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A complete check-up of European eel after eight years of restocking in an upland river: Trends in growth, lipid content, sex ratio and health status

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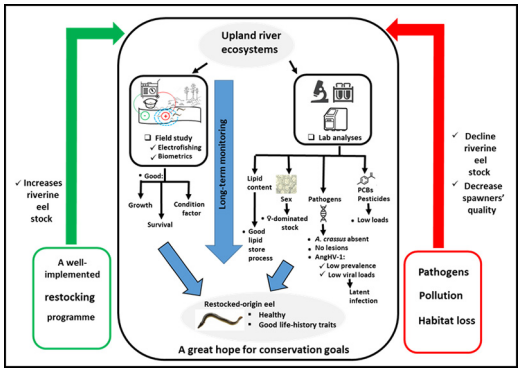
HIGHLIGHTS

- Life history traits and health status of restocked eels tested in rivers.
- Female-dominated eel stocks had good condition factor (K) and lipid stores.
- No parasites or internal lesions observed and eel survival was high.
- Low AngHV-1 prevalence detected and viral loads positively correlated with K.
- Low pollutant loads found negatively correlated with K, lipid and eel density.

GRAPHICAL ABSTRACT



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ABSTRACT

By combining field research and careful laboratory analysis of samples over the course of an eight-year study, we met the challenge of assessing the life history traits and health status of eels restocked in freshwater ecosystems. We found that restocked eels exhibited good growth performance; moreover, the stocks were female-dominated, showed a good Fulton's condition factor (K) and lipid stores and had high survival probability estimated using the best model of Jolly-Seber stock assessment method for open populations. A necropsy revealed the absence of internal lesions. A swim bladder examination revealed the absence of the parasite *Anguillicola crassus*. Polymerase chain reaction (PCR) analyses revealed an increase of *Anguillid herpesvirus*-1 (AngHV-1) prevalence throughout the study. Most positive subjects expressed viral loads compatible with a latent infection and correlated positively with K. All restocked eels were contaminated by at least one of the organic pollutant congeners studied, but the pollution loads corresponded to the lowest range of pollutant concentrations reported in the available literature for European eels and did not exceed the maximum residue and contaminant limits in food and feed of several national and international regulations. Pollutant loads were negatively correlated with K, lipid content and eel density for polychlorinated biphenyls PCB 138, 153 and 180 and K for pesticides p.p'-DDE, p.p'-DDD, p.p'-DDT and PBDE47. This study highlights the potential role played by upland aquatic

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ecosystems in enhancing riverine silver eel production from the perspective of species conservation. To be successful, restocking must be accompanied by improved ecosystem quality and migration routes for eels in inland freshwaters. We also provide some recommendations for future research to improve the management of restocking programmes.

1. Introduction

The European eel *Anguilla anguilla* is a benthic carnivorous fish species that has a high fat content and a long life span. Adults reproduce once in the Sargasso Sea after their catadromous migration; larvae then migrate back to continental waters, where they grow (Tesch and Thorpe, 2003; Degerman et al., 2019). Catadromous life history may be facultative in some situations for this species (Righton et al., 2021). Within the growth phase in continental waters, the yellow eel stage is characterised by sedentarism; at this time and given their combination of high longevity and feeding behaviour, eels are exposed to high risks of persistent pollutant accumulation and pathogen contamination from water, food and sediment compared with other inland freshwater fish species. Pollution and pathogens represent serious threats to these individuals' health while they are developing in continental areas and may impair their return as silver eels to the Sargasso Sea by forcing them to use the fat accumulated during the continental phase. Since around 1980 there has been serious concern over the drastic stock decline of the European eel, which has led to it being included on the Red List of the International Union for Conservation of Nature and it being classified as a critically endangered species (Jacoby and Gollock, 2014).

Several causes have been suggested to explain the decline in eel stocks, including habitat loss, pathogens and pollution (Feunteun, 2002; Robinet and Feunteun, 2002; Haenen et al., 2012; Muñoz et al., 2019; Delrez et al., 2021). Habitat loss essentially owes to the anthropogenic modification of land such as through the construction of hydropower dams and weirs. These changes have led to the fragmentation of aquatic ecosystems and a reduction in eel movements, affecting the sex ratio (Kettle et al., 2011; Drouineau et al., 2018), wetland drainage and river channelisation. Consequently, there are fewer suitable habitats available (Basset et al., 2013), while revetments along rivers lower the condition factor (K) of eels through a loss of prey diversity (Itakura et al., 2015a, 2015b). These factors can restrict eels' growth performance and ultimately affect their reproductive success via good age/size at maturity, fecundity and the period to which they are susceptible to size-selective predators. According to Helfman et al. (1987) and Vøllestad (1992), in an attempt to find a trade-off between survival and fecundity, females of this species follow a size-maximising strategy which leads them to adapt their length at silvering – an onset of sexual maturation – to local conditions of growth and mortality. Better individual growth is linked with access to suitable local habitats for growth and survival, which may be beneficial for the recovery of altered stocks of endangered fish species like the European eel (Werner and Gilliam, 1984; Wootton, 1990; Rose et al., 2001; Boulenger et al., 2014; Nzau Matondo et al., 2021).

Eels are affected by pollution through increasing amounts of organic contaminants, pesticides and toxic metal loads released into freshwater ecosystems by industry, agriculture and urbanisation. The deleterious effects include altered metabolism, immunotoxicity, endocrine disruption and neurotoxicity (Maes et al., 2005; Byer et al., 2013; Köhler and Triebskorn, 2013; Kammann et al., 2014; Privitera et al., 2014; ICES, 2016; Pannetier et al., 2016; Drouineau et al., 2018). Many contaminants are ecologically damaging because persistent chemicals have been shown to biomagnify and to accumulate in food webs at high trophic levels (Köhler and Triebskorn, 2013; Drouineau et al., 2018). Contaminants can directly kill eels, but most researchers have reported the impacts of sublethal concentrations of contaminants that lead to tissue damage, stress, effects on osmoregulation, behavioural alterations, hormonal disruption and genotoxic effects (Couillard et al., 2014; Geeraerts and Belpaire, 2010). Contaminants can also be transferred to eels' offspring, causing larval malformation, accumulating in stored lipids and altering lipid metabolism in this fatty fish species, which is particularly sensitive to pollutants. Lipid accumulation reaches its maximum level in the silver eel stage (over 13% in muscle tissues on a wet weight basis; Palstra et al., 2006a, 2006b), enabling the species' transoceanic spawning migration to the Sargasso Sea. Females must spend 67% of their fat stores during spawning migration and oocyte maturation (Palstra and van den Thillart, 2010). It is during the lipid mobilisation process that contaminants are mostly released at high concentrations into the blood, adversely affecting gonad maturation and oocyte production and thereby hindering successful spawning migration (Jürgens et al., 2015).

Pathogens include a wide range of parasites, such as the haematophagous nematode *Anguillicola crassus* of the eel swim bladder as well as a wide range of infectious diseases, including the haemorrhagic disease caused by *Anguillid herpesvirus*-1 (AngHV-1). *A. crassus* causes inflammation of the swim bladder, leading to bacterial infections, stress and appetite loss (Lefebvre et al., 2013). The infection can alter the gas composition, obstruct the pneumatic duct and lead to swim bladder necrosis, thereby impairing this organ's function (Würtz and Taraschewski, 2000; Lefebvre et al., 2013). Swim bladder alteration directly affects eels' swimming capacity, thereby threatening their successful transoceanic spawning migration because they need to control their buoyancy during important vertical migrations that occur during the daytime (Palstra et al., 2007; Righton et al., 2016; Simon et al., 2018). The high-energy requirement for spawning migration and the dysfunction of the swim bladder have an adverse effect on individuals with reduced lipid stores due to infection (Marohn et al., 2013). The AngHV-1 infection causes the following symptoms: lethargy; swimming near the surface; swimming in a circular motion; mottled skin; inflammation; haemorrhaging in the fins; throat, operculum, head, lower jaw, pectoral fin, tail and skin lesions; and necrosis within the kidney, gills and liver (Haenen et al., 2012; Armitage et al., 2014; McConville et al., 2018; Donohoe et al., 2021). Armitage et al. (2014) reported dead eels measuring 50–90 cm (17–26 years old) due to AngHV-1 infection in two English still-water fisheries, but experimental infections did not produce eel mortality (see also Kobayashi and Miyazaki, 1996; Hangalapura et al., 2007). It has been suggested that eel viral infections, mainly the AngHV-1 and the rhabdovirus Eel Virus European X (EVEX), have a negative effect on the eel spawning migration (Van Ginneken et al., 2005; Haenen et al., 2009). These issues collectively highlight the danger of a combination of habitat loss, pollutant contamination and viral/parasite infection on the health and reproductive migration success and fitness as well as the recruitment success of a species that is declining throughout its entire distribution range.

As a consequence of the massive recruitment decline of the European eel since the 1980s, the European Union has adopted multiple measures to stimulate the recovery of this species' stocks. These include restocking, namely the reallocation of naturally recruited eels from donor aquatic environments with high eel densities to recipient freshwater habitats where there is little or no natural colonisation of eels (Pedersen, 2000; Ovidio et al., 2015; Nzau Matondo et al., 2019, 2020, 2021). For long-distance transfers of young eels, Stacey et al. (2015) have suggested that the life history characteristics of donor and recipient sites should be matched to increase the likelihood of achieving

conservation goals. The European eel is a single panmictic population that is unstructured in meta-population through its entire range, so the translocation of eels within its distribution area or between river basins for restocking purposes is genetically unrestricted (van Ginneken and Maes, 2005; Palm et al., 2009). This practice depends on catching wild glass eels and elvers, because eel reproduction in captivity has not yet been achieved (Pedersen and Rasmussen, 2016). Recent outcomes from restocking in inland freshwaters have revealed successful adaptation, survival, dispersal, growth and silvering in restocked eels (Félix et al., 2020a, 2020b; Nzau Matondo et al., 2020, 2021). These findings provide great hope for inland freshwaters to enhance local stocks of eels and eventually increase their contribution to panmictic stocks of genitors reproducing in the Sargasso Sea. However, restocked eels encounter the same threats in terms of habitat loss, pollution and pathogens as naturally recruited eels in inland waters. Restocking is carried out early in the species' life cycle, when these fish are often naturally absent in upstream recipient inland rivers. According to eels' biology, this approach implies a forced freshwater lifespan and a risk of exposure to the above-mentioned adverse environmental conditions for restocked eels that differs from that faced by ascending wild eels which reach the upstream parts of inland waters much later. The practice of restocking has raised many concerns about the quality of the genitors produced with regard to length at silvering, the sex ratio due to high-density artificial generation, fat content during spawning migration and health status (Belpaire et al., 2011; Clevestam et al., 2011; Couillard et al., 2014; Geffroy and Bardonnet, 2015; Bonnineau et al., 2016; Miller et al., 2016; Nzau Matondo et al., 2020).

In the Belgian Meuse River basin (> 320 km from the North Sea), local eel stocks have declined drastically. The number of wild yellow eels ascending the Meuse River from the North Sea via the Dutch Meuse dropped by about 3.6% per year from 1992 to 2020; in 2020, this number represented 0.6% of the level recorded in 1992 (Nzau Matondo and Ovidio, 2016, 2018; Nzau Matondo et al., 2019, 2020, 2021). This decline owes to the decrease in glass eel recruitment in the North Sea, which in 2019 fell to 1.4% of the average level of 1960–1979 (ICES, 2020). Without restocking, eels will probably disappear from the Belgian Meuse River basin in the next decade. Hence, there is an urgent need to optimise this practice and to evaluate the quality of the spawners produced by restocking, as freshwater eels must often encounter negative factors like pathogens, pollution, barriers and turbine mortality. In this study, we selected an upland river belonging to the Meuse River basin, which has been intensively monitored since restocking in 2013 using imported glass eels. Over the course of eight years, we have surveyed in the field the growth of the restocked eels each year. Moreover, we have analysed in a laboratory the sex ratio, persistent organic pollutant loads and pathogen loads on two occasions: at five and eight years post-restocking. The ultimate goal of the study was to perform an in-depth assessment of the quality of restocked eels at this step of the survey to better predict and understand their quality in the future at the spawning migration stage. We hypothesised the following: (1) there is poor growth performance due to the effects of negative factors encountered in freshwater habitats; (2) there is a highly unbalanced sex ratio in favour of male production because of overdensity from releasing glass eels at a single point; (3) there is a high pollution load, particularly comprising persistent organic pollutants; and (4) there is a high prevalence and load of pathogens like *A. crassus* and AngHV-1, especially in larger individual eels, because restocked eels face a greater risk of contagion with increasing total length, Fulton's condition factor, lipid content, density, age and silvering stage.

