Global Ecology and Conservation 24 (2020) e01245

Contents lists available at ScienceDirect

Global Ecology and Conservation

journal homepage: http://www.elsevier.com/locate/gecco

Original Research Article

# Environmental DNA analysis indicates that migration barriers are decreasing the occurrence of European eel (*Anguilla anguilla*) in distance from the sea

Silje Halvorsen [a, *], Lars Korslund [a], Per Ø. Gustavsen [b], Audun Slettan [a]

[a] Centre of Coastal Research, Department of Natural Sciences, University of Agder, P.O. Box 422, Kristiansand, NO-4604, Norway
[b] Gustavsen Naturanalyser, Varpevegen 26, 3728, Skien, Norway

ARTICLE INFO

*Article history:*
Received 19 March 2020
Received in revised form 23 August 2020
Accepted 23 August 2020

*Keywords:*
Environmental DNA
*Anguilla Anguilla*
Passage barriers
Conservation
Real-time PCR

ABSTRACT

The European eel (*Anguilla* L.) is considered critically endangered by the IUCN Red List, and recruitment remains low. One of the challenges for the species today is migration barriers that limit their habitat. Along the Norwegian coast, like in other countries, the abundance of eels appears to decrease with distance from the sea. This pattern may be a result of factors like water temperature, water quality, competition, and habitat suitability and availability. This study aims to use environmental DNA (eDNA) analysis to investigate the potential relationship between migration barriers and the decreasing occurrence of eels in distance from the sea by the coast of southern Norway. Sixty locations with potential migration barriers are investigated by collecting water samples upstream and downstream from each construction before eDNA from each sample is isolated and analyzed by real-time PCR with specific primers and probes matching *A. anguilla*. The results reveal that the probability of detecting eel eDNA decreases significantly with number of hydroelectric power stations and their associated basins, even when the effect of distance to sea is accounted for. In addition, there is a clear border at which eel eDNA could no longer be detected upstream of the major watercourses. Therefore, it is likely that the migration of eels is prohibited by these constructions, which seem to constitute a much greater challenge than every other type of potential migration barrier investigated in this study.


## 1. Introduction

Freshwater ecosystems provide homes for a considerable number of the world's species but are threatened by human activities that change the environment on local and global scales. Many of these alterations have led to dramatic changes in biodiversity, and few fish stocks have been more affected than the European eel (*Anguilla* L.) (Dekker and Beaulaton, 2016). The European eel stock has been reduced by approximately 90% since the 1980s, and they are now considered critically endangered by thssee IUCN Red List (Jacoby and Gollock, 2014). The negative development has been attributed to a combination of factors like overfishing and aquaculture (Castonguay et al., 1994), parasites (Feunteun, 2002), poisoning (Belpaire et al., 2009), seaic changes and global warming (Drouineau et al., 2018; Friedland et al., 2007), and the destruction of habitat and building of dams and barriers (Kettle et al., 2011). In 2007, the European Union (EU) proposed a regulation plan regarding

* Corresponding author.
*E-mail address:* silje.halvorsen@uia.no (S. Halvorsen).

https://doi.org/10.1016/j.gecco.2020.e01245

the restoration of the eel stock (Council regulation No 1100/2007). Every member country of the EU imposed secure conservation plans to reduce human-made mortality with the aim of increasing the stock by 40% of the historical biomass of silver eels (Anonymous, 2007). The conservation effort has contributed to a stop in the decreasing trend of the stock since 2011, and time-series from 1980 to 2019 reveal an increasing trend from 2011 to 2019 (ICES, 2019). However, in 2019, the recruitment of glass eel from the sea was only 1.4% of the historical level in the North Sea and 6.0% elsewhere in Europe compared to 1960–1979 (ICES, 2019). Considering the low recruitment in 2019, improvements and continuous conservation efforts are still needed.

The European eel larvae migrate over 5000 km from the spawning area in the Sargasso Sea to Europe and North Africa and metamorphose into glass eel (Righton et al., 2016). Most of the eels migrate upstream to freshwater systems, but some stay in marine and brackish water. Here they start their pigmentation and are called "elvers" until fully pigmented into yellow eels. The eels undergo the last metamorphosis into silver eels on their final migration back to the spawning area after approximately 20 years in the coastal habitats (Vøllestad, 1992).

In the freshwater systems, the probability of eel occurrence decreases significantly with increasing distance from the sea (Degerman et al., 2019; Ibbotson et al., 2002). In Norway, 46% of every registered area with an abundance of eels is located within 5 km from the sea, and 42% of lakes with eels are found less than 50 m above sea level (Thorstad et al., 2010). Distance is acknowledged as an explanation for the distribution pattern, in addition to factors like temperature (Ogden, 1970) and water quality (Degerman et al., 2019). Competition can also explain the pattern in which low population density leads to low competition for resources, thus failing to drive individuals further upstream (Arai, 2016; Ibbotson et al., 2002). Also, suitability and accessibility of habitats defines the inland abundance of eels (Laffaille et al., 2009). Habitat accessibility depends on free-flowing rivers and streams and could be restricted by migration barriers. The number of migration barriers has shown to have a more significant reduction effect on eel abundance than distance alone (White and Knights, 1997). Construction in freshwater systems escalated in the 1950–1960s, leading to about 50–90% habitat loss for eels in Europe (Feunteun, 2002; Tesch, 1977). Migration barriers taller than 50–60% of the eels' body length, or barriers that cause high flow rate or velocity, could prohibit further upstream migration (Porcher, 2002; Thorstad et al., 2010). Examples of such barriers are fish ladders, pipelines, basins, and hydroelectric power stations. Migration downstream is especially threatened by turbines connected to power stations. The results of several studies indicate that 52% of eels passing the turbines suffer injury or death (Thorstad et al., 2010).

Human-made modifications of freshwater systems constitute a threat to the European eel that should be possible to reduce. The first step in the conservation effort is to investigate possible connections between eel distribution and potential barriers. Traditional methods for species detection in aquatic environments are fishing, traps, or observation of the organisms. These methods can be challenging when investigating species with different life stages (like eggs or larvae), especially for rare species. Studies of environmental DNA (eDNA) could solve the challenges associated with traditional methods. eDNA is DNA that organisms shed into the environment, such as cells or tissue, and analyses of water for species-specific eDNA have increasingly become a tool for the detection of aquatic organisms (Rees et al., 2014). Because eDNA is shed into the water, the organism can potentially be detected in an analysis of a water sample with polymerase chain reaction (PCR) with specific primers and probes (Thomsen and Willerslev, 2015). The method gives more precise and objective results than many of the traditional methods (Strickler et al., 2015; Thomsen and Willerslev, 2015). It is also suited for the detection of rare species like the European eel, which could be difficult to catch or observe. eDNA could, therefore, be an advisable tool for investigating the abundance and distribution of the European eel.

This study aims to use eDNA analysis to investigate the potential relationship between migration barriers and the decreasing occurrence of eels in distance from the sea by the coast of southern Norway.

