Distribution of Eurasian otter biliary parasites, *Pseudamphistomum truncatum* and *Metorchis albidus* (Family Opisthorchiidae), in England and Wales

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SUMMARY

Gall bladders from 273 otter carcasses, collected throughout England and Wales, were screened to assess the status of gall bladder parasites in the Eurasian otter, *Lutra lutra*. The digenean *Pseudamphistomum truncatum* had previously been found in UK otters collected between 2000 and 2007. The parasite was established in Somerset and Dorset but its distribution elsewhere in the UK was largely unknown. In the current study, *P. truncatum* was also found to be abundant in south Wales, with occasional cases elsewhere, but appears to be absent from the north of England. Overall, 11·7% of otters were infected with 1–238 *P. truncatum*. A second digenean, *Metorchis albidus*, previously unreported in British otters, was found in the biliary system of 6·6% of otters. *M. albidus* appears well established in Suffolk, Norfolk and north Essex but was recorded elsewhere rarely. Both parasites are associated with pathological damage to the otter gall bladder. The recent discovery of these two non-native parasites provides a unique opportunity to assess their impact on native British fauna.

Key words: Opisthorchiid, *Pseudamphistomum truncatum*, *Metorchis albidus*, *Lutra lutra*, UK.

INTRODUCTION

Pathogens may cause dramatic declines even in healthy populations, and small or endangered populations are especially vulnerable (Gozlan *et al*. 2005). Particularly problematic for conservationists is the occurrence of novel host-parasite interactions that arise if a parasite extends its host or geographical range. For example, *Anguillicola crassus*, a parasite from the Far East, caused few serious health problems to its native host but since expanding its geographical range into Europe, has been found to be highly pathogenic to European eels (Kirk, 2003). Despite strict legislation controlling the transfer of fish stocks for food or ornamental purposes this may also lead to the introduction of novel parasites to native hosts. For example, *Gyrodactylus salaris* is relatively benign on Baltic salmon stocks but has decimated North Atlantic stocks of this fish following its accidental introduction into Norway (reviewed by Bakke *et al*. 2007). Sunbleak (*Leucaspius delineatus*) have been established in the UK since their introduction in the mid-1980s (Gozlan *et al*. 2003), and are reportedly intermediate hosts for certain opisthorchiid digeneans. Recently 2 exotic crustacean parasites, *Neoergasilus japonicus* from Asia and *Ergasilus briani* from mainland Europe, have been detected on this fish (Beyer *et al*. 2005). The ability of sunbleak to spread rapidly probably contributes to the dispersal of novel parasites throughout UK watercourses. Simpson *et al*. (2005) suggested that the digenean, *Pseudamphistomum truncatum*, was introduced into the UK with ornamental non-native fish, namely the sunbleak and topmouth gudgeon (*Pseudorasbora parva*), and could easily spread to other teleosts.

Pseudamphistomum truncatum is a gall bladder digenean native to Eastern Europe and common in a range of wild carnivores (Simpson *et al*. 2005). It was first reported in Britain following examination of otter carcasses collected in Somerset in 2004 (Simpson *et al*. 2005). Otters have been examined post-mortem since 1988, and retrospective assessment of previous records in the southwest of England showed abnormal gall bladders in a further 7 cases, the first of which was collected in 2000 (Simpson *et al*. 2005). In contrast, *Metorchis albidus* is native to Europe (Bowman *et al*. 2002) but is rarely reported in otters (but see Sidorovich and Anisimova, 1999). This parasite has not previously been recovered from otters in the UK. Life-cycles specific to *P. truncatum* and *M. albidus* are not described in the literature but an account of similar species is given in Chandler and Read (1961). Opisthorchiids typically have 2 intermediate hosts; the first is commonly a species-specific gastropod (Prosobranchia) mollusc and the second a freshwater fish. The parasites reach the

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definitive host through consumption of infected fish and migrate to the gall bladder where they mature (Sukhdeo and Sukhdeo, 2004). Both *P. truncatum* and *M. albidus* are generalists, potential parasites of a wide range of carnivores, including the red fox (Saeed *et al.* 2006), mink, otters (Simpson *et al.* 2005) and domestic cats (Nielsen and Guidal, 1974), with the potential to infect any piscivore including man (Simpson *et al.* 2005).

