INTRODUCTION

Invasion by non-native parasites can affect the viability of novel hosts (Daszak et al., 2000; Peeler et al., 2011), posing strong selection pressure on the host to adapt (Penczykowski et al., 2011). Adaptation to novel parasites has been suggested for a number of species. For example, a Hawaiian honeycreeper species shows signs of increased tolerance to avian malaria after severe population declines following the parasite's introduction (Atkinson et al., 2013). Both increased tolerance and increased resistance were reported in blue mussels after the introduction of a parasitic copepod (Feis et al., 2016). Increased resistance was also observed in a rainbow trout population in response to an invasive myxozoan parasite (Miller & Vincent, 2008).

Anguillicola crassus Kuwahara, Niimi & Hagaki is a parasitic swim bladder nematode that is invasive in the European eel (Anguilla anguilla, L.). It was first detected in European freshwaters in 1982, and it has rapidly spread across the entire range of its new host (Kirk, 2003). Infections with the parasite were suggested to hamper the trans-oceanic spawning migration and reproduction of the European eel (A. anguilla L.), and there is evidence that encapsulation frequency has increased since the introduction of A. crassus. We examined whether encapsulation of A. crassus provides an advantage to its novel host in Lake Müggelsee, NE Germany. We provide the first evidence that encapsulation was associated with reduced abundance of adult A. crassus. This pattern was consistent in samples taken 3 months apart. There was no influence of infection on the expression of the two metabolic genes studied, but the number of capsules was negatively correlated with the expression of two mhc II genes of the adaptive immune response, suggesting a reduced activation. Interestingly, eels that encapsulated A. crassus had higher abundances of two native parasites compared with non-encapsulating eels. We propose that the response of A. anguilla to infection by A. crassus may interfere with its reaction to other co-occurring parasites.

KEYWORDS
Anguilla anguilla, Anguillicola crassus, gene expression, invasive parasite, parasite community
in defending against A. crassus, resulting in killing and encapsulating a large proportion of larvae (Knöpf & Lucius, 2008; Knöpf & Mahnke, 2004; Weclawski et al., 2013). Encapsulation of helminth parasites has been described as a defence mechanism involving an immune response in several fish species, including eels (Dezfuli et al., 2015, 2016). Naturally infected European eels can encapsulate A. crassus larvae, and the immune system was shown to take part in this process (Molnár, 1994). However, the associated costs to the host are not known. Nonetheless, the frequency of encapsulation increased from 0% in 1990 to 20% in 1997 and 2000 in Flanders, Belgium (Audenaert et al., 2003). This suggests that the European eel may be capable of developing strategies to cope with the novel parasite.

European eels infected with A. crassus are susceptible to adverse environmental conditions (Molnár et al., 1991). Mortality during hypoxia increases with severity of infection (Lefebvre et al., 2007; Molnár, 1993). Infected eels consume more oxygen during activity than non-infected, thus having a higher energy demand (Palstra et al., 2007). This may be due to increased allocation of resources to the immune system and resource consumption by the parasite itself. Consistent with higher oxygen demand with infection severity, the expression of the haemoglobin α gene was correlated with parasite biomass in experimental infections (Fazio et al., 2009). Down-regulation of several cytochrome genes of the cell respiration pathway indicated that energy provision may be compromised in experimentally infected European eels, which may be due to alteration of resource allocation in infected individuals (Bracamonte, Johnston, Monaghan, et al., 2019).

Populations regularly differ in their resistance to parasites, and differences appear to be related to the degree of exposure and adaptation (MacColl & Chapman, 2010; Weber et al., 2017). Populations that are adapted to a particular parasite have been observed to mount a stronger immune response when challenged with that parasite (Kalbe & Kurtz, 2006; Scharsack & Kalbe, 2014). Increased immune gene expression has been associated with higher resistance in fish (Lenz et al., 2013; Lohman et al., 2017), birds (Bonneaud et al., 2011) and mammals (Guo et al., 2016). At the same time, there is evidence that the expression of non-immune genes is differentially affected in populations that differ in parasite resistance (Bonneaud et al., 2011). Additionally, individuals that are better at coping with a particular parasite tend to grow more, have better body conditions and have higher metabolic condition when infected, suggestive of reduced metabolic and energetic costs (Kalbe & Kurtz, 2006; Kurze et al., 2016; MacColl & Chapman, 2010). Based on these observations in other species, we hypothesize that encapsulating A. crassus leads to lower infection intensities, an increased immune response and reduced metabolic costs in the European eel.

From a natural population of European eels in Lake Müggelsee, Berlin, Germany, we identified A. crassus infection intensity and macroparasite community composition and compared them between eels encapsulating A. crassus and those not encapsulating it. We further tested for temporal variation of these parameters between August and October. We chose these two dates to determine whether the observed pattern was temporarily stable, because A. crassus infections varied temporally in some locations (Lefebvre et al., 2002; Schabuss et al., 2005) but not in others (Kennedy & Fitch, 1990; Würtz et al., 1998) and we had no information about the situation in Lake Müggelsee. We used quantitative PCR to test whether eels encapsulating and those not encapsulating the parasite differed in immune (mhc II), energy-related (cox1) and haematopoietic (epor) gene expression, suggestive of an increased immune response and reduced metabolic costs (see Section 2 for information on target genes). As for infection intensity and parasite community, we tested whether gene expression responses showed temporal variation. Genes were selected based on differential expression in transcriptome-wide expression studies on European eels and Japanese eels experimentally infected with A. crassus. Mhc IIA and mhc IIB both had altered expression profiles in experimentally infected European eels (Bracamonte, Johnston, Knöpf, et al., 2019; Bracamonte, Johnston, Monaghan, et al., 2019). We hypothesized that encapsulation in a natural population may trigger an immune response and lead to increased expression of mhc II genes. Cox1 expression was reduced in European eels experimentally infected with A. crassus (Bracamonte, Johnston, Monaghan, et al., 2019). We hypothesized that the disruption of the energy balance would be mitigated by encapsulation, resulting in higher expression of cox1. The expression of epor was increased in Japanese eels following A. crassus infections (Bracamonte, Johnston, Monaghan, et al., 2019). We expected increased expression in more heavily infected European eels, especially in the presence of blood-feeding adults. Furthermore, we expected reduced expression in individuals encapsulating A. crassus if encapsulation led to reduced infection intensity.