## 2. Materials and methods

### 2.1. Water sampling

We sampled water at 60 locations with potential migration barriers in the county Agder, in southern Norway. The sites were sorted into five categories of potential migration barriers—pipelines, tunnels and bridges; fish ladders; basins (separated with small dams, such as those for drinking water); hydroelectric power stations with associated water basins; or natural/unknown barriers. The positions for each site are shown in Fig. 2; GPS coordinates are listed in Table 1, Appendix A. We collected samples 1–26 in June 2018, the remaining in June–August 2019, both upstream and downstream of each potential barrier. One sample consists of 1 L of water, collected by combining 20 samples of each 50 ml from surface water. Each sample is collected within an area of 1–50 m along the river, stream, or lake. New 50 ml sterile Falcon centrifuge tubes and gloves were used for each sampling to prohibit contamination. The samples were stored on ice until filtration, which was performed within 5 h. The 1 L bottles were rinsed in 10% chlorine for 10 min followed by several washes with water before sampling. We used water from the location where samples were collected if the bottles were reused in the field. We filtered 150–900 ml water (as much as possible) from the samples through a cellulose nitrate filter with 0.45 μm pore size (Thermo Scientific Nalgene) by an ILMVAC vacuum pump (GmbH). After filtration, the filters were folded three times via tweezers and stored in a 1.5 ml Microcentrifuge tube at −20 °C until DNA was isolated.

### 2.2. eDNA isolation

We extracted eDNA from the filters by DNeasy® Blood and Tissue Kit (Qiagen) and bead beating, using the method described by Thomsen et al. (2012). After isolation, the eDNA was stored in microcentrifuge tubes at −20 °C. We performed the eDNA isolation in a separate room from the PCR amplification. Every sample was analyzed by a spectrophotometer (NanoDrop™ One, Thermofisher) after isolation to examine purity and eDNA-concentration.

### 2.3. PCR amplification

We examined eDNA extract for *A. anguilla* DNA using a real-time PCR assay with TaqMan®-probe and primers specific for a section of the *cytb* gene in the mitochondria. The primers were designed with Primer-BLAST at the web page of the National Center for Biotechnology Information (NCBI) and the program Primer Express 3.0.1 (Thermo Fisher). The sequences were as follows: "Alcyt forward": 5′-CACCCATACTTCTCCTACAAAGACCTA-3′, and "Alcyt reverse": 5′-TCTGGGTCTCCAAGCAGGTT-3' (101 bp product) and the probe: 5-FAM-TTCATTATCATGCTCACC-MGBEQ-3'. The primers and probe were tested for species-specificity by searching for homology to DNA sequences from species that could be found in the same area by using Clustal Omega (European Bioinformatics Institute) and NCBI's GenBank.

PCR-mix had the following ingredient concentrations: 1 x TaqMan Environmental Master Mix (Applied Biosystems), 0.9 μM Alcyt forward, 0.9 μM Alcyt reverse, and 0.55 μM probe. 20 μl PCR mix with 5 μl template was transferred to a 0.1 ml Micro Fast Tube Strips (Thermo Fisher). We conducted real-time PCR on a StepOnePlus™ Real-time PCR System (Applied Biosystem) with a temperature profile of 50 °C in 2 min and 95 °C in 10 min, followed by 60 cycles of 96 °C in 15 s, 57 °C in 30 s, and 72 °C in 30 s, with fluorescence detection after each cycle. We analyzed every sample in triplicates. A sample containing tissue of genomic DNA from *A. anguilla* was used as a positive control, and PCR-grade $H_2O$ was used as a negative control. Positive and negative controls were included in all runs. A selected number (26) of the locations were analyzed for the abundance of brown trout (*Salmo trutta*) as an additional control for false negatives, as trout are expected to be present in almost every location. If a sample is negative for eel DNA and positive for trout DNA, the result is not a false negative. These analyses had the same PCR conditions as for European eel but with species-specific primers and probes matching *S. trutta*. The primers, specific for a section of the *cytb* gene in the mitochondria, and probe were designed with the same tools as for European eel. The sequences were as follows: Stcyt-F: 5′-CCACCCCTACTTCTCATA-3′, Stcytb-R: 5′-GGAGGTTGGGTGCGAA-TAGA-3' (88 bp product), and probe: 5′- FAM-CTTGGATTCGTAGCTAT-MGBEQ -3'.

The real-time PCR results were analyzed by the software provided by the StepOnePlus™ Real-time PCR System.

To conclude that the analyzed sample contains *A. anguilla* DNA, at least one of the PCR triplicates needs to be positive. One amplicon from a selected sample was Sanger-sequenced (by Eurofins Genomics, Germany) to verify that *A. anguilla* is detected.

### 2.4. Statistical analysis

To investigate if the probability of *A. Anguilla* eDNA presence at each location could be explained by the occurrence of migration barriers, we tested a set of logistic regression models, and a stepwise forward model selection procedure was applied. We started with the null-model (with no variables) and tested if the addition of each new variable improved the model sufficiently. We wanted to separate the effect of barriers from the effect of the distance eels needed to migrate to reach a location. Therefore, the distance from the location to the sea, in km, was included as a co-variate and kept in all subsequent models. The cumulative number of each of the five types of barriers (see Table 1) downstream of the location (i.e. the number of each type of barrier an eel would have to pass to reach the location) was used as potential variables in the model selection. The separate effect of each type of barrier was tested by including each of these variables in the model, one by one, and comparing the performance of the model with and without the variable in question, by comparing the residual deviance and degrees of freedom of the two models using a Chi-square test and a significance level of 0.05). This procedure was repeated until all variables that significantly improved the model fit was included in the model. The statistical analysis was performed in R ("R Core Team," 2017).

**Table 1**
Locations where eDNA from European eel is detected and not detected, sorted by the different types of migration barriers.

| Type of barrier | Detected upstream and downstream | Not detected upstream, but detected downstream | Not detected upstream or downstream |
|---|---|---|---|
| Pipelines, tunnels, and bridges | 1, 4, 5, 6, 8, 9, 10, 11, 14, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 30, 49 | 15, 50 | 2, 13, 17 |
| Fish ladders | 3, 7, 12 | | |
| Basins | 27, 43, 45, 46 | | |
| Hydroelectric power stations and associated water basins | 34, 38, 59 | 28, 29, 31, 35, 58 | 32, 33, 36, 37, 57 |
| Natural barriers/unknown | 41, 42, 44, 47, 48, 51, 56, 60 | 39, 40, 52, 53, 54, 55 | |

## 3. Results

A summary of the results, where the detection of eDNA from *A. anguilla* is sorted by the different types of migration barriers, is provided in Table 1. In the five different categories of migration barriers, most locations are found in "Pipelines, tunnels and bridges" (26) followed by "Natural barriers/unknown" (14) and "Hydroelectric power stations and associated water basins" (13). There were few locations in the category of "Basins" (4) and "Fish ladders" (3).

