Received: 17 January 2022 | Revised: 25 April 2022 | Accepted: 27 April 2022

DOI: 10.1111/jfd.13658

Journal of Fish Diseases WILEY

RESEARCH ARTICLE

Identification of virus infections of European eels intended for stocking measures

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Funding information
This study was funded by the European Maritime and Fisheries Fund (EMFF).

Abstract

The spread of viral diseases in eels is suggested to severely affect the European eel (*Anguilla anguilla*) panmictic population. The European Commission has initiated the Eel Recovery Plan (Council Regulation No. 1100/2007) to try to return the European eel stock to more sustainable levels within that measures eel restocking. However, scientific evidence evaluating the efficacy of stocking remains scarce. In addition, knowledge about the impact and contribution of eel stocking on the distribution of infectious diseases is insufficient. In this study, we aimed to investigate virus infections in batches of eels intended for restocking. We analysed samples of glass eels from certified fisheries and farmed European eels from different aquaculture farms. All analysed eels were purchased within a North Rhine Westphalian conservation program. Via a combination of cell culture and qPCR-based techniques, we detected infections of glass eels with the rhabdovirus Eel Virus European X and anguillid herpesvirus 1 infections in farmed eels (10–15 cm).

KEYWORDS
AngHV-1, European eel, EVEX, farmed eel, glass eel, restocking

1 | INTRODUCTION

The European eel (*Anguilla anguilla*) has existed on the earth for millions of years. However, for several decades the species has been critically endangered (ICES, 2016, 2021). Unsolved questions regarding reproduction and anthropogenic influences on *A. anguilla* complicate the circumstances of its rescue. The European eel has an obligatory catadromous life cycle, living in fresh water and spawning in the Sargasso Sea, more than 5.000–7.000 km apart (Kleckner & McCleave, 1988; Miller et al., 2015; Miller et al., 2019; Munk et al., 2010; Schoth & Tesch, 1982). The eel larvae migrate from the Sargasso Sea to continental waters (Figure 1). When arrived at the coasts they metamorphose to glass eels, which migrate up the inland waters. According to current findings, glass eels migrate upstream by an intrinsic magnetic compass memorizing the magnetic direction of tidal flows at the estuaries (Cresci et al., 2019). The adult European eels (yellow eels) spend several years in freshwaters before they undergo further maturation to silver eels, which migrate back to the Sargasso Sea for mating and reproduction (Cresci, 2020; Dekker, 2000; Tesch & Thorpe, 2003; van Ginneken & Maes, 2005). After spawning silver eels die.

The indices (measurements of annual abundances) of both, glass and yellow eels strongly declined during the past decades (ICES, 2021). Therefore, the European Commission released the Eel Regulation (EC 1100/2007) in 2007. This regulation aims to provide a framework for the recovery of the *A. anguilla* panmictic population. Based on the Eel Regulation, EU countries have to implement management plans for the recovery of this species. However, glass eel recruitment remains at a very low level (ICES, 2021). During their life eels are exposed to many threats. Throughout the past years, there have been many scientific

attempts to explore and prevent the dramatic decline. Currently, the International Council for the Exploration of the Sea (ICES) advised zero catches of eels in all habitats in 2022. To counteract the anthropogenic decline, the EU management plans include different measures; among these, restocking activities. Restocking measures of European eels are challenging, as they cannot be reproduced in captivity to date. Thus, restocking of eels relies on wild glass eel catches (Figure 1). In spring, after the glass eel arrival, they are caught by the fishery using several methods (Harrison et al., 2014). The caught eel then are traded by specialized eel traders (Figure 1(A)). Hereby, a high number of individuals is brought together, leading to very high densities, which do not happen under natural conditions. Some of the eels are sold directly by traders for restocking purposes. In addition, glass eels are further traded, and then ongrown for several weeks or months in aquaculture facilities, until they reach a size of 15–20 cm (Figure 1(B)). Both types of restocking procedures seem to run unique risks of spreading environmental pathogens. Direct transport of caught glass eels to the restocking destination (assisted migration), proceeds without intermediary keeping (Figure 1(C)).

Regarding the dramatic decline of the European Eel, the issue of eel health is increasingly coming into research focus. More and more studies on infectious diseases of adult European eels were conducted. However, in the past, the process of restocking eels was rarely in the focus of scientific investigations. Thus, several open questions regarding infectious diseases of larvae or glass eels remain. A few virus-induced infectious diseases of eels are well studied, among them infections with the alloherpesvirus anguillid herpesvirus 1 (AngHV-1) (Sano et al., 1990), the eel rhabdovirus Eel virus European X (EVEX) (Sano et al., 1977) or the aquabirnavirus Eel virus European (EVE) (Sano, 1976). Infections with the mentioned viruses can result in severe hemorrhagic diseases or anaemia, chronical infections and high mortality rates (Kobayashi & Miyazaki, 1996; Okamoto et al., 1983; van Beurden et al., 2012; van Nieuwstadt et al., 2001) and are assumed to be triggered by stress conditions (Haenen et al., 2009). In 2013 a study demonstrated the infection of a diseased yellow eel with a novel picornavirus (EPV-1) in Lake Constance (Fichtner et al., 2013). Currently, EPV-1 was isolated from elvers and yellow eels caught in the Rhine system in Germany (Danne et al., 2021). Knowledge about some virus infections of eels is still at the beginning and to date, some findings of eel viruses come from other eel species. For example, polyomavirus infections of *Anguilla marmorata* have been reported (Wen et al., 2016) and reovirus infections (Pao et al., 2019). Reovirus-like particles have been isolated from European eels (Haenen et al., 2009) without further investigation. In 2014 circovirus infections of adult European eels have been reported (Doszpoly et al., 2014) and circovirus-infected eels were detected in Hungary (Borzák et al., 2017).

