CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Towards an in-situ non-lethal rapid test to accurately detect the presence of the nematode parasite, Anguillicoloides crassus, in European eel, Anguilla anguilla
Authors	De Noia, M.;Poole, R.;Kaufmann, Joshka;Waters, C.;Adams, C.;McGinnity, Philip;Llewellyn, M.
Publication date	2022-01-19
Original Citation	De Noia, M., Poole, R., Kaufmann, J., Waters, C., Adams, C., McGinnity, P. and Llewellyn, M. (2022) 'Towards an in-situ non-lethal rapid test to accurately detect the presence of the nematode parasite, Anguillicoloides crassus, in European eel, Anguilla anguilla', Parasitology, 149(5), pp. 605-611. doi: 10.1017/S0031182021002146
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1017/S0031182021002146
Rights	© 2022, the Authors. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited. - https://creativecommons.org/licenses/by/4.0/
Download date	2026-10-31 17:46:52
Item downloaded from	https://hdl.handle.net/10468/12468

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Towards an *in-situ* non-lethal rapid test to accurately detect the presence of the nematode parasite, *Anguillicoloides crassus*, in European eel, *Anguilla anguilla*

De Noia M.[1], Poole R.[3], Kaufmann J.[2,3], Waters C.[2,3], Adams C.[1], McGinnity P.[2,3], Llewellyn M.[1]

[1] Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow, G20 6NB , Glasgow, UK; [2] School of Biological, Earth and Environmental Sciences, University College Cork, T23 N73K, Cork, Ireland and [3] Marine Institute, Foras na Mara, F28 PF65, Newport, Ireland.

*** Author for correspondence:** Michele De Noia, E-mail: MicheleDeNoia@glasgow.ac.uk

Accepted Manuscript

This is an Accepted Manuscript for Parasitology. This version may be subject to change during the production process. DOI: 10.1017/S0031182021002146

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

Abstract

Anguillicoloides crassus is an invasive nematode parasite of the critically endangered European eel, *Anguilla anguilla*, and possibly one of the primary drivers of eel population collapse. The presence of the parasite has been shown to impact many features of eel physiology and life history. Early detection of the parasite is vital to limit the spread of A. crassus, and to assess its potential impact on spawning biomass. However, until recently, accurate diagnosis of infection could only be achieved via necropsy. To support *A. anguilla* fisheries management in the context of *A. crassus* we developed a rapid, non-lethal, minimally invasive and *in-situ* DNA-based method to infer the presence of the parasite in the swim bladder. Screening of 131 wild eels was undertaken between 2017 and 2019 in Ireland and UK to validate the procedure. DNA extractions and PCR were conducted using both a Qiagen Stool kit at Glasgow University and *in situ* using Whatman qualitative filter paper No. 1 and a miniPCR DNA Discovery System™. Primers were specifically designed to target the cytochrome oxidase mtDNA gene region and *in situ* extraction and amplification takes approximately 3h for up to 16 individuals. Our *in situ* diagnostic procedure demonstrated Positive Predictive Values at 96% and Negative Predictive Values at 87% by comparison to necropsy data. Our method could be a valuable tool in the hands of fisheries managers to enable infection control and help protect this iconic but critically endangered species.

Key words: Non-lethal test, fish parasite, genetic, eDNA

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

Introduction

Anguillicoloides crassus, (Kuwahara, Niimi & Itagaki 1974) is a nematode parasite of the *Anguilla japonica* that also infects other *Anguilla* species, including the European eel *Anguilla anguilla* (Lefebvre, Wielgoss, Nagasawa, & Moravec, 2012). *A. crassus* originates from East Asia, having been introduced into Europe in the early 1980s as a result of the trade in live Japanese eels, *Anguilla japonica,* (Temminck & Schlegel, 1847*)* (Laetsch, Heitlinger, Taraschewski, Nadler, & Blaxter, 2012; Weclawski *et al*., 2013). *A. crassus* is now well established in the Western Hemisphere and can be found in almost all European rivers and lakes, where it can tolerate salinities up to 12 ppt (Aguilar, Álvarez, Leiro, & Sanmartín, 2005; Becerra-Jurado *et al*., 2014). While *A. crassus* was unlikely to have been the primary cause of the *Anguilla anguilla* recruitment collapse since the 1980s, in conjunction with low recruitment, infections of the parasite may have contributed to declining adult stocks (Henderson, Plenty, Newton, & Bird, 2012) and to the quality of emigrating silver eels, thereby potentially impacting on effective spawner biomass and the ability of the stock to recover (Kirk, 2003).

Anguillicoloides crassus reproduces sexually in the swim bladder of the eels. The eggs hatch in the female worm inside the swim bladder and L2 larvae migrate to the intestinal tract via the pneumatic duct to be excreted with the faeces (Didžiulis, 2013). As part of its life cycle *A. crassus* is then trophically transmitted to various intermediate hosts including several zooplankton species (especially copepods of the orders Cyclopoidea and Calanoidea) as well as planktivorous fish such as the three-spined sticklebacks, *Gasterosteus aculeatus*, Linnaeus, 1758 (Kuwahara, 1999). In the intermediate host, the parasite develops into the infectious phase L4 larvae, which, once ingested, parasitize the eel as the final host. The parasite migrates from the gut system perforating the connective tissue and muscles reaching the swim bladder (Heitlinger, Laetsch, Weclawski, Han, & Taraschewski, 2009). The number of

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0031182021002146

parasites found in the swim bladder can vary from less than ten to greater than 70 individuals per eel (Jousseaume *et al*., 2021). The presence of the parasite has been shown to detrimentally affect many features of eel physiology and life history (Newbold *et al*., 2015). Adult nematodes feed on blood supplied to the swim bladder wall and can result in increased eel mortality as a consequence of damage caused to the organ (Schneebauer, Dirks, & Pelster, 2017). The swim bladder wall becomes thicker, opaque and less elastic due to the perforation caused by the parasite feeding habit with an impact on buoyancy control (Barry *et al*., 2014; Newbold *et al*., 2015; Weclawski *et al*., 2013). *A. crassus* infection is also thought to alter the physiological mechanisms involved in silvering – the process by which freshwater sub-adults adapt to life in the ocean. In this respect, infected eels have also been found to silver faster as a result of an over-production of cortisol, which seems to have a stimulatory effect on GTH2 synthesis (Di Biase *et al*., 2017; Muñoz, Peñalver, Ruiz de Ybañez, & Garcia, 2015). Moreover, cortisol is the key hormone produced during fasting, typical of the silvering phase stage (Fazio, Sasal, Mouahid, Lecomte-Finiger, & Moné, 2012). During the silvering phase occur a normal increase of erythropoiesis but the parasite, due to their blood feeding behaviour, increase erythropoiesis in infected eels prior their silvering (Churcher *et al*., 2015). The presence of the parasite may impact on the eel's migration speed in rivers (Newbold *et al*., 2015) and in the ocean as the energy demand increases (Pelster, 2015), due to the reduction of the swim bladder elasticity. The presence of the parasite appears not to affect the speed and migratory behaviour during the first phase of the migration in shallow water (Simon, Westerberg, Righton, Sjöberg, & Dorow, 2018). However, where deep diving is required in the ocean, damage to the integrity of the swim bladder is believed to seriously impact on an infected eel's chances of survival (Lefebvre *et al*., 2012; Righton *et al*., 2016).