2 | MATERIALS AND METHODS

2.1 | Sampling

European eels were caught by electrofishing near Surferwiese (52.448°N 13.656°E) in Lake Müggelsee, Germany, on 8 August 2017 (n = 13) and on 10 and 17 October 2017 (n = 25). Electrofishing for sampling the fish for this study was approved by the responsible fisheries authority (Fischereiamt Berlin). Eels were immediately decapitated, immobilized by destruction of the spinal cord and kept on ice for transportation back to the laboratory. Dissections were carried out approximately 1 hr after electrofishing. The spleen and the head kidney were removed and stored at −20°C in RNaLater (Life Technologies) following the manufacturer’s instructions. For all individuals, weight was determined to the nearest g and total length (TL) to the nearest 0.5 cm. Relative condition factor (Ks) was calculated according to Le Cren (1951) using the values available from FishBase (Froese & Pauly, 2019). Anguillicola crassus in the swim bladder and other parasites on the gills, in the gut, the anal fin and the eyes were counted using a stereomicroscope (7×–70× magnification). The intestinal cestodes Bothrioccephalus claviceps and Proteocephalus macrocephalus were combined, because they were assumed to affect
their host in a similar way and because they could not always be distinguished during dissection. Similarly, the gill monogeneans *Pseudodactylogyrus bini* and *Pseudodactylogyrus anguilae* were not distinguished. Cysts formed by the myxozoans *Myxobolus portocalensis* on the anal fin and *Myxidiurn giardi* on the gills and the gill monogenean *Pseudodactylogyrus* spp. were categorized into abundance classes of 0, 1–5, 6–20 and >20 (on the anal fin or per gill arch; Table S1). The number of encapsulated *A. crassus* larvae (Figure S1) in the swim bladder wall was recorded. We performed PCRs on a subset of capsules following Heitlinger et al. (2009). We checked the size of PCR products on a gel to confirm that the capsules contained *A. crassus* tissue.

### 2.2 Analysis of parasite communities

All analyses were done in R v3.5.3 (R Core Team, 2019). Prevalence, mean infection intensity and mean abundance of larval *A. crassus* in the third (*L* 3) and fourth (*L* 4) larval stages, adults and all stages combined (simply referred to as *A. crassus*) were calculated for all eels and separately for each month. We determined the prevalence of encapsulated larvae. We also calculated infection intensity and abundance of larval and adult stages only including eels that contained living *A. crassus* in the swim bladder and for which the encapsulation status (presence/absence of capsules) could be determined unambiguously (*n* = 32). For these eels, we used Wilcoxon rank-sum tests to estimate whether weight and length differed between encapsulation status or sampling month. Six eels either did not contain living *A. crassus* in their swim bladders or were of uncertain encapsulation status and were excluded from further analyses (i.e. parasite community analysis and gene expression analysis; Table S1).

We assessed differences in total abundance, larval abundance and adult abundance of *A. crassus* with generalized linear models (GLMs) that included encapsulation status, sampling month and their interaction as factors, each with a negative binomial distribution with a log-link function using the glm.nb function of the MASS package. Model assumptions were tested with the DHARMa package v0.3.2.0 (Hartig, 2020). We further used GLMs with the same parameters to assess whether abundances were a function of the number of capsules rather than the encapsulation status. We ran these latter models including all individuals and including only individuals encapsulating *A. crassus*. We correlated the total number of *A. crassus*, the number of larval *A. crassus* and the number of adult *A. crassus* with weight and TL using Spearman’s rank correlation tests with the cor.test function in base R. We performed these tests once for all eels and then separately for eels sampled in August and October and for eels of the non-encapsulating group (i.e. without capsules, NC) and the encapsulating group (i.e. with at least one capsule, C). GLMs were used to test whether prevalences of each parasite differed between encapsulation status and sampling month applying a binomial distribution with a logistic link function. Similarly, GLMs with a Poisson distribution with a log-link function were used to test for differences in abundances. Differences in parasite community composition, excluding *A. crassus* and species with <10% overall prevalence, were determined with an analysis of similarity (Clarke, 1993) on Bray–Curtis distances using the function anosim of the vegan package v2.4-4 (Oksanen et al., 2019) with 100,000 permutations. Parasite communities were compared between the two months and the two encapsulation status. Species that contributed most to parasite community dissimilarities were identified with a similarity percentage analysis (simper) implemented in the vegan package. For visualization, non-metric multidimensional scaling plots were produced with the function metaMDS of the vegan package.

### 2.3 RNA extraction and cDNA synthesis

RNA was extracted from spleen and head kidney tissue as described in Bracamonte, Johnston, Monaghan, et al. (2019) and quantified on a NanoDrop 1000 Spectrophotometer (Thermo Scientific). Remnant DNA was removed from RNA extracts with DNase I, Amplification grade (Thermo Fisher Scientific) following the manufacturer’s instructions. Purified RNA was reverse-transcribed in duplicates with MMLV High Performance Reverse Transcriptase (Biozym) following the manufacturer’s instructions. For quantitative real-time PCR (qPCR), duplicate reverse transcriptions were pooled.

### 2.4 Target genes

The selected genes responded to experimental infection with *A. crassus* in previous studies, based on transcriptome-wide gene expression (Bracamonte, Johnston, Knopf, et al., 2019; Bracamonte, Johnston, Monaghan, et al., 2019). Genes had either increased expression (*mhc IIB* and *epor*) or decreased expression (*mhc IIA* and *cox1*) in the European eel or the Japanese eel (divergence time approx. 20 Mya; Minegishi et al., 2005). Furthermore, the genes are involved in physiological processes that were previously shown to respond to infection in the European eel (Fazio et al., 2009; Knopf & Lucius, 2008; Palstra et al., 2007). The major histocompatibility complex class II (MHC II) is essential for initiating an adaptive immune response against extracellular parasites, which ultimately results in highly specific antibody production (Morris et al., 1994). An MHC II molecule is composed of two chains encoded by genes *mhc IIA* and *mhc IIB*. *Mhc IIA* is usually more polymorphic, providing higher antigen specificity (Brown et al., 1993; Reche & Reinherz, 2003). However, in European eels *mhc IIA* may be equally variable (Bracamonte et al., 2015). Cytochrome c oxidase subunit 1 (COX1) is a core protein of the respiratory chain, which is responsible for energy generation (Hosler et al., 2006). The erythropoietin receptor (EPOR) is expressed on the progenitors of erythrocytes during...
their maturation and promotes their proliferation and differentiation (Elliott et al., 2014).