In comparison to research about infectious diseases of adult European eels, scientific knowledge of virus infections of glass eels remains scarce. In addition, nothing is known whether vertical transmission of eel viruses is of importance and should be taken into consideration for planning stocking measures. In this study, we designed qPCR assays for the detection of AngHV-1, EVEX and EPV-1 and investigated virus infections of batches of glass eels and farmed eels that were intended for stocking measures in North Rhine Westphalia Germany.

2 | MATERIAL AND METHODS

2.1 | Eel purchase and sample preparation

As part of a species conservation project, European eels were purchased for stocking measures in North Rhine-Westphalia, Germany. The purchase was made via an international tender. Eels were purchased from certified eel traders (Sustainable Eel Group [SEG] certified). Purchase conditions included proof of a quality and health

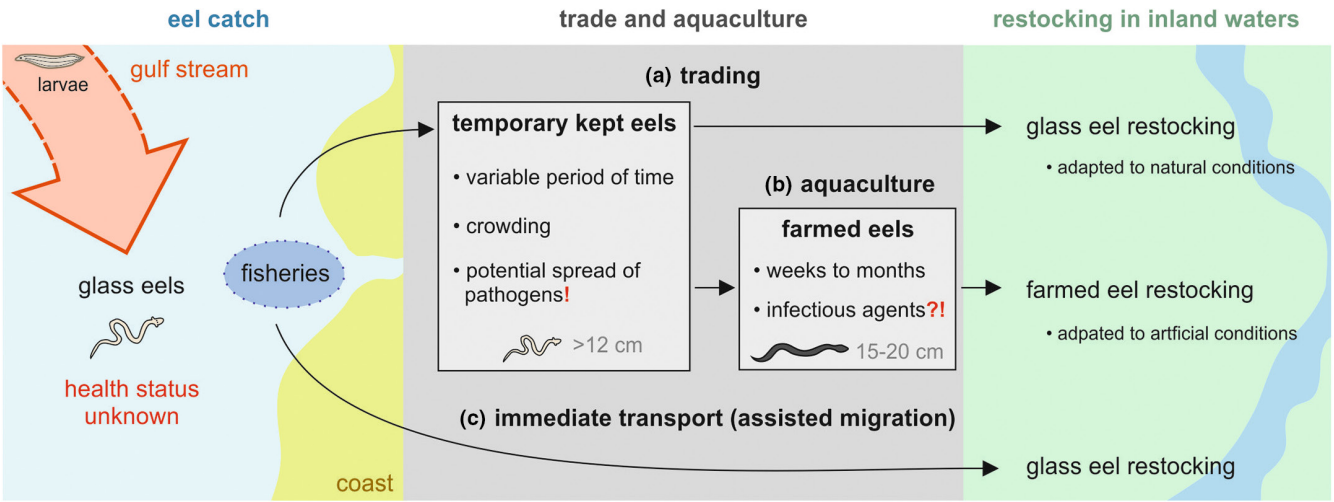

FIGURE 1 Schematic overview of eel restocking practices. (a) Glass eels are caught and sold by professional glass eel fishers and are then further traded by fish trading companies for stocking measures and aquaculture. The glass eels traded to aquaculture are kept and farmed for a period of time (b) before these ongrown eels are restocked to inland waters. (c) Glass eels are directly transported to the restocking destination after being caught

check, and verification of AngHV-1 free animals. A France company was contracted for the delivery of glass eels. The company bought the glass eels from local professional SEG-certified glass eel fisheries. The eels originated from the coasts of South France (Bay of Biscay). Due to delays in the procurement procedure for the supply of stocking material, the contract was awarded late at the end of the glass eel season. Therefore, the purchased eels were kept for unknown period of time. Glass eels were transported in polystyrene boxes in which the glass eels were transported without water (but moistened). For cooling conditions, the transport boxes were supplemented with ice packs. Each box contained about 3600 glass eels. Samples that were analysed in our laboratory were taken each stocking day. Glass eel stocking measures started on 08 May 2020.

Farmed eels (10–15 cm) originated from three different commercial eel traders or farms. In total, virus infections of 804 eels have been investigated (354 glass eels, 450 farmed eels).

For the preparation of pool samples, 10 individuals were used. For sample preparation of glass eels tails were removed to sample virus relevant organs. Tissue samples of farmed eels (pools of spleen, heart, gills, kidney) were taken from each individual and prepared in pools (10 individuals). Samples were stored at −80°C.

