Effect of an energy turbine on fish eDNA as indicator for species composition

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NIOZ Royal Netherlands Institute for Sea Research

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Summary

The effect of energy turbines on the species composition of the fish community in the nearby environment of the turbine was tested using environmental (eDNA). Both turbines in the Marsdiep (Wadden Sea) and Lake IJssel were included. The turbines are expected to influence the behaviour of fish by either disturbance due to sounds or physical damage due to contact with the turbine.

Water samples for eDNA were taken close to the installations and analysed for fish community composition. For the Marsdiep turbine, samples were taken during turbine operations in summer 2015 and winter 2016 and in the same period in the consecutive year. Lake IJssel samples were taken during autumn 2017. DNA was extracted from the water samples and 12S fish eDNA was amplified. Community composition was inferred from sequences generated by Illumina sequencing.

For the Wadden Sea samples, species composition during the operation and the year after significantly differed, however, normal year-to-year variation could not be excluded. Although year-to-year variations could not be excluded, the turbine effect seems to be more strong on pelagic species, for which prey species avoid the turbine and predator species are more attracted by the turbine. The water samples taken at Lake IJssel showed no significant differences at samples taken nearby the discharge turbine and reference samples.

Contents

1. Introduction

The foremost aim of this report is to test whether the operation of an energy turbine affects the species composition of fish community in the nearby environment, whereby environmental DNA (eDNA) of fish during and after the operation of the energy turbine was taken as indicator for the presence and abundance of fish species.

The energy turbine are expected to influence the behaviour of fish in the close vicinity of the installation. Fish can either avoid the installation as a result of disturbance. However, fishes which do approach the turbine can get injured or killed, which might result in attracting predator species.

The energy turbine was situated in the Marsdiep tidal inlet between July 2015 until March 2016. At the start, a small turbine was installed and operated until October 2015 (mostly summer), whereas a bigger turbine was operational during January and February 2016 (winter). In order to measure the effects of the turbines, three assumptions were made: 1) the relative abundance of eDNA of a specific fish species represents its abundance (mass); 2) the year to year effects on fish community composition are subordinate to the effect of the turbine; 3) the turbine differently affects fish species featuring dissimilar ecological traits (i.e, demersal/pelagic, predator/prey).

The second aim of this report is to test for the presence of three species of interest (Atlantic salmon - Salmon salar, European sea sturgeon – Acipenser sturio and European eel – Anguilla anguilla) near the discharge turbine at Den Oever in Lake Ijssel.

2. Metabarcoding

The analysis of environmental DNA (eDNA), i.e., the extraction and analysis of genetic material obtained directly from environmental samples, is a relatively new approach used to monitor the distribution of fish species. The method implies identification of species based on their DNA-sequences, also called barcoding. The eDNA can be extracted from different environmental samples such as soil, water, faeces etc..

Environmental DNA
The term eDNA describes all extra organismal genetic materials that have been released in the environment via faeces, shrubs, mucus, etc., and may consist of both free DNA, cellular debris and particle bound DNA. The concentration of eDNA depends on both the rate of release, as well as the rate of decay. The release rate is believed to be relatively constant and mainly influenced by eDNA particles from the gut lining of fish (Hansen et al., 2017). Decay rates were found to be rather high in marine ecosystems; i.e., eDNA released from a specific organism can only be detected on the short time scale (e.g., days) (Sassoubre et al., 2016). The combination of a constant release and relatively fast decay make eDNA a useful monitoring tool for short-term effect studies.

Metabarcoding
The study of the complete genetic material obtained from environmental samples is called eDNA metabarcoding. Metabarcoding combines two technologies:[1] high throughput DNA sequencing using NGS platforms and [2] identification of species based on standardized DNA barcodes. These DNA barcodes were introduced by Hebert et al. (2003) who recommended a part of the mitochondrial cytochrome c oxidase gene I (COI) for sequencing. However, many more barcode regions have been introduced since then. Barcodes should be specifically chosen for a target species and should contain both a variable region for discrimination between species, as well as two adjacent highly conserved regions, so-called 'marker' sites. The mitochondrial 12S rRNA gene was found to encompass both of these features for fish species (Kelly et al, 2014; Miya et al., 2015; Thomsen et al., 2016).
A metabarcoding study usually starts with a DNA extraction on the sampled material. During this step, DNA is isolated and purified. The specific barcodes can then be amplified from the extracted DNA using a polymerase chain reaction (PCR) and universal primers resulting in numerous copies, or amplicons, from the specific barcode present in the sample. These numerous copies serve as starting material for the analyses via high throughput DNA sequencing.