2.5 Primer design and qPCR

We carried out qPCR using a combination of newly designed and published primers (Table 1). We newly designed primers for cox1, epor and the housekeeping gene β-actin (actb) using sequences from two European eel transcriptome assemblies (Bracamonte, Johnston, Knopf, et al., 2019; Bracamonte, Johnston, Monaghan, et al., 2019) and other data as follows. For cox1, we included sequences of European eels and American eels (A. rostrata) available on NCBI (acc. nos: NC_006531.1 and NC_006547.2) and sequences from EeelBase (Coppe et al., 2010). For epor, no anguillid sequences were available in public databases; therefore, we included sequences of a Japanese eel transcriptome assembly for primer design (Bracamonte, Johnston, Monaghan, et al., 2019). For actb, we used a published reverse primer (Fazio, Mone, et al., 2008) and a new forward primer designed using European eel and Japanese eel sequences available on NCBI (acc. nos: DQ286836.1, KJ021893.1 and GU001950.1) and EeelBase (Coppe et al., 2010). Primers for both mhc II genes were modified from Bracamonte et al. (2015).

Primers were validated in regular PCRs using the Biozym Probe qPCR Kit (Biozym). PCRs were carried out in a volume of 20 μl containing 10 μl of 2x qPCR Probe Mix, 400 nM of forward and reverse primer, and 2 μl of cDNA for the genes actb, cox1, mhc IIA and mhc IIB. PCR for epor contained 500 nM of each primer. For genes actb, cox1, mhc IIA and mhc IIB, cycling conditions were as follows: initial denaturation at 95°C for 2 min, 30 cycles of 95°C for 5 s and 65°C for 30 s, and a final elongation at 65°C for 10 min. For epor, the number of cycles was increased to 40. A Mastercycler nexus GSX1 (Eppendorf) was used for all PCRs. PCR products were purified and sequenced at Macrogen Europe. Sequences were aligned back to those used for primer design, and they were blasted against the nr protein database of NCBI for identity confirmation.

For the qPCRs, the reaction mix was identical to that used for regular PCRs (above) except that 0.0006 μl 10,000× SYBR Green I Nucleic Acid Gel Stain (Invitrogen) was added. Reactions were run on a Stratagene Mx3005P qPCR System (Agilent Technologies) with the cycling conditions described above, but omitting the final elongation. The number of cycles was set to 40 for all genes. qPCRs were run in duplicates, and a fivefold dilution series was added on each plate as standard curve. One plate was prepared for each gene and tissue containing all samples and duplicates. Ct values were determined with MxPro QPCR Software (Agilent Technologies) using default parameters. Samples for which Ct values between duplicates differed by more than 0.5 were repeated (mixed plates for genes and tissues).

2.6 Gene expression analysis

Relative expression of the target genes was calculated for every sample following the ΔΔCt method of Pfaffl (2001) using actb as reference gene. The 1:5 dilution of the standard curve was used as a calibrator to standardize among plates. Since tissues are known to differ in gene expression, the spleen and the head kidney were analysed separately. Analyses were performed separately for every gene in R. GLMs were used to analyse relative gene expression changes as a function of encapsulation status, sampling month and their interaction. Model assumptions were tested as above. Tukey’s HSD post hoc tests were performed for models with significant factors using the multcomp v1.4-8 (Hothorn et al., 2008) package. Relative gene expression changes were further analysed with infection intensity of A. crassus as continuous predictor, sampling month as a factor and their interaction. The same analyses were performed with the number of adult A. crassus as continuous predictor, sampling month as

| Gene | Primer name | Sequence 5’→3’ | Amplicon size | Source |
|------|-------------|---------------|--------------|--------|
| Actb | ACTBF2      | GAGACCACCTTCAACTCC | 196 bp | Present study |
|      | Actin R     | TCCAGACGGGAGTATTTCG | | Fazio, Mone, et al. (2008) |
| Cox1 | COX1F2      | CTACTCTCTCTCTCGCATGT | 150 bp | Present study |
|      | COX1R2      | GTATCTTCTGGTGGGCCGA | | Present study |
| Epor | EPORF1      | ACAATGACCGAGACGGAGA | 142 bp | Present study |
|      | EPORR1      | CCTTCACCAATTCGCCGTTG | | Present study |
| Mhc IIA | MHCIIAE3F | GATCCTCTCAGAGGACACATCT | 250 bp | Modified from Bracamonte et al. (2015) |
|      | MHCIIAE3R | TGTCCTCAGGCAGGGA | | Modified from Bracamonte et al. (2015) |
| Mhc IIB | MHCIIBE3F | TTACTCCCCAGAGGAATCACAATGAC | 167 bp | Bracamonte et al. (2015) |
|      | MHCIIBE3R | TGCTCCACCTGAGAGGATTT | | Modified from Bracamonte et al. (2015) |

Abbreviation: bp, base pairs.
TABLE 2 Prevalence (P, %), mean intensity ± SE (I) and mean abundance ± SE (A) of Anguillicola crassus stages. The prevalence of encapsulated A. crassus is indicated. Parameters are given for all sampled eels (overall) and separately for each sampling month and each encapsulation status.

