Wiley
Journal of Applied Ichthyology
Volume 2025, Article ID 2328039, 19 pages
https://doi.org/10.1155/jai/2328039

WILEY

Research Article

Parasitic and Viral Infection Rates in European Eels (*Anguilla anguilla*) in Four Spanish Mediterranean Wetlands

Elena Bereziuk[1], **Alonso Pérez-Vegas**[1], **Elena Barcala**[2], **Diego Romero**[3] **and Pilar Muñoz**[1]

[1] *Department of Animal Health, Faculty of Veterinary Medicine, Universidad de Murcia, Murcia, Spain*
[2] *National Centre Spanish Institute of Oceanography, Oceanographic Centre of Murcia, CSIC, San Pedro del Pinatar, Murcia, Spain*
[3] *Toxicology Department, Regional Campus of International Excellence "Campus Mare Nostrum", Universidad de Murcia, Murcia, Spain*

Correspondence should be addressed to Diego Romero; diegorom@um.es

Received 21 March 2025; Revised 4 November 2025; Accepted 13 November 2025

Academic Editor: Gournaga Biswas

The European eel (*Anguilla anguilla* L. 1758) is a migratory fish species whose populations have undergone a severe collapse in numbers in recent decades. Infectious diseases are one of the factors contributing to the decline of European eels. One hundred and eighteen eels from four Spanish Mediterranean sites were captured and analysed for *Anguillicoloides crassus*, anguillid herpesvirus 1 (AngHV1), rhabdovirus Eel Virus European X (EVEX) and aquabirnavirus Eel Virus European (EVE). The nematode *A. crassus* and AngHV1 were present in eels from all four sampled wetlands, which indicates that these two pathogens are commonly occurring in Mediterranean ecosystems. The global prevalence (eels from the four wetlands) of the later parasite stages (L3, L4 and adults) was 74.58%. The global prevalence of all parasite stages (L2, L3, L4 and adults) was 93.22%. The global prevalence of the AngHV1 virus was 27.7%. EVEX was only detected in eels from two of the four wetlands (12.87% global prevalence), while EVE was not detected in any sample. At the population level, pathogens seem to represent a serious threat to eels in L'Albufera de Valencia: 92.31% of specimens were co-infected by AngHV1 and EVEX and 84.62% of specimens were triple co-infected with AngHV1, EVEX and any stage of *A. crassus*. Further studies are necessary to understand the impact of diseases, together with other anthropogenic factors (pollution, salinity, climatic change, etc.), on eel status in Mediterranean ecosystems.

Keywords: *Anguillicoloides crassus*; European eel; EVE; EVEX; herpesvirus; Mediterranean areas

1. Introduction

The European eel (*Anguilla anguilla* L. 1758) is a migratory catadromous fish species with an extraordinary life cycle. The great depths of the Sargasso Sea are believed to be its breeding grounds, from where the recently emerged leaf-shaped eel larvae initiate their migration across the Atlantic Ocean. When they reach European continental environments, the larvae transform first into eel-shaped glass eels and then into yellow eels, the growth phase that occupies most of their lifespan (5–25 years) [1]. Finally, the eels undergo a second metamorphosis—the silvering process—and migrate back to the Sargasso Sea to reproduce and die [2].

The distribution area of the European eel extends from northern Norway to northern Africa, including the continental fluvial zone and the Mediterranean Basin [3, 4]. Nevertheless, the decline of eel populations in recent decades has pushed this species beyond safe biological limits [5], and so *A. anguilla* is now classified on the IUCN Red List of Threatened Species as Critically Endangered [6]. Despite the implementation of recovery plans and monitoring programmes in the European Union [7], recent reports indicate that no improvement in the status of this species has occurred to date [8]. The causes of the decline of this fish are multiple and synergistic [9, 10] and include habitat destruction, the construction of large dams, overfishing, climate and oceanic changes, environmental contamination and infectious diseases. Several studies have indicated that the parasite *Anguillicoloides crassus*, the anguillid herpesvirus 1 (AngHV1) and the rhabdovirus Eel Virus European X (EVEX), along with the aquabirnavirus Eel Virus European (EVE), represent a serious threat to European eel [11–13].

Anguillicoloides crassus [14], previously identified as *Anguillicola crassus*, is a nematode (Nematoda, Dracunculoidea) originating from Asia and presently distributed across four continents [15]. *A. crassus* larvae inhabit the wall of the eel's swim bladder, while haematophagous adults live in the lumen of the swim bladder. The presence of the parasite in the swim bladder, in addition to the severe tissue damage it causes, can impair the ability of eels to perform vertical movements in the water column during migration, which could greatly increase energetic costs [16, 17]. At the same time, this highly procreative nematode [18] contributes to reducing the eel's immune response, which may increase susceptibility of eels to other diseases [19].

Regarding viral infection, the abovementioned viruses are prone to cause severe haemorrhagic disease, immunosuppression and mortality [13, 20]. Asymptomatic virus-carrying fish may even develop disease symptoms when faced with triggering factors such as high water temperatures, low oxygen concentrations, migration, spawning and infections by other infectious agents [20]. Through an experiment, Van Ginneken et al. [13] demonstrated that asymptomatic EVEX-carrying eels developed haemorrhages and anaemia and even died during a simulated migration in large swimming tunnels. Furthermore, double or triple co-infections with these or other viruses, parasites and bacteria have been reported, which can have a serious negative impact on eels' transoceanic migratory capacity [11, 21].

In light of the recommendations from ICES [22], this research was designed to improve understanding of the current status of pathogens in the European Mediterranean eel population. To this end, the objective was to assess the infection rates of the parasite *A. crassus* and the viruses AngHV1, EVEX and EVE in eels inhabiting four wetlands along the eastern coast of Spain, three of which had no previous data available. These wetlands were selected due to their geographic position and varying degrees of anthropogenic impact. The initial hypothesis was that pathogens would be present in eels at all the studied sites and that reporting of the current prevalence levels would provide useful information for the management of European eel stocks.

2. Materials and Methods

2.1. Sampling Area. Eels were obtained from four Spanish Mediterranean ecosystems with different degrees of anthropogenic pressure: (1) L'Albufera de Valencia Natural Park; (2) El Hondo-Salinas de Santa Pola Natural Park; (3) S'Albufereta Natural Reserve; and (4) S'Albufera des Grau Natural Park (Figure 1). These ecosystems have great ecological value since they are Special Protection Areas for Birds (L'Albufera de Valencia and El Hondo-Salinas de Santa Pola), Sites of Community Interest (L'Albufera de Valencia, S'Albufereta and S'Albufera des Grau), Natura 2000 areas (L'Albufera de Valencia, S'Albufereta and S'Albufera des Grau) and important wetlands under the RAMSAR convention (L'Albufera de Valencia, El Hondo-Salinas de Santa Pola and S'Albufereta).

L'Albufera de Valencia Natural Park (39°19′54″N 0°21′08″W) is a shallow hypertrophic lagoon (salinity 1–2 PSU and depth 1–3 m), situated 15 km south of the city of Valencia, with a surface area of 23.2 km^2 (the second largest coastal lagoon in Spain). It is a brackish water lagoon due to the regulation of its connection with the sea, and, according to the quality variables used by the EU Water Framework Directive [23], its ecological status is 'worse than good' [24, 25]. Its bad trophic state is due to the pressure exercised by urban and agricultural systems, from which nutrients and phytosanitary products originate and enter into the water flowing into the lagoon [24, 26].

El Hondo-Salinas de Santa Pola Natural Park (38°11′00″N 0°45′00″W), situated in a rural environment in the south of Alicante province, consists of two natural parks crisscrossed and linked by an extensive network of canals and ditches. El Hondo is a complex of mesosaline and polysaline (salinity 8.5–9.4 PSU) semi-artificial wetlands used to store irrigation water, while the neighbouring Las Salinas de Santa Pola consists of saltpans that receive inflow from surrounding agricultural areas. These ecosystems comprise one of the most important wetlands in south-eastern Spain, given the great variety of fauna and flora found in the area and its relevance for migrating birds. This ecosystem is threatened mostly by low water levels and poor water quality, which is considered 'bad' [27].

S'Albufereta Natural Reserve (39°51′47″N 3°05′19″E), located in the Bay of Pollença, in the northeast of the island of Mallorca (Illes Balears), is one of the most important wetlands in Mallorca (salinity 3.27–3.78 PSU). At this site, 2.1 km^2 are protected as a Special Natural Reserve, and there is also a buffer zone of 3.02 km^2 and a 100 m security strip around the strictly protected zone. The wetland is located at the confluence of four freshwater rivers, the largest of which is the Torrent del Rec, and is bordered by dunes. In the east, it is connected to the sea by the Es Grau channel, which crosses a 100 m wide sandbar. The Mediterranean climate of the area results in an average annual precipitation of 708 mm with a dry summer period. Inland, this wetland is surrounded by partially abandoned farmland. According to the EU Water Framework Directive [23], its ecological status is 'moderate', the main threats being spillage from sewage treatment and infiltration from septic tanks [28].

Finally, S'Albufera des Grau Natural Park (39°58′35″N 4°14′23″E), located on the Balearic Island of Menorca, has a surface area of 0.78 km^2. The average depth of its water is 1.37 m, with a maximum depth of 3 m. The lagoon receives freshwater from two streams that drain an area of 56 km^2,

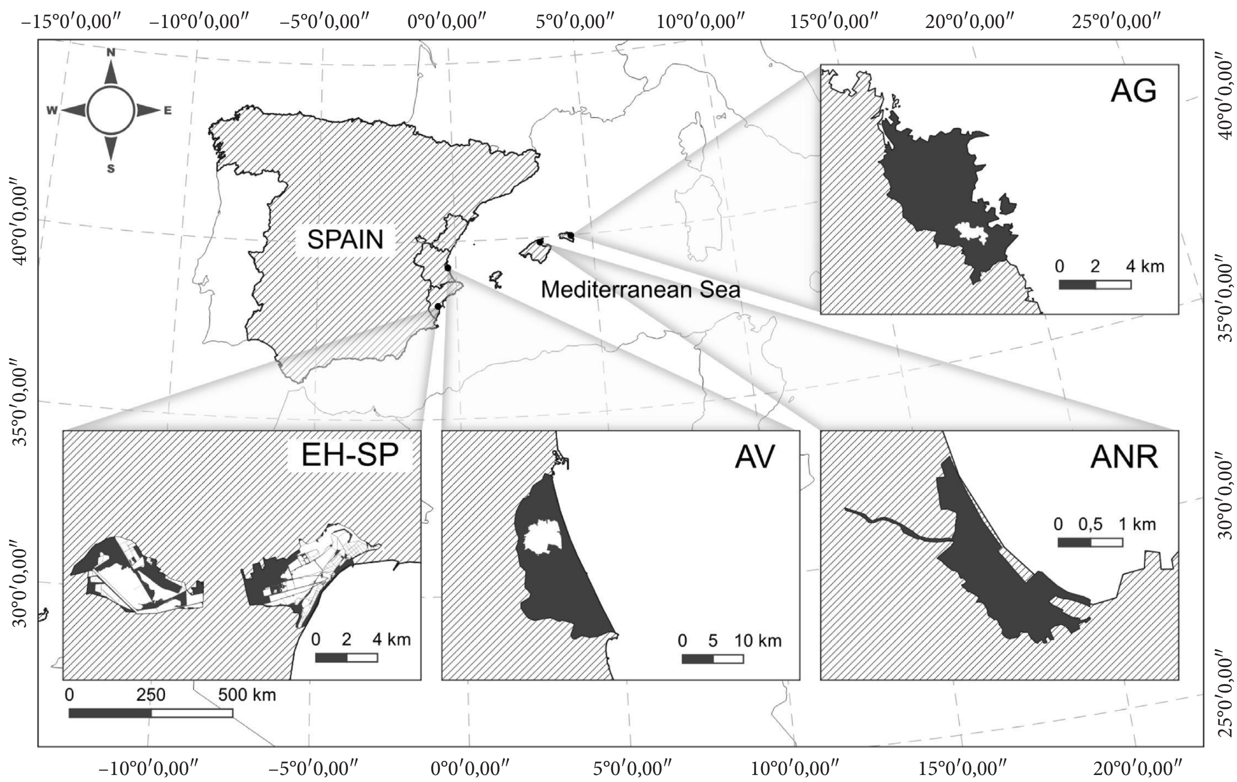

Figure 1: Location of the studied wetlands. AG = S'Albufera des Grau natural park, ANR = S'Albufereta natural reserve, AV = L'Albufera de Valencia natural park, EH-SP = El Hondo-Salinas de Santa Pola natural park.

whose flow is irregular and concentrated in spring and autumn. The lagoon is connected to the sea by a narrow, 500-m long channel, Sa Gola, in which a small floodgate allows the lagoon-sea connection to be regulated when the sand barrier is opened. The water of the lagoon is mesohaline with a salinity of 6–20 PSU. There is a marked seasonality in the salinity and water levels due to the Mediterranean evaporation/precipitation regime. According to the EU Water Framework Directive [23], its ecological status is considered 'good' or 'very good', its main threats being the spillage of slurry and a fall in the freshwater supply [28].