2.2 | Virus isolation

The isolation and propagation of viruses were performed using the eel kidney 1 cell line (EK-1; (Chen et al., 1982)). EK-1 cell line (CCLV Rie 809) was provided by the Friedrich-Loeffler-Institute (Federal Research Institute for Animal Health, Germany). The cells were propagated in cell culture flasks for confluent monolayer in Leibovitz L-15 medium (10% fetal bovine serum; 100 µg/ml tylosin; 100 µg/ml streptomycine/spectinomycine) at 20°C and 26°C. Glass eels (without tail) or organ samples of farmed eels were prelyzed using a Tissue Lyser (Qiagen). The homogenate was resuspended in a cell culture medium, and samples were centrifuged to remove cell debris (15 min; 2360 × *g*; 10°C). The inoculation of EK-1 cells with cleared homogenate occurred at 20°C and 26°C. In total, three passages were performed. Passaging was performed over 7 days. Samples showing distinct changes in cell morphology (cytopathic effects [CPEs]) compared with the negative control were defined as virus-positive cell cultures. Negative defined samples were comparable in morphology to mock-infected cells. Screening for CPEs was carried out every 24 h using a Primovert microscope (Zeiss). Cell cultures were subjected to centrifugation (15 min; 2360 × *g*; 10°C) and supernatants were stored at −80°C.

TABLE 1 Virus strains used in this study

Description	Reference/origin; date
AngHV-1	LANUV NRW; 2014
EVEX	Olga Haenen Wageningen University & Research
EPV-1	LANUV NRW, FLI; 2017

2.3 | Nucleic acid isolation

Virus identification/quantification of viral load in cell culture supernatants, organ pools or glass eel samples was performed via qPCR. Total DNA and RNA were extracted from 100 µl of cell culture supernatants or tissue samples in accordance with the manufacturer's instructions using the *Mini kit for DNA from cells and tissue* (Macherey & Nagel) and the *Mini kit for viral RNA/DNA purification* (NucleoSpin Virus; Macherey & Nagel), respectively. The quality and quantity of DNA and RNA isolates were assessed using the NanoDrop Onec (ThermoFisher). Virus strains are listed in Table 1. Mock-infected cells were used as a negative control.

TABLE 2 Primer and probes used in this study

Designation	Oligonucleotide sequence (5′→3′)	Reference
HVA-Pol1-F	CTACCTGAGCATGCGAAACA	Wonnemann et al., 2010
HVA-Pol1-R	ATCTCGTTCTGCAGCTGGTT	Wonnemann et al., 2010
HVA-Pol1-P	6FAM-AATACAAGGGCGACATGAAGACGG-BHQ-1	Wonnemann et al., 2010
EPV.2C_F	GTGTTGAAGGTTTTGGCATTAG	Danne et al., 2021
EPV.2C_R	GTCAACATACTCAGCAATACC	Danne et al., 2021
EPV.2C_P	6FAM-TCGCAGTCGACGATGAGCAGTGT—BHQ1	this study
EVEX-190-F	GAGAGGGAACTAAAAAAAGCTG	this study
EVEX-190-R	CATCCTCTCCTTGTCCTTGAC	this study
EVEX-190-P	Tamra-CTCTAATGTCCTGGGAATTGAGGGAGT-BHQ2	this study
ACT-1030-F	AGCGCAAGTACTCCGTGTG	Toussaint et al., 2007
ACT-1135-R	CGGACTCATCGTACTCCTGCTT	Toussaint et al., 2007
ACT-1081-YAK	YAK-TCGCTGTCCACCTTCCAGCAGATGT-BHQ1	Toussaint et al., 2007
EVEX.L_F	CTCTAATGTCCTGGGAATTGAGGG	Danne et al., 2021
EVEX.L_R	GATTTTAGATTCTCTTTTGATGACCAACAGGT	Danne et al., 2021