|         | Sum | Larvae (L₃ + L₄) | Adults | Capsules |
|---------|-----|-----------------|--------|----------|
|         | n   | P    | I     | A      | P    | I    | A    | A      | P    |
| Overall | 38  | 89   | 8.7 ± 1.6 | 7.8 ± 1.5 | 79   | 6.8 ± 1.3 | 5.4 ± 1.2 | 55 | 4.3 ± 1.0 | 2.4 ± 0.8 | 39  |
| August  | 13  | 92   | 7.8 ± 2.0 | 7.2 ± 1.9 | 85   | 6.1 ± 2.1 | 5.2 ± 2.0 | 46 | 4.3 ± 1.4 | 2.0 ± 1.0 | 46  |
| October | 25  | 88   | 9.2 ± 2.2 | 8.1 ± 2.1 | 76   | 7.3 ± 1.7 | 5.5 ± 1.5 | 60 | 4.3 ± 1.3 | 2.6 ± 1.0 | 36  |
| NC      | 19  | 100  | 10.2 ± 2.6 | 10.2 ± 2.6 | 84   | 7.2 ± 2.0 | 6.1 ± 1.8 | 79 | 5.1 ± 1.6 | 4.1 ± 1.4 | 0   |
| C       | 13  | 100  | 5.6 ± 1.5 | 5.6 ± 1.5 | 92   | 5.2 ± 1.6 | 4.8 ± 1.5 | 38 | 2.0 ± 0.5 | 0.8 ± 0.3 | 100 |

Abbreviations: C, encapsulating eels; n, number of eels examined; NC, non-encapsulating eels.

aIncludes only infected individuals for which the encapsulation status could be determined unambiguously.

TABLE 3 GLM statistics for (a) infection intensity with Anguillicola crassus and (b) gene expression. Only parameters of interest are shown. Significant deviance values (p < .05) are indicated with bold text.

| Response variable | Group | Month | Group × month |
|-------------------|-------|-------|---------------|
|                   | Deviance | p-value | Deviance | p-value | Deviance | p-value |
| a Sum             | 3.65 | 0.056 | 0.19 | 0.66 | 1.18 | 0.28 |
| Larvae            | 0.39 | 0.53 | 0.65 | 0.42 | 2.20 | 0.14 |
| Adults            | 9.24 | 0.002 | 0.03 | 0.87 | 0.01 | 0.93 |
| b Cox1 spleen     | 0.29 | 0.17 | 0.40 | 0.11 | 0.01 | 0.74 |
| Cox1 head kidney  | 2.29 | 0.14 | 6.56 | 0.012 | 1.29 | 0.27 |
| Epor spleen       | 0.49 | 0.56 | 7.17 | 0.027 | <0.01 | 0.99 |
| Epor head kidney  | 0.38 | 0.37 | 0.04 | 0.76 | <0.01 | 0.97 |
| Mhc IIA spleen    | 0.95 | 0.11 | 5.49 | 0.001 | 1.19 | 0.07 |
| Mhc IIA head kidney | 0.41 | 0.44 | 0.20 | 0.59 | 5.84 | 0.003 |
| Mhc IIB spleen    | 0.29 | 0.44 | 2.94 | 0.014 | 0.06 | 0.72 |
| Mhc IIB head kidney | 1.09 | 0.12 | 0.09 | 0.65 | 2.18 | 0.028 |

Note: Group = non-encapsulating versus encapsulating and Month = August versus October.

3 | RESULTS

Mean weight ± SE, mean TL ± SE and mean K_rel ± SE were 122 ± 15 g, 40.6 ± 1.5 cm and 0.948 ± 0.014, and they did not differ significantly between C and NC eels (Wilcoxon’s rank-sum test, weight: W = 97, p = .32; length: W = 94.5, p = .27; K_rel; W = 135, p = .67). Weight and TL were greater in August than in October (W = 171, p = .029 for both weight and TL, August: 166 ± 26 g and 45.5 ± 2.6 cm, October: 100 ± 16 g and 38.0 ± 1.6 cm), but there were no differences in K_rel (W = 111, p = .87, August: 0.941 ± 0.022, October: 0.951 ± 0.018).

3.1 | Parasite community

The abundance of A. crassus in the swim bladder ranged from 0 to 46, with 0–35 larvae and 0–25 adult worms. Encapsulated A. crassus
were found in 39% of the eels. Infection parameters are summarized in Table 2. Mean infection intensity was 1.8 times higher in NC eels than in C eels, although the difference was not significant (Tables 2 and 3). Mean abundance of adult *A. crassus* was 5.3 times lower in C eels than in NC eels (Figure 1a; Table 3). Mean abundance of larval *A. crassus* did not differ with encapsulation status (Figure 1b; Table 3). Neither infection intensity nor abundance differed with sampling month. Infection intensity and adult abundance decreased with an increasing number of capsules when including both NC and C eels (intensity: Dev = 5.42, *p* = .020; abundance: Dev = 9.52, *p* = .002). However, this relationship did not hold in C eels only (intensity: Dev = 2.59, *p* = .11; abundance: Dev = 1.37, *p* = .24). There was no relationship between larval abundance and the number of capsules (NC + C: Dev = 1.15, *p* = .28; C: Dev = 1.40, *p* = .24). Heavier and larger individuals did not harbour more *A. crassus* or more larval stages for any encapsulation status or sampling month. The number of adult parasites was positively correlated with eel weight and TL for the NC group (Spearman’s rank correlation test, rho = 0.50, *p* = .028, for both weight and TL) but not the C group (weight: rho = 0.14, *p* = .64; TL: rho = 0.20, *p* = .50). There was no correlation between the number of adult parasites and weight or TL in August or October.