2.2. Sampling. A total of 118 eels were sampled between December 2019 and March 2020. Eels were either bought from local fishermen at L'Albufera de Valencia ($N = 30$) and El Hondo-Salinas de Santa Pola ($N = 25$) or fished using traditional gear at El Hondo-Salinas de Santa Pola ($N = 5$), S'Albufereta ($N = 28$) and S'Albufera des Grau ($N = 30$). The traditional fishing methods used in these regions for many generations are of Arab origin and consist of setting traps, known as 'butrones', at a depth of approximately 2.5 m. The butrones are spiral metal cones covered with netting that are inserted one inside the other, with the eels retained alive in the last cone.

Eels were euthanised by sedation followed by an overdose of tricaine methanesulfonate (MS222) at 100 mg/L, according to current legislation. The protocol and procedures used have been approved by the Ethical Committee for Animal Experimentation of the University of Murcia. The eels were then dissected. The carcasses and organs of eels were macroscopically inspected for signs of viral infection, such as skin lesions, petechiae, haemorrhages, nodules and necrosis [29–32]. Samples from the spleen, liver and gills (approximately 50 mg of each organ) from all eels were obtained and stored at −80°C until processed. The swim bladder of each eel was removed, macroscopically inspected and stored at −20°C until processed.

In addition, total body length (TL), eviscerated body weight (W), weight of the liver (Wl) and gonads (Wg), maximum pectoral fin length (PFL), horizontal (Dh) and vertical (Dv) diameters of left eyes (using a digital calliper 0.01–150 mm) were measured. The fin index (FI), hepatosomatic index (HSI) and gonadosomatic index (GSI) were calculated [33]. The HSI indicates the condition of the eel's energy reserves. The GSI estimates the degree of gonad development. The eye index (EI) was calculated using the equations of Pankhurst [34]. In addition, the scaled mass index (SMI) of each eel was calculated following the method described by Peig and Green [35].

Three stages were considered to estimate the DS of eels: yellow eels, silver eels and eels undergoing the silvering process. The latter stage is an intermediate phase between yellow and silver eels, in which not all silvering parameters are present, but maturation has already started. The degree of silvering was determined using a multicriteria methodology, according to the EI, FI and GSI values, the pectoral fin colour, the presence of a clearly differentiated lateral line with characteristic black corpuscles (neuromasts), and the presence of contrasting colour between dorsal and ventral surfaces [33, 36, 37]. The 'silver' status was reserved for eels that showed all the required characteristics ($EI \geq 6.5$, $GSI > 0.6$, presence of contrasting colour and neuromasts). Those eels that only exhibited some of the characteristics were considered 'silvering', and eels that did not exhibit any of the characteristics were considered 'yellow' eels [37].

Both sagittal otoliths were extracted from each eel, cleaned with water and dried. The otoliths were then stored in Eppendorf Tubes® until further processing. Since most of the eels were less than 5 years old, the otoliths were examined entirely using a small black container of 96% ethanol to increase the contrast of the growth marks. Readings were taken without considering the zero band as the first annual ring [38].

2.3. Parasitological Analysis. Once defrosted, the swim bladders were macroscopically inspected. The lumen was opened, and adult and pre-adult stages of *A. crassus* were removed. The larvae were extracted from the swim bladder wall following the method described by Martínez-Carrasco et al. [39]. The swim bladders were digested at 40°C for 1 h in 1.5% (w/v) hydrochloric acid. After centrifugation and washing (500*g* for 5 min), the larval stages L2, L3 and L4 of *A. crassus* were counted in a Favatti chamber at magnifications 4 and 10 using an OLYMPUS BX41 optical microscope. The larvae were distinguished from one another using the keys of Rolbiecki [40].

In accordance with the terms of Bush et al. [41], the prevalence of each pathogen was calculated as the number of infected eels divided by the total number of sampled eels (expressed as a percentage). Furthermore, the mean parasite intensity was calculated as the number of parasites recovered from sampled eels divided by the number of infected eels.

Wetland-specific and total prevalence and mean intensity for each larval stage (the second, third and fourth larvae, that is, L2, L3 and L4), for adult worms and for advanced parasitic stages (L3, L4 and adults) were calculated. The L2 count included eggs containing L2 and hatched L2. Also, the total prevalence (all parasitic stages) was calculated.

In addition, the swim bladder degenerative index (SDI) values were calculated according to Lefebvre et al. [42]. A score of 0 corresponds to an intact and transparent swim bladder; 1–3 to medium-low to medium inflammation with partial or total opacity of the swim bladder; 4–5 to severely damaged swim bladders with medium-high to very high inflammation (5); and 6 if no lumen was left.

2.4. Virological Analysis. Pools of spleen, liver and gill samples from the tested eels were sent to the National Reference Laboratory for Fish Diseases (The Netherlands), where the presence or absence of AngHV1, EVEX and EVE viruses was determined. The real-time PCR assay was used for the detection of the herpesvirus AngHV1 [43]. The two-step real-time RT-PCR method was applied in order to detect the rhabdovirus EVEX [44]. Finally, the methodology of Orpetveit et al. [45], in particular isolating the real-time reverse transcription polymerase chain reaction method, was used for the detection of EVE. Samples were analysed in pools of a maximum of 10 eels; if any pool was positive for any of the tested viruses, all specimens in the pool were evaluated individually. Finally, the wetland-specific and total prevalences were calculated for each virus, for double co-infection (AngHV1-EVEX, AngHV1-*A. crassus* or EVEX-*A. crassus*) and for triple co-infection (AngHV1-EVEX-*A. crassus*), in which any parasitic stage of *A. crassus* was considered.

2.5. Statistical Analysis. Eel biometric data, age and measured indices, as well as parasite intensity, were expressed as geometric mean, standard error, minimum and maximum. The data for the prevalence of infection were expressed as a percentage with 95% confidence limits (CL). The Kolmogorov–Smirnov test was used to determine whether the distribution of the variables age, biometric data, measured indices and parasite intensity was normal. Due to the number of independent variables to consider in each area (degree of silvering, presence or absence of each pathogen), when no differences were found between wetlands, the statistical analysis was carried out on the whole eel population (the four wetlands).

The relationship between capture site and degree of eel silvering (yellow or silvering eels) was estimated using Pearson's chi-square test, together with a pairwise comparison (p values were not corrected for multiple comparisons). The nonparametric Kruskal–Wallis test and pairwise comparisons were used to assess the influence of capture site or degree of silvering (independent variables) on eel age, biometric data and measured indices. The relationship between age, biometric data and measured indices of eels was estimated by Spearman's correlation test.

The relationship between the presence or absence of each pathogen (*A. crassus*, virus or co-infection) and capture site or degree of silvering was estimated using Pearson's chi-square test (Yates-corrected in analysis X2) and pairwise comparisons. To identify statistical differences in eel age, biometric data and computed indices between parasite-infected, virus-infected or co-infected eels and non-infected eels (independent variables), a Kruskal–Wallis test (followed by *post hoc* pairwise comparisons) was performed. In addition, the same test was used to assess the influence of capture site and degree of eel silvering (independent variables) on parasite intensity. The relationship between parasite intensity and eel age, biometric data and computed indices was assessed using Spearman's correlation test. Differences were considered significant or marginally significant for $p < 0.05$ and $p < 0.1$, respectively. The statistical analysis was conducted using SPSS software Version 24.0.

3. Results

3.1. Biometric Data. Table 1 presents the descriptive statistics for eel age, biometric data, measured indices and degree of eel silvering. Upon macroscopic inspection, all individuals were apparently healthy. The TL and eviscerated

Table 1: Descriptive statistics (geometric mean, standard error, minimum and maximum) of biometric data, age and measured indices, and percentage of each stage of European eels from four Spanish Mediterranean sites.

	AV	EH-SP	ANR	AG	Total
N	30	30	28	30	118
Age	5 ± 2 (2–7)	5 ± 1 (3–8)	4 ± 1 (2–6)	4 ± 1 (3–7)	5 ± 1 (2–8)
TL	743 ± 42 (662–838)	738 ± 75 (642–915)	545 ± 103 (344–760)	519 ± 74 (195–600)	638 ± 129 (195–915)
W	743 ± 124 (554–1017)	744 ± 201 (457–1201)	271 ± 155 (79–716)	240 ± 55 (132–332)	503 ± 284 (79–1201)
PFL	41 ± 6 (33–65)	36 ± 6 (25–53)	26 ± 5 (16–35)	26 ± 3 (20–33)	32 ± 8 (16–65)
HSI	1.48 ± 0.37 (0.95–2.52)	1.26 ± 0.31 (0.95–2.52)	1.22 ± 0.17 (0.9–1.49)	1.33 ± 0.32 (0.2–1.64)	1.33 ± 0.32 (0.2–2.52)
GSI	1.73 ± 0.28 (1.05–2.24)	1.68 ± 0.39 (0.78–2.3)	0.44 ± 0.28 (0.02–0.96)	0.58 ± 0.28 (0.2–1.64)	1.11 ± 0.68 (0.02–2.24)
FI	5.50 ± 0.64 (4.41–8.03)	4.81 ± 0.45 (3.66–5.81)	4.77 ± 0.80 (3.94–4.43)	5.15 ± 1.98 (3.83–15.37)	5.06 ± 1.17 (3.66–15.37)
EI	10.06 ± 1.65 (7.48–13.33)	9.34 ± 2.56 (5.41–16.61)	5.89 ± 1.92 (2.48–9.6)	5.42 ± 1.98 (3.83–15.33)	7.71 ± 2.89 (2.48–16.61)
SMI	423.09 ± 38.78 (366.43–510.24)	435.85 ± 79.19 (248.63–596.09)	423.83 ± 88.48 (280.84–721.9)	462.48 ± 63.74 (330.74–611.54)	436.41 ± 70.53 (248.63–721.9)
DS (Y/S)	20/80	30/70	85.71/14.29	100/0	58.47/41.53

Note: Age = eel age (years), *N* = sample size, S = percentage of silvering eels, Total = total eel population of the four wetlands, W = eviscerated body weight (g), Y = percentage of yellow eels.
Abbreviations: AG = S'Albufera des Grau natural park, ANR = S'Albufereta natural reserve, AV = L'Albufera de Valencia natural park, DS = degree of eel silvering, EH-SP = El Hondo-Salinas de Santa Pola natural park, EI = eye index, FI = fin index, GSI = gonadosomatic index, HSI = hepatosomatic index, PFL = pectoral fin length (mm), SMI = scaled mass index, TL = total body length (mm).

body weight of the sampled eels (hereafter simply 'length and weight') ranged from 195 to 915 mm (average 638 ± 129 mm) and 79 to 1201 g (average 503 ± 284 g), respectively. The age ranged from 2 to 8 years, with an average of 5 ± 1 years. The percentage of yellow and silvering eels at the moment of capture was 58.47% and 41.53%, respectively. No silver eels were caught.

Pearson's chi-squared test ($p < 0.001$) detected differences in the distribution of yellow and silvering eels between wetlands (pairwise comparisons in Table S1 of supporting files). As illustrated in Table 1, most of the eels from L'Albufera de Valencia (80%) and El Hondo-Salinas de Santa Pola (70%) were identified as silvering eels. Conversely, most of the eels from S'Albufereta (85.71%) and 100% of the eels from S'Albufera des Grau were yellow eels.

Eel length, weight and biometric indices differed significantly between capture sites, with larger and heavier eels found in the peninsular wetlands of L'Albufera de Valencia and El Hondo-Salinas de Santa Pola, and smaller eels in the Balearic wetlands of S'Albufereta and S'Albufera des Grau (Table 2 and pairwise comparisons in Table S1 of supporting files).

Eel age did not differ significantly between yellow and silvering eels and was not related to the capture site. Nevertheless, eel age was correlated with eel weight, PFL and GSI in S'Albufereta; with eel length and SMI in three of the four wetland sites; and with the EI index in El Hondo-Salinas de Santa Pola (Table 2).