Reference databases
Identification of DNA sequences derived from the eDNA metabarcoding approach depends crucially on reliable reference databases. Most current studies rely solely on the DNA sequences available in public databases such as GenBank™. The current databases are incomplete and strongly biased in geographical and taxonomic coverage (Thomsen et al., 2015). Therefore, at the NIOZ, a local reference database of common Wadden Sea fish species has been compiled over the last few years. This database, combined with sequences from public databases allows for an accurate taxonomic assignment.

3. Methods

3.1. Sampling

3.1.1. Marsdiep

Water samples were taken both during around high water and low water from the Jetty of the Royal Netherlands Institute of Sea Research (NIOZ), situated in the Marsdiep tidal inlet (Figure 1a). The BlueTec Energy turbine was situated approximately 200 meters offshore from the Jetty.

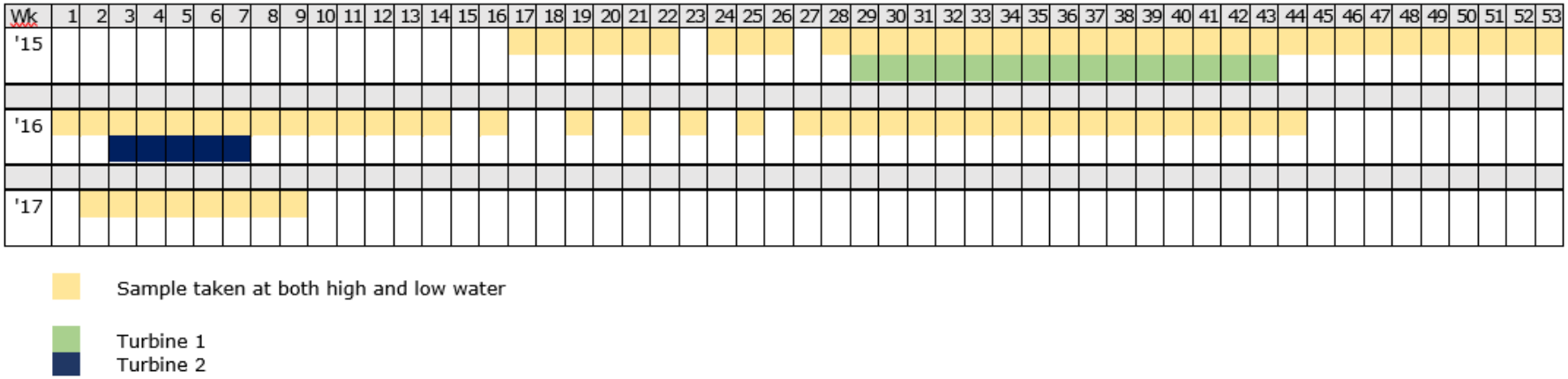
Samples were taken weekly on Wednesday during the operation of the BlueTec turbines and in the corresponding weeks the year after. Also, reference samples were taken before, in between and after turbine operations (Table 1). The water samples were taken from the surface water with a bucket. Water was then transfused to a clean 1L glass bottle and directly stored in a cool box. The sampling procedure was repeated three times. Of each of the three water samples, 150-250 ml was vacuum-filtered on a 0.2µm polycarbonate filter. Filters were then folded inwards, placed in 2ml tubes and stored at -80ºC.

3.1.2. Lake IJssel

Water samples were collected at two locations in Lake IJssel, next to Den Oever and at Breezanddijk (Figure 1b & 1c). Samples were taken weekly for seven consecutive weeks starting from 15th September 2017. Sampling and filtration procedures were similar to the Marsdiep sampling.

Figure 1: Sampling locations: Marsdiep (A), Den Oever (B) and Breezanddijk (C).

Table 1: Overview of samples taken from the NIOZ Jetty and turbine operation during 2015, 2016 and 2017



3.2. Metabarcoding

3.2.1. DNA extraction and amplification

Filters were cut in small pieces with sterile scissors and DNA collected on the filters was extracted using the MoBIO Powersoil™ DNA isolation kit (MoBio Inc.) following the manufacturer's instructions. DNA from all extractions, as well as a mock sample, were used as template to amplify, a fragment from the mitochondrial 12S rRNA gene. Primers were an adapted version of the primers used by Kelly et al. (2014) and PCR settings were similar to the original ones. All forward and reverse primers were extended with 6nt unique barcodes. Triplo samples were labelled with similar barcode combinations. The PCR products were visually inspected after electrophoresis through a 1% agarose gel, excised from the gel and purified using the Qiaquick Gel Extraction Kit (Qiagen, Inc.). Amplicons of the triplicates were pooled and quantified with a Qubit™ 3.0 fluorometer (Qiagen, Inc.). Subsequently, pooled amplicons of all samples were combined in equimolar quantities together with blank PCR controls. The pooled sample was then subjected to a final purification using MinElute™ PCR purification columns (Qiagen, Inc.) as described by the manufacturer. The pooled sample was submitted for PE sequencing at GATC-Biotech (Ebersberg, Germany) on a Illumina HiSeq using the 2x 150bp package.