Overall prevalence of the native parasites across both sampling months and encapsulation status ranged from 6% for *Camallanus lacustris* to 78% for *Myxidium giardi* (Table 4). The invasive *Pseudodactylogyrus* species (*P. bini* and *P. anguillae*) had a prevalence of 100%. Overall mean infection intensities ± SE ranged from 1 for *Diplostomum* sp. to 1.5 ± 0.3 for cestodes (*B. claviceps* and *P. macrocephalus*) and *Ergasilus gibbus* and were thus low compared with *A. crassus*. Infections with *Pseudodactylogyrus* spp., *M. giardi* and *Myxobolus portucalensis* were categorized into unequally sized intervals; therefore, abundances and intensities could not be calculated. *Diplostomum* sp. and *C. lacustris* had low prevalences (<10%) and were excluded from analyses of parasite community composition. The prevalence of cestodes and *E. gibbus* was 2.6 times higher in C group eels compared with NC group eels and that of *M. portucalensis* was 2.2 times higher, though none of the differences was significant (Dev = 3.68, *p* = .055, for cestodes and *E. gibbus* and Dev = 2.25, *p* = .13, for *M. portucalensis*; Table 4). The prevalence of *M. giardi* did not differ between groups. *M. portucalensis* was 7.6 times more prevalent in August than in October (Dev = 12.37, *p* = .0004; Table 4). The abundance of cestodes and *E. gibbus* was significantly higher in C group eels than NC group eels (Dev = 4.00, *p* = .046, for both). Although the categorization of *Pseudodactylogyrus* spp., *M. portucalensis* and *M. giardi* did not allow for the testing of differences in

![Graph showing infection intensities of (a) larval Anguillicola crassus and (b) adult A. crassus in the swim bladder of eels with unambiguous encapsulation status.](image)

**Table 4** Prevalence (P, %) of parasites other than Anguillicola crassus in eels that were included in the gene expression analyses. Parameters are given for all eels and separately for each encapsulation status and each sampling month.

| Parasite                | Location | Overall | NC    | C    | August | October |
|-------------------------|----------|---------|-------|------|--------|---------|
| *Camallanus lacustris*  | Intestine| 34      | 21    | 54   | 36     | 33      |
| *Pseudodactylogyrus* sp.| Intestine| 6       | 5     | 8    | 9      | 5       |
| *Myxidium giardi*       | Gill     | 78      | 79    | 77   | 64     | 86      |
| *Ergasilus gibbus*      | Gill     | 34      | 21    | 54   | 45     | 29      |
| *Myxobolus portucalensis* | Fins    | 31      | 21    | 46   | 73     | 10      |
| *Diplostomum* sp.       | Eyes     | 9       | 11    | 8    | 18     | 5       |
| n                       |          | 32      | 19    | 13   | 11     | 21      |

Abbreviations: C, encapsulating eels; n, number of eels examined; NC, non-encapsulating eels.

*Bothriocephalus claviceps* and *Proteocephalus microcephalus.*
abundance, categories suggest higher abundance in August than October for *Pseudodactylogyrus* spp. and *M. portucalensis*, but not *M. giardi*, and no difference between C and NC group eels for any of the three parasites. An analysis of similarity revealed moderate differences in parasite community composition between the two months (\(R = 0.30, p = .001\), Figure 2a), but no significant difference between C and NC group eels (\(R = 0.09, p = .066\), Figure 2b). Myxobolus portucalensis and *Pseudodactylogyrus* spp. contributed significantly to the parasite community differences between August and October (\(p = .001\) for both taxa).

3.2 | Gene expression

Encapsulation status had no significant effect on relative expression of any studied gene in spleen or head kidney (Figure 3; Table 3). Cox1 was about 1.8 times more highly expressed in the head kidney of eels sampled in August than in those sampled in October (Figure 3b; Table 3). Post hoc tests indicated higher expression in the NC group in August than in October (Tukey’s HSD, \(z = 2.64, p = .041\)). Relative expression of *epor* and both *mhc II* genes in the spleen differed between sampling months (Figure 3c,e,g; Table 3). The expression of *epor* was elevated 2.5-fold, *mhc IIA* 2.2-fold and *mhc IIB* 1.8-fold in August compared with October. For both *mhc II* genes, temporal expression patterns in the head kidney differed with encapsulation status (Figure 3f,h).

The expression of both *mhc II* genes in the head kidney correlated negatively with the number of capsules (\(mhc IIA: \text{Dev} = 101.8, p = .016\), *mhc IIB*: \(\text{Dev} = 3.22, p = .008\)). There was no correlation between gene expression and either infection intensity or the number of adult *A. crassus* in either organ. For *mhc IIA* and *mhc IIB*, there was a strong overall correlation between relative expression levels (Spearman’s rank correlation, \(p = 0.749, p < .001\)). The correlation remained highly significant when analysing the spleen and the head kidney separately (spleen: \(\rho = 0.529, p < .001\), head kidney: \(\rho = 0.542, p < .001\)).

4 | DISCUSSION

European eels are infected by a wide variety of macroparasites. The number of parasite taxa that we observed in eels of Lake Müggelsee was comparable to those reported from other European locations (e.g. Gérard et al., 2013; Jakob et al., 2009; Sures et al., 1999; Sures & Streit, 2001). Similarly to those reports, the invasive parasites *Anguillicola crassus* and *Pseudodactylogyrus* spp. were the most prevalent. Prevalence and mean infection intensity of *A. crassus* were in the upper range of those reported across Europe (e.g. Audenaert et al., 2003; Becerra-Jurado et al., 2014; Gérard et al., 2013; Knopf, 2006). We found no difference in abundance between August and October. Seasonal dynamics have now been reported in some locations (e.g. Lefebvre et al., 2002; Schabuss et al., 2005) but not others (e.g. Kennedy & Fitch, 1990; Würtz et al., 1998). We observed no relationship between eel size and infection intensity or abundance of *A. crassus*. This was similar to the findings of Norton et al. (2005), although both positive (Becerra-Jurado et al., 2014; Neto et al., 2010; Schabuss et al., 2005) and negative (Barry et al., 2017; Fazio, Sasal, et al., 2008) relationships have been reported.

Invasive parasites can impose strong selective pressures on their novel hosts, which are expected to lead to adaptation of the host (Penczykowski et al., 2011). We found that European eels encapsulating *A. crassus* had fewer adult-stage parasites in their swim bladders compared with eels that did not encapsulate, but all eels

![Figure 2](https://example.com/figure2.png)  
**Figure 2** Non-metric multidimensional scaling plot of parasite communities in eels with unambiguous encapsulation status (a) in August (●) and October (♦) and (b) for non-encapsulating (NC, ■) and encapsulating (C, ▲) eels.
had similar numbers of larval-stage parasites. Encapsulation may therefore prevent the parasite's development to adulthood rather than prevent its establishment. Adult stages are proposed to have the strongest impact on the European eel (Würtz et al., 1996), and reducing their abundance may diminish adverse effects of severe A. crassus infections. This may indicate a first step towards adapting to the novel parasite. However, there was no relationship between the abundance of adult A. crassus and the number of capsules for eels encapsulating the parasite and it is possible that both depend on additional factors.