Currently accurate detection of the parasite can only be achieved via post-mortem dissection and thus requires the eel to be dissected. However, several non-lethal techniques

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

are under development (Frisch, Davie, Schwarz, & Turnbull, 2016). Anal redness can be used as indicator for presence or absence of the parasite, but this approach lacks both specificity and objectivity (Crean, Dick, Evans, Elwood, & Rosell, 2003). A radio diagnostic method has been developed to detect inflammation caused by the nematode's feeding habits (Beregi, Molnár, Békési, & Székely, 1998). The method uses X-ray to scan the pneumatic duct and can detect swim bladder damage and parasite presence. The quality of the images has a large margin of error so the accuracy of detection can be low and swim bladder alterations can be caused by other factors (Beregi *et al*., 1998). Frisch *et al*. 2016 made improvements to the method developed by Beregi *et al*. (1998). Using compound radiography, they were able to detect small alterations to the thickness of the swim bladder wall and to inflations of the lumen. However, to perform a full body scan using this method, the animal has also to be euthanized. Recently, attempts have been made to develop a molecular test for *A. crassus* infection based in nuclear microsatellite markers (Jousseaume *et al*., 2021), however, reported sensitivity and specificity was below 71%, and the test, which involves fine-scale size discrimination of microsatellite locus sizes between target and off-target nematode species, is not easily transferred to field conditions. Finally, it is not clear from this molecular study whether faeces could be sampled non-lethally (Jousseaume *et al*., 2021).

To support the assessment of eel stocks and ultimately fisheries management in the context of *A. crassus*, sensitive, specific and rapid non-lethal and *in situ* methods for pathogen detection are urgently required. Screening of translocated eel populations could, for example, limit the spread of the pathogen. Furthermore, non-lethal screening of silver eels alongside satellite tagging studies could reveal the impact of infection on the migratory and breeding success. Several non-lethal and/or molecular methods have been proposed to detect parasites in various fish species related to the food health safety chain and conservation management (Cavallero *et al*., 2017; Levsen *et al*., 2018). A non-lethal qPCR based eDNA

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

approach has also been optimized to detect the cestode *Schistocephalus solidus* in samples taken by needle from the intra-peritoneal cavity of a fish (Berger & Aubin-Horth, 2018).

In the current study, we developed an alternate, rapid, non-lethal and portable, *in situ* PCR-based approach to detect *A. crassus* in the European eel using parasite DNA traces in faecal material. We tested the specificity and sensitivity of two different DNA extraction methods, the former lab-based extraction protocol, the latter more suited to the field. Using necropsy data, we were also able to explore any link between host condition and parasite infection load.

Materials and methods

Sample collection

The study was conducted at two different locations in UK and Ireland. In the Burrishoole catchment, Ireland 53°55'27.6"N 9°34'27.0"W, yellow eel (feeding stage) were collected from Loughs Feeagh (freshwater) and Furnace (tidal brackish water) using unbaited fyke nets deployed overnight in chains of 10 nets set at different lake depths in summer 2017, 2018 and 2019. Eels undergoing silvering were collected in autumn 2019 using permanent downstream river Wolf-type traps. The study was carried out under a Health Products Regulatory Authority (HPRA) license number AE19130-P096. In Lough Neagh, UK-Northern Ireland 54°36'05.5"N 6°24'55.5"W, yellow eels were collected with baited long lines fished overnight in the lake in summer 2018. Between capture and the procedures, eels were kept in holding tanks at the Marine Institute of the Burrishoole catchment. Under mild anaesthesia, colonic irrigation with 2ml 0.09 % sterile saline solution was performed to collect faecal material using a 5 ml syringe and a modified (needle removed) Terumo Surflo Winged Infusion Set (Figure 1). Each colonic irrigation procedure lasted less than 30 seconds. The eels were then euthanized with an overdose of MS-222 (10 min in a 100 mg/L Tricaine

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

methane sulfonate solution, FVG Ireland) followed by a cervical separation of the spinal cord. A total number of 131 eels were sampled and weight and length was recorded to the nearest 0.1 cm and gram (Supplementary 1). A drop of the collected wash material was placed on 1 cm^2 of Whatman qualitative filter paper No. 1 and air dried for 5 minutes at room temperature. The air-dried paper was used to perform instant *in situ* DNA extraction or preserved at -80◦C. The remaining wash was stored in 100% ethanol (1 wash: 9 ETOH) at -20◦C. Subsequently, all eels were dissected, swim bladder inspected and the number of *A. crassus* present counted. *A. crassus* were collected and stored in 100% ethanol before being stored at -20◦C. A eel was considered infected if at least one parasite, regardless of its lifecycle stage, was found in the swim bladder.

DNA collection and extraction methods development

DNA extraction

A total of 131 eels were sampled and faecal material was collected from all. DNA was extracted from faecal wash of 104 eels using the Qiagen stool. The Whatman extraction protocol was used *in situ* for eels sampled in 2019 (N= 55). To enable direct comparison between the two protocols, DNA was extracted from 28 eels caught in 2019 (14 in Lough Furnace and 14 in Lough Feeagh) using both Qiagen and Whattman extraction methods for each eel. DNA concentrations for Qiagen extractions are included in Supplementary table 3.

Laboratory genomic DNA extraction

DNA from 200 µl of stored faecal material was centrifuged for 5 min at 12000 rpm to concentrate the pellet. For each sample, 180 µl of supernatant was removed and the remaining material was extracted with a slight modification to the suggested protocol of the

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0031182021002146

QIAamp Stool Kit (Qiagen). ATL tissue lysis buffer volume was increased to 350 µl, proteinase K up to 20 µl , AL lysis buffer up to 300µl and Ethanol 100% up to 400µl.

In situ genomic DNA extraction

A small sample of the filter paper (were faecal material had been previously deposited) of 1mm diameter was removed by a punch from the Whatman qualitative filter paper No. 1 and DNA was extracted using adjusted extraction protocol of DNA from Whatman™ FTA™ cards (Santos *et al*., 2006) (Figure 1) (Supplementary Material 1).