As the distance to sea increased, the probability of detecting European eel eDNA decreased (Fig. 1A), and this effect was highly significant ($\chi^2 = 21.8$, df = 1, $p < 0.001$). The one category of barriers that had the strongest effect on the probability of detecting eel eDNA was hydroelectric power stations, even when the effect of distance to sea was accounted for. As the number of such barriers increased, the probability of detecting eel eDNA decreased significantly ($\chi^2 = 9.19$, df = 1, $p = 0.002$). The probability of detection dropped 26% going from 3 to 4 such barriers, and the probability of detection dropped close to zero when the number of such barriers reached seven (Fig. 1B). As the number of natural barriers increased, the probability of detecting eel eDNA decreased, but the pattern was not consistent (Fig. 1C) and this effect was not significant ($\chi^2 = 3.22$, df = 1, $p = 0.07$). There was a slight decrease in the probability of detecting eel eDNA with an increasing number of pipelines, tunnels or bridges (Fig. 1D), but this pattern was far from clear and the effect was not strong enough for this variable to be significant ($\chi^2 = 2.25$, df = 1, $p = 0.13$). In fact, at the only two locations positioned upstream of two such barriers, the water sample was positive (Fig. 1D). The number of basins downstream of a location had no effect on the probability of detecting eel eDNA ($\chi^2 = 0.01$, df = 1, $p = 0.95$). Only seven locations were positioned above one or two basins, and among these, only one was negative (Fig. 1E). The total number of fish ladders in the dataset was only three, and due to lack of data the models with number of fish ladders included as a variable did not converge. Therefore, only the observed data are presented (Fig. 1F).

The probability of detecting *A. Anguilla* eDNA was significantly reduced as one moved inward from the coast (Fig. 2). The water area upstream from site 34 is colored yellow in the figure, despite positive PCR-results (Appendix A), because eDNA

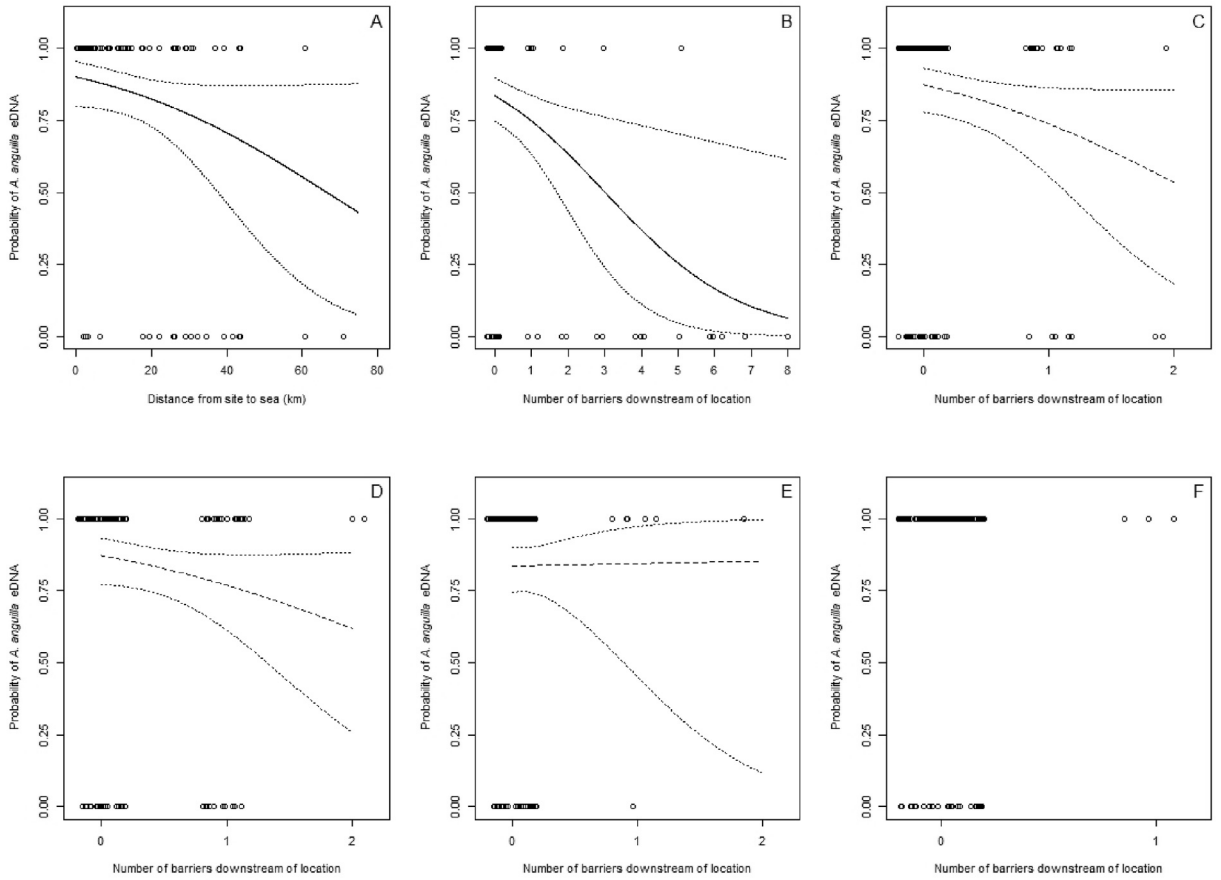


**Fig. 1:.** Predicted change in probability of detecting *A. anguilla* eDNA with the different predictor variables. Solid and dashed lines represent mean probability of significant and non-significant explanatory variables, respectively. Dotted lines represent the 95% confidence interval limits. Circles show observed data (positive or negative eDNA sample). A small amount of random noise is added to the observed data along the x-axis in order to prevent overplotting. A: Distance to sea in km, B: Number of hydroelectric power stations, C: Number of natural barriers, D: Number of pipelines, tunnels or bridges, E: Number of basins, F: Number of Fish ladders. Due to lack of data on fish ladders the model with this variable did not converge, and therefore only observed data are presented here.

