Received: 15 February 2023 | Revised: 30 May 2023 | Accepted: 31 May 2023

DOI: 10.1111/jfd.13822

Journal of Fish Diseases WILEY

RESEARCH ARTICLE

Effects of the invasive swim bladder parasite *Anguillicola crassus* on health and condition indicators in the European eel

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Funding information
European Commission´s Data Collection Framework; SLU Aqua, Department of Aquatic Resources, Swedish University of Agricultural Sciences

Abstract

Parasites negatively affect biological processes within their hosts, which may alter for example health, growth, and reproductive ability. Non-native invasive parasites, in particular, may have large effects on the endemic hosts, given that the hosts lack evolved specific defences against such parasites. The swim bladder nematode *Anguillicola crassus*, an invasive parasite originating from Asia, is found in the European eel (*Anguilla anguilla*, L. 1758), since the 1980s. We investigated whether *A. crassus* affected several indicators related to health of the European eel (spleen- and liver size, body fat content and relative condition). Our results indicate that during the continental residency of the eels, infection by *A. crassus* had no major negative impacts on the investigated health indicators at the generally low infection intensities present in this study (median 2–3 visible parasites). Given that many of the adult eels were found to have swim bladder damage, concerns about their spawning migration through deeper oceanic environments can still be raised. To allow further investigations, we suggest that quantification of swim bladder damage should be implemented in eel-monitoring programs. Compared to other parasite pressure parameters, swim bladder damage provides additional information about past infections and future problems.

KEYWORDS
Anguilla anguilla, fat, nematode, relative condition, swim bladder damage

1 | INTRODUCTION

Parasitism is a successful mode of life and parasites play a major role in food web dynamics and organismal energy flows (Lafferty et al., 2008; Poulin & Morand, 2000). Parasites affect processes within their hosts that may alter their health, growth, and reproductive ability, which, in turn, can affect population dynamics (Tompkins et al., 2002) and lead to indirect effects on the ecosystem through trophic cascades (Dunn et al., 2012). Non-native invasive parasites, in particular, can have large effects on hosts in their new habitat as well as on parasite communities (Hohenadler et al., 2019). Due to lack of a shared evolutionary history between invasive parasites and endemic hosts, the hosts may have less efficient immunological defences against such parasites, as compared to native parasites to which the hosts have had opportunities to evolve specific defence mechanisms (Knopf & Mahnke, 2004; Torchin et al., 2002). Hence, human introductions of parasites and other infectious agents into new geographic areas risk leading to detrimental environmental and socio-economic impacts (Lafferty et al., 2015). On the individual level of the host, parasite infection generally activates the immune system to protect and minimize the host's costs of being infected (Gunn & Pitt, 2022). Changes in the environmental demands due to parasite infection can, therefore, require allocation of energy from e.g., growth and reproduction to immune defence (Bradshaw

& McMahon, 2008; Nelson, 2011; Schreck & Tort, 2016). If growth and energy storage are affected by the infection, the host's ability to cope with additional stressors can be negatively affected, sometimes with detrimental effects (Lefebvre et al., 2007; Molnár, 1993).

The eel swim bladder nematode *Anguillicola crassus* (Nematoda, Dracunculoidea, Kuwahara et al., 1974) is a parasite found in several species of anguillid eels (Kuwahara et al., 1974). It originates from Asia, where it parasitizes the Japanese eel, *Anguilla japonica* (Kuwahara et al., 1974), but it is an invasive parasite to five other anguillid eel species around the world (Lefebvre et al., 2012; Moravec, 2006; Sasal et al., 2008). *A. crassus* can be found in several intermediate or paratenic host species during its different life stages but it grows into its adult life stage, reproduce, and close its life cycle in the swim bladder of anguillid eels (De Charleroy et al., 1990; Haenen & Van Banning, 1990). Eels get infected by *A. crassus* through their diet; by feeding on infected hosts like copepods (for juvenile eels) or infected fish and invertebrates (for adult eels), which can contain large numbers of the parasite without being severely affected themselves (Haenen & Van Banning, 1990; Thomas & Ollevier, 1992a). Once consumed by the eel, the parasite migrates through the layers of the gastrointestinal tract, whereafter it penetrates into the wall of the swim bladder, causing tissue damage on the way (De Charleroy et al., 1990). Juvenile and preadult larval stages stay inside the walls of the swim bladder and feed on the wall tissue (Würtz et al., 1996). In its adult life stage, the parasite resides in the lumen of the swim bladder and feeds on blood (Kirk, 2003). Consequences of infection include inflammation, fibrosis, and necrosis of the swim bladder (Würtz & Taraschewski, 2000). In addition, *A. crassus* also cause damage to the gas glands in the swim bladder, leading to decreased oxygen levels in the bladder, likely affecting control of the buoyancy regulation of the eels (Würtz et al., 1996; Würtz & Taraschewski, 2000). Lowered swimming speed and impaired endurance have been demonstrated in swim tunnel experiments for both eels with an ongoing parasite infection and eels with a damaged swim bladder (Palstra et al., 2007). Due to immune and stress responses, infected eels may also have higher energy consumption and higher oxygen demand (Palstra et al., 2007) and they may reallocate stored energy into these responses. Increased oxygen demand has been demonstrated by experimental evidence showing that infected eels have a shorter lifespan under hypoxia (Molnár, 1993), indicative of either an increased metabolic rate or a decreased ability for oxygen uptake. In addition, loss of appetite has been shown in infected eels in laboratory experiments (Haenen & van Banning, 1990).

The European eel (*Anguilla anguilla* L. 1758) is one of the five species for which *A. crassus* is an invasive parasite. *Anguillicola crassus* was introduced to Europe in the 1980s (Ashworth & Blanc, 1997; Kirk, 2003), most likely due to the intercontinental eel trade and transfer of ballast water (Barry et al., 2017; Lefebvre et al., 2012). The spread through Europe was rapid and the nematode has been common in most European countries for several decades (Moravec, 1992). In Sweden, *A. crassus* was first recorded in 1987 and within a few years it was found both along the coast and in freshwater lakes (Wickström et al., 1998). *Anguillicola crassus* could potentially be problematic for the European eel since this species undertakes a very long spawning migration, with adults migrating over 5000 km from European waters to their spawning grounds in the Sargasso Sea (Schmidt, 1912; Wright et al., 2022). Considering this distance in combination with observed diel vertical migration patterns, which cover several hundred meters of depth (Aarestrup et al., 2009), any negative effects from a parasite infection on swimming ability and/or buoyancy control of the eels could be detrimental (Pelster, 2015). In addition, during migration, the adult eels cease to feed and rely on their body reserves (Durif et al., 2005; Freese et al., 2019). If the body condition of infected eels is negatively affected, this too could have implications for migration success. Any negative impact on spawning migration, or spawning success, is particularly worrying in this panmictic species (Enbody et al., 2021) since it has decreased to very low levels (Dekker, 2003; Magnusson & Dekker, 2021). Since 2008, the European eel is listed as critically endangered in the International Union for Conservation of Nature's Red List of threatened species (Pike et al., 2020). Hence, there is an urgent need to clarify the factors causing the decline, and the effects of the invasive parasites may be one of them. The joint working group on eels ['WGEEL', the joint European Inland Fisheries and Aquaculture Advisory Commission (EIFAAC) and International Council for the Exploration of the Sea (ICES) and General Fisheries Commission for the Mediterranean (GFCM) working group] has recently called for more research to fully understand the fitness implications of these parasites on the European eel (ICES, 2021).

In this study, we investigated the effects of *A. crassus* on the European eel by measuring several health indicators and factors related to condition. Parasitization rate was assessed using four different measures with the intention to cover several health-related aspects, and to identify the most biologically meaningful measure of parasitization within monitoring. The four measures were: (i) presence, to test whether presence or absence of parasites in the lumen of the swim bladder has an impact, (ii) intensity, to test whether the number of parasites in the lumen of the swim bladder scales with impact, and (iii) mass of parasites, to test whether mass of parasites scales with impact, (iv) swim bladder degenerative index (SDI), to test whether the morphological change the parasite causes to the swim bladder has an impact. All measures of presence, intensity, and mass refers to visually detectable parasites in the lumen and does not include any potentially present larval stages in the swim bladder wall. Firstly, we used existing data from a database containing dissection data from thousands of individual eels to investigate the effects of parasite presence and intensity on body condition (mass at a given length). We predicted that infected eels would have a lower body condition compared to uninfected eels, since infected eels may spend energy on an immunological response, use more energy when swimming (due to damaged swim bladder), and potentially suffer from impaired appetite and stress. Secondly, we utilized eels sampled within the ongoing EU data collection program to enable more in-depth analyses on the effects of parasite presence, intensity, mass of parasites, and SDI on spleen- and liver mass, and body fat content, as well as

of SDI on body condition. We predicted that infected eels would have a larger relative spleen size, as a sign of an active immune response to parasitism (Lamková et al., 2007; Ottová et al., 2007; Owens & Wilson, 1999), and a smaller relative liver size due to parasite-related depletion of the energy reserve typically stored in the liver (Dave et al., 1975). We also predicted that the body fat content would be lower in parasitized eels due to energy demands related to hosting the parasite (Khan, 1988; Shanebeck et al., 2022).