Primer design, PCR conditions and species specificity

A pair of specific customised primers were designed using all 467 cytochrome c oxidase subunit 1 (COI) gene sequences available for *A. crassus* (NCBI). All sequences were aligned to build a consensus sequence using BioEdit version 7.0.5.3 (Thomas A. Hall, 2017). The obtained consensus sequence was used to identify a conserved region within *A. crassus* suitable for primer design (Table 1). The designed primer pair was assayed for cross-reactivity *in silico* against common fish nematode parasites *Camallanus* sp. (NCBI Accession: EU598889), *Contracaecum* sp. (NCBI Accession: FJ866816) and *Capillaria* sp (NCBI Accession: AJ288168) (Pouder, Curtis, & Yanong, 2009). The total length of the expected amplicon is 187 bp. The same PCR conditions and mastermix were used to test the efficiency and specificity of the primer using a mini PCR DNA Discovery System. The PCR Mastermix was made with of 10 µl of Q5® High-Fidelity DNA Polymerase, 1 µl of FWD Primer [10 nM], 1 µl of RV primer [10 nM], 0.5 µl MgCl2 (0.5 M), 6,5 µl of RNA and DNA free water and 1 µl of extracted DNA. The total volume of the PCR reaction was 20 µl per sample. The cycle used for the PCR started with 5 minutes at 95 °C, followed by 35 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds and 72 °C for 30 seconds and a last step of 10

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

minutes at 72 °C. 5 µl of PCR products was visualized on a 2% agarose gel using SYBR safe staining (Invitrogen). Each sample was amplified in technical triplicate alongside negative controls (ddH_20) and a positive control of either 20ng/ul DNA (Qiagen extraction) or *A. crassus* tissue crushed onto Whatman FTA card. Species specificity of the primer set was confirmed using a series of parasites collected in the same study system, various non-nematode parasites (*Anisakis* sp.) and other animal taxa, including the European eel, to assay cross-reactivity (*Lepeophtheirus salmonis*, *Anguilla anguilla*, *Neoparamoeba perurans*, *Scombrus scombrus*, *Diphyllobothrium* sp., *Schistocephalus* sp., *Dentitruncus truttae*). For each organism 1 µl of DNA was used. To identify amplicons as *A. crassus*, a subset of positive amplifications were Sanger-sequenced at MRC PPU DNA Sequencing and Services, Dundee, UK.

Sensitivity, Specificity, PPV and NPV

Several parameters were calculated to assay the validity of the test. Here *sensitivity* is defined the ability of the test to correctly classify an individual as infected (i.e. a true positive). The ability of a test to correctly classify an individual as non-infected (i.e. a true negative) is called the test's *specificity*. The Positive Predictive Value (PPV) is the percentage of eels with a positive test which are actually infected on dissection and the Negative Predictive Value (NPV) is the percentage of eels with a negative test which do not have the parasite on dissection. Positive and negative predictive values are directly related to the prevalence of the disease in the population (Stojanović *et al*., 2014) (Table 2).

Biological validation of eel infection status

Eggs count in faecal wash

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0031182021002146

Nematode larvated and unlarvated eggs and L2 larvae were counted with a modified McMaster Salt Flotation Technique. 200 µg of faecal material was diluted in 1,5 ml of distilled water. After mechanical homogenisation, the suspension was poured through a 250-micron aperture sieve and the filtrate collected. After thorough mixing, the solution was transferred to a centrifuge tube and spun for five minutes at 2500 rpm. The supernatant was discarded and the remaining faecal pellet covered and homogenised with 300 µl of saturated sodium chloride solution, mixed by inverting slowly six times. Then, using a Pasteur pipette, the mixture was transferred to a McMaster slide. Each chamber holds 0.15 ml beneath the gridded area. The preparation is then examined using the 25x objective of a stereoscopic microscope, the number of eggs present in the grids of both chambers were counted to give an estimate of the numbers of eggs/gram of faecal material.

A. crassus count in swim bladder necropsy

All dissected eels were checked for *A. crassus*, and where present, they were counted. The swim bladder was extracted whole from the animal and stored at 4°C until the procedure was completed for all the specimens. The swim bladder was then opened and nematodes were counted and classified to adults and larval stages. A Mann-Whitney test was performed in R studio between years of infection to test if there was significant change in the parasitic load.

Accepted Manuscript

Results

Rapid test in-situ

The *in situ* non-lethal test was performed on 55 eels collected in 2019. Individuals were anally catheterised to enable colonic irrigation with a soft silicon tube without causing internal lesions. The amount of saline solution used (between 0.5 ml and 2 ml) varied in

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

approximate proportion to the size of the tested animals. The procedure was deployed to minimize the

invasiveness of the collection of the faecal material. *In-situ* DNA extraction took 20 minutes for 16 samples, PCR reaction was undertaken over a period of 2 hours and electrophoresis with gel visualization took a further 17 minutes. Thus, the test can be performed for 16 individuals in approximately 3 hours (Figure 1).

Comparison of different DNA extraction methods

The DNA Whatman paper extraction method provides a rapid and more reliable assessment of infection compared to the method based on the Qiagen Stool and Blood kit. Both specificity ($p<0.006$) and sensitivity ($p<0.003$) where shown to be significantly higher using the Whatman protocol (Figure 2, Table 3). The resulting improvement of using the Whatman test in specificity was 46%, in sensitivity 45%, in PPV is 30% and in NPV 41% (Figure 2). Additionally, the time for DNA extraction from 1 sample using the Whatman paper as compared to a Qiagen extraction was reduced from c.80 minutes to c.20 minutes.

Parasite count in swim bladder and eggs count in faecal material.

Faecal material from all 131 samples was tested to detect the presence of eggs and/or L2 larvae using the McMaster floatation protocol. All 131 collected swim bladders were then screened under the microscope and number of nematode eggs reported (Supplementary 1). No eggs or larvae were detected in any samples. Number of nematodes in the swim bladder showed and increasing trend in infection across the three sampling years and a significant increase in the silver eels collected in 2019 (Figure 3).

Discussion

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0031182021002146

Our study represents the first attempt to develop a sensitive, non-lethal and, importantly, i*n situ* method to establish *A. crassus* infection in *A. anguilla* via the detection of parasite DNA in faecal material. High values for NPV (87%) and PPV (95%) suggest the test may have a useful role in both veterinary and fisheries management contexts. We found inter-annual differences in the prevalence of infected eels in the three years we sampled in the Burrishoole catchment with the total number of infected animals significantly higher in 2019.

Sensitivity is a key consideration for any molecular test. Mitochondrial genes are the major target genes in PCR-based detection systems because they are highly conserved and present in multiple copies (Paoletti *et al*., 2018). High target copy number may explain the high sensitivity of the test we deployed. The mitochondrial gene COI has been widely used to detect the presence of nematode parasites in commercially important fish species (Godínez-González, Roca-Geronès, Cancino-Faure, Montoliu, & Fisa, 2017; Herrero, Vieites, & Espiñeira, 2011; Paoletti *et al*., 2018; Santos *et al*., 2006). The use of microsatellites is also a well-established method for parasite detection (Vieira, Santini, Diniz, & Munhoz, 2016), although these nuclear markers can suffer from lower sensitivity that their mitochondrial counterparts, which may contribute to their poorer performance in detecting *A. crassus* in eels (Jousseaume *et al*. in 2021).