TABLE 3 Results of glass eel examinations

Sample designation	Developmental stage	Pool size (Individuals)	condition	size (cm)	weight (g)	nucleic acids from tissue			Copy numbers	cell culture	nucleic acids from cell culture supernatant		
						qAngHV-1	qEPV-1	qEVEX			qAngHV-1	qEPV-1	qEVEX
267/20	Glass eel	7	1	7.31	0.30	neg (u)	neg (u)	neg (u)	–	neg	neg	neg	neg
270/20	Glass eel	10	0	6.71	0.21	neg (u)	neg (u)	neg (u)	–	neg	neg	neg	neg
272/20	Glass eel	6	1	6.85	0.27	neg (u)	neg (u)	neg (u)	–	neg	neg	neg	neg
281/20	Glass eel	10	1	7.00	0.31	neg (u)	neg (u)	neg (u)	–	neg	neg	neg	neg
290/20	Glass eel	8	1	7.06	0.31	neg (u)	neg (u)	neg (u)	–	neg	neg	neg	neg
298/20	Glass eel	10	1	7.06	0.28	neg (u)	neg (u)	pos (Ct 26)	89	pos	neg	neg	pos
299/20	Glass eel	10	1	7.15	0.27	neg (u)	neg (u)	pos (Ct 30)	2	pos	neg	neg	pos
300/20	Glass eel	10	1	7.19	0.29	neg (u)	neg (u)	pos (Ct 26)	83	pos	neg	neg	pos
301/20	Glass eel	10	1	6.91	0.25	neg (u)	neg (u)	pos (Ct 26)	111	pos	neg	neg	pos
302/20	Glass eel	10	1	6.90	0.26	neg (u)	neg (u)	pos (Ct 25)	314	pos	neg	neg	pos
347/20	Glass eel	10	0	6.74	0.19	neg (u)	neg (u)	neg (u)	–	neg	neg	neg	neg
348/20	Glass eel	10	0	6.93	0.22	neg (u)	neg (u)	neg (u)	–	neg	neg	neg	neg
349/20	Glass eel	10	0	6.97	0.22	neg (u)	neg (u)	neg (u)	–	neg	neg	neg	neg
350/20	Glass eel	10	0	6.83	0.23	neg (u)	neg (u)	neg (u)	–	neg	neg	neg	neg
346/20	Glass eel	10	0	6.67	0.18	neg (u)	neg (u)	neg (u)	–	neg	neg	neg	neg
268/20	Glass eel	13	0	6.68	0.25	neg (u)	–	–	–	–	–	–	–
269/20	Glass eel	10	0	6.84	0.26	neg (u)	–	–	–	–	–	–	–
273/20	Glass eel	10	0	6.72	0.26	neg (u)	–	–	–	–	–	–	–
274/20	Glass eel	10	0	6.78	0.26	neg (u)	–	–	–	–	–	–	–
282/20	Glass eel	10	0	6.87	0.29	neg (u)	–	–	–	–	–	–	–
283/20	Glass eel	10	0	7.16	0.27	neg (u)	–	–	–	–	–	–	–
351/20	Glass eel	10	0	6.82	0.21	neg (u)	–	–	–	–	–	–	–
352/20	Glass eel	10	0	6.92	0.20	neg (u)	–	–	–	–	–	–	–
353/20	Glass eel	10	0	6.86	0.22	neg (u)	–	–	–	–	–	–	–
354/20	Glass eel	10	0	6.57	0.18	neg (u)	–	–	–	–	–	–	–
355/20	Glass eel	10	0	6.90	0.22	neg (u)	–	–	–	–	–	–	–
356/20	Glass eel	10	0	6.73	0.19	neg (u)	–	–	–	–	–	–	–
357/20	Glass eel	10	0	6.85	0.19	neg (u)	–	–	–	–	–	–	–
291/20	Glass eel	10	0	6.91	0.29	neg (u)	–	–	–	–	–	–	–
322/20	Glass eel	10	0	–	–	neg (u)	–	–	–	–	–	–	–

TABLE 3 (Continued)

Sample designation	Developmental stage	Pool size (Individuals)	condition	size (cm)	weight (g)	nucleic acids from tissue			Copy numbers	cell culture	nucleic acids from cell culture supernatant		
						qAngHV-1	qEPV-1	qEVEX			qAngHV-1	qEPV-1	qEVEX
323/20	Glass eel	10	0	-	-	neg (u)	-	-	-	-	-	-	-
324/20	Glass eel	10	0	-	-	neg (u)	-	-	-	-	-	-	-
325/20	Glass eel	10	0	-	-	neg (u)	-	-	-	-	-	-	-
326/20	Glass eel	10	0	-	-	neg (u)	-	-	-	-	-	-	-
327/20	Glass eel	10	0	-	-	neg (u)	-	-	-	-	-	-	-
328/20	Glass eel	10	0	-	-	neg (u)	-	-	-	-	-	-	-

The samples were examined for viral infections by cell culturing and qPCR. Ct values and normalized copy numbers (template copies per ~60 ng extracted DNA or RNA) of positive samples are indicated.
Condition 0: Alive, condition 1: Dead during transport.
Abbreviations: (u), Undetectable; Neg, Negative; pos, Positive.

2.4 | Real-time PCR

Detection of AngHV-1, EPV-1 and EVEX was performed via qPCR using the TaqMan™ Fast Virus 1-Step Master Mix kit (Applied Biosystems™). PCR reactions were performed in a QuantStudio 3 (Thermo Fisher Scientific) and normalized template copies were calculated using artificial gene fragments (Eurofins Genomics). As an internal control, the β-actin housekeeping gene was amplified according to Toussaint et al., 2007 (Table 2; primer 0.2 μM and probe 0.1 μM). Samples up to a Ct value of 38 were considered positive.

AngHV-1 was detected by amplification of a fragment of the polymerase gene in accordance with Wonnemann et al., 2010. Briefly, primers and probes were deduced from AF333066 with a resulting amplification product of 93 base pairs (Table 2). The final reaction mixture contained a total volume of 20 μl consisting of 5 μl kit solution, primers and probe in a final concentration of 0.4 μM and 0.2 μM, respectively. Reactions mixtures contained 2 μl of template DNA (5–30 ng/μl). The thermal profile consisted of activation at 95°C for 15 min and PCR cycling for 60 sec at 95°C and for 60 sec at 60°C. Non-template control (water control) and isolated DNA from mock-infected cells were used as a negative control. DNA isolated from AngHV-1 isolate was used as positive control and a synthetic DNA fragment was used as a standard for quantification.

Detection of EPV-1 and EVEX was carried out as duplex RT-qPCR. Primers and probes sequences are listed in Table 2. The target for detection of EPV-1 is a fragment of the 2C gene, primers were described recently (Danne et al., 2021). EPV-1 probe was deduced from the published EPV-1 sequence NC_022332.1 (Fichtner et al., 2013). EVEX was detected via amplification of a 190 bp fragment of the L gene. Primers and probes were designed for published sequence NC_022581.1 (Galinier et al., 2012). The reaction mixtures (20 μl) contained 5 μl kit solution and EVEX primers and probe in a final concentration of 1 μM. EPV-1 primers and probe were used in a concentration of 1 μM and 0.5 μM, respectively. The reactions were supplemented with 2 μl template RNA (10–30 ng/μl). The thermal profile consisted of reverse transcription for 5 min at 50°C, inactivation for 20 s at 95°C and 45 PCR cycles for 5 sec at 95°C and for 60 sec at 58°C.