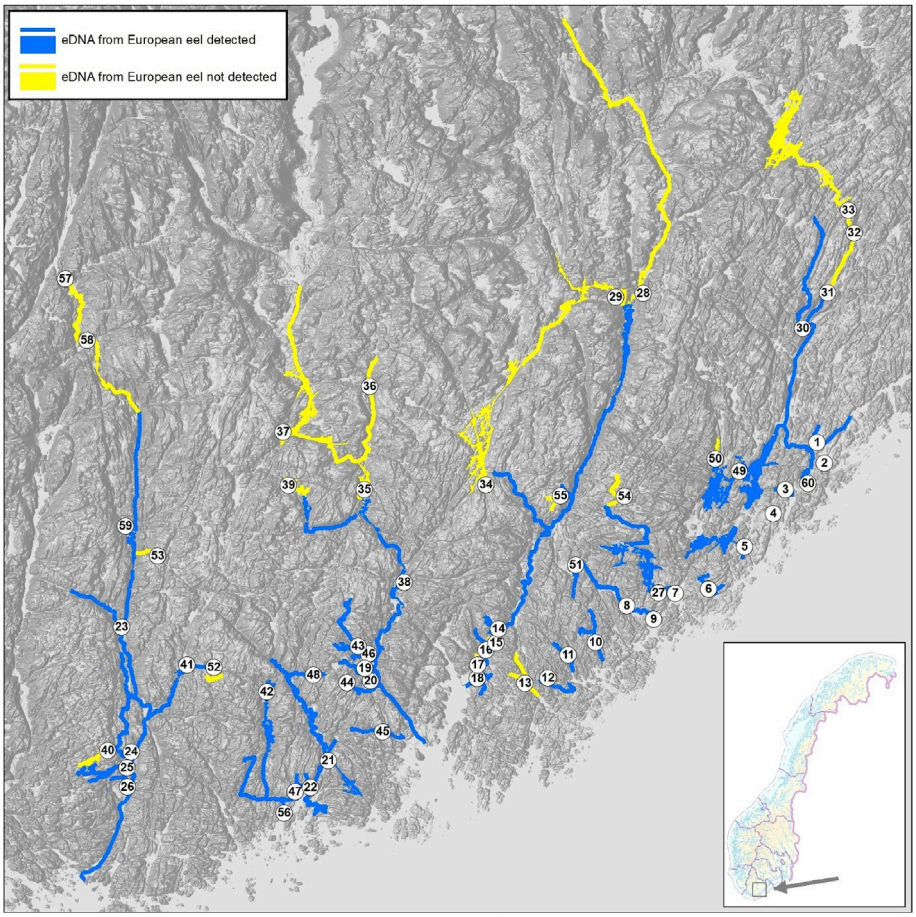


**Fig. 2.** Water samples collected at 60 locations in the county Agder, south of Norway. Watercourses where eDNA from European eel is detected are colored blue. Watercourses where eDNA from European eel is not detected are colored yellow. Illustration: Per Ø. Gustavsen. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

from European eel was not detected in the connecting waters upstream (at locality 29). Also, eDNA from eels was only recorded in one of the three samples in the PCR-triplicate upstream of location 34. Site 58 is near a basin at a hydroelectric power station. The downstream sample (eel detected) was collected downstream of the basin and upstream sample (eel not detected) was, due to difficult terrain, collected some hundred meters above the basin. The river was flowing slowly above the basin with no barriers. Therefore, the change in color at this site in Fig. 2 is placed by the barrier, not the upstream sample collection site. Detailed data for every location is found in Table 1, Appendix A.

The DNA sequence of a selected amplicon matched 100% with the corresponding region of the *cytb* gene of *A. anguilla* found in the NCBI GenBank (data not shown). There was a low degree of homology between the primers (Alcyt forward and Alcyt reverse) and probe with the corresponding sequence of other species that might be found in the same locations (Table 2, Appendix A) which secures a high degree of uniqueness. eDNA from trout was detected in 25 of the 26 water samples selected as controls. The concentration and purity of the samples analyzed in the spectrophotometer vary but had acceptable values (data not shown).

## 4. Discussion

### *4.1. Water basins connected to hydroelectric power stations constitute the greatest upstream migration challenge for European eel*

We have shown that in general, as distance from the sea to a given location decreases the probability to record eel eDNA increases. In addition, the probability of detecting eDNA from European eel decreases significantly with number of hydroelectric power stations and their water basins, even when the effect of distance to sea is accounted for. The probability of detecting eDNA from eels upstream of seven of such barriers are close to zero. Therefore, these constructions seem to constitute upstream migration challenges for eels.

There are four major watercourses examined in this study in which eDNA from European eel is detected to a specific border upstream. Location 31 is a hydroelectric power station called Evenstad (~26 km from the sea) where eels are detected downstream but not upstream of the construction. This indicates that eel migration is prohibited. eDNA from European eel is not recorded in the upstream samples of locations 32 and 33 either, which are upstream from location 31.

In the second major watercourse, eDNA from eels is not detected in the upstream samples of sites 28 and 29. These sites are located upstream of the hydroelectric power station Hanefoss (~43 km from the sea), which therefore constitutes a passage barrier. Location 28 and 29, in addition to 34, empties into the same river downstream (Topdalselva). Even though there is approximately 6–9 km between sites 28–29 and 34, they are also connected by waterways upstream. Therefore, the power station Hanefoss also prohibits eel migration to location 34. However, it is also possible for eels to reach site 34 from the opposite direction (where the upstream sample is collected), which has possible natural barriers downstream. eDNA from European eel is detected in only one of three samples in the PCR-triplicate upstream of location 34, which may indicate low eel eDNA-concentration and a possible low abundance of eels. It is likely that some eels can migrate to this water area while most are unable.

At location 35, in the third major watercourse, eDNA from European eel is detected downstream of the power station Nomeland (~35 km from the sea). This indicates that eel migration is prohibited. There are, however, two additional stations further downstream the river system—Steinsfoss and Hunsfoss—where eDNA from eels is recorded upstream. These constructions are likely to cause challenges for migrating eels, even though some can pass.

In the fourth major watercourse in this study, we have detected a potential barrier hindering eel migration downstream of location 58. This location is linked to the power stations Bjelland and Håverstad (~61 km from the sea). eDNA from European eel is not recorded at location 57 either, which is upstream from location 58. Downstream of the mentioned power stations, there is an additional station called Laudal where eels can pass.

### 4.2. Constructions can constitute passage barriers even though eDNA from eels is recorded upstream

European eel can pass some of the power stations and water basins but are prohibited by others, probably due to differences between the constructions and between individual eels. The different designs of the structures, such as high or low walls of the water basins, can create distinct degrees of challenges for migrating eels. In addition, some power stations will have possibilities to pass by alternative routes, e.g., nearby streams or fish ladders, while some will not. Individual differences between eels, especially body size, will also affect their ability to migrate past the barriers. Obstacles creating high water velocity will prevent migration of the smallest individuals, and even barriers taller than 50–60% of the eel's body length is likely to prohibit migration (Thorstad et al., 2010). The differences between the constructions and between individual eels may be the reason why eel abundance decreases with number of hydroelectric power stations and their basins. Accordingly, some constructions limit migration despite the detection of eDNA from the species upstream. Knowledge regarding biomass of eels upstream of the installations could illuminate the degree of challenges connected to each migration barrier. Still, such information is not obtained in this study. Quantification of populations by eDNA has been performed by comparing eDNA concentration with biomass data from fishing or trapping methods. Such correlations are found in studies of different species, such as in the case of Japanese eel (*Anguilla japonica*), where a weak but significant correlation between eDNA concentration and biomass of eels was determined by electrofishing (Itakura et al., 2019). Another example is relationship between "eDNA rates" and six years of mark-recapture population estimates for eulachon (*Thaleichthys pacificus*) (Pochardt et al., 2020). However, this type of quantification is still debated (Lacoursiere-Roussel et al., 2016). Several studies do not find significant correlations between concentration and biomass, such as in a study of brown trout in the river Wehebach in Germany (Deutschmann et al., 2019). Estimating biomass by eDNA in water can be biased by multiple factors, e.g. differences in eDNA shedding among species, water flow, temperature, microbial activity, UV-light, in addition to different match with primers, and technical parameters (e.g., polymerase mixes and the number of PCR cycles) (Ficetola et al., 2019; Strickler et al., 2015). When estimating the abundance of a new set of species, it could be useful to calibrate the relationship between eDNA and species abundance, for instance by traditional methods (Ficetola et al., 2019). Future knowledge and development of eDNA-methods that could quantify populations would be highly valuable in conservation efforts connected to migration barriers.

