *Gasterosteus aculeatus*. Male sticklebacks had a higher frequency of benthic items in their diet, and had a higher prevalence of parasites which dominate benthic habitats, whereas female sticklebacks had a higher frequency of pelagic items in their diet, and subsequently had a higher prevalence of the pelagic cestode *Schistocephalus solidus* (see Reimchen & Nosil 2001). The sex-biased parasitism in *L. gibbosus* may also have an ecological basis. The oviparous lifecycle of *O. dispar* is unknown; however, closely related species release eggs which are expelled from the host by respiratory currents and sink to the substrate (Olsen 1974, Cone & Burt 1981, 1985, King & Cone 2008). Eggs of most monopisthocotylean parasites hatch into ciliated oncomiracidia which infect new hosts close to the site of hatching (Smyth & Halton 1983). The oncomiracidia attach to the skin of new hosts before losing their ciliated cells and migrating to the gills by entry through the opercular and buccal cavities (Cone & Burt 1981, Euzet & Combes 1998, Vadstein et al. 2004). Therefore, male *L. gibbosus* will have an increased exposure to the free-living stages of *O. dispar* when constructing nests on the lake bed.

A physiological basis may also explain the observed sex-biased parasitism. During the breeding season, male *Lepomis gibbosus* construct nests where they encourage several females to lay their eggs following an elaborate courtship display. The males are highly territorial and will fend off other males, keeping guard of the nest until the young hatch and disperse (Maitland & Campbell 1992). As a consequence of this intensive parental care, males will be subject to higher stress levels, producing elevated levels of glucocorticoid hormones, such as cortisol. Such hormones are immunosuppressive (Fast et al. 2008), increasing host susceptibility to disease (Zuk & McKean 1996). However, further studies (e.g. Wedekind & Jakobsen 1998, Ferrari et al. 2007, Grear et al. 2009) are needed to determine whether the differing levels of infection in *L. gibbosus* reflect differences in behaviour and/or physiology.

Onchocleidus dispar infection was significantly higher in adult compared to immature *Lepomis gibbosus*, possibly reflecting longer exposure of the adults to infective stages. However, there was no correlation between host size and infection intensity, and we did not investigate the effect of host age on *O. dispar* intensity. Young *L. gibbosus* tend to dwell in shallow waters, swimming close to the surface, whereas adult *L. gibbosus* are more commonly found

in deeper parts of the lakes (Becker 1983). Thus, differences in host habitat preference may have influenced parasitism.

Microhabitat specificity appears to be a common trait among monogeneans. In this study, *Onchocleidus dispar* demonstrated some degree of habitat selection for the first (outer) gill arch of its fish host, with decreasing preference towards the second, third and fourth. Similarly, other authors have recorded a higher number of monogeneans on the first (Hanek & Fernando 1978, Ramasamy et al. 1985) or second gill arches (Gutiérrez & Martorelli 1999, Chapman et al. 2000, Raymond et al. 2006). An apparent preference for this location may merely reflect the first point of contact for the larvae when they crawl into the gill chamber. Alternatively, there may be a habitat trade-off between high oxygen availability and avoiding becoming dislodged in the centre of the gill cavity (gill arches 2 and 3) where there is a higher respiratory current (Hanek & Fernando 1978, Gutiérrez & Martorelli 1999). Raymond et al. (2006) concluded that microhabitat specificity in *Afrodiplozoon polycotyleus* for gill arch 2 on 4 species of *Barbus* was highest when oxygen availability was low, and site specificity was more relaxed when oxygen was more available. The increased number of *O. dispar* may also be reflective of the larger size of the outer gill arches, therefore providing a greater gill surface for attachment (Gutiérrez & Martorelli 1999).

In the majority of fish sampled, as *Onchocleidus dispar* intensity increased on a host, the number of parasites on all 9 regions of the gills increased. However, the rate of increase was highest in the anterior and central regions, which are closest to the gill rakers. Selection for the anterior gill regions may be a consequence of parasites being dislodged from the posterior regions, which are more unstable due to greater movement of the filaments in this region. This will be particularly apparent when intensity increases and competition for stable sites on the gills is increased. Clustering of parasites in specific areas in the gills will also increase intraspecific contact. This will increase the likelihood of locating and selecting mates of higher fitness to facilitate cross fertilisation. The lack of competing parasite species rules out interspecific interaction influencing the microhabitat of *O. dispar*. Furthermore, on other hosts there is little evidence that site segregation is caused by interspecific competition (Lo & Morand 2000, Simková et al. 2000, Rohde 1991, Bagge et al. 2005, Karvonen et al. 2007), and is more likely to be related to intraspecific interaction and extrinsic factors as discussed.

Awareness of non-native parasite introductions has important implications for the protection and management of freshwater fisheries. From this study, we concluded that *Onchocleidus dispar* appears to have limited impact on native species. As commented by Kennedy (1994), only a small proportion of non-native parasites is likely to cause serious disease. However, there are examples, including the recent discovery of the rosette agent *Sphaerothecum destruens* in topmouth gudgeon *Pseudorasbora parva* (see Gozlan et al. 2005), which highlight the dangers that can result from the introduction of non-native parasites with infected hosts (Bauer 1991, Johnsen & Jensen 1991). Risk assessments developed for the management of non-native fish in the UK include recognition of exotic parasites and disease threats (Copp et al. 2005), and these need to be carefully considered before translocations of fish take place to minimise the spread of disease.

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