Environmental Pollution
December 2022, Volume 314 Pages 120207 (13p.)
https://doi.org/10.1016/j.envpol.2022.120207
https://archimer.ifremer.fr/doc/00796/90750/

Impact of environmental micropollutants and diet composition on the gut microbiota of wild european eels (*Anguilla anguilla*)

Bertucci Anthony [1, *], Hoede Claire [2, 3], Dassié Emilie [4], Gourves Pierre-Yves [4], Suin Amandine [5], Le Menach Karine [4], Budzinski Hélène [4], Daverat Françoise [1]

[1] EABX, INRAE, 50 Avenue de Verdun 33612, Cestas, France
[2] Université de Toulouse, INRAE, UR MIAT, PF GenoToul Bioinfo, 31320, Castanet-Tolosan, France
[3] Université Fédérale de Toulouse, INRAE, BioinfOmics, GenoToul Bioinformatics Facility, 31326, Castanet-Tolosan, France
[4] Univ. Bordeaux, CNRS, EPOC, EPHE, UMR 5805, 33600, Pessac, France
[5] Genome & Transcriptome - Plateforme GeT-PlaGe, INRAE, 31326, Castanet-Tolosan, France

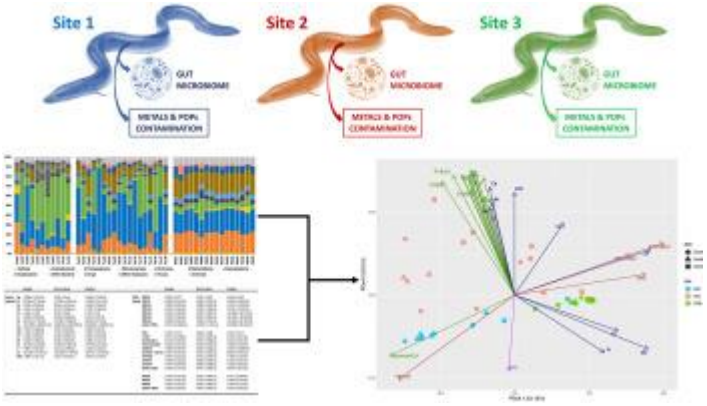
* Corresponding author : Anthony Bertucci, email address : anthony.bertucci@ifremer.fr

Abstract :

In fish, the gut microbiome plays a crucial role in homeostasis and health and is affected by several organic and inorganic environmental contaminants. Amphidromous fish are sentinel species, particularly exposed to these stressors. We used whole metagenome sequencing to characterize the gut microbiome of wild European eels (Anguilla anguilla) at a juvenile stage captured from three sites with contrasted pollution levels in term of heavy metals and persistent organic pollutants. The objectives were to identify what parameters could alter the gut microbiome of this catadromous fish and to explore the potential use of microbiota as bioindicators of environment quality.

We identified a total of 1079 microbial genera. Overall, gut microbiome was dominated by Proteobacteria, Firmicutes and Actinobacteria. Alpha and beta diversity were different amongst sites and could be explained by a reduced number of environmental and biological factors, specifically the relative abundance of fish preys in eels' diet, PCB101, γHCH (lindane), transnonachlor and arsenic. Furthermore, we identified a series of indicator taxa with differential abundance between the three sites. Changes in the microbial communities in the gut caused by environmental pollutants were previously undocumented in European eels. Our results indicate that microbiota might represent another route by which pollutants affect the health of these aquatic sentinel organisms.

Graphical abstract



Highlights

► The gut microbial diversity of wild European eels differed amongst sites of catching. ► POPs and metals levels showed strong associations with gut microbiota composition. ► The relative abundance of fish in eels' diet also impacted gut microbiota. ► 323 microbial genera were differentially abundant between the catching sites.

Keywords : European eel, Gut microbiome, Heavy metals, Persistent organic pollutants, Diet

INTRODUCTION:

In fish, as in mammals, the collection of commensal, symbiotic and pathogenic microorganisms (eukaryotes, bacteria, archaea, and viruses) that occupy a particular environment, like the internal and external epidermal surfaces of metazoans, has the ability to fulfil key functions in nutritional provisioning, metabolic homeostasis, and immune defence. These microbial partners are referred to as "microbiota", whereas the term "microbiome" often refers to the collection of genomes within this microbiota (Burokas et al., 2015). Owing to the ability of the microbiota to modulate the host physiology, an increasing number of studies have been carried out to characterize and determine the mechanisms of action of these microbes, particularly concerning the gut microbiota. This importance is highlighted by the fact that some authors consider gut microbiota as an "extra organ" (Feng et al., 2018) or a "second genome" (Grice and Segre, 2012). Though well behind mammalian models, teleost microbiome research is a quickly developing field (Llewellyn et al., 2014). Despite early works published in the 1920's and 1930's, this topic has known a recent expansion (for a review, see Egerton et al., 2018). More recently, next-generation sequencing techniques has represented a rapid and cost-effective method (i) to obtain data that provide more accurate information on the composition as well as the genetic and metabolic potential of a microbial community and (ii) to compare communities and test hypotheses. The microbiota associated with the fish intestine is supposedly dominated by Bacteria, mostly due to the caveat that they have been almost the only focus of research so far. The fish intestine may harbour 10^7 to 10^{11} bacteria per gram of intestinal content. In the different fish species studied, *Proteobacteria*, *Bacteroidetes* and *Firmicutes*, represent 90% of the intestinal microbiota (Ghanbari et al., 2015). This composition shows substantial inter-species and inter-individual variation depending on various factors such as life stage, trophic level, diet, season, habitat, sex and genetics of the host (Butt and Volkoff, 2019; Egerton et al., 2018; Tarnecki et al., 2017). Gut bacterial communities have been assessed in approximatively 150 fish species covering a diverse range of physiology and ecology. However, most of the research effort is being carried out on economically important species such as salmonids, carp or tilapia (Perry et al., 2020), to meet the needs of the expanding aquaculture industry.

The European eel *Anguilla anguilla* is a catadromous fish with a complex life cycle shared between marine (reproduction, larval growth, and sexual maturation) and continental (growth) environments. European eels spawn in the Sargasso Sea before larvae drift towards the continental shelf of Europe and North Africa where they first metamorphose into unpigmented juvenile (glass eel) before reaching the growth phase (yellow eel) in continental water bodies where they remain up to 15-20 years, often moving across different habitat types. The yellow eel stage ends with a second metamorphosis into maturing eels (silver eels) that will migrate back to the Sargasso Sea to reproduce and die. This life cycle makes *A. anguilla* particularly vulnerable to pollution (Feunteun,

2002; Guimaraes et al., 2009). The species has been considered as critically endangered since 2008 and added to the IUCN red list (Jacoby and Gollock, 2014). Its catadromous migratory behaviour, long life, drastic habitat reduction, migration barriers, pollution, human-introduced diseases, overfishing, as well as climatic events may be amongst the causes of the catastrophic collapse of the European eel population observed over the past decades (Bevacqua et al., 2015; Knights, 2003; van den Thillart et al., 2009). Whereas the sensitivity of *A. anguilla* to metal and persistent organic pollutants is well documented (*e.g.* Baillon et al., 2015; Belpaire et al., 2008; Bertucci et al., 2019; Perrier et al., 2020; Pujolar et al., 2012), metagenomic studies in this species are scarce. Some were performed on the microbiome associated with skin mucosal surfaces (Carda-Dieguez et al., 2017; Carda-Diéguez et al., 2014). They showed the dominance of *Gammaproteobacteria*, especially the genus *Vibrio,* in eels from estuary and wetland while a mixture of genera predominated in lake and river eels. Which evidenced the role of salinity in shaping microbiota in fish. To our knowledge, community composition of intestinal microbiota of European eels was only assessed in cultivated individuals (Huang et al., 2018). The results showed the dominance *Proteobacteria* and *Fusobacteria*. However, more recently, gut microbiota was studied in other *Anguilla* species like the giant mottled eel *A. marmorata* (Lin et al., 2019), the Indonesian shortfin eel *A. bicolor* (Kusumawaty et al., 2020), and the Japanese eel *A. japonica* (Zhu et al., 2021).

The function of the gut microbiota and the subsequent physiological responses of the host depend on which microbes are present in the intestinal tractus and the interaction with the immune system. Then, the alteration of the microbiota composition and / or its metabolic functions might impact host's health and survival (Claus et al., 2016). This phenomenon is called dysbiosis and its links with environmental stressors suggest that the microbiome has the potential to provide innovative biomarkers (Boutin et al., 2013; Webster et al., 2019) and the identification of dysbiosis might be informative regarding adverse outcomes. Still, processes that drive microbial community in fish are poorly understood. The incorporation of the microbiome in studies investigating the effects of chemical compounds could then be a promising approach in ecotoxicology (Adamovsky et al., 2018; Duperron et al., 2020). In this study we investigated the impact of environmental pollutants, diet composition, and biometric parameters on the composition of intestinal microbiota of wild European eels sampled from three sites with contrasted anthropogenic pollution profiles. We measured a total of 15 metals in fish liver, and 28 persistent organic pollutants including polychlorinated biphenyls (PCBs), polybromodiphenylethers (PBDEs) and organochlorine pesticides in fish muscle. PCBs and PBDEs are widespread contaminants. PCBs are man-made chlorinated industrial chemicals that were used in electrical equipment hydraulic fluids, heat transfer fluids, lubricants, plasticizers, surface coatings and inks. PBDEs are used in plastics, electronics, building materials and textiles as flame retardants. Pesticides are used in agriculture and are rarely selective

enough to prevent effect on non-target organisms. Many studies have documented the accumulation of these xenobiotics in aquatic organisms and more recently their toxicity for gut microbiota (see Evariste et al., 2019). Diet composition was assessed through DNA sequencing of the intestinal content. Our aim was not only to characterize the microbiota of these migratory fish but also to determine what factors can drive the taxonomy of their microbial partners. The results of this study allowed the identification of a limited number of pollutants and diet component that could explain dysbiosis in European eels.

MATERIAL AND METHODS:

Sample collection – Wild European eels (*Anguilla anguilla*) specimens were collected in Batejin (BAT: 44°55'53.87"N, 01°07'22.94"W; 12 individuals in July 2019), Pas-du-Bouc (PDB: 44°50'27.03"N, 01°09'07.58"W; 20 individuals in July 2019) and Pauillac (PAU: 45°14'00.26"N, 00°44'01.80"W; 20 individuals in September 2019). Immature yellow eels of a pre-defined size (20-40 cm) were targeted in order to have the same range for all sites. BAT and PDB samples were collected by electro-fishing according to the authorizations from the "Direction Départementale des Territoires et de la Mer de la Gironde" edicted on May 22, 2019 and June 7, 2019. PAU samples were trapped by a professional fisherman. Fish were kept in tanks with aerated water from the site for few minutes after collection until being sacrificed and dissected at the proximity of the sampling site. Individuals were measured (cm) and weighted (g). Liver, spleen, and kidney were collected and weighted for organo-somatic indices calculation and stored at -20°C. The intestinal section located shortly down the stomach and up the colon was dissected, transferred in RNA-later and placed at 4 °C overnight before storage at −20 °C until DNA extraction. Otoliths were collected for age determination. For all sites, temperature, salinity, and dissolved oxygen concentrations were measured using a multi-parameter portable device or by using data coming from the automated observation network of the Gironde (MAGEST network).

Biometric indices – Fulton index (K), hepato-somatic index (HSI) and spleno-somatic index (SSI) were calculated as $K = (\text{total weight} / \text{length}^3) \times 100$; HSI = (liver weight / total weight) x 100; and SSI = (spleen weight / total weight) x 100; where weight is in grams and length is in centimetres.

Age determination – Age was determined according to the methods described in Annex 4 of the report of the ICES Workshop on Age Reading of European and American Eel (ICES, 2009). Briefly, sagittal otoliths were extracted, cleaned with distilled water, dried, and stored in plastic Eppendorf tubes at ambient room temperature until processing. Otoliths were embedded in epoxy resin; grinded on 1200-grit paper with water until the nucleus is reached and polished on 4000-grit paper; etched for 3 minutes with a 5% EDTA solution, washed with distilled water and stained with 0.01%

toluidine blue for 3 minutes. Otoliths were rinsed with distilled water and observed with a binocular microscope under transmitted light.

Trace metal analyses – Liver tissues were dried, weighted, and digested in polypropylene tubes using nitric acid (67%). Digesta were heated at 100 °C for three hours. After complete mineralization, samples were diluted with ultrapure water. In order to verify the accuracy of the method blank samples, Internationals Certified Reference Materials (CRM) for trace metals elements: DOLT-5 (fish liver), TORT-3 (lobster hepatopancreas), and IAEA-413 (algae) were prepared in similar conditions as the samples. A series of 15 elements were analysed simultaneously by Inductively Coupled Plasma Optical Emission Spectrometer (700 Series ICP-OES, Agilent): Silver (Ag), Aluminium (Al), Arsenic (As), Cadmium (Cd), Cobalt (Co), Chrome (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Lead (Pb), Selenium (Se), Vanadium (V), and Zinc (Zn). Metals concentrations in tissue samples were expressed in $\mu g.mg^{-1}$ dry weight (dw). Average recovery rates were between 97% and 102%. When not detectable, metal concentrations were replaced by the maximum theoretical detection limit (DL_{max}) to allow multivariate analysis. DL_{max} of a sample is calculated as (DL x W) / V; where DL is the detection limit for the metal in $\mu g.L^{-1}$, W the mass of the sample in g, and V the volume of the sample in ml. DL values are given in supplementary file 1. The metal pollution index was calculated as in Khan et al. (2020).