2 | MATERIALS AND METHODS

2.1 | European eel database extraction

For the first part of the study, we used existing dissection data from a database called 'Sötebasen', kept by the Institute of Freshwater Research, Swedish University of Agricultural Sciences (SLU Aqua). Data on parasite presence and intensity has been collected over many years, stemming from various aquatic environments (lakes, rivers, and a few coastal areas) across Sweden as part of several different monitoring programs, with the most recent being the EU data collection framework (DCF) in the fisheries and aquaculture sector (Regulation (EU) 2017/1004). The great number of individual eels in this dataset (more than 17,000) allowed us to investigate overall effects of parasite presence and intensity on eel condition (mass at a given length). Data was extracted on August 10, 2022. After filtering out individuals with an unrepresentative body mass (with notes about e.g., being partially decomposed, rotten, or amputated in hydropower turbines), 17,674 individuals remained in the dataset. For the statistical analyses, we needed data on parasite intensity (data collected as the number of visible parasites in the swim bladder), parasite presence (yes/no), total body length (±1 mm, measured using a standard measuring board), body mass (±0.1 g), year collected, and collection site, for each eel. Additionally, the mean length of the left and right side pectoral fin and mean eye diameter of the left and right side eye was needed (together with mass and total length) to calculate the Durif's silvering index, i.e. the degree of metamorphosis (Durif et al., 2005, 2009; see Section 2.4). Individuals in the database with missing data for any of the morphometric variables needed for calculation of Durif's silvering index were classified as having 'unknown' maturation status. The majority of the eels were females, but males ($n = 341$) and unsexed ($n = 2771$) individuals were also included. Many of the unsexed individuals were classified as elvers ($n = 2424$) and most lacked eye- and pectoral fin measurements (hence classified as 'unknown' maturation status). The data was collected between 1991 and 2021, and the eels had been measured in the field (alive and sedated) or in the laboratory (alive and sedated, dead and fresh, or dead frozen and thawed). Note that the database does not contain any data on mass of parasites, swim bladder degenerative index (SDI), spleen- or liver mass, or fat content, wherefore none of these aspects could be analysed using this dataset.

2.2 | Fish collection and morphometrics

For the second part of the study, we utilized eels sampled within the ongoing EU data collection program to collect more in-depth data, including on swim bladder degenerative index (SDI), mass of parasites, spleen size, liver size, and body fat content. The eels were sampled in 2021 from a fisheries-independent monitoring program in a freshwater lake (Mälaren), where mainly younger eels in their growth phase were caught, and the known parasitization rate (based on data from previous sampling years) was relatively low. We also sampled from a commercial fisher in another freshwater lake (Ringsjön), where mainly older premigrating and migrating eels were caught, and the known parasitization rate (based on data from previous sampling years) was relatively high. By utilizing eels sampled within ongoing data collection programs, this study did not require any additional lethal sampling.

In Mälaren (59°20′04.3″ N, 17°52′30.6″ E), a scientific fishing survey was conducted May–July, in a shallow soft bottom bay with partial macrophyte coverage (mean pH in 2021: 7.9 (Lännergren, 2022) and mean temperature May–July: 15°C (data from the Institute of Freshwater Research, SLU Aqua)). Five unbaited sets of knotless, circular fyke nets were used (opening 385 mm in diameter and mesh size ranging from min. 18 mm to max. 30 mm at their centre points). Each set had 16 pairwise traps (each trap ~2 m long) with a leading net between each pair. In total, each set was approximately 68 m long. Each set was placed approximately 50 m apart, starting close to the shore at a depth of 0.5 m and ending at a depth of 2 m. The fyke nets were emptied twice weekly, and all eels caught ($N = 112$), irrespective of their size, were collected and kept in net bags in a barrel until all fyke nets had been checked and emptied (maximum holding time: 3 h). The eels were then brought to the laboratory at the Institute of Freshwater Research at Drottningholm (near Stockholm, Sweden), situated just next to the fishing location (i.e., no transportation time). At the laboratory, the eels were sedated with benzocaine (0.12 g L^{-1} water, Benzocaine E1501-100G, Sigma-Aldrich) for ca. 15 min, after which total body length (±1 mm, measured using a standard measuring board) and body mass (±0.1 g) was measured. We also measured body fat content on a sub-sample ($n = 45$) of eels using a fat meter (Distell Fish Fat Meter Model FFM-992; Distell). The fat meter, calibrated to eel tissue by the manufacturer (program EEL-1), uses a microstrip sensor and calculates the fat content based on the amount of water in the body. The measurements might, due to differences in skin thickness, vary in the accuracy between different life stages of the eel (Pohlmann et al., 2019). It has, however, been shown that using a fat meter to estimate fat content in muscles can be more precise than other methods, and it is an easy and cheap method (Klefoth et al., 2013; Pohlmann et al., 2019). We, therefore, concluded that it would be

a suitable method for measuring fat content if life stage was included in the analyses. For each individual eel, two average values were recorded as percentage lipid concentration; one as an average along the lateral line on the right side of the eel (average of four measurements), and the other as an average (of four measurements) taken at one spot by the lateral line anterior to the anal opening on the right side. Fat content is usually estimated based on measurements taken along the lateral line, but since there was a strong positive rank correlation between these two measurements, we used the fat measurement taken at one spot for the statistical analyses (see Appendix: Figure A1). After these measurements, the eels were frozen at −18°C, one and one in plastic bags (they were not flash frozen), until further examination (see dissection data collection below). The eels had to be frozen for later dissection due to time constraint reasons. Out of the 112 eels collected, 22 were kept in indoor tanks at the Institute of Freshwater Research for 3 days after collection, prior to being sedated and measured (kept in 1250-L tanks, ≤ 3 eels per tank, with flow-through water from Mälaren). These 22 eels were originally kept for blood sampling, which had to be cancelled due to equipment shipping delays (due to the global COVID-19 pandemic occurring in 2021), and were instead treated as mentioned above for the eel sampled in Mälaren. Since we only kept the eels for 3 days in captivity, this additional handling is assumed to have no impact on the data collected for this study.

In Ringsjön, commercial fishing was conducted in June–August, in the eastern basin of the lake ('Östra Ringsjön', 55°51′49.8″ N, 13°31′55.0″ E; mean pH in 2021: 8.2, mean temperature June–August in 2021: 17°C; Calluna, 2022). Stationary pound nets were used, set at ca. 1–5 m depth. The pound nets were emptied three times per week and all eels above the fisheries regulation minimum size limit (≥700 mm) were frozen (ca. 10 individuals per plastic bag, they were not flash frozen) at −18°C directly after landing. The eels had to be frozen for later dissection due to time constraint reasons, and for practical reasons, since the dissections cannot be performed on site at the fisher. A subset of the (frozen) landed catch ($N = 90$, representing the total landed size range) was sent to the Institute of Freshwater Research at Drottningholm for dissection (see Dissection protocol below).

All eels included in this study were collected within the Swedish monitoring program for the European eel, which is part of the EU data collection framework in the fisheries and aquaculture sector (Regulation (EU) 2017/1004). Current national legislation on animal welfare was followed, and the scientific fish monitoring in Mälaren was conducted in accordance with ethical permit Dnr 6229-2020 (Swedish Board of Agriculture). Sampling from landed catch from commercial fishing does not require an animal ethical permit.

2.3 | Dissection protocol

Post-mortem dissections of all eels were conducted after freezing and thawing (freezing time: 6–133 days). For each individual eel, we measured total body length (±1 mm, measured using a standard measuring board), body mass (±0.1 g), and body fat content (as described above, collecting the average of four measurements taken at one spot, body fat data collected on frozen and thawed individuals did not differ from data collected on alive sedated individuals, Appendix: Figure A1). For the purpose of calculating Durif's silvering index (see Section 2.4 below) we measured vertical and horizontal diameter of the left and right eye (±0.01 mm, measured using callipers; Mitutoyo Absolute IP67; Mitutoyo Corp.), and length of the left and right pectoral fin (±0.01 mm, measured using callipers). The eels were then dissected, and spleen and liver wet mass was measured (±0.001 g, using a KERN ABS/ABJ balance, version 1.3, Kern & Sohn GmbH) after having removed excess water. Sex was determined by gonad inspection and all eels were female. This was expected in Ringsjön since the fisheries regulations bias the catch towards females, since male eels are typically smaller (350–400 mm in length; Durif et al., 2009) than the minimum size limit (700 mm). In Mälaren, it was also expected to catch mainly females, since data from this survey from 2003–2021 show ~2% males in the catch, and only ~2.6% of the eels were smaller than 400 mm (data from the database 'Sötebasen', Institute of Freshwater Research, SLU Aqua). The reason for this is most likely explained by environmental sex determination (Davey & Jellyman, 2005; Geffroy & Bardonnet, 2016). The condition of the swim bladder was noted using a swim bladder degenerative index (SDI), similar to Lefebvre et al. (2002). The SDI is a qualitative assessment index and another length–ratio index (called length ratio index (LRI) or swim bladder index (SBI); Palstra et al., 2007) has been proposed to be less subjective (Lefebvre et al., 2011). The LRI require some validation before being used since there are contradicting results in the literature regarding whether a more damaged swim bladder actually results in lower index values (Frisch et al., 2016). Therefore, we decided to use the more common SDI. To minimize the potential risk of inter-observer variability, SDI was measured by one observer (EM) in this study. We assume that using frozen and thawed eels to calculate the SDI index is unproblematic since the study where the SDI index was established (Lefebvre et al., 2002) used frozen and thawed eels. The degree of damage caused by parasite infection was based on three criteria of the gross pathology (transparency, pigmentation, and thickness), each graded on a three-level scale (Table 1). Transparency was assessed first, before opening the swim bladder. After carefully opening and removing the parasites, the pigmentation was noted and the thickness of the swim bladder wall was measured.

After summation of the grading criteria, the SDI ranged from 0 to 6 (0 = non-affected swim bladder; 6 = severely affected swim bladder). The categorization of SDI was reduced from the original six classes into four classes (0, 1, 2–3 and 4–6; Figure 1) to achieve relatively homogenous sample sizes for the statistical analyses, similar to Lefebvre et al. (2013). Then the number of parasites was counted and the total wet mass of the parasites was measured to the nearest 0.001 g (using the same balance as for spleen- and liver mass) after carefully having removed excess water with a moist paper.

TABLE 1 Criteria (transparency, pigmentation and thickness) and grading (0, 1 or 2) for the Swim bladder Degenerative Index (SDI) based on the gross pathology changes caused by *Anguillicola crassus* infection on the swim bladder. Criteria and grading were based on Lefebvre et al. (2002).