### 4.3. Few migration barriers associated with pipelines, tunnels, bridges, basins and natural barriers

The probability of detecting eel eDNA decreases slightly with number of pipelines, tunnels or bridges, in addition to natural barriers, but not significantly. The number of basins had no effect on the probability of detecting eDNA from European eel, and there was not enough data regarding fish ladders in this study to draw a conclusion. Eel eDNA is detected in most locations upstream from the human made constructions (pipelines, tunnels, bridges and basins), which are mostly located about 0–25 km from the sea. Only in 5 of these 30 locations, eDNA from European eel is not detected. This means that the majority of these potential barriers do not prohibit the migration of eels upstream.

However, since eel spend approximately 20 years in the rivers and lakes, individuals might have migrated upstream from construction before it was built. Thus, eel might be recorded by eDNA analysis upstream of a potential barrier, and one may erroneously conclude that the barrier does not stop eel migration. European eel are, to a certain degree, able to migrate (horizontally and vertically) on a moist substrate (Vøllestad, 1992) and could therefore reach upstream waters by alternative

routes like wet grass or flood streams. In these cases, the constructions would probably prohibit the migration of other species, like Atlantic salmon (*Salmo salar*) and trout, which depend on flowing water.

Migration barriers could explain the 5 locations where eDNA from European eel is not detected. However, only at sites 15 and 50 in the category of pipelines, tunnels, and bridges is eDNA detected downstream of the specific barrier but not upstream. These locations could have barriers that prohibit the migration of eels. At the other locations in this category, eDNA could be detected neither upstream nor downstream from the constructions. Therefore, it is not possible to conclude whether these are migration barriers or not. Lack of suitable habitat could be the answer in locations 2 and 13 where the streams are tiny and do not lead to any lake upstream. The water quality could be the reason on site 17, where there is a known high concentration of aluminum (NVE, 2019).

The remaining locations (39, 40, 52, 53, 54, and 55) where eDNA from eels could not be detected are categorized as natural or unknown barriers. These are all located a bit further from the sea or main river and could have natural barriers downstream (e.g., steep waterfalls). The idea that low population density leads to low competition for resources and thus does not drive individuals further upstream (Ibbotson et al., 2002) could also explain why eDNA from eels is not detected in these locations. However, we have not focused on these issues in the study.

Trout is recorded in 25 of 26 selected locations, which indicates acceptable quality of the isolated eDNA. At the site where eDNA from trout and eel is not detected (site 2), the eDNA-concentration and purity were adequate, so the area was probably not suitable for these species.

Furthermore, European eel may be present in an area where eDNA is not recorded due to the long distance between eels and the location of sample collection, which can lead to low (not detectable) eDNA-concentrations. However, the eDNA-method is sensitive, and studies have detected eDNA 12 km downstream from the source (Shogren et al., 2017). Errors regarding potential low eDNA-concentrations are also minimized in this study because several sub-samples along a section of every location were collected (and pooled into one sample) at every possible site. Therefore, it is likely that eels are present in locations where eDNA is detected and not where eDNA is not detected. In future studies, one might collect samples both at a higher number of locations in a specific area, as well as regular samples from each site throughout the season.

## 5. Conclusions

The probability of detecting eDNA from European eel decreases with number of hydroelectric power stations and their associated water basins, even when distance to the sea is accounted for. There is a clear border to where eel eDNA could no longer be detected in the major watercourses along the coast of southern Norway, and this border seem to be associated with the presence of hydroelectric power stations and their associated water basins. Therefore, it is likely that these constructions prohibit the migration of eels. To continue with and improve the conservation effort for the European eel, we recommended facilitating migration past upstream and downstream human-made barriers. We also advise having a precautionary approach and facilitating passage where constructions are likely to limit migration, even if eels are recorded upstream.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A

**Table 1**
Localities where water samples are collected and results of real-time PCR-triplicate ("+" indicates that eDNA is detected, and "-" indicates that eDNA is not detected), location name, type of barrier, distance to sea and GPS-coordinates.

| Location number, upstream/ downstream | Real-time PCR triplicate *A.anguilla* | Location name | Category/type of barrier | Distance to sea (km) | GPS-coordinates |
|---|---|---|---|---|---|
| 1 downstream | + + + | Nidelva | | | |
| 1 upstream | + + + | | Pipelines, tunnels, and bridges | 4,77 | 59.4099°N 8.6566°E |
| 2 downstream | - - - | Birketveit | | | |
| 2 upstream | - - - | | Pipelines, tunnels, and bridges | 2,49 | 58.3933°N 8.6673°E |
| 3 downstream | + + + | Sævelibekken | | | |
| 3 upstream | + + + | | Fish ladder | 1,12 | 58.3689°N 8.6047°E |
| 4 downstream | + + + | Frivoll | | | |

(*continued on next page*)

**Table 1** (*continued*)

| Location number, upstream/ downstream | Real-time PCR triplicate *A.anguilla* | Location name | Category/type of barrier | Distance to sea (km) | GPS-coordinates |
|---|---|---|---|---|---|
| 4 upstream | + + + | | Pipelines, tunnels, and bridges | 2,51 | 58.3488°N 8.5855°E |
| 5 downstream | + + + | Reddalskanalen | | | |
| 5 upstream | + + + | | Pipelines, tunnels, and bridges | 3,42 | 58.3192°N 8.5388°E |
| 6 downstream | + + + | Pendalen | | | |
| 6 upstream | + + + | | Pipelines, tunnels, and bridges | 1,42 | 58.2822°N 8.4784°E |
| 7 downstream | + + + | Kaldvell | | | |
| 7 upstream | + + + | | Fish ladder | 0,20 | 58.2796°N 8.4200°E |
| 8 downstream | + + - | Moelva | | | |
| 8 upstream | + + - | | Pipelines, tunnels, and bridges | 5,21 | 58.263°N 8.344°E |
| 9 downstream | + + + | Tingsaker | | | |
| 9 upstream | + + + | | Pipelines, tunnels, and bridges | 0,04 | 58.2552°N 8.3881°E |
| 10 downstream | + + - | Fjelldalselva | | | |
| 10 upstream | + + - | | Pipelines, tunnels, and bridges | 2,36 | 58.2348°N 8.2936°E |
| 11 downstream | + + + | Steindalsbekken | | 1,92 | |
| 11 upstream | + + + | | Pipelines, tunnels, and bridges | 1,92 | 58.2227°N 8.2503°E |
| 12 downstream | + + - | Urevann | | | |
| 12 upstream | + + + | | Fish ladder | 4,48 | 58.2032°N 8.2150°E |
| 13 downstream | - - - | Studedalen | | | |
| 13 upstream | - - - | | Pipelines, tunnels, and bridges | 1,94 | 59.1988°N 8.1758°E |
| 14 downstream | + + + | Blegehåla | | | |
| 14 upstream | + + + | | Pipelines, tunnels, and bridges | 6,75 | 58.236°N 8.128°E |
| 15 downstream | + - - | Østerbekk | | | |
| 15 upstream | - - - | | Pipelines, tunnels, and bridges | 6,40 | 58.2367°N 8.1288°E |
| 16 downstream | + + + | Prestebekken | | | |
| 16 upstream | + + + | | Pipelines, tunnels, and bridges | 5,15 | 58.2290°N 8.1165°E |
| 17 downstream | - - - | Bøen | | | |
| 17 upstream | - - - | | Pipelines, tunnels, and bridges | 3,16 | 58.2°N 8.09°E |
| 18 downstream | + + + | Vesbekken | | | |
| 18 upstream | + + + | | Pipelines, tunnels, and bridges | 1,60 | 58.2031°N 8.0986°E |
| 19 downstream | + + + | Augland | | | |
| 19 upstream | + + + | | Pipelines, tunnels, and bridges | 8,89 | 58.20087°N 7.9254°E |
| 20 downstream | + + + | Øvre Strai | | | |
| 20 upstream | + + - | | Pipelines, tunnels, and bridges | 8,90 | 58.2055°N 7.9251°E |
| 21 downstream | + + + | Rosslandsbekken | | | |
| 21 upstream | + + + | | Pipelines, tunnels, and bridges | 12,97 | 58.1290°N 7.8554°E |
| 22 downstream | + + + | Kleplandsbekken | | | |
| 22 upstream | + + + | | Pipelines, tunnels, and bridges | 8,62 | 58.1045°N. 7.8232°E |
| 23 downstream | + + + | Mjålandsbekken | | | |
| 23 upstream | + + + | | Pipelines, tunnels, and bridges | 29,30 | 58.2420°N 7.5117°E |
| 24 downstream | + + + | Smelandsbekken | | | |
| 24 upstream | + + + | | Pipelines, tunnels, and bridges | 14,90 | 58.1296°N. 7.5300°E |
| 25 downstream | + - - | Fodnebøbekken | | | |
| 25 upstream | + + - | | Pipelines, tunnels, and bridges | 13,84 | 58.1197°N. 7.5255°E |
| 26 downstream | + + + | Vådnebekken | | | |
| 26 upstream | + + + | | Pipelines, tunnels, and bridges | 11,29 | 58.1022°N. 7.5307°E |