Organic pollutants analyses – Analysis of the seven polychlorinated biphenyls (CB28; CB52; CB101; CB118; CB138; CB153 and CB180), thirteen organochlorine pesticides (hexachlorobenzene or HCB; lindane or γ-HCH; heptachlor; heptachlor epoxide; *cis*-chlordane; *trans*-nonachlor; mirex; 2,4'DDE; 2,4'DDD; 4,4'DDE; 4,4'DDD; 2,4'DDT and 4,4'DDT), and 8 polybromodiphenylethers (BDE28, BDE47, BDE99, BDE100, BDE153, BDE154, BDE183 and BDE209) were performed on individual muscle samples by liquid / liquid extraction followed by purification by adsorption chromatography as described previously (Tartu et al., 2015). PCB and OCP were analysed by gas chromatography coupled to an electron capture detector (GC-ECD), while PBDEs were determined by GC hyphenated with mass spectrometry (GC-MS). Results were expressed in $ng.g^{-1}$. When not detectable, concentrations were replaced by the quantification limit of the compound to allow multivariate analysis (supplementary file 1).

DNA extraction, sequencing and filtering – In order to capture the widest microbial diversity, three gut sections of approximatively 5 millimetres each were cut out at the beginning, the middle and the end of the intestine sample dissected previously. These three pieces were sliced open and minced with a sterile blade, then mixed and processed with the QIAamp PowerFecal Pro DNA Kit (Qiagen) following the supplier's instructions.

DNAseq was performed at the GeT-PlaGe core facility, INRAE Toulouse. DNA-seq libraries were prepared according to Illumina's protocols using the Illumina TruSeq Nano DNA HT Library Prep Kit.

Briefly, DNA was fragmented by sonication, size selection was performed using SPB beads (kit beads) and adaptors were ligated to be sequenced. Library quality was assessed using an Advanced Analytical Fragment Analyzer and libraries were quantified by qPCR using the Kapa Library Quantification Kit. DNA-seq experiments have been performed on an Illumina NovaSeq6000 using a paired-end read length of 2x150 pb with the Illumina NovaSeq6000 Reagent Kits.

Each sample was sequenced twice on separate lanes of the same flowcell and the two paired files were merged. Raw data were cleaned as follows by using first step of metagWGS v1.1 available at: https://forgemia.inra.fr/genotoul-bioinfo/metagwgs/-/tree/master (Fourquet et al., 2020): (i) we checked quality of raw reads by fastQC v0.11.9, (ii) we remove adaptors with cutadapt v2.10, (iii) trimmed paired reads if nucleotide quality is lesser than 20 with sickle v1.33, (iv) we used bwa mem v0.7.17-r1188 to map trimmed reads against *Anguilla anguilla* genome (GCA_013347855.1), samtools view v1.10 and bam2fastq from bedtools v2.29.2 were used to obtain cleaned paired reads fastq files without host reads, (v) finally we used fastQC again on cleaned reads to obtain quality metrics.

Taxonomic profiling **–** Because we used gut sections, we anticipated that bacteria and other microbial partners would represent a small portion of our libraries. For each sample, taxonomic classification of quality trimmed and filtered metagenomic paired-end reads was performed using Kaiju (v.1.7.4) ("greedy" heuristic approach, -e 3), with the NCBI nr database (release dated May 25th, 2020; containing all proteins belonging to Archaea, Bacteria and Viruses and proteins from fungal and microbial eukaryotes) as reference. We used kaiju2table to build taxonomy table at each taxonomy levels (superkingdom, phylum, class, order, family, genus, species). To obtain one unique matrix for all samples we used the home-made script merge_kaiju_results.py available in metagWGS (https://forgemia.inra.fr/genotoul-bioinfo/metagwgs/-/tree/master/bin). Counts of sequences successfully assigned and taxonomic information were imported into RStudio (v. 1.2.5019), based on R (v. 3.6.1), for further processing with the phyloseq (v. 1.30.0) and vegan (v. 2.5.5) packages. Filtering of the most abundant Genera were based on a minimum abundance threshold of 100 cpm (counts per million reads). Taxa not exceeding these thresholds in at least half the samples of one site (50% prevalence) were removed. The core microbiota was determined with a 0.10% relative frequency threshold and a prevalence of at least 80%. This was performed using the core function in microbiome R package version 1.17.42 (Leo and Sudarshan, 2017). In order to assess the diet of the fish, taxonomic classification of quality trimmed and filtered metagenomic paired-end reads was performed using Kraken2 (v. 2.0.8-beta) with '--paired' parameter against the entire NCBI nt database downloaded on January 24, 2022. Metazoan sequences were classified at the Class level and taxa not exceeding 1% of the reads in half of the samples from at least one site were removed.

The numbers of reads used in both kaiju and kraken analysis for each sample are presented in supplementary file 2.

Statistics and data analysis – Alpha diversity was calculated using the R package "vegan" (Oksanen et al., 2020) based on observed, Shannon, Chao1 and Pielou's Evenness indices calculated from non-normalized genus-level read counts. Beta diversity between the sites were visualized using nonmetric multidimensional scaling (NMDS) and Principal Coordinates Analysis (PCoA) plots based on the Bray-Curtis dissimilarity. Beta diversity analysis were performed on cpm-transformed counts at the genus level to control for sequencing depth between libraries. Pairwise differences were tested using PERMANOVA (n=1000 permutations) based on Bray-Curtis dissimilarity and adjusted P values (FDR ≤ 0.05 were considered as statistically significant. Beta dispersion was assessed by an ANOVA-like permutation test (n=1000 permutations) on the Bray-Curtis distance matrix. A Linear discriminant analysis Effect Size (LEfSe) was used to identify bacterial taxa responsible for differences between sampling sites at the genus level (Segata et al., 2011) with a LDA score threshold of 2.0 and a FDR of 0.01.

The statistical analysis of morphometric data and pollutant levels were performed using RStudio (v.1.2.5019). Metal concentrations and morphologic variables were expressed as mean ± SE. The comparison among sites was performed using a linear model after checking for normality and homoscedasticity of residuals. When necessary, post-hoc analysis was performed by the Tukey test. A p-value ≤ 0.05 was considered significant.

Covariates of gut microbiome β-diversity were identified by performing linear correlations to calculate the association between pollutant content, diet, morphometry and genus-level community coordinate with the envfit() function in the "vegan" R-package. Then, in order to identify non-redundant determinants of microbiota variation, covariates selected by envfit() function were sub-selected by forward stepwise redundancy analysis on genus-level community ordination calculated by Bray-Curtis distance with the ordiR2step() function in the "vegan" R-package.

Data availability – The molecular data set generated and analyzed for this study were deposited in NCBI repository under the BioProject accession number PRJNA825732.

RESULTS:

Differences among sampling sites – Three sites located along the Gironde estuarian ecosystem were selected. Batejin (BAT) and Pas-du-Bouc (PDB) were located on the 'canal des étangs' located between the Lacanau lake and the Arcachon lagoon, while Pauillac (PAU) was located in the Gironde estuary. The major difference was salinity, but variability also exists in term of dissolved oxygen level and temperature on the date of sampling (Table 1). According to one-way ANOVAs followed by Tukey's HSD tests (p≤0.05), differences were observed in eels' morphologic features even though fish

were initially selected according to their size range (20-40 cm). PDB and PAU individuals were significantly longer and heavier than BAT individuals. PDB eels were older and exhibited a higher Fulton index. These individuals also had lower HSI, and higher SSI values compared to BAT and PAU.

Heavy metal pollution in eels' liver – Fifteen trace metal concentration were measured in hepatic tissue. Co was the only element for which no difference was observed between the 3 sites ($p \leq 0.05$). BAT eels are characterized by the highest levels of Cr (0.426 ± 0.094 $\mu g.mg^{-1}$) and the lowest levels of Cu, Mn and Zn compared to eels from the other sites ($p \leq 0.05$). PDB eels are characterized by the highest level of Fe (2,936.914 ± 298.146 $\mu g.mg^{-1}$), Mo (1.581 ± 0.082 $\mu g.mg^{-1}$), Se (39.264 ± 6.647 $\mu g.mg^{-1}$) and V (2.01 ± 0.198 $\mu g.mg^{-1}$) and the lowest levels of Pb (0.05 ± 0.003 $\mu g.mg^{-1}$) in their livers ($p \leq 0.05$). PAU individuals showed the highest levels of Ag (0.968 ± 0.104 $\mu g.mg^{-1}$), As (4.444 ± 0.438 $\mu g.mg^{-1}$) and Cd (1.729 ± 0.184 $\mu g.mg^{-1}$) and the lowest levels of Al (13.489 ± 1.373 $\mu g.mg^{-1}$) and Ni (0.052 ± 0.019 $\mu g.mg^{-1}$) compared to BAT and PDB ($P \leq 0.05$). Moreover, PAU samples (3.014 ± 0.178 $\mu g.mg^{-1}$) showed a significantly higher MPI value than BAT (1.689 ± 0.217 $\mu g.mg^{-1}$) and PDB (2.022 ± 0.193 $\mu g.mg^{-1}$), suggesting a higher total content of metals in their livers. Details are given in Table 2.

Organic contaminants in eels' muscle – Four OCPs (2,4'DDE; heptachlor; heptachlor epoxide and mirex) and five BDEs (BDE100; BDE153; BDE154; BDE183 and BDE209) were not detected in any sample and were then removed from the analysis. Except for γ-HCH (0.551 ± 0.058 $ng.g^{-1}$) and BDE28 (0.353 ± 0.055 $ng.g^{-1}$) that are significantly more present in BAT eels, all organic pollutants showed the highest levels in PAU fish ($p \leq 0.05$). With a mean ± SE of 678.480 ± 93.419 $ng.g^{-1}$, ΣPCBs is 20- and 25-times higher in PAU than in PDB and BAT, respectively; ΣOCPs in PAU (56.997 ± 8.13 $ng.g^{-1}$) is 16- and 22-times higher than in PDB and BAT, respectively; and ΣBDEs in PAU (2.623 ± 0.422 $ng.g^{-1}$) is 4- and 3-times higher than in PDB and BAT, respectively (Table 2).

A Principal Component Analysis (PCA) was carried out on the whole pollutants' levels (Figure 1). Eigenvalues in components one and two represent 51% and 10% of the variation in the data, respectively and the site of origin appears as the major explanatory variable. The first component is associated with POPs levels and separates the more contaminated estuarian samples (Pauillac) from the freshwater samples (Batejin and Pas-du-Bouc). The second component is rather associated with metals and discriminates Batejin samples and Pas-du-Bouc samples within the freshwater cluster.

Assessment of eels' diet – Following quality trimming, filtering, taxonomic classification with Kraken2 (v. 2.0.8), and removal of unclassified sequences we analysed a total of 18,182,766 metazoan sequences from eels' intestine, more than 90% of which were classified within five Classes: *Insecta*, *Actinopteri* (referred to as Fish thereafter), *Mammalia*, *Aves* (referred to as Birds thereafter), *Bivalvia*, *Gastropoda*, and *Malacostraca*. Based on individual percentages, 3 groups were created through k-mean clustering (R command "kmeans", the optimal number of clusters was determined with the Elbow method, supplementary file 3). Group A contained only 3 individuals that were

characterized by a higher percentage of Malacostraca. Group B contained 15 individuals with higher percentages of insects, bivalves and gastropods. Group C contained 27 individuals whose intestines contained more fish, mammals, and birds as preys (Table 3).

Differences in microbial community structure between sampling sites – Following quality trimming, filtering, taxonomic classification with Kaiju (v.1.7.4), and removal of unclassified sequences we analysed a total of 7,416,386 microbial sequences. Library sizes ranged from 28,877 to 726,035 with an average size of 151,354 (details are given in supplementary file 2). After removal of unassigned reads, rarefaction curves at the species-level indicated that the sequencing depth was sufficient to capture microbial complexity in each library (supplementary file 4). The gut microbial community was mainly composed of Bacteria, accounting for an average of 69% of the sequences, while the Eukaryota, Archaea and viruses accounted for 26%, 1.3% and 2.7% of the sequences, respectively (Figure 2A and Supplementary file 5).