3.2. Anguillicoloides crassus Parasitological Data. The nematode *A. crassus* (at any stage) was detected in the swim bladders of 110 out of 118 sampled eels. The estimated total prevalence (95% CL) was 93.22 (88.68–97.76)% and exceeded 80% across all sampling sites. The prevalence of advanced stages (L3, L4 and adults) was 74.58 (66.72–82.43)% and exceeded 60% across all wetlands tested. Wetland-specific and total data on prevalences (95% CL), mean intensity, and minimum and maximum of different stages of the *A. crassus* parasite infecting eels, together with SDI values, are given in Table 3.

The mean intensity of adult parasites was 4.42 ± 4.5 (1–21) parasites per infected eel. The mean intensity of advanced stages was 99.66 ± 328.07 (1–2654) nematodes per infected eel. Figure 2, with the total larval burden of *A. crassus* ln-transformed, shows the plots of the distribution of adult and larval parasite intensity.

Table 4 shows the results of the relationships between parasite presence/absence or intensity and capture site, degree of eel silvering, eel biometric data and measured indices. Table S2 of the supporting files shows pairwise comparisons by capture site.

No significant differences were found in the distribution of parasite infection with respect to eel age, although the presence or absence of adult parasites differed significantly between yellow and silvering eels ($p = 0.020$), with silvering eels being more infected (77.55%) than yellow eels (50.07%) (Table S3 of supporting files). Furthermore, the prevalence of adult parasites was higher among larger and heavier eels compared to smaller and lighter eels (Table 4).

The intensity of *A. crassus* differed significantly between capture sites, with eels from L'Albufera de Valencia and El Hondo Salinas de Santa Pola accumulating a higher number of parasites (Table 4 and pairwise comparisons in Table S2 of supporting files). Figure 3, which shows the wetland-specific distribution of adult and advanced stages of *A. crassus* among yellow and silvering eels, highlights the El Hondo-Salinas de Santa Pola, where silvering eels harboured a greater number of advanced stages than yellow eels ($p = 0.001$). In the same wetland, the number of adult parasites correlated positively with eel length ($p = 0.019$) and the number of advanced stages correlated positively with eel length ($p = 0.014$) and weight ($p = 0.044$). No relationship between eel age and infection rate was found. In S'Albufera des Grau, GSI index values were higher in eels infected with advanced stages compared to uninfected eels ($p = 0.012$), and were positively correlated with the number of advanced stages ($p = 0.002$).

3.3. Virological and Co-Infection Data. The AngHV1 virus was detected in eels inhabiting all tested wetlands, while the EVEX virus was only detected in eels from L'Albufera de Valencia and S'Albufereta. No evidence of the EVE virus was found. Compared to EVEX, the prevalence of AngHV1 was much higher in all observed wetlands. Comparing the wetlands, the prevalence of AngHV1 and EVEX viruses was much higher in L'Albufera de Valencia (100% and 92.31%, respectively), also exceeding 80% prevalence for double (AngHV1-EVEX, AngHV1-*A. crassus*, EVEX-*A. crassus*) and triple (AngHV1-EVEX-*A. crassus*) co-infection in eels from this wetland (Table 5).

Table 6 shows that the presence and absence of AngHV1, EVEX and co-infections were significantly related to the capture site. Table S2 of the supporting files shows pairwise comparisons between capture sites. Table S3 of the supporting files shows the distribution of pathogen infection rates among yellow and silvering eels. A wetland-specific analysis showed that only eels from L'Albufera de Valencia exhibit a trend towards a higher prevalence of EVEX virus and its co-infections in silvering eels. No relationship between eel age and infection rate was found. In S'Albufereta Natural Reserve, FI index values were lower in eels infected with AngHV1 and AngHV1-*A. crassus* ($p = 0.016$), as well as eel weight, which was marginally significantly higher in eels infected with EVEX and EVEX-*A. crassus*.

4. Discussion

4.1. Anguillicoloides crassus. The presence of a pathogen represents a potential threat to the health and breeding status of an endangered species [11]. Consequently, a comprehensive understanding of how the most dangerous pathogens affect a species is imperative. Adult European eels require good body condition and immune status, together with high energy reserves, to complete their return migration to the Sargasso Sea and reproduce [13, 46]. The presence of pathogens may impair the migratory capacity of eels infected by *A. crassus* or viruses and therefore reduce their reproductive rate.

Table 2: Relationship between capture site, degree of silvering, age, biometric data and measured indices of European eels in four Spanish Mediterranean sites, when significant.

		Test	TL	W	PFL	HSI	GSI	FI	EI	SMI
Site		Kruskal–Wallis	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.004$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.023$
DS	AV	Kruskal–Wallis	$p = 0.049$	$p = 0.029$	—	M	—	—	—	—
	EH-SP		$p = 0.001^a$	$p = 0.003^a$	M^a	$p = 0.028$	M^a	—	—	$p = 0.044^a$
	ANR		—	—	—	—	—	—	M^a	—
	AG		x	x	x	x	x	x	x	x
	Total		$p < 0.001^a$	$p < 0.001^a$	$p < 0.001^a$	—	$p < 0.001^a$	$p = 0.022^a$	$p < 0.001^a$	$p = 0.028$
Age	AV	Spearman	—	—	—	—	—	—	—	—
	EH-SP		0.442^b ($p = 0.014$)	M^b	—	M^b	—	—	0.594^b ($p = 0.001$)	−0.394 ($p = 0.031$)
	ANR		0.753^b ($p < 0.001$)	0.724^b ($p < 0.001$)	0.698^b ($p < 0.001$)	—	0.529^b ($p = 0.004$)	—	M^b	−0.527 ($p = 0.005$)
	AG		0.451^b ($p = 0.012$)	—	—	—	—	—	—	−0.430 ($p = 0.020$)
	Total		0.358^b ($p < 0.001$)	0.298^b ($p = 0.001$)	0.280^b ($p = 0.002$)	—	0.244^b ($p = 0.008$)	—	0.267^b ($p = 0.004$)	−0.341 ($p < 0.001$)

Note: (a) = higher in silvering eels, Age = eel age (years), (b) = higher in older eels, Kruskal–Wallis = Kruskal–Wallis nonparametric test p values, M = marginally significant; Site = capture site, Spearman = Spearman's correlation coefficient values and p values, Total = total eel population of the four wetlands, W = eviscerated body weight (g), (x) = estimation was not possible, (—) = nonsignificant data.

Abbreviations: AG = S'Albufera des Grau natural park, ANR = S'Albufereta natural reserve, AV = L'Albufera de Valencia natural park, DS = degree of eel silvering (yellow or silvering eel), EH-SP = El Hondo-Salinas de Santa Pola natural park, EI = eye index, FI = fin index, GSI = gonadosomatic index, HSI = hepatosomatic index, PFL = pectoral fin length (mm), SMI = scaled mass index, TL = total body length (mm).

Table 3: Wetland-specific and total prevalence, mean intensity, minimum and maximum of *Anguillicoloides crassus* in European eels from four Spanish Mediterranean sites.

		AV ($N=30$)	EH-SP ($N=30$)	ANR ($N=28$)	AG ($N=30$)	Total ($N=118$)
L2	*p* (95% CL)	43.33 (25.6–61.07)	76.67 (61.53–91.8)	82.14 (67.96–96.33)	90 (79.26–100)	72.88 (64.86–80.9)
	MI ± SD (min–max)	22,911.54 ± 45,680.06 (100–156,250)	7895.65 ± 15,839.66 (50–56,950)	4330.36 ± 12,068.66 (50–58,500)	2194 ± 2706.17 (50–10,950)	7673.84 ± 21,291.12 (50–156,250)
L3	*p* (95% CL)	13.33 (1.17–25.5)	50 (32.11–67.89)	14.29 (1.32–27.25)	16.67 (3.33–30)	23.73 (16.05–31.4)
	MI ± SD (min–max)	40 ± 45.34 (2–100)	243.53 ± 312.01 (50–1200)	3 ± 1.83 (1–5)	130.2 ± 207.81 (50–500)	159.86 ± 258.71 (1–1200)
L4	*p* (95% CL)	40 (22.47–57.53)	46.67 (28.81–64.52)	14.29 (1.32–27.25)	23.33 (8.2–38.47)	31.36 (22.98–39.73)
	MI ± SD (min–max)	22.25 ± 32.49 (1–100)	258.64 ± 654.49 (50–2500)	2 ± 1.41 (1–4)	8.86 ± 18.2 (1–50)	106.97 ± 411.7 (1–2500)
Adult parasites	*p* (95% CL)	76.67 (61.53–91.8)	73.33 (57.51–89.16)	60.71 (42.62–78.8)	46.67 (28.81–64.52)	64.41 (55.77–73.05)
	MI ± SD (min–max)	5.61 ± 5.2 (1–21)	5.95 ± 5.28 (1–16)	2.41 ± 2.21 (1–10)	2.5 ± 2.1 (1–9)	4.42 ± 4.5 (1–21)
Advanced parasitic stage count	*p* (95% CL)	83.33 (70–96.67)	90 (79.26–100)	60.71 (42.62–78.80)	63.33 (46.09–80.58)	74.58 (66.72–82.43)
	MI ± SD (min–max)	22.24 ± 30.21 (1–106)	274.26 ± 551.52 (1–2654)	3.59 ± 2.83 (1–10)	39.34 ± 114.06 (1–503)	99.66 ± 328.07 (1–2654)
Total parasite burden	*p* (95% CL)	93.33 (84.41–100)	96.67 (90.24–100)	85.71 (72.75–98.38)	96.67 (90.24–100)	93.22 (88.68–97.76)
SDI	Mean ± SD (min–max)	2.23 ± 1.28 (0–4)	2.32 ± 0.85 (0.5–3.5)	0.88 ± 1.03 (0–3)	1.23 ± 1.17 (0–3.5)	1.68 ± 1.25 (0–4)

Note: advanced parasitic stage count = L3, L4 and adult parasites, L2 = eggs containing L2 or hatched L2, N = sample size, p (95% CL) = prevalence, expressed as the percentage with 95% confidence limits, Total = total eel population of the four wetlands, Total parasite burden = L2, L3, L4 and adult parasites.
Abbreviations: AG = S'Albufera des Grau natural park, ANR = S'Albufereta natural reserve, AV = L'Albufera de Valencia natural park, EH-SP = El Hondo-Salinas de Santa Pola natural park, MI = mean intensity, SD = standard deviation, SDI = swim bladder degenerative index.

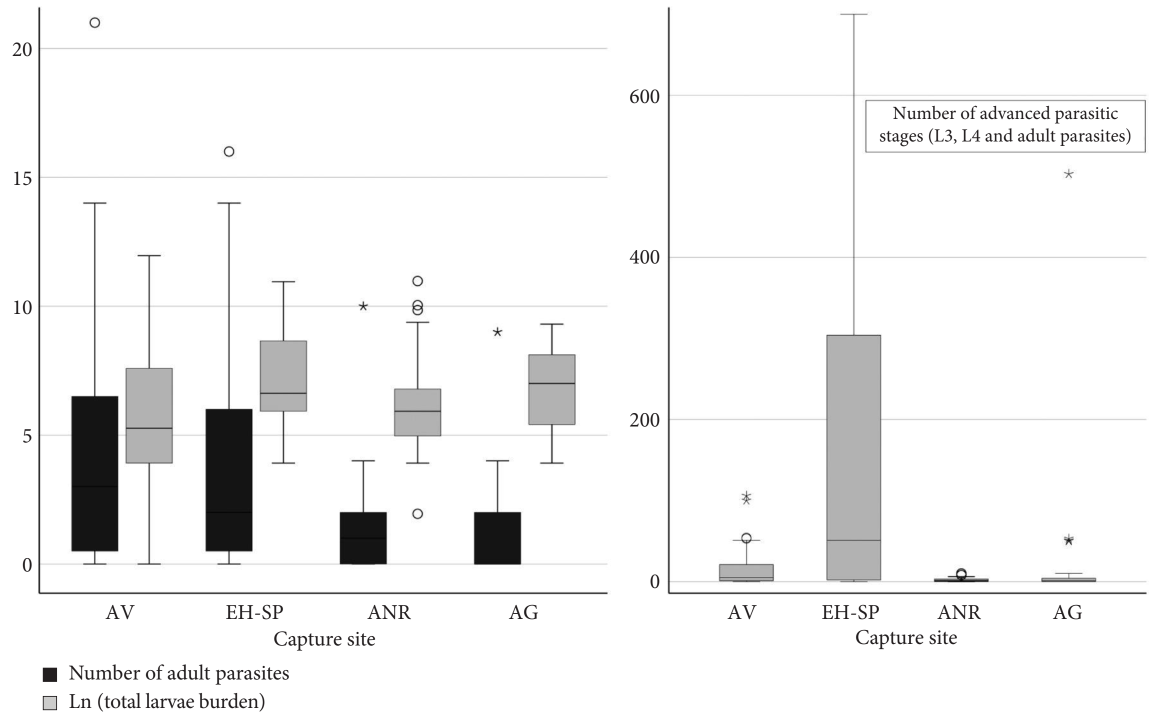

Figure 2: Box plot depicting the distribution of the mean parasite intensity of *Anguillicoloides crassus* among European eels from four Spanish Mediterranean environments. On the left, the number of adult parasites and the logarithmic transformation (ln) of the number of larvae (any stage). On the right, the number of advanced stages of the parasite (L3, L4 and adults). Circles represent outliers, and asterisks represent extreme outliers. AG = S'Albufera des Grau natural park, ANR = S'Albufereta natural reserve, AV = L'Albufera de Valencia natural park, EH-SP = El Hondo-Salinas de Santa Pola natural park.