**FIGURE 3** Expression of target genes relative to the expression of the reference gene β-actin in the spleen (a, c, e, g) and the head kidney (b, d, f, h). Aug and Oct indicate the sampling months, and NC and C indicate absence and presence of capsules.
The co-infecting parasite community can determine the outcome of infections and its impact on the host (Abbate et al., 2018; Benesh & Kalbe, 2016; Johnson & Hoverman, 2012). Co-infecting parasite species can interact with each other either directly via competition, for example for resources, or indirectly, for example via host immune response, which can suppress or facilitate co-infections (Pedersen & Fenton, 2007; Poulin, 1999). We found abundances of two native parasites to be higher in eels encapsulating A. crassus, suggesting that the ability to encapsulate and the establishment of other parasites may interfere with each other.

The number of capsules was negatively correlated with mhc II expression among eels encapsulating A. crassus. Western blot analysis of the antibody response of experimentally infected European eels suggested that antigens of adult parasites trigger an adaptive immune response (Knopf et al., 2000) and that this response is stronger with increasing infection intensity (Knopf & Lucius, 2008). Increased mhc II expression may thus indicate higher susceptibility and encapsulation may lead to a reduction in the adaptive immune response by reducing the number of adult worms. Here, mhc II expression did not correlate with adult A. crassus load; however, it may be correlated more strongly with encapsulation and the number of adults than was detected. Our primers do not discriminate the alleles of European eel mhc II (Bramont et al., 2015). European eels contain at least four expressed mhc II A and mhc II B alleles, and if A. crassus-specific alleles exist, their change in expression may be masked by expression changes in other alleles induced by the other parasites. The need to respond to co-infecting parasites could also explain why we did not find a difference in mhc II expression between encapsulating and non-encapsulating eels.

Adult A. crassus are sanguivorous (De Charleroy et al., 1990), and a high load of adults may stimulate erythrocyte production in eel hosts. In experimental infections, Fazio et al. (2009) found increased expression of a gene associated with red blood cells (haemoglobin a), suggesting an increase in red blood cells with increasing parasite biomass. Our results based on expression of epo, a gene involved in erythropoiesis, did not indicate such an increase. Furthermore, we did not find a correlation of expression with the number of adult A. crassus or the number of capsules. Additional biotic and abiotic factors may influence the effect of A. crassus on red blood cells in the wild. Similarly, we did not find an association between the expression of the energy-associated gene cox1 and the presence of capsules, their number or the number of adult A. crassus. Hence, there was no evidence for energetic benefits of encapsulating A. crassus. One reason could be that such benefits only become evident during the spawning migration (Palstra et al., 2007; Palstra & van den Thillart, 2010) and none of the eels we studied were migrating. Another could be that the higher abundance of native parasites (see above) may counteract any energetic benefits of harbouring fewer adult A. crassus.

Whole transcriptome gene expression analyses of infected and uninfected individuals indicated reduced cell respiration and the induction of both innate and adaptive immune responses in the presence of A. crassus larvae (Bracamonte, Johnston, Knopf, et al., 2019; Bracamonte, Johnston, Monaghan, et al., 2019). Increased immune gene expression in naturally infected eels compared to those without an active infection was also observed after acclimation to a common, stress-free environment (Schneebauer et al., 2017). Because all of our individuals were infected with A. crassus, we cannot exclude the possibility that the mere presence of A. crassus determines the physiological status and the initiation of an immune response, regardless of infection intensity. Extending the analysis to non-infected individuals may offer further insight into the importance of A. crassus on the physiological status of wild continental European eels.

Independently of A. crassus or its encapsulation, the expression of all genes varied between August and October. This suggests that environmental factors may affect expression of the selected genes. All genes showed higher expression in August than in October in one of the two tissues. Fish are ectothermic; hence, colder water in October could be one factor driving the difference (Brown et al., 2016; Logan & Somero, 2010). Parasite community composition also differed between sampling dates, and this could lead to variation in gene expression. Translocated sticklebacks adjust the expression profiles of immune genes to those of the local population, which is likely a response to encountering different parasite communities (Stutz et al., 2015). Temporally changing parasite communities can be expected to cause similar adjustments of the response. However, eels caught in August were significantly larger than eels caught in October. Hence, we cannot exclude that body size has an effect on relative gene expression.

In conclusion, the invasive parasite A. crassus was one of the most prevalent parasites of eels in Lake Müggelsee. Eels that encapsulated A. crassus had fewer blood-feeding adults. We argue that this may be a first step towards adaptation by the novel host, although we have no information on the genetic basis of encapsulation. The lower number of adults may then contribute to the negative relationship between mhc II gene expression and the number of capsules because the adaptive immune system was shown to respond to adult A. crassus. However, we found that the abundance of two native parasites was higher in eels that encapsulated, suggesting that there may be interference among responses to different parasites or among the parasites themselves. This interference, together with a potentially weak effect of A. crassus on the continental stage of eels, may be one reason for the absence of a clear pattern in gene expression, particularly of the energy-related and the haematopoietic genes.