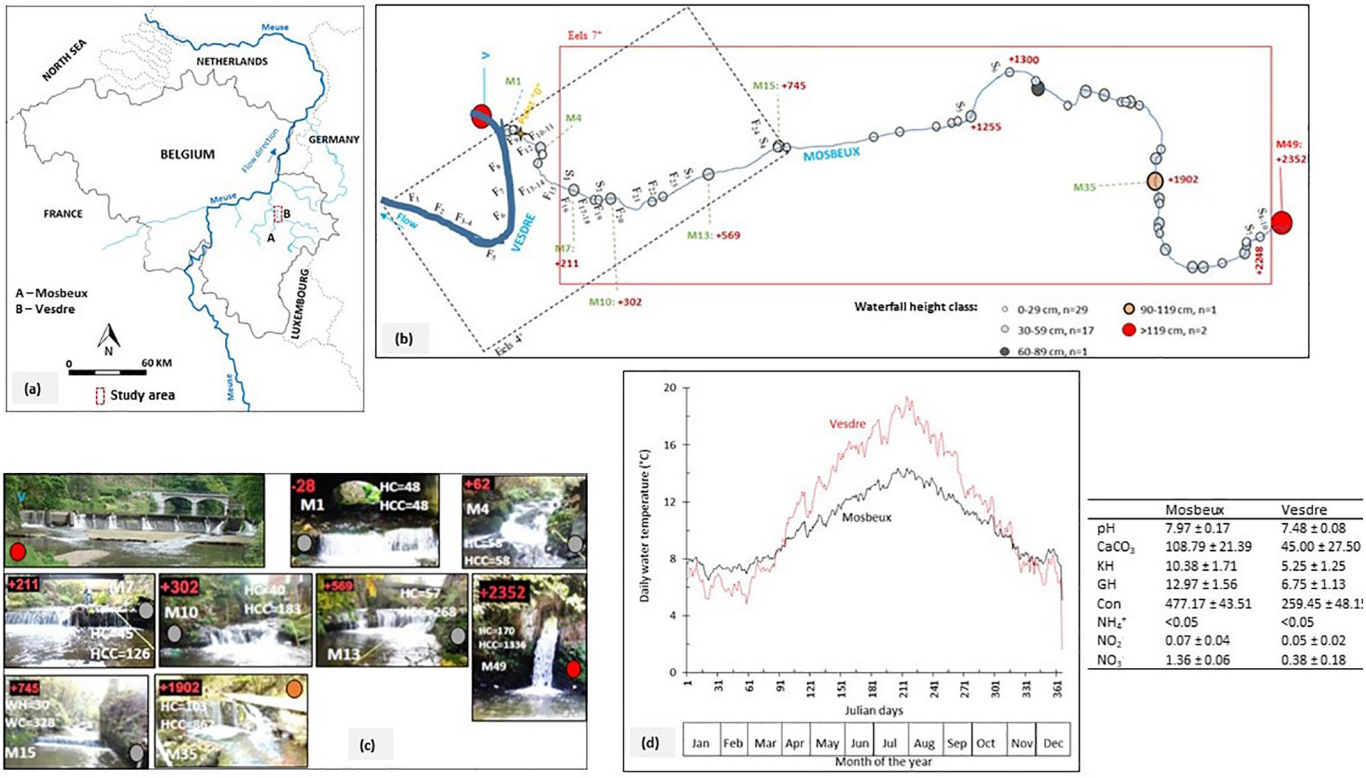
2. Material and methods

2.1. Study area

The study area is part of the Belgian Meuse River basin in southern Belgium, 359.3 km upstream from the Meuse River estuary in the Netherlands (Fig. 1). It includes a 2.380-km-long river stretch of the Mosbeux River from its mouth and a 0.584 km stretch of the Vesdre River downstream of the Mosbeux/Vesdre confluence. The Mosbeux River has a catchment area of 19.16 km^2, a length of 6.36 km, a mean width of 2.7 m and a mean depth of 15.2 cm. It is a typical brown trout fish zone and flows directly into the Vesdre River, which is a barbell fish zone (Huet, 1949). At the study site, the Vesdre River has a mean width of 14.9 m and a mean depth of 44.0 cm and is a direct tributary of the Ourthe River, which drains into the Meuse River. On 22 May 2013, the study area was subjected to restocking using glass eels (n = 4155, total length [TL] = 70 ± 4 mm, weight = 0.26 ± 0.07 g) released at a single point located in the Mosbeux River, 0.040 km upstream of its confluence (Ovidio et al., 2015; Nzau Matondo et al., 2019, 2020). These glass eels – identified as VI_{A1} to VI_{A2} pigment stages according to Elie et al. (1982) – were caught in a freshwater environment in the United Kingdom and imported via a commercial eel trade company (UK Glass Eels Ltd., Gloucester, UK). From 2013 to 2020, the water temperature in the two rivers was continuously recorded using TidbiT data loggers (version 2, Onset Computer Corporation, MA, USA). The water of the Vesdre (mean daily temperature = 11.5 °C and maximum = 23 °C recorded on 3–4 August 2018) is warmer than that of the Mosbeux (mean daily temperature = 10.2 °C and maximum = 16.4 °C recorded on 4–5 August 2018). Seasonal changes in temperature revealed a growing period (> 8 °C) from late March to the end of November in the Vesdre and from early March to late December in the Mosbeux (Fig. 1) (Sadler, 1979; Morin et al., 1999; Nzau Matondo et al., 2019, 2021). Several water physicochemical parameters (monthly measurements: 40 m upstream of the Mosbeux/Vesdre confluence in both rivers) were within the eels' normal tolerance range with regard to their vital requirements (Esteve and Garay, 1991; Angelidis et al., 2005). The parameters revealed that the Vesdre is a less alkaline river than the Mosbeux. The riverbed at the study site mainly consisted of large stones and blocks. The stretch monitored (2.964 km long) was highly fragmented by 49 small human-made obstacles with a cumulative waterfall height of 13.86 m (Nzau Matondo et al., 2019). The fish community was identified by electrofishing surveys performed in the study area before restocking and was found to contain 11 species, including abundant bullhead *Cottus rhenanus* and brown trout *Salmo trutta*. Old resident eels (TL, mm: mean = 666 and range = 515–910) were easily identified, tagged and monitored by telemetry. However, they were very few in number (density, number of eels per m^2: Mosbeux = 0.0010 and Vesdre = 0.0003) and were disappearing over time without being replaced by new, naturally recruited eels (Nzau Matondo et al., 2019). This was due to the shutdown of natural immigration of wild eels from the Meuse River, because glass eel recruitment in the North Sea has declined drastically (Nzau Matondo and Ovidio, 2016; Nzau Matondo and Ovidio, 2018; ICES, 2020).

2.2. Fish sampling

Since 2013, restocked eels have been caught each year using electrofishing (EFKO, 3.0 kVA FEG 5000, 150–300/300–600 V DC, in accordance with VDE 0686, IEC 60335–2-86, Leutkrich im Allgäu) and hand nets with a 40 × 40 cm diameter and a 2 × 2 mm mesh size following the technique used by Ovidio et al. (2015). From October 2013 to July 2020, seven electrofishing sessions were performed to study the growth performance of the restocked eels. Electrofishing sessions were conducted over a 160-m-long river stretch, including 120 m of the Mosbeux and 40 m of the Vesdre. In 2017, to examine growth in combination with sex determination, pollutant loads and pathogen infections, this monitored stretch was extended to 350 m downstream and 645 m upstream of the annually surveyed stretch in the Vesdre and the Mosbeux, respectively. In 2020, growth was examined in combination with sex determination and pathogen infections in an electrofished stretch extended to 350 m and 2250 m in the Vesdre and the Mosbeux, respectively. In 2017 and 2020, electrofishing sessions outside the annually



	Mosbeux	Vesdre
pH	7.97 ± 0.17	7.48 ± 0.08
$CaCO_3$	108.79 ± 21.39	45.00 ± 27.50
KH	10.38 ± 1.71	5.25 ± 1.25
GH	12.97 ± 1.56	6.75 ± 1.13
Con	477.17 ± 43.51	259.45 ± 48.1
NH_4^+	<0.05	<0.05
NO_2^-	0.07 ± 0.04	0.05 ± 0.02
NO_3^-	1.36 ± 0.06	0.38 ± 0.18

Fig. 1. Map showing the location of the study area in southern Belgium (a); map of the sampling sectors regarding sex identification and AngHV-1 detection in eels 4^+ (Sectors F_1–F_{24}: outlined by a dotted black rectangle) and eels 7^+ (S_1–S_{10}: delimited by a red rectangle) (b); photos of the major obstacles (c); and graph of daily mean temperature from 2013 to 2020 and mean and standard error values of monthly water physicochemical parameter measurements (d). The mean values of the waterfall height (HC) and the cumulative waterfall height (HCC) from point 0, the release point of glass eels in 2013 are given in centimetres. M (Mosbeux) or V (Vesdre) with a number refers to the river and the number of obstacles from point 0. A number preceded by - or + indicates the distance in metres downstream or upstream of the release point. Units: carbonate hardness (°KH); total hardness (°GH); conductivity (Con, µs.cm^{-1}); calcium carbonate ($CaCO_3$); and ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-), all in mg.L^{-1}.

surveyed stretch were conducted in productive eel habitats, which were generally located downstream of the obstacles.

Caught eels were anaesthetised with a 1:10 ratio of eugenol to alcohol (0.5 mL.L^{-1}), examined macroscopically for the presence of pathogens, measured in total length (± 1 mm) and weighed (W, ± 0.01 g). They were also scanned to identify the eels that had been tagged during previous electrofishing sessions. The untagged eels were fitted with small biocompatible radio frequency identification (RFID) tags (half duplex, 134.2 kHz, size/weight in air: 12 × 2 mm/0.095 g; Texas Instruments Inc., Dallas, TX, USA), which were inserted into a 2-mm-long incision in the eel's visceral cavity using a scalpel in the pre-anal position (Nzau Matondo et al., 2019, 2020). The inserted tags weighed on average 1.73% (Q50 = 0.89%; Q95 = 5.94%; range = 0.01–10.56%) of the eel's body mass. Once fully recovered, the tagged eels were released into the river at their point of capture. No mortality was observed during tagging.

In 2017 and 2020, restocked eels caught in the environment were sampled and introduced alive at the laboratory of Immunology-Vaccinology (Fundamental and Applied Research for Animal and Health, University of Liège), where they were euthanised by an overdose of benzocaine (10 mL.L^{-1}) for sex determination, pollutant load assessment and pathogen infection examination. In total, 24 river stretches (mean ± standard error [SE]: stretch = 16.7 ± 2.5 m long; distance between stretches = 31.5 ± 31.7 m) were electrofished in 2017 against 10 stretches (stretch = 15.7 ± 2.5 m; distance between stretches = 208.8 ± 187.7 m) in 2020. In each stretch, the number of caught eels (N) was counted and the stretch length (L) and width (l) were measured to evaluate the density of eels (D) at fishing using the following formula: D [ind.m^{-2}] = N [ind.]/(L [m] × l [m]). During these two years, additional morphometrical measurements were made. Pectoral fin length (PFL, ± 1 mm) and mean eye diameter (ED, ± 1 mm) were measured to estimate the stage of eels as a function of the TL, PFL and ED according to Durif et al. (2005) and Edeline et al. (2009). In combination with the degree of eel silvering, Fulton's condition factor (K) was also calculated using the following formula: K = 100 × [(W [g]/(TL [cm])^3] (Fulton, 1904; Simon, 2007). In 2020, the annual growth rate (G) was assessed for the restocked eels aged 7^+ as a function of the TL at their capture, the TL of glass eels at release (TL_0) and the age after restocking (T) using the following equation: G [mm. year^{-1}] = (TL [mm] – TL_0 [mm])/T$^-$ [year]) (Kumai et al., 2020).

To assess demographic parameters of eels from 0^+ to 7^+ age, the *Jolly-Seber* method based on multiple time-spaced capture-mark-recapture sessions was used the annually surveyed stretch using the Program MARK 8.0 POPAN module (Schwarz and Arnason, 1996; White and Burnham, 1999; Pledger et al., 2010). The selected model of {p(.), ϕ{.}, pent{t}, N(.)} had capture probability p(.) and survival ϕ{.} being constant over time and arrival probability pent{t} varying with time or age of eels (Nzau Matondo et al., 2020). It was the most parsimonious model based on the quasi-likelihood Akaike information criterion (QAICc) and corresponded to the species' biology and the survey design as a same sampling site was electrofished over an eight-year period.

2.3. Phenotypic sex determination

To determine the phenotypic sex, subjects were euthanised and dissected. Gonads were located after dissection using a dissecting microscope. In 2017, histological sections of gonadal tissue were removed, stored in liquid nitrogen, frozen, packaged in a dry ice pack and shipped to Behavioural Ecology and Fish Population Biology (ECOBIOP, French National Institute for Agriculture, Food and Environment [INRAE], Saint-Pée-sur-Nivelle, France) for sex determination. These sections were

fixed in Bouin's solution for 21 h, dehydrated in solutions with increasing alcohol content, embedded in paraffin, sectioned at 4 μm thick, stained with Mayer's haematoxylin–eosin and observed under a microscope using 50–100× magnification (Montchowui et al., 2012; Cho et al., 2014).

In 2020, a portion of gonadal tissue was placed on a glass slide, a drop of aceto-carmine was added, the tissue was squashed with a cover slip and the mount was observed under a microscope using 20–100× magnification following the technique used by Guerrero and Shelton (1974). Aceto-carmine solution was prepared by adding 0.5 g of carmine to 100 mL of 45% acetic acid and then boiled for 2–4 min. This aceto-carmine squash method for sexing restocked eels was performed at the laboratory of Immunology-Vaccinology.