Reverse transcription PCR was performed as described recently (Danne et al., 2021) in a Mastercyler Pro S (Eppendorf) using the OneStep RT-PCR Kit (Qiagen). Resulting PCR products were sequenced at Eurofins Genomics. All PCR reaction mixtures were prepared with the molecular grade, nuclease-free water (ThermoFisher Scientific).

3 | RESULTS

3.1 | Glass eels

The mortality rate of glass eels during the transport process from the stocking destination to the laboratory was 25% (n = 89/354). 354 glass eels were prepared in 36 pool samples (Table 3). Pools

TABLE 4 Results of examinations of farmed eels

Origin	Individuals total	Pool size (individuals)	Amount of pools	Amount of positive qAngHV-1 pools	Amount of positive qEPV-1 pools	Amount of positive qEVEX-1 pools
Eel farm 1	100	10	10	10 (Ct 23 to 36) Copy numbers: 3240–2	none	none
Eel farm 2	320	10	32	26 (Ct 30 to 38) Copy numbers: 41–1	none	none
Eel farm 3	30	5	5	5 (Ct 30 to 32) Copy numbers: 100–41	none	none

The samples were examined for viral infections by cell culturing and qPCR. Ranges of Ct values and normalized copy numbers (template copies per ~60 ng extracted DNA or RNA) of positive samples are indicated.

have been analysed via cell culture and qPCR. Nucleic acid isolates of organ pools and cell culture supernatants were subjected to qPCR. Five pool samples (14%) were positive in cell culture and exhibited a CPE in the second passage of cell culturing (Table 3, Figure S1). However, the CPE did not remain in the third passage. Supernatants of first, second and third cell culture passage were subjected to qPCR and EVEX has been detected in the samples with occurring CPE. EVEX has been detected in the corresponding five pool samples via nucleic acid extraction from organ pools as well (Table 3, Figure S1). EVEX-positive pool samples contained animals, which died during the glass eel transport process to their stocking destination. The Ct values in these samples ranged from 25 to 30. The results were verified via Sanger sequencing. EPV-1 has not been detected in any sample (Table 3, Figure S2). Further, in none of the investigated pool samples, AngHV-1 has been detected via qPCR (Table 3, Figure S3).

3.2 | Farmed eels

Three sources for the farmed eels were tested: (i) 100 farmed eels (10–15 cm) from an eel farm located in the Netherlands have been investigated in ten pool samples. Every pool sample (100%) was positive for AngHV-1 (Table 4, Figure S4). The Ct values are indicated in Table S1 and ranged from 23 to 36. The samples were negative for EPV-1 or EVEX particles (Table 4, Figure S5).

(ii) Farmed eels from a company located in Germany have been checked for virus infections ($n = 320$). 26 of 32 pools (81%) were AngHV-1 positive (Table 4, Figure S6). The Ct values ranged from 30 to 38. EVEX or EPV-1 have not been detected in any sample (Table 4, Figure S5).

(iii) Further, 30 farmed eels from a third trader located in Germany have been checked for infectious diseases (Table 4). Five pool samples (100%) were positive for AngHV-1 (Table 4, Figure S7). The Ct values ranged from 30 to 32. The samples were negative for EPV-1 or EVEX particles (Table 4, Figure S5).

4 | DISCUSSION

To date, it is possible to reproduce European eels under experimental conditions (Politis et al., 2018; Sørensen et al., 2016), but further development of larval culture technology is necessary. It will take much more time until eels for stocking measures will be available from aquaculture. Hence, the stocking of eels relies on wild-caught individuals, which are directly implemented into the environment or are grown before stocking as farmed eels (Cabezas et al., 2006; Pedersen et al., 2017). Recent studies provide evidence for successful adaptation, survival, dispersal, growth and silvering of restocked eels in inland freshwaters and there are suggestions that eels will probably disappear without restocking (Félix et al., 2021; Nzau Matondo et al., 2019; Nzau Matondo et al., 2020; Nzau Matondo et al., 2021). However, in addition, a number of publications raised questions about the way in which the stocking measures are carried out and emphasized the importance of detailed planning and precise control of eel stocking. More and more considerations arise whether stocking can have negative effects on the population. A crucial aspect is the spread of pathogens through the stocking measures (Delrez et al., 2021; Haenen et al., 2009; ICES, 2018).

At this point detailed information about the health of larvae or glass eels regarding virally caused diseases is insufficient. Currently, examinations of glass eels originating from different geographic origins over 2 years revealed an excellent sanitary status (Delrez et al., 2021). But, EVEX was detected in a batch of glass eels from France intended for restocking measures in 2017 (ICES, 2018). Here, we report glass eel infection with EVEX. Strikingly, we detected EVEX in glass eel intended for restocking in 2020, which died during the transport process. Whether the virus load detected in many of these fish might indicate the clinical relevance needs to be investigated by further experiments. However, these results clearly point out the need for separating and screening of the fish batches at the capture site.