In the present study, the health status of European eels from four Spanish Mediterranean wetlands—two of which are situated in the southeast of the Iberian Peninsula and the other two in the Balearic Islands— was assessed. The nematode *A. crassus* was present at high prevalence levels in wild eels inhabiting four wetlands under investigation. To the best of our knowledge, this is the first time that parasitological data on *A. crassus* have been published for three of the analysed wetlands (El Hondo-Salinas de Santa Pola Natural Park, S'Albufereta Natural Reserve and S'Albufera des Grau Natural Park).

After monitoring *A. crassus* on the Mediterranean coast of France for 2 decades, Lefebvre and Crivelli [47] concluded that the introduction of this parasite into a new environment led to a rapid spread and a peak in its prevalence, followed by a subsequent stabilisation at around 60%–70% prevalence levels in adults and pre-adults. In L'Albufera de Valencia Natural Park, the presence of the parasite *A. crassus* was first recorded by Asturiano et al. [48] in a study on the reproduction of European eels. Although these authors did not measure the prevalence of the parasite, they suggested that the presence of this nematode could interfere with the gonadal development of eels. Subsequent investigations by Esteve and Alcaide [49] on wild eels captured between 2003 and 2005 revealed an infestation rate of 11.5% in adults and pre-adults of *A. crassus*, while Martínez-Carrasco et al. [50] reported a rate of 100% in eels sampled in 2008. Our study indicates that the prevalence of adult and preadult parasites in eels from L'Albufera de Valencia reached 76.67%, suggesting that the infection rate had already peaked and stabilised at the time of our observation.

In the 1980s, the presence of *A. crassus* was incidentally detected in eels at the wetland S'Albufera des Grau Natural Park (unpublished data, personal communication from Dr. Cardona Pascual, former Director of this protected area). Unfortunately, there are no parasitological data available on this discovery. Given the four-decade interval between the initial detection of the parasite and the current study, along with the current adult and pre-adult prevalence data (46.67%), it is likely that the infection rate has peaked here and has established at lower levels than those reported by other authors (e.g., [47]). Nonetheless, continuous monitoring of prevalence is advisable to confirm this hypothesis.

The prevalence of adult parasites at El Hondo Salinas de Santa Pola Natural Park (73.33%) and S'Albufereta Natural Reserve (60.71%) cannot be compared with other studies since no previous data on the presence of these parasites in these wetlands were available. Consequently, we cannot conclude whether the infection rate in these sites has already peaked or, conversely, has not yet reached peak prevalence.

The present study found that the prevalence of advanced stages of *A. crassus* (L3, L4 and adults) differed between capture sites. This could be explained by variations in salinity—an increase in salinity can induce a decrease in both egg hatching rate and larval survival in intermediate hosts, although parasite infectivity is not totally inhibited [51]—and the wetlands' ecological status [52]. The lower prevalence rates in S'Albufereta (60.71%) and S'Albufera des Grau (63.33%) could be

Table 4: Relationship between *Anguillicoloides crassus* presence/absence or intensity and capture site, degree of silvering, age, biometric data and measured indices of European eels from four Spanish Mediterranean sites (when significant).

	Sample	Site	DS	TL	W	HSI	GSI	FI	EI	SMI
P/A L2	Total	18.495 ($p < 0.001$)	9.184 ($p = 0.002$)$^{(Y)}$	$p = 0.039^{(Uninfected)}$	$p = 0.020^{(Uninfected)}$	—	$p = 0.042^{(Uninfected)}$	$p = 0.002^{(Uninfected)}$	$p = 0.004^{(Uninfected)}$	—
	EH-SP	x	—	—	—	—	—	$M^{(Uninfected)}$	—	—
	AG	x	x	—	—	—	—	—	—	$p = 0.021^{(Uninfected)}$
P/A adults	Total	—	5.373 ($p = 0.020$)$^{(S)}$	$p = 0.032^{(Infected)}$	$p = 0.023^{(Infected)}$	—	$M^{(Infected)}$	$M^{(Infected)}$	$p = 0.030^{(Infected)}$	—
	EH-SP	x	—	$M^{(Infected)}$	—	—	—	—	—	$M^{(Uninfected)}$
	ANR	x	—	—	—	—	$M^{(Uninfected)}$	—	—	—
	AG	x	x	—	$M^{(Infected)}$	—	$M^{(Infected)}$	—	—	—
P/A (L3, L4, adults)	Total	9.815 ($p = 0.020$)	8.911 ($p = 0.002$)$^{(S)}$	$p = 0.023^{(Infected)}$	$p = 0.011^{(Infected)}$	—	$p = 0.010^{(Infected)}$	—	$p = 0.013^{(Infected)}$	—
	EH-SP	x	—	$M^{(Infected)}$	—	—	—	—	—	—
	ANR	x	—	—	—	—	$M^{(Uninfected)}$	—	—	—
	AG	x	x	—	$M^{(Infected)}$	—	$p = 0.012^{(Infected)}$	—	—	—
Test		Chi-square coefficient values and p values		Kruskal–Wallis non-parametric test p values						
Intensity L2	Total	$p = 0.015$	$M^{(Y)}$	—	—	—	$M^{(Increase)}$	$M^{(Decrease)}$	−0.193 ($p = 0.037$)	—
	AG	x	x	$M^{(Increase)}$	—	—	0.372 ($p = 0.043$)	—	—	−0.362 ($p = 0.049$)
Intensity adults	Total	$p = 0.004$	$p = 0.001^{(S)}$	0.349 ($p < 0.001$)	0.336 ($p < 0.001$)	—	0.274 ($p = 0.003$)	—	0.253 ($p = 0.006$)	$M^{(Decrease)}$
	EH-SP	x	—	0.425 ($p = 0.019$)	—	$M^{(Decrease)}$	—	—	—	$M^{(Decrease)}$
	AG	x	x	—	—	—	$M^{(Increase)}$	—	—	—
Intensity (L3, L4, adults)	Total	$p < 0.001$	$p < 0.001^{(S)}$	0.418 ($p < 0.001$)	0.421 ($p < 0.001$)	—	0.409 ($p < 0.001$)	—	0.365 ($p < 0.001$)	—
	EH-SP	x	$p = 0.001^{(S)}$	0.446 ($p = 0.014$)	0.370 ($p = 0.044$)	—	—	—	—	—
	ANR	x	—	—	—	—	$M^{(Decrease)}$	—	—	—
	AG	x	x	—	$M^{(Decrease)}$	—	0.541 ($p = 0.002$)	—	—	—
Test		Kruskal–Wallis test p values		Spearman's correlation coefficient values and p values						

Note: (Decrease) = the parameter value decreases with the number of parasites, (Increase) = the parameter value increases with the number of parasites, (Infected) = the parameter value was higher in infected eels, M = marginally significant, P/A = presence or absence, (S) = higher percentage of infected individuals in silvering eels or higher number of parasites in silvering eels, sample = the population of eels to which the analysis was applied, Site = capture site, Total = total eel population, (Uninfected) = the parameter value was higher in uninfected eels, W = eviscerated body weight (g), x = the estimate would not be appropriate, (Y) = higher percentage of infected individuals in yellow eels or higher number of parasites in yellow eels, (—) = nonsignificant data.
Abbreviations: AG = S'Albufera des Grau natural park, ANR = S'Albufereta natural reserve, EH-SP = El Hondo-Salinas de Santa Pola natural park, EI = eye index, FI = fin index, GS = degree of eel silvering (respect to total eel population), GSI = gonadosomatic index, HSI = hepatosomatic index, SMI = scaled mass index, TL = total body length (mm).

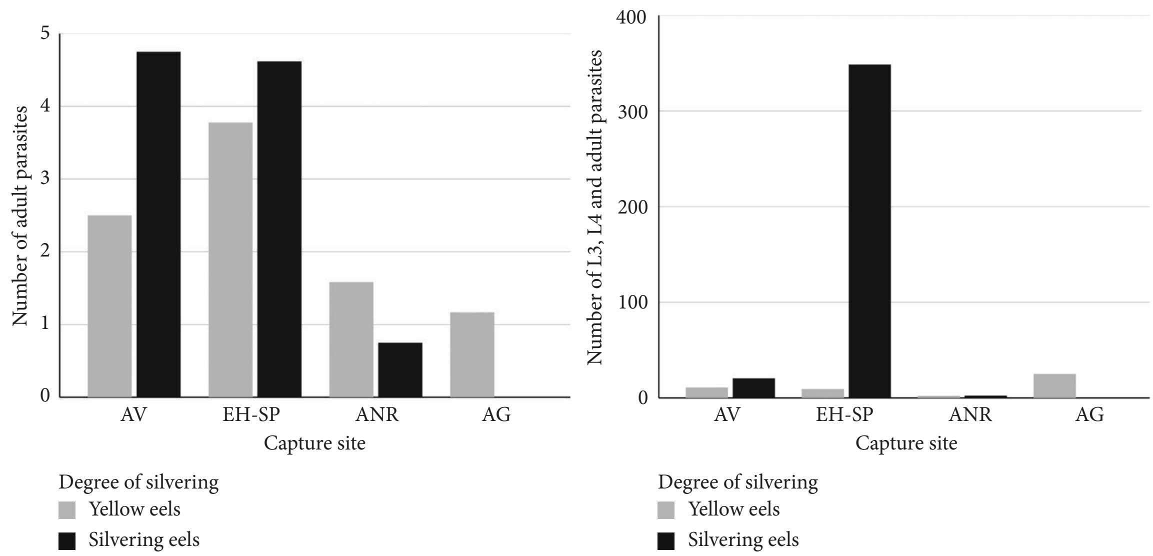

Figure 3: Bar chart showing the distribution of adult and advanced (L3, L4 and adults) stages of *Anguillicoloides crassus* among yellow and silvering European eels from four Spanish Mediterranean environments. AG = S'Albufera des Grau natural park, ANR = S'Albufereta natural reserve, AV = L'Albufera de Valencia natural park, EH-SP = El Hondo-Salinas de Santa Pola natural park.

Table 5: Wetland-specific and total prevalences (expressed as percentages with 95% confidence limits) of AngHV1 and EVEX viruses, double virus co-infection and virus-*Anguillicoloides crassus* co-infections in European eels from four Spanish Mediterranean sites.

	AV (*N* = 13)	EH-SP (*N* = 30)	ANR (*N* = 28)	AG (*N* = 30)	Total (*N* = 101)
AngHV1	100	23.33 (8.2–38.47)	10.71 (0–22.17)	16.67 (3.33–30)	27.70 (19–36.45)
EVEX	92.31 (77.82–100)	0	3.57 (0–10.45)	0	12.87 (6.34–19.4)
EVE	0	0	0	0	0
AngHV1-EVEX	92.31 (77.82–100)	0	0	0	11.88 (5.57–18.2)
AngHV1-*A. crassus*	92.31 (77.82–100)	23.33 (8.2–38.47)	10 (0–20.74)	16.67 (3.33–30)	26.73 (18.1–35.36)
EVEX-*A. crassus*	84.62 (65–104.23)	0	3.33 (0–9.76)	0	11.88 (5.57–18.19)
AngHV1-EVEX-*A. crassus*	84.62 (65–100)	0	0	0	10.89 (4.82–16.97)

Note: A. crassus = any parasitic stage, *N* = sample size, total = total eel population of the four wetlands.
Abbreviations: AG = S'Albufera des Grau natural park, ANR = S'Albufereta natural reserve, AV = L'Albufera de Valencia natural park, EH-SP = El Hondo-Salinas de Santa Pola natural park.

attributed to the relatively high salinity levels (6–20 PSU and 3.27–3.78 PSU, respectively) and better ecological status of these wetlands [53]. The lower anthropogenic pressure experienced by eels from S'Albufera des Grau may have contributed to their better physical and immunological condition when facing the parasite, which could have established the prevalence of *A. crassus* within a lower range [54]. Higher prevalence rates in L'Albufera de Valencia (83.33%) and El Hondo-Salinas de Santa Pola (90%) could be explained by poor ecological status, urban and agricultural pollution and low water quality and salinity (1–2 PSU in L'Albufera de Valencia) [26, 27].