	Grading criteria		
Indicator	**0**	**1**	**2**
Transparency of the wall, established by visual inspection	Transparent with a naturally yellowish colour	Semi-transparent milky white	Completely opaque
Pigmentation of and exudate in the swim bladder, established by visual inspection	Neither pigmentation nor exudate	Only pigmentation or exudate	Both pigmentation and exudate
Thickness of the swim bladder wall, measured with a calliper	<1 mm	1–3 mm	>3 mm

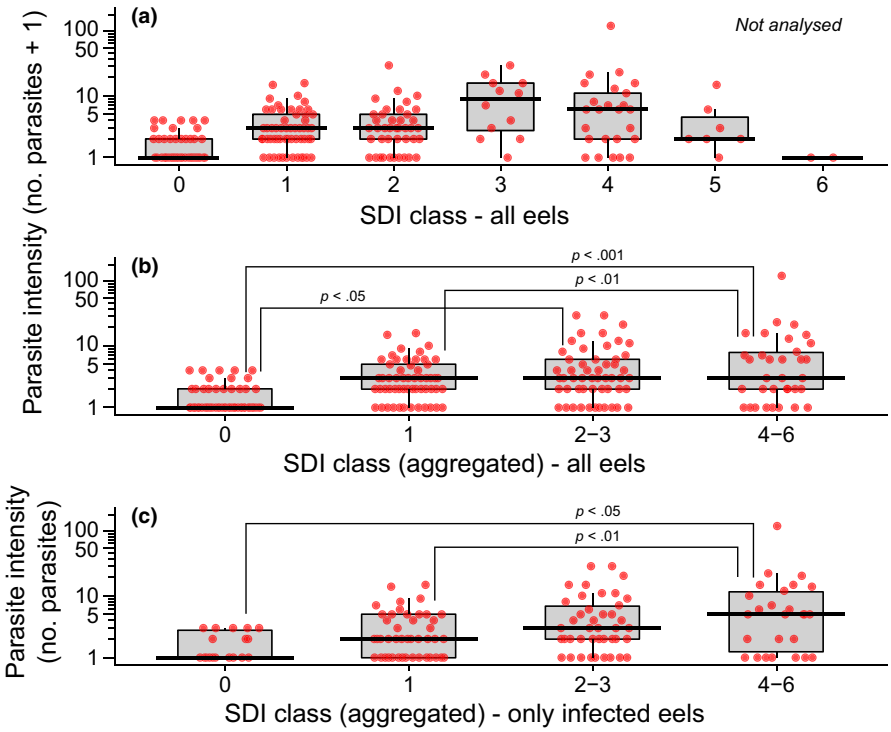

FIGURE 1 *Anguillicola crassus* parasite intensity (number of parasites) in European eel for (a) each class of swim bladder degenerative index (SDI), for all eels (data was not analysed divided over all SDI classes due to low sample size in class 5 and 6, instead see analyses on aggregated classes in panel (b), (b) aggregated SDI classes (aggregated due to low sample sizes), all eels, and (c) aggregated SDI classes, only including eels with current presence of visible *A. crassus*, referred to as 'only infected eels'. Boxplots: grey boxes range from the first (Q1) to the third (Q3) quantile of the data distribution, median (Q2) is represented by a line across the box. Whiskers extend up to 1.5 times the interquartile range (IQR). Red points show raw data points. *p*-values stem from pairwise contrasts, based on a quasi-Poisson regression model (for details see Table 2).

2.4 | Data analyses

All analyses were performed in R 4.1.2 (R Core Team, 2021) using R Studio (R Studio Inc., Boston). Results were considered statistically significant when $p < .05$.

The life stage of each individual eel was determined by calculating Durif's silvering index (Durif et al., 2005, 2009) using the R-package stacomiR (Briand & Legrand, 2022). The silvering index results in six maturation stages, where I denotes resident undifferentiated males and females, FII denotes residential females still in their growth phase, MII are migrant males, FIII are premigrant females, and FIV and FV are migrant females. The original six life stage classes derived from Durif's silvering index were reduced into three classes to achieve relatively homogenous sample sizes for the statistical analyses; FI, FII or I = 'growth stage', FIII = 'premigrating' and FIV, FV or MII = 'migrating'.

For the first part of the study, the effects of parasite presence and intensity on body condition were analysed using the extracted

dataset from the database 'Sötebasen'. We analysed body condition based on the $\log_e$-$\log_e$ length-mass relationship (i.e., comparing mass at a given length). Such regressions effectively include the same parameters as the 'Le Cren condition factor' (Le Cren, 1951), but do not turn them into an index. The more commonly used cube law ('Fulton condition factor') is not suitable due to the allometric growth of eels (Froese, 2006). We analysed the effects of *A crassus* presence and intensity using two regression models with the same structure, where $\log_e$(body mass) was the dependent variable, and body length [$\log_e$(LENGTH)], life stage (STAGE), preservation method (PRESERVE; three levels: fresh, ethanol, and frozen), and either parasite presence (PRESENCE) or –intensity [$\log_{10}$(INTENSITY+1)] were included as independent variables. The interaction $\log_e$(LENGTH)×STAGE was included to allow for different life stages to have different length-mass relationships and the interaction STAGE × [PRESENCE or $\log_{10}$(INTENSITY+1)] was included to allow for different effects of parasites at different life stages. Finally, the water body in which the eels were captured was included as a random factor '(1|WATERBODY)'. Models were evaluated based on Analysis of Deviance (ANODEV), with type III Wald χ^2-tests. For the model investigating the effects of parasite intensity, we also derived residuals from a model excluding $\log_{10}$ (INTENSITY+1) and STAGE, to examine potential non-linear effects of intensity for each separate life stage by means of loess regression.

In the second part of the study, body condition (relative body mass) was analysed based on the $\log_e$-$\log_e$ length-mass relationship, as described above for the first part of the study, with the difference that we only focussed on effects of SDI, since intensity and presence was analysed using the much larger dataset from the database. The models (one for each lake) included $\log_e$ (body mass) as the dependent variable, and [$\log_e$ (LENGTH)] and SDI as independent variables. Ad hoc, we also explored whether adding life stage (STAGE) to the models would improve the model fit, which it did not (see Section 3). The effects of *A. crassus* on spleen mass, liver mass and body fat were analysed using a single procedure. Linear models were constructed with $\log_e$-transformed spleen wet mass as the dependent variable, in separate models investigating effects of parasite presence ('PRESENCE'—2 levels: yes or no), parasite intensity ('INTENSITY'—number of visible parasites, continuous variable), and SDI class ('SDI'—4 levels: 0, 1, 2–3, and 4–6). Each model included $\log_e$-transformed body wet mass ('BM') as a covariate. Lake ('LAKE'—2 levels: Mälaren and Ringsjön) was included as a factor in the PRESENCE- and INTENSITY-models. Interactions including LAKE and parasite-related independent variables were initially included but removed if the interaction models were not indicating a better fit than the additive main factor model, based on likelihood ratio tests (threshold: $p < .1$). For analyses of effects of SDI, separate models were run on lake-specific data subsets, due to certain SDI classes being absent or severely under-represented in each of the lakes (Mälaren: class 4–6 absent; Ringsjön: class 0 with $n = 1$). Model results were further evaluated based on pairwise contrasts of estimated marginal means (R package: emmeans; Lenth, 2022) for the PRESENCE- and SDI-models, and using loess regression of residuals derived from a model excluding INTENSITY to detect possible non-linear patterns in the relationship between parasite intensity and spleen size. Liver mass ($\log_e$-transformed) analyses also included life-stage ('STAGE') for the PRESENCE model (all STAGE-related parameters were non-significant, hence not included in INTENSITY- or SDI-models). For the global models of body fat levels, STAGE was included and BM excluded. The SDI-models for each lake only included SDI.

In the second part of the study, we also evaluated if all four measurements of parasitization pressure show a similar picture of parasitization. A series of comparative analyses were run using Spearman's rank tests (R package: pspearman; Savicky, 2022), Phi correlation test (R package: psych; Revelle, 2022) or χ^2 test of independence (R package: MASS; Ripley, 2022). Differences in average parasite intensity among the categories of SDI were analysed using a quasi-Poisson regression (log link function), suitable for overdispersion count data (R package: stats; R Core Team, 2021). Pairwise contrasts of SDI classes were conducted, adjusting *p*-values using the Tukey method for comparing a family of four estimates (R package: emmeans; Lenth, 2022).

3 | RESULTS

3.1 | Overall infection, descriptive results

For the first part of the study, visible *A. crassus* was found in 60.2% of all eels in the database. Among all eels ($N = 17{,}674$), the intensity of visible parasites per eel ranged from 0 to 167 (with the maximum intensity recorded in one individual), and an overall median of 1 parasite (IQR: 4; Q1–Q3: 0–4 parasites). Among infected eels (i.e., eels with current presence of visible *A. crassus*, $n = 10{,}646$), the median intensity was 3 parasites (IQR: 5; Q1–Q3: 2–7 parasites). The total body length of the eels ($N = 17{,}674$) ranged from 63 to 1230 mm, with a median of 696 mm and an interquartile range (IQR) of 308 mm (Q1–Q3: 484–792 mm). Total wet mass of the eels ($N = 17{,}674$) ranged from 0.07 to 3672 g, with a median of 672 g (IQR: 864 g; Q1–Q3: 182–1046 g). In comparison, in the second part of the study, the total body length of the eels ($N = 202$) ranged from 305 to 1085 mm, with a median of 702 mm and an interquartile range (IQR) of 207 mm (Q1–Q3: 563–770 mm). Total wet mass of the eels ($N = 202$) ranged from 45.1 to 3560 g, with a median of 688 g (IQR: 585 g; Q1–Q3: 314–899 g). In total, visible *A. crassus* was found in 64% of all eels analysed (Ringsjön: $n = 90$; 82%; Mälaren: $n = 112$; 50%). Among all eels, the intensity of visible parasites per eel ranged from 0 to 134 (with the maximum intensity recorded in one individual), and an overall median of 1 parasite (IQR: 3; Q1–Q3: 0–3 parasites). Among infected eels (i.e., eels with current presence of visible *A. crassus*, $n = 130$), the median intensity was 2 parasites (IQR: 4; Q1–Q3: 1–5 parasites). The median total mass of parasites per eel was 0.099 g (IQR: 0.287; Q1–Q3: 0.012–0.299 g) while the maximum total recorded in a single eel was 5.94 g. Damaged swim bladders, i.e., SDI of category 1 and above, were observed in 71% of all eels ($N = 202$); 15% of the eels without visible parasites present had a damaged swim bladder, indicating previous infections or larval stages in the wall causing the damage. All SDI classes were represented in Ringsjön, but only

one eel had a swim bladder with no damage, i.e., SDI of category 0 ($n_{SDI:0}=1$, $n_{SDI:1}=20$, $n_{SDI:2-3}=35$, $n_{SDI:4-6}=34$). In Mälaren, there were no eels in the highest SDI damage class ($n_{SDI:0}=58$, $n_{SDI:1}=36$, $n_{SDI:2-3}=18$, $n_{SDI:4-6}=0$).