A total of 1,079 taxa were identified at the genus level. Among which 645 bacterial genera from 24 phyla were detected. The most dominant bacterial phyla in the eel gut samples, averaged for all samples, were *Proteobacteria* (36.97% of the Bacteria), *Firmicutes* (30%) and *Actinobacteria* (16.17%) followed by *Bacteroidetes*, *Fusobacteria,* and *Cyanobacteria*. The remaining 18 phyla combined represented only 10% of the relative abundance of Bacteria. Amongst microbial eukaryotic sequences, a total of 335 fungal genera from 8 phyla were detected. Fungi represented most of the microbial Eukaryotes (58%). The most abundant fungal phyla were *Ascomycota* (48%) and *Basidiomycota* (35%) followed by *Mucoromycota* (6%) and *Chytridiomycota* (5%). The remaining microbes belonged to viruses (2.75%) and Archaea (1%). The *A. anguilla* core microbiota is made of 33 bacterial genera that were identified at an 80% prevalence threshold (Figure 2B and Supplementary file 5) from the Phyla *Actinobacteria* (10 genera), *Proteobacteria* (9 genera) and *Firmicutes* (6 genera). *Streptomyces* (*Actinobacteria*); *Clostridium* and *Bacillus* (*Firmicutes*); and *Vibrio* and *Pseudomonas* (*Proteobacteria*) were the only genera found in all the samples. This core microbiota was completed by *Fungi* from the phylum *Ascomycota*, other Eukaryotes and Viruses.

Differences in microbial community structures were observed between sites (Figure 2 and Supplementary file 5). BAT samples were dominated by *Firmicutes* (58%) with *Clostridiales* being the major Order (88.5% of *Firmicutes*). *Actinobacteria* were dominant in PDB samples (34%) with *Streptomycetales* being the major Order (36.8%) and *Proteobacteria* dominated PAU samples (52%) with a majority of *Vibrionales* (63.3%). We also observed large differences in Fungi abundance that represents an average of 6.5%, 12.4% and 24% of sequences in BAT, PAU and PDB, respectively.

We calculated four within-sample (alpha) diversity indices: number of observed OTUs, Shannon index, Chao1 and Pielou's Evenness (Figure 3). Significant differences at the genus level were observed among sites. BAT eels had significantly higher observed number of OTUs and Chao1 index,

while PDB eels had significantly higher Shannon index and Evenness values. This suggests that species richness was higher in BAT samples while relative taxonomic abundance was more homogeneous in PDB samples, with less dominant genera.

A Principal Coordinate Analysis (PCoA) was used to study the genus composition and the effect of the sampling site on the β-diversity of microbial communities (Figure 4). The site of origin of the fish accounted for a substantial variation in β-diversity of the gut microbiome at the genus level (PERMANOVA R^2 = 0.429, p = 0.001). This effect was detected at all taxonomic levels (Supplementary file 6 and Supplementary file 7). Moreover, the microbial composition of PDB appeared more homogeneous since these samples showed a significantly lower beta dispersion compared to BAT (permuted p-value = 0.009) and PAU (permuted p-value = 0.001). BAT and PAU showed a similar beta dispersion (permuted p-value = 0.175). Following site, diet also had an impact on microbial β-diversity (PERMANOVA R^2 = 0.277, p = 0.001) as the microbiome of fish harbouring Diet B significantly differed from those with Diet C (p = 0.003). Salinity (*i.e.* BAT and PDB = freshwater and PAU = estuary) also had an effect, though smaller, on β-diversity (PERMANOVA R^2 = 0.150, p = 0.001).

Identification of covariates impacting gut microbiota in European eels – Site of origin (42.9%) and diet (27.7%) were identified to be the main factors impacting microbial diversity. A univariate analysis revealed that amongst all the covariates associated to these parameters (15 metals, MPI, 9 biometric values and indices, 19 POPs, 3 sums of POPs, and proportion of 7 classes of preys), 32 were significantly associated with microbiome composition (FDR ≤ 0.01) (Figure 4). POPs levels (18 covariates) showed strong associations with gut microbiota especially PCBs, followed by metals (9 covariates). Four out of the seven classes of prey in eels' diet influenced microbiota. The Hepato-somatic index was the only morphometric variable to associate with gut microbiota of eels. Some variables showed overlapping directionalities for the ordination of gut microbiota composition suggesting a redundancy amongst covariates. Five non-redundant covariates with a cumulative effect size (r^2) of 0.534 were identified by a forward stepwise redundancy analysis. The relative abundance of fish in eels' diet was the main non-redundant factor impacting gut microbiota (p-value = 0.002) with an effect size of 0.220, followed by the levels of PCB101 (r^2 = 0.154, p-value = 0.002), γHCH (r^2 = 0.090, p-value = 0.002), transnonachlor (r^2 = 0.044, p-value = 0.004) and Arsenic (r^2 = 0.023, p-value = 0.014).

Differential abundance of microbial genera between sites – Here we focused on differences amongst sites since it appeared as the main factor explaining microbiome composition in eels' intestines. Within the 1079 genera we identified previously, a LEfSe analysis identified 726 discriminant features (p-value ≤ 0.01, LDA score > 2), amongst which 323 at the Genus level (details in Supplementary file 8). As previously suggested by lower Pielou's evenness values (Figure 3), a limited number of dominant taxa were identified in BAT and PAU samples. Most of the 32

significantly enriched genera in BAT samples belonged to the Phylum *Firmicutes* (25 genera), class *Clostridia* (17 genera). Amongst the 8 significantly enriched genera in PAU samples, 7 were the class *Gammaproteobacteria* with the order *Vibrionales* accounting for most of them (5 out of 7). Dominant taxa were harder to identify in PDB samples which had 283 enriched genera. Most of those (132 genera) are Fungi with 50.0% of *Ascomycota* and 33.3% of *Basidiomycota*. Bacteria enriched in PDB (135 genera) were in majority from the phyla *Actinobacteria* (42 genera) and *Proteobacteria* (63 genera). These results are in agreement with what was previously suggested by the taxonomic composition in Figure 2 (and Supplementary file 5). No enrichment was observed in relation to the diet group. The most differentially abundant taxa (LDA > 4.0) enriched in each site are presented in Figure 5. A Spearman correlation analysis based on the most abundant genera in each site showed significant associations with pollution- and diet-related covariates (Figure 6).

DISCUSSION:

Gut microbiomes of fish are complex, dynamic communities influenced by a wide variety of extrinsic factors such as environmental parameters, and intrinsic factors such as physiology or genetic background of the host (Butt and Volkoff, 2019). The characterization of the microbial communities that are present in fish and the comprehension of what factors influence that composition are equally important and may contribute to the diagnostic of habitat health condition, especially in sentinel organisms. Micropollutants of the environment are now recognized as strong and pivotal factors shaping the gastrointestinal microbiome (Adamovsky et al., 2018; Claus et al., 2016; Duperron et al., 2020). Altogether, our results indicate that environmental parameters, particularly pollutants, may alter microbial community in the gut of eels and that microbial taxa may represent innovative biomarkers of environmental quality. Moreover, as gut microbiota may differ between wild and captured / cultured fish (Egerton et al., 2018; Uren Webster et al., 2018), the use of wild specimens increases the degree of ecological relevance. One should note that our work was based on observations of both the gut digesta and mucosa of fish and that both resident and transient microbial communities (also referred to as autochtonous and allochthonous, respectively) were studied simultaneously. Most of the current literature on teleost microbiomes was obtained through 16S rRNA barcoding (Llewellyn et al., 2014). In this study, we used whole metagenome sequencing (WMS) that has revealed an effective method for analysing both the feeding habits and gut microbiomes of fish (Pan et al., 2021). In addition to taxa information down to species and even strain levels that are not restricted to procaryotes, the use of WMS can allow analysis of functional potentials of microbiomes. Unfortunately, the sequencing depth here was not sufficient to assemble a metagenome and we were able to annotate only a small proportion of kaiju reads (Supplementary files 9 and 10). Sample preparation and DNA extraction methods should be improved toward this

objective in the future. However, shallow whole-metagenome shotgun sequencing may still provide highly similar data to WMS and more accurate taxonomic assignments than rRNA targeted sequencing (Xu et al., 2021).

We identified 1,079 taxa at the genus level. Unsurprisingly, Bacteria were the main component of eels' intestinal microbiota. This result is similar to other fish species that received more interest from the microbiome research community in which the phyla *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Fusobacteria*, and *Tenericutes* are commonly reported to be amongst the most dominant members present in the gastrointestinal tracts of fish species (Ghanbari et al., 2015; Tarnecki et al., 2017). A small proportion of the microbiome was made of *Archaea* and viruses. Though they are common components of vertebrates' gut microbiota, information in fish is scarce, either in terms of composition or functionality. Finally, amongst eukaryotes, *Ascomycota* and *Basidiomycota* have been previously identified in several marine and freshwater fish. Various yeasts have been identified in the normal intestinal microbiota of fish in aquaculture. Their presence is generally considered as commensalism, even though opportunistic strains may reveal pathogenic in some case (Gatesoupe, 2007). In our results, Fungi were particularly abundant in eels collected from Pas-du-Bouc (Figures 2 and 5). This result is a first hint on the potential bioindicative role that some microbial taxa could play regarding the links between European eels' health and/or ecosystem quality.

Our results showed similarities with previous studies in European eel such as the dominance of *Proteobacteria* (Huang et al., 2018) in all the samples or the abundance of *Fusobacteria* and *Firmicutes* in larger individuals (Shi et al., 2020). However, we must remain careful before we extrapolate from previous literature since we used wild animals and a larger number of individuals compared to previous literature. In addition, this discrepancy may come from the much larger use of bacterial 16S rRNA gene amplicon targeted sequencing in fish literature to date (Talwar et al., 2018). PCR-based sequencing, unlike WMS, may indeed bias results toward certain bacterial phyla depending on the primer that were used (Hansen et al., 1998).

Numerous drivers of gut microbiome diversity have been reported, amongst which external environment and diet (Johny et al., 2021; Legrand et al., 2020; Sullam et al., 2012; Tarnecki et al., 2017). In our study, differences in alpha-diversity were observed between sites (Figure 3). This first result seems in accordance with Kim *et al.* (2021) who showed that the gut microbiota is primarily determined by host habitat, rather than by genetic factors. Fish from Batejin, that were less contaminated by micropollutants (Table 2 and Figure 1), exhibited a significantly higher microbial richness as indicated by higher numbers of observed OTUs and Chao1 values. As Chao1 evaluate the total richness, the fact that these two values were similar confirm that we were able to capture most of the microbial diversity in all our samples. This was also evidenced by saturation curves

(Supplementary file 4). Pas-du-Bouc samples exhibited a higher microbial diversity demonstrated by a higher value of Shannon index and more indicator taxa identified by LEfSE in this group of fish. The site of origin was also the main factor impacting beta diversity. We observed a larger dissimilarity in beta-diversity of PAU samples (Figure 4) that were characterized by higher pollution levels (*i.e.* significantly higher MPI, ∑PCBs, ∑OCPs, and ∑BDEs). This result might be an example of the Anna Karenina principle (AKP) (Zaneveld et al., 2017). The AKP predicts that stressors have stochastic rather than deterministic effects on community composition and that healthy microbiomes are similar whereas each dysbiotic microbiome is dysbiotic in its own way. To explain these differences, we performed linear correlations that identified a series of persistent organic pollutants, metals, as well as diet components that are drivers of this beta diversity (Figure 4). Amongst those, we identified five non-redundant determinants with a relatively high cumulative effect size on community variation (53.4%).

The proportion of fish preys in eels diet showed the largest effect (22.0%). Fish can employ a large variety of feeding strategies, and numerous studies have shown diet can influence intraspecies differences in fish gut microbiota (*e.g.* Desai et al., 2012). For instance, the guts of carnivorous fish was dominated by protease producing bacteria whereas herbivorous fish guts by cellulose degrading bacteria (Liu et al., 2016). In *A. anguilla* at the yellow eel stage, dietary differences may result in two morphotypes amongst individuals from the same population and of similar size. Eels feeding on hard food developed broader heads than soft feeders with narrow-heads (De Meyer et al., 2016; Proman and Reynolds, 2000), with consequences on growth rate (De Meyer et al., 2017). According to its physiological role, it would then be interesting to test whether these phenotypic differences could be linked with alterations of the microbiome. The intestinal microbial diversity usually increases sequentially from carnivores to herbivores and to omnivores (Kashinskaya et al., 2018). *A. anguilla* is a generalist predator. Though it feeds mainly on invertebrates and fish, some individuals exhibit scavenger behaviour feeding on dead animals (Sporta Caputi et al., 2020). Our results meet this assumption since eels from Pas-du-Bouc, which have a microbiome composition influenced by the proportion of fish, mammals and bird preys in their diet, also showed a lower microbial alpha-diversity (Observed and Chao1). However, cautions must be taken regarding the impact of diet on microbiota in wild individuals. First, most studies linking diet and microbiome composition were performed in culture or controlled conditions (Perry et al., 2020; Tarnecki et al., 2017). Then, in fish, gut microbiome composition and its predicted metabolic function may vary shortly after a feeding event and/or during the digestion cycle (Parris et al., 2019), and finally, the DNA detected in the dietary material can either come from the environment (eDNA), from the food eaten by the eels, or from remnant DNA contained in the diet of eels' prey. However, many authors reported a nocturnal feeding activity in *A. anguilla (Tesch and Thorpe, 2003)* suggesting that, since sampling was

performed during daytime, the differences in microbiota composition observed here unlikely resulted from a recent food intake.