**Table 1** (*continued*)

| Location number, upstream/ downstream | Real-time PCR triplicate *A.anguilla* | Location name | Category/type of barrier | Distance to sea (km) | GPS-coordinates |
|---|---|---|---|---|---|
| 27 downstream | + + + | Austre Grimevannet | | | |
| 27 upstream | + + + | | Basin | 1,82 | 58.2789°N 8.4017°E |
| 28 downstream | + - - | Herefoss | | | |
| 28 upstream | - - - | | Hydroelectric power stations and associated water basins | 43,60 | 58.5386°N 8.3546°E |
| 29 downstream | + - - | Hanefoss | | | |
| 29 upstream | - - - | | Hydroelectric power stations and associated water basins | 43,30 | 58.5356°N 8.3317°E |
| 30 downstream | + + + | Songeelva | | | |
| 30 upstream | + + + | | Pipelines, tunnels, and bridges | 21,94 | 58.5110°N 8.6330°E |
| 31 downstream | + + + | Evenstad | | | |
| 31 upstream | - - - | | Hydroelectric power stations and associated water basins | 25,88 | 58.54°N 8.6738°E |
| 32 downstream | - - - | Bøylefoss | | | |
| 32 upstream | - - - | | Hydroelectric power stations and associated water basins | 32,40 | 58.5924°N 8.7185°E |
| 33 downstream | - - - | Kilandsfjorden | | | |
| 33 upstream | - - - | | Hydroelectric power stations and associated water basins | 34,70 | 58.6129°N 8.7074°E |
| 34 downstream | + + + | Oggevatn | | | |
| 34 upstream | + - - | | Hydroelectric power stations and associated water basins | 27,01 | 58.3703°N 8.1094°E |
| 35 downstream | + + + | Nomelandsdammen | | | |
| 35 upstream | - - - | | Hydroelectric power stations and associated water basins | 30,42 | 58.3829°N 7.9103°E |
| 36 downstream | - - - | Birketveitstjønna | | | |
| 36 upstream | - - - | | Hydroelectric power stations and associated water basins | 41,66 | 58.4550°N 7.9127°E |
| 37 downstream | - - - | Kilefjorden | | | |
| 37 upstream | - - - | | Hydroelectric power stations and associated water basins | 43,30 | 58.4141°N 7.7711°E |
| 38 downstream | + + + | Venneslafjorden | | | |
| 38 upstream | + + + | | Hydroelectric power stations and associated water basins | 17,82 | 58.2852°N 7.9774°E |
| 39 downstream | + + + | Eikelandsvatn | | | |
| 39 upstream | - - - | | Natural barriers/unknown | 39,35 | 58.3678°N 7.7808°E |
| 40 downstream | + + + | Kårstøylveien | | | |
| 40 upstream | - - - | | Natural barriers/unknown | 17,53 | 58.1341°N 7.4980°E |
| 41 downstream | + + + | Høyevatnet | | | |
| 41 upstream | + + + | | Natural barriers/unknown | 26,48 | 58.2099°N 7.6204°E |
| 42 downstream | + + + | Birkelandsvannet | | | |
| 42 upstream | + + - | | Natural barriers/unknown | 14,01 | 58.1877°N 7.7521°E |
| 43 downstream | + + + | Sagtjønn | | | |
| 43 upstream | + - - | | Basin | 12,45 | 58.2262°N 7.9058°E |
| 44 downstream | + + + | Aurebekkvatnet | | | |
| 44 upstream | + + + | | Natural barriers/unknown | 10,90 | 58.1972°N 7.8839°E |
| 45 downstream | + + + | Grotjønn | | | |
| 45 upstream | + + + | | Basin | 3,01 | 58.1550°N 7.9421°E |
| 46 downstream | + + + | Stemmen | | | |
| 46 upstream | + + - | | Basin | 11,70 | 58.2240°N 7.9144°E |
| 47 downstream | + + + | Stemvann | | | |
| 47 upstream | + + + | | Natural barriers/unknown | 3,95 | 58.1078°N 7.8052°E |
| 48 downstream | + + + | Røyrvatnet | | | |
| 48 upstream | + + - | | Natural barriers/unknown | 31,34 | 58.2033°N 7.8291°E |
| 49 downstream | + + + | Syndle | | | |

(*continued on next page*)