ACKNOWLEDGEMENTS

We thank Mathias Kunow for assistance with eel sampling and Eva Kreuz and Elisabeth Funke for advice on qPCRs. Kate Laskowski assisted with statistical analyses. Funding was provided by the IMPact-Vector Graduate School of the Leibniz Association (Senate Competition Committee Grant SAW-2014-SGN-3). Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.
Gérard, C., Tranctart, C., Amlhat, E., Faliex, E., Virag, L., Feunteun, E., & Acou, A. (2013). Influence of introduced vs. native parasites on the body condition of migrant silver eels. *Parasite*, 20, 10. https://doi.org/10.1051/parasite/20130140
Guo, Z. Y., Gonzalez, J. F., Hernandez, J. N., McNeilly, T. N., Corripio-Miyar, Y., Frew, D., Morrison, T., Yu, P., & Li, R. W. (2016). Possible mechanisms of host resistance to *Haemonchus contortus* infection in sheep breeds native to the Canary Islands. *Scientific Reports*, 6, 14. https://doi.org/10.1038/srep26200
Hartig, F. (2020). DHARMa: Residual diagnostics for hierarchical (multi-level / mixed) regression models (0.3.2.0). Retrieved from https://CRAN.R-project.org/package=DHARMa
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 & Vectors*, 2, 11. https://doi.org/10.1186/1753-3350-2-48
Hosler, J. P., Ferguson-Miller, S., & Mills, D. A. (2006). Energy transduction: Proton transfer through the respiratory complexes. *Annual Review of Biochemistry*, 75, 165–187. https://doi.org/10.1146/annurev.biochem.75.062003.101730
Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in mixed regression models (0.3.2.0). https://doi.org/10.1038/srep26200
Kaifu, K., Kurwie, T., Sasal, P., Silfvergrip, A. M. C., Smith, K. G., and Kurze, C., Mayack, C., Hirche, F., Stangl, G. I., Le Conte, Y., Kryger, P., & Moritz, R. F. A. (2016). Nosema spp. infections cause no energetic stress in tolerant honeybees. *Parasitology Research*, 115(6), 2381–2388. https://doi.org/10.1007/s00436-016-4988-3
Le Cren, E. D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *Journal of Animal Ecology*, 20, 201–219. https://doi.org/10.2307/1540
Lefebvre, F., Contournet, P., & Crivelli, A. J. (2007). Interaction between the severity of the infection by the nematode *Anguillicola crassus* and the tolerance to hypoxia in the European eel *Anguilla anguilla*. *Acta Parasitologica*, 52(2), 171–175. https://doi.org/10.2478/s11686-007-0013-4
Lefebvre, F., Contournet, P., Priour, F., Soulas, O., & Crivelli, A. J. (2002). Spatial and temporal variation in *Anguillicola crassus* counts: Results of a 4 year survey of eels in Mediterranean lagoons. *Diseases of Aquatic Organisms*, 50(3), 181–188. https://doi.org/10.3354/dao050181
Lenz, T. L., Eizaguirre, C., Rotter, B., Kalbe, M., & Milinski, M. (2013). Exploring local immunological adaptation of two stickleback eco-types by experimental infection and transcriptome-wide digital gene expression analysis. *Molecular Ecology*, 22(3), 774–786. https://doi.org/10.1111/j.1365-294X.2012.05756.x
Logan, C. A., & Somero, G. N. (2010). Transcriptional responses to thermal acclimation in the eurythermal fish *Gillichthys mirabilis* (Cooper 1864). *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, 299(3), R843–R852. https://doi.org/10.1152/ajpregu.00306.2010
Lohman, B. K., Steinel, N. C., Weber, J. N., & Bolnick, D. I. (2017). Gene expression contributes to the recent evolution of host resistance in a model host parasite system. *Frontiers in Immunology*, 8, 12. https://doi.org/10.3389/fimmu.2017.01071
MacColl, A. D. C., & Chapman, S. M. (2010). Parasites can cause selection against migrants following dispersal between environments. *Functional Ecology*, 24(4), 847–856. https://doi.org/10.1111/j.1365-2435.2010.01691.x
Miller, M. P., & Vincent, E. R. (2008). Rapid natural selection for resistance to an introduced parasite of rainbow trout. *Evolutionary Applications*, 1(2), 336–341. https://doi.org/10.1111/j.1752-4571.2008.00018.x
Minegishi, Y., Aoyama, J., Inoue, J. G., Miya, M., Nishida, M., & Tsukamoto, K. (2005). Molecular phylogeny and evolution of the freshwater eels (Anguillidae) based on the whole mitochondrial genome sequences. *Molecular Phylogenetics and Evolution*, 34(1), 134–146. https://doi.org/10.1016/j.ympev.2004.09.003
Molnár, K. (1993). Effect of decreased oxygen-content on eels (*Anguilla anguilla*) infected by *Anguillicola crassus*. *Nematoda, Dracunculoidea*. *Acta Veterinaria Hungarica*, 41(3–4), 349–360.
Molnár, K. (1994). Formation of parasitic nodules in the swim bladder and intestinal walls of the eel *Anguilla anguilla* due to infections with larval stages of *Anguillicola crassus*. *Diseases of Aquatic Organisms*, 20(3), 163–170.
Molnár, K., Székely, C., & Baska, F. (1991). Mass mortality of eel in Lake Balaton due to *Anguillicola crassus* infection. *Bulletin of the European Association for Fish Pathology*, 11(6), 211–212.
Morris, A., Hewitt, C., & Young, S. (1994). The major histocompatibility complex: Its genes and their roles in antigen presentation. *Molecular Aspects of Medicine*, 15(5), 377–503. https://doi.org/10.1016/0108-9819(94)90041-8
Münderle, M., Taraschewski, H., Klar, B., Chang, C. W., Shiao, J. C., Shen, K. N., He, J. T., Lin, S. H., & Tzeng, W. N. (2006). Occurrence of *Anguillicola crassus* (Nematoda: Dracunculoidea) in Japanese eels *Anguilla japonica* from a river and an aquaculture unit in SW Taiwan. *Diseases of Aquatic Organisms*, 71(2), 101–108. https://doi.org/10.3354/dao071101
Neto, A. F., Costa, J. L., Costa, M. J., & Domingos, I. (2010). Epidemiology and pathology of Anguillicoloides crassus in European eel Anguilla anguilla from the Tagus estuary (Portugal). Diseases of Aquatic Organisms, 88(3), 225–233. https://doi.org/10.3354/dao02166

Norton, J., Rollinson, D., & Lewis, J. W. (2005). Epidemiology of Anguillicola crassus in the European eel (Anguilla anguilla) from two rivers in southern England. Parasitology, 130, 679–686. https://doi.org/10.1017/S0031182004007139

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Minchin, P. R., O’Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). vegan: Community ecology. Journal of Statistical Software, 84(2), 1–21. https://doi.org/10.18637/jss.v084.i02