In the present study, the prevalence of adult parasites (mean of 64.41%) did not vary significantly between wetlands. Comparable prevalences have been reported in eels from the Mediterranean rivers and lagoons of Andalusia (30%–75%; [55]), from two low-salinity lakes in Algeria (58.47%; [56]), from Lake El Broullus in Egypt (54.1%; [57]), and from three Turkish river estuaries (67.5%; [58]). In contrast, prevalences were generally lower in eels from other ecosystems on the Iberian Peninsula [39, 59–62], as well as in European eels from two hypersaline estuaries in Morocco (34% and 54%; [63]), from the Gediz Delta in Turkey (mean of 6.7%; [64]), and from three lagoons in Tunisia (2.3%, 15.5% and 46.3%; [65]). The wide range of prevalence observed across nearby locations could be attributed to the presence or absence of suitable intermediate hosts or to the timing of the parasite's introduction into each environment [39].

In this study, mean parasite intensity at any stage also differed between capture sites. Eels from L'Albufera de Valencia and El Hondo-Salinas de Santa Pola (wetlands subjected to high anthropogenic pressure) had the highest intensity of adult parasites (5.61 and 5.95, respectively), while the lowest intensity of adult parasites (2.41 and 2.5) was found in eels from two Balearic wetlands (Figure 2). At the same time, silvering eels harboured more advanced parasitic stages than yellow eels, especially in El Hondo-Salinas de Santa Pola (Figure 3). At the time of sampling, European eels from L'Albufera de Valencia and El Hondo-Salinas de Santa Pola were probably at greater risk than eels from the Balearic Islands, since adult parasites are more harmful to migratory eels than to sedentary ones and reduce

Table 6: Relationship between the presence or absence of AngHV1 and EVEX virus infection, double virus co-infection and virus-*Anguillicoloides crassus* co-infections and capture site, degree of silvering, biometric data and measured indices of European eels from Spanish Mediterranean sites (when significant).

	Sample	**Site**	**DS**	**TL**	**W**	**HSI**	**GSI**	**FI**	**EI**
AngHV1	Total	40.054 ($p = 0.001$)	7.333 ($p = 0.007$)$^{(S)}$	$p = 0.003^{(\text{Infected})}$	$p = 0.003^{(\text{Infected})}$	$M^{(\text{Infected})}$	$p = 0.002^{(\text{Infected})}$	$p = 0.002^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$
	ANR	x	—	—	—	—	—	$p = 0.016^{(\text{Uninfected})}$	—
EVEX	Total	84.170 ($p < 0.001$)	9.728 ($p = 0.002$)$^{(S)}$	$p < 0.001^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$	$p = 0.013^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$
	AV	x	$M^{(S)}$	—	—	—	—	—	—
	ANR	x	—	—	$M^{(\text{Infected})}$	—	—	—	—
AngHV1-EVEX	Total	66.588 ($p < 0.001$)	11.916 ($p = 0.001$)$^{(S)}$	$p < 0.001^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$	$p = 0.012^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$
	AV	x	$M^{(S)}$	—	—	—	—	—	—
AngHV1-*A. crassus*	Total	33.938 ($p < 0.001$)	8.426 ($p = 0.004$)$^{(S)}$	$p = 0.003^{(\text{Infected})}$	$p = 0.003^{(\text{Infected})}$	$M^{(\text{Infected})}$	$p = 0.002^{(\text{Infected})}$	$p = 0.002^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$
	AV	x	$M^{(S)}$	—	—	—	—	—	—
	ANR	x	—	—	—	—	—	$p = 0.016^{(\text{Uninfected})}$	—
EVEX-*A. crassus*	Total	75.626 ($p < 0.001$)	11.916 ($p < 0.001$)$^{(S)}$	$p < 0.001^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$	$p = 0.022^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$
	AV	x	7.879 ($p = 0.005$)$^{(S)}$	—	—	—	—	—	—
	ANR	x	—	—	$M^{(\text{Infected})}$	—	—	—	—
AngHV1-EVEX-*A. crassus*	Total	58.373 ($p < 0.001$)	14.577 ($p < 0.001$)$^{(S)}$	$p = 0.001^{(\text{Infected})}$	$p = 0.001^{(\text{Infected})}$	$p = 0.020^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$	$p < 0.001^{(\text{Infected})}$
	AV	x	7.879 ($p = 0.005$)$^{(S)}$	—	—	—	—	—	—
Test		Chi-square		Kruskal–Wallis					

Note: Chi-square = Pearson's chi-square coefficient values and p values, (Infected) = the parameter value was higher in infected eels, Kruskal–Wallis = Kruskal–Wallis non-parametric test p values, M = marginally significant, (S) = higher percentage of infected individuals in silvering eels, Sample = the population of eels to which the analysis was applied, Site = capture site, Total = total eel population, (Uninfected) = the parameter value was higher in uninfected eels, W = eviscerated body weight (g), Spearman = Spearman's correlation coefficient values and p values, x = the estimate would not be appropriate, (—) = nonsignificant data. TL = total body length (mm), HSI = hepatosomatic index and GSI = gonadosomatic index.

Abbreviations: ANR = S'Albufereta natural reserve, AV = L'Albufera de Valencia, DS = degree of eel silvering, EI = eye index and FI = fin index.

the ability of eels to move vertically within the water column, endangering the reproductive success of the species [16, 17].

Data from the present investigation also revealed that 10.17% of the eels tested had more than 10 adult parasites, and one eel from L'Albufera de Valencia even had 21 adult parasites in its swim bladder. Dezfuli et al. [66] reported that heavily infected swim bladders showed hyperplasia, cellular swelling and abundant vacuolisation, along with numerous mast cells and macrophage aggregations. Muñoz et al. [67] demonstrated a less effective response in the macrophages of eels infected with more than 10 adult parasites. Thus, more than one-tenth of the eels sampled in our study may have had the function of their swim bladders impaired and may have been at risk of not completing migration and breeding or even dying, as host mortality and morbidity are a function of the parasitic intensity [68].

The variations in parasite aggregation pattern may be related to individual differences in host (behaviour, immunity, presence of other diseases) or habitat conditions (water quality and pollution, salinity, distribution of intermediate hosts) [68–70], but there may be other causes that need to be explored.

The mean intensity of adult parasites detected in eels from L'Albufera de Valencia and El Hondo-Salinas (5.61 and 5.95 parasites per infected eel, respectively) was similar to that reported in eels from the Mediterranean rivers and lagoons of Andalusia (5.8; [55]), from the Gediz Delta in Turkey (5.05; [64]), and from other Iberian ecosystems (5.5–9; [59–61]). In contrast, the mean intensity of adult parasites was lower in eels from Algerian estuaries (3.41 and 3.267; [56]) and from Lake El Broullus in Egypt (4.2; [57]). The mean intensity of adult parasites in eels from S'Albufereta and S'Albufera des Grau (2.41 and 2.5), detected in the present study, was much lower.

Moreover, data on L2-larva infection rates were relevant, especially in S'Albufera des Grau (Table 3). Unlike adult-parasite rates, L2-prevalence was the highest there (90%), with 10 eels harbouring L2 but not advanced stages. Nevertheless, L2 mean intensity was the lowest (2,194). In contrast, in L'Albufera de Valencia, L2 prevalence was the lowest (43.33%), while L2 mean intensity was the highest (22,911.54). In the latter case, most of the L2 were concentrated in a few eels (13), which agrees with the common parasitological postulate on over-dispersion [68, 70, 71]. This pattern matched the findings of Martínez-Carrasco et al. [39] and Palikova and Navratil [72], who reported thousands of L2 larvae per eel, often in the absence of advanced stages of the parasite. Martínez-Carrasco et al. [39] state that excluding L2 stages from parasitological analyses may lead to an underestimation of infection prevalence. It would be reasonable to assume that, despite L2 not being an infectious stage of *A. crassus* for eels, such extremely high infection rates suggest that a relatively small number of eels could be largely responsible for parasite transmission. Furthermore, fish harbouring a large number of L2 larvae had likely been previously infected by numerous large haematophagous adult parasites, which may have negatively affected their body condition.

In the present study, wetland-specific analysis revealed that in El Hondo-Salinas de Santa Pola, larger and heavier silver eels accumulated a greater number of advanced stages of the parasite than smaller yellow eels (Table 4). These findings support the theory that parasite burden increases with the age and size of the host [68] and align with the results of Esteve and Alcaide [49], who reported significantly lower intensity and abundance of adult stages in smaller, younger yellow eels compared to larger silvering eels in L'Albufera de Valencia. In 2002, Lefebvre et al. reported similar results, observing that the intensity of adult parasites was strongly related to eel length [73], with larger eels accumulating significantly more parasites than smaller eels or elvers. Nevertheless, these authors did not observe any relationship between the presence/absence of parasites and eel length. On the contrary, several authors, who have investigated eels inhabiting upstream freshwater, have reported a significantly higher incidence of *A. crassus* in smaller eels [55, 59, 74]. The differences between the results could be explained by the fact that the present study was performed with eels from brackish lagoons located near the sea, where feeding conditions and salinity are different.

Regarding the biometric measured indices, a negative correlation was observed between HSI index values and the number of adult parasites in El Hondo-Salinas de Santa Pola, although it was marginally significant. Nonetheless, in S'Albufera des Grau, GSI values were higher in eels infected with advanced stages of *A. crassus* ($p = 0.012$) compared to uninfected eels and were also positively correlated with the number of advanced stages ($p = 0.002$). In the same wetland, however, the SMI values were lower in L2-infected eels ($p = 0.021$), compared to uninfected eels and were negatively correlated with the number of L2 ($p = 0.049$). These findings contrast with those of Myrenås et al. [75], who found no substantial negative effects of *A. crassus* infection rates on health indicators (spleen and liver size, body fat content and body condition) in continental European eels, attributing this to low infection intensities (median of 2–3 visible parasites). Even so, Habbechi et al. [65] also found no influence of the adult stages of *A. crassus* on the development of the liver, gonads and intestines during the continental lifespan of eels in Tunisia, although they detected higher intensities (1.6–5.6), very similar to the present study. Non-uniformity in sample size or methods used could influence differences in results. Further studies are needed to improve our understanding of the impact of pathogens on biometric parameters and derived indices, many of which reflect information about energy reserves (HSI), reproductive status (GSI), silvering development (FI and EI) and body condition (SMI).

Regarding eel age, no age-related differences were found between infected and uninfected eels, which could contradict reports of a higher prevalence rate in younger and older eels but lower in middle-aged eels [76]. Eel age did not vary between yellow and silvering eels, which was consistent with the claim that eel age at silvering time varied enormously, depending on multifactorial conditions [37].

Furthermore, in the present study, no significant relationship was observed between SDI values and eel infection rates. This finding was consistent with studies reporting intact swim bladders in infected eels [56], but contrasted with that of Wariaghli and Yahyaoui [63], who found a significant positive relationship between SDI values and the number of adult stages of *A. crassus*. This could be explained by the difference in the rate at which damage accumulates in the swim bladder, which has been shown to increase dramatically during the first year and then slow down significantly with age [76].

In the present study, eels from the Iberian Peninsula were sampled between December and February, and those from the Balearic Islands between February and March. Boughaba et al. [56] observed that the abundance and intensity values of *A. crassus* were higher in winter, but other authors reported that the prevalence of adult parasites was higher in summer [57, 64]. To better understand the seasonal variations between eels from the Iberian Peninsula and those from the Balearic Islands, more detailed studies would be necessary.

4.2. Virus and Co-Infection. In addition to parasites, it has been suggested that several viruses negatively affect the health of European eels and could contribute to the decline in eel populations [20]. International trade helps spread pathogens from farmed fish to healthy wild fish [77].

The herpesvirus AngHV1 was first described from wild *Anguilla japonica* in Japan [78] and is considered by many authors to be highly pathogenic and the most widely distributed of all viruses affecting the genus *Anguilla* [20, 79, 80]. AngHV1 infects several eel species, with moderate-to-high mortality rates leading to apathy, gill congestion, haemorrhages or necrosis of the skin and fins, and liver necrosis, and often remains latent in the tissues of asymptomatic carriers [11, 30, 31, 79].

In the present research, AngHV1 was detected in wild eels in all the sampled ecosystems, with extremely variable prevalences depending on the capture site ($p = 0.001$). The prevalence of AngHV1 at L'Albufera de Valencia (100%) was the highest of all the studied wetlands. Bandín et al. [81] tested wild eels from the same wetland in 2004 and 2008 and reported an AngHV1 prevalence of 18% and 83%, respectively. The present study indicates that herpesvirus infection is currently at its peak in this wetland. According to the best of our knowledge, this represents the first description of herpesvirus AngHV1 epidemiological data in eels from El Hondo-Salinas de Santa Pola Natural Park, S'Albufereta Natural Reserve and S'Albufera des Grau Natural Park.