It was proposed earlier that the farming process of eels is associated with an increasing risk of viral infections and AngHV-1 is usually detected in eels originating from a commercial eel farm (Haenen et al., 2009; Kullmann et al., 2017). Suitable to these assumptions we detected AngHV-1 with a high prevalence in animals purchased from three companies of interest. Conspicuously, despite a valid health certificate. The viral loads in these animals were divergent, but some of them were harbouring the significant number of virus particles, which could indicate the lytic phase of the infection, which is associated with virus shedding. As a consequence, animals were not used for stocking measures. These results demonstrate the need for precise investigations of eels with appropriate molecular

methods and a suitable sample size. Recently, a study by Kullmann et al. (2017) provides evidence that uncontrolled stocking measures with AngHV-1 positive farmed eels in the Schlei fjord in Germany introduced AngHV-1 into the local population.

A recent study investigated the impact of short-term quarantine conditions on the general conditions of glass eels from different geographical origins in order to force optimization and reduction in associated risks of restocking (Delrez et al., 2021). A quarantine program is already implemented in stocking measures in other countries for example Sweden. Delrez et al. found that a short-term quarantine did not affect the general fitness conditions of the animals. As we found, that a significant number of animals died during the process of the glass eel transport, a restocking strategy based on a quarantine-precaution measure might have prevented the stocking of animals carrying pathogenic eel viruses. However, the key point is that it is currently unknown how, and at what stage of development, eels are infected with pathogens. Answering this question seems to be critical for future planning of restocking measures and combating the anthropogenic spread of infectious diseases. As we detected pathogenic eel viruses in glass eels, another decisive question arises: are the animals infected during the transport process from the catch location? Spreading of infectious agents might be caused by contaminated water, transport vehicles or keeping tanks, and virus-positive individuals.

Taken together, restocking measures aim to counteract the decline of the European Eel panmictic population. It needs to take into account that if not properly controlled the stocking might carry a risk of unintentional spread of pathogens. Future research involving the modelling of risk factors is likely to provide guidance on reducing stocking-associated disease risks. This would help minimize the possibility that stocking practices would interfere with native populations via accidental disease introduction.

ACKNOWLEDGEMENTS

We thank Karin Camara for the critical reading of the manuscript and Jakob Gährken for his commitment during fieldwork and sample collection. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

There are no conflicts of interest to declare for any of the authors.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

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REFERENCES

Borzák, R., Sellyei, B., Székely, C., & Doszpoly, A. (2017). Molecular detection and genome analysis of circoviruses of European eel (*Anguilla Anguilla*) and sichel (*Pelecus cultratus*). *Acta Veterinaria Hungarica*, *65*(2), 262–277. https://doi.org/10.1556/004.2017.026

Cabezas, S., Calvete, C., & Moreno, S. (2006). Vaccination success and body condition in the European wild rabbit: Applications for conservation strategies. *Journal of Wildlife Management*, *70*(4), 1125–1131. https://doi.org/10.2193/0022-541X(2006)70[1125:VSABCI]2.0.CO;2

Chen, S.-N., Ueno, Y., & Kou, G.-H. (1982). A cell line derived from Japanese eel (*Anguilla japonica*) kidney. *Proceedings of the National Science Council, Republic of China/a*, *6*, 93–100.

Cresci, A. (2020). A comprehensive hypothesis on the migration of European glass eels (*Anguilla Anguilla*). *Biological Reviews of the Cambridge Philosophical Society*, *95*(5), 1273–1286. https://doi.org/10.1111/brv.12609

Cresci, A., Durif, C. M., Paris, C. B., Shema, S. D., Skiftesvik, A. B., & Browman, H. I. (2019). Glass eels (*Anguilla Anguilla*) imprint the magnetic direction of tidal currents from their juvenile estuaries. *Communications Biology*, *2*, 366. https://doi.org/10.1038/s42003-019-0619-8

Danne, L., Horn, L., Feldhaus, A., Fey, D., Emde, S., Schütze, H., Adamek, M., & Hellmann, J. (2021). Virus infections of the European eel in North Rhine Westphalian rivers. *Journal of Fish Diseases*, *45*, 69–76. https://doi.org/10.1111/jfd.13536

Dekker, W. (2000). The fractal geometry of the European eel stock. *ICES Journal of Marine Science*, *57*(1), 109–121. https://doi.org/10.1006/jmsc.1999.0562

Delrez, N., Zhang, H., Lieffrig, F., Mélard, C., Farnir, F., Boutier, M., Donohoe, O., & Vanderplasschen, A. (2021). European eel restocking programs based on wild-caught glass eels: Feasibility of quarantine stage compatible with implementation of prophylactic measures prior to scheduled reintroduction to the wild. *Journal for Nature Conservation*, *59*, 125933. https://doi.org/10.1016/j.jnc.2020.125933

Doszpoly, A., Tarján, Z. L., Glávits, R., Müller, T., & Benkő, M. (2014). Full genome sequence of a novel circo-like virus detected in an adult European eel *Anguilla Anguilla* showing signs of cauliflower disease. *Diseases of Aquatic Organisms*, *109*(2), 107–115. https://doi.org/10.3354/dao02730

Félix, P. M., Costa, J. L., Quintella, B. R., Almeida, P. R., Monteiro, R., Santos, J., Portela, T., & Domingos, I. (2021). Early settlement and growth of stocked European glass eels in a fragmented watercourse. *Fisheries Management and Ecology*, *28*(1), 91–100. https://doi.org/10.1111/fme.12461