Some improvement in detection sensitivity and specificity was achieved here by adopting a more 'crude' nucleic acid extraction approach using a Whatman paper. Nucleic acid extraction is increasingly recognised as a major rate limiting step in molecular diagnostics, however, paper-based options offer several advantages in terms of speed and cost (Zou *et al*., 2017), as seems to be the case in our study. Nonetheless, our final protocol did show both false positives and false negatives, albeit at a low rate. False negatives likely relate to issues with template purification and PCR amplification, or potentially the reduced biomass of younger worms (Barry *et al*., 2014). Similarly juvenile, un-mated worms are

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

likely to shed less genetic material in the form of larvae. False positives could indicate that presence of early infections, not yet detectable via necropsy – and further investigation of such cases is warranted. It is not clear whether the DNA we are detecting originates from embryonated eggs, cellular material or free DNA shed from the worms. Our inability to microscopically detect the presence of *A. crassus* larvae or eggs in faecal material suggests DNA or cellular fragments from worms are the likely source. However, our use of ethanol as fixative for storing samples could have played a role in our low success in detecting eggs or larvae (Crawley, Chapman, Lummaa, & Lynsdale, 2016).

The test we present relies on a simple PCR, not qPCR. Nonetheless, validation against 'real' infection levels assayed via necropsy reveals excellent specificity and sensitivity. Point-of-care qPCR for viral pathogens can now deliver a result in less than 20 minutes (Melchers, Kuijpers, Sickler, & Rahamat-Langendoen, 2017). Similarly, several mobile qPCR instruments have been brought to the market and have been successfully deployed to deliver veterinary diagnoses in remote locations on actionable timescales for cattle (Hole & Nfon, 2019). However, the cost of such approaches may be prohibitive in respect to their application to the detection of pathogens fish. Our experimental set-up, which fits in a carry-on suitcase and can be performed in the field powered with a portable battery shows that standard PCR, using low-cost reagents and equipment may be just as portable, and informative epidemiologically, as 'higher-end' devices, although benchmarking against a portable qPCR device could be a focus of future study.

Stocking, as part of eel population enhancement, is likely a major contributor to *A. crassus* dispersal, as are translocations associated with the trade in live eels (Laetsch *et al.*, 2012; Weclawski *et al.*, 2013). Screening of such individuals and early detection with a non-lethal method could be a powerful tool to avoid the spread of the parasite. However, there

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

remains a need to clarify whether our approach has enough sensitivity to detect infection in glass eels and elvers. *A. crassus* is known to infect the elvers or European eels (Haenen, Grisez, De Charleroy, Belpaire, & Ollevier, 1989) and natural infection has been detected in late-stage glass eels as well as elvers of the American eel *Anguilla rostrata* (Hein *et al*., 2016). Both juvenile stages are a major component of stocking biomass. Advances in sample pooling designs and detection algorithms during the recent coronavirus epidemic can achieve individual-level identification using a seven-fold lower number of tests than the number of individuals (Shental *et al*., 2020). Such algorithms could also be adapted to screen large cohorts of eels, but regulation and legislation may be required before industry agrees to bear the associated cost.

The increase in parasite load we noted from 2017 to 2019 follows a trend that is also found all over Europe, where the parasite is established and is fast colonizing all the fresh water basins (Aguilar *et al*., 2005; Schabuss *et al*., 2005; Selim & El-ashram, 2012; Wielgoss, Taraschewski, Meyer, & Wirth, 2008). *A. crassus* has a very recent history in the Burrishoole catchment. First detection occurred in 2010 in a yellow eel in brackish water and in 2016 for the first time in a silver eel from freshwater (R. Poole, pers. comm.). In contrast to the rising burdens across much of Europe, in some lakes where the parasite had been detected since first discovery there is stabilization and even a slight decline in nematode abundance and intensities (Wielgoss *et al*., 2008). There is a possibility of an increased resistance towards the parasites in the long term (Schabuss *et al*., 2005). Although some evidence of increasing tolerance of *A. anguilla* to parasite infection, the overall impact of the parasite on the eel's mortality has been severe and is likely a contributor to the European population's steep decline impeding a recovery (Kirk, 2003; Molnar, England, & Martinod, 1993; Schabuss *et al*., 2005). Treatment of infected eels with anti-helminthic has not been trialled and a single vaccination study aimed at reducing development of adult from irradiated

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

L3 larvae was unsuccessful and revealed the antibody response is not a key element in resistance of *A. anguilla* against *A. crassus* (Knopf & Lucius, 2008). Infection control via physically blocking transmission, which requires extensive diagnostic testing, therefore represents the only feasible route to reducing population-wide parasite burden.

In this study, we developed potentially useful tool that can be deployed for specific parasite screening for the European eel. Cost per sample is low and the time to run a test comprising 16 samples is under 3 hours. Our test offers managers the opportunity to engage in infection control by assessing the disease status of adult eels before allowing transfers between river systems, although further work is required to establish whether in can survey juveniles. Nonetheless, the rapid test represents an important contribution to the conservation and management of this critically endangered species.

Accepted Manuscript

Supplementary material

All supplementary material is available alongside the article.

Data

All data generated or analysed during this study are included in this published article. The dataset supporting the conclusions of this article is available in the Supplementary material section. Supplementary table 1 reports all the data collected, Supplementary table 2 the DNA extraction protocol and Supplementary table 3 the DNA concentration of the faecal material extracted with the Qiagen method.

Acknowledgements

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

We would like to express our sincere gratitude to Dr Derek Evans who provided the samples from Lough Neagh, to Sara Gandy for the support in statistical analysis and to all the members of the Marine Institute in Newport, Ireland, for the immense help during sampling.

Author Contribution

MDN collected the data, analysed and wrote the manuscript draft. RP and JK helped with data collection and analysis, CW supported the data collection and experimental set up. CA and PM supervised the data collection and analysis. ML supervised the overall project, data analysis and writing of the manuscript. All authors read and approved the final manuscript.

Financial Support

This research was supported in part by a research grant from the BBSRC (grant number BB/P001203/1), by Science Foundation Ireland, the Marine Institute, Ireland (Beyond 2020 program) and the Department for the Economy, Northern Ireland, under the Investigators Program grant number SFI/15/IA/3028.

Conflicts of Interest

The authors declare there are no conflicts of interest.

Ethical Standards

The study was carried out under a Health Products Regulatory Authority (HPRA) license number AE19130-P096.