Compared to other nearby wetlands, the prevalence of AngHV1 in wild European eels from Andalusia (35.2%) was considerably lower than at L'Albufera de Valencia [55] but higher than the prevalences at El Hondo-Salinas de Santa Pola (23.33%), S'Albufereta (10.71%) and S'Albufera des Grau (16.67%). The prevalence of AngHV1 in wild eels from the Mar Menor was similar (12.67%) to those from El Hondo-Salinas de Santa Pola, S'Albufereta and S'Albufera des Grau in the present study [82].

The virus EVEX was first isolated in *A. anguilla* elvers imported into Japan from France [83] and later detected in eels from France, Germany, the United Kingdom, Denmark, Sweden, Italy and the Netherlands [20]. EVEX is phylogenetically related to Eel Virus America (EVA) [84], trout rhabdovirus 903/87 [85] and the genus *Vesiculovirus* [86]. The most frequent clinical signs of EVEX are haemorrhages and red areas on the skin [79]. The fact that EVEX infection can seriously threaten the migratory capacity of eels was demonstrated by a simulation of long-distance migration using swimming tunnels, where it was observed that eels subclinically infected with EVEX developed symptoms and died after swimming between 1000 and 1500 km, while uninfected eels were able to swim successfully up to 5500 km [13].

In the present study, EVEX virus was only detected in eels from L'Albufera de Valencia Natural Park (92.31%) and S'Albufereta Natural Reserve (3.57%), showing L'Albufera de Valencia an extremely high prevalence, similar to very high prevalences reported in eels from other Mediterranean ecosystems [79]. By contrast, lower prevalences [82, 87] or even absences [55] have been reported elsewhere. The high prevalence at L'Albufera de Valencia could be related to the poor ecological status of the environment at this site [88].

EVE virus, a highly contagious virus of the genus *Aquabirnavirus* (Birnaviridae) serologically connected with the Infectious Pancreatic Necrosis Virus (IPNV) type AB [45, 89], was not detected in the eels tested in the present survey. This virus, chiefly detected in farmed eels [20, 90], has not been described in wild eels in Spain, although recently McConville et al. [91] did report its presence in the wild population of European eels from Lough Neagh (Northern Ireland).

In the present investigation, the presence or absence of viral infections differed significantly according to the capture site ($p \leq 0.001$). Unfortunately, wetland-specific assays were not always possible (e.g., all eels from L'Albufera de Valencia were infected with AngHV1, while none of the eels from S'Albufera des Grau were infected with EVEX). Even so, silvering eels from L'Albufera de Valencia showed a higher prevalence of the EVEX virus and its co-infections than yellow eels. Eels from S'Albufereta Natural Reserve, infected with EVEX or EVEX-*A. crassus* co-infected, had a higher weight (marginal significance) than uninfected eels, which coincided with results reported by other authors [55]. In the same wetland, eels that were infected with AngHV1 or AngHV1-*A. crassus* co-infected had lower FI index values ($p = 0.016$), possibly suggesting that the infection had a cost in terms of the development of certain body parts and silvering [92].

In the present study, eels from L'Albufera de Valencia were the only ones to exhibit double co-infections with prevalences exceeding 80% for combinations of AngHV1-EVEX, AngHV1-*A. crassus* and EVEX-*A. crassus*. Additionally, only eels from this wetland showed triple co-infections (AngHV1-EVEX-*A. crassus*) with a prevalence of 84.62% (Table 5). According to data from the EU Water Framework Directive [26], L'Albufera de Valencia experiences significant anthropogenic pressures from urban and agricultural activities. However, a previous study using samples from several organs of the eels tested in this investigation found no or very low levels of various organochlorine pesticides in L'Albufera de Valencia [93]. This suggests that other anthropogenic and environmental factors are affecting European eels in this wetland. These factors may include elevated nutrient levels from agricultural runoff leading to eutrophication, discharge of untreated or partially treated sewage, alterations in the hydrological cycle due to irrigation water extraction, introduction of invasive species or pathogens, and other human activities. These factors can create an environment that reduces water quality, increases stress on eel populations and finally increases the proliferation of pathogens, making eels more susceptible to disease. These results, however, should be interpreted with caution because of the limited number of samples.

The high prevalence of triple co-infection observed in the present study (AngHV1-EVEX-*A. crassus*) may have profound implications for the health and fitness of European eels. Co-infections are known to produce synergistic pathological effects that exceed those caused by single agents, as the primary pathogen induces immunosuppression and prevents the host from developing an immune response against subsequent infections, leading to increased symptom severity [94]. In eels, *A. crassus* causes severe chronic stress and lesions, which could lead to viral diseases in the presence of viruses [21], potentially becoming a primary pathogen in the parasite-virus relationship. In addition, AngHV1 and EVEX viral infections in eels have been associated with impaired swimming performance and increased mortality during migration [13], while *A. crassus* compromises the swim bladder, a key organ for buoyancy control. The simultaneous presence of these agents is therefore likely to produce severe additive effects, potentially reducing swimming endurance and migratory success. Evidence from other fish species supports this view: Co-infection with parasites and viruses has been shown to increase disease severity, reduce growth rates, and impair reproductive output compared to single infections [94]. Thus, the high prevalence of multiple infections in eels raises concern about their cumulative impact on both migratory behaviour and reproductive success, ultimately contributing to the decline of this endangered species.

5. Conclusions

Diseases have been suggested as one of the causes of the European eel decline. At the population level, the pathogen threat was very high in eels from L'Albufera de Valencia Natural Park, a site recognised by its poor ecological status: 92.31% of specimens were co-infected by EVEX and AngHV1, while 84.62% of specimens showed triple co-infections (EVEX, AngHV1 and any stage of *A. crassus*). In general, despite not having many physical barriers obstructing the movement of eels from L'Albufera de Valencia Natural Park towards the sea, these eels are probably not in good enough condition to contribute to the global eel population.

The present study highlights the complex situation of European eels in Mediterranean wetlands. The significant presence of *A. crassus* and several viruses, especially in L'Albufera de Valencia, underlines the urgent need for holistic management strategies that address both ecological and anthropogenic factors. This should include the implementation of regular monitoring programmes for *A. crassus* and viral agents in wild populations, together with routine assessments of water quality. Measures to prevent the introduction and spread of invasive species must also be reinforced. Although eel restocking remains a contentious issue, it could be considered conditionally acceptable if a set of strict preconditions is met. In this context, sanitary controls in eel translocations and restocking programmes to avoid the spread of infections should be strictly required. In addition, policy actions should prioritise habitat restoration, improvements in wastewater treatment, and tighter control of agricultural runoff and irrigation practices. Future research should also focus on the life cycles of key pathogens, the potential effects of climate change, and eel immunity and genetic resistance. Ultimately, advancing eel conservation will require ecosystem-based approaches and coordinated efforts among scientists, policymakers, and stakeholders across multiple sectors.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

This work was supported by "Programa Estatal de I + D + I Orientada a los Retos de la Sociedad, Ministerio de Ciencia, Innovación y Universidades, Gobierno de España" (grant RTI2018-097228-B-I00).

Acknowledgements

The authors thank the personnel of the L'Albufera de Valencia Natural Park, El Hondo-Salinas de Santa Pola Natural Park, S'Albufereta Natural Reserve and S'Albufera des Grau Natural Park and the local fishermen for their friendly cooperation. This work was supported by "Programa Estatal de I + D + I Orientada a los Retos de la Sociedad, Ministerio de Ciencia, Innovación y Universidades, Gobierno de España" (grant RTI2018-097228-B-I00).

Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)*

Table S1: Relationship between capture site and degree of silvering, age, biometric data and measured indices of European eels from four Spanish Mediterranean sites (p values from pairwise comparisons).

Table S2: Relationship between capture site and intensity of *Anguillicoloides crassus*, presence/absence of AngHV1 virus, EVEX virus and co-infections in European eels from four Spanish Mediterranean sites (p values from pairwise comparisons).

Table S3: Relationship between the degree of eel silvering and presence or absence of *Anguillicoloides crassus*, AngHV1 or EVEX viruses and co-infections in European eels from four Spanish Mediterranean sites (p values of chi-square test and cross-tabulation).

References

[1] J. Schmidt, "The Breeding Places of the Eel," *Philosophical Transactions of the Royal Society of London – Series B: Containing Papers of a Biological Character* 211 (1923): 179–U38, https://doi.org/10.1098/rstb.1923.0004.

[2] C. Durif, A. Guibert, and P. Elie, "Morphological Discrimination of the Silvering Stages of the European Eel," in *Proceedings of the 2009 American Fisheries Society Symposium*, 58 (Nashville, TN, 2009).

[3] W. Dekker, *Slipping Through Our Hands* (Universiteit van Amsterdam, 2004).

[4] J. Schmidt, "On the Distribution of the Fresh-Water Eels (*Anguilla*) Throughout the World. I. Atlantic Ocean and Adjacent Regions," *Meddelelser Fra Kommissionen for Havundersogelser, Seri Fiskeri* (Copenhagen: Danish Commission for the Study of the Sea, 1909), 1–45.

[5] ICES, in *European Eel (Anguilla anguilla) Throughout Its Natural Range* (ICES, 2022).

[6] C. Pike, V. Crook, and M. Gollock, *Anguilla anguilla. The IUCN RED List of Threatened Species 2020* (IUCN, 2020).

[7] EU, "Council Regulation (EC) No 1100/2007 of 18 September 2007 Establishing Measures for the Recovery of the Stock of European Eel," *Official Journal of the European Union* 248, no. 1100 (2007): 17–23.

[8] European Commission, *Commission Staff Working Document Executive Summary of the Evaluation of Council Regulation (EC) No 1100/2007 of 18 September 2007 Establishing Measures for the Recovery of the Stock of European Eel. Brussels, 17.2.2020 SWD 36* (European Commission, 2020).

[9] H. Drouineau, C. Durif, M. Castonguay, et al., "Freshwater Eels: A Symbol of the Effects of Global Change," *Fish and Fisheries* 19, no. 5 (2018): 903–930, https://doi.org/10.1111/faf.12300.

[10] T. Wirth and L. Bernatchez, "Decline of North Atlantic Eels: A Fatal Synergy?" *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270, no. 1516 (2003): 681–688, https://doi.org/10.1098/rspb.2002.2301.

[11] O. L. M. Haenen, I. Mladineo, R. Konecny, et al., "Diseases of Eels in an International Perspective: Workshop on Eel Diseases at the 15th International Conference on Diseases of Fish and Shellfish, Split, Croatia, 2011," *Bulletin of the European Association of Fish Pathologists* 32, no. 3 (2012): 109–115.

[12] ICES, "Report of the EIFAC/ICES Working Group on Eels (WGEEL)," *Fisheries Management* 139, no. 43 (2009).

[13] V. J. T. Van Ginneken, B. Ballieux, R. Willemze, et al., "Hematology Patterns of Migrating European Eels and the Role of EVEX Virus," *Comparative Biochemistry and Physiology – C Toxicology and Pharmacology* 140, no. 1 (2005): 97–102, https://doi.org/10.1016/j.cca.2005.01.011.

[14] A. Kuwahara, A. Niimi, and H. Itagaki, "Studies on a Nematode Parasitic in the Air Bladder of the Eel. I. Description of *Anguillicola crassa* n. Sp.(Philometridea, Anguillicolidae)," *Japanese Journal of Parasitology* 23, no. 5 (1974): 275–279.

[15] V. Kantzoura, A. Sapounidis, M. K. Kouam, M. N. Kolygas, G. Krey, and E. Koutrakis, "*Anguillicola (Anguillicoides) crassus*: Morphometric Characteristics and Pathogenicity in Eels (*Anguilla anguilla*) in Greece," *Veterinary Parasitology-Regional Studies and Reports* 25 (2021): 100586, https://doi.org/10.1016/j.vprsr.2021.100586.

[16] R. S. Kirk, "The Impact of *Anguillicola crassus* on European Eels," *Fisheries Management and Ecology* 10, no. 6 (2003): 385–394, https://doi.org/10.1111/j.1365-2400.2003.00355.x.

[17] A. P. Palstra, D. F. M. Heppener, V. J. T. Van Ginneken, C. Székely, and G. Van den Thillart, "Swimming Performance of Silver Eels is Severely Impaired by the Swim-Bladder Parasite *Anguillicola crassus*," *Journal of Experimental Marine Biology and Ecology* 352, no. 1 (2007): 244–256, https://doi.org/10.1016/j.jembe.2007.08.003.