Fichtner, D., Philipps, A., Groth, M., Schmidt-Posthaus, H., Granzow, H., Dauber, M., Platzer, M., Bergmann, S. M., Schrudde, D., Sauerbrei, A., & Zell, R. (2013). Characterization of a novel picornavirus isolate from a diseased European eel (*Anguilla Anguilla*). *Journal of Virology*, *87*(19), 10895–10899. https://doi.org/10.1128/JVI.01094-13

Galinier, R., van Beurden, S., Amilhat, E., Castric, J., Schoehn, G., Verneau, O., Fazio, G., Allienne, J.-F., Engelsma, M., Sasal, P., & Faliex, E. (2012). Complete genomic sequence and taxonomic position of eel virus European X (EVEX), a rhabdovirus of European eel. *Virus Research*, *166*(1–2), 1–12. https://doi.org/10.1016/j.virusres.2012.02.020

Haenen, O., van Ginneken, V., Engelsma, M., & van den Thillart, G. (2009). Impact of eel viruses on recruitment of European eel. In G. van den Thillart, S. Dufour, & J. C. Rankin (Eds.), *Spawning migration of the European eel* (pp. 387–400). Springer Netherlands. https://doi.org/10.1007/978-1-4020-9095-0_16

Harrison, A. J., Walker, A. M., Pinder, A. C., Briand, C., & Aprahamian, M. W. (2014). A review of glass eel migratory behaviour, sampling techniques and abundance estimates in estuaries: Implications for assessing recruitment, local production and exploitation. *Reviews in Fish Biology and Fisheries*, *24*(4), 967–983. https://doi.org/10.1007/s11160-014-9356-8

ICES (2016). *Report of the working group on eels (WGEEL), 15–22 September 2016, Cordoba, Spain*. ICES CM 2016/ACOM:19. 107 pp. https://doi.org/10.17895/ICES.PUB.5626

ICES (2018). *Report of the joint EIFAAC/ICES/GFCM working group on eels (WGEEL), 5–12 September 2018, Gdańsk, Poland*. ICES CM 2018/ACOM:15. 152 pp.

ICES (2021). *European eel (Anguilla Anguilla) throughout its natural range*. In report of the ICES advisory committee, 2021. ICES advice 2021, ele.2737. Nea https://doi.org/10.17895/ICES.ADVICE.7752

Kleckner, R. C., & McCleave, J. D. (1988). The northern limit of spawning by Atlantic eels (*Anguilla spp.*) in the Sargasso Sea in relation to thermal fronts and surface water masses. *Journal of Marine Research*, *46*(3), 647–667. https://doi.org/10.1357/002224088785113469

Kobayashi, T., & Miyazaki, T. (1996). Rhabdoviral dermatitis in Japanese eel, Anguilla japonica. *Fish Pathology*, *31*(4), 183–190. https://doi.org/10.3147/jsfp.31.183

Kullmann, B., Adamek, M., Steinhagen, D., & Thiel, R. (2017). Anthropogenic spreading of anguillid herpesvirus 1 by stocking of infected farmed European eels, *Anguilla Anguilla* (L.), in the Schlei fjord in northern Germany. *Journal of Fish Diseases*, *40*(11), 1695–1706. https://doi.org/10.1111/jfd.12637

Miller, M. J., Bonhommeau, S., Munk, P., Castonguay, M., Hanel, R., & McCleave, J. D. (2015). A century of research on the larval distributions of the Atlantic eels: A re-examination of the data. *Biological Reviews of the Cambridge Philosophical Society*, *90*(4), 1035–1064. https://doi.org/10.1111/brv.12144

Miller, M. J., Westerberg, H., Sparholt, H., Wysujack, K., Sørensen, S. R., Marohn, L., Jacobsen, M. W., Freese, M., Ayala, D. J., Pohlmann, J.-D., Svendsen, J. C., Watanabe, S., Andersen, L., Møller, P. R., Tsukamoto, K., Munk, P., & Hanel, R. (2019). Spawning by the European eel across 2000 km of the Sargasso Sea. *Biology Letters*, *15*(4), 20180835. https://doi.org/10.1098/rsbl.2018.0835

Munk, P., Hansen, M. M., Maes, G. E., Nielsen, T. G., Castonguay, M., Riemann, L., Sparholt, H., Als, T. D., Aarestrup, K., Andersen, N. G., & Bachler, M. (2010). Oceanic fronts in the Sargasso Sea control the early life and drift of Atlantic eels. *Proceedings Biological Sciences*, *277*(1700), 3593–3599. https://doi.org/10.1098/rspb.2010.0900

Nzau Matondo, B., Benitez, J.-P., Dierckx, A., Rollin, X., & Ovidio, M. (2020). An evaluation of restocking practice and demographic stock assessment methods for cryptic juvenile European eel in upland Rivers. *Sustainability*, *12*(3), 1124. https://doi.org/10.3390/su12031124

Nzau Matondo, B., Delrez, N., Bardonnet, A., Vanderplasschen, A., Joaquim-Justo, C., Rives, J., Benitez, J., Dierckx, A., Séleck, E., Rollin, X., & Ovidio, M. (2021). 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. *The Science of the Total Environment*, *807*, 151020. https://doi.org/10.1016/j.scitotenv.2021.151020

Nzau Matondo, B., Séleck, E., Dierckx, A., Benitez, J.-P., Rollin, X., & Ovidio, M. (2019). What happens to glass eels after restocking in upland rivers? A long-term study on their dispersal and behavioural traits. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *29*(3), 374–388. https://doi.org/10.1002/aqc.3062