2.4. Pathogen detection

Pathogen detection of sampled individual eels was performed immediately after euthanasia in a lethal bath of benzocaine (1%). Skin and fins were examined for external lesions. Following dissection, internal organs were examined for the presence of lesions and macroscopic parasites. Notably, the swim bladder was carefully examined to detect the nematode *A. crassus*.

DNA was extracted from tissues of fish gills and hearts (in 2017) and gills and brains (in 2020) using a QIAamp DNA Mini Kit (Qiagen). In 2017, AngHV-1 polymerase chain reaction (PCR) diagnosis was performed on DNA extracted from gills and hearts using AngHV-1 specific primers (HVAPOLVPSD 5′-GTGTCGGGCCTTTGTGGTGA-3′; HVAPOLOOSN 5′-CATGCCGGGAGTCTTTTTGAT-3′) as described previously (Rijsewijk et al., 2005) and by using the following thermal cycle: 95 °C for 30 s; 35 cycles of 95 °C for 30 s, 54 °C for 30 s and 68 °C for 30 s; and a final extension at 65 °C for 5 min. The PCR products were then submitted to electrophoresis (2% agarose gel). A positive result was denoted by a band of 394 base pairs (bp). In 2020, virus genome copies in tissues were quantified by TaqMan real-time polymerase chain reaction (qPCR) targeting AngHV-1 ORF57, as described by van Beurden et al. (2016). In addition, a second PCR was performed targeting a sequence of the eel 18S ribosomal RNA (rRNA) gene (Sunarto et al., 2014), a cellular gene known to be highly conserved (Frankowski and Bastrop, 2010). This second TaqMan assay served as a genomic DNA extraction control for quantifying the number of cellular genomes present in the samples. For each target sequence, two primers and an internal fluorescently labelled TaqMan probe (Table 1) were ordered from Eurogentec (Seraing, Belgium). The amplicons were cloned into the pGEM-T Easy Vector and the resulting plasmids were used to generate standard curves by running reactions with 10^1 to 10^9 plasmid molecules. DNA was isolated using a QIAamp DNA Mini Kit (Qiagen) from ~25 mg of organs stored at −80 °C in 80 μL of phosphate-buffered saline (PBS). Before qPCR, DNA samples were diluted to reach an average concentration of 50 ng.μL^{-1}. Each reaction was set up in triplicates. The reaction mix contained 1× iQ Supermix (Bio-Rad, Hercules, CA, USA), 200 nM of each primer, 400 nM of fluorescent probe and 250 ng of DNA. The TaqMan qPCR programme used to detect AngHV-1 ORF57 started with 10 min at 37 °C and then 10 min at 95 °C, followed by 40 cycles of 3 s at 95 °C and 30 s at 60 °C. The 18S rRNA gene was detected using the following profile: 1 cycle at 48 °C for 30 min and then 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The analyses were performed using a C1000 Touch Thermal cycler (CFX96 Touch Real-Time PCR Detection System, Bio-Rad). The viral load in positive samples corresponded to the number of viral genome copies expressed per 10^9 eel 18S gene copies.

Table 1
Primers and probes used for real-time PCR assays.

Gene amplified	Primer name	5′ – 3′ Sequence
AngHV-1 ORF57	AngHV1.CapProt. F06	TGCTCTTGGAGTCGGTTGATG
	AngHV1.CapProt. R06	CCGTGTGGGAAAAGACTATTTGA
	AngHV1.CapProt. p06	(6FAM) TCTGAAAACCCGCTCGCCCTGA (BHQ1)
Eel 18S rRNA	18S rRNA F	CGGCTACCACATCCAAGGAA
	18S rRNA R	GCTGGAATTACCGCGGCT
	18S rRNA P	(6FAM) TGCTGGCACCAGACTTGCCCTC (BHQ1)

2.5. Organic pollutant loads

In 2020, no tissue was collected for organic pollutant load assessment. In 2017, the muscles and livers of the individual eels euthanised at the laboratory of Immunology-Vaccinology were collected and then transported to the laboratory of Animal Ecology and Ecotoxicology (Freshwater and Oceanic Science Unit of Research [FOCUS], University of Liège) to assess the lipid content and organic pollutant loads. Muscle and liver tissues were freeze-dried (Cryotec cosmos; Saint-Gély-du-Fesc, France). Approximately 200 mg (wet weight) of tissue was spiked with 50 μL of a hexane solution (100 pg.μL^{-1}) of PCB 112 (Dr Ehrenstorfer GmgH, Augsburg, Germany) as an internal standard (surrogate) to determine the recovery efficiency. The freeze-dried samples were submitted to accelerated solvent extraction (ASE; Dionex 200; Thermo Fisher Scientific, Waltham, MA, USA) using a mixture of hexane:dichloromethane (90:10; V:V) at 125 °C and 1.304×10^7 Pa. The lipid content was determined gravimetrically after solvent evaporation with a TurboVap LV evaporator (Zymark, Charlotte, NC). Lipids were then dissolved in 4 mL of hexane. These extracts were cleaned up using H_2SO_4 98% followed by Florisil solid-phase purification (Supelco; Envi-Florisil, Bellefonte, PA, USA), as described by Damseaux et al. (2017). Nonane (5 μL) was added to the purified extracts as a keeper. Each extract was evaporated under a gentle stream of nitrogen until only the keeper remained in the vial. The final extract was reconstituted with 45 μL of *n*-hexane and 50 μl of Mirex (100 pg.μL^{-1} in hexane) as the injection volume internal standard (Dr Erhenstorfer GmbH). Neither Mirex nor PCB112 was detected in the samples during the pre-test analysis, allowing their use as standards. Finally, the extracts were analysed by high-resolution gas chromatography (Trace 2000 Gas Chromatograph, ThermoQuest, Milan, Italy) equipped with a 63Ni electron capture detector (ECD) and on-column injector to prevent *p,p*'-DDT from being degraded in split/splitless injectors (Muir and Sverko, 2006). Targeted contaminant (polychlorinated biphenyls [PCBs], organochlorine pesticides [OCPs] and polybrominated diphenyl ethers [PBDEs]) indicators were analysed on a 60 m × 0.25 mm (0.25 mm film) DB-5 ms capillary column (J&W Scientific, now Agilent). Other analytical parameters have been described by Debier et al. (2003). Quantification was performed using internal standards. A calibration curve (1.5–400 pg.μL^{-1}) was established for each compound of interest. The limit of detection (LOD) and the measured limit of quantification (LOQ) determined with PCB-spiked cod were 0.02 ng.g^{-1} wet weight (ww) and 0.06 n.g^{-1} ww, respectively.

The collected tissues were analysed for PCB congeners (28, 52, 101, 118, 138, 153 and 180) and OCP indicators (α-HCH, β-HCH, γ-HCH, HCB, *p,p*'-DDE, *p,p*'-DDD, *p,p*'-DDT and *o,p*'-DDT) as well as for PBDEs (47 and 99). The ΣPCB included the seven indicator PCBs 28, 52, 101, 118, 138, 153 and 180 as suggested by the ICES, which are recognised by the wider scientific community as a representative index of PCB contamination (Macgregor et al., 2018). For comparison of PCB levels with some environmental norms, conversion of PCB concentrations to Toxic Equivalent Concentrations (TEQ) was achieved by multiplying each congener concentration by the corresponding Toxic Equivalency Factor for fish (TEF) given by Van den Berg et al. (2006). The PCBs have been used as isolation fluids in converters, as plasticisers, as paint additives and in oils and appear as combustion by-products. The ΣDDT included the two isomers *p,p*'-DDT and *o,p*'-DDT as well as the metabolites *p,p*'-DDE and *p,p*'-DDD (Maes et al., 2008). All contaminants analysed are persistent organic pollutants regulated by international agreements to reduce or eliminate their use and release into the environment.

2.6. Statistical analysis

Pearson's chi-squared (χ^2) test was performed to test differences in abundance between sexes in the eels 4^+ and the eels 7^+, within the small eel group, the low-density eel group and the high-density eel group. This test was also used to identify differences in abundance between individual eels testing positive or negative for AngHV-1. The relative abundances between the observed six silvering stages as well as between the three TL classes or the three density classes of virus-positive and virus-negative individual eels 7^+ were compared using Fisher's exact test. To evaluate the effect of eels' longitudinal dispersal in the river based on their density, a regression linear model was used, which included density as a response variable and distance from the glass eel release point as a predictor variable.

Owing to the small sample sizes, we used non-parametric statistical methods. The gamma rank correlation coefficient (r_G) was used to separately assess the potential relationships between the viral load and eels' TL, K, density at fishing and silvering stage. We applied the same method to evaluate the relationship between the organic pollutant load and eels' TL, K, density at fishing and lipid content. We used the non-parametric Kruskal–Wallis (KW) test and the post hoc Dunn's (DN) test with the Bonferroni correction for multiple pairwise comparisons of mean rank sums to compare the TL and K between eels' silvering stages as well as the pollutant load between PCB indicators and between OCPs and PBDEs. The one-sided Wilcoxon (W) signed-rank test for paired samples with normal continuity correction was applied to compare the life history traits of eels 4^+ between the two rivers. All statistical analyses were performed using R statistical software version 3.3.2 packages Rcmdr 2.3.-2, Hmisc and dunn.test (R Development Core Team, 2016; Fox, 2017; Harrell, 2018). The results were described as statistically significant when the estimated probability of error (p) was <0.05.

3. Results

3.1. Life history traits

The mean TL of restocked eels significantly increased over time (Pearson's correlation, $r = 0.968$ and $p = 3.348 \times 10^{-4}$), with an annual growth rate estimate of 61 mm.year^{-1} (range = 25–99 mm.year^{-1}) over the eight-year span following their release as glass eels in 2013 (Fig. 2). At age 4^+, these eels were 99% ($n = 96$) at the FI silvering stage against 1% ($n = 1$) at FII. Eels 4^+ were caught at a mean density of 0.049 ind.m^{-2} (range = 0.004–0.099 ind.m^{-2}) and had a mean TL of 268 mm (K, range = 124–586 mm), with a mean K of 0.166 (range = 0.123–0.266).

Eels 7^+ were caught at a mean density of 0.060 ind.m^{-2} (range = 0.005–0.173 ind.m^{-2}) and had a mean TL of 495 mm (range = 243–760 mm), with a K of 0.197 (range = 0.085–0.259). The relationship between their length (TL) and weight (W) was described by log W (g) = $-3.005 + 3.174 \times$ log TL (cm), $R^2 = 0.981$ and $p < 0.0001$. These eels presented six silvering stages, with more than half being in the migratory stages (54%, $n = 33$) (Fig. 3a). Of the six eel stages observed, the most abundant was FV (Fisher's exact test: $p = 0.04969$ to $<2.2 \times 10^{-16}$), which is the last migratory stage before eels migrate towards the sea. The FV silvering stage had a significantly higher TL and K than FI, a growth stage (KW test: df = 5, $\chi^2 = 31.256$–62.149, $p = 8.348 \times 10^{-6}$ to 1.726×10^{-8}; DN test: $t = 3.472$–7.767, $p = 0.0351$ to <0.0001) (Fig. 3b and c). However, eels 7^+ belonging to FV showed a significantly lower density at fishing than those of FI and FII (KW test: df = 5, $\chi^2 = 39.080$, $p = 2.282 \times 10^{-7}$; DN test: $t = 3.586$–4.064, $p = 0.021$–3.140×10^{-3}) (Fig. 3d).

Using the best model of the *Jolly-Seber* method according to QAICc, the results of demographic parameters on the reference stretch revealed that the survival probability $\phi(.)$ on monthly basis was estimated at 0.91 (± standard error = 0.01, 95% confidence interval (CI) limits = 0.88–0.93). The capture probability $p(.)$ was estimated at 0.30 (± 0.07) with 95% CI of 0.18–0.46. The observed mean arrival probability *pent*{t} was 0.095 (± 0.07). The overall population (N) was all eel individuals that occupied the reference site during the entire study period was estimated at 665 (± 124) individuals with 95% CI of 485–990. The superpopulation (*N*-hat*) defined as all eel individuals who occasionally used the reference stretch and they were present on the site between the sampling sessions but they left it before they were counted and were estimated at 864 (± 135) individuals with 95% CI of 637–1172.

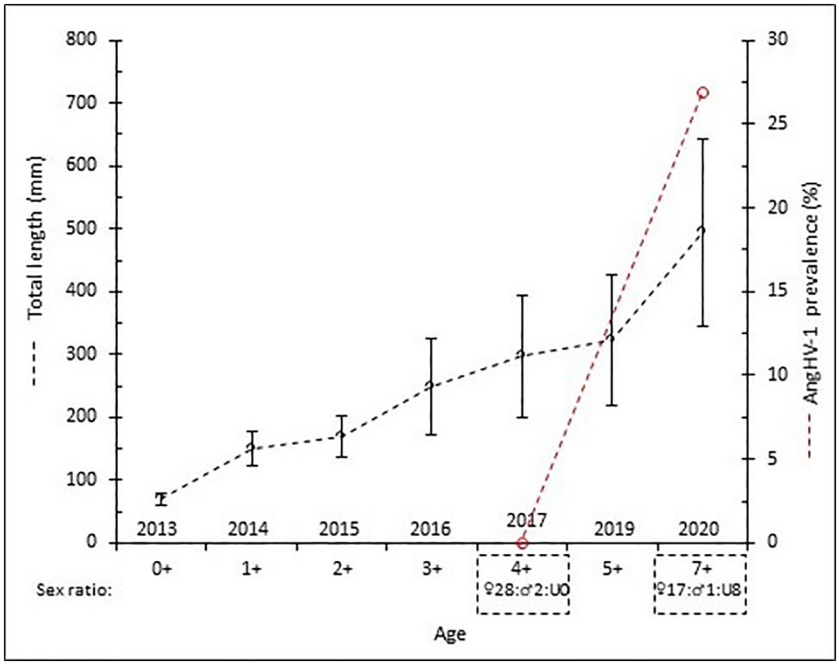


Fig. 2. Total length, virus prevalence and sex ratio evolution in restocked eels (a). Mean and standard error values. Sexes are: ♀, female; ♂, male; and U, undetermined. Sample sizes (n) are: 0^+, $n = 83$; 1^+, $n = 38$; 2^+, $n = 87$; 3^+, $n = 85$; 4^+, $n = 97$; 5^+, $n = 11$; 7^+, $n = 63$.