If chemicals can alter microbial communities, the microbiota also has the ability to biotransform a wide variety of chemicals, hence influencing fundamental properties of toxicants (Claus et al., 2016). The comprehension of how chemicals may perturb the host-microbiome relationship is crucial to establish links between chemical exposures and adverse effects. This can have long-term implications for adaptation of organisms in highly contaminated environments (Adamovsky et al., 2018). In the present study, three POPs (PCB101, γHCH, and Transnonachlor) and arsenic showed a cumulative effect size of 31.3%. Recent studies have investigated the impact of POPs exposure on the gut microbiota in mammals and the potential consequences on health and disease (Chiu et al., 2020; Tian et al., 2020). But in fish, even though POPs are known to impair endocrine functions, early development, growth / condition / energy reserves, reproduction, immunity, and behaviour (Johnson et al., 2013), links with the microbiome are still poorly investigated. The fact that, in the present study, the level of many POPs showed strong association with gut microbiota should encourage research toward this direction. Amongst all the environmental and biological parameters that we measured in each individual eel; our work allowed the identification of a reduced number of factors that drive microbial communities in this particular context. Those should be further investigated in controlled conditions to better characterize their mode of action and specific consequences on fish microbiome and health.

Migratory amphidromous fish experience extreme modifications of environmental conditions, especially salinity (Sullam et al., 2012), during transit from freshwater to seawater, along with physiological changes associated with metamorphosis that may impact gut microbiota (*e.g.* Dehler et al., 2017; Llewellyn et al., 2016; Zhao et al., 2020). This suggests that gut microbial community adaptations allow fish to cope with changing environmental conditions, even though differences may exist between species. In our study, PERMANOVA analysis revealed that salinity had a smaller effect on microbiota structure (10.5 %) than site of origin (42.9 %) and diet composition (27.7 %). At yellow stage, *A. anguilla* has the ability to move back and forth between seawater, estuaries and freshwater mostly for feeding purposes. Some individuals may even never live in freshwater (Daverat et al., 2006). This phenotypic plasticity could partly result from the limited impact of salinity on gut microbial composition that we observed here. The effects of salinity and metamorphosis on gut microbiota should certainly be further investigated in mature individuals (silver eels) prior or during their catadromous migration.

Owing to their involvement in crucial processes in all environmental compartments such as wastewaters, continental and coastal surface water, groundwater, and sediments, along with their sensitivity to alterations, their quick and early responses, microorganisms are now commonly used as

early-warning bioindicators in ecotoxicological studies (Ghiglione et al., 2016; Pesce et al., 2020). The recent coupling of host-associated microbiome research with ecotoxicology in multicellular organisms is a promising approach (Duperron et al., 2020). Here, 323 genera showed differential abundance amongst sites (Figure 5). Due to a larger variability in their beta-diversity (see above), *Vibrionales* was the only enriched taxon (LDA = 5.14, p-value ≤ 0.001) identified in the fish from the Gironde estuary (Pauillac). *Vibrionales* bacteria are abundant in marine fish (*e.g.* Egerton et al., 2018; Sullam et al., 2012) and contain beneficial and pathogenic representatives. In chinook salmon (*Oncorhynchus tshawytscha*), juveniles exposed to organic chemical pollutants in a contaminated urban estuary were immunosuppressed and more susceptible to pathogenic strains, such as *Vibrio anguillarum* (Arkoosh et al., 1998). In Teleosts, the spleen is the major organ of immunity and fish with smaller spleen-somatic index show lower immune response and disease resistance (Hadidi et al., 2008). SSI values were smaller (0.08 ± 0.01) in PAU eels (Table 1) and micropollutants, particularly POPs, had an important effect on the abundance of *Vibrionales* (*e.g.* Transnonachlor, Figure 6). Thus, we cannot exclude the hypothesis that these compounds may increase the risk of infection by pathogenic members of *Vibrionales* by impairing the immune system of European eels.

In Batejin, that could be considered the less contaminated of our three study sites, taxa of the class *Clostridia* (Phylum *Firmicutes*) were significantly more abundant. Within *Clostridia*, the order *Clostridiales* is associated with the enhanced production of the short chain fatty acids acetate, butyrate, and propionate and with the degradation and metabolism of sugars in animals. In the common carp (*Cyprinus carpio L.*), a greater proportion of *Clostridiales* was associated with higher food intake and growth rate (Li et al., 2013) suggesting the good health of eels there. This hypothesis was partly confirmed by the higher values of Hepatosomatic Index (HSI) in BAT eels. In fisheries science, this index is used as an indicator of energy reserves in the liver. Correlation studies showed that, in our eels, the abundance of *Clostridiales* was negatively impacted by heavy metals (Mo and Zn) and diet composition (Fish and Mammal preys). The physiological role of these bacteria and the impact of pollutants should be further investigated by functional studies.

Basidiomycota and *Ascomycota* phyla were enriched in our fish from Pas-du-Bouc (Figure 5 and Supplementary file 5). Fungi colonizing animal gut, also referred to as mycobiota, is an important, yet largely neglected component of animal microbiome (Lavrinienko et al., 2021), partly because in wild animals, resident gut mycobiota is often overwhelmed by the large proportion of food-borne *Fungi*. However, strong and consistent covariation between fungal and bacterial communities were identified across animal kingdom by Harrison et al. (2021) suggesting an important role in host physiology through metabolic interactions with *Bacteria*. For instance, In *Actinopterygii*, these authors found that *Basidiomycota* and *Ascomycota* showed positive co-occurrence with *Firmicutes* and *Proteobacteria*. In PDB fish, genus *Aspergillus* was the indicator taxon with the larger LDA score,

and was positively correlated to Mo, Zn and the proportion of fish in diet. *Aspergillus* sp. is a saprophyte fungus occurring on decaying vegetation that could have been more abundant in the PDB area facilitating ingestion by the fish. Likewise, the free-living soil bacteria *Streptomyces* sp. was significantly more present in the gut of PDB eels. The presence of this genus as symbiont in gut microbiota has been previously reported in animals (Seipke et al., 2012), including freshwater fish (Jami et al., 2015) where they could help in the digestion of insoluble organic polymers, like chitin or cellulose but their precise role remains unclear. The class *Actinobacteria* are known to produce many secondary metabolites, including anti-inflammatory cytokines. *Actinobacteria*, that were more abundant in PDB (LDA = 4.85, p-value ≤ 0.001), are usually found on the bottom mud of lakes and water streams. In bighead carp (*Aristichthys nobilis*), they were more abundant in the gut of omnivorous juvenile fish living in lower water layers than in adult filter feeders that live in middle to upper water layers (Lu et al., 2022). In European eels also, these modifications could hence reflect a difference in feeding habits.

CONCLUSION:

Microbiome response to pollutants is crucial but remains an underestimated element to better understand the toxicity of environmental contaminants on aquatic organisms (Duperron et al., 2020; Evariste et al., 2019). The sensitivity of microbiomes to environmental stressors suggests that microbial communities could represent novel ecological indicators for biomonitoring of ecosystem or host health (Sehnal et al., 2021). In the present study, we were able to associate environmental contamination and biologic variables with modifications in taxonomic composition of intestinal gut microbiome of European eels from three contrasted sites. The comprehension of how this species responds to contamination is crucial for environmental risk assessment. We also identify microbial genera specifically enriched in each of these sites. That suggests that changes in the structure of microbial communities associated with the gut of European eels could be an underestimated route by which the health of this endangered species is threatened by pollutants present in the environment. To the best of our knowledge, this work constitutes the first example trying to link gut microbiome modifications with the ecotoxicological landscape in wild fish. Due to the conservation status of *A. anguilla*, the use of gut microbial modifications as novel environmental biomarkers of contamination shall be coupled to non invasive sampling methods.

In the future, it will be necessary to complement the description of microbiome changes with the measurement of other endpoints of toxicological relevance in the host such as markers of the immune status or histology, as well as with functional studies of host-microbiota interactions.

ACKNOWLEDGEMENTS:

559 This study has been carried out with financial support from the French National Research Agency
560 (ANR) in the frame of the Investments for the future Programme, within the Cluster of Excellence
561 COTE (ANR-10-LABX-45). The GeT core facility was supported by France Génomique National
562 infrastructure (ANR-10-INBS-09). We thank the analytical platforms “Majeurs et Métaux traceS”
563 (M&Ms) and PLATINE at UMR 5805 EPOC (University of Bordeaux) for performing the quantification
564 of trace elements and POPs in fish tissues, respectively. This work benefited from the French GDR
565 “Aquatic Ecotoxicology” framework which aims at fostering stimulating scientific discussions and
566 collaborations for more integrative approaches. The authors thank Romaric Le Barh and Gwilherm
567 Jan as well as Jean-Christophe Huguet, and all the volunteers from UMR 5805 EPOC and
568 FDAAPPMA33 for their field support in fishing and dissections.
569

BIBLIOGRAPHY:

Adamovsky, O., Buerger, A.N., Wormington, A.M., Ector, N., Griffitt, R.J., Bisesi, J.H., Martyniuk, C.J., 2018. The gut microbiome and aquatic toxicology: An emerging concept for environmental health. Env. Toxicol Chem 37, 2758–2775. https://doi.org/10.1002/etc.4249

Arkoosh, M.R., Casillas, E., Huffman, P., Clemons, E., Evered, J., Stein, J.E., Varanasi, U., 1998. Increased Susceptibility of Juvenile Chinook Salmon from a Contaminated Estuary to *Vibrio anguillarum*. Trans. Am. Fish. Soc. 127, 360–374. https://doi.org/10.1577/1548-8659(1998)127<0360:ISOJCS>2.0.CO;2

Baillon, L., Pierron, F., Coudret, R., Normendeau, E., Caron, A., Peluhet, L., Labadie, P., Budzinski, H., Durrieu, G., Sarraco, J., Elie, P., Couture, P., Baudrimont, M., Bernatchez, L., 2015. Transcriptome profile analysis reveals specific signatures of pollutants in Atlantic eels. Ecotoxicology 24, 71–84. https://doi.org/10.1007/s10646-014-1356-x

Belpaire, C., Goemans, G., Geeraerts, C., Quataert, P., Parmentier, K., 2008. Pollution fingerprints in eels as models for the chemical status of rivers. ICES J. Mar. Sci. 65, 1483–1491. https://doi.org/10.1093/icesjms/fsn112

Bertucci, A., Pierron, F., Ye, T., Gonzalez, P., Couture, P., Baudrimont, M., 2019. Identification and expression of microRNAs in european eels Anguilla anguilla from two natural sites with different pollution levels. Environ. Pollut. 250, 274–283. https://doi.org/10.1016/j.envpol.2019.04.029

Bevacqua, D., Melia, P., Gatto, M., De Leo, G.A., 2015. A global viability assessment of the European eel. Glob Chang Biol 21, 3323–35. https://doi.org/10.1111/gcb.12972

Boutin, S., Bernatchez, L., Audet, C., Derôme, N., 2013. Network Analysis Highlights Complex Interactions between Pathogen, Host and Commensal Microbiota. PLoS ONE 8, e84772. https://doi.org/10.1371/journal.pone.0084772

Burokas, A., Moloney, R.D., Dinan, T.G., Cryan, J.F., 2015. Microbiota Regulation of the Mammalian Gut–Brain Axis, in: Advances in Applied Microbiology. Elsevier, pp. 1–62. https://doi.org/10.1016/bs.aambs.2015.02.001

Butt, R.L., Volkoff, H., 2019. Gut Microbiota and Energy Homeostasis in Fish. Front. Endocrinol. 10, 9. https://doi.org/10.3389/fendo.2019.00009

Carda-Dieguez, M., Ghai, R., Rodriguez-Valera, F., Amaro, C., 2017. Wild eel microbiome reveals that skin mucus of fish could be a natural niche for aquatic mucosal pathogen evolution. Microbiome 5, 162. https://doi.org/10.1186/s40168-017-0376-1

Carda-Diéguez, M., Ghai, R., Rodriguez-Valera, F., Amaro, C., 2014. Metagenomics of the Mucosal Microbiota of European Eels. Genome Announc. 2. https://doi.org/10.1128/genomeA.01132-14

Chiu, K., Warner, G., Nowak, R.A., Flaws, J.A., Mei, W., 2020. The Impact of Environmental Chemicals on the Gut Microbiome. Toxicol. Sci. 176, 253–284. https://doi.org/10.1093/toxsci/kfaa065

Claus, S.P., Guillou, H., Ellero-Simatos, S., 2016. The gut microbiota: a major player in the toxicity of environmental pollutants? Npj Biofilms Microbiomes 2, 16003. https://doi.org/10.1038/npjbiofilms.2016.3
https://www.nature.com/articles/npjbiofilms20163#supplementary-information

Daverat, F., Limburg, K., Thibault, I., Shiao, J., Dodson, J., Caron, F., Tzeng, W., Iizuka, Y., Wickström, H., 2006. Phenotypic plasticity of habitat use by three temperate eel species, Anguilla anguilla, A. japonica and A. rostrata. Mar. Ecol. Prog. Ser. 308, 231–241. https://doi.org/10.3354/meps308231

De Meyer, J., Christiaens, J., Adriaens, D., 2016. Diet-induced phenotypic plasticity in European eel (*Anguilla anguilla*). J. Exp. Biol. 219, 354–363. https://doi.org/10.1242/jeb.131714