3.2.2. 12S amplicon sequence data work flow

Read pears were merged and quality filters using pear (Zhang et al., 2014). Reads required a minimum overlap of 7nt, a minimum individual read length of 20nt and a p-value threshold of 0.05 for the statistical test to decide whether to merge the reads. The 6nt unique barcodes were extracted using the barcode_paired_stitched script in QIIME and reads were assigned to samples using the split_libraries_fastq.py script allowing zero mismatches in both the forward and reverse barcodes (Caporaso et al., 2010). Only reads with a minimum Phred score of 24 at all positions and a read length between 134bp and 154bp were left in the analysis. The remaining reads were aligned against a custom database of 12S sequences of local fish and common contaminants using mother (v. 1.34.4) (Schloss et al., 2009). Aligned sequences were front, and end clipped to remove the primers using cutadapt (v. 1.15) and alignment characters were removed with degapseq from EMBOSS 6.6.0.0 (Rice et al., 2000). Sequences which became shorter than 90nt due to poor alignment were discarded. OTU's were generated using tree_method in QIIME and from each OTU's the most abundant sequence was selected as representative sequence. Representative OTU's were run against a costume reference database using blastn (BLAST 2.6.0+ (Morgulis et al., 2008) with a 98% sequence identity in the aligned part and 98% query sequence coverage. The best hit was used for taxonomic assignment. A total of 50 taxonomic species were recovered from the entire dataset. The costume reference database used was a combination of sequences derived from the NCBI database as well as newly derived sequences from 53 local fish species. The genetic distance at the 12S gene was sufficient to discriminate most species. However, those species sharing identical genotypes were clustered.

3.2.3. Biofinformatics

Read numbers varied between 5 and 670000 reads per sample. Samples with a total amount of reads lower than 5.000 were discarded from the analysis. Also, the corresponding sample taken at the same date was discarded (i.e., if the low water sample produced less than 5.000 reads, the high tide sample at the same day was discarded). This resulted in the removal of data for week 39 and 40 from the 2015 sampling (also see Table 1). All remaining reads were transformed into relative abundance of the total read numbers, i.e. the number of reads per OTU divided by the total numbers of reads in the corresponding sample. A cut-off of 0.02% (which corresponds to a minimum of 1 read in a sample of 5.000 reads) was taken for false-positives and all OTUs below that threshold were deleted. For the final analysis, relative abundance was transformed to a relative abundance of the total amount of fish reads. If multiple OTUs were present for one taxonomic species, read counts were summed up. Subsequently, an average was calculated for each week resulting in an average relative abundance of reads for the combined low tide and high tide samples.

3.3. Data analysis

3.3.1. Marsdiep

For the Marsdiep samples, three periods in time were selected. To determine the effects of Turbine 1, samples from week 29 until week 43 in 2015 (T1_on) were compared to samples from week 29 until week 43 in 2016 (T1_off). Similarly, to determine the effects of Turbine 2, samples from week 3 until week 7 in 2016 (T2_on) were compared to samples from week 3 until week 7 in 2017 (T2_off). To determine the year to year effects in fish abundancies, an extra set of samples from week 16 until week 28 for both 2015 and 2016 (Ref_2015 and Ref_2016) was analysed. We selected 15 fish species or species groups based on their relatively high abundance and/or importance in the Dutch Wadden Sea (Table 2). A nested anova was performed, in which species were considered as subgroups, to test for significant differences in abundancies between turbine operations (on/off) and the year to year differences (2015/2016). For each of the species, the relative percentage of increase and/or decrease was calculated by dividing the increase/decrease by the average abundancy.

Also, a nonmetric multidimensional scaling using Bray-Curtis dissimilarity distances was performed for the relative abundance of all species in the dataset. The Bray-Curtis dissimilarity matrix was used for analysis of variance between the turbine operations (on/off) and the year to year differences (2015/2016) and a simper analysis to discriminate the effect of each species.