**Table 1** (*continued*)

| Location number, upstream/ downstream | Real-time PCR triplicate *A.anguilla* | Location name | Category/type of barrier | Distance to sea (km) | GPS-coordinates |
|---|---|---|---|---|---|
| 49 upstream | + - - | | Pipelines, tunnels, and bridges | 17,52 | 58.3849°N 8.5300°E |
| 50 downstream | + + + | Holvannet | | | |
| 50 upstream | - - - | | Pipelines, tunnels, and bridges | 22,05 | 58.3957°N 8.4882°E |
| 51 downstream | + + - | Moelva | | | |
| 51 upstream | + + - | | Natural barriers/unknown | 9,03 | 58.3020°N 8.2577°E |
| 52 downstream | + + - | Heddekjerrvatnet | | | |
| 52 uptream | - - - | | Natural barriers/unknown | 29,20 | 58.2084°N 7.6640°E |
| 53 downstream | + + + | Folltjørn | | | |
| 53 upstream | - - - | | Natural barriers/unknown | 39,39 | 58.3041°N 7.5680°E |
| 54 downstream | + + + | Hundlandsvatnet | | | |
| 54 upstream | - - - | | Natural barriers/unknown | 19,51 | 58.3622°N 8.3380°E |
| 55 downstream | + + + | Høgkleivvatnet | | | |
| 55 upstream | - - - | | Natural barriers/unknown | 26,07 | 58.3620°N 8.2331°E |
| 56 downstream | + + + | Lundeelva | | | |
| 56 upstream | + + + | | Natural barriers/unknown | 0,47 | 58.0837°N 7.7846°E |
| 57 downstream | - - - | Eikerapen | | | |
| 57 upstream | - - - | | Hydroelectric power stations and associated water basins | 71,12 | 58.5440°N 7.4065°E |
| 58 downstream | + + + | Øre | | | |
| 58 upstream | - - - | | Hydroelectric power stations and associated water basins | 60,86 | 58.4907°N 7.4431°E |
| 59 downstream | + + + | Mannflåvannet | | | |
| 59 upstream | + + + | | Natural barriers/unknown | 36,92 | 58.3306°N 7.5132°E |
| 60 downstream | + + + | Temse | | | |
| 60 upstream | + + + | | Natural barriers/unknown | 8,39 | 58.3822°N 8.6415°E |

**Table 2**
Primers Alcyt-F, Alcyt-R and probe compared to mtDNA from species possible to find at location 1–60. The comparisons were conducted with Clustal Omega, and the sequences where found in the NCBI's Genbank. Grey areas in the sequences indicates nucleotides that are similar between the primers and probe of *A. anguilla* and the other species.

| Species | Match between primers and probe of *A. anguilla* and mtDNA of the other species | |
|---|---|---|
| **Brown trout** | Probe | T**TCA**TTA**TCATGCTCACC** |
| *Salmo trutta* | *S. trutta* | G**TCC**TAT**TCCTGCTCACC** |
| GenBank: MF621760.1 | Alcytb −F | **CACCCATACTTCTC**C**TACAAAGACCT**A |
| | *S. trutta* | **CACCCATACTTCTC**A**TACAAAGACCT** |
| | Alcytb −R | **TCTGGGTCTCCAAGCA**G**GTT** |
| | *S. trutta* | **AAGCAAGTT** |
| **Arctic char** | Probe | **TTCATT**ATCATGCTCACC |
| *Salvelinus alpinus* | *S. alpinus* | **TTCATT**TC**C** |
| GenBank: MF621743.1 | Alcytb-F | **CACCC**A**TACTTCTC**C**TACAAAGACCT**A |
| | *S. alpinus* | **CACCCC**T**ACTTCTC**G**TACAAAGACCT**C |
| | Alcytb-R | **TCTGGGTCTCCAAGCAGGTT** |
| | *S. alpinus* | **TCTGGCTCTCCCA** |
| **River lamprey** | Probe | **TTCATT**A**TCATGCTCAC**C |
| *Lampetra fluviatilis* | *L. fluviatilis* | **TTCATT**T**TCATGATCAC**A |
| GenBank: Y18683.1 | Alcytb −F | **CACCCATACTTCTCCTACAAAGACCTA** |
| | *L. fluviatilis* | **CACCCATACTTCTCTTTCAAAGACATT** |
| | Alcytb −R | **T**CT**GGGTCTCC**AAGCAGGTT |
| | *L. fluviatilis* | **TTGGGGCCTCC** |
| **Common minnow** | Probe | **TTCATT**ATC**ATGCTCACC** |
| *Phoxinus* | *P. phoxinus* | **TTAATT**GCC**ATGCCCTCC** |
| GenBank: Y18683.1 | Alcytb −F | **CA**CCCATA**CTT**CTCCTA**CAAAGACCT**A |
| | *P. phoxinus* | **CA**TCCATATTTTTCTTATA**AAAGACCT**T |

**Table 2** (*continued*)

| Species | Match between primers and probe of *A. anguilla* and mtDNA of the other species | |
|---|---|---|
| | Alcytb-R | TCTGGGTCT**CCAAGCAG**GTT |
| | *P. phoxinus* | **CCAAGCAG**TTA |
| **European whitefish** | Probe | T**TCATTATCATGCTCACC** |
| *Coregonus lavaretus* | *C. lavaretus* | G**TCCTTACCTTGCTCACC** |
| GenBank: AB034824.1 | Alcyt-F | **CACCCATACTTCTCCTACAAAGACCT**A |
| | *C. lavaretus* | **CACCCCTACTTCTC**A**TACAAAGACCT**G |
| | Alcyt-R | TCTG**GGTCTCCAA**GCAGG**TT** |
| | *C. lavaretus* | TAT**GGGCCCCCA**TGCCA**TT** |
| **European smelt** | Probe | T**TCATTATC**AT**GCTCACC** |
| *Osmerus eperlanus* | *O. eperlanus* | **CTTATTATC**CA**GATCACC** |
| GenBank: MH238073.1 | Alcyt-F | CACCCATAC**TTCT**C**CTACAA**AG**ACCT**A |
| | *O. eperlanus* | A**TTCAACTACAA**GA**ACCT** |
| | Alcyt-R | **TCT**G**GGTCTCCAAG**CA**GGTT** |
| | *O. eperlanus* | **TCT**TGCC**CTAAAAGT**G**GGTT** |
| **European perch** | Probe | **TTCATTATCATGCTCA**CC |
| *Perca fluviatilis* | *P. fluviatilis* | **TTCATTTTCACGACCA** |
| GenBank: VHII01000304.1 | Alcyt-F | **CACCCATACTTCTCCTACAAAGACCT**A |
| | *P. fluviatilis* | **CATCCTTATTTTTCTTACAAAGACCT**C |
| | Alcyt-R | T**CTGGGTCT**CCAAGCAGGTT |
| | *P. fluviatilis* | C**CGGGGTCT**A |

## References


Anonymous, 2007. Council Regulation (EC) No 1100/2007 of 18 September 2007 establishing measures for the recovery of the stock of European eel. Retrieved from Official Journal of the European Union L. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/.

Arai, T., 2016. Biology and Ecology of Anguillid Eels. CRC Press.

Belpaire, C.G.J., Goemans, G., Geeraerts, C., Quataert, P., Parmentier, K., Hagel, P., De Boer, J., 2009. Decreasing eel stocks: survival of the fattest? Ecol. Freshw. Fish 18 (2), 197–214. https://doi.org/10.1111/j.1600-0633.2008.00337.x.

NVE (Cartographer), 2019. Vann-nett portal. Retrieved from. https://vann-nett.no/portal/#/mainmap.

Castonguay, M., Hodson, P.V., Moriarty, C., Drinkwater, K.F., Jessop, B.M., 1994. Is there a role of ocean environment in American and European eel decline? Fish. Oceanogr. 3 (3), 197–203.