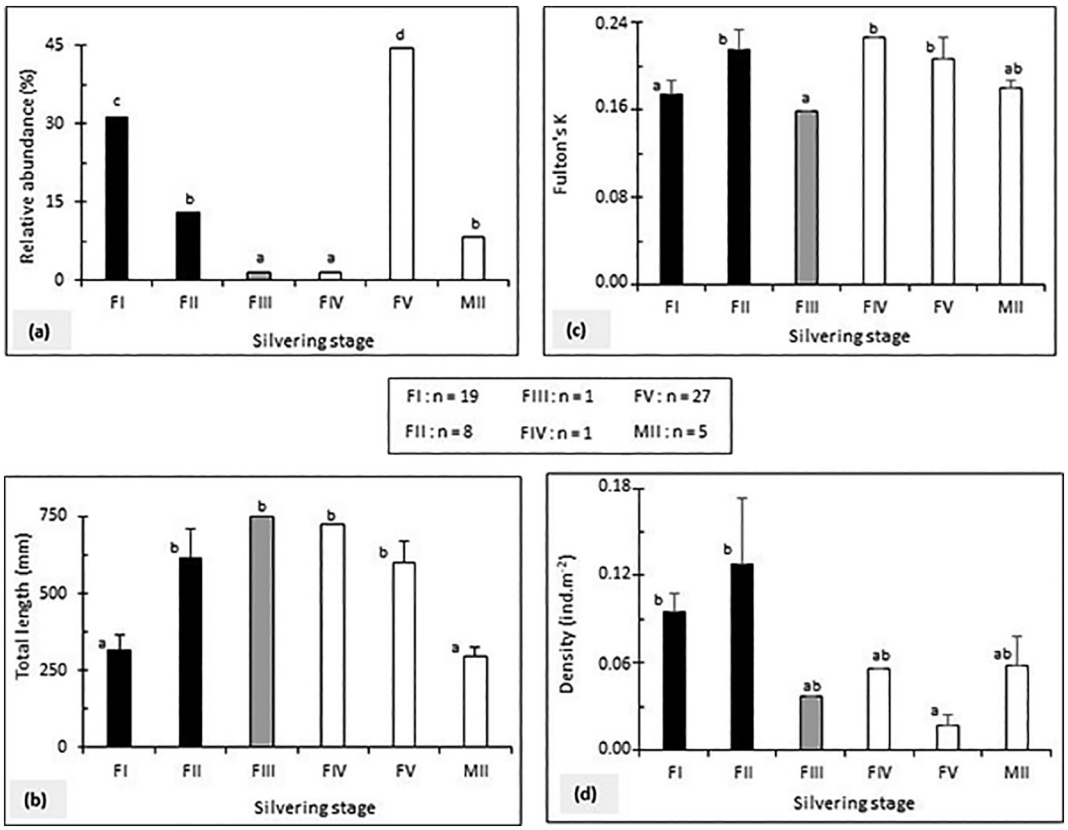


Fig. 3. Relative abundance (a), total length (b), Fulton's condition factor (c) and density at fishing (d) according to the silvering stage of the eels 7^+. The data represent the mean and standard error values. n is sample size. Silvering stages with the same letter are not significantly different (KW and DN tests: $p < 0.05$).

3.2. Sex identification

Histological examinations of the gonads and the aceto-carmine gonadal squashes were used to determine the sex of restocked eels $4^+$and eels 7^+, respectively. They revealed the presence of 100% (n = 16) females with regard to large eels (TL > 399 mm) (Fig. 4a). Small eels (< 300 mm) were successfully sexed via histological examinations of their gonads, showing 91% (n = 10) female and 9% (n = 1) male individuals 4^+ (χ^2 test: df = 1, χ^2 = 14.727, $p = 1.242 \times 10^{-4}$). These examinations also showed germ cells at different stages of gametogenesis, spermatogonia cysts in the testes and previtellogenic primary growth oocytes with a nucleus and a cytoplasm in the ovaries (Fig. 5a–f). Using the gonadal squash method, only 37.5% (n = 3) of the small eels were successfully identified as females 7^+, while most (62.5%; n = 5) were undetermined. The squash method revealed stained gonadal tissues showing oocytes (Fig. 5e). The gonads had an elongated

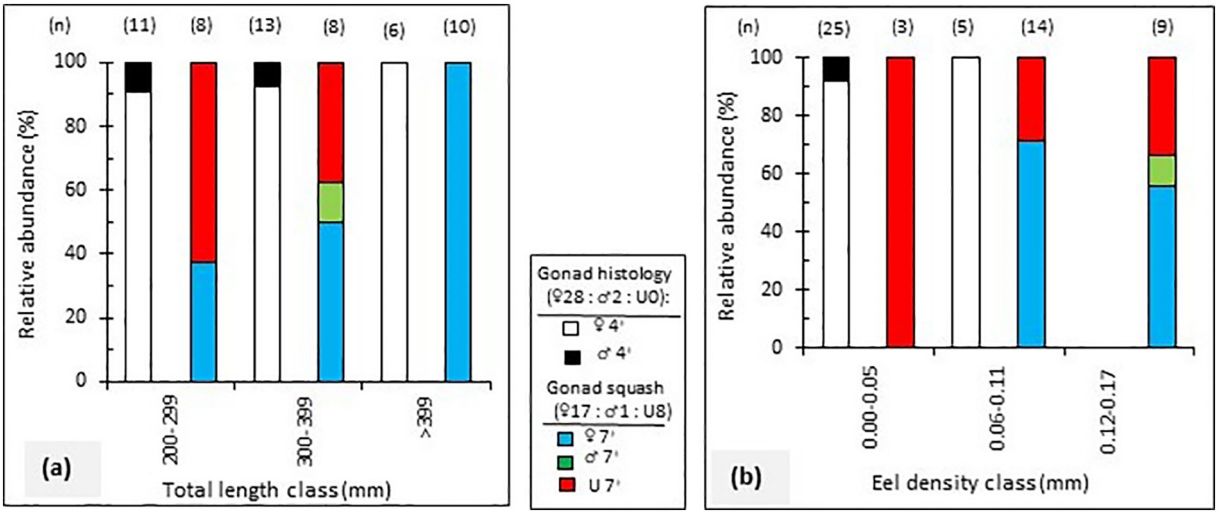


Fig. 4. Sex ratio abundance of restocked eels 4^+ and eels 7^+ according to the total length (a) and the density at fishing (b). Sexes are: ♀, female; ♂, male; and U, undetermined. (n) is sample size.

paired structure attached dorsally to the body wall (Fig. 5f). In these two sexing methods, male individuals were scarce, representing only 5% (n = 3) of the 56 sampled eels (χ^2 test: df = 1, χ^2 = 95.22, $p < 2.2 \times 10^{-16}$).

The linear regression model showed that the density of eels at fishing was not significantly associated with the distance from the glass eel release point for eels 4^+ (Vesdre/Mosbeux: R^2 = 4%/0.05%, n = 8/16, p = 0.639/0.930) and for eels 7^+ (Mosbeux: R^2 = 4%, n = 10, p = 0.113). At low density (< 0.06 ind.m^{-2}), eels 4^+ were 92% (n = 25) females, a level of abundance that was significantly higher than males (8%, n = 2), while eels 7^+ were all sexually undetermined individuals (100%, n = 3) (Fig. 4b). At a high density (≥0.06 ind.m^{-2}), eels 4^+ were all females (100%, n = 5), while eels 7^+ were 65.2% (n = 15) females, 4.4% (n = 1) male and 30.4% (n = 7) sexually undetermined individuals. The abundance level of female eels 7^+ was significantly higher than that of males (χ^2 test: df = 1, χ^2 = 7.58, $p = 5.919 \times 10^{-3}$).

3.3. Pathogen detection

A necropsy revealed the absence of internal lesions. Importantly, examination of the swim bladder revealed the absence of the parasite *A. crassus*. In 2017, the conventional PCR analysis performed on the gills and heart samples revealed the absence of AngHV-1 genome copies in sampled DNA belonging to individual eels aged 4^+ (TL: mean = 295 mm, range = 156–586 mm, n = 48). At age 7^+ (TL: mean = 404 mm, range = 243–749 mm, n = 26) in 2020, the qPCR analyses revealed that 26.9% (n = 7) (χ^2 test: df = 1, χ^2 = 11.08, $p = 8.741 \times 10^{-4}$) of the subjects were infected by AngHV-1. Infected

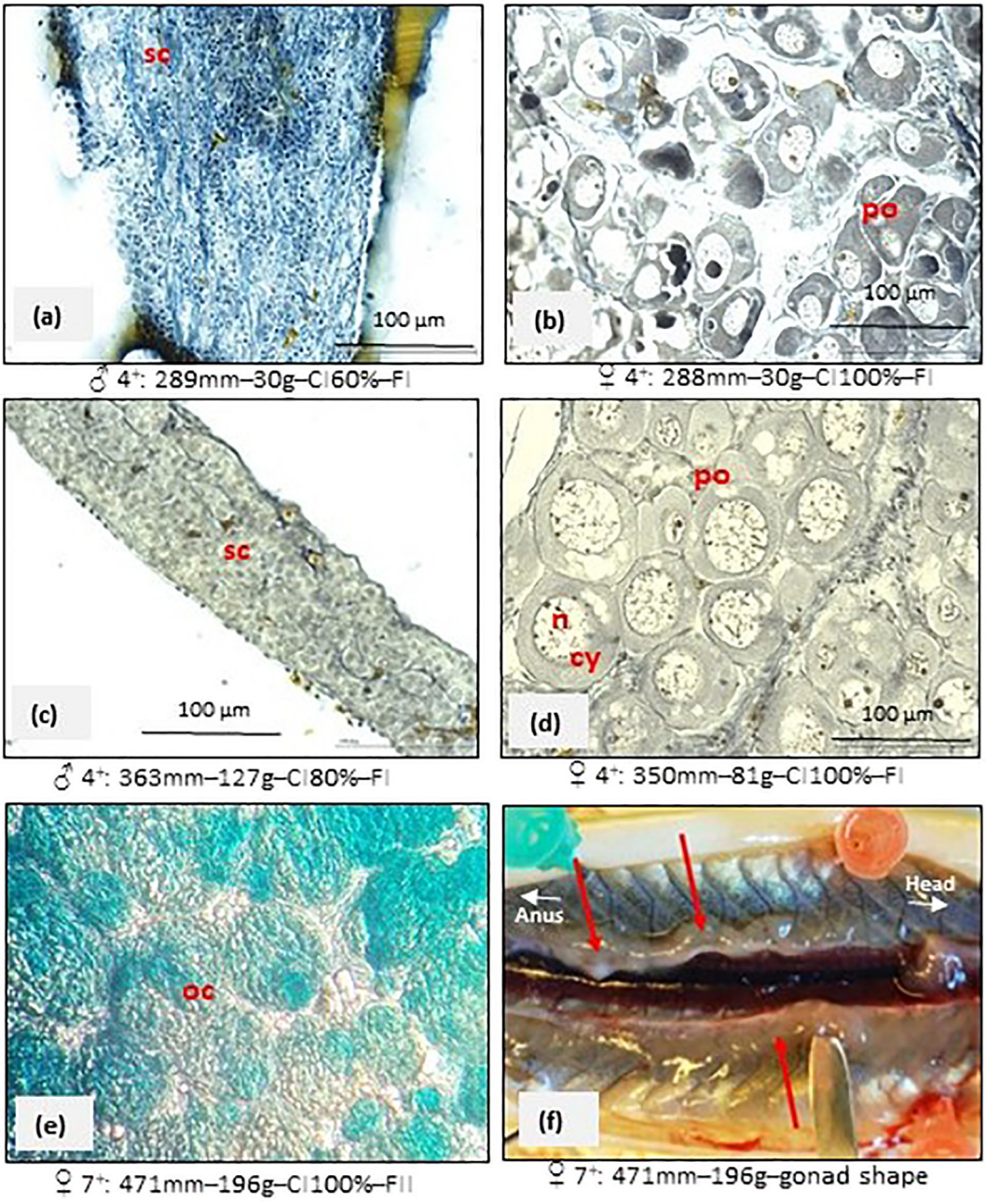


Fig. 5. Histological sections of testes showing spermatogonia cysts (a and c), histological sections of ovaries showing previtellogenic oocytes with nucleus and cytoplasm (b and d), stained ovary from squash method showing oocytes (e, magnification x 10) and macroscopic observation of ovarian shape (f, red arrow)0.280 mm–30 g–CI60%–FI mean TL–weight–confidence interval for sexing-silvering stage of eels. Sexes are: ♀, female; ♂, male; and U, undetermined. po, previtellogenic oocytes; oc, oocytes; sc, spermatogonia cysts; n, nucleus; and cy, cytoplasm.