Table 2: Selection of 15 fish species based on their relative high abundance and/or importance in the Dutch Wadden Sea.

Order	Family	Species	Common name
Anguilliformes	Anguillidae	*Anguilla anguilla*	European eel
Clupeiformes	Clupeidae	*Sardina pilchardus*	European pilchard
Clupeiformes	Clupeidae	*Sprattus sprattus* / *Clupea harengus*	European sprat / Herring
Gadiformes	Gadidae	*Pollachius virens* / *Merlangus merlangus*	Saithe / Whiting
Gadiformes	Lotidae	*Ciliata mustela*	Fivebeard rockling
Perciformes	Ammodytidae	*Ammodytes tobianus* / *Hyperoplus lancolata*	Lesser sand eel / Great sand eel
Perciformes	Gobiidae	*Pomatoschistus minutus*	Sand goby
Perciformes	Gobiidae	*Pomatoschistus microps*	Common goby
Perciformes	Moronidae	*Dicentrarchus labrax*	European bass
Perciformes	Mugilidae	*Chelon labrosus*	Thicklip grey mullet
Perciformes	Pholidae	*Pholis gunnellus*	Rock gunnel
Perciformes	Zoarcidae	*Zoarces viviparus*	Eelpout
Pleuronectiformes	Pleuronectidae	*Pleuronectes platessa* / *Platichthys flesus* / *Limanda limanda*	European plaice / Flounder / Common dab
Salmoniformes	Salmonidae	*Salmo trutta*	Sea trout
Scorpaeniformes	Cottidae	*Taurulus bubalis* / *Myoxocephalus scorpius*	Sea scorpion / Bull-rout

3.3.2. Lake IJssel

Three species were selected as species of special interest (rare and red list) for Lake IJssel (Table 3). For each of these species, the relative abundance at both Den Oever and Breezanddijk was analysed. A nested anova, in which species were considered as subgroups, was performed to test for differences between their abundance at the two sample locations.

Table 3: Selection of 3 fish species for the Lake IJssel analysis based on their scientific relevance (rare and red list species).

Order	Family	Species	Common name
Anguilliformes	Anguillidae	*Anguilla anguilla*	European eel
Salmoniformes	Salmonidae	*Salmon salar*	Atlantic Salmon
Acipenseriformes	Acipenseridae	*Acipenser sturio*	European sea sturgeon