[18] F. Lefebvre, S. Wielgoss, K. Nagasawa, and F. Moravec, "On the Origin of *Anguillicoloides crassus*, the Invasive Nematode of Anguillid Eels," *Aquatic Invasions* 7, no. 4 (2012): 443–453, https://doi.org/10.3391/ai.2012.7.4.001.

[19] E. Terech-Majewska, P. Schulz, and A. K. Siwicki, "Influence of Nematode *Anguillicoloides crassus* Infestation on the Celular and Humoral Innate Immunity in European Eel (*Anguilla anguilla* L.)," *Central-European Journal of Immunology* 40, no. 2 (2015): 127.

[20] S. J. Van Beurden, M. Y. Engelsma, I. Roozenburg, et al., "Viral Diseases of Wild and Farmed European Eel *Anguilla anguilla* With Particular Reference to the Netherlands," *Diseases of Aquatic Organisms* 101, no. 1 (2012): 69–86, https://doi.org/10.3354/dao02501.

[21] O. L. M. Haenen, J. Lehmann, M. Engelsma, et al., "The Health Status of European Silver Eels, *Anguilla anguilla*, in the Dutch River Rhine Watershed and Lake IJsselmeer," *Aquaculture* 309, no. 1–4 (2010): 15–24, https://doi.org/10.1016/j.aquaculture.2010.08.026.

[22] ICES, "Joint EIFAAC/ICES/GFCM Working Group on Eels, and Country Reports 2018–2019 (Version 1). ICES Scientific Reports," *International Council for the Exploration of the Sea ICES Scientific Reports* 1, no. 50 (2019): 177, https://doi.org/10.17895/ices.pub.5545.

[23] WFD, "The EU Water Framework Directive. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy," *EC Directive* 60 (2000).

[24] CHJ, "Seguimiento del Plan Hidrológico del Júcar," (2022), https://www.chj.es/es-es/medioambiente/planificacionhidrologica/Documents/Informe-Seguimiento-PHC/InformeSeguimiento2022_POSTCAD.pdf.

[25] CHJ, "Plan Hidrológico de la Demarcación Hidrográfica del Júcar 2022–2027," (2023), https://www.chj.es/es-es/medioambiente/planificacionhidrologica/Documents/Plan-Hidrologico-cuenca-2021-2027/PHC/VersionFinal/PHJ2227_Anejo12_Estado_2023_01_24.pdf.

[26] CHJ-GV-AV, *Borrador del Plan Especial de L'Albufera* (Confederación Hidrográfica Del Júcar, Generalitat Valenciana y Ajuntament de València, 2019).

[27] CHS, "Desarrollo del Programa de Seguimiento Para Determinar el Estado de las Aguas Continentales y el Control de las Zonas Protegidas en la Demarcación Hidrográfica del Segura," (2023), https://www.chsegura.es/es/cuenca/redes-de-control/calidad-en-aguas-superficiales/informes/.

[28] Govern de les Illes Balears, "Plan Hidrológico de la Demarcación Hidrográfica de las Illes Balears," (2024), https://www.caib.es/sites/aigua/es/proposta_projecte/.

[29] C. Caruso, S. Peletto, A. Gustinelli, et al., "Detection of a Phylogenetically Divergent Eel Virus European X (EVEX) Isolate in European Eels (*Anguilla anguilla*) Farmed in Experimental Tanks in Italy," *Aquaculture* 434 (2014): 115–120, https://doi.org/10.1016/j.aquaculture.2014.07.024.

[30] A. Davidse, O. L. M. Haenen, S. G. Dijkstra, et al., "First Isolation of Herpesvirus of Eel (*Herpesvirus anguillae*) in Diseased European Eel (*Anguilla anguilla* L.) in Europe," *Bulletin of the European Association of Fish Pathologists* 19, no. 4 (1999): 137–141.

[31] O. L. M. Haenen, S. G. Dijkstra, P. W. Van Tulden, et al., "*Herpesvirus anguillae* (HVA) Isolations From Disease Outbreaks in Cultured European Eel, *Anguilla anguilla* in the Netherlands Since 1996," *Bulletin of the European Association of Fish Pathologists* 22, no. 4 (2002): 247–257.

[32] B. N. Hangalapura, R. Zwart, M. Y. Engelsma, and O. L. M. Haenen, "Pathogenesis of *Herpesvirus anguillae* (HVA) in Juvenile European Eel *Anguilla anguilla* After Infection by Bath Immersion," *Diseases of Aquatic Organisms* 78, no. 1 (2007): 13–22, https://doi.org/10.3354/dao01849.

[33] C. Durif, S. Dufour, and P. Elie, "The Silvering Process of *Anguilla anguilla*: A New Classification From the Yellow Resident to the Silver Migrating Stage," *Journal of Fish Biology* 66, no. 4 (2005): 1025–1043, https://doi.org/10.1111/j.0022-1112.2005.00662.x.

[34] N. W. Pankhurst, "Relation of Visual Changes to the Onset of Sexual Maturation in the European Eel *Anguilla anguilla* (L.)," *Journal of Fish Biology* 21, no. 2 (1982): 127–140, https://doi.org/10.1111/j.1095-8649.1982.tb03994.x.

[35] J. Peig and A. J. Green, "New Perspectives for Estimating Body Condition From Mass/Length Data: The Scaled Mass Index as an Alternative Method," *Oikos* 118, no. 12 (2009): 1883–1891, https://doi.org/10.1111/j.1600-0706.2009.17643.x.

[36] A. Acou, P. Boury, P. Laffaille, A. J. Crivelli, and E. Feunteun, "Towards a Standardized Characterization of the Potentially Migrating Silver European Eel (*Anguilla anguilla* L.)," *Archiv fur Hydrobiologie* 164, no. 2 (2005): 237–255, https://doi.org/10.1127/0003-9136/2005/0164-0237.

[37] E. Barcala, D. Romero, C. Bulto, et al., "An Endangered Species Living in an Endangered Ecosystem: Population Structure and Growth of European Eel *Anguilla anguilla* in a Mediterranean Coastal Lagoon," *Regional Studies in Marine Science* 50 (2022): 102163, https://doi.org/10.1016/j.rsma.2022.102163.

[38] ICES, *Workshop on Age Reading of European and American Eel (WKAREA), 20-24 April 2009, Bordeaux, France* (ICES, 2009).

[39] C. Martínez-Carrasco, R. Ruiz De Ybañez, J. Peñalver, E. Mayo-Hernández, A. García-Ayala, and P. Muñoz, "Prevalence of *Anguillicoloides crassus* (Nematoda, Dracunculoidea) in Wild European Eels (*Anguilla anguilla* L.) From Mar Menor Lagoon (Western Mediterranean, Spain)," *Revue de Medecine Veterinaire* 162, no. 3 (2011): 154–158.

[40] L. Rolbiecki, "New Data on the Biology of the Introduced Exotic Nematode *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 in the Eel *Anguilla anguilla* in Lake Wdzydze (Polish Waters)," *Oceanological and Hydrobiological Studies* 37, no. 3 (2008): 37–48, https://doi.org/10.2478/v10009-008-0006-0.

[41] A. O. Bush, K. D. Lafferty, J. M. Lotz, and A. W. Shostak, "Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited," *The Journal of Parasitology* 83, no. 4 (1997): 575–583, https://doi.org/10.2307/3284227.

[42] F. Lefebvre, P. Contournet, and A. J. Crivelli, "The Health State of the Eel Swimbladder as a Measure of Parasite Pressure by *Anguillicola crassus*," *Parasitology* 124, no. 4 (2002): 457–463, https://doi.org/10.1017/S0031182001001378.

[43] S. J. Van Beurden, M. A. Voorbergen-Laarman, I. Roozenburg, J. Van Tellingen, O. L. M. Haenen, and M. Y. Engelsma, "Development and Validation of a Real-Time PCR Assay for the Detection of Anguillid Herpesvirus 1," *Journal of Fish Diseases* 39, no. 1 (2016): 95–104, https://doi.org/10.1111/jfd.12330.

[44] S. J. Van Beurden, M. A. Voorbergen-Laarman, I. Roozenburg, A. S. Boerlage, O. L. M. Haenen, and M. Y. Engelsma, "Development and Validation of a Two-Step Real-Time RT-PCR for the Detection of Eel Virus European X in European Eel, *Anguilla anguilla*," *Journal of Virological Methods* 171, no. 2 (2011): 352–359, https://doi.org/10.1016/j.jviromet.2010.11.019.

[45] I. Orpetveit, A. B. Mikalsen, H. Sindre, O. Evensen, B. H. Dannevig, and P. J. Midtlyng, "Detection of Infectious Pancreatic Necrosis Virus in Subclinically Infected Atlantic Salmon by Virus Isolation in Cell Culture or Real-Time Reverse Transcription Polymerase Chain Reaction: Influence of Sample Preservation and Storage," *Journal of Veterinary Diagnostic Investigation* 22, no. 6 (2010): 886–895, https://doi.org/10.1177/104063871002200606.

[46] A. P. Palstra and G. Van den Thillart, "Swimming Physiology of European Silver Eels (*Anguilla anguilla* L.): Energetic Costs and Effects on Sexual Maturation and Reproduction," *Fish Physiology and Biochemistry* 36, no. 3 (2010): 297–322, https://doi.org/10.1007/s10695-010-9397-4.

[47] F. Lefebvre and A. J. Crivelli, "Anguillicolosis: Dynamics of the Infection Over Two Decades," *Diseases of Aquatic Organisms* 62, no. 3 (2004): 227–232, https://doi.org/10.3354/dao062227.

[48] J. F. Asturiano, L. Pérez, A. Tomás, S. Zegrari, F. J. Espinós, and M. Jover, "Inducción Hormonal de la Maduración Gonadal y la Puesta en Hembras de Anguila Europea *Anguilla anguilla* L., 1758: Cambios Morfológicos y Desarrollo Oocitario," *Boletin – Instituto Espanol de Oceanografia* 18, no. 1–4 (2002): 127–137.

[49] C. Esteve and E. Alcaide, "Influence of Diseases on the Wild Eel Stock: The Case of Albufera Lake," *Aquaculture* 289, no. 1-2 (2009): 143–149, https://doi.org/10.1016/j.aquaculture.2008.12.015.

[50] C. Martínez-Carrasco, E. Serrano, R. Ruiz de Ybáñez, et al., "The European Eel: The Swim Bladder-Nematode System Provides a New View of the Invasion Paradox," *Parasitology Research* 108, no. 6 (2011): 1501–1506, https://doi.org/10.1007/s00436-010-2200-8.

[51] C. R. Kennedy and D. J. Fitch, "Colonization, Larval Survival and Epidemiology of the Nematode *Anguillicola crassus*, Parasitic in the Eel, *Anguilla anguilla*, in Britain," *Journal of Fish Biology* 36, no. 2 (1990): 117–131, https://doi.org/10.1111/j.1095-8649.1990.tb05588.x.

[52] B. Sures, "Environmental Parasitology. Interactions Between Parasites and Pollutants in the Aquatic Environment," *Parasite* 15, no. 3 (2008): 434–438, https://doi.org/10.1051/parasite/2008153434.

[53] Govern de les Illes Balears, "Plan Hidrológico de la Demarcación Hidrográfica de las Illes Balears (2022–2027)," (2023), https://www.caib.es/sites/aigua/es/propuesta_proyecto_/.

[54] G. E. Maes, J. A. M. Raeymaekers, B. Hellemans, et al., "Gene Transcription Reflects Poor Health Status of Resident European Eel Chronically Exposed to Environmental Pollutants," *Aquatic Toxicology* 126 (2013): 242–255, https://doi.org/10.1016/j.aquatox.2012.11.006.

[55] R. Ruiz de Ybáñez, L. Del Río, C. Flores-Flores, et al., "Monitoring for *Anguillicoloides crassus*, Anguillid Herpesvirus 1, Aquabirnavirus EVE and Rhabdovirus EVEX in the European Eel Population of Southern Spain," *Journal of Fish Diseases* 46, no. 4 (2023): 417–431, https://doi.org/10.1111/jfd.13754.

[56] O. Boughaba, M. Tahri, N. Djebbari, and M. Bensouilah, "Seasonal Patterns and Host Factors Influencing *Anguillicoloides crassus* Infestation in Eels from Northeastern Algeria," *African Journal of Biological Sciences* 6, no. 16 (2024): https://doi.org/10.48047/AFJBS.6.16.2024.1694-1710.