DNA samples appeared in all TL classes of eels, but their occurrences were low and limited to a maximum of 1–3 virus-positive individual eels per class (Fig. 6a). By contrast, negative results were observed in individual eels 7^+ living at low eel density < 0.06 ind.m^{-2}. At high eel density ≥ 0.06 ind.m^{-2}, a significantly higher prevalence of virus-positive individual eels was observed at an eel density of 0.06–0.11 ind.m^{-2} (abundance = 64.5%, $n = 9$) than at 0.12–0.17 ind.m^{-2} (22.2%, $n = 2$) (Fisher's exact test, $p = 2.670 \times 10^{-9}$) (Fig. 6b). The DNA samples of 22.2% (silvering stage FI, n = 4), 33.3% (FII, n = 2) and 100% (FIV, $n = 1$) of individual eels 7^+ were tested positive for AngHV-1 (Fig. 6c). Viral genome detection was higher in the brain (virus prevalence = 26.9%, n = 7) than in the gills (virus prevalence = 7.7%, n = 2) (Fig. 6d). The TL, K and density at collection did not differ significantly between the virus-positive (mean ± SE: TL = 440 ± 162 mm, K = 0.191 ± 0.033, density = 0.117 ± 0.056 ind.m^{-2}) and the virus-negative (TL = 391 ± 157 mm, K = 0.182 ± 0.015, density = 0.090 ± 0.043 ind.m^{-2}) individual eels (W test: df = 1, V = 7–9, p = 0.1359–0.2234) (Fig. 7a, b and c).

Of the individual eels 7^+ used for the AngHV-1 prevalence investigation, females dominated, accounting for 85.7% ($n = 6$), of which 42.9% ($n = 3$) belonged to stage FI and 28.6% (n = 2) belonged to FII, the growth phases of eels, while 14.3% ($n = 1$) belonged to stage FIV, the migratory phase. The individual eel 7^+ (14.3%, n = 1) tested positive for AngHV-1 was sexually undetermined at FI. The viral load in positive samples was presented as the number of viral genome copies expressed per 10^2 eel 18S gene copies. In the brain, the viral load was positively correlated with K (gamma rank correlation: $r_G = 0.429$, $p = 9.344 \times 10^{-3}$) (Fig. 8a), but it did not significantly correlate with density ($r_G = 0.412$, $p = 0.243$), TL ($r_G = 0.429$, $p = 0.150$) and silvering stage ($r_G = 0.429$, $p = 0.255$). Two individual eels were virus-positive in both the brain and the gills (Fig. 8). However, the observed viral loads in both the brain and the gills were low and consistent with a latent AngHV-1 infection (Kullmann et al., 2017).

3.4. Organic pollutant bioaccumulation

These analyses took place only in 2017 and concerned 4^+-year-old fish. The 10 eels sampled for this analysis showed average values of 329 ± 44 mm TL, 63 ± 24 g weight, 8.3 ± 3.5% lipid content in the muscles, 3.8 ± 1.0% lipid content in the liver, 0.167 ± 0.026 K and 0.04 ± 0.03 ind.m^{-2} density at fishing (Supplementary Table 1). These eels belonged to stage FI and included 70% ($n = 7$) females identified by ovarian histological sections and 30% (n = 3) that were not sexed. They were all contaminated by at least one of the organic pollutants studied, although the contamination prevalence and load varied among the pollutant congeners (Fig. 9).

The contamination prevalence ranged in terms of PCB indicators from 10% ($n = 1$) for PCB 101 to 100% (n = 7) for PCB 118 in the liver; and from 0% ($n = 0$) for PCBs 28 and 52 to 100% ($n = 9$) for PCB 118 in the muscles (Fig. 9a). The PCBs 118, 138, 153 and 180 were most prevalent, reaching 90%–100% of the eels tested, whereas PCB 101 was detected in no more than 10% of the eels. There was variability with respect to OCPs, ranging from 10% (n = 1) for HCB and β-HCH to 90% (n = 9) for *o,p*'-DDE in the liver and 0% ($n = 0$) for β-HCH and γ-HCH to 100% (n = 9) for *p,p*'-DDE and *p,p*'-DDT in the muscles (Fig. 9b). The two isomers *p,p*'-DDT and *o,p*'-DDT and their metabolites *p,p*'-DDE and *p,p*'-DDD were frequently detected in up to 80%–100% of eels, while β-HCH reached a maximum prevalence of only 10% of eels. The contamination prevalence to PBDEs fluctuated between 10% (n = 1) for PBDE 99 and 100% ($n = 10$) for PBDE 47 in the liver and

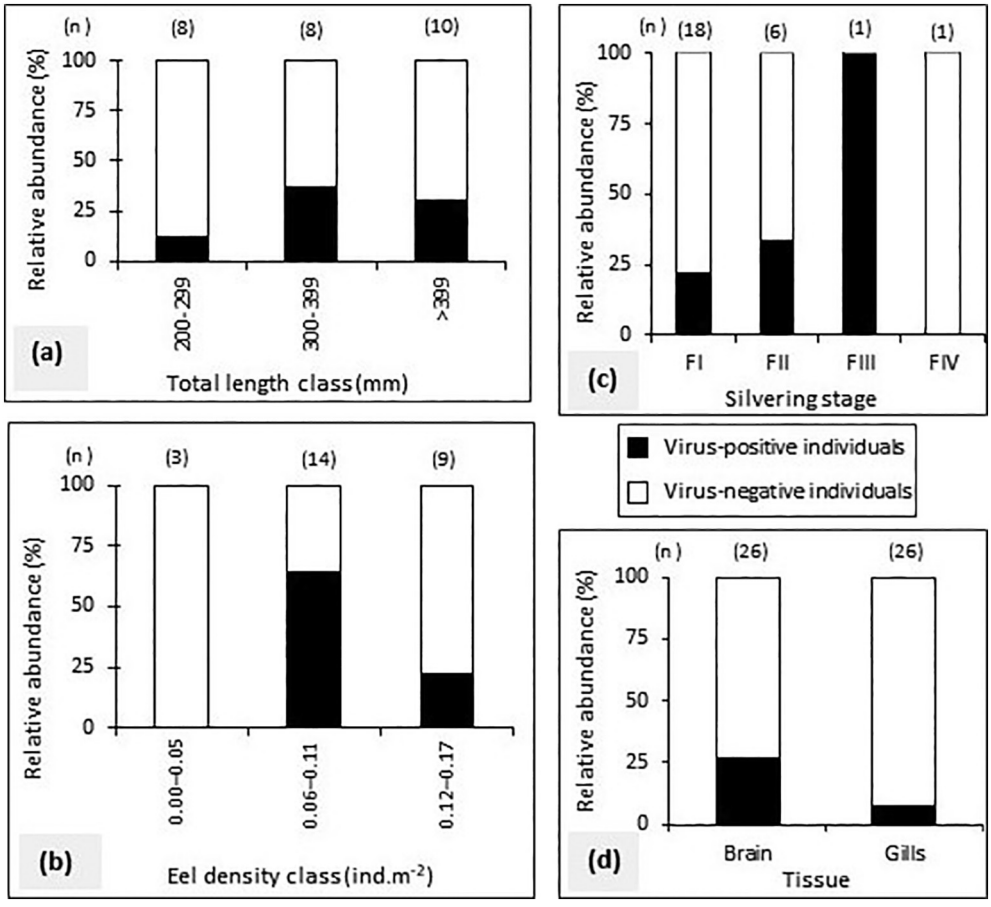


Fig. 6. Proportion of virus-positive and virus-negative individual eels 7^+ according to the total length class (a), the eel density class (b), the silvering stage (c) and the sampled tissues (d). (n) is sample size.

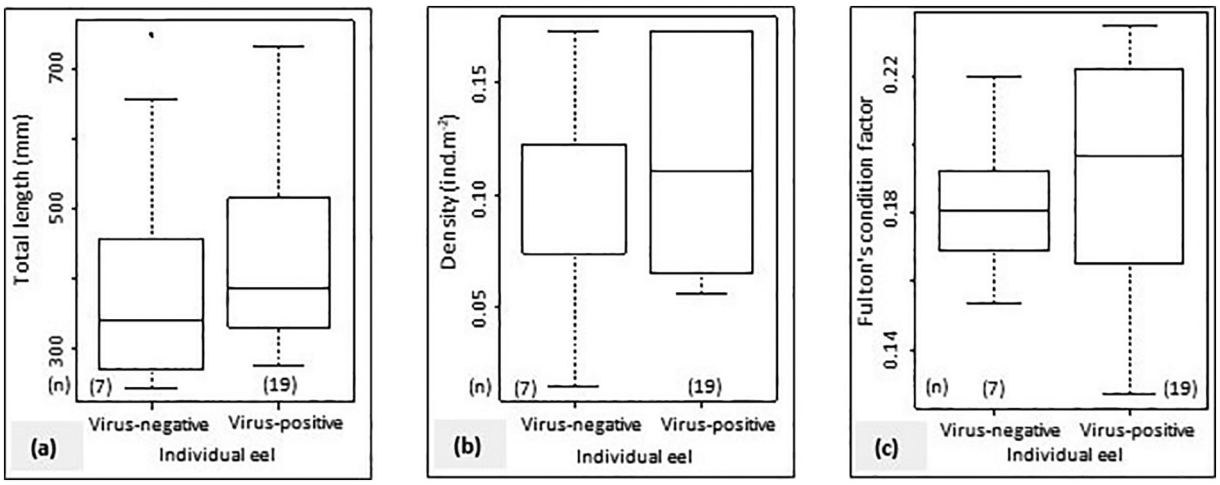


Fig. 7. Boxplots of total length (a), density (b) and Fulton's condition factor of virus-positive and virus-negative individual eels 7^{+}. Boxplots are presented with median values; the hinges indicate the first and third quartiles and circles indicate outliers. Individual eel groups did not differ significantly (W test: $p > 0.05$). (n) is the sample size.

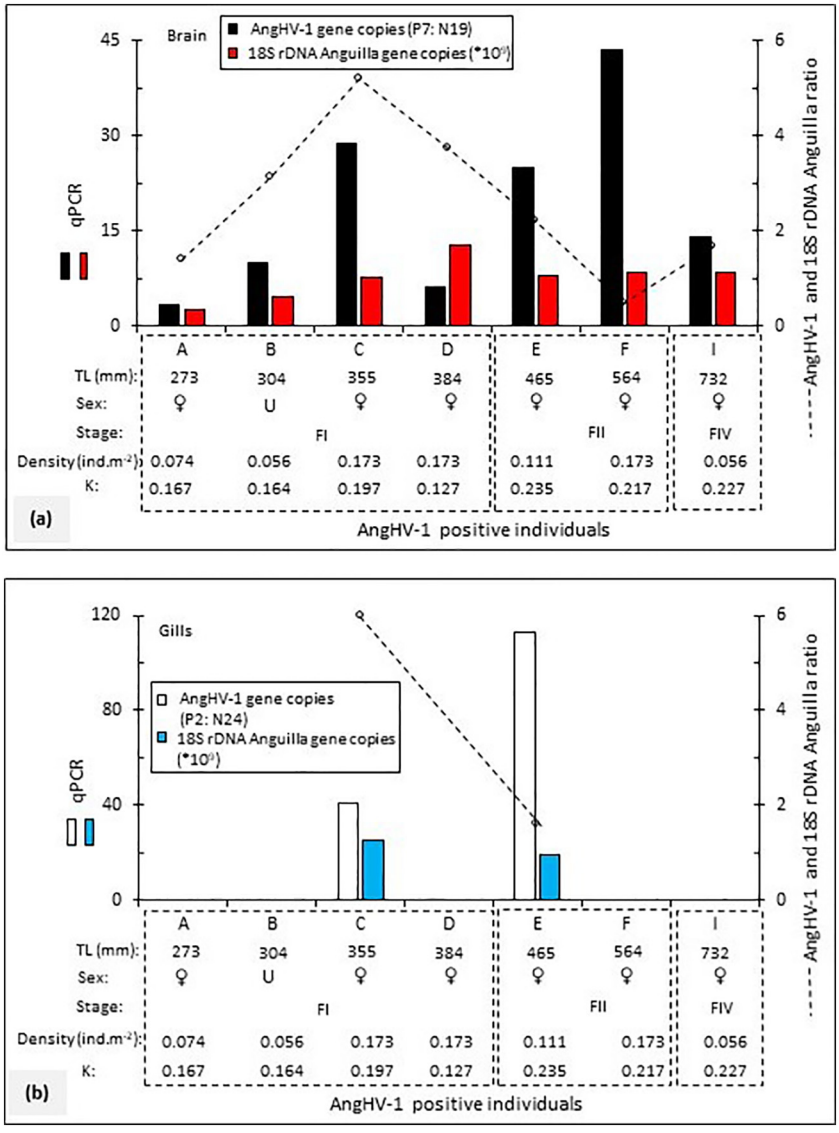


Fig. 8. The AngHV-1 and 18S rDNA Anguilla genome copies and their ratios in virus-positive individual eels 7^{+} according to the brain (a) and gills (b). Sexes are: ♀, female; ♂: male; and U, undetermined. Silvering stages of eels are: FI, FII and FIV. (P7:N19) indicates 7 virus-positive (P) and 19 virus-negative (N) individual eels 7^{+}.