The otter (*Lutra lutra*) is a European protected species, listed as near threatened on the IUCN Red List of Threatened Species. Populations declined severely during the 1960s and 70s (Chanin and Jefferies, 1978), but are generally believed to have made a strong recovery over the past 2 decades. Long-term studies reveal otter populations to be healthy with little indication of disease (Simpson, 2007), but recent detection of *P. truncatum* has raised concern (Simpson *et al.* 2005). The Cardiff University Otter Project (CUOP) receives otter carcasses from across England and Wales for post-mortem (1994 to present). As part of a long-term study on otter ecology, we report here for the first time the presence of *M. albidus* in the UK and present a more detailed account of the distribution of *P. truncatum*.

MATERIALS AND METHODS

Sample collection

Following Simpson *et al.*'s (2005) publication, 273 gall bladders were retained from otter carcasses received by CUOP, and stored at −18 °C. These otters were found dead between January 2003 and June 2008, most as road kills, and all from England and Wales.

Microscopy

Gall bladders were defrosted, immersed in 0·6% saline in a Petri dish, punctured and inverted, and examined under a Nikon dissecting microscope at ×30 magnification with fibre optic illumination. In humans and experimental animals, opisthorchiid parasites cause symptomatic damage to the gall bladder and bile ducts over the time-course of infection (Sripa *et al.* 2007). Sripa and Kaewkes (2000) infected hamsters with *Opisthorchis viverrini* and found that an initial inflammatory response (due to a host cellular response and/or mechanical damage by parasite movement) was followed by periductal fibrosis in chronic infections. In the current study, histopathology of the gall bladder and bile ducts was not possible due to cell damage inflicted by the freezing process. However, a condition score of 1 to 5 (1 corresponding to a healthy gall bladder lining, 2 characterized by low level inflammation and 3–5 representing progressively more fibrous tissues) was used to describe the internal lining of the gall bladder. Parasites were then removed, counted and stored in 90% ethanol at −18 °C, for identification and archiving. Samples were only recorded as positive if a parasite was found, because other factors (such as gall stones) related to dietary or genetic conditions, may also cause thickening and inflammation of this tissue.

Preliminary parasite identification was based on morphometric characteristics following Yamaguti (1958) before multiple samples were sent to the Natural History Museum, London, for specialist identification.

Statistical analysis

Parasite prevalence was plotted using ArcMap GIS and this was used to describe the distribution of each parasite. A bootstrap confidence interval for mean intensity was calculated for both parasites using the statistical package R version 2.8. The Environment Agency separates England and Wales into 8 ecological regions (Fig. 1) based on river catchments. Parasite prevalence across regions and between seasons was examined using the Chi-square test for association; parasite intensity across regions and seasons was examined using Kruskal-Wallis tests.

Due to the large number of tied observations in the prevalence and intensity data, neither variable could be normalized, so it was not possible to fit linear models. Chi-squared tests were used to compare prevalence of infections in male and female hosts, and adult, subadult and juvenile hosts. Kruskal-Wallis tests were used to examine differences in the intensity of infection between sexes and age-classes. Data for both sexes were subsequently combined after testing for differences between male and female infections. Spearman's Rank Correlations were used to examine the relationship between host size (body weight and total length) and parasite intensity. Temporal variation in parasitic infection levels were compared using Chi-squared tests, between 2005 and 2008, excluding those animals from Dorset, Somerset, Avon, Wiltshire and Hampshire because hosts from these areas were only included in the study from 2007 onwards. Finally, the same tests were used to examine the relationship between prevalence and intensity of infections with gall bladder condition.

The spatial distribution of each parasite was assessed for clustering using a modified Ripley's K statistic, K[i.](r), with Ripley's isotropic edge correction (Ripley, 1988). Boundaries were defined as a simplified coastal border of England and Wales. Chance distribution of the location of infections was simulated (for 1000 iterations) by maintaining the position of events whilst randomizing labels (presence or absence of parasite) to examine the extent of spatial clustering at the 95% confidence level. The