De Meyer, J., Maes, G.E., Dirks, R.P., Adriaens, D., 2017. Differential gene expression in narrow- and broad-headed European glass eels (*Anguilla anguilla*) points to a transcriptomic link of head shape dimorphism with growth rate and chemotaxis. Mol. Ecol. 26, 3943–3953. https://doi.org/10.1111/mec.14155

Dehler, C.E., Secombes, C.J., Martin, S.A.M., 2017. Seawater transfer alters the intestinal microbiota profiles of Atlantic salmon (Salmo salar L.). Sci. Rep. 7, 13877. https://doi.org/10.1038/s41598-017-13249-8

Desai, A.R., Links, M.G., Collins, S.A., Mansfield, G.S., Drew, M.D., Van Kessel, A.G., Hill, J.E., 2012. Effects of plant-based diets on the distal gut microbiome of rainbow trout (Oncorhynchus mykiss). Aquaculture 350–353, 134–142. https://doi.org/10.1016/j.aquaculture.2012.04.005

Duperron, S., Halary, S., Gallet, A., Marie, B., 2020. Microbiome-Aware Ecotoxicology of Organisms: Relevance, Pitfalls, and Challenges. Front. Public Health 8, 407. https://doi.org/10.3389/fpubh.2020.00407

Egerton, S., Culloty, S., Whooley, J., Stanton, C., Ross, R.P., 2018. The Gut Microbiota of Marine Fish. Front. Microbiol. 9, 873–873. https://doi.org/10.3389/fmicb.2018.00873

Evariste, L., Barret, M., Mottier, A., Mouchet, F., Gauthier, L., Pinelli, E., 2019. Gut microbiota of aquatic organisms: A key endpoint for ecotoxicological studies. Environ. Pollut. 248, 989–999. https://doi.org/10.1016/j.envpol.2019.02.101

Feng, Q., Chen, W.-D., Wang, Y.-D., 2018. Gut Microbiota: An Integral Moderator in Health and Disease. Front. Microbiol. 9, 151. https://doi.org/10.3389/fmicb.2018.00151

Feunteun, E., 2002. Management and restoration of European eel population (Anguilla anguilla): An impossible bargain. Ecol. Eng. 18, 575–591. https://doi.org/10.1016/S0925-8574(02)00021-6

Fourquet, J., Noirot, C., Klopp, C., Pinton, P., Combes, S., Hoede, C., Pascal, G., 2020. Whole metagenome analysis with metagWGS [Poster]. JOBIM2020.

Fouz, B., Toranzo, A.E., Milan, M., Amaro, C., 2000. Evidence that water transmits the disease caused by the fish pathogen Photobacterium damselae subsp. damselae. J. Appl. Microbiol. 88, 531–535. https://doi.org/10.1046/j.1365-2672.2000.00992.x

Gatesoupe, F.J., 2007. Live yeasts in the gut: Natural occurrence, dietary introduction, and their effects on fish health and development. Aquaculture 267, 20–30. https://doi.org/10.1016/j.aquaculture.2007.01.005

Ghanbari, M., Kneifel, W., Domig, K.J., 2015. A new view of the fish gut microbiome: Advances from next-generation sequencing. Aquaculture 448, 464–475. https://doi.org/10.1016/j.aquaculture.2015.06.033

Ghiglione, J.-F., Martin-Laurent, F., Pesce, S., 2016. Microbial ecotoxicology: an emerging discipline facing contemporary environmental threats. Environ. Sci. Pollut. Res. 23, 3981–3983. https://doi.org/10.1007/s11356-015-5763-1

Grice, E.A., Segre, J.A., 2012. The human microbiome: our second genome. Annu. Rev. Genomics Hum. Genet. 13, 151–170. https://doi.org/10.1146/annurev-genom-090711-163814

Guimaraes, L., Gravato, C., Santos, J., Monteiro, L.S., Guilhermino, L., 2009. Yellow eel (Anguilla anguilla) development in NW Portuguese estuaries with different contamination levels. Ecotoxicology 18, 385–402. https://doi.org/10.1007/s10646-008-0294-x

Hadidi, S., Glenney, G.W., Welch, T.J., Silverstein, J.T., Wiens, G.D., 2008. Spleen Size Predicts Resistance of Rainbow Trout to *Flavobacterium psychrophilum* Challenge. J. Immunol. 180, 4156–4165. https://doi.org/10.4049/jimmunol.180.6.4156

Hansen, M.C., Tolker-Nielsen, T., Givskov, M., Molin, S., 1998. Biased 16S rDNA PCR amplification caused by interference from DNA flanking the template region. FEMS Microbiol. Ecol. 26, 141–149. https://doi.org/10.1111/j.1574-6941.1998.tb00500.x

Harrison, X.A., McDevitt, A.D., Dunn, J.C., Griffiths, S.M., Benvenuto, C., Birtles, R., Boubli, J.P., Bown, K., Bridson, C., Brooks, D.R., Browett, S.S., Carden, R.F., Chantrey, J., Clever, F., Coscia, I., Edwards, K.L., Ferry, N., Goodhead, I., Highlands, A., Hopper, J., Jackson, J., Jehle, R., da Cruz Kaizer, M., King, T., Lea, J.M.D., Lenka, J.L., McCubbin, A., McKenzie, J., de Moraes, B.L.C., O'Meara, D.B., Pescod, P., Preziosi, R.F., Rowntree, J.K., Shultz, S., Silk, M.J., Stockdale, J.E., Symondson, W.O.C., de la Pena, M.V., Walker, S.L., Wood, M.D., Antwis, R.E., 2021. Fungal microbiomes are determined by host phylogeny and exhibit widespread associations with the bacterial microbiome. Proc. R. Soc. B Biol. Sci. 288, 20210552. https://doi.org/10.1098/rspb.2021.0552

Huang, W., Cheng, Z., Lei, S., Liu, L., Lv, X., Chen, L., Wu, M., Wang, C., Tian, B., Song, Y., 2018. Community composition, diversity, and metabolism of intestinal microbiota in cultivated European eel (Anguilla anguilla). Appl. Microbiol. Biotechnol. 102, 4143–4157. https://doi.org/10.1007/s00253-018-8885-9

ICES, 2009. Workshop on Age Reading of European and American Eel (WKAREA), 20-24 April 2009, Bordeaux, France. ICES CM 2009\ACOM: 48. 66 pp.

Itoi, S., Okamura, T., Koyama, Y., Sugita, H., 2006. Chitinolytic bacteria in the intestinal tract of Japanese coastal fishes. Can. J. Microbiol. 52, 1158–1163. https://doi.org/10.1139/w06-082

Jacoby, D., Gollock, M., 2014. Anguilla anguilla. IUCN Red List Threat. Species e.T60344A45833138.

Jami, M., Ghanbari, M., Kneifel, W., Domig, K.J., 2015. Phylogenetic diversity and biological activity of culturable Actinobacteria isolated from freshwater fish gut microbiota. Microbiol. Res. 175, 6–15. https://doi.org/10.1016/j.micres.2015.01.009

Johnson, L.L., Anulacion, B.F., Arkoosh, M.R., Burrows, D.G., da Silva, D.A.M., Dietrich, J.P., Myers, M.S., Spromberg, J., Ylitalo, G.M., 2013. Effects of Legacy Persistent Organic Pollutants (POPs) in Fish—Current and Future Challenges, in: Fish Physiology. Elsevier, pp. 53–140. https://doi.org/10.1016/B978-0-12-398254-4.00002-9

Johny, T.K., Puthusseri, R.M., Bhat, S.G., 2021. A primer on metagenomics and next- generation

688 sequencing in fish gut microbiome research. Aquac. Res. 52, 4574–4600.
689 https://doi.org/10.1111/are.15373

690 Kashinskaya, E.N., Simonov, E.P., Kabilov, M.R., Izvekova, G.I., Andree, K.B., Solovyev, M.M.,
691 2018. Diet and other environmental factors shape the bacterial communities of fish gut in an eutrophic
692 lake. J. Appl. Microbiol. 125, 1626–1641. https://doi.org/10.1111/jam.14064

693 Khan, Mohd.S., Javed, M., Rehman, Md.T., Urooj, M., Ahmad, Md.I., 2020. Heavy metal pollution
694 and risk assessment by the battery of toxicity tests. Sci. Rep. 10, 16593.
695 https://doi.org/10.1038/s41598-020-73468-4

696 Khurana, H., Singh, D.N., Singh, A., Singh, Y., Lal, R., Negi, R.K., 2020. Gut microbiome of
697 endangered Tor putitora (Ham.) as a reservoir of antibiotic resistance genes and pathogens associated
698 with fish health. BMC Microbiol. 20, 249. https://doi.org/10.1186/s12866-020-01911-7

699 Kim, P.S., Shin, N.-R., Lee, J.-B., Kim, M.-S., Whon, T.W., Hyun, D.-W., Yun, J.-H., Jung, M.-J.,
700 Kim, J.Y., Bae, J.-W., 2021. Host habitat is the major determinant of the gut microbiome of fish.
701 Microbiome 9, 166. https://doi.org/10.1186/s40168-021-01113-x

702 Knights, B., 2003. A review of the possible impacts of long-term oceanic and climate changes and
703 fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. Sci. Total Environ. 310,
704 237–244. https://doi.org/10.1016/S0048-9697(02)00644-7

705 Kusumawaty, D., Surtikanti, H.K., Hernawati, Tallei, T.E., 2020. Data on community structure and
706 diversity of the intestinal bacteria in elver and fingerling stages of wild Indonesian shortfin eel
707 (Anguilla bicolor bicolor). Data Brief 29, 105299. https://doi.org/10.1016/j.dib.2020.105299

708 Lavrinienko, A., Scholier, T., Bates, S.T., Miller, A.N., Watts, P.C., 2021. Defining gut mycobiota for
709 wild animals: a need for caution in assigning authentic resident fungal taxa. Anim. Microbiome 3, 75.
710 https://doi.org/10.1186/s42523-021-00134-z

711 Legrand, T.P.R.A., Wynne, J.W., Weyrich, L.S., Oxley, A.P.A., 2020. A microbial sea of possibilities:
712 current knowledge and prospects for an improved understanding of the fish microbiome. Rev. Aquac.
713 12, 1101–1134. https://doi.org/10.1111/raq.12375

714 Leo, L., Sudarshan, S., 2017. Tools for Microbiome Analysis in R, Version 1.17.42. Available at:
715 http://microbiome.github.com/microbiome.

716 Li, X., Yan, Q., Xie, S., Hu, W., Yu, Y., Hu, Z., 2013. Gut Microbiota Contributes to the Growth of
717 Fast-Growing Transgenic Common Carp (Cyprinus carpio L.). PLoS ONE 8, e64577.
718 https://doi.org/10.1371/journal.pone.0064577

719 Lin, M., Zeng, C.X., Jia, X.Q., Zhai, S.W., Li, Z.Q., Ma, Y., 2019. The composition and structure of
720 the intestinal microflora of *Anguilla marmorata* at different growth rates: a deep sequencing study. J.
721 Appl. Microbiol. 126, 1340–1352. https://doi.org/10.1111/jam.14174

722 Liu, H., Guo, X., Gooneratne, R., Lai, R., Zeng, C., Zhan, F., Wang, W., 2016. The gut microbiome
723 and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. Sci. Rep.
724 6, 24340. https://doi.org/10.1038/srep24340

725 Llewellyn, M.S., Boutin, S., Hoseinifar, S.H., Derome, N., 2014. Teleost microbiomes: the state of the
726 art in their characterization, manipulation and importance in aquaculture and fisheries. Front.

Microbiol. 5. https://doi.org/10.3389/fmicb.2014.00207

Llewellyn, M.S., McGinnity, P., Dionne, M., Letourneau, J., Thonier, F., Carvalho, G.R., Creer, S., Derome, N., 2016. The biogeography of the atlantic salmon (Salmo salar) gut microbiome. ISME J. 10, 1280–1284. https://doi.org/10.1038/ismej.2015.189

Lu, Y., Zhang, P., Li, W., Liu, J., Shang, X., Cheng, Y., Li, Y., 2022. Comparison of gut microbial communities, free amino acids or fatty acids contents in the muscle of wild Aristichthys nobilis from Xinlicheng reservoir and Chagan lake. BMC Microbiol. 22, 32. https://doi.org/10.1186/s12866-022-02440-1

Oksanen, J., Blanchet, L.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2020. vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan.