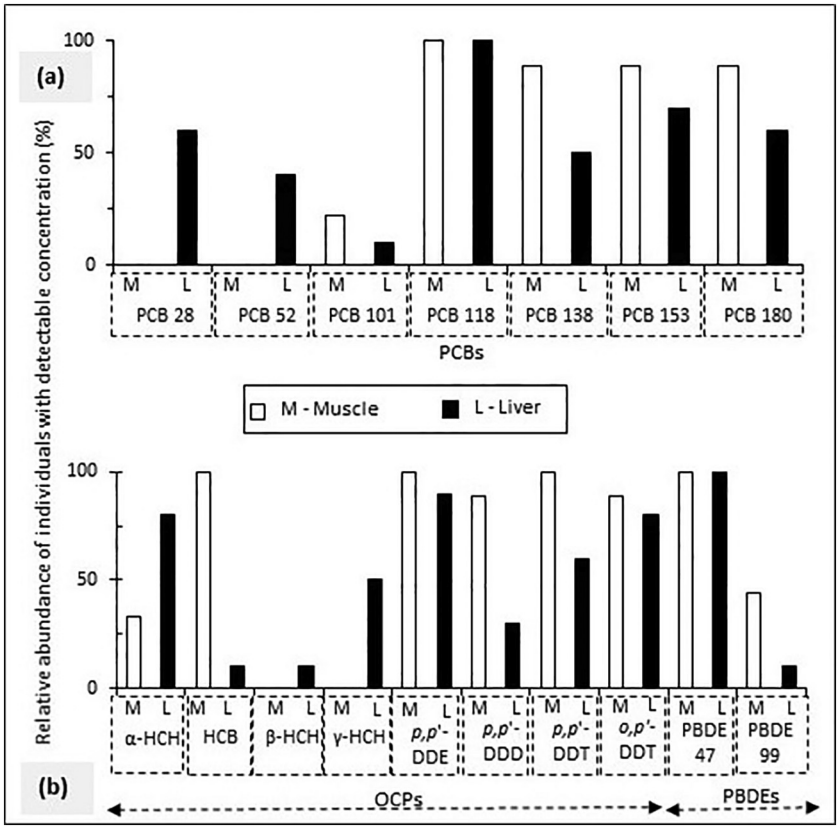


Fig. 9. Relative abundances of individual eels 4^+ with detectable PCBs (a) and OCPs and PBDEs (b) in the muscle (M) and liver (L). In the two rivers pooled, $n = 9$ and $n = 10$ muscle and liver samples, respectively. For PCB 118, $n = 7$ in muscle.

between 44% ($n = 4$) for PBDE 99 and 100% (n = 9) for PBDE 47 in the muscles, hence PBDE 47 was present in all samples. The pollution loads of eels also varied significantly among PCB congeners (Fig. 10a, KW test, range: df = 13, $\chi^2 = 63.515$, $p = 1.225 \times 10^{-8}$) as well as among OCP and PBDE indicators (Fig. 10b, KW test, range: df = 19, $\chi^2 = 135.64$, $p < 2.2 \times 10^{-16}$). For PCBs, congeners 118 and 153 were detected in higher loads in the liver, while for OCPs, *p,p*'-DDE and *p,p*'-DDT showed higher loads in both the liver and the muscles (DN test, range: $t = -4.802$ to -3.713, $p = 0.0123$–0.0001). Regarding PBDE congener loads, PBDE 47 was more common than PBDE 99. The mean loads of ΣPCB were 140 ± 129 ng.g^{-1} ww in the liver and 51 ± 51 ng.g^{-1} ww in the muscles; the latter did not exceed the Belgian limit in fish for human consumption (75 ng.g^{-1} ww, European Commission – EC/199, 2006). However, we observed that 20% (n = 1) of the five eels sampled in the Mosbeux River and 75% ($n = 3$) of the four eels sampled in the Vesdre River exceeded the Belgian ΣPCB limit for fishery products. The mean loads of ΣDDT were 24 ± 8 and 14 ± 7 ng.g^{-1} ww in the muscles and the liver, respectively. We observed that 0% (n = 0) of the five eels tested in the Mosbeux River and 75% (n = 3) of the four eels tested in the Vesdre River showed loads of ΣDDT in the liver that exceeded the maximum limit of 14 ng.g^{-1} ww established for the tissue of diet for wildlife predators by the Canadian Tissue Residue Guideline for the Protection of Wildlife Consumers of Aquatic Biota (CTRGPWCAB, 1999). In the muscles, 100% ($n = 5$) of eels in the Mosbeux River and 75% (n = 3) of eels in the Vesdre exceeded the CTRGPWCAB threshold.

The pollution loads were higher in the liver than in the muscles for PCBs 118 and 153 (DN test: $t = -3.486$ to -4.074, $p = 0.0028$–0.0294), while they did not significantly differ between the two tissues for each OCP or PBDE congener (Fig. 10). Eels caught in the Vesdre contained more PCBs 118, 138, 153 and 180, ΣPCB and PDBE 47 than those caught in the Mosbeux (W test, range: df = 1, V = 0–2, $p = 0.006$–0.009), but regarding the other pollution indicators the two rivers did not differ significantly (W test: $p > 0.05$). The TL, lipid content, K and density at fishing of eels 4^+ also did not differ significantly between the two rivers (W test: p > 0.05).

The gamma rank correlation coefficient used to separately assess the potential relationships between eels' organic pollutant load and their TL, K, density or lipid content revealed that PCBs 138, 153 and 180 in the liver were negatively related with K ($r_G = -0.857$ to -0.897, $p < 0.000001$) and eel density ($r_G = -0.590$ to -0.667, $p < 0.05$), they were also in the muscles negatively related with lipid content ($r_G = -0.333$ to -0.500, $p < 0.01$) (Table 2). The PCB 118 in the muscles and the liver was negatively correlated with K (in muscles, $r_G = -0.524$, p < 0.05; liver, $r_G = -0.833$, p < 0.000001), while the eel density was negatively correlated with PCB 118 in the muscles($r_G = -0.500$, p < 0.05) and positively correlated with PCB 118 in the liver ($r_G = 0.885$, p < 0.000001).The PCBs 28, 52 and 101 in the liver were positively correlated with lipid content ($r_G = 0.667$ to 0.778, $p < 0.01$). The PCB 101 in the liver was positively correlated with eel density and TL ($r_G = 0.897$, p < 0.000001), while it was in the muscle negatively correlated with K ($r_G = -0.867$, $p < 0.0001$). The ΣPCB in the liver was positively related with K ($r_G = 4.414$, p < 0.000001) and negatively correlated with eel density ($r_G = -0.714$, p < 0.01). The ΣPCB in the muscle was also negatively correlated with lipid content ($r_G = -0.333$, p < 0.01). Regarding OCPs, HCB and α- HCB in the muscle were positively correlated with K ($r_G = 0.661$ to 0.810, p < 0.05) and in the liver, they were negatively correlated with lipid content ($r_G = -0.727$ to -0.787, p < 0.01). The HCB in the liver was also positively correlated with TL ($r_G = 0.881$, p < 0.000001) and in the muscles with lipid content ($r_G = 0.611$, p < 0.05). The β-HCH in the liver was positively correlated with K, lipid content and TL ($r_G = 0.778$ to 0.877, p < 0.01). The *p,p*'-DDT, *p,p*'-DDE and *p,p*'-DDD and ΣDDT in the liver were negatively correlated with K ($r_G = -0.467$ to 0.689, p < 0.05). The *p,p*'-DDT and ΣDDT in the liver were also negatively correlated

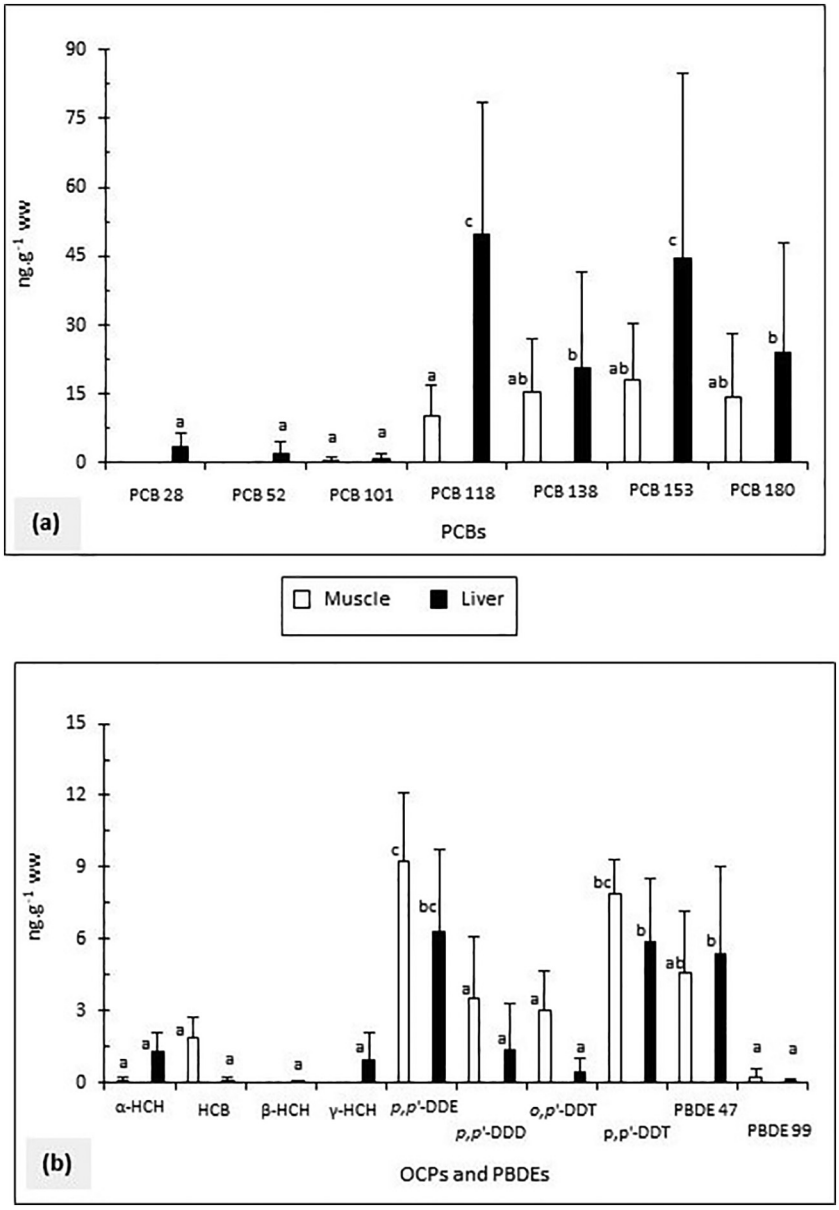


Fig. 10. Loads of PCBs (a) and OCPs and PBDEs (b) in the muscle and liver of individual eels 4^+. In the two rivers pooled, n = 9 and 10 muscle and liver samples, respectively. For PCB 118, n = 7 in the muscle. The data are the mean and standard error. Pollutant congeners with the same letter are not significantly different (KW and DN tests: $p < 0.05$).

with eel density ($r_G = -0.476$, $p < 0.05$). Regarding PBDEs, PBDE 47 in the liver was negatively correlated with K and eel density ($r_G = -0.476$ to -0.822, $p < 0.01$), while PBDE 99 in the muscles was positively correlated with eel density and lipid content ($r_G = 0.539$ to 0.615, $p < 0.05$) and it was also positively correlated in the liver with TL ($r_G = 0.897$, $p < 0.000001$). The PBDE 99 in the muscles was negatively correlated with TL ($r_G = -0.840$, $p < 0.001$) and in the liver, it was also negatively correlated with eel density ($r_G = -0.778$, $p < 0.01$).

4. Discussion

By combining field research to examine the evolution of eels' growth, K, silvering stage and density with careful laboratory analysis to determine the sex, evaluate the lipid content, detect pathogens and assess the organic pollutant load of samples over an eight-year period, the present work has met the challenge of assessing the life history traits and health status of restocked eels in freshwater ecosystems. The restocked eels were monitored from the glass eel stage to the silver eel stage. The innovative character of these findings was further amplified by the geographic location of the ecological model used in this study, which involved an upland river situated far from the sea.

The restocked eels in these upland river conditions showed strong growth performance, specifically an annual growth rate of 61 mm. year^{-1} (range = 25–99 mm.year^{-1}), thereby falling into the upper limits of the reported annual growth rates of eels restocked in European eutrophic aquatic environments (20–69 mm.year^{-1}; Bisgaard and Pedersen, 1991; Pedersen, 2000; Mazel et al., 2013; Simon et al., 2013; Ovidio et al., 2015; Silm et al., 2017; Nzau Matondo et al., 2019, 2020, 2021). These results contradict the common assumption that eel growth is poor in freshwaters where eels are often confronted with negative environmental factors that cause reduced growth (hypothesis 1). The growth performance observed in this study was accompanied by a high level of success in the recovery of eel stocks, as revealed by the high density of eels 4^+ (up to 10 individuals per 100 m^2) as well as eels 7^+ (up to 17 individuals per 100 m^2).