[57] A. E. Mahmoud, "Some Studies on Parasitic *Anguillicoliosis* in Wild *Anguilla anguilla* Offsprings in Lake El Broullus, Egypt," *Egyptian Journal of Chemistry and Environmental Health* 2, no. 2 (2016): 617–625, https://doi.org/10.21608/ejceh.2016.254632.

[58] D. Innal, O. Ozmen, and E. Genc, "Infection of European Eel, *Anguilla anguilla* With the Nematode *Anguillicoloides crassus* From Some Estuarine Systems in Turkey," *Turkish Journal of Fisheries and Aquatic Sciences* 19, no. 11 (2019): 899–905, https://doi.org/10.4194/1303-2712-v19_11_01.

[59] A. Aguilar, M. F. Alvarez, J. M. Leiro, and M. L. Sanmartin, "Parasite Populations of the European Eel (*Anguilla anguilla* L.) in the Rivers Ulla and Tea (Galicia, Northwest Spain)," *Aquaculture* 249, no. 1–4 (2005): 85–94, https://doi.org/10.1016/j.aquaculture.2005.04.052.

[60] C. Eira, J. Torres, J. Miquel, J. Vaqueiro, A. Soares, and J. Vingada, "Trace Element Concentrations in *Proteocephalus macrocephalus* (Cestoda) and *Anguillicola crassus* (Nematoda) in Comparison to Their Fish Host, *Anguilla anguilla* in Ria De Aveiro, Portugal," *Science of the Total Environment* 407, no. 2 (2009): 991–998, https://doi.org/10.1016/j.scitotenv.2008.10.040.

[61] I. Gallastegi, A. Rallo, and M. F. Mulcahy, "A Report of *Anguillicola crassus* From Spain," *Bulletin of the European Association of Fish Pathologists* 22, no. 4 (2002): 283–284.

[62] P. A. Maíllo, M. A. Vich, H. Salvadó, A. Marqués, and M. P. Gracia, "Parasites of *Anguilla anguilla* (L.) From Three Coastal Lagoons of the River Ebro Delta (Western Mediterranean)," *Acta Parasitologica* 50, no. 2 (2005): 156–160.

[63] F. Wariaghli and A. Yahyaoui, "*Anguillicoloides crassus* (Nematoda: Dracunculoidea) Infection in Eels in Moroccan Estuaries," *AACL Bioflux* 11, no. 1 (2018): 194–202.

[64] M. Salman, "Infection of the Swim Bladder Parasite *Anguillicoloides crassus* in the European Eel (*Anguilla anguilla*) Caught From the Gediz Delta (Aegean Sea)," *EGE Journal of Fisheries and Aquatic Sciences* 40, no. 1 (2023): 15–19, https://doi.org/10.12714/egejfas.40.1.02.

[65] B. H. Habbechi, M. M. Kraiem, and P. Elie, "Etude de la Contamination de l'Anguille Européenne (*Anguilla anguilla* L., 1758) Par *Anguillicoloides crassus* Dans Quelques Hydrosystèmes de la Tunisie Septentrionale: Analyse de Son Impact sur les Paramètres de Croissance," *Cybium: International Journal of Ichthyology* 36, no. 3 (2012): 417–433.

[66] B. S. Dezfuli, C. Maestri, M. Lorenzoni, A. Carosi, B. J. Maynard, and G. Bosi, "The Impact of *Anguillicoloides crassus* (Nematoda) on European Eel Swimbladder: Histopathology and Relationship Between Neuroendocrine and Immune Cells," *Parasitology* 148, no. 5 (2021): 612–622, https://doi.org/10.1017/s0031182021000032.

[67] P. Muñoz, J. Peñalver, R. Ruiz De Ybañez, and J. Garcia, "Influence of Adult *Anguillicoloides crassus* Load in European Eels Swimbladder on Macrophage Response," *Fish & Shellfish Immunology* 42, no. 2 (2015): 221–224, https://doi.org/10.1016/j.fsi.2014.11.011.

[68] K. Wilson, O. N. Bjørnstad, A. P. Dobson, et al., "Heterogeneities in Macroparasite Infections: Patterns and Processes," *The Ecology of Wildlife Diseases* 44 (2002): 6–44, https://doi.org/10.1093/oso/9780198506201.003.0002.

[69] P. Galli, G. Crosa, L. Mariniello, M. Ortis, and S. D'Amelio, "Water Quality as a Determinant of the Composition of Fish Parasite Communities," *Hydrobiologia* 452, no. 1–3 (2001): 173–179, https://doi.org/10.1023/a:1011958422446.

[70] D. J. Shaw, B. T. Grenfell, and A. P. Dobson, "Patterns of Macroparasite Aggregation in Wildlife Host Populations," *Parasitology* 117, no. 6 (1998): 597–610, https://doi.org/10.1017/s0031182098003448.

[71] M. E. J. Woolhouse, C. Dye, J. F. Etard, et al., "Heterogeneities in the Transmission of Infectious Agents: Implications for the Design of Control Programs," *Proceedings of the National Academy of Sciences* 94, no. 1 (1997): 338–342, https://doi.org/10.1073/pnas.94.1.338.

[72] M. Palikova and S. Navratil, "Occurrence of *Anguillicola crassus* in the Water Reservoir Koryčany (Czech Republic) and Its Influence on the Health Condition and Haematological Indices of Eels," *Acta Veterinaria Brno* 70, no. 4 (2001): 443–449, https://doi.org/10.2754/avb200170040443.

[73] F. Lefebvre, P. Contournet, F. Priour, O. Soulas, and A. J. Crivelli, "Spatial and Temporal Variation in *Anguillicola crassus* Counts: Results of a 4 Year Survey of Eels in Mediterranean Lagoons," *Diseases of Aquatic Organisms* 50, no. 3 (2002): 181–188, https://doi.org/10.3354/dao050181.

[74] J. Barry, M. Newton, J. A. Dodd, D. Evans, J. Newton, and C. E. Adams, "The Effect of Foraging and Ontogeny on the Prevalence and Intensity of the Invasive Parasite *Anguillicola crassus* in the European Eel *Anguilla anguilla*," *Journal of Fish Diseases* 40, no. 9 (2017): 1213–1222, https://doi.org/10.1111/jfd.12596.

[75] E. Myrenås, J. Näslund, J. Persson, and J. Sundin, "Effects of the Invasive Swim Bladder Parasite *Anguillicola crassus* on Health and Condition Indicators in the European Eel," *Journal of Fish Diseases* 46, no. 10 (2023): 1029–1047, https://doi.org/10.1111/jfd.13822.

[76] Z. T. Warshafsky, "Impact of the Parasitic Nematode *Anguillicoloides Crassus* on American Eels (*Anguilla rostrata*) in Chesapeake Bay," (2017), https://scholarworks.wm.edu/server/api/core/bitstreams/4e97b568-0d2f-4e28-b451-0e50de9d492d/content.

[77] O. L. M. Haenen, V. J. T. Van Ginneken, M. Engelsma, and G. Van den Thillart, "Impact of Eel Viruses on Recruitment of European Eel," in *Spawning Migration of the European Eel: Reproduction Index, a Useful Tool for Conservation Management*, ed. G. van den Thillart, S. Dufour, and J. C. Ranakin (Amsterdam: Springer, 2009), 387–400, https://doi.org/10.1007/978-1-4020-9095-0_16.

[78] M. Sano, H. Fukuda, and T. Sano, "Isolation and Characterization of a New Herpesvirus From Eel," in *Pathology in Marine Science*, 3rd ed., ed. F. O. Perkins and T. C. Cheng (New York, NY: Academic Press, 1990), 15–31.

[79] V. J. T. Van Ginneken, O. L. M. Haenen, K. Coldenhoff, et al., "Presence of Eel Viruses in Eel Species From Various

Geographic Regions," *Bulletin of the European Association of Fish Pathologists* 24, no. 5 (2004): 268–272.

[80] A. P. Van Nieuwstadt, S. G. Dijkstra, and O. L. M. Haenen, "Persistence of Herpesvirus of Eel *Herpesvirus anguillae* in Farmed European Eel *Anguilla anguilla*," *Diseases of Aquatic Organisms* 45, no. 2 (2001): 103–107, https://doi.org/10.3354/dao045103.

[81] I. Bandín, S. Souto, J. M. Cutrín, et al., "Presence of Viruses in Wild Eels *Anguilla anguilla* L., From the Albufera Lake (Spain)," *Journal of Fish Diseases* 37, no. 7 (2014): 597–607, https://doi.org/10.1111/jfd.1392.

[82] P. Muñoz, J. Peñalver, E. Barcala, et al., "Prevalence of Virus in Wild European Eels (*Anguilla anguilla*) From Mar Menor (Western Mediterranean, Spain)," (2018), https://digital.csic.es/bitstream/10261/320542/1/24172.pdf.

[83] T. Sano, T. Nishimura, N. Okamoto, and F. H, "Studies on Viral Diseases of Japanese Fishes 7. A Rhabdovirus Isolated From European Eel, *Anguilla anguilla*," *Bulletin of the Japanese Society of Scientific Flsheries* 43, no. 5 (1977): 491–495, https://doi.org/10.2331/suisan.43.491.

[84] T. Kobayashi and T. Miyazaki, "Rhabdoviral Dermatitis in Japanese Eel, *Anguilla japonica*," *Fish Pathology* 31, no. 4 (1996): 183–190, https://doi.org/10.3147/jsfp.31.183.

[85] R. Galinier, S. J. Van Beurden, E. Amilhat, et al., "Complete Genomic Sequence and Taxonomic Position of Eel Virus European X (EVEX), a Rhabdovirus of European Eel," *Virus Research* 166, no. 1-2 (2012): 1–12, https://doi.org/10.1016/j.virusres.2012.02.020.

[86] D. M. Stone, R. C. Kerr, M. Hughes, A. D. Radford, and A. C. Darby, "Characterisation of the Genomes of Four Putative Vesiculoviruses: Tench Rhabdovirus, Grass Carp Rhabdovirus, Perch Rhabdovirus and Eel Rhabdovirus European X," *Archives of Virology* 158, no. 11 (2013): 2371–2377, https://doi.org/10.1007/s00705-013-1711-x.

[87] E. Amilhat, G. Fazio, G. Simon, et al., "Silver European Eels Health in Mediterranean Habitats," *Ecology of Freshwater Fish* 23, no. 1 (2014): 49–64, https://doi.org/10.1111/eff.12077.

[88] C. Geeraerts and C. Belpaire, "The Effects of Contaminants in European Eel: A Review," *Ecotoxicology* 19, no. 2 (2010): 239–266, https://doi.org/10.1007/s10646-009-0424-0.

[89] R. P. Hedrick, J. L. Fryer, S. N. Chen, and G. H. Kou, "Characteristics of Four Birnaviruses Isolated From Fish in Taiwan," *Fish Pathology* 18, no. 2 (1983): 91–97, https://doi.org/10.3147/jsfp.18.91.

[90] P. E. V. Jørgensen, J. Castric, B. Hill, O. Ljungberg, and P. De Kinkelin, "The Occurrence of Virus Infections in Elvers and Eels (*Anguilla anguilla*) in Europe With Particular Reference to VHSV and IHNV," *Aquaculture* 123, no. 1-2 (1994): 11–19, https://doi.org/10.1016/0044-8486(94)90115-5.

[91] J. McConville, E. Fringuelli, D. Evans, and P. Savage, "First Examination of the Lough Neagh European Eel (*Anguilla anguilla*) Population for Eel Virus European, Eel Virus European X and Anguillid Herpesvirus-1 Infection by Employing Novel Molecular Techniques," *Journal of Fish Diseases* 41, no. 12 (2018): 1783–1791, https://doi.org/10.1111/jfd.12885.

[92] G. Fazio, P. Sasal, G. Mouahid, R. Lecomte-Finiger, and H. Moné, "Swim Bladder Nematodes (*Anguillicoloides crassus*) Disturb Silvering in European Eels (*Anguilla anguilla*)," *The Journal of Parasitology* 98, no. 4 (2012): 695–705, https://doi.org/10.1645/ge-2700.1.

[93] A. Pérez-Vegas, M. Pérez-López, E. Barcala, D. Romero, and P. Muñoz, "Organochlorine Residues in Muscle of European Eels (*Anguilla anguilla*) From Four Spanish Mediterranean Wetlands and Coastal Lagoons," *Marine Pollution Bulletin* 186 (2023): 114408, https://doi.org/10.1016/j.marpolbul.2022.114408.

[94] M. H. Kotob, S. Menanteau-Ledouble, G. Kumar, M. Abdelzaher, and M. El-Matbouli, "The Impact of Co-Infections on Fish: A Review," *Veterinary Research* 47, no. 1 (2016): 98, https://doi.org/10.1186/s13567-016-0383-4.