Pan, W., Qin, C., Zuo, T., Yu, G., Zhu, W., Ma, H., Xi, S., 2021. Is Metagenomic Analysis an Effective Way to Analyze Fish Feeding Habits? A Case of the Yellowfin Sea Bream Acanthopagrus latus (Houttuyn) in Daya Bay. Front. Mar. Sci. 8, 634651. https://doi.org/10.3389/fmars.2021.634651

Parris, D.J., Morgan, M.M., Stewart, F.J., 2019. Feeding Rapidly Alters Microbiome Composition and Gene Transcription in the Clownfish Gut. Appl. Environ. Microbiol. 85. https://doi.org/10.1128/AEM.02479-18

Perrier, F., Bertucci, A., Pierron, F., Feurtet- Mazel, A., Simon, O., Klopp, C., Candaudap, F., Pokrovski, O., Etcheverria, B., Mornet, S., Baudrimont, M., 2020. Transfer and Transcriptomic Profiling in Liver and Brain of European Eels (*Anguilla anguilla*) After Diet- borne Exposure to Gold Nanoparticles. Environ. Toxicol. Chem. etc.4858. https://doi.org/10.1002/etc.4858

Perry, W.B., Lindsay, E., Payne, C.J., Brodie, C., Kazlauskaite, R., 2020. The role of the gut microbiome in sustainable teleost aquaculture. Proc. R. Soc. B Biol. Sci. 287, 20200184. https://doi.org/10.1098/rspb.2020.0184

Pesce, S., Ghiglione, J.-F., Topp, E., Martin-Laurent, F., 2020. Editorial: Microbial Ecotoxicology. Front. Microbiol. 11, 1342. https://doi.org/10.3389/fmicb.2020.01342

Proman, J.M., Reynolds, J.D., 2000. Differences in head shape of the European eel, *Anguilla anguilla* (L.). Fish. Manag. Ecol. 7, 349–354. https://doi.org/10.1046/j.1365-2400.2000.007004349.x

Pruzzo, C., Huq, A., Colwell, R.R., Donelli, G., 2005. Pathogenic Vibrio Species in the Marine and Estuarine Environment, in: Belkin, S., Colwell, R.R. (Eds.), Oceans and Health: Pathogens in the Marine Environment. Springer US, Boston, MA, pp. 217–252. https://doi.org/10.1007/0-387-23709-7_9

Pujolar, J.M., Marino, I.A., Milan, M., Coppe, A., Maes, G.E., Capoccioni, F., Ciccotti, E., Bervoets, L., Covaci, A., Belpaire, C., Cramb, G., Patarnello, T., Bargelloni, L., Bortoluzzi, S., Zane, L., 2012. Surviving in a toxic world: transcriptomics and gene expression profiling in response to environmental pollution in the critically endangered European eel. BMC Genomics 13, 507. https://doi.org/10.1186/1471-2164-13-507

Ray, A.K., Ghosh, K., Ringø, E., 2012. Enzyme-producing bacteria isolated from fish gut: a review. Aquac. Nutr. 18, 465–492. https://doi.org/10.1111/j.1365-2095.2012.00943.x

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12, R60. https://doi.org/10.1186/gb-2011-12-6-r60

Sehnal, L., Brammer-Robbins, E., Wormington, A.M., Blaha, L., Bisesi, J., Larkin, I., Martyniuk, C.J., Simonin, M., Adamovsky, O., 2021. Microbiome Composition and Function in Aquatic Vertebrates: Small Organisms Making Big Impacts on Aquatic Animal Health. Front. Microbiol. 12, 567408. https://doi.org/10.3389/fmicb.2021.567408

Seipke, R.F., Kaltenpoth, M., Hutchings, M.I., 2012. *Streptomyces* as symbionts: an emerging and widespread theme? FEMS Microbiol. Rev. 36, 862–876. https://doi.org/10.1111/j.1574-6976.2011.00313.x

Shi, Y., Ma, D.-Y., Shao-Wei Zhai, S.-W., 2020. Revealing the difference of intestinal microbiota composition of cultured European eels (Anguilla anguilla) with different growth rates. Isr. J. Aquac. - Bamidgeh. https://doi.org/10.46989/001c.21169

Sporta Caputi, S., Careddu, G., Calizza, E., Fiorentino, F., Maccapan, D., Rossi, L., Costantini, M.L., 2020. Changing Isotopic Food Webs of Two Economically Important Fish in Mediterranean Coastal Lakes with Different Trophic Status. Appl. Sci. 10, 2756. https://doi.org/10.3390/app10082756

Sullam, K.E., Essinger, S.D., Lozupone, C.A., O'Connor, M.P., Rosen, G.L., Knight, R., Kilham, S.S., Russell, J.A., 2012. Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis: FISH GUT BACTERIAL COMMUNITIES. Mol. Ecol. 21, 3363–3378. https://doi.org/10.1111/j.1365-294X.2012.05552.x

Talwar, C., Nagar, S., Lal, R., Negi, R.K., 2018. Fish Gut Microbiome: Current Approaches and Future Perspectives. Indian J. Microbiol. 58, 397–414. https://doi.org/10.1007/s12088-018-0760-y

Tarnecki, A.M., Burgos, F.A., Ray, C.L., Arias, C.R., 2017. Fish intestinal microbiome: diversity and symbiosis unravelled by metagenomics. J. Appl. Microbiol. 123, 2–17. https://doi.org/10.1111/jam.13415

Tartu, S., Angelier, F., Bustnes, J.O., Moe, B., Hanssen, S.A., Herzke, D., Gabrielsen, G.W., Verboven, N., Verreault, J., Labadie, P., Budzinski, H., Wingfield, J.C., Chastel, O., 2015. Polychlorinated biphenyl exposure and corticosterone levels in seven polar seabird species. Environ. Pollut. 197, 173–180. https://doi.org/10.1016/j.envpol.2014.12.007

Tesch, F.-W., Thorpe, J.E., 2003. The eel, 3rd ed. ed. Blackwell Science, Oxford, UK.

Tian, Y., Gui, W., Rimal, B., Koo, I., Smith, P.B., Nichols, R.G., Cai, J., Liu, Q., Patterson, A.D., 2020. Metabolic impact of persistent organic pollutants on gut microbiota. Gut Microbes 12, 1848209. https://doi.org/10.1080/19490976.2020.1848209

Uren Webster, T.M., Consuegra, S., Hitchings, M., Garcia de Leaniz, C., 2018. Interpopulation Variation in the Atlantic Salmon Microbiome Reflects Environmental and Genetic Diversity. Appl. Environ. Microbiol. 84, e00691-18, /aem/84/16/e00691-18.atom. https://doi.org/10.1128/AEM.00691-18

van den Thillart, G., Dufour, S., Rankin, J.C. (Eds.), 2009. Spawning Migration of the European Eel: Reproduction Index, a Useful Tool for Conservation Management. Springer Netherlands, Dordrecht. https://doi.org/10.1007/978-1-4020-9095-0

Webster, T.M.U., Rodriguez-Barreto, D., Consuegra, S., Garcia de Leaniz, C., 2019. Cortisol-induced signatures of stress in the fish microbiome (preprint). Microbiology. https://doi.org/10.1101/826503

Xu, W., Chen, T., Pei, Y., Guo, H., Li, Z., Yang, Yanan, Zhang, F., Yu, J., Li, X., Yang, Yu, Zhao, B., Wu, C., 2021. Characterization of Shallow Whole-Metagenome Shotgun Sequencing as a High-Accuracy and Low-Cost Method by Complicated Mock Microbiomes. Front. Microbiol. 12, 678319. https://doi.org/10.3389/fmicb.2021.678319

Zaneveld, J.R., McMinds, R., Vega Thurber, R., 2017. Stress and stability: applying the Anna Karenina principle to animal microbiomes. Nat. Microbiol. 2, 17121. https://doi.org/10.1038/nmicrobiol.2017.121

Zhao, R., Symonds, J.E., Walker, S.P., Steiner, K., Carter, C.G., Bowman, J.P., Nowak, B.F., 2020. Salinity and fish age affect the gut microbiota of farmed Chinook salmon (Oncorhynchus tshawytscha). Aquaculture 528, 735539. https://doi.org/10.1016/j.aquaculture.2020.735539

Zhu, P., Wong, M.K. - S., Lin, X., Chan, T.F., Wong, C.K.C., Lai, K.P., Tse, W.K.F., 2021. Changes of the intestinal microbiota along the gut of Japanese Eel (*Anguilla japonica*). Lett. Appl. Microbiol. 73, 529–541. https://doi.org/10.1111/lam.13539

Figure 1

Click here to access/download;Figure;Figure1.pdf

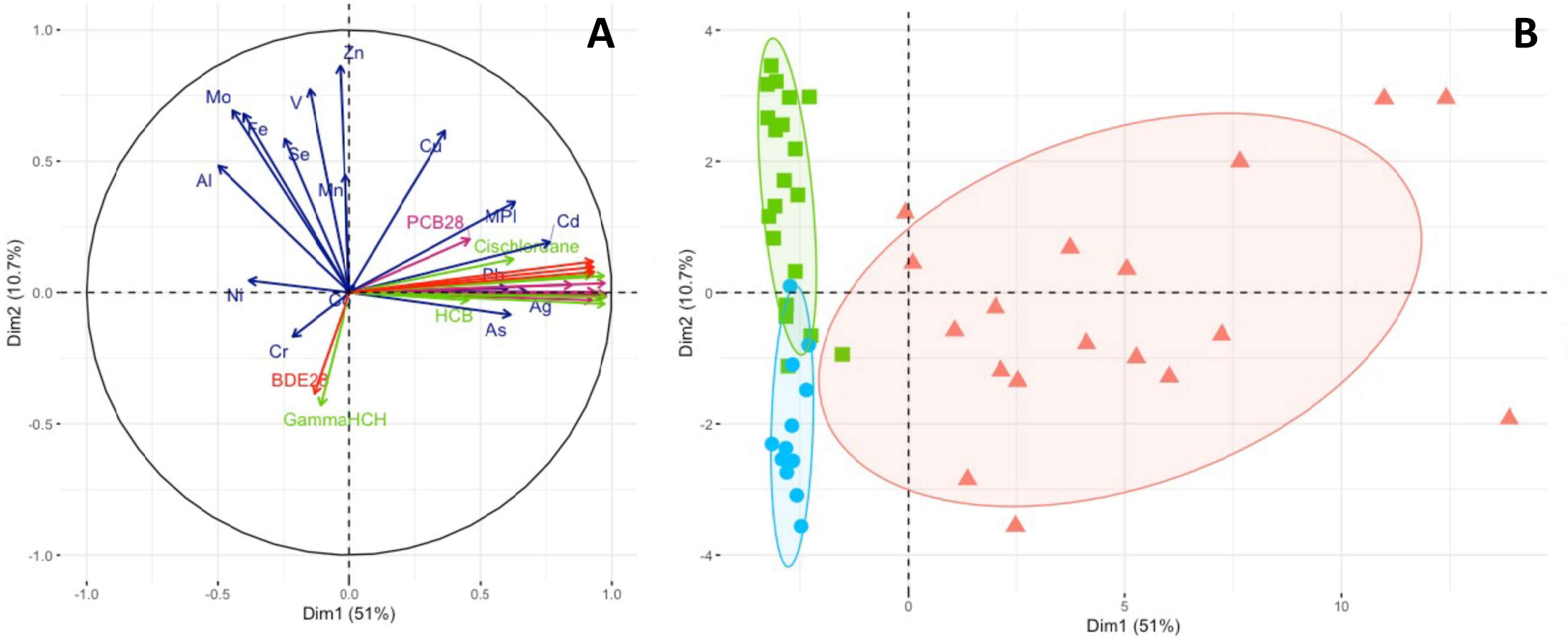


Figure 1: Principal Component Analysis results for the pollutants levels. (A) Correlation circle for all variables. Blue, purple, green and red arrows represent metals, PCBs, OCPs and BDEs, respectively. (B) Distribution of individuals and grouping according to the site of origin. Blue dots = Batejin, green squares = Pas-du-Bouc, red triangles = Pauillac. Ellipses are represented with a 0.99 confidence level.