4. Results

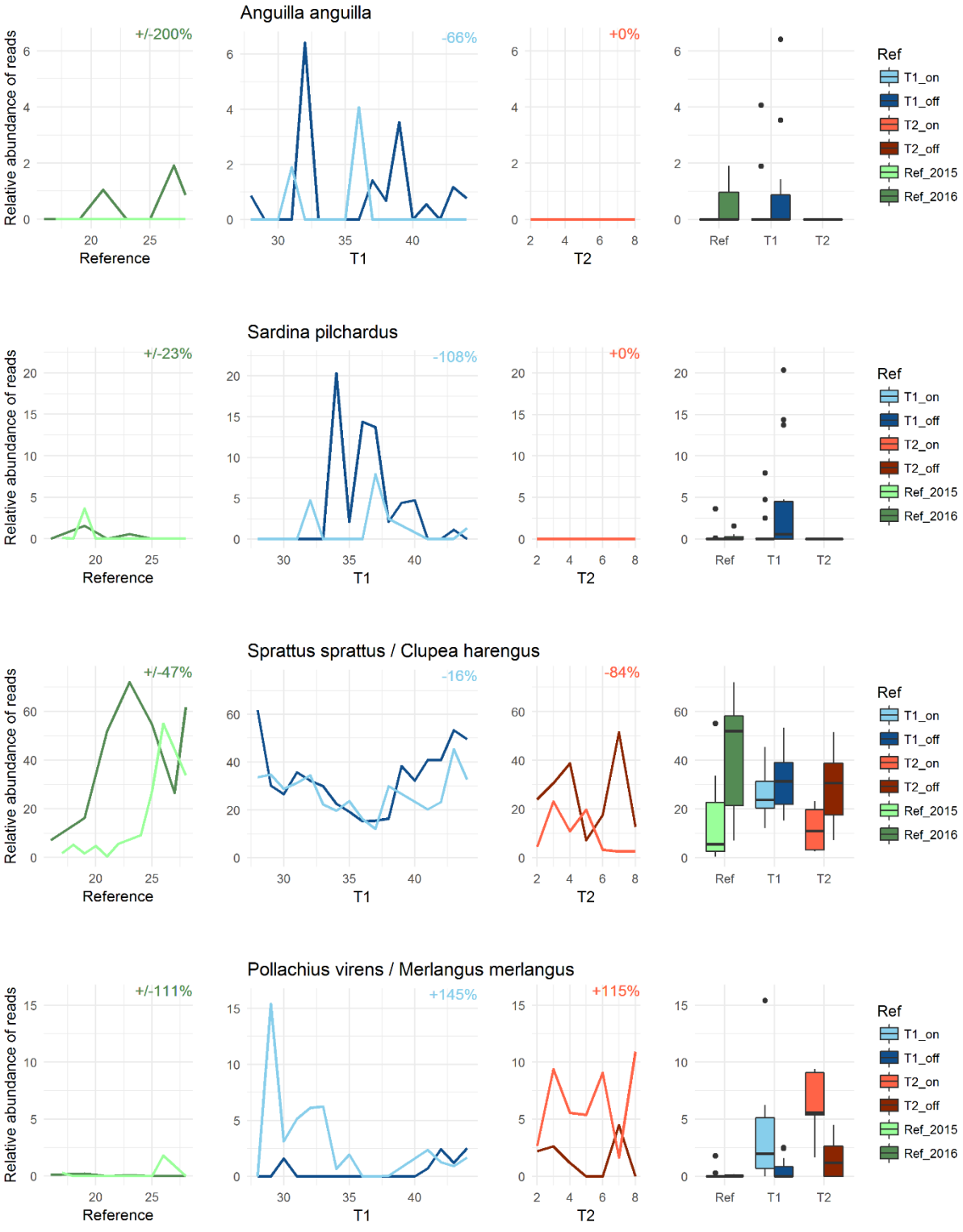
4.1. Marsdiep

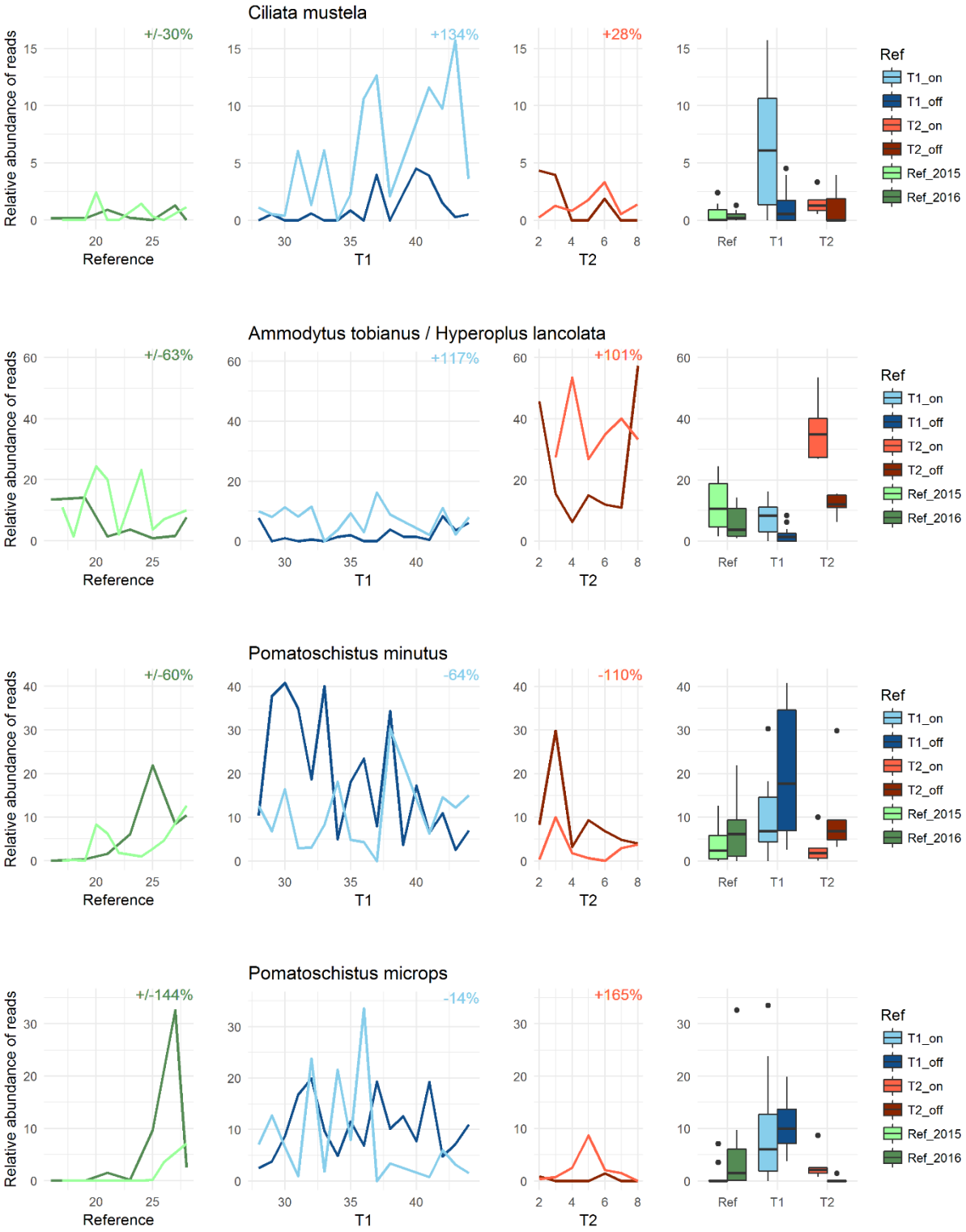
For each of the 15 species, the relative abundance and the overall variance in these abundancies is shown in Figure 2.1 – 2.15. Species abundancies differed between the summer and winter period. Most species showed a higher abundance in the summer period, only the species from the Ammoditydae family (lesser sand eel/greater sand eel – A. tobianus/H. lanceolata) and the Cottidae family (Sea scorpion/Bull-rout - T. bubalis/M. scorpius) were more abundant in winter. Most species were present both in summer and winter, only the European eel (A. anguilla), European pilchard (S. pilchardus) and the thicklip grey mullet (C. labrosus) were absent in winter.