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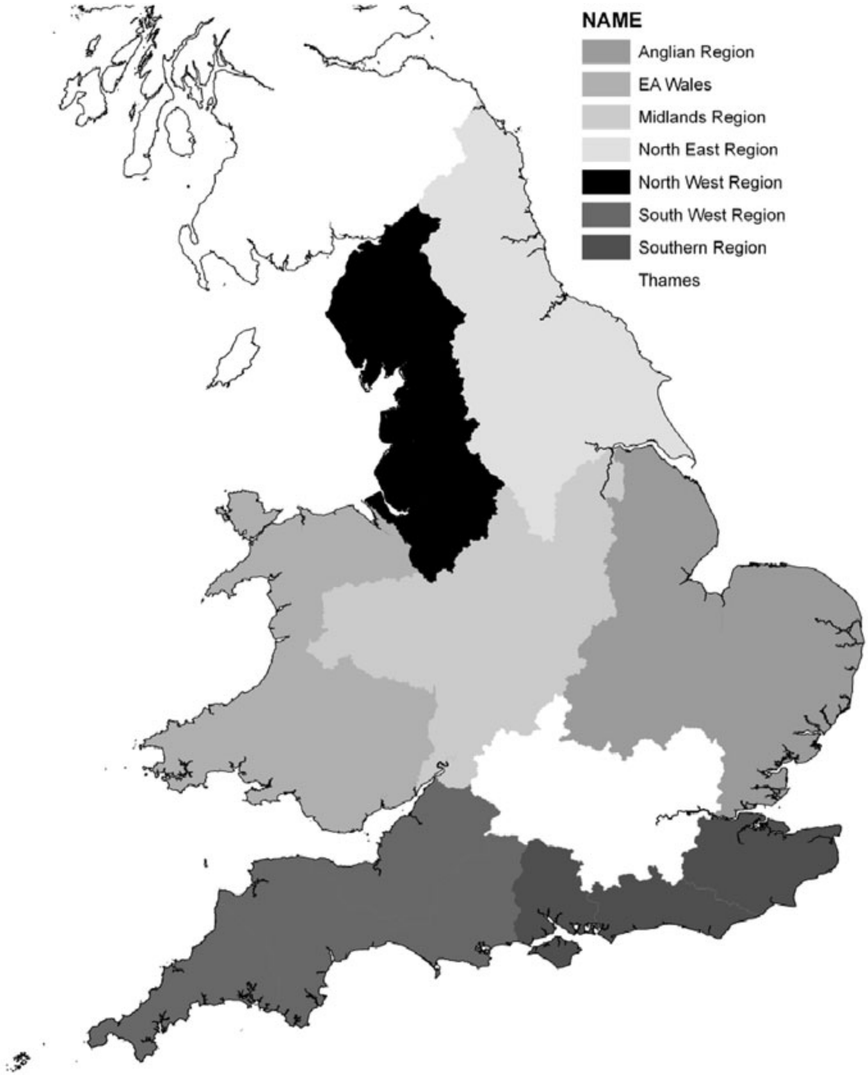

Fig. 1. Environment Agency defined regions in England and Wales.

estimated isotropic function is compared to the theoretical function under the null hypothesis of complete spatial randomness of parasite presence in the data set. Positive autocorrelation in parasite distribution was quantified by testing for the presence of clustering in sequentially increasing radii (1–131 km) around each data point. All statistical analysis was conducted using ArcGIS 9.1 and R version 2.8 (R Development Core Team, 2008).

RESULTS

Two parasite species, *Pseudamphistomum truncatum* (11·7%) and *Metorchis albidus* (6·6%), were detected in the gall bladders of otters collected from across England and Wales, with an overall prevalence of 18·3% (50 out of 273 samples). Table 1 shows the prevalence, mean intensities and geographical ranges for each parasite species. Figure 2 describes the frequency distribution of the 2 parasite species. A co-infection of *P. truncatum* and *M. albidus* was recorded in just 1 host, which harboured 1 *P. truncatum* and 7 *M. albidus* adult specimens. This female otter had been collected in East Anglia (NGR TG304087), and interestingly only 1 other otter from East Anglia was infected with *P. truncatum*.

Host sex and age

There was no significant difference in prevalence of *P. truncatum* or *M. albidus* between male and female hosts (D.F. $=1$; $\chi^2 < 0{\cdot}6$; $P > 0{\cdot}4$ for each parasite). Similarly, there was no significant difference in median intensity of infections between sexes or age-classes for either parasite (Kruskal-Wallis test; D.F. $=1$; $P > 0{\cdot}05$ in each case). Prevalence of parasitic infection in adults ($N = 147$) (15% infected with *P. truncatum*; 8·2% infected with *M. albidus*) was greater than subadults ($N = 114$) (8·8% *P. truncatum*; 5·3% *M. albidus*), but this was not significant (D.F. $=2$; $\chi^2 < 4$; $P > 0{\cdot}1$ for each parasite). No juvenile otters were infected ($N = 14$). There was no significant correlation between parasite intensity and host weight ($r_s = -0{\cdot}067$; $P = 0{\cdot}646$; $N = 31$), or length

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Table 1. Overall parasite load of gall bladders from otters collected from across England and Wales between 2003 and 2008 ($N=273$)

(Shown are sample size, prevalence, mean intensity with upper and lower 95% bootstrap confidence interval (10 000 iterations), median intensity and main geographical range.)