3.2 | Relationships between measures of parasite pressure

The comparison of the four measurements of parasitization pressure indicates that they do not show a completely similar picture. There was a moderate correlation between swim bladder damage (binary: damage or no damage) and parasite presence (binary: present or absent) (Phi correlation: $\varphi=0.45$), showing that the two dichotomous variables were not independent of each other (χ^2-test of independence: $\chi^2=39.57$, $p<.0001$; Table 2a). There was a moderate increase in damage to the swim bladders with an increasing number of parasites, as indicated by a significant rank correlation between parasite intensity and swim bladder degenerative index (SDI) (Spearman's $\rho=.43$, $p<.001$). For analyses of both all eels and infected eels only (i.e., eels with visible parasites), the parasite intensity was found to be significantly different among the SDI classes (all eels: $\chi^2=32.58$, $p<.001$; infected eels: $\chi^2=15.73$, $p=.001$; Figure 1b,c). The pairwise comparisons of the SDI classes indicated significant differences for the contrasts SDI_0 vs. SDI_{4-6} and SDI_1 vs. SDI_{4-6} in both analyses, and for SDI_0 and SDI_{2-3} in the analysis including all eels (Table 2b). There was no significant rank correlation between SDI and total parasite mass (Spearman's $\rho=.16$, $p=.06$), but a significant rank correlation was found between total parasite mass and parasite intensity among infected eels (Spearman's $\rho=.69$, $p<.01$). Weighing the parasites proved to be difficult; sometimes the parasites would break while being handled (leading to loss of body fluid and thereby most likely reduced mass), and the accuracy in this parameter might not have been as good as intended. Due to this fact, and since there was a strong correlation between parasite mass and parasite intensity, only parasite presence, intensity, and SDI were used in the remaining analyses.

TABLE 2 Relationships between *Anguillicola crassus* parasite presence and swim bladder damage in the European eel. (A) Contingency table showing frequencies of parasite presence and swim bladder damage. (B) *p*-values for pairwise contrasts (*p*-value adjustment using the Tukey method for a family of 4 estimates) of quasi-Poisson regressions of number of parasites as dependent on swim bladder degenerative index (SDI) categories: 0, 1, 2–3, and 4–6.

		Parasites	
(A)		**Absent**	**Present**
Swim bladder:	No damage (SDI = 0)	41	18
	Damage (SDI > 0)	31	112

(B)	**All eels (Figure 1b)**		**Only parasitized eels (Figure 1c)**	
Comparison: SDI classes	**Ratio (SE)**	***p***	**Ratio (SE)**	***p***
0 vs. 1	0.20 (0.15)	.135	0.52 (0.40)	.832
0 vs. 2–3	0.11 (0.08)	.013	0.30 (0.22)	.362
0 vs. 4–6	0.06 (0.04)	<.001	0.15 (0.11)	.049
1 vs. 2–3	0.57 (0.22)	.469	0.57 (0.23)	.520
1 vs. 4–6	0.29 (0.11)	.007	0.29 (0.11)	.008
2–3 vs. 4–6	0.52 (0.17)	.179	0.50 (0.17)	.172

3.3 | Body condition

For the first part of the study, we did not detect any effects of parasite presence on relative body mass (i.e., body condition) (ANODEV: PRESENCE: $\chi^2=0.070$; $p=.792$; STAGE × PRESENCE: $\chi^2=3.090$; $p=.378$; Figure 2a). All other fixed factor terms (i.e., length, life stage, and preservation method) had a significant effect on body mass [$\log_e$(LENGTH): $\chi^2>4.8\times10^4$; $p<.001$; STAGE: $\chi^2=357.3$; $p<.001$; PRESERV: $\chi^2=172.7$; $p<.001$; $\log_e$(LENGTH) × STAGE: $\chi^2=328.6$; $p<.001$)]. There were also no detectable effects of parasite intensity on relative body mass ($\log_{10}$(INTENSITY +1): $\chi^2=1.572$; $p=.210$; STAGE × $\log_{10}$(INTENSITY +1): $\chi^2=4.738$; $p=.192$; Figure 2b). For the rest of the fixed factors, the results were largely equivalent with the parasite presence model (χ^2 and *p*-values differing very marginally), as expected given that both models analyse body mass using the same terms except for the parasite variables. No apparent indications of non-linear effects of parasite intensity were detected (Figure 2b). For both models, the random factor (1|WATERBODY) contributed to a better fit as compared to a model excluding the term (likelihood ratio test: $\chi^2>3.6\times10^3$; $p<.001$). Parameter estimates for both models are found in Table 3.

The investigation of body condition during the second part of the study was limited to effects of SDI. No effects of SDI class were detected for either of the two sampled lakes (Table 4). Adding life-stage to the models, either as only an additive effect or also including the interaction with lake, did not improve the model fit. Instead, the simpler models excluding these terms had significantly better fit, as assessed by likelihood ratio tests (all $p<.008$).

3.4 | Spleen size

Spleen size was not clearly affected by parasite presence, intensity, or SDI class (Table 5a, Figure 3). For presence, potential differences between eels with and without parasites were virtually undetectable in relation to the overall variation (Figure 3a) and estimated marginal means support no effects, with largely overlapping 95% confidence intervals for the estimates (Figure 3b). Parasite intensity showed no visible tendency for effects, neither linear (Table 5a) nor non-linear (Figure 3c,d). For SDI, no statistical tendencies for effects were detected in the pairwise contrasts, in either lake (Mälaren: all $p>.36$; Ringsjön: all $p>.85$; Figure 3e–f). Parameter estimates from the PRESENCE- and INTENSITY-models indicated that eels from Lake

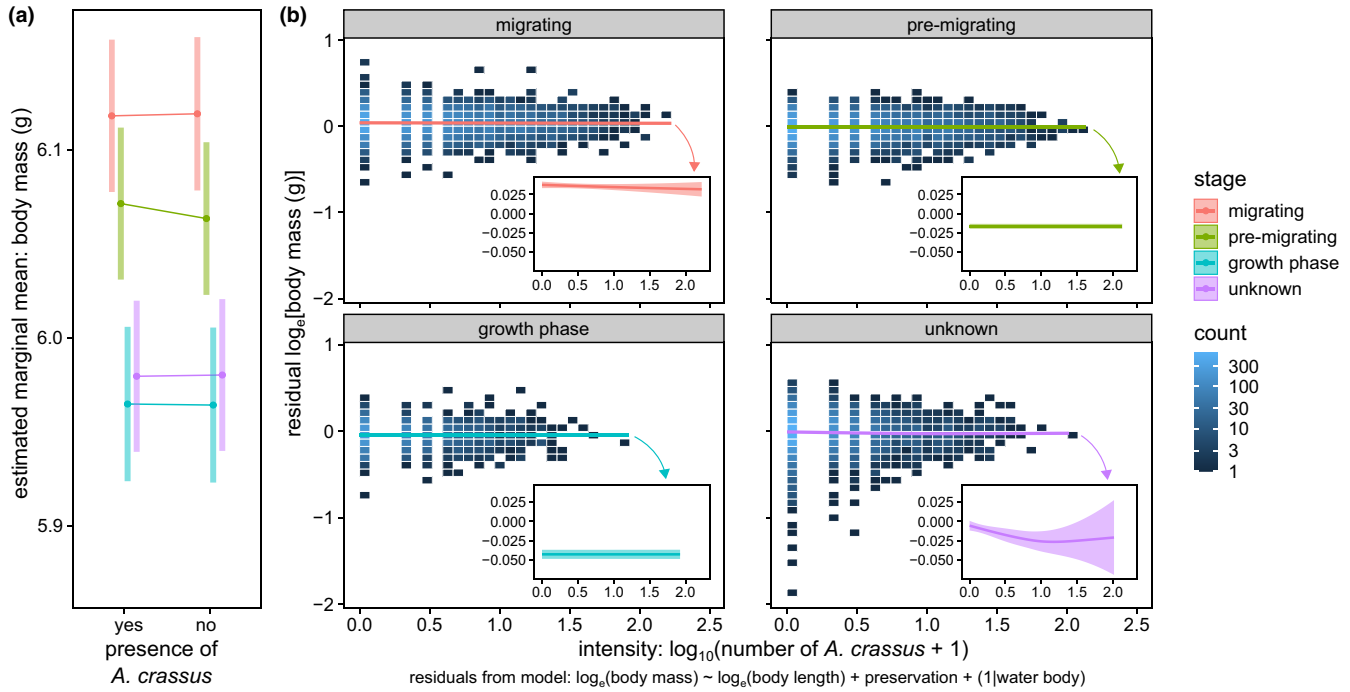

FIGURE 2 Effects of *Anguillicola crassus* on the relative body mass of European eel (indicative of body condition, i.e., mass at a given body length). (a) Effects of parasite presence, presented as estimated marginal means, evaluated at the overall mean body length. Error bars represent the 95% confidence interval of the predictions for each presented estimate. (b) Relationship between parasite intensity (number of parasites) and body condition, for each life stage category, based on analyses of residuals from a reduced model (specified in the figure). The main figures show the linear relationships, and the inset figures show the non-linear fit (loess regression, with 95% confidence intervals; note the difference in the *y*-axis scaling compared to the main figure). For plots of raw data, see Figure S1. Parameter estimates are presented in Table 3.

Ringsjön had lower relative spleen mass than eels from Mälaren, on average (*p*-values ranging from 0.038 to 0.053; Table 5a, Figure 3a).