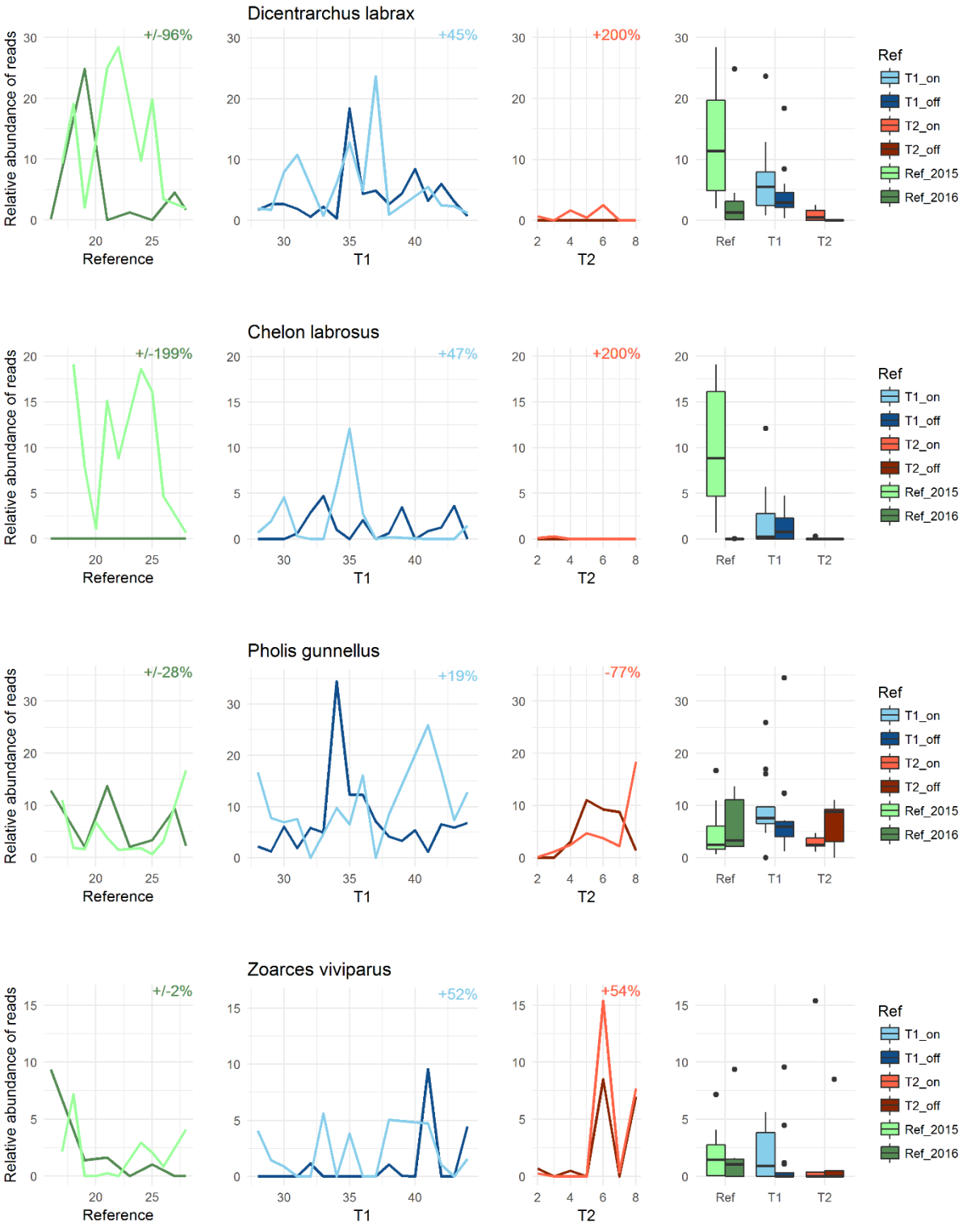
Overall, there were no significant differences in abundancies as determined by the nested ANOVA related to the functioning of either turbine 1 ($F_{1,419} = 0.011$, $p = 0.916$) or turbine 2 ($F_{1,134} = 0.347$, $p = 0.557$). Also, no significant differences in abundances were found for the references samples ($F_{1,239} = 0.173$, $p = 0.678$).