Figure 2

Click here to access/download;Figure;Figure2_revised.pdf

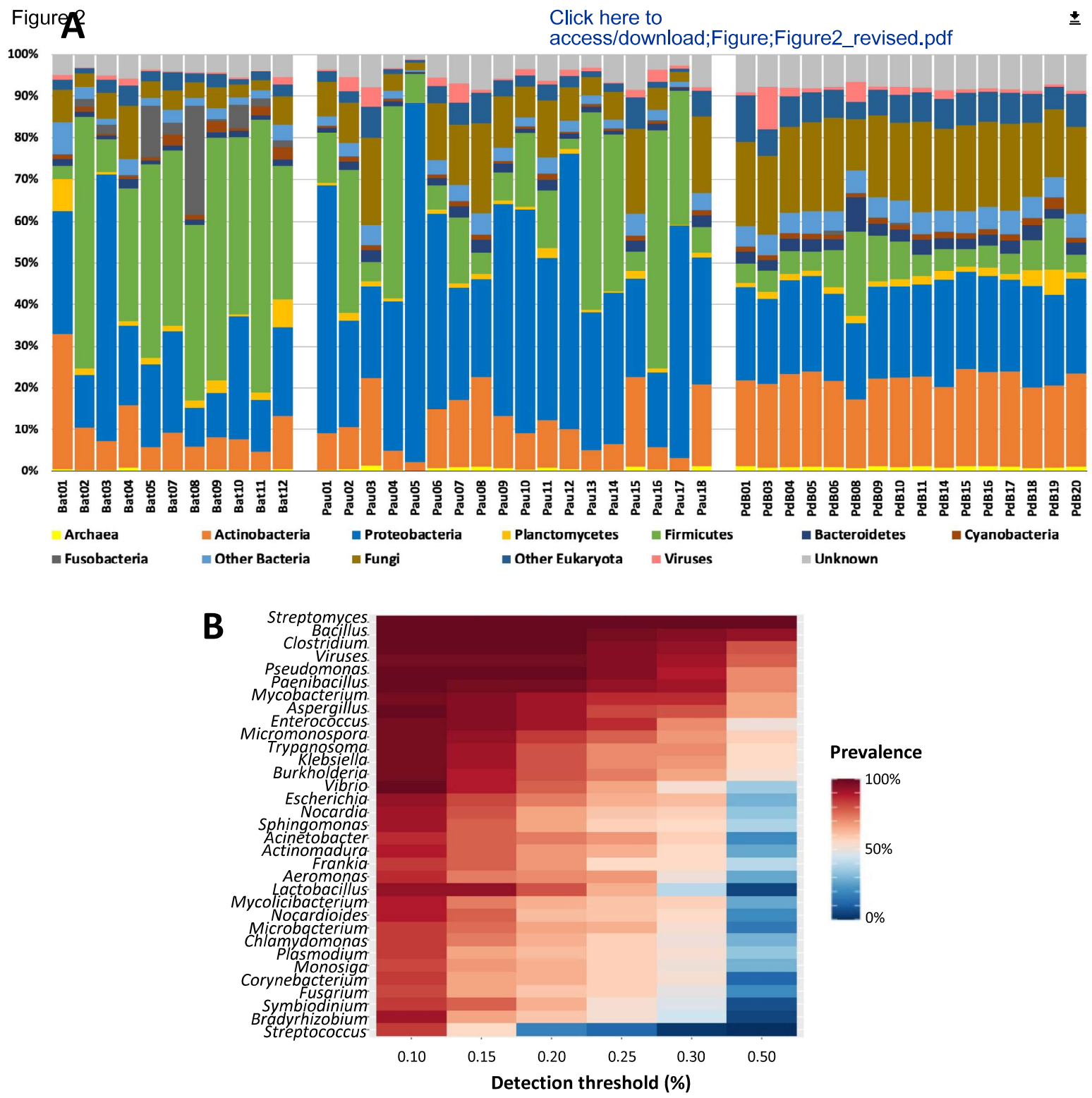


Figure 2: Taxonomic composition of the eels' intestine microbiome. **(A)** Relative abundance of metagenomic shotgun sequences classified at the domain (Archaea, Bacteria, Eukaryota and virus) and Phylum levels in all samples. Only the most abundant bacterial phyla are presented. **(B)** Core gut microbiota of *A. anguilla* at the Genus level. Selected taxa were above 0.1% or relative abundance in 80% of samples.

Figure 3

Click here to access/download;Figure;Figure3_revised.pdf

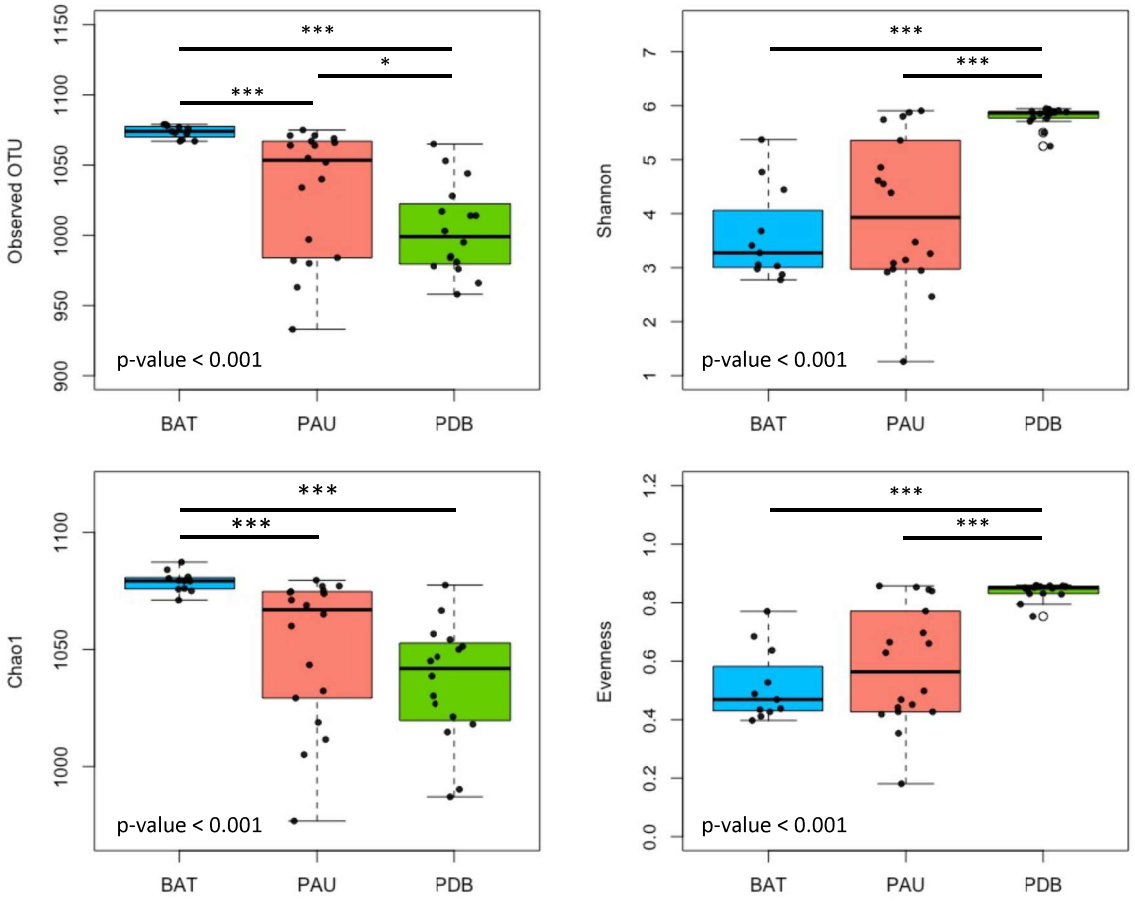


Figure 3: Within-sample microbial alpha diversity in eels' intestines at the genus level. Boxplots of Observed OTU, Shannon, Chao1 and Pielou's Evenness diversity indices calculated from non-normalized counts of reads. Individuals are represented by a point and grouped by sampling site. Statistical analysis was performed using Kruskal-Wallis tests (global p-value indicated on each panel) followed by pairwise Wilcoxon rank sum tests (* p≤0.05; ** p≤0.01; *** p≤0.001).

Figure 4

Click here to access/download;Figure;Figure4_revised.pdf

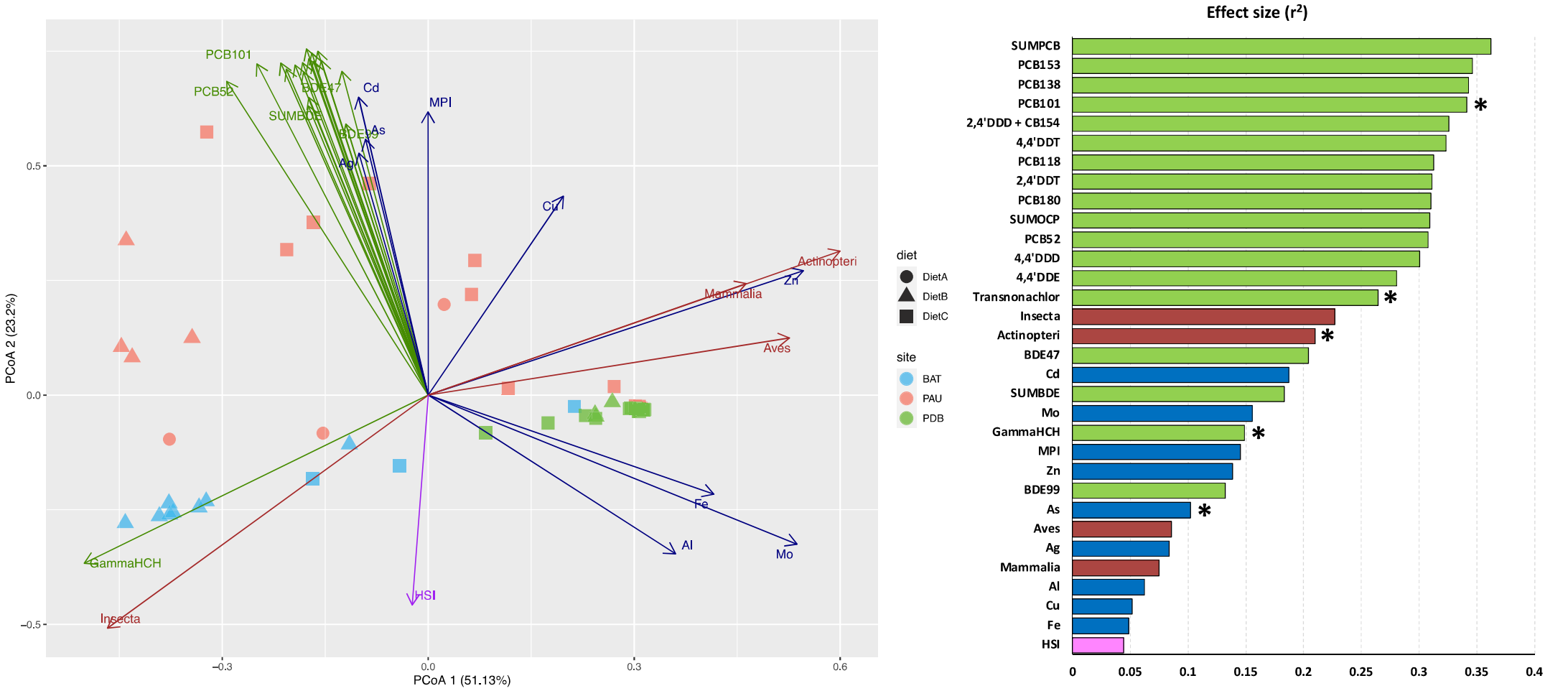


Figure 4: PCoA-based on Bray-Curtis distance (left panel) and effect size of microbiome covariates (right panel). Arrows show the ordination of 32 covariates for overall microbiome community variation in eels' gut. Factors are coloured based on their category (blue = metals, green = POPs, red = diet composition, and purple = biometry). Individuals are grouped by site (colour) or diet (shape). * symbol indicates non-redundant covariates.

Figure 5

Click here to access/download;Figure;Figure5_revised.pdf

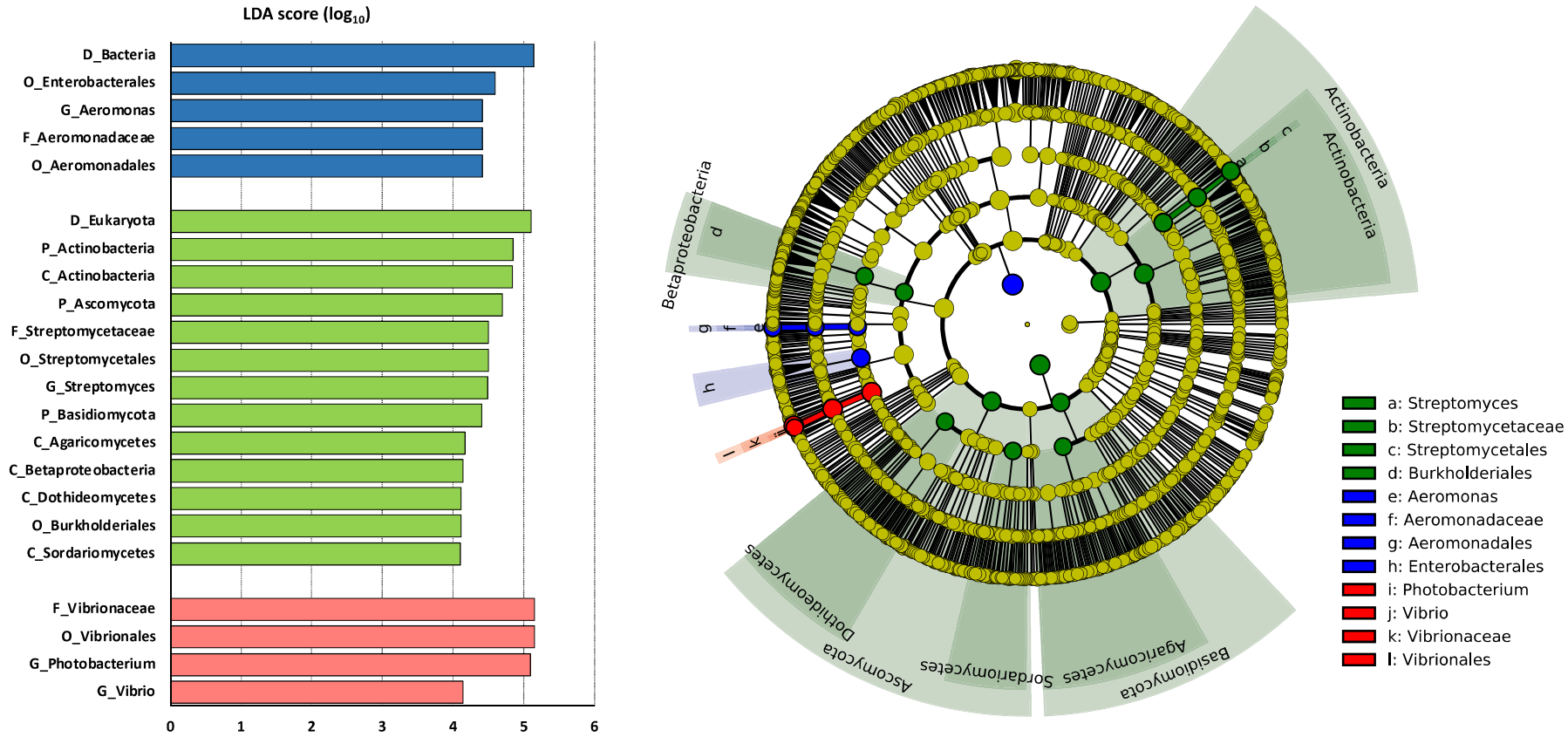


Figure 5: Linear discriminant analysis (LDA) effect size (LEfSe) results. Left panel: Indicator taxa with scores of 4 or greater in the gut microbiota of eels from Batejin (blue), Pas-du-Bouc (green) and Pauillac (red). D_domain; P_phylum; C_class; O_order; F_family; G_genus. Right panel: circular cladogram of the microbiome components classified from Phylum to genus. Yellow nodes represent genera with no significant differences. Blue, green, and red nodes represent the microbial groups that are significantly more present in Batejin, Pas-du-Bouc and Pauillac groups, respectively. Abbreviations of lower taxonomic levels are displayed in the bottom right legend.