3.5 | Liver size

Liver size was not clearly affected by parasite presence, intensity, or SDI class (Table 5b, Figure 4). For presence, potential differences between eels with and without parasites were virtually undetectable in relation to the overall variation (Figure 4a). Parasite intensity showed no visible tendency for effects, neither linear (Table 5b) nor non-linear (Figure 4b,c). For SDI, no statistical tendencies for effects were detected in the pairwise contrasts, in either lake (Mälaren: all $p > .98$; Ringsjön: all $p > .39$; Figure 4d,e). Lake had a significant effect on relative liver size, with eels from Ringsjön having relatively smaller livers than eels from Mälaren (Table 5b). Life stage had no effect on liver size in the PRESENCE model (Table 5b).

3.6 | Body fat

No effects of parasite presence or SDI on body fat were detected (Figure 5, Table 6). For parasite intensity, the global model was non-significantly different from the intercept-only model ($p = .097$; Table 6). However, ANOVA indicated a tendency for an interaction effect between parasite intensity and life stage ($p = .052$). Parameter estimates suggest that the growth-stage eels may have a decreasing fat level with increasing parasite intensity, as compared with premigratory and migratory eels (Figure 5b, Table 6). Since growth-stage eels were relatively poorly represented for eels with high intensity, and since the data are still within the range of the other life stages, the biological relevance of the interaction tendency can be reasonably questioned.

4 | DISCUSSION

In this study, the effects of parasitization of the non-native invasive nematode *Anguillicola crassus* on the European eel were studied with a focus on the health status and condition of eels during their continental residency. No major effects on spleen size, liver size, fat content or body condition could be detected in relation to parasite presence, intensity (number of parasites in the lumen) or swim bladder degenerative Index (SDI).

4.1 | Spleen size

If parasitization by *A. crassus* activates the immune system, then increased lymphocyte production and consequently larger spleens

TABLE 3 Summary tables for linear mixed models of mass [$\log_e$(mass)] in relation to length [$\log_e$(length)], parasite variables ['X' in table; either presence (yes or no; reference = yes) or intensity ($\log_{10}$-transformed number of parasites)], and nuisance factors [stage (reference = migrating) and preservation method (reference = ethanol)].

	X = presence		*X* = $\log_{10}$(intensity + 1)	
	Estimate (±SE)	***p***	**Estimate (±SE)**	***p***
Intercept	−14.04 (0.097)	<.001	−14.04 (0.097)	<.001
$\log_e$(Length)	3.135 (0.014)	<.001	3.135 (0.014)	<.001
Stage (premigrating)	1.050 (0.146)	<.001	1.036 (0.145)	<.001
Stage (growth phase)	−0.797 (0.144)	<.001	−0.796 (0.144)	<.001
Stage (unknown)	−1.125 (0.100)	<.001	−1.128 (0.100)	<.001
X	0.001 (0.004)	.792	−0.005 (0.004)	.210
Preservation (fresh)	−0.004 (0.012)	.751	−0.004 (0.012)	.756
Preservation (thawed)	0.070 (0.009)	<.001	0.070 (0.009)	<.001
$\log_e$(Length) × Stage (premigrating)	−0.171 (0.022)	<.001	−0.170 (0.022)	<.001
$\log_e$(Length) × Stage (growth phase)	0.100 (0.022)	<.001	0.099 (0.022)	<.001
$\log_e$(Length) × Stage (unknown)	0.154 (0.015)	<.001	0.154 (0.015)	<.001
Stage (premigr.) × *X*	0.009 (0.006)	.117	0.012 (0.006)	.039
Stage (growth phase) × *X*	−0.002 (0.008)	.832	0.012 (0.010)	.241
Stage (unknown) × *X*	−0.000 (0.006)	.943	0.004 (0.008)	.579
Random effects	**Variance**	**SD**	**Variance**	**SD**
Water body	0.0183	0.1352	0.0187	0.1367
Residual	0.0188	0.1372	0.0188	0.1372

TABLE 4 Model results with parameter estimates for the analysis of body condition of the European eel. The table presents ANOVA results for the global model fit (adjusted R^2, degrees of freedom, *F*-value and *p*-value) and parameter estimates, with standard errors, for the independent variable swim bladder degenerative index (SDI) categories. Note that separate models were run on lake-specific data subsets (Mälaren and Ringjsön).

		Adj. R^2	df	*F*	Estimate (±SE)	*p*
Body condition (Relative body mass)						
SDI:Mälaren	Global model	−.02	2, 53	0.38		.685
	Intercept				21.841 (1.321)	<.001
	SDI (1)				0.559 (1.698)	.743
	SDI (2–3)				−1.057 (2.006)	.601
SDI:Ringsjön	Global model	−.02	3, 70	0.49		.689
	Intercept				24.300 (5.065)	<.001
	SDI (1)				−2.567 (5.204)	.623
	SDI (2–3)				−0.797 (5.152)	.878
	SDI (4–6)				−1.208 (5.162)	.816

would be expected in infected eels as have been seen in other species (Arnott et al., 2000; Morand & Poulin, 2000; Owens & Wilson, 1999). In our study, larger relative spleen size was detected in eels with a mildly damaged swim bladder (SDI = 1), compared to eels without any damage. The effect was statistically significant when analysing the data subset containing eels from Mälaren, but not in Ringsjön and SDI was not significantly related to spleen size in the overall model. In addition, eels with the highest SDI (SDI = 2–3 in Mälaren, 4–6 in Ringsjön), did not have larger spleens compared to those with no damage. One potential explanation for this pattern could be that presence of the parasite activates the immune response initially after infection, with a subsequent fading response (Sures et al., 2001). Eels with a high SDI would then already have passed the stage where the immune effect on the spleen is detectable. The relationship between spleen size and parasite intensity would thereby be non-linear, with effects on spleen size primarily detected at low-intensity levels. This was not seen in Mälaren. Sures et al. (2001) observed increased cortisol levels, indicative of a stress response, in the early stages of experimental infection of *A. crassus*, but no chronically elevated cortisol levels were seen as the parasites grew and matured within the eels. Consequently, the authors proposed that it is mainly the initial infection by the

TABLE 5 Model results with parameter estimates for (A) spleen mass (log_e-transformed) and (B) liver mass (log_e-transformed) analyses. The table presents ANOVA results for the global model fit (adjusted R^2, degrees of freedom, *F*-value and *p*-value) and parameter estimates, with standard errors, for each included independent variable (*A. crassus* parasite presence, parasite intensity (number of parasites), and swim bladder degenerative index (SDI) categories). Note that separate models were run on lake-specific data subsets (Mälaren and Ringsjön) for the analyses of effects of SDI.

		Adj. R^2	df	*F*	Estimate (SE)	*p*
(A) Spleen						
Presence	Global model	.72	3, 198	170.3		<.001
	Intercept				−5.361 (0.249)	<.001
	log_e(body mass)				+0.809 (0.042)	<.001
	Presence (yes)				+0.047 (0.054)	.383
	Lake (Ringsjön)				+0.127 (0.061)	.038
Intensity	Global model	.60	3, 126	64.84		<.001
	Intercept				−5.186 (0.387)	<.001
	log_e(body mass)				+0.792 (0.064)	<.001
	Parasite intensity				−0.000 (0.003)	.887
	Lake (Ringsjön)				−0.157 (0.080)	.053
SDI: Mälaren	Global model	.80	3, 108	146.1		<.001
	Intercept				−5.328 (0.238)	<.001
	log_e(body mass)				+0.799 (0.041)	<.001
	SDI (1)				+0.149 (0.069)	.032
	SDI (2–3)				−0.010 (0.086)	.907
SDI: Ringsjön	Global model	.12	4, 85	4.041		.005
	Intercept				−5.242 (1.387)	<.001
	log_e(body mass)				+0.752 (0.189)	<.001
	SDI (1)				+0.155 (0.390)	.693
	SDI (2–3)				+0.185 (0.386)	.633
	SDI (4–6)				+0.193 (0.389)	.621
(B) Liver						
Presence	Global model	.93	5, 196	578.4		<.001
	Intercept				−3.857 (0.161)	<.001
	log_e(body mass)				+0.900 (0.029)	<.001
	Presence (yes)				+0.015 (0.026)	.555
	Stage (premigrating)				+0.001 (0.047)	.991
	Stage (migrating)				+0.006 (0.050)	.902
	Lake (Ringsjön)				−0.084 (0.032)	.009
Intensity	Global model	.91	3, 126	463.2		<.001
	Intercept				−4.086 (0.178)	<.001
	log_e(body mass)				+0.941 (0.029)	<.001
	Parasite intensity				+0.000 (0.001)	.838
	Lake (Ringsjön)				−0.108 (0.037)	.004
SDI: Mälaren	Global model	.94	3, 108	560.2		<.001
	Intercept				−3.888 (0.134)	<.001
	log_e(body mass)				+0.908 (0.023)	<.001
	SDI (1)				−0.006 (0.039)	.886
	SDI (2–3)				+0.004 (0.048)	.938
SDI: Ringsjön	Global model	.63	4, 85	38.22		<.001
	Intercept				−3.212 (0.531)	<.001
	log_e(body mass)				+0.827 (0.072)	<.001
	SDI (1)				−0.231 (0.150)	.126
	SDI (2–3)				−0.194 (0.148)	.194
	SDI (4–6)				−0.246 (0.149)	.102