We have demonstrated that restocked glass eels can progress to the silver eel stage. At eight years old, more than half (54%) of individuals 7^+ were silver eels in the migratory phase, of which FV, the final

Table 2
Synthesis of the relationships between pollutant loads ($ng \times g^{-1}$), total length (LT, mm), density ($ind.m^{-2}$), Fulton's condition factor (K) and lipid content (%) using the Gamma rank correlation coefficient. n = 9 and 10 muscle and liver samples, respectively. For PCB 118, n = 7 in the muscle. Values are Gamma rank coefficient and p. *p < 0.05; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; and ***** $p < 0.000001$.

Parameters	Muscle coefficient (p)				Liver coefficient (p)			
	Density	Fulton's K	Lipid content	Total length	Density	Fulton's K	Lipid content	Total length
a) Polychlorinated biphenyls (PCBs)								
PCB 28	–	–	–	–	0.135 (0.669)	0.250 (0.457)	0.744 ($6.328 \times 10^{-4***}$)	0.135 (0.669)
PCB 52	–	–	–	–	−0.071 (0.838)	0.200 (0.523)	0.667 ($5.645 \times 10^{-3**}$)	0.500 (0.082)
PCB 101	−0.571 (0.073)	−0.867 ($2.404 \times 10^{-5****}$)	−0.333 (0.337)	−0.067 (0.855)	0.897 ($6.117 \times 10^{-7******}$)	−0.111 (0.745)	0.778 ($4.827 \times 10^{-3**}$)	0.887 ($3.775 \times 10^{-7******}$)
PCB 118	−0.500 (0.035*)	−0.524 (0.029*)	−0.143 (0.609)	0.053 (0.901)	0.885 ($6.409 \times 10^{-7******}$)	−0.833 ($6.928 \times 10^{-5****}$)	−0.278 (0.283)	0.000 (1.000)
PCB 138	−0.529 (0.070)	−0.278 (0.258)	−0.333 (0.003**)	0.235 (0.225)	−0.636 ($8.095 \times 10^{-3**}$)	−0.886 ($6.726 \times 10^{-7******}$)	−0.200 (0.475)	−0.059 (0.863)
PCB 153	−0.471 (0.090)	−0.222 (0.298)	−0.389 (0.002**)	0.294 (0.127)	−0.590 (0.042*)	−0.857 ($7.931 \times 10^{-6*****}$)	−0.095 (0.707)	0.220 (0.384)
PCB 180	−0.471 (0.086)	−0.333 (0.173)	−0.500 (0.002**)	0.412 (0.022*)	−0.667 ($7.387 \times 10^{-3**}$)	−0.897 ($1.020 \times 10^{-6*****}$)	−0.180 (0.531)	0.105 (0.725)
ΣPCB	−0.529 (0.070)	−0.278 (0.258)	−0.333 (0.003**)	0.235 (0.225)	−0.714 ($4.932 \times 10^{-3**}$)	4.414 ($1.020 \times 10^{-6*****}$)	−0.067 (0.821)	0.023 (0.935)
b) Pesticides (OCPs and PBDEs)								
HCB	0.529 (0.020*)	0.611 (0.019*)	0.778 ($6.991 \times 10^{-4***}$)	−0.300 (0.571)	0.879 ($4.672 \times 10^{-7******}$)	−0.111 (0.745)	0.787 ($4.827 \times 10^{-3**}$)	0.881 ($2.762 \times 10^{-7******}$)
α-HCH	0.600 (0.070)	0.810 ($1.294 \times 10^{-3**}$)	0.524 (0.071)	−0.353 (0.306)	0.122 (0.718)	0.273 (0.327)	0.727 ($6.435 \times 10^{-5****}$)	0.190 (0.476)
β-HCH	–	–	–	–	0.556 (0.076)	0.778 ($4.827 \times 10^{-3**}$)	0.889 ($4.645 \times 10^{-7******}$)	0.877 ($5.702 \times 10^{-7******}$)
γ-HCH	–	–	–	–	0.071 (0.808)	0.333 (0.293)	0.667 ($5.645 \times 10^{-3**}$)	0.357 (0.216)
PBDE 47	0.412 (0.094)	−0.278 (0.231)	−0.222 (0.108)	0.176 (0.353)	−0.476 ($5.632 \times 10^{-3**}$)	−0.822 ($2.660 \times 10^{-5****}$)	−0.244 (0.232)	0.023 (0.941)
PBDE 99	0.539 (0.029*)	0.615 (0.106)	0.615 (0.034*)	−0.840 ($4.927 \times 10^{-4***}$)	−0.778 ($4.828 \times 10^{-3**}$)	1.000 (0.000)	−0.244 (0.232)	0.897 ($6.117 \times 10^{-7******}$)
p,p'-DDE	0.176 (0.459)	0.333 (0.256)	0.389 (0.101)	−0.176 (0.521)	−0.476 (0.097)	−0.467 (0.045*)	0.289 (0.291)	−0.023 (0.931)
p,p'-DDD	−0.059 (0.826)	0.056 (0.846)	0.000 (1.000)	−0.118 (0.643)	−0.273 (0.400)	−0.583 (0.033*)	−0.167 (0.580)	0.083 (0.762)
p,p'-DDT	0.235 (0.445)	0.500 (0.055)	0.444 (0.138)	−0.112 (0.744)	−0.476 ($5.631 \times 10^{-3**}$)	−0.556 (0.012*)	−0.022 (0.898)	−0.070 (0.814)
o,p'-DDT	−0.176 (0.482)	−0.056 (0.835)	−0.111 (0.359)	0.000 (1.000)	0.273 (0.436)	0.200 (0.533)	0.143 (0.739)	−0.059 (0.843)
ΣDDT	0.000 (1.000)	0.111 (0.725)	0.167 (0.320)	−0.294 (0.292)	−0.476 (0.028*)	−0.689 ($7.487 \times 10^{-3**}$)	0.067 (0.807)	0.070 (0.786)

migratory stage before seaward migration, was the most abundant silvering stage group, accounting for 44% (mean TL = 602 mm, range = 450–760 mm) of eels 7^+. The age and the TL of eels at the FV silvering stage matched the mean age of eight-year-old eels (range = 3–18 years) and the mean length of 605 mm (range = 325–1060 mm) of seaward-migrating silver eels caught at the mouth of the Imsa River located in south-western Norway (Vollestad and Jonsson, 1988). This river has a mean water temperature higher than the country's mean. The present study also yielded other encouraging outcomes, namely a greater K for eels 7^+ (mean = 0.197, maximum = 0.259) and a positive K (mean = 0.166, maximum = 0.266) for eels 4^+, with a good lipid store (mean = 8.3%, maximum = 20%) only five years after restocking. These K are consistent with the values reported in an Estonian lake (mean K = 0.19) in eels with a mean age of eight years as well as with those recorded in European eels (K = 0.16–0.22) that had completed their growth phase (van Ginneken et al., 2007; Silm et al., 2017). Given the lack of lipid content data for eels 7^+, the data for eels 4^+ are an indicator of a good accumulation process of lipid reserves, because lipid stores in this fatty fish species increase with age and developmental stage and a lipid content >13% is needed to cover the cost of the return journey for successful reproduction in the Sargasso Sea (Palstra et al., 2006a, 2006b). Male individuals in this study were scarce while females were dominant, allowing us to reject hypothesis 2 that overdensity restocking by releasing glass eels at a single point in a freshwater body can lead to an overproduction of males in this cryptic species tending to stay within the vicinity of the restocking and catch locations (Järvalt et al., 2010; Verhelst et al., 2018). The sex ratio (male: female) was 1: 14 for eels 4^+ and 1: 17 for eels 7^+, reflecting the possibility of producing, through restocking, female-dominated stocks upstream of catchment areas as during the colonisation process of naturally recruited eels. With the low abundance of males and their presence exclusively in the low-density group, we have been unable to establish a link between male production and eel density. This observation is consistent with findings of a positive correlation between the male: female ratio and eel density (Acou et al., 2011; Geffroy and Bardonnet, 2015; Boulenger et al., 2016). By contrast, with a significantly higher occurrence of females in the low-density group, we have demonstrated that density plays a role in the feminisation of eel individuals as well as their optimal living habitats and food resources (Acou et al., 2011; Geffroy and Bardonnet, 2015; Boulenger et al., 2016). Similarly, given the lack of influence of riverine longitudinal gradient on eels' density and sex, we consider that the overdensity restocking practice involving the release of glass eels at a single point facilitates the effective dispersal of eels, meaning that the eels' density depends on the dispersal process and the ratio between eels' number or biomass and productivity of the aquatic system, as well as the methodology used does not affect the final sex of eels, as observed by Nzau Matondo et al. (2019). This sounds very interesting because the methodology is easy to implement

due to its reduced human resource requirements (Nzau Matondo et al., 2019, 2020). The success of this restocking method has also been shown in our regularly monitored river stretch through a particularly high monthly survival rate (> 90%) of these eels over the eight years after restocking, using a more appropriate model for open population assessments, namely the Jolly-Seber Model. The absence of sexually undetermined eels when using histological sections of younger eel gonads clearly shows the advantage of this method over gonadal squash for sexing eels, which could enable its use for sexing very young eels after restocking.

Notably, we have demonstrated the absence of the threatening parasite *A. crassus* (or associated lesions) and the lower prevalence of viral pathogens such as AngHV-1 (eels 4^+: 0% and eels 7^+: 27%, $n = 7$ AngHV-1-positive individuals) in the restocked eels. This success is consistent with that reported in the Mediterranean area, where wild silver eels escaping from an Italian euhaline lagoon were found to be free of *A. crassus* and their swim bladders showed no symptoms of alteration, due to the disconnection of the lagoon from the riverine system and its high water salinity values, preventing successful parasite transmission to the host (Kirk et al., 2000; Capoccioni et al., 2020). These escaping silver eels also showed a low prevalence of AngHV-1 (25%, $n = 5$) (Capoccioni et al., 2020). There is therefore hope that pathogen-free eels can be produced from restocking in inland freshwaters (hypothesis 4), where eels often encounter negative factors such as high parasite prevalence (Belpaire et al., 2011). This is especially true in the case of the present study because the eels were monitored up to age 7^+ (eight years old) in a Western European freshwater environment, where it is known that sexual maturity and the associated metamorphosis of silver eels are reached later (at six to ten years old) than in the Mediterranean region (three to six years old) (Cattrijsse and Hampel, 2000; Acou et al., 2003; Desprez et al., 2013). The observed viral prevalence trend from 0% (age 4^+) to 27% (age 7^+) may indicate that the glass eels were healthy at restocking and that the AngHV-1 infection occurred during the freshwater growing period. Delrez et al. (2021) have recently demonstrated by anatomopathological, bacterial, parasitical and viral analyses the good health status of glass eels. They suggest that glass eels enter estuaries free of pathogens and that their capture at this early stage reduces the risk of the anthropogenic introduction of pathogens through restocking programmes. The observed higher AngHV-1 DNA prevalence of eels 7^+ in the present study may be related to the older sampling age (eight years old), the later period of the year (16 July 2020, hence in the summer), the higher water temperature (12.8 °C) and the larger body size (mean TL of 404 mm and K of 0.197). By contrast, the sampling for eels 4^+ occurred when they were younger (five years old), earlier in the year (2–3 May 2017, hence in the spring), at 9.7 °C and with a smaller body size (mean TL of 295 mm and K of 0.166). This higher viral prevalence in eels 7^+ than in eels 4^+ is sustained by the fact that AngHV-1 genome copies were found to be positively correlated with the K of the virus-positive individuals. However, the viral loads in the DNA of the sampled eels were low, corresponding to latent AngHV-1 infections in virus-positive eels. The threshold value for consideration of a clinically relevant viral load is $\geq 1 \times 10^4$ genomic copies of AngHV-1 found in eels (Kullmann et al., 2017) as well as *Cyprinid herpesvirus 3* (CyHV-3) in the common carp *Cyprinus carpio* (Adamek et al., 2014), exhibiting clinical signs such as skin and fin haemorrhages. Kullmann et al. (2017) have also reported an AngHV-1 prevalence of 68% in restocked eels (range: TL = 243–729 mm, age = 1–6 years, $n = 100$) caught in the Schlei Fjord in northern Germany after a restocking programme conducted as part of a seven-year study beginning in 2010. Elsewhere in Europe, the prevalence of AngHV-1 varies among regions (McConville et al., 2018), being estimated at about 40% in the Netherlands (Haenen et al., 2010) and in Poland (Nguyen et al., 2016a), 48% in southern Germany (Scheinert and Baath, 2006) and 51-57% in Spain (Bandin et al., 2014). McConville et al. (2018) and Nguyen et al. (2016b) have mentioned a possible non-eel vector or reservoir of AngHV-1, because viral DNA has been detected in wild native ichthyofauna in Poland. Kullmann et al. (2017) have observed that an increase in body length is accompanied by an increased risk of clinically relevant viral loads, thus larger specimens being more often tested positive for the virus. It has been shown that lower water temperature can lead to lower viral DNA replication (Gilad et al., 2004). Although in the present study the sampling of eels 4^+ occurred in spring at a lower water temperature, this condition did not prove stressful for the eels and the conventional PCR method used is known to be the gold standard for detecting viruses (Hanson et al., 2016; van Beurden et al., 2016), meaning that eels 4^+ were healthy. By contrast, the silvering process may be stressful for eels 7^+, as revealed by their virus prevalence of 27% as well as the prevalence of 100% (n = 1) for the FIV individual at an eel migratory stage. The low representation of eels in the migratory stages in eels sampled for the AngHV-1 investigation, despite the high abundance of these stages in eels 7^+ caught, was a missed opportunity for a robust assessment of their health status. This low representation probably owed to bias in the field sampling. The absence of virus-positive individuals at low eel density in our study could also demonstrate that a high eel density would promote the spread of the AngHV-1 through intense agonistic interactions between individuals in this naturally solitary and territorial species (Knights, 1987; Huertas and Cerda, 2006). It must be highlighted that two DNA amplification methods were used in this study. While conventional PCR is considered the gold standard (van Beurden et al., 2016), qPCR has the advantage of providing fast and high-throughput detection and quantification of target DNA sequences. Moreover, qPCR provides an appropriate specificity and sensitivity for viral DNA detection (Kralik and Ricchi, 2017). Thus, the method and the choice of tissue samples were optimised in 2020, as the brain seems more accurate than the heart for herpesvirus latent infection detection (Boutier et al., 2015, 2021). In this situation, the possibility of having missed latent carriers in individual eels 4^+ cannot be ruled out. In our study, the virus was found to be hosted more in the brain than in the gills, consistent with Gilad et al. (2004) regarding *Cyprinid carpio koi* experimentally exposed to CyHV-3 at 13 °C water temperature for 10 days. Perhaps alloherpesviruses establish a neuronal latency, potentially rendering this organ a good candidate for sampling. However, the major portal of entry for AngHV-1 and its mode of transmission remain unexplained in eels, a benthic carnivorous fish species. Costes et al. (2009) have clearly identified the skin and not the gills as the major portal of entry for CyHV-3 in common carp *Cyprinus carpio carpio*, an omnivorous fish species. In our study, adult *A. crassus* nematodes were detected neither in 2017 nor in 2020. Furthermore, we did not observe any acute or chronic inflammation (wall thickening) of the swim bladder during the necropsies. In contrast, Kullmann et al. (2017) observed in the eel sample described above an *A. crassus* prevalence of 50% and a parasite load of 5 *A. crassus* individuals per eel independently of their body length, therefore supporting the good health of the eels in our study.