Parasitic species	Prevalence (%)	Mean intensity (95% CI)	Median intensity	Main geographical range
Pseudamphistomum truncatum	11·7	29 (12·3–50·4)	8·5	SE Wales, SW England
Metorchis albidus	6·6	5 (2·6–8·2)	3	East Anglia

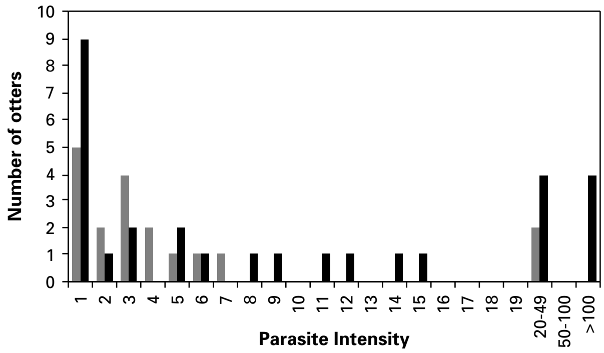

Fig. 2. Frequency distribution of *Pseudamphistomum truncatum* (black bars) and *Metorchis albidus* (grey bars) in the Eurasian otter population of England and Wales.

($r_s=-0{\cdot}186$; $P=0{\cdot}233$; $N=43$). The lack of significant results may be due to the small sample sizes in this study.

Geographical, seasonal and temporal distribution

Binary logistic regression revealed that *P. truncatum* was over-represented in the Southwest Region (Table 2). *Metorchis albidus* was over-represented in the Anglian region and more likely to be found in winter (Table 2). Intensity of parasite infections did not vary significantly between regions or seasons (Kruskal-Wallis test; D.F.$=7$; $P>0{\cdot}4$ for regions and D.F.$=3$; $P>0{\cdot}3$ for seasons for each parasite). Spatial analysis indicated that neither *P. truncatum* nor *M. albidus* infections showed evidence of spatial clustering.

Parasites were not detected in the North West region. During the course of the study, only 4 otters were received from the Southern region, 2 from Thames region and none from west of Somerset (Cornwall and Devon) so parasite prevalence in these areas is unknown (Fig. 3) although Simpson *et al.* (2009) found no parasites in Cornwall in a parallel study ($n=22$). There was no difference in parasite prevalence between 2005 and 2008 for *M. albidus* ($\chi^2=2{\cdot}194$; D.F.$=3$; $P=0{\cdot}533$) or *P. truncatum* ($\chi^2=2{\cdot}228$; D.F.$=3$; $P=0{\cdot}526$).

Host pathology

A large proportion (70%) of infected gall bladders were thickened, fibrous or inflamed. Infected hosts had gall bladders in the poorest condition ($\chi^2=54{\cdot}48$; D.F.$=4$; $P<0{\cdot}001$); the more fibrous gall bladders, condition classes 4 and 5, having most parasites (Fig. 4). Gall bladder condition degraded with parasite intensity ($\chi^2=132{\cdot}26$; D.F.$=4$; $P<0{\cdot}001$). This relationship held true even after removing the most heavily infected gall bladder ($N=238$ parasites) that doubled the total number of parasites in class 4. However, 18% of gall bladders with pathological damage lacked parasites and 30% of infected hosts showed no signs of gall bladder pathology.

DISCUSSION

Two species of opisthorchiid digeneans were found in UK otters. *Pseudamphistomum truncatum* has previously been described (Simpson *et al.* 2005, 2009), but here *Metorchis albidus* is recorded in the UK for the first time. As far as we are aware there is only one other report of *M. albidus* in otters; Sidorovich (1997) found the parasite in Belarusian otters autopsied between 1960 and 1980. In a more recent study of mustelids autopsied between 1987 and 1995 in Belarus, *M. albidus* was found in a single mink (*Mustela lutreola*) ($n=17$), but was absent from the otters examined ($n=38$) (Sidorovich and Anisimova, 1999).