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

References

Aguilar, A., Álvarez, M. F., Leiro, J. M., & Sanmartín, M. L. (2005). Parasite populations of the European eel (Anguilla anguilla L.) in the Rivers Ulla and Tea (Galicia, northwest Spain). *Aquaculture*, *249*(1–4), 85–94. https://doi.org/10.1016/j.aquaculture.2005.04.052

Barry, J., Mcleish, J., Dodd, J. A., Turnbull, J. F., Boylan, P., & Adams, C. E. (2014). Introduced parasite Anguillicola crassus infection significantly impedes swim bladder function in the European eel Anguilla anguilla (L.). *Journal of Fish Diseases*, *37*(10), 921–924. https://doi.org/10.1111/jfd.12215

Becerra-Jurado, G., Cruikshanks, R., O'Leary, C., Kelly, F., Poole, R., & Gargan, P. (2014). Distribution, prevalence and intensity of Anguillicola crassus (Nematoda) infection in Anguilla anguilla in the Republic of Ireland. *Journal of Fish Biology*, *84*(4), 1046–1062. https://doi.org/10.1111/jfb.12344

Beregi, A., Molnár, K., Békési, L., & Székely, C. (1998). Radiodiagnostic method for studying swimbladder inflammation caused by Anguillicola crassus (Nematoda: Dracunculoidea). *Diseases of Aquatic Organisms*, *34*(2), 155–160. https://doi.org/10.3354/dao034155

Berger, C. S., & Aubin-Horth, N. (2018). An eDNA-qPCR assay to detect the presence of the parasite Schistocephalus solidus inside its threespine stickleback host. *Journal of Experimental Biology*, *221*(9). https://doi.org/10.1242/jeb.178137

Cavallero, S., Bruno, A., Arletti, E., Caffara, M., Fioravanti, M. L., Costa, A., … D'Amelio, S. (2017). Validation of a commercial kit aimed to the detection of pathogenic anisakid nematodes in fish products. *International Journal of Food Microbiology*, *257*(March), 75–79. https://doi.org/10.1016/j.ijfoodmicro.2017.06.011

Churcher, A. M., Pujolar, J. M., Milan, M., Huertas, M., Hubbard, P. C., Bargelloni, L., … Canário, A. V. M. (2015). Transcriptomic profiling of male European eel (Anguilla

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

anguilla) livers at sexual maturity. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, *16*, 28–35. https://doi.org/10.1016/j.cbd.2015.07.002

Crawley, J. A. H., Chapman, S. N., Lummaa, V., & Lynsdale, C. L. (2016). Testing storage methods of faecal samples for subsequent measurement of helminth egg numbers in the domestic horse. *Veterinary Parasitology*, *221*, 130–133. https://doi.org/10.1016/j.vetpar.2016.03.012

Crean, S. R., Dick, J. T. A., Evans, D. W., Elwood, R. W., & Rosell, R. S. (2003). Anal redness in European eels as an indicator of infection by the swimbladder nematode, Anguillicola crassus. *Journal of Fish Biology*, *62*(2), 482–485. https://doi.org/10.1046/j.1095-8649.2003.00034.x

Di Biase, A., Lokman, P. M., Govoni, N., Casalini, A., Emmanuele, P., Parmeggiani, A., & Mordenti, O. (2017). Co-treatment with androgens during artificial induction of maturation in female eel, Anguilla anguilla: Effects on egg production and early development. *Aquaculture*, *479*(November 2016), 508–515. https://doi.org/10.1016/j.aquaculture.2017.06.030

Didžiulis, V. (2013). NOBANIS – Invasive Alien Species Fact Sheet – Anguillicoloides crassus, (Weber 2000), 1–11. Retrieved from http://www.nobanis.org/files/factsheets/Pacifastacus_leniusculus.pdf

Fazio, G., Sasal, P., Mouahid, G., Lecomte-Finiger, R., & Moné, H. (2012). Swim Bladder Nematodes (Anguillicoloides crassus) Disturb Silvering In European Eels (Anguilla anguilla). *Journal of Parasitology*, *98*(4), 695–705. https://doi.org/10.1645/GE-2700.1

Frisch, K., Davie, A., Schwarz, T., & Turnbull, J. F. (2016). Comparative imaging of European eels (Anguilla anguilla) for the evaluation of swimbladder nematode (Anguillicoloides crassus) infestation. *Journal of Fish Diseases*, *39*(6), 635–647. https://doi.org/10.1111/jfd.12383

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

Godínez-González, C., Roca-Geronès, X., Cancino-Faure, B., Montoliu, I., & Fisa, R. (2017). Quantitative SYBR Green qPCR technique for the detection of the nematode parasite Anisakis in commercial fish-derived food. *International Journal of Food Microbiology*, *261*(May), 89–94. https://doi.org/10.1016/j.ijfoodmicro.2017.05.012

Haenen, O., Grisez, L., De Charleroy, D., Belpaire, C., & Ollevier, F. (1989). Experimentally induced infections of European eel Anguilla angutila with Anguillicola crassus (Nematoda, Dracunculoidea) and subsequent migration of larvae. *Diseases of Aquatic Organisms*, *7*, 97–101. https://doi.org/10.3354/dao007097

Hein, J. L., Buron, I. De, Roumillat, W. A., Post, W. C., Hazel, A. P., & Arnott, S. A. (2016). Infection of newly recruited American eels (Anguilla rostrata) by the invasive swimbladder parasite Anguillicoloides crassus in a US Atlantic tidal creek. *ICES Journal of Marine Science*, *73*, 14–21.

Heitlinger, E. G., Laetsch, D. R., Weclawski, U., Han, Y. S., & Taraschewski, H. (2009). Massive encapsulation of larval Anguillicoloides crassus in the intestinal wall of Japanese eels. *Parasites and Vectors*, *2*(1), 1–11. https://doi.org/10.1186/1756-3305-2-48

Henderson, P. A., Plenty, S. J., Newton, L. C., & Bird, D. J. (2012). Evidence for a population collapse of European eel (Anguilla anguilla) in the Bristol Channel. *Journal of the Marine Biological Association of the United Kingdom*, *92*(4), 843–851. https://doi.org/10.1017/S002531541100124X

Herrero, B., Vieites, J. M., & Espiñeira, M. (2011). Detection of anisakids in fish and seafood products by real-time PCR. *Food Control*, *22*(6), 933–939. https://doi.org/10.1016/j.foodcont.2010.11.028

Hole, K., & Nfon, C. (2019). Foot-and-mouth disease virus detection on a handheld real-time polymerase chain reaction platform. *Transboundary and Emerging Diseases*, *66*(4),

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

1789–1795. https://doi.org/10.1111/tbed.13227

Jousseaume, T., Roussel, J. M., Beaulaton, L., Bardonnet, A., Faliex, E., Amilhat, E., … Launey, S. (2021). Molecular detection of the swim bladder parasite Anguillicola crassus (Nematoda) in fecal samples of the endangered European eel Anguilla anguilla. *Parasitology Research*, *120*(5), 1897–1902. https://doi.org/10.1007/s00436-021-07100-3

Kirk, R. S. (2003). The impact of Anguillicola crassus on European eels. *Fisheries Management and Ecology*, *10*(6), 385–394. https://doi.org/10.1111/j.1365-2400.2003.00355.x

Knopf, K., & Lucius, R. (2008). Vaccination of eels (Anguilla japonica and Anguilla anguilla) against Anguillicola crassus with irradiated L3. *Parasitology*, *135*(5), 633–640. https://doi.org/10.1017/S0031182008004162

Kuwahara, N. & I. (1999). Anguillicola crassus DAISIE (Delivering, Alien Invasive Species Inventories for Europe). *DAISIE*, *34*(2), 81–82.