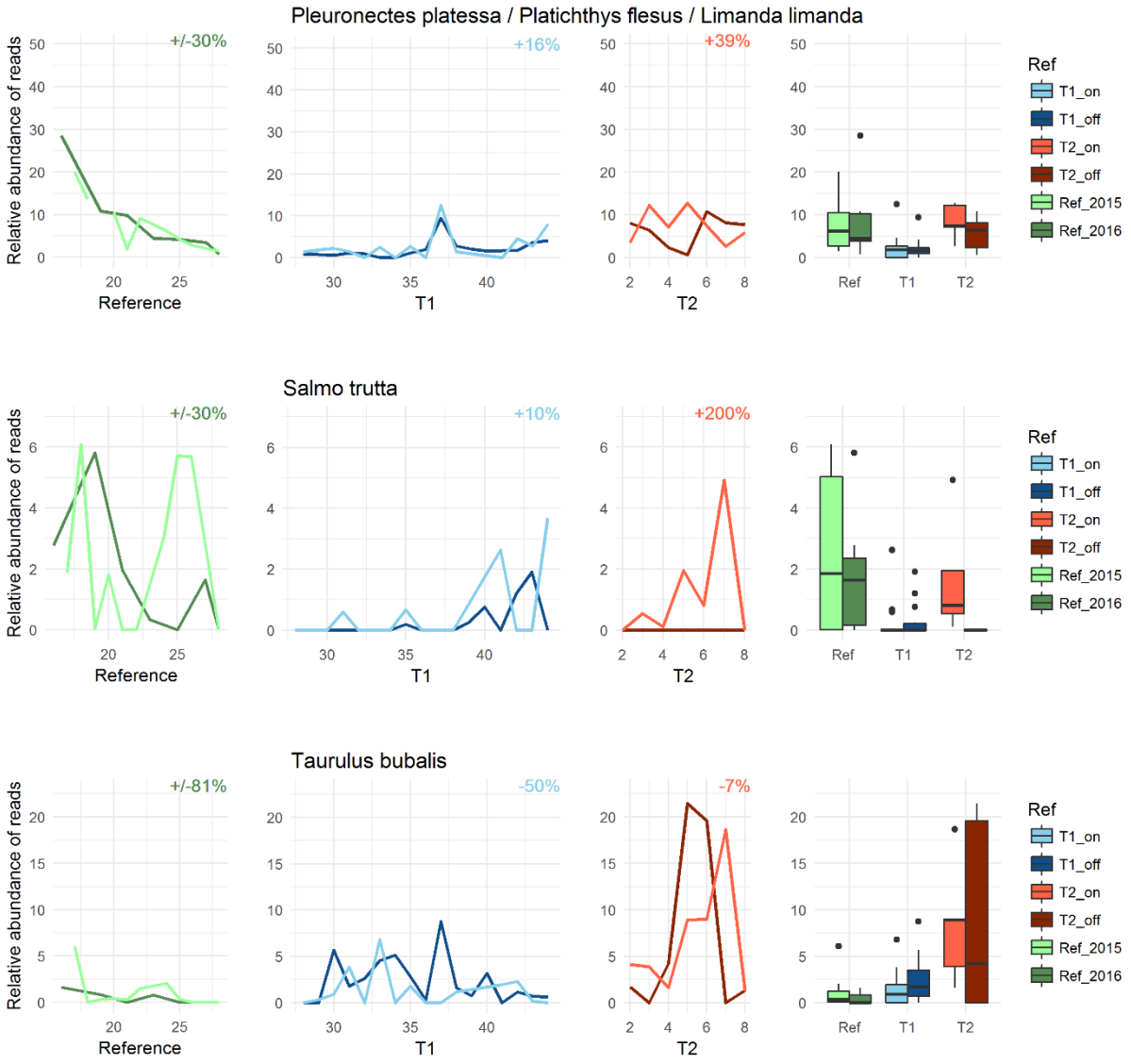
For each of the species, the percentage of increase and/or decrease of the relative abundance of reads was calculated and presented in Figures 2.1 - 2.15. During the operation of turbine 1, the European pilchard (S. pilchardus) and sand goby (P. minutus) showed the largest decrease in abundancy compared to the abundancy in the same period a year after. The saithe/whiting (P. virens/M.merlangus) and the fivebeard rockling (C. mustela) showed an increase during the operation of turbine 1.