There is a marked difference in the spatial distribution of *P. truncatum* and *M. albidus* suggesting they were independently introduced to different locations across the UK and, possibly, on multiple occasions. The lack of clustering may be, in part, due to the limited data-points across central areas of England. Simpson *et al.*'s (2005) retrospective examination of post-mortem reports from otters in the southwest indicated that *P. truncatum* was not present in that area prior to 2000. Unfortunately, no gall bladder samples were retained by Cardiff University Otter Project prior to 2005 so it is not possible to infer whether the infections in Wales pre- or

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Table 2. Composition of the binary logistic regression model and significant predictors for infection with gall bladder digeneans *Pseudamphistomum truncatum* and *Metorchis albidus* (based on Morgan *et al*. 2008)

(HR, Hosmer-Lemeshow goodness of fit Chi-square statistic, D.F., degrees of freedom, CI, 95% confidence intervals.)

Variable	D.F.	Category	*n*
a. Model constituents			
Region	7	Anglian	40
		EA Wales	110
		Midlands	28
		North East Region	41
		North West Region	22
		South West Region	24
		Southern	4
		Thames	2
Season	4	Winter (December–February)	99
		Spring (March–May)	60
		Summer (June–August)	30
		Autumn (September–October)	79
		No data	3
Sex	1	Male	157
		Female	114
Age-class	2	Juvenile	14
		Sub-adult	113
		Adult	144

Factor	Significant levels	*P*	Odds ratio	CI (95%)
b. Significant predictors (95% confidence level) of *Pseudamphistomum truncatum* infection, HR = 3·3517, 8 D.F., *P* = 0·910				
Region	South West region	0·002	14·60	2·62–81·39
c. Significant predictors (95% confidence level) of *Metorchis albidus* infection, HR = 2·2129, 8 D.F., *P* = 0·974				
Region	Anglian region	< 0·001	21·79	4·40–107·92
Season	Winter	0·047	0·21	0·05–0·98

post-date those in the Southwest. The cases of *M. albidus* in Anglian region are interesting; during the 1990s there were multiple re-introductions of otters into this region (Copp and Roche, 2003). Although little is known about the origin of these otters, it is possible that some were relocated from or exposed in captivity to food sourced from mainland Europe, where *M. albidus* is prevalent in red fox (*Vulpes vulpes*). The most recent case, outside East Anglia, was in the Derwent Catchment in the north east of England (in March 2008) where rehabilitated otters are known to have been released in the past (Woodroffe, 1998). Movement of fish stocks, sediment (containing parasite eggs or gastropods reported to host larval digeneans, such as *Bithynia* spp. (Morley *et al*. 2004)), or otters (and other definitive hosts) moving between river systems could increase the geographical range of either parasite. As noted by Kennedy (1993) re-introductions and natural colonizations contribute to the spread of British freshwater helminths, and the effect of wildlife movement is generally underestimated.

There was no relationship between parasite prevalence and otter gender; this is unsurprising as both sexes are likely to feed on the intermediate hosts, probably small cyprinids such as sunbleak (*Leucaspius delineatus*) or topmouth gudgeon (*Pseudorasbora parva*) (see Simpson *et al*. 2005). No juvenile otters were infected with either parasite, possibly because they are fed on milk until roughly 2 months old when the mother begins to bring fish (Kruuk, 2006). However, only 14 juveniles ($\sim$5% of the sample) were examined during this study, and so the lack of parasites may just reflect the low sample size. Simpson *et al*. (2009) did not recover any parasites from juvenile otters in a similar study on *P. truncatum*.

Opisthorchiids are renowned for their pathogenic effects. *Opisthorchis viverrini* causes several hepatobiliary diseases in humans and other mammals (Sripa, 2003), and human populations in Southeast Asia experience serious health problems, such as fatal cholangiocarcinoma, following infection (Müller *et al*. 2007). All opisthorchiid species are thought to cause symptoms associated with obstruction of the biliary system (Müller *et al*. 2007). We found that parasite presence negatively affects gall bladder condition; a large number of infected gall bladders were inflamed, thickened or fibrous; conditions possibly relating to varying time stages of infection (Sripa *et al*. 2007). Our study agrees with previous work; Simpson *et al*. (2005) concluded that cholecystitis resulted from the presence of *P. truncatum* in mink and otters in southwest England. Nielsen and Guidal (1974) found infection with *M. albidus* caused progressive icterus (jaundice) and cholangitis (inflammation of the bile ducts) in a cat from Copenhagen,