Laetsch, D. R., Heitlinger, E. G., Taraschewski, H., Nadler, S. A., & Blaxter, M. L. (2012). The phylogenetics of Anguillicolidae (Nematoda: Anguillicoloidea), swimbladder parasites of eels. *BMC Evolutionary Biology*, *12*(1). https://doi.org/10.1186/1471-2148-12-60

Lefebvre, F., Wielgoss, S., Nagasawa, K., & Moravec, F. (2012). On the origin of Anguillicoloides crassus, the invasive nematode of anguillid eels. *Aquatic Invasions*, *7*(4), 443–453. https://doi.org/10.3391/ai.2012.7.4.001

Levsen, A., Svanevik, C. S., Cipriani, P., Mattiucci, S., Gay, M., Hastie, L. C., … Pierce, G. J. (2018). A survey of zoonotic nematodes of commercial key fish species from major European fishing grounds—Introducing the FP7 PARASITE exposure assessment study. *Fisheries Research*, *202*(September 2017), 4–21.

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

https://doi.org/10.1016/j.fishres.2017.09.009

Melchers, W. J. G., Kuijpers, J., Sickler, J. J., & Rahamat-Langendoen, J. (2017). Lab-in-a-tube: Real-time molecular point-of-care diagnostics for influenza A and B using the cobas® Liat® system. *Journal of Medical Virology*, *89*(8), 1382–1386. https://doi.org/10.1002/jmv.24796

Molnar, P., England, P., & Martinod, J. (1993). Mantle dynamics, uplift of the Tibetan Plateau, and the Indian Monsoon. *Reviews of Geophysics*, *31*(4), 357–396. https://doi.org/10.1029/93RG02030

Muñoz, P., Peñalver, J., Ruiz de Ybañez, R., & Garcia, J. (2015). Influence of adult Anguillicoloides crassus load in European eels swimbladder on macrophage response. *Fish and Shellfish Immunology*, *42*(2), 221–224. https://doi.org/10.1016/j.fsi.2014.11.011

Newbold, L. R., Hockley, F. A., Williams, C. F., Cable, J., Reading, A. J., Auchterlonie, N., & Kemp, P. S. (2015). Relationship between European eel Anguilla anguilla infection with non-native parasites and swimming behaviour on encountering accelerating flow. *Journal of Fish Biology*, *86*(5), 1519–1533. https://doi.org/10.1111/jfb.12659

Paoletti, M., Mattiucci, S., Colantoni, A., Levsen, A., Gay, M., & Nascetti, G. (2018). Species-specific Real Time-PCR primers/probe systems to identify fish parasites of the genera Anisakis, Pseudoterranova and Hysterothylacium (Nematoda: Ascaridoidea). *Fisheries Research*, *202*(February 2017), 38–48. https://doi.org/10.1016/j.fishres.2017.07.015

Pelster, B. (2015). Swimbladder function and the spawning migration of the European eel Anguilla anguilla. *Frontiers in Physiology*, *6*(JAN), 1–10. https://doi.org/10.3389/fphys.2014.00486

Pouder, D. B., Curtis, E. W., & Yanong, R. P. E. (2009). Common Freshwater Fish Parasites

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

Pictorial Guide, 109–112.

Righton, D., Westerberg, H., Feunteun, E., Økland, F., Gargan, P., Amilhat, E., … Aarestrup, K. (2016). Empirical observations of the spawning migration of European eels: The long and dangerous road to the Sargasso Sea. *Science Advances*, *2*(10), 1–14. https://doi.org/10.1126/sciadv.1501694

Santos, A. T., Sasal, P., Verneau, O., & Lenfant, P. (2006). A method to detect the parasitic nematodes from the family Anisakidae, in Sardina pilchardus, using specific primers of 18 S DNA gene. *European Food Research and Technology*, *222*(1–2), 71–77. https://doi.org/10.1007/s00217-005-0052-8

Schabuss, M., Kennedy, C. R., Konecny, R., Grillitsch, B., Schiemer, F., & Herzig, A. (2005). Long-term investigation of the composition and richness of intestinal helminth communities in the stocked population of eel, Anguilla anguilla, in Neusiedler See, Austria. *Parasitology*, *130*(2), 185–194. https://doi.org/10.1017/S0031182004006444

Schneebauer, G., Dirks, R. P., & Pelster, B. (2017). Anguillicola crassus infection affects mRNA expression levels in gas gland tissue of European yellow and silver eel. *PLoS ONE*, *12*(8), 1–26. https://doi.org/10.1371/journal.pone.0183128

Selim, K. M., & El-ashram, A. M. (2012). STUDIES ON ANGUILLICOLIASIS OF CULTURED EEL (ANGUILLA ANGUILLA) IN EGYPT., (5), 409–425.

Shental, N., Levy, S., Wuvshet, V., Skorniakov, S., Shalem, B., Ottolenghi, A., … Hertz, T. (2020). Efficient high-throughput SARS-CoV-2 testing to detect asymptomatic carriers. *Science Advances*, *6*(37). https://doi.org/10.1126/sciadv.abc5961

Simon, J., Westerberg, H., Righton, D., Sjöberg, N. B., & Dorow, M. (2018). Diving activity of migrating silver eel with and without Anguillicola crassus infection. *Journal of Applied Ichthyology*, (December 2017), 1–10. https://doi.org/10.1111/jai.13626

Stojanović, M., Apostolović, M., Stojanović, D., Milošević, Z., Toplaović, A., Lakušić, V.