Okamoto, N., Sano, T., Hedrick, R. P., & Fryer, J. L. (1983). Antigenic relationships of selected strains of infectious pancreatic necrosis virus and European eel virus. *Journal of Fish Diseases*, *6*(1), 19–25. https://doi.org/10.1111/j.1365-2761.1983.tb00047.x

Pao, H.-Y., Wu, C.-Y., & Wen, C. M. (2019). Persistent development of adomavirus and aquareovirus in a novel cell line from marbled eel with petechial skin haemorrhage. *Journal of Fish Diseases*, *42*(3), 345–355. https://doi.org/10.1111/jfd.12939

Pedersen, M. I., Jepsen, N., & Rasmussen, G. (2017). Survival and growth compared between wild and farmed eel stocked in freshwater ponds. *Fisheries Research*, *194*, 112–116. https://doi.org/10.1016/j.fishres.2017.05.013

Politis, S. N., Sørensen, S. R., Mazurais, D., Servili, A., Zambonino-Infante, J. L., Miest, J. J., Clemmesen, C. M., Jonna Tomkiewicz, J., & Butts, I. A. E. (2018). Molecular ontogeny of first feeding European eel larvae. *Frontiers in Physiology*, *9*, 1477. https://doi.org/10.3389/fphys.2018.01477

Sano, T. (1976). Viral diseases of cultured fishes in Japan. *Fish Pathology*, *10*(2), 221–226. https://doi.org/10.3147/jsfp.10.221

Sano, M., Fukuda, H., & Sano, T. (1990). Isolation and characterization of a new herpesvirus from eel. In *Pathology in marine science* (pp. 15–31). Elsevier. https://doi.org/10.1016/B978-0-12-550755-4.50008-2

Sano, T., Nishimura, T., Okamoto, N., & Fukuda, H. (1977). Studies on viral diseases of Japanese fishes. VII. A rhabdovirus isolated from European eel, *Anguilla Anguilla*. *Nippon Suisan Gakkaishi*, *43*(5), 491–495. https://doi.org/10.2331/suisan.43.491

Schoth, M., & Tesch, F.-W. (1982). Spatial distribution of 0-group eel larvae (Anguilla sp.) in the Sargasso Sea. *Helgoländer Meeresuntersuchungen*, *35*(3), 309–320. https://doi.org/10.1007/BF02006139

Sørensen, S. R., Tomkiewicz, J., Munk, P., Butts, I. A. E., Nielsen, A., Lauesen, P., & Graver, C. (2016). Ontogeny and growth of early life stages of captive-bred European eel. *Aquaculture*, *456*, 50–61. https://doi.org/10.1016/j.aquaculture.2016.01.015

Tesch, F.-W., & Thorpe, J. E. (2003). *The eel*. Wiley. https://doi.org/10.1002/9780470995389

Toussaint, J. F., Sailleau, C., Breard, E., Zientara, S., & De Clercq, K. (2007). Bluetongue virus detection by two real-time RT-qPCRs targeting two different genomic segments. *Journal of Virological Methods*, *140*(1–2), 115–123. https://doi.org/10.1016/j.jviromet.2006.11.007

Van Beurden, S. J., Engelsma, M. Y., Roozenburg, I., Voorbergen-Laarman, M. A., van Tulden, P. W., Kerkhoff, S., van Nieuwstadt, A. P., Aart Davidse, A., & Haenen, O. L. M. (2012). Viral diseases of wild and farmed European eel *Anguilla Anguilla* with particular reference to The Netherlands. *Diseases of Aquatic Organisms*, *101*(1), 69–86. https://doi.org/10.3354/dao02501

Van Ginneken, V., & Maes, G. E. (2005). The European eel (*Anguilla Anguilla*, Linnaeus), its lifecycle, evolution and reproduction: A literature review. *Reviews in Fish Biology and Fisheries*, *15*(4), 367–398. https://doi.org/10.1007/s11160-006-0005-8

Van Nieuwstadt, A. P., Dijkstra, S. G., & Haenen, O. L. (2001). Persistence of herpesvirus of eel herpesvirus anguillae in farmed European eel *Anguilla Anguilla*. *Diseases of Aquatic Organisms*, *45*(2), 103–107. https://doi.org/10.3354/dao045103

Wen, C. M., Chen, M. M., Wang, C. S., Liu, P. C., & Nan, F. H. (2016). Isolation of a novel polyomavirus, related to Japanese eel endothelial cell-infecting virus, from marbled eels, *Anguilla marmorata* (Quoy & Gaimard). *Journal of Fish Diseases*, *39*(7), 889–897. https://doi.org/10.1111/jfd.12423

Wonnemann, H., Lemcke, R., & Bergmann, S. M. (2010). *Untersuchung zur Verbreitung von Herpesvirus Anguilla (HVA) in Schleswig-Holstein mittels real-time PCR: AVID-Tagung Virologie 15-17 September 2010, Kloster Banz*.

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How to cite this article: Danne, L., Adamek, M., Wonnemann, H., Pieper, T., Fey, D., & Hellmann, J. (2022). Identification of virus infections of European eels intended for stocking measures. *Journal of Fish Diseases*, *45*, 1259–1266. https://doi.org/10.1111/jfd.13658