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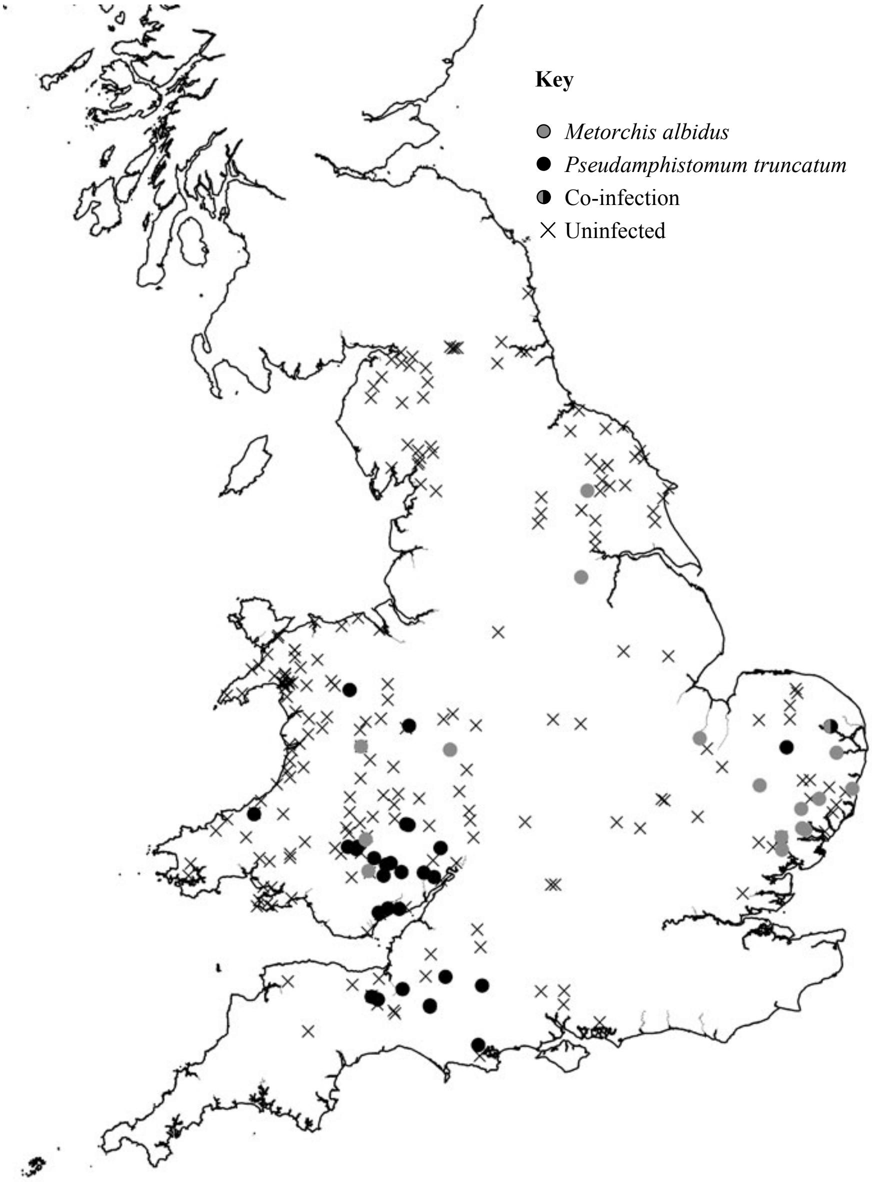

Fig. 3. Location of infected and uninfected otters across England and Wales: *Metorchis albidus* infections are shown in grey, *Pseudamphistomum truncatum* infections are shown in black and uninfected hosts are marked with a cross.

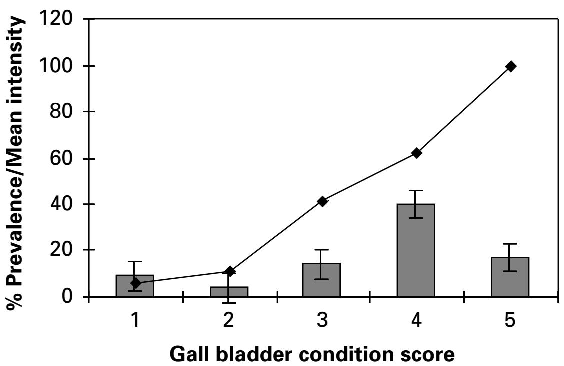

Fig. 4. The percentage prevalence (line) and mean intensity (grey bars with standard error bars) of parasite infections (both *Pseudamphistomum truncatum* and *Metorchis albidus*) in UK otters (*Lutra lutra*) in relation to gall bladder condition (ranging from 1 – healthy – to 5 – extremely fibrous and thickened).