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

M., & Golubović, M. (2014). Understanding sensitivity, Specificity and predictive values. *Vojnosanitetski Pregled*, *71*(11), 1062–1065. https://doi.org/10.2298/VSP1411062S

Thomas A. Hall. (2017). BioEdit: user-friendly biological sequebce alignment editor and analysis program for windows 95/98/NT. *Nucleic Acid Symposium*. https://doi.org/10.1039/c7qi00394c

Vieira, M. L. C., Santini, L., Diniz, A. L., & Munhoz, C. de F. (2016). Microsatellite markers: What they mean and why they are so useful. *Genetics and Molecular Biology*, *39*(3), 312–328. https://doi.org/10.1590/1678-4685-GMB-2016-0027

Weclawski, U., Heitlinger, E. G., Baust, T., Klar, B., Petney, T., Han, Y. S., & Taraschewski, H. (2013). Evolutionary divergence of the swim bladder nematode Anguillicola crassus after colonization of a novel host, Anguilla anguilla. *BMC Evolutionary Biology*, *13*(1). https://doi.org/10.1186/1471-2148-13-78

Wielgoss, S., Taraschewski, H., Meyer, A., & Wirth, T. (2008). Population structure of the parasitic nematode Anguillicola crassus, an invader of declining North Atlantic eel stocks. *Molecular Ecology*, *17*(15), 3478–3495. https://doi.org/10.1111/j.1365-294X.2008.03855.x

Zou, Y., Mason, M. G., Wang, Y., Wee, E., Turni, C., Blackall, P. J., … Botella, J. R. (2017). Nucleic acid purification from plants, animals and microbes in under 30 seconds. *PLoS Biology*, *15*(11), 1–22. https://doi.org/10.1371/journal.pbio.2003916

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

Table 1. Primer name, direction of amplification, primer size expressed in base pairs and specific designed sequence.

Primer Name	Direction	Primer size bp	Primer sequence
AcrasCO1_fwd	5′ -> 3′	26	CCATTCTGGTATAAGTGTTGATCTCG
AcrasCO1_rev	3′ -> 5′	30	ACAACCTCATATGTTCTARAGTAATAGAT

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0031182021002146

Table 2. The criteria for Specificity, Sensitivity NPV (Negative Predictive Value) and PPV (Positive Predictive Value) as applied to a rapid test for *A. crassus*.

		Dissection result			
Test result		Infected	Not infected		
	Positive	True Positive (TP)	False Positives (FP)	Sensitivity=(TP)/(TP+FN)	PPV=(TP)/(TP+FP)
	Negative	False Negatives (FN)	True Negatives (TN)	Specificity=(TN)/(TN+FP)	NPV=(TN)/(TN+FN)

Animals that are infected and test positive are considered True Positive (TP). Animals that are infected and test negative are described as False Negative (FN). Animals that have no visible parasites, but test positive are False Positive (FP), and those that have no visible parasites but test negative are True Negative (TN).

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0031182021002146

Table 3. Relative *A. crassus* detection for the two different extractions methods across the 3 sampling seasons.

Extraction Method	Year	No. samples	Observed Infected	Test positive	Test Negative	False Positive	False Negative
Qiagen	2017	12	8	3	2	3	4
Qiagen	2018	23	12	4	8	2	9
Qiagen	2018n	41	28	20	10	4	7
Qiagen	2019	28	18	16	4	6	2
Whatman paper	2019	28	18	19	9	2	1
Whatman paper	2019s	27	23	22	4	0	1

For 2019 the same samples were tested with both methods. 2018n considers samples collected in Lough Neagh and 2019s silver eels collected in the Burrishoole catchment.

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0031182021002146

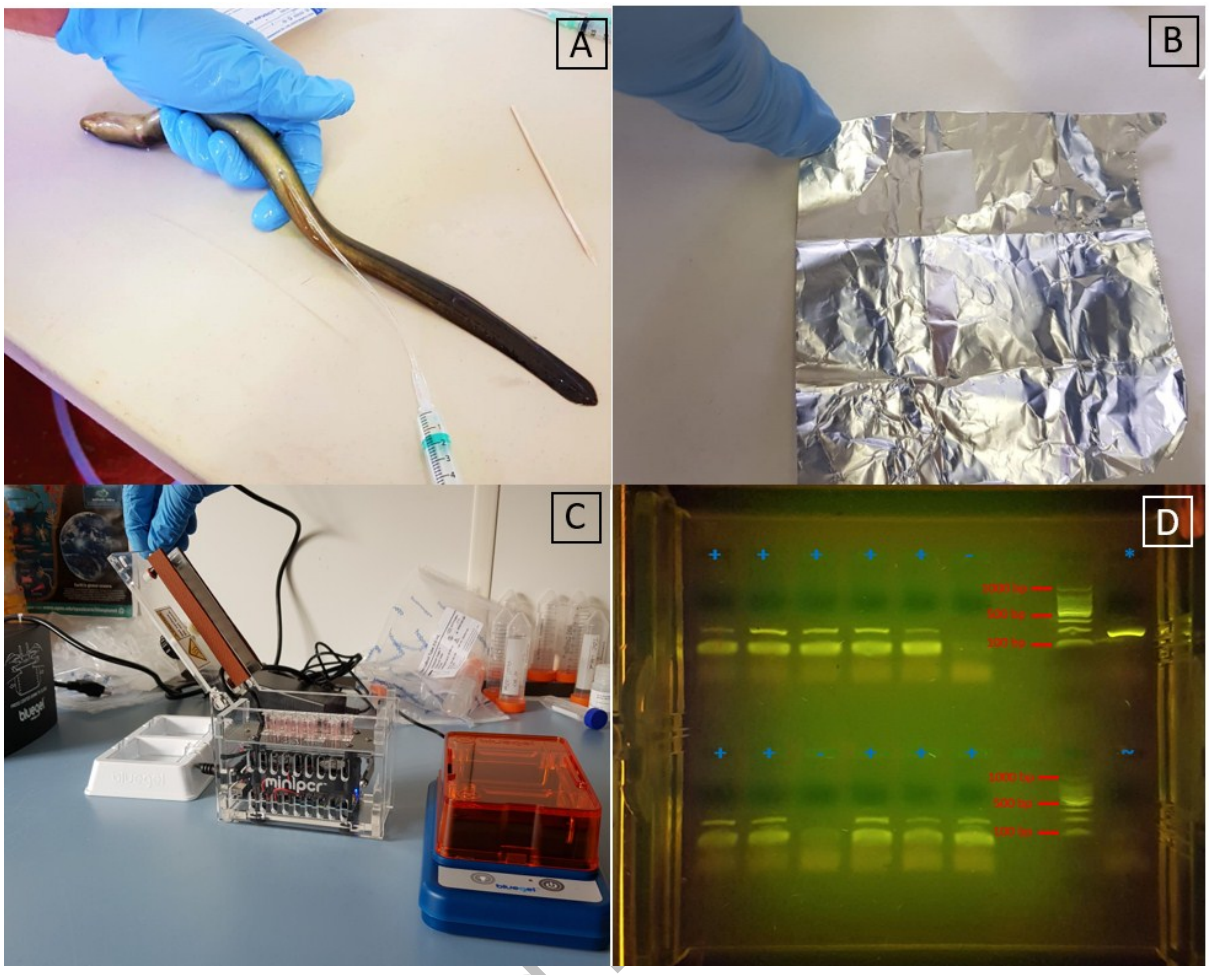


Figure 1. Experimental procedure for rapid, *in situ* and non-lethal molecular detection of *A. crassus* from the European eel. A) Colonic irrigation with sterile saline solution (9 ‰) on an anesthetized yellow eel. B) Collection of a drop of faecal material on a piece of Whatman qualitative filter paper No. 1. C) *In situ* DNA extraction and diagnostic PCR with MiniPCR thermocycler. D) *In situ* visualization on electrophoresis agarose gel 2 % on amplified target CO1 gene. "+" Positive amplification from faecal extracted DNA, "-" Negative amplification from faecal extracted DNA, "*" Positive control, "~" Negative control. The amplified fragment can be visualized around 187 bp. The band below represents resultant primer dimer.