Figure 6

Click here to access/download;Figure;Figure6.pdf

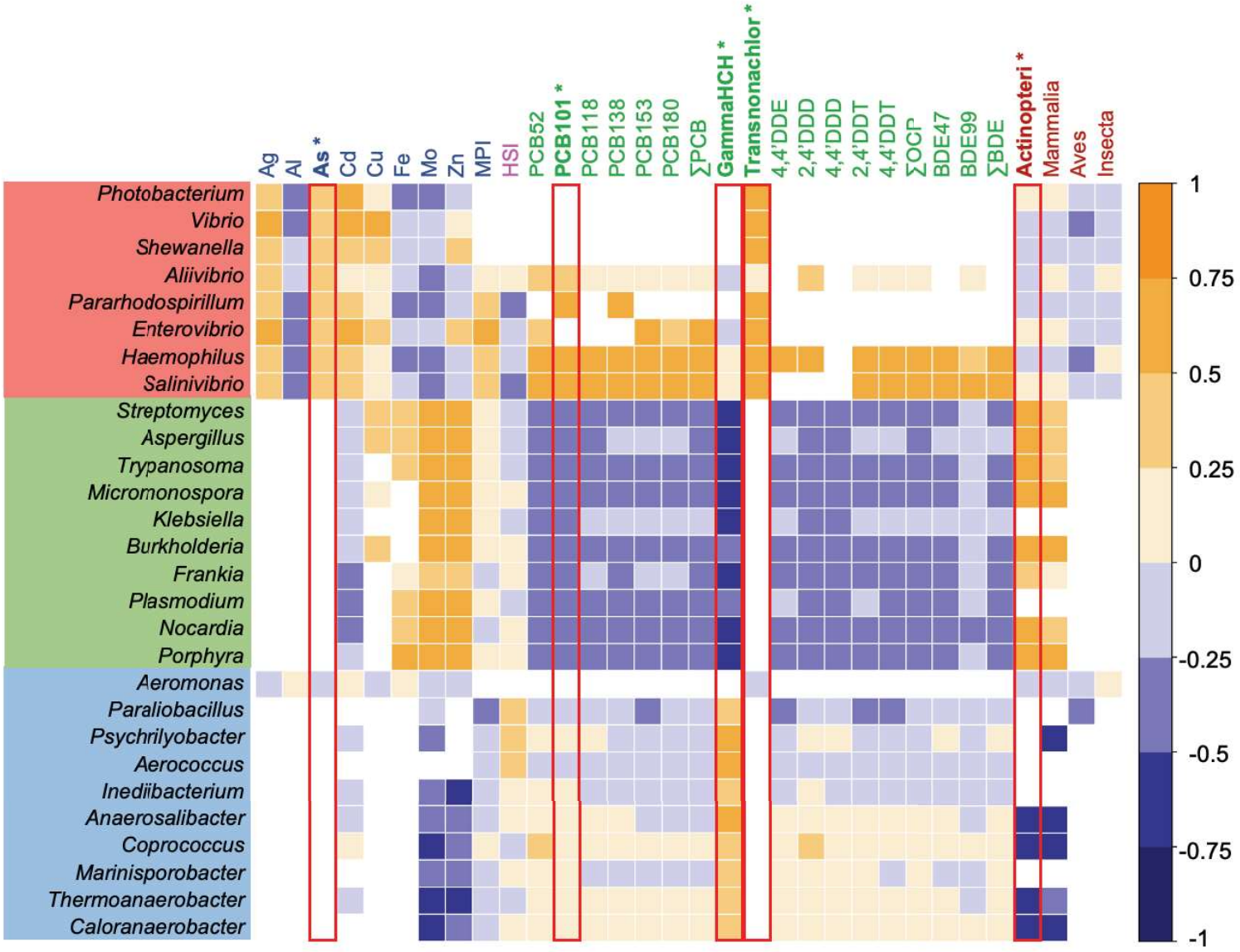


Figure 6: Spearman correlation matrix heatmap between dominant (LDA > 3) indicator taxa at the genus level as identified by LEfSe in each group (blue: Batejin, green: Pas-du-Bouc, red: Pauillac) and covariate significantly associated with microbiome composition. Non-redundant covariates selected by stepwise redundancy analysis are indicated by the * symbol. Only significant correlations ($p \leq 0.05$) are indicated.

Table 1: Summary of environmental and morphologic measurements on each sampling site. Values are means ± SE. Letters indicate significant differences between groups (p-value < 0.05).

		BAT	PDB	PAU
	Location	44°55'53.87"N	44°50'27.03"N	45°14'00.26"N
	(GPS coordinates)	01°07'22.94"W	01°09'07.58"W	00°44'01.80"W
	Sampling date	04-JUL-2019	22-JUL-2019	16-SEP-2019
Environmental	**Temperature (°C)**	28.00	23.80	22.36
	Salinity (‰)	0.12	0.12	10.36
	pH	8.19	6.42	nd
	Dissolved O_2 (mg/l)	8.24	7.67	5.84
Eels characteristics	**n samples**	11	16	18
	Age	4.75 ± 0.55 (a)	5.25 ± 0.29 (b)	3.89 ± 0.22 (a)
	Length (cm)	26.57 ± 1.92 (a)	29.44 ± 0.90 (b)	31.54 ± 0.83 (b)
	Mass (g)	31.16 ± 8.74 (a)	44.70 ± 4.20 (b)	46.89 ± 3.97 (b)
	Fulton K	0.16 ± 0.01 (ab)	0.16 ± 0.01 (b)	0.14 ± 0.01 (a)
	HSI	1.36 ± 0.08 (b)	0.93 ± 0.05 (a)	1.20 ± 0.07 (b)
	SSI	0.19 ± 0.04 (b)	0.25 ± 0.03 (c)	0.08 ± 0.01 (a)

Table 2: Concentrations of heavy metal in the liver (left panel) and organic pollutants in muscles (right panel) of European eels from the different sampling sites. Mean ± SE, letters indicate significant differences between groups (p-value < 0.05).

		Batejin	Pas-Du-Bouc	Pauillac			Batejin	Pas-Du-Bouc	Pauillac
Metals (µg/mg)	**Ag**	0.008 ± 0.001 (a)	0.005 ± 0 (a)	0.968 ± 0.104 (b)	**POPs (ng/g)**	**PCB28**	0.823 ± 0.077	1.651 ± 0.567	6.784 ± 4.649
	Al	27.684 ± 3.768 (a)	35.782 ± 2.553 (a)	13.489 ± 1.373 (b)		**PCB52**	4.838 ± 0.634 (a)	4.296 ± 0.519 (a)	13.864 ± 2.601 (b)
	As	0.036 ± 0.004 (a)	0.440 ± 0.156 (a)	4.444 ± 0.438 (b)		**PCB101**	5.583 ± 0.865 (a)	4.698 ± 0.633 (a)	31.132 ± 5.983 (b)
	Cd	0.762 ± 0.168 (a)	0.667 ± 0.085 (a)	1.729 ± 0.184 (b)		**PCB118**	4.354 ± 0.569 (a)	5.290 ± 1.046 (a)	37.347 ± 5.291 (b)
	Co	0.232 ± 0.059	0.149 ± 0.042	0.151 ± 0.020		**PCB138**	6.659 ± 0.682 (a)	8.249 ± 0.567 (a)	281.848 ± 34.575 (b)
	Cr	0.426 ± 0.094 (a)	0.258 ± 0.043 (b)	0.221 ± 0.010 (b)		**PCB153**	3.990 ± 0.365 (a)	8.651 ± 3.651 (a)	165.551 ± 24.128 (b)
	Cu	26.472 ± 1.769 (a)	49.738 ± 4.568 (b)	60.049 ± 4.740 (b)		**PCB180**	0.966 ± 0.094 (a)	1.236 ± 0.114 (a)	141.954 ± 22.759 (b)
	Fe	1721.904 ± 204.857 (a)	2936.914 ± 298.146 (b)	1128.601 ± 128.825 (a)		**SUM of PCBs**	27.214 ± 2.980 (a)	34.071 ± 6.711 (a)	678.480 ± 93.419 (b)
	Mn	7.013 ± 0.468 (a)	8.793 ± 0.320 (b)	8.578 ± 0.410 (b)					
	Mo	1.154 ± 0.048 (a)	1.581 ± 0.082 (b)	1.043 ± 0.073 (a)		**HCB**	0.446 ± 0.074	0.482 ± 0.104	0.630 ± 0.111
	Ni	0.355 ± 0.033(a)	0.268 ± 0.067 (a)	0.052 ± 0.019 (b)		**γ-HCH**	0.551 ± 0.058 (b)	0.223 ± 0.047 (a)	0.310 ± 0.031 (a)
	Pb	0.570 ± 0.240 (a)	0.050 ± 0.003 (b)	0.906 ± 0.113 (a)		**Cischlordane**	0.016 ± 0.005 (a)	0.037 ± 0.008 (a)	0.221 ± 0.051 (b)
	Se	15.000 ± 1.238 (a)	39.264 ± 6.647 (b)	17.448 ± 1.012 (a)		**Transnonachlor**	0.126 ± 0.015 (a)	0.279 ± 0.070 (a)	1.006 ± 0.142 (b)
	V	0.887 ± 0.183 (a)	2.010 ± 0.198 (b)	1.116 ± 0.158 (a)		**4,4'DDE**	0.789 ± 0.067 (a)	1.492 ± 0.162 (a)	31.581 ± 4.889 (b)
	Zn	113.280 ± 5.176 (a)	163.304 ± 5.796 (b)	143.791 ± 9.135 (b)		**2,4'DDD + CB154**	0.197 ± 0.034 (a)	0.259 ± 0.024 (a)	8.830 ± 0.941 (b)
	MPI	1.689 ± 0.217 (a)	2.022 ± 0.193 (a)	3.014 ± 0.178 (b)		**4,4'DDD**	0.095 ± 0.013 (a)	0.231 ± 0.079 (a)	7.796 ± 1.424 (b)
						2,4'DDT	0.138 ± 0.016 (a)	0.189 ± 0.036 (a)	4.701 ± 0.501 (b)
						4,4'DDT	0.147 ± 0.044 (a)	0.284 ± 0.028 (a)	1.922 ± 0.302 (b)
						SUM of OCPs	2.504 ± 0.176 (a)	3.477 ± 0.369 (a)	56.997 ± 8.130 (b)
						BDE28	0.353 ± 0.055 (b)	0.263 ± 0.001 (a)	0.271 ± 0.008 (a)
						BDE47	0.214 ± 0.001 (a)	0.214 ± 0.001 (a)	1.648 ± 0.314 (b)
						BDE99	0.192 ± 0.001 (a)	0.192 ± 0.001 (a)	0.703 ± 0.114 (b)
						SUM of BDEs	0.759 ± 0.055 (a)	0.670 ± 0.001 (a)	2.623 ± 0.422 (b)

Table 3: Mean percentage (± SD) of metazoan sequences in the 5 classes used to assess diet for each group of eels created by k-mean clustering.

Diet group	N	Actinopteri	Mammalia	Aves	Insecta	Malacostraca	Bivalvia	Gastropoda
A	3	22.54 ± 1.93	11.26 ± 0.59	3.74 ± 0.25	39.80 ± 1.40	13.76 ± 3.47	1.36 ± 0.06	1.34 ± 0.01
B	15	26.76 ± 2.34	12.53 ± 0.45	4.26 ± 0.18	45.24 ± 2.91	1.70 ± 1.85	1.44 ± 0.05	1.39 ± 0.06
C	27	31.25 ± 0.71	13.29 ± 0.29	4.55 ± 0.13	40.39 ± 0.81	1.16 ± 0.65	1.41 ± 0.04	1.33 ± 0.04