Denmark. Interestingly, 18% of the damaged gall bladders in our study were uninfected, so either the parasites are not the sole cause of biliary pathology in otters, or these hosts had previously been infected and subsequently shed their parasites. Those animals with infections and no sign of pathology may have only recently acquired the parasites.

Unfortunately, sampling using predominantly road-killed animals often presents a biased sample of the population. It has been suggested that healthy, male and young otters tend to be over-represented in road-killed samples (Bradshaw, 1999; Chadwick, 2007). The infected otters in this study were all killed on the road with the exception of 2 individuals that died from fighting injuries and 2 that were severely emaciated. This is important to consider because if parasitic infection correlates with age, the animals sampled here may under-estimate parasite loads in

UK otters. Although freezing and re-freezing samples has been suggested to affect the reliability of parasite intensity data (Joy and Pennington, 1998), in this study the majority of parasites found were structurally normal suggesting that the freezing process did not significantly affect detection of these macroparasites.

In conclusion, this study reports 2 biliary parasites in UK otters. Infection with *P. truncatum* or *M. albidus* appears to significantly damage the biliary system in these mustelids. This study continues the work of Simpson *et al*. (2005) in establishing the prevalence of *P. truncatum* in the UK.

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REFERENCES

Bakke, T. A., Cable, J. and Harris, P. D. (2007). The biology of Gyrodactylid Monogeneans: the ''Russian-Doll Killers''. *Advances in Parasitology* **64**, 161–376. doi:10.1016/S0065-308X(06)64003-7

Beyer, K., Kochanowska, D., Longshaw, M., Feist, S. W. and Gozlan, R. E. (2005). A potential role for invasive sunbleak in the further dissemination of a non-native parasite. *Journal of Fish Biology* **67**, 1730–1733. doi: 10.1111/j.1095-8649.2005.00859.x

Bowman, D. D., Hendrix, C. M., Lindsey, D. S. and Barr, S. C. (2002). *Feline Clinical Parasitology*. Blackwell Publishing, UK.

Bradshaw, A. V. (1999). Aspects of otter *Lutra lutra* mortality in England and Wales. Ph.D. thesis Cardiff University, Cardiff, UK.

Chadwick, E. A. (2007). *Post-mortem Study of Otters in England and Wales 1992–2003*. Environment Agency Science Report, UK.

Chandler, A. C. and Read, C. P. (1961). *Introduction to Parasitology*. 10th Edn. John Wiley and Sons, New York, USA and London, UK.

Chanin, P. R. F. and Jefferies, D. J. (1978). Decline of otter *Lutra lutra* L. in Britain – Analysis of hunting records and discussion of causes. *Biological Journal of the Linnean Society* **10**, 305–328.

Copp, G. H. and Roche, K. (2003). Range and diet of Eurasian otters *Lutra lutra* (L.) in the catchment of the River Lee (south-east England) since re-introduction. *Aquatic Conservation: Marine and Freshwater Ecosystems* **13**, 65–76. doi: 10.1002/aqc.561

Gozlan, R. E., Flower, C. J. and Pinder, A. C. (2003). Reproductive success in male sunbleak, a recent invasive fish species in the UK. *Journal of Fish Biology* **63**, 131–143. doi: 10.1111/j.1095-8649.2003.00210.x

Gozlan, R. E., St-Hilaire, S., Feist, S. W., Martin, P. and Kent, M. L. (2005). Biodiversity: disease threat to European fish. *Nature, London* **435**, 1046. doi: 10.1038/4351046a

Joy, J. E. and Pennington, J. L. (1998). Ecology of *Megalodiscus temperatus* (Digenea: Paramphistomatidae) in red-spotted newts, *Notophthalmus v. viridescens*, from West Virginia. *Journal of the Helminthological Society of Washington* **65**, 205–211.

Kennedy, C. R. (1993). Introductions, spread and colonization of new localities by fish helminth and crustacean parasites in the British Isles: a perspective and appraisal. *Journal of Fish Biology* **43**, 287–301. doi: 10.1111/j.1095-8649.1993.tb00429.x

Kirk, R. S. (2003). The impact of *Anguillicola crassus* on European eels. *Fisheries Management and Ecology* **10**, 385–394. doi: 10.1111/j.1365-2400.2003.00355.x

Kruuk, H. (2006). *Otters: Ecology, Behaviour and Conservation*. Oxford University Press, Oxford, UK.