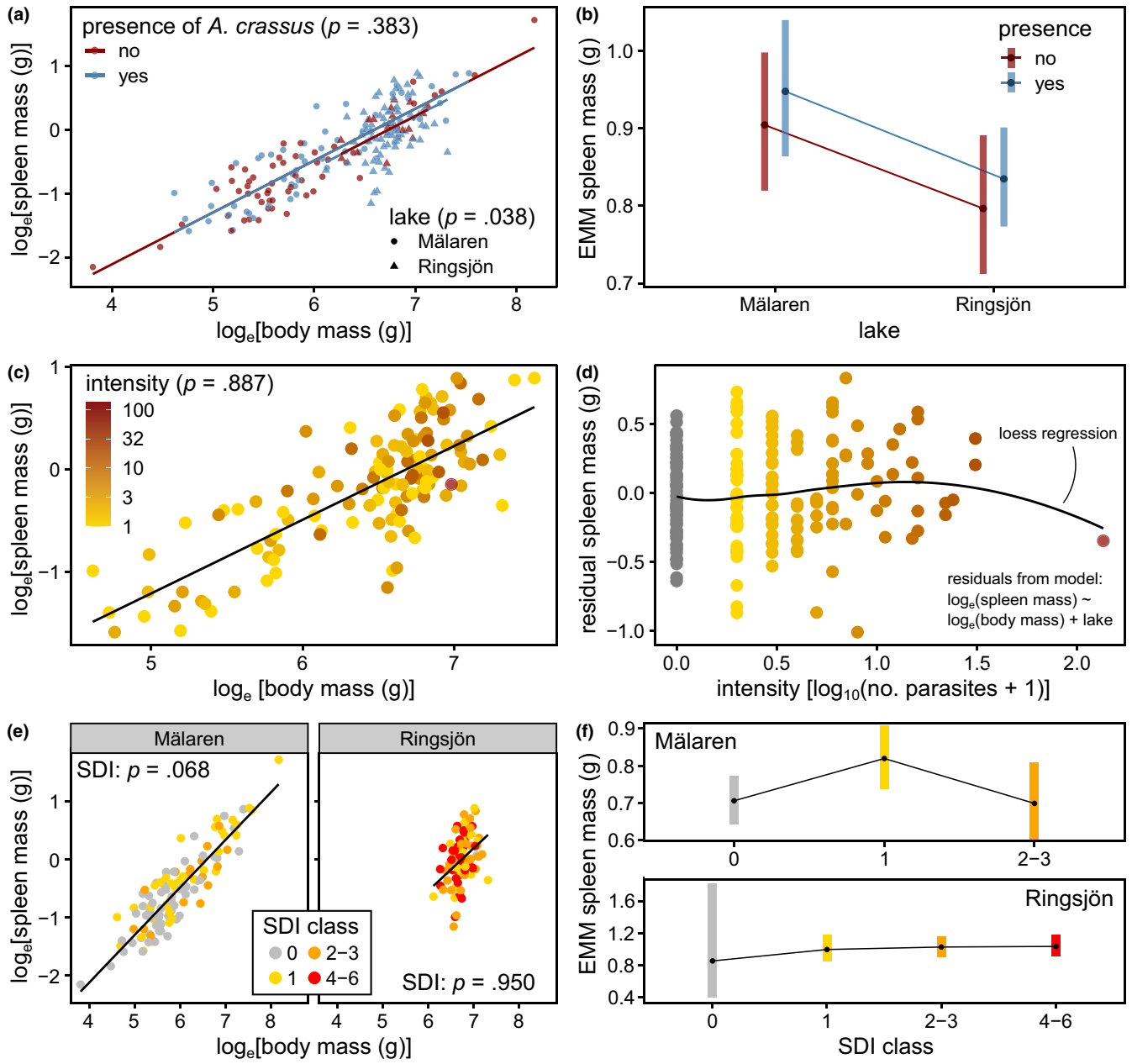

FIGURE 3 Spleen mass of European eel in relation to *Anguillicola crassus* parasite-related variables. (a, b) Effects of parasite presence presented in relation to body mass (a) and as estimated marginal means, with 95% confidence intervals, evaluated at the mean body mass (b). (c, d) Effects of parasite intensity (number of parasites) presented in relation to body mass (c) and as residuals from a model excluding intensity as an independent variable, to detect possible non-linear patterns (d; also including uninfected individuals, i.e., eels with no current presence of visible *A. crassus*, as grey data points, for illustrative purpose). (e, f) Effects of swim bladder damage (swim bladder degenerative index—'SDI class') for each investigated lake (Mälaren and Ringsjön), presented in relation to body mass (e) and as estimated marginal means, with 95% confidence intervals, evaluated at the mean body mass (f). Note that separate models were run on lake-specific data subsets for the analyses of effects of SDI. Parameter estimates for each modelled effect are presented in Table 3a.

larval stages in the swim bladder wall tissue that causes damage and triggers the stress response (Sures et al., 2001). This could be the reason why increased spleen size was only observed in relation to swim bladder damage and not to parasite numbers in the lumen in this study, as the immune response may decline when the parasites move from the wall tissue to the lumen. The larval stages of *A. crassus* in the swim bladder walls were not counted in this study, and future studies would, therefore, be needed to confirm this theory. We observed a moderate correlation between the damage to the bladders and the presence or intensity of parasites in the lumen in this study. Some of the eels that had visible parasites did not have a damaged swim bladder, and some of the most damaged swim bladders did not contain any visible parasites. This supports a hypothesis that the parasites are not able to survive in the eel when

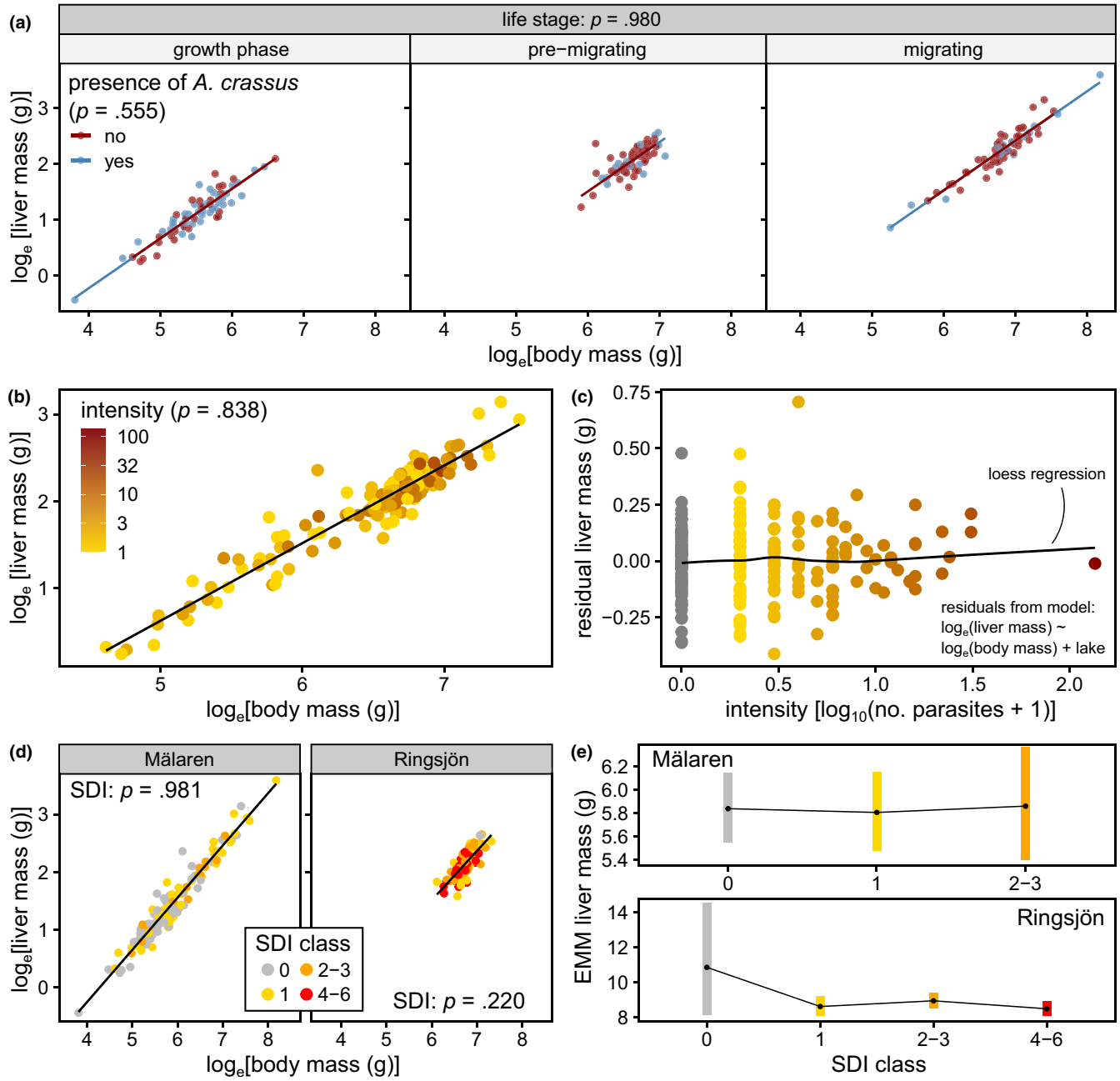

FIGURE 4 Liver mass of European eel in relation to *Angillicola crassus* parasite-related variables. (a) Effects of parasite in relation to body mass for different life stages. (b, c) Illustration of effects of parasite intensity on relative liver mass (b) and distribution of residuals from the predicted mean $\log_e$-$\log_e$ relationship between liver- and body mass (c). (d) Illustration of relative liver mass as dependent on swim bladder damage (swim bladder degenerative index—'SDI class') and lake origin (Mälaren or Ringsjön). (e) Estimated marginal means of liver mass, evaluated at the geometric mean body mass, for different SDI classes in the two sampled lakes (error bars = 95% confidence intervals). Note that separate models were run on lake-specific data subsets for the analyses of effects of SDI. Parameter estimates for each modelled effect are presented in Table 5b.

the swim bladder becomes too damaged (Lefebvre et al., 2002). If the damage of the swim bladder is a remnant of past infections in some cases, another possible explanation for why larger spleens were not observed in eels with a more damaged bladder could be a confounding effect of no ongoing infection. In turn, this makes it difficult to use the SDI as a measure of ongoing parasitization. This needs to be considered when studying naturally infected animals. Studies where the test animal has been experimentally infected could be used to confirm results from studies based on naturally infected animals. Yet another hypothesis is that the most severely damaged swim bladder is a result of repeated infections (Lefebvre et al., 2002). If the eel react differently immunologically to a repeated infection compared to a novel infection, this could further explain the larger relative spleens in eels with a low SDI, and why