Degerman, E., Tamario, C., Watz, J., Nilsson, P.A., Calles, O., 2019. Occurrence and habitat use of European eel (Anguilla anguilla) in running waters: lessons for improved monitoring, habitat restoration and stocking. Aquat. Ecol. 53 (4), 639–650. https://doi.org/10.1007/s10452-019-09714-3.

Dekker, W., Beaulaton, L., 2016. Climbing back up what slippery slope? Dynamics of the European eel stock and its management in historical perspective. ICES J. Mar. Sci. 73 (1), 5–13. https://doi.org/10.1093/icesjms/fsv132.

Deutschmann, B., Muller, A.K., Hollert, H., Brinkmann, M., 2019. Assessing the fate of brown trout (Salmo trutta) environmental DNA in a natural stream using a sensitive and specific dual-labelled probe. Sci. Total Environ. 655, 321–327. https://doi.org/10.1016/j.scitotenv.2018.11.247.

Drouineau, H., Durif, C., Castonguay, M., Mateo, M., Rochard, E., Verreault, G., Lambert, P., 2018. Freshwater eels: a symbol of the effects of global change. Fish Fish. 19 (5), 903–930. https://doi.org/10.1111/faf.12300.

Feunteun, E., 2002. Management and restoration of European eel population (Anguilla anguilla): an impossible bargain. Ecol. Eng. 18 (5), 575–591. https://doi.org/10.1016/s0925-8574(02)00021-6.

Ficetola, G.F., Manenti, R., Taberlet, P., 2019. Environmental DNA and metabarcoding for the study of amphibians and reptiles: species distribution, the microbiome, and much more. Amphibia-Reptilia 40 (2), 129–148. https://doi.org/10.1163/15685381-20191194.

Friedland, K.D., Miller, M.J., Knights, B., 2007. Oceanic changes in the Sargasso Sea and declines in recruitment of the European eel. ICES J. Mar. Sci. 64 (3), 519–530. https://doi.org/10.1093/icesjms/fsm022.

Ibbotson, A., Smith, J., Scarlett, P., Aprhamian, M., 2002. Colonisation of freshwater habitats by the European eel Anguilla anguilla. Freshw. Biol. 47 (9), 1696–1706. https://doi.org/10.1046/j.1365-2427.2002.00930.x.

ICES, 2019. Joint EIFAAC/ICES/GFCM Working Group on Eels (WGEEL) (2618-1371). Retrieved from.

Itakura, H., Wakiya, R., Yamamoto, S., Kaifu, K., Sato, T., Minamoto, T., 2019. Environmental DNA analysis reveals the spatial distribution, abundance, and biomass of Japanese eels at the river-basin scale. Aquat. Conserv. Mar. Freshw. Ecosyst. 1–13.

Jacoby, D., Gollock, M., 2014. Anguilla anguilla. The IUCN red list of threatened species. Retrieved from. http://www.iucnredlist.org/details/60344/0.

Kettle, A.J., Vollestad, L.A., Wibig, J., 2011. Where once the eel and the elephant were together: decline of the European eel because of changing hydrology in southwest Europe and northwest Africa? Fish Fish. 12 (4), 380–411. https://doi.org/10.1111/j.1467-2979.2010.00400.x.

Lacoursiere-Roussel, A., Dubois, Y., Normandeau, E., Bernatchez, L., 2016. Improving herpetological surveys in eastern North America using the environmental DNA method. Genome 59 (11), 991–1007. https://doi.org/10.1139/gen-2015-0218.

Laffaille, P., Lasne, E., Baisez, A., 2009. Effects of improving longitudinal connectivity on colonisation and distribution of European eel in the Loire catchment, France. Ecol. Freshw. Fish 18 (4), 610–619. https://doi.org/10.1111/j.1600-0633.2009.00378.x.

Ogden, J.C., 1970. Relative abundance, food habits, and age OF AMERICAN eel, Anguilla-rostrata (lesueur), IN certain new-Jersey streams. Trans. Am. Fish. Soc. 99 (1), 54. https://doi.org/10.1577/1548-8659(1970)99<54:Rafhaa>2.0.Co;2.

Pochardt, M., Allen, J.M., Hart, T., Miller, S.D.L., Yu, D.W., Levi, T., 2020. Environmental DNA facilitates accurate, inexpensive, and multiyear population estimates of millions of anadromous fish. Molecular Ecology Resources 20 (2), 457–467. https://doi.org/10.1111/1755-0998.13123.

Porcher, J.P., 2002. Fishways for Eels. Bulletin Francais De La Peche Et De La Pisciculture, vol. 364, pp. 147–155. https://doi.org/10.1051/kmae/2002099.

R Core Team, 2017. Vienna, Austria: R Foundation for Statistical Computing.

Rees, H.C., Maddison, B.C., Middleditch, D.J., Patmore, J.R.M., Gough, K.C., 2014. REVIEW: the detection of aquatic animal species using environmental DNA – a review of eDNA as a survey tool in ecology. J. Appl. Ecol. 51 (5), 1450–1459. https://doi.org/10.1111/1365-2664.12306.

Righton, D., Westerberg, H., Feunteun, E., Okland, F., Gargan, P., Amilhat, E., Aarestrup, K., 2016. Empirical observations of the spawning migration of European eels: the long and dangerous road to the Sargasso Sea. Science Advances 2 (10). https://doi.org/10.1126/sciadv.1501694.

Shogren, A.J., Tank, J.L., Andruszkiewicz, E., Olds, B., Mahon, A.R., Jerde, C.L., Bolster, D., 2017. Controls on eDNA movement in streams: transport, retention, and resuspension. Sci. Rep. 7 https://doi.org/10.1038/s41598-017-05223-1.

Strickler, K.M., Fremier, A.K., Goldberg, C.S., 2015. Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. Biol. Conserv. 183, 85–92. https://doi.org/10.1016/j.biocon.2014.11.038.

Tesch, F., 1977. The Eel: Biology and Management of Anguillid eels.(J. Greenwood, Translator). John Wiley & Sons, New York, New York, USA.
Thomsen, P.F., Willerslev, E., 2015. Environmental DNA - an emerging tool in conservation for monitoring past and present biodiversity. Biol. Conserv. 183, 4–18. https://doi.org/10.1016/j.biocon.2014.11.019.
Thomsen, P.F., Kielgast, J., Iversen, L.L., Moller, P.R., Rasmussen, M., Willerslev, E., 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PloS One 7 (8). https://doi.org/10.1371/journal.pone.0041732.
Thorstad, B.E., Larsen, M., Hesthagen, T., Næsje, F.T., Poole, R., Aarestrup, K., Sandlund, T.O., 2010. Ål Og Konsekvenser Av Vannkraftutbygging (Retrieved from).
Vøllestad, L. A. (1992). Ålefisker. I Jonsson, B. & Semb-Johansen, A. (red) Norges dyr: J.W. Cappelens forlag.
White, E.M., Knights, B., 1997. Dynamics of upstream migration of the European eel, Anguilla anguilla (L.), in the Rivers Severn and Avon, England, with special reference to the effects of man-made barriers. Fish. Manag. Ecol. 4 (4), 311–324. https://doi.org/10.1046/j.1365-2400.1997.00050.x.