With the contamination of all eels 4^+ by at least one of the organic pollutant congeners studied, it is important that the evaluation of the eels' riverine contamination by pollutants is considered when assessing the quality of the restocked silver eels produced (hypothesis 3). This is necessary for European inland waters, especially Belgian freshwaters given the country's industrial history, urbanisation and agricultural activity as well as possible trans-boundary aquatic pollution from neighbouring countries through the main rivers. Eels colonise a very wide range of habitats, including those with high organic loads and low oxygen content (Jürgens et al., 2015; Ovidio et al., 2015; Nzau Matondo et al., 2019, 2020, 2021). However, we know very little about the critical life stages of sexual maturation and spawning, which occur during periods of prolonged fasting when pollutants stored in lipids can be remobilised and affect the eels themselves or their offspring through mother-to-child transfer (Robinet and Feunteun, 2002; Jürgens et al., 2015). In this study, eels 4^+ showed lower pollutant loads for all the assessed PCB and PBDE congeners as well as pesticides than the pollutant levels reported in eels of other riverine ecosystems in

Belgium (multi-river experience, larger sample size of eels: Maes et al., 2008; Malarvannan et al., 2014) and elsewhere in Europe (only contaminated sites or single river experiences and smaller sample sizes: Van Der Oost et al., 1996; Dauberschmidt and Hoffmann, 2001; Bordajandi et al., 2003). The higher pollutant concentrations reported by Maes et al. (2008) and Malarvannan et al. (2014) in two Flemish studies in Belgium can be related to the higher local pollution pressure in Flanders than in Wallonia, due to the former being located in the lowland part of Belgium near the North Sea, which is a highly industrialised area with a dense population and intensive agricultural and industrial activities. The lower level of contamination in our study undertaken in Wallonia is an additional argument for enhancing eel restocking in suitable and less anthropised areas, like Walloon upland rivers (Nzau Matondo et al., 2021). Our results also showed trends towards lower values from 2002 to 2017 in the time series of PCB load monitoring data for the eels in the Meuse River basin in Wallonia (Table 3). The PCBs have been banned in Europe since 1985 and their decreasing trends have also been reported in other long-term monitoring studies performed on eels in the Netherlands (since 1977: de Boer et al., 2010) and Flanders/Belgium (since 1994: Maes et al., 2008; since 2000: Malarvannan et al., 2014) as well as on other teleost fish species (Lamon et al., 1998; Lindell et al., 2001), demonstrating that bans and environmental policies are leading to a decrease in fish pollutant concentrations.

The ΣPCB concentrations in the muscles of the restocked eels in this study are problematic for human consumption, as about 56% of the analysed individuals exceeded the maximum level allowed in fish in Belgium, set at 75 ng.g^{-1} ww. However, this figure is below that reported in Flemish river basins (76% of the analysed eels: Maes et al., 2008) and all eels were below the European limit for human consumption of wild-caught eels, which is 300 ng.g^{-1} ww (EC, 2011). The Belgian limit for ΣPCB concentrations was set after the Belgian dioxin crisis in 1999, when dioxin was introduced into the Belgian food chain through contaminated animal fats used in animal feeds. Due to the subsequent public awareness regarding food safety issues, the consumption of eels caught in Walloon rivers in southern Belgium is now prohibited. Since 2006, eel fishing has also been banned in Wallonia as a result of high concentrations of PCBs found in the flesh of this fish species and the collapse of local eel stocks (Chalon et al., 2006; Nzau Matondo and Ovidio, 2016).

When we expressed the PCB loads we measured in toxic equivalents (TEQ) using the factors defined by Van den Berg et al. (2006), we obtained values of up to 0.6 and 3.36 ng TEQ.kg^{-1} ww in the muscles and the liver, respectively, for consumption by humans and mammals. Compared with the TEQ limit above which it is considered that there is a threat to the mammalian wildlife that consume aquatic biota, which is 0.79 ng TEQ.kg^{-1} diet ww according to the Canadian Council of Ministers of the Environment (CCME, 2001), it appears that natural predators of eels may be at risk when ingesting the most contaminated eels 4^{+}. Regarding the impact of these PCB loads on the health of eels, Palstra et al. (2006a, 2006b) observed that the TEQ levels of dioxin-like pollutants, at least 86% of which were PCBs (de Vries, 2002, in Palstra et al., 2006a, 2006b) and which were as low as 1 ng TEQ.kg^{-1} ww in gonads (contamination of which was very similar to that of muscles), led to decreased survival in embryos. It is thus possible that the survival of future embryos from genitors produced in the sampled rivers will not be significantly affected by their parental PCB contamination (maximum 0.56 ng TEQ.kg^{-1} ww in the liver).

The higher pollution loads observed in the Vesdre River (catchment area 702 km^2) than in the Mosbeux River (19.2 km^2) in terms of PCBs 118, 138, 153 and 180, ΣPCB and PDBE 47 may be related to its large catchment area – associated with previously significant industrial, livestock and agricultural activities – as well as its riverbed substrate, which is less rich in alkaline cations. Negative relationships identified between PCBs 138, 153 and 180 and K, lipid content and eel density as well as between the pesticides p.p'-DDE, p.p'-DDD, p.p'-DDT and PBDE47 and K, support the good quality of the restocked eels in our study. However, these findings must be interpreted with caution given the absence of any repetition over time for the assessment of organic pollutants. Nevertheless, it is a strong signal of a possible production of spawners, the silver eel individuals in our upland river conditions; which would be less loaded with organic pollutants.

The good quality of eels was further supported by the absence of eels exceeding the maximum residue and contaminant limits in food and feed as adopted by several national and international regulations (Table 4). All of the pesticides analysed were lower than the limits for human consumption, considering a daily fish consumption of 0.033 kg.day^{-1} (NYCRR, 1998). However, all of the eels contained ΣDDT concentrations above the limit of 14 ng.g^{-1} ww in the muscles, presenting a threat for wildlife feeding on these fish (CTRGPWCAB, 1999).

Our findings highlight the importance of restocking upland aquatic ecosystems to enhance both riverine silver eel production and, in the long term, the panmictic spawner stocks that reproduce in the Sargasso Sea (Nzau Matondo et al., 2019, 2020, 2021). To be successful, restocking must be accompanied by improved ecosystem quality for eels in inland freshwaters, which implies the availability of contaminant- and pathogen-free growing habitats as well as safe migration routes for catadromous spawners towards spawning grounds and for future offspring towards rivers (Verbiest et al., 2012; Nzau Matondo and Ovidio, 2016; Nzau Matondo et al., 2017; Nzau Matondo and Ovidio, 2018; Delrez et al., 2021). Future work should better assess the quality of genitors produced in inland freshwaters through routine monitoring of the life history traits of restocked silver eels in the seaward migration stage as well as their health status. The latter should include contamination by organic and chemical pollutants and toxic metals as well as infection by pathogens, given the considerable importance of this species' conservation and management in Europe (Armitage et al., 2014). Future experimental studies identifying AngHV1 portal of entry as well as modes of transmission in the field may also help to understand the epidemiology of the virus in the eel population. The AngHV-1 DNA have been detected in other fish species in Poland (Nguyen et al., 2016b), but it remains to be determine whether these non-eel species can contribute to the persistence and the spread of this virus species. Further experiences in a controlled environment would be useful to define the precise impacts of organic and chemical pollutants as well as toxic metals on the development and biology of restocked eels. It would also be necessary to identify polluted environments and their sources for remediation.

Table 3
Time trends of main pollutant loads in the Meuse River basin (mean values).

Persistent organic pollutants	Maximum residue and pollutant limit			Loads observed (ng.g^{-1} ww)		
Substance	Time-series	Rivers	Tissues	Mean values	n	References
Σ 7 PCB	2002	Vesdre	Muscle	1915	1	Thomé et al., 2003
	2002	Meuse	Muscle	1730	3	Thomé et al., 2003
	2011	Meuse	Muscle	252	3	Thomé et al., 2011
	2017	Vesdre	Muscle	85	4	This study
	2017	Mosbeux	Muscle	33	5	This study
Σ DDT	2011	Meuse	Muscle	16	3	Thomé et al., 2011
	2017	Vesdre	Muscle	25	4	This study
	2017	Mosbeux	Muscle	23	5	This study

Table 4
Comparison between the maximum residue and pollutant limits in eel or fish as adopted by different international regulations and abundance of non-compliant eels in this study.

Persistent organic pollutants	Maximum residue and pollutant limits		This study	
Substance	Levels	References	Maximal loads	Non-compliant eel (%)
ΣPCB	75 $ng.g^{-1}$ ww for human consumers	EC/199, 2006	337 $ng.g^{-1}$ ww	56
	300 $ng.g^{-1}$ ww for human consumers	EU/1259, 2011		0
	0.79 ng $TEQ.kg^{-1}$ diet ww for mammalian predators	TEQ limit, CCME, 2001	0.6 ng $TEQ.kg^{-1}$ diet ww[a] in muscle 3.36 $ng.kg^{-1}$ diet ww[a] in liver	–
	2.4 $ng.kg^{-1}$ diet ww for avian predators	TEQ limit, CCME, 2001		–
Pesticides				
γ-HCH (lindane)	10 $ng.g^{-1}$ ww in flesh of fish for human consumption 33 $ng.g^{-1}$ ww for secondary poisoning	FAO Codex Alimentarus, 2021 EC/396, 2005	5.2 $ng.g^{-1}$ ww in liver < 0.02 $ng.g^{-1}$ ww in muscle	0
α-HCH	20 $ng.g^{-1}$ ww for human consumption	EC/86, 1986	0.27 $ng.g^{-1}$ ww in muscle 3.04 $ng.g^{-1}$ ww in liver	0
HCB	10 $ng.g^{-1}$ diet ww for human consumption	EC/86, 1986/NYSHHFC, 1998	3.6 $ng.g^{-1}$ ww in muscle	0
ΣDDT	42 $\mu g.g^{-1}$ ww[b] for human consumption	Vermeire et al., 1991		0
	14 $ng.g^{-1}$ ww in muscle of diet for wildlife predators	CTRGPWCAB, 1999	41 $ng.g^{-1}$ ww (muscle) 36 $ng.g^{-1}$ ww (liver)	100 (muscle) 30 (liver)
	1000 $ng.g^{-1}$ ww	EC/86, 1986		0

[a] Depends on whether the liver is ingested by a predator.
[b] For a 70 kg adult who consumes 0.033 $kg.day^{-1}$ (NYCRR, 1998).

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CRediT authorship contribution statement

B.N.M., M.O., V.A., D.N., A.B., J.J.C. and X.R. designed research, analysed data's and wrote the paper. All authors participated to data collection in the field and the laboratory.

Declaration of competing interest

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