Accepted

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146

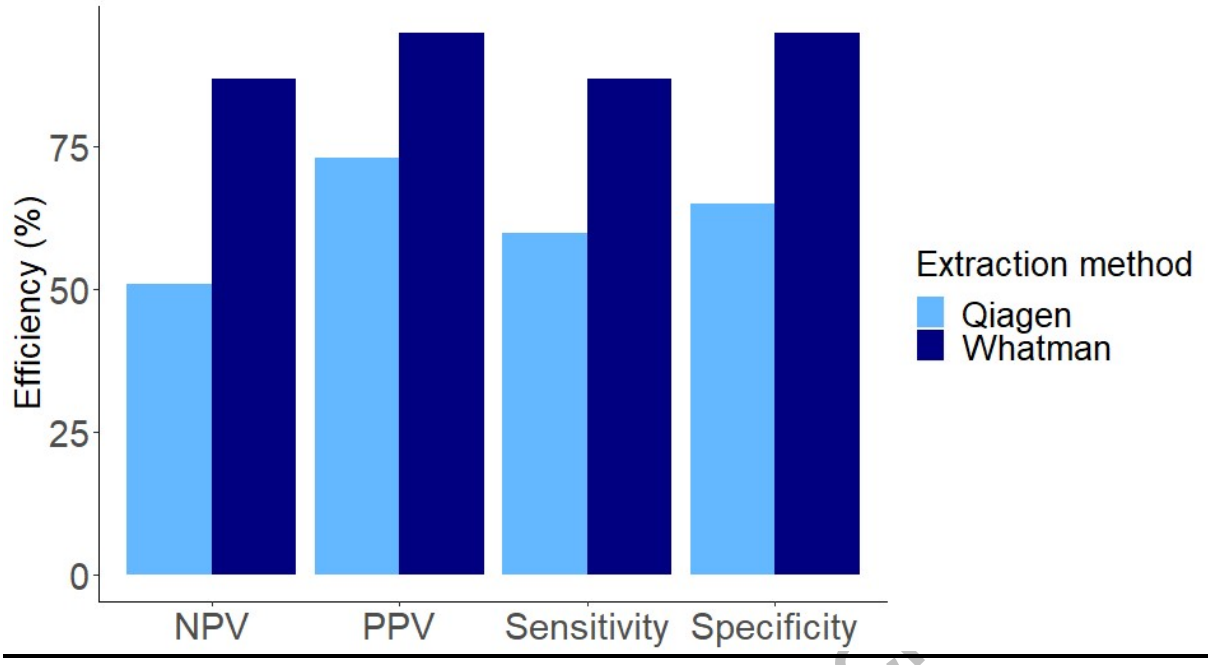


Figure 2. Relative *A. crassus* parasite detection efficiency for the two DNA extraction methods. The Whatman DNA extraction method (dark blue bar) performs better in all the categories with an average improvement of 41% over the Qiagen method (pale blue bar). NPV= Negative predictive value, PPV = Positive predictive value. A Welch two-sample T-test indicates both sensitivity ($p<0.006$) and specificity ($p<0.003$) were significantly improved by using the Whatman protocol.

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/S0031182021002146

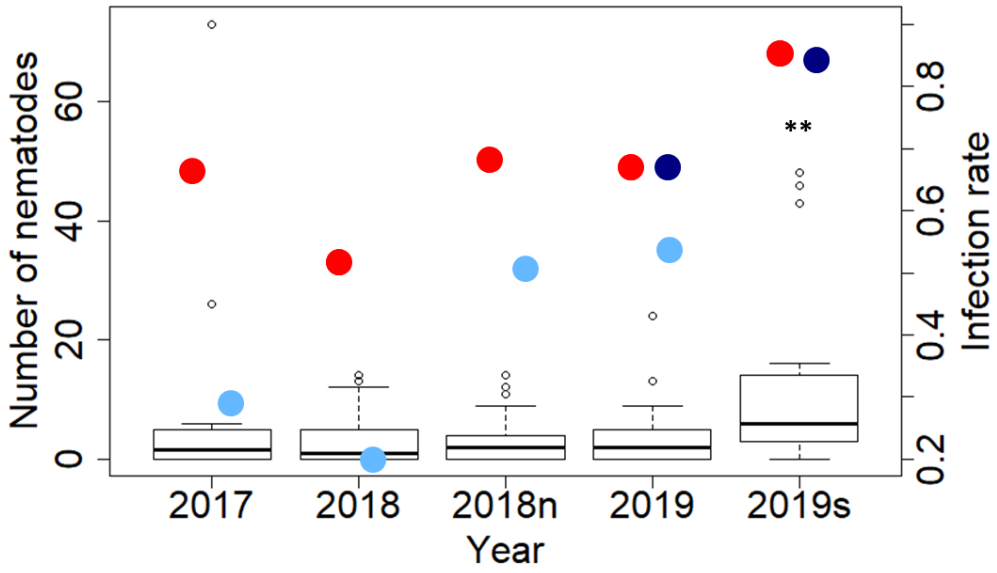


Figure 3. Number of *A. crassus* counted in dissected animals and infection rate in different years of sampling. Infection prevalence represents the number of animal infected compared to the total number of animals. Dark line in each box stands for the mean number of nematode per cohort of sampling. Red dots show the actual infection rate based on average parasite load in dissected animals, each empty dots stands for a single dissected eel. Light Blue dots indicate the infection rate derived from the extraction using Qiagen Blood and Stool kit. Dark Blue dots indicates the infection rate observed with Whatman. A Mann-Whitney test shows that silver eels in 2019 were significantly more infected than other eels (P value < 0.05). "2018n" refers to samples collected in Lough Neagh and "2019s" to silver eels collected in the Burrishoole system.

Accepted Manuscript

Downloaded from https://www.cambridge.org/core. University College Cork, on 25 Jan 2022 at 13:18:47, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0031182021002146