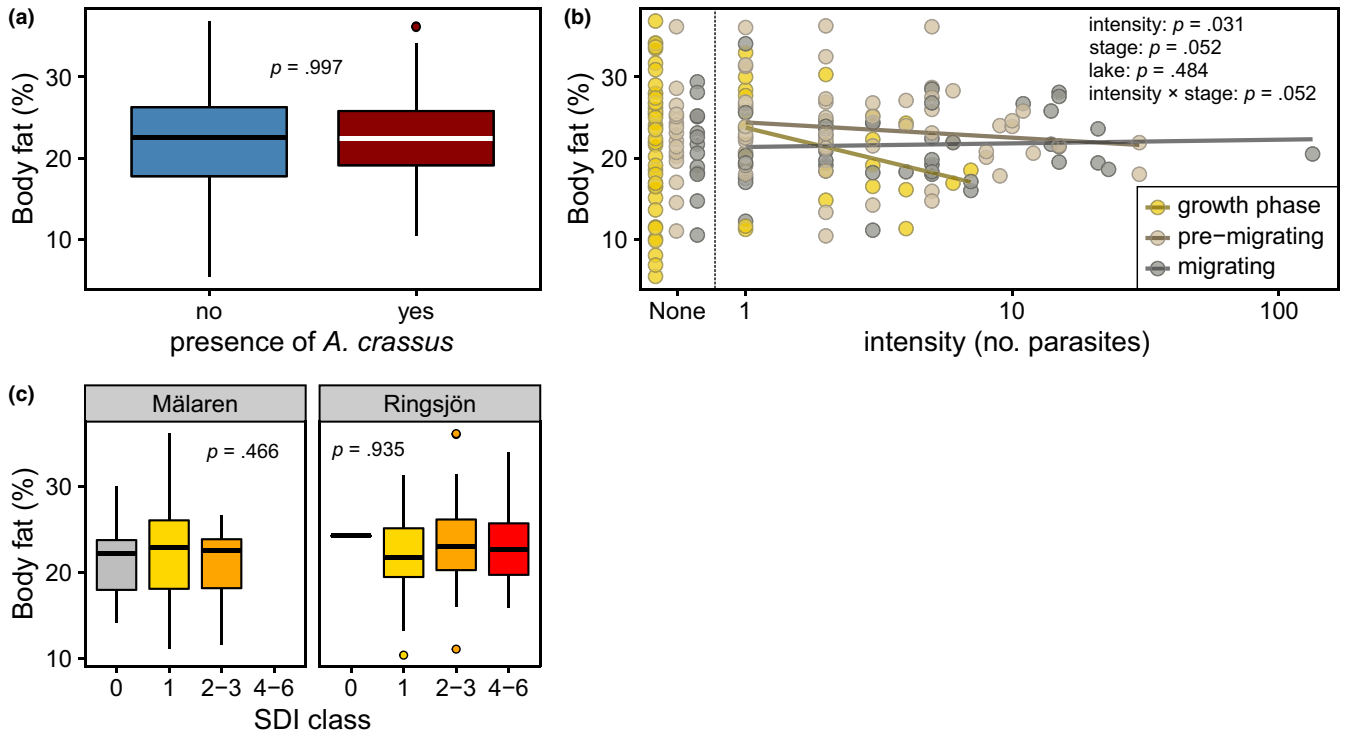

FIGURE 5 Effects of *Anguillicola crassus* parasitization on the body fat content (%) of European eel. (a) Tukey boxplots showing fat content in relation to parasite presence. (b) Effects of parasite intensity on body fat content (%). Regression lines relate to eels with parasites present in the swim bladder, separated by life stage; data points for eels without parasites (i.e., eels with no current presence of visible *A. crassus*) are presented for an overview of their body fat levels. (c) Tukey boxplots illustrating effects of swim bladder degenerative index (SDI) class on body fat content (%) in the two sampled lakes (Mälaren and Ringsjön). Note that separate models were run on lake-specific data subsets for the analyses of effects of SDI.

it was not found in eels with a high SDI. This is supported by the results from Ringsjön, where the sample mainly consisted of premigrating and migrating eels with a relatively high degree of damage to the swim bladders, yet no significant relation between spleen size and SDI was observed, while in Mälaren, where the sample mainly consisted of eels in their growth phase, there was a significant relationship between spleen size and SDI. The detected effect on spleen size in relation to swim bladder damage was not large compared to the overall variation of spleen size and it is possible that *A. crassus* does not impose any major reaction of the immune system related to the spleen in the European eel. Nevertheless, a recent study showed that infected European eel had a higher immune-related gene expression in the kidney compared to the Japanese eel (the native host to *A. crassus*), and argued that the immunological response in the European eel has not yet been adapted to co-existence with *A. crassus* (Bracamonte et al., 2019). Several mechanisms for a fading immunological response have been described in fish host–parasite interactions and could be driven by immune evasion by the parasite (Sitjà- Bobadilla, 2008), energy-balance trade-offs by the host (Sheldon & Verhulst, 1996), or immunological trade-offs in the presence of other parasites (Bracamonte et al., 2021). Further work is needed to investigate what mechanisms are at play in the interaction between the European eel and *A. crassus*.

4.2 | Energy storage—Liver size, body condition and fat reserves

No effects of parasite presence, parasite intensity, or SDI were detected on liver size in this study. Liver mass could be affected negatively if energy is allocated to the immune system (Houston et al., 2007). Since no effects on either liver size or any clear effects on spleen size were found, this study provides no support for the hypothesis that energy storage depletion can be caused by an immunological response to parasitism of *A. crassus*. Since damaged swim bladders could be associated with problems related to buoyancy regulation, it could in turn lead to inefficient swimming and impaired foraging ability. Hence, we expected to see a decrease in liver mass in relation to damaged swim bladders, given that the liver is the first energy storage tissue to be affected during energy deprivation (Dave et al., 1975). No such effects were found. Effects were neither observed in relation to body condition nor fat content regarding any of the parasite pressure variables. There were some indications that eels in the growth phase with a higher parasite intensity had a lower fat content, but the same pattern was not found for eels in the premigrating or migrating phase. The fat meter used for these analyses tends to overestimate fat content for eels in their growth phase since they have thinner skin than matured migrating eels (Pohlmann et al., 2019). Here we assume that this overestimation

TABLE 6 Model results with parameter estimates for the analysis of body fat content of the European eel. The table presents ANOVA results for the global model fit (adjusted R^2, degrees of freedom, *F*-value and *p*-value) and parameter estimates, with standard errors, for each included independent variable (*Anguillicola crassus* parasite presence, parasite intensity (number of visible parasites), and swim bladder degenerative index (SDI) categories). Note that separate models were run on lake-specific data subsets (Mälaren and Ringsjön) for the analyses of effects of SDI.

		Adj. R^2	df	*F*	Estimate (±SE)	*p*
Body fat						
Presence	Global model	.01	4, 197	1.48		.209
	Intercept				21.770 (0.799)	<.001
	Presence				0.004 (0.941)	.997
	Life stage (premigrating)				0.095 (1.330)	.943
	Life stage (migrating)				−1.135 (1.254)	.367
	Lake (Ringsjön)				1.877 (1.131)	.099
Intensity	Global model	.038	6123	1.84		.097
	Intercept				24.701 (1.635)	<.001
	Intensity				−1.329 (0.607)	.031
	Life stage (premigrating)				−1.104 (2.057)	.592
	Life stage (migrating)				−3.543 (1.952)	.072
	Lake (Ringsjön)				0.816 (1.163)	.485
	Intensity × Stage (premigr.)				1.178 (0.618)	.059
	Intensity × Stage (migrating)				1.322 (0.608)	.032
SDI:Mälaren	Global model	−.02	2, 53	0.38		.685
	Intercept				21.841 (1.321)	<.001
	SDI (1)				0.559 (1.698)	.743
	SDI (2–3)				−1.057 (2.006)	.601
SDI:Ringsjön	Global model	−0.02	3, 70	0.49		.689
	Intercept				24.300 (5.065)	<.001
	SDI (1)				−2.567 (5.204)	.623
	SDI (2–3)				−0.797 (5.152)	.878
	SDI (4–6)				−1.208 (5.162)	.816

is reasonably consistent for all eels in this life stage, and hence the analyses should still show any potential effects of parasite intensity. In general, the median number of parasites was low in this study and the sample size for eels in their growth phase with many parasites was low. Since body condition and fat content both reflect the lipid levels in the muscles (Boëtius & Boétius, 1985; Parzanini et al., 2021), differences between life stages in fat content should reasonably be reflected in body condition as well. This was not the case for the dataset used in the first part of this study, including many eels in their growth phase, in relation to parasite presence or parasite intensity, nor in the second part of this study, with eels from the two lakes, in relation to SDI. There was also a significant effect indicating that relationships between relative body mass and parasite intensity differ depending on life stage, but in these analyses, it was the migrating eels that had a slightly steeper negative slope. However, the effect is minor and appear to be biologically insignificant given the overall variation observed in the dataset. Overall, we interpret the results as not providing any strong evidence for generally negative effects of *A. crassus* on eel body condition or fat content. Our study spanned over a period of high feeding (Westerberg & Sjöberg, 2015), with possible increase in both growth and parasitization over time, and it is possible that a collection of individuals later in the season would have given different results. Nevertheless, our conclusion is in line with several previous studies also failing to find negative effects of *A. crassus* on body condition of eels in their natural habitat (e.g., Giari et al., 2021; Lefebvre et al., 2013; Sjöberg et al., 2009; Würtz et al., 1998). It is possible that any energy cost caused by parasitization can be compensated for by a higher food intake, as Lefebvre et al. (2013) proposed, who also found higher food content in the stomachs of parasitized eels. Muscle lipids have been suggested to be the most important energy source when it comes to reaching the spawning grounds and having enough energy left for successful reproduction (Svedäng & Wickström, 1997; Van Den Thillart et al., 2004). Arguably, the migrating stage is the most important stage to study from a population viability perspective, rather than the body condition of earlier life stages. However, a study of American eel (*Anguilla rostrata*) found that eels with signs of previous infections with *A. crassus* (damage to the swim bladder) were shorter at a given age compared to eels that did not show any signs of previous infections (no damage to the swim bladder) (Zimmerman

& Welsh, 2012). It is important to mention that our results showing no major observed effects of *A. crassus* concern only selected health indicators. If eels infected with *A. crassus* invest more in storing fat, rather than in growth, the effect of the parasites would not show as a lower relative condition or as low values of fat content in their muscles. Eels feeding a lot are also potentially at higher risk of being repeatedly infected, possibly leading to more damaged swim bladders. Therefore, knowledge about how earlier life stages are affected by the parasite is also important. In addition, sub-lethal infections by parasites that affect growth might lead to a delayed age at maturity, as shown in other parasite—host systems (Vollset & Krkosek, 2021), which in turn can have implications for the viability of the species. Further research is needed to investigate if *A. crassus* could affect growth also in the European eel since already conducted studies show contradicting results (Lefebvre et al., 2013; Thomas & Ollevier, 1992b).