Morgan, E. R., Tomlinson, A., Hunter, S., Nichols, T., Roberts, E., Fox, M. T. and Taylor, M. A. (2008). *Angiostrongylus vasorum* and *Eucoleus aerophilus* in foxes (*Vulpes vulpes*) in Great Britain. *Veterinary Parasitology* **154**, 48–57. doi: 10.1016/j.vetpar.2008.02.030

Morley, N. J., Adam, M. E. and Lewis, J. W. (2004). The role of *Bithynia tentaculata* in the transmission of larval digeneans from a gravel pit in the Lower Thames Valley. *Journal of Helminthology* **78**, 129–135. doi: 10.1079/JOH2003223

Müller, B., Schmidt, J. and Mehlhorn, H. (2007). PCR diagnosis of infections with different species of Opisthorchiidae using a rapid clean-up procedure for stool samples and specific primers. *Parasitology Research* **100**, 905–909. doi: 10.1007/s00436-006-0321-x

Nielsen, J. C. L. and Guildal, J. A. (1974). Infestation with *Metorchis albidus* in a cat. *Nordisk Veterinaer Medicin* **26**, 467.

R Development Core Team (2008). *R: a Language and Environment for Statistical Computing*. *R Foundation for Statistical Computing*. Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Ripley, B. D. A. (1988). *Statistical Inference for Spatial Processes*. Cambridge University Press, Cambridge, UK.

Saeed, I., Maddox-Hyttel, C., Monrad, J. and Kapel, C. M. O. (2006). Helminths of red foxes (*Vulpes vulpes*) in Denmark. *Veterinary Parasitology* **139**, 168–179. doi: 10.1016/j.vetpar.2006.02.015

Sidorovich, V. and Anisimova, E. I. E. (1999). Comparative analysis of the helminthocenoses of the native semiaquatic mustelids (*Lutra lutra*, *Mustela lutreola*) in connection with the width of food spectra. *IUCN Otter Specialist Group Bulletin* **16**, 76–78.

Sidorovich, V. E. (1997). *Mustelids in Belarus: Evolutionary Ecology, Demography and Interspecific Relationships*. Zolotoy Uley, Minsk, Belarus.

Simpson, V. R. (2007). *Health Status of Otters in Southern and South West England 1996–2003*. Environment Agency Science Report SC10064/SR1.

Simpson, V. R., Gibbons, L. M., Khalil, L. F. and Williams, J. L. R. (2005). Cholecystitis in otters (*Lutra lutra*) and mink (*Mustela vison*) caused by the fluke *Pseudamphistomum truncatum*. *Veterinary Record* **157**, 49–52.

Simpson, V. R., Tomlinson, A. J., and Molenaar, F. M. (2009). Prevalence, distribution and pathological significance of the bile fluke *Pseudamphistomum truncatum* in Eurasian otters (*Lutra lutra*) in Great Britain. *Veterinary Record* **164**, 397–401.

Sripa, B. (2003). Pathobiology of opisthorchiasis: an update. *Acta Tropica* **88**, 209–220. doi: 10.1016/j.actatropica.2003.08.002

Sripa, B. and Kaewkes, S. (2000). Localisation of parasite antigens and inflammatory responses in experimental opisthorchiasis. *International Journal for Parasitology* **30**, 735–740. doi: 10.1016/S0020-7519(00)00054-0

Sripa, B., Kaewkes, S., Sithithaworn, P., Mairiang, E., Laha, T., Smout, M., Pairojkul, C., Bhudhisawasdi, V., Tesana, S., Thinkamrop, B., Bethony, J. M., Loukas, A. and Brindley, P. J. (2007). Liver fluke induces cholangiocarcinoma. *Public Library of Science Medicine* **4**, 1148–1155. doi: 10.1371/journal.pmed.0040201

Sukhdeo, M. V. K. and Sukhdeo, S. C. (2004). Trematode behaviours and the perceptual world of parasites. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **82**, 292–315. doi: 10.1016/j.actatropica.2003.08.002

Woodroffe, G. (1998). Reinforcing otter populations of the Derwent and Esk catchments in north Yorkshire. *British Wildlife* **9**, 145–153.

Yamaguti, S. (1958). *Systema Helminthum Vol 1: The Digenetic Trematodes of Vertebrates, Parts 1 and 2*. Interscience Publishers, London, UK and New York, USA.