Palstra, A. P., & van den Thillart, G. (2007). Swimming performance of silver eels is severely impaired by the swim-bladder parasite Anguillicola crassus. Journal of Experimental Marine Biology and Ecology, 352(1), 244–256. https://doi.org/10.1016/j.jembe.2007.08.003

Palstra, A. P., & van den Thillart, G. (2010). Swimming physiology of European silver eels (Anguilla anguilla L): Energetic costs and effects on sexual maturation and reproduction. Fish Physiology and Biochemistry, 36(3), 297–322. https://doi.org/10.1007/s10695-010-9397-4

Palstra, A. P., & van den Thillart, G. (2010). Swimming performance of silver eels in European eel (Anguilla anguilla) from two rivers in southern England. Parasitology, 130, 679–686. https://doi.org/10.1017/S0031182004007139

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O’Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). Vegan: Community ecology package (2.5-4). Retrieved from https://CRAN.R-project.org/package=vegan

Palstra, A. P., Heppener, D. F. M., van Ginneken, V. J. T., Székely, C., & van den Thillart, G. (2007). Swimming performance of silver eels is severely impaired by the swim-bladder parasite Anguillicola crassus. Journal of Experimental Marine Biology and Ecology, 352(1), 244–256. https://doi.org/10.1016/j.jembe.2007.08.003

Palstra, A. P., & van den Thillart, G. (2010). Swimming physiology of European silver eels (Anguilla anguilla L): Energetic costs and effects on sexual maturation and reproduction. Fish Physiology and Biochemistry, 36(3), 297–322. https://doi.org/10.1007/s10695-010-9397-4

Pedersen, A. B., & Fenton, A. (2007). Emphasizing the ecology in parasite community ecology. Trends in Ecology & Evolution, 22(3), 133–139. https://doi.org/10.1016/j.tree.2007.01.003

Peeler, E. J., Oldtman, B. C., Midtlying, P. J., Miossec, L., & Gozlan, R. E. (2011). Non-native aquatic animals introductions have driven disease emergence in Europe. Biological Invasions, 13(6), 1291–1303. https://doi.org/10.1007/s10530-011-0088-y

Pelster, B. (2015). Swinbladder function and the spawning migration of the European eel Anguilla anguilla. Frontiers in Physiology, 5, 10. https://doi.org/10.3389/fphys.2014.00486

Penceykowski, R. M., Forde, S. E., & Duffy, M. A. (2011). Rapid evolution as a possible constraint on emerging infectious diseases. Freshwater Biology, 56(4), 689–704. https://doi.org/10.1111/j.1365-2427.2010.02499.x

Pfaff, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Research, 29(9), 6. https://doi.org/10.1093/nar/29.9.e45

Poulin, R. (1999). The functional importance of parasites in animal communities: Many roles at many levels? International Journal for Parasitology, 29(6), 903–914. https://doi.org/10.1016/s0020-7519(99)00045-4

R Core Team. (2019). R: A language and environment for statistical computing (3.5.3). R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Reche, P. A., & Reinherz, E. L. (2003). Sequence variability analysis of human class I and class II MHC molecules: Functional and structural correlates of amino acid polymorphisms. Journal of Molecular Biology, 331(3), 623–641. https://doi.org/10.1016/s0022-2836(03)00750-2

Schabuss, M., Kennedy, C. R., Konecny, R., Grillitsch, B., Reckendorfer, W., Schiemer, F., & Herzig, A. (2020). Dynamics and predicted decline of Anguillicola crassus infection in European eels, Anguilla anguilla, in Neusiedler See, Austria. Journal of Helminthology, 79(2), 159–167. https://doi.org/10.1079/joh2005281

Scharsack, J. P., & Kalbe, M. (2014). Differences in susceptibility and immune responses of three-spined sticklebacks (Gasterosteus aculeatus) from lake and river ecotypes to sequential infections with the eye fluke Diplostomum pseudospathaceum. Parasites & Vectors, 7, 10. https://doi.org/10.1186/1756-3305-7-109

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

Stutz, W. E., Schmerer, M., Coates, J. L., & Bolnick, D. I. (2015). Among-lake reciprocal transplants induce convergent expression of immune genes in threespine stickleback. Molecular Ecology, 24(18), 4629–4646. https://doi.org/10.1111/mec.13295

Sures, B., & Knopf, K. (2004). Parasites as a threat to freshwater eels? Science, 304(5668), 208–209.

Sures, B., Knopf, K., Würtz, J., & Hirt, J. (1999). Richness and diversity of parasite communities in European eels Anguilla anguilla of the River Rhine, Germany, with special reference to helminth parasites. Parasitology, 119(Pt 3), 323–330. https://doi.org/10.1017/s003118200008465

Sures, B., & Streit, B. (2001). Eel parasite diversity and intermediate host abundance in the River Rhine, Germany. Parasitology, 123, 185–191. https://doi.org/10.1017/s003118200008356

Weber, J. N., Kalbe, M., Shim, K. C., Ern, N. I., Steinel, N. C., Ma, L., & Bolnick, D. I. (2017). Resist globally, infect locally: A transcontinental test of adaptation by stickleback and their tapeworm parasite. The American Naturalist, 189(1), 43–57. https://doi.org/10.1086/689597

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, https://doi.org/10.1186/1471-2148-13-78

Würzt, J., Knopf, K., & Taraschewski, H. (1998). Distribution and prevalence of Anguillicola crassus (Nematoda) in eels Anguilla anguilla of the rivers Rhine and Naab, Germany. Diseases of Aquatic Organisms, 32(2), 137–143. https://doi.org/10.3354/dao032137

Würzt, J., Taraschewski, H., & Pelster, B. (1996). Changes in gene composition in the swimbladder of the European eel (Anguilla anguilla) infected with Anguillicola crassus (Nematoda). Parasitology, 112, 233–238. https://doi.org/10.1017/s003118200008481X

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Bracamonte SE, Knopf K, Monaghan MT. Encapsulation of Anguillicola crassus reduces the abundance of adult parasite stages in the European eel (Anguilla anguilla). J Fish Dis. 2020;00:1-12. https://doi.org/10.1111/jfd.13301