4.3 | Possible effects of swim bladder damage

The most severely damaged swim bladders were only seen in the older premigrating and migrating eels, supporting the hypothesis that it takes multiple infections before the bladder becomes severely damaged (Lefebvre et al., 2002). A damaged swim bladder is not trivial for the survival of the eel since buoyancy control is considered to be important for the diurnal vertical migration observed in ocean-migrating adult eels (Aarestrup et al., 2009). One study, however, found that an eel infected with *A. crassus* had a similar swimming and diving pattern as uninfected eels, suggesting that infection had no effect on buoyancy control (Simon et al., 2018). It should be noted that those results are based on observations of a single infected individual and three uninfected individuals (Simon et al., 2018). The one infected individual was recaptured within a period of 2 months (Simon et al., 2018), which is a relatively short time given the long migration of the European eel. It might still have had enough energy stored to retain normal behaviour, as compared to if it had been recaptured after several months of migration. More research is hence needed to determine the effects of *A. crassus* on migratory behaviour. Molnár et al. (1993) studied the mechanical damage caused by *A. crassus*, and considered a thickening of the swim bladder wall of 2 mm to be severe. In this study, few eels reached the highest criteria for swim bladder wall thickness (>3 mm), even though a thickening of the wall was observed in many eels from Ringsjön. As indicated by a reduced elasticity of the wall shown in experimental studies (Barry et al., 2014; Currie et al., 2020), this criterion of the swim bladder degenerative index (SDI) could turn out to be of great importance for migratory behaviour, and even a minor thickening of the wall might have an impact. If so, wall thickness of less than the lower limit for the highest criteria of the SDI (>3 mm) should potentially also be considered severe. It is also unknown if all criteria in the swim bladder degenerative index (transparency, pigmentation, thickness) are equally important. If they are not, then SDI might not give the most representative indication of damage caused by the parasites. In addition, with respect to the natural data distribution across the categories of SDI in our samples, with few cases of the higher SDI classes compared to the lower classes, and the following need for amalgamation of these categories for comparative analyses, there is a need for evaluating detailed effects of different degrees of severe swim bladder damage. Data on parasite presence and/or intensity is collected as part of the European eel-monitoring programs in many countries (Regulation (EU) 2017/1004) and it is recommended by the joint working group on eel, WGEEL, to include it (ICES, 2021). Considering that there was only a moderate correlation between parasite presence or intensity and the damaged swim bladder categories, swim bladder damage gives us important additional information about past infections and future problems but is not a reliable measure of present parasitization. We argue that damage to the swim bladder would be a good complement to parasite presence in monitoring. However, if SDI, or a similar measure of swim bladder damage, would be included in monitoring programs, it is important that the method is thoroughly evaluated since measurement methods and precision are important for an effective stock assessment (Sundin et al., 2022). It is also important that the monitoring is well organized to minimize lethal samplings, considering the threatened status of the European eel.

5 | CONCLUSIONS

Our results show that infection with *A. crassus* had no major negative impacts on the investigated indicators of health status and body condition of European eels during their continental residency at the investigated sites across Sweden. This study provides no support for the hypothesis that energy storage depletion can be caused by an immunological response in the spleen, but effects may still be detectable in other immunological indicators. If the swim bladder damage, caused by the parasites, does have implications for the swimming abilities, which, in turn, affect the condition of the eel, then this seems to be compensated for as long as they reside in shallow waters. However, *A. crassus* is still a concern for the European eel fitness, since the many of the adult eels were found to have damaged swim bladders. Such damage could lead to problems during the Atlantic migration, where they dive to great depths and have seized to feed. Considering that there was only a moderate correlation between parasite presence or intensity and the damaged swim bladder categories, swim bladder damage gives us important additional information about past infections and future problems.

AUTHOR CONTRIBUTIONS

EM and JS, conceived and designed the study. EM and JP collected the data. EM and JN conducted the statistical analyses and created the figures. EM and JS drafted the manuscript, with contributions from all co-authors. All authors approved the final submission.

ACKNOWLEDGEMENTS

We thank all staff that have conducted data collection over the years, leading to the important and substantial database Sötebasen. Thanks to the staff that assisted during the scientific fishing survey in Mälaren, and to the commercial fisher in Ringsjön. Thanks to Sandra A Binning for valuable discussions and advice on measurements of parasitization pressure. Thanks to four reviewers, whose comments greatly improved the manuscript.

FUNDING INFORMATION

Collection of monitoring data was funded within the European Commission's Data Collection Framework (DCF). The drafting of the manuscript was funded by an internal writing grant from SLU Aqua, Department of Aquatic Resources, Swedish University of Agricultural Sciences.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data and script for this study are archived in the figshare repository: https://doi.org/10.6084/m9.figshare.22068371, following best practices (Roche et al., 2015), and was available to editors and reviewers upon initial submission.

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How to cite this article: Myrenås, E., Näslund, J., Persson, J., & Sundin, J. (2023). Effects of the invasive swim bladder parasite *Anguillicola crassus* on health and condition indicators in the European eel. *Journal of Fish Diseases*, *46*, 1029–1047. https://doi.org/10.1111/jfd.13822

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

APPENDIX 1

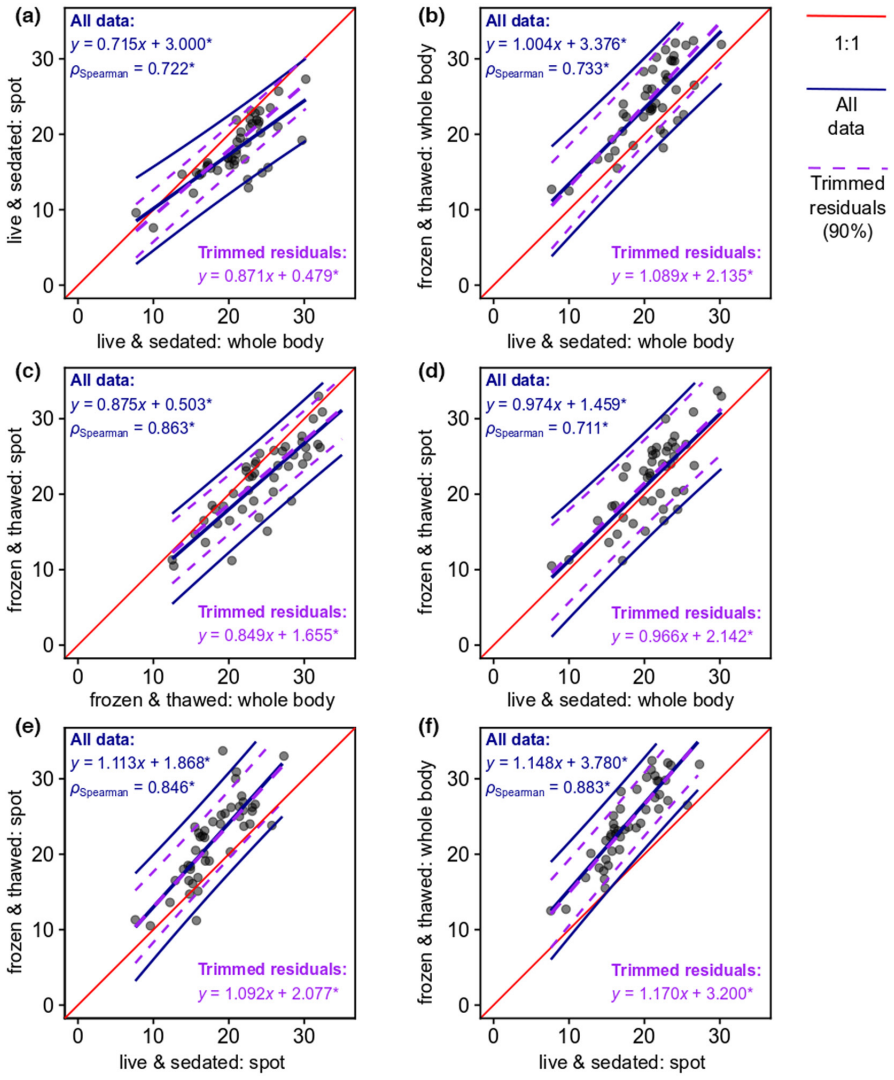

FIGURE A1 Comparison of fat measurements, using different measurement strategies: 'live & sedated' = measurements on fresh live eels; 'frozen & thawed' = measurements on frozen and subsequently thawed eels; 'whole body' = average of measurements at four different places along the body; 'spot' = average of four measurements at the same spot of the body. Red diagonal line indicate the 1:1 relationship of *x* vs. *y*. Blue lines indicate predicted values of *y* given *x* (thick line) and their 95% prediction interval (thin lines), based on all data available. Magenta coloured hatched lines indicate predicted values of *y* given *x* (thick line) and their 95% prediction interval (thin lines), based on data where the 10% most extreme residuals were trimmed from the data set. Formulas in the graphs specify the parameters for the regression lines; for complete-data analyses, the correlation between *x* and *y* is presented based on Spearman rank correlations (Spearman's ρ).