During the operation of turbine 2, during winter, again the abundance of the sand goby decreased compared to its abundance in the same period the year after. Next to the sand goby, also the abundance of the European sprat/herring (S. sprattus/C.harengus) decreased during the operation of turbine 2. The saithe/whiting showed, as found during the operation of turbine 1, also a decrease of abundance during the operation of turbine 2. Also the lesser sand eel/greater sand eel showed a decrease in the latter period.


Anguilla anguilla
+/-200%
-66%
+0%
Relative abundance of reads
Reference
T1
T2
Ref
Ref
T1_on
T1_off
T2_on
T2_off
Ref_2015
Ref_2016
Sardina pilchardus
+/-23%
-108%
+0%
Sprattus sprattus / Clupea harengus
+/-47%
-16%
-84%
Pollachius virens / Merlangus merlangus
+/-111%
+145%
+115%


Ciliata mustela
+/-30%
+134%
+28%
Relative abundance of reads
Reference
T1
T2
Ref
T1_on
T1_off
T2_on
T2_off
Ref_2015
Ref_2016
Ammodytus tobianus / Hyperoplus lancolata
+/-63%
+117%
+101%
Pomatoschistus minutus
+/-60%
-64%
-110%
Pomatoschistus microps
+/-144%
-14%
+165%


Dicentrarchus labrax
+/-96%
+45%
+200%
Relative abundance of reads
Reference
T1
T2
Ref
T1_on
T1_off
T2_on
T2_off
Ref_2015
Ref_2016
Chelon labrosus
+/-199%
+47%
+200%
Pholis gunnellus
+/-28%
+19%
-77%
Zoarces viviparus
+/-2%
+52%
+54%



Figures 2.1 – 2.15: Relative abundance of reads for a selection of 15 species. The relative abundance of reads is shown for the reference samples from 2015 and 2016, for the period during operation of turbine 1 in 2015 and the corresponding weeks the year after (T1) as well as for the period during operation of turbine 2 in 2016 and the corresponding weeks the year after (T2). The right panel shows a boxplot of the assembled relative abundance of reads for the reference samples, T1 and T2.

An MDS ordination was calculated from the relative abundance of reads of all fish species found in the samples. These ordinations, showed a deviating composition of fish species between the sample subset, i.e., between the period during the operation and the year after as well as between the two reference periods (Figure 3). A permanova analysis indicated a statistically significant different between the species composition during the operation of turbine 1 and the species composition in the same period the year after ($F_{1,24} = 3.546$, $p = 0.001$) as well as for turbine 2 ($F_{1,8} = 4.642$, $p = 0.005$). A statistically significant difference was also found between the two years of reference samples ($F_{1,15} = 4.255$, $p = 0.004$).

Simper analysis showed that the differences for turbine 1 were mainly due to a lower abundance of the sand goby (P. minutus) and the European sprat/herring (S. sprattus/C. harengus) during the operation of the turbine. During the operation of turbine 2 an increased abundance of the lesser sand eel/great sand eel (A. tobianus/H. lanceolata) and a decreased abundance of the European sprat/herring (S. sprattus/C. harengus) were the main cause of the deviating species composition compared to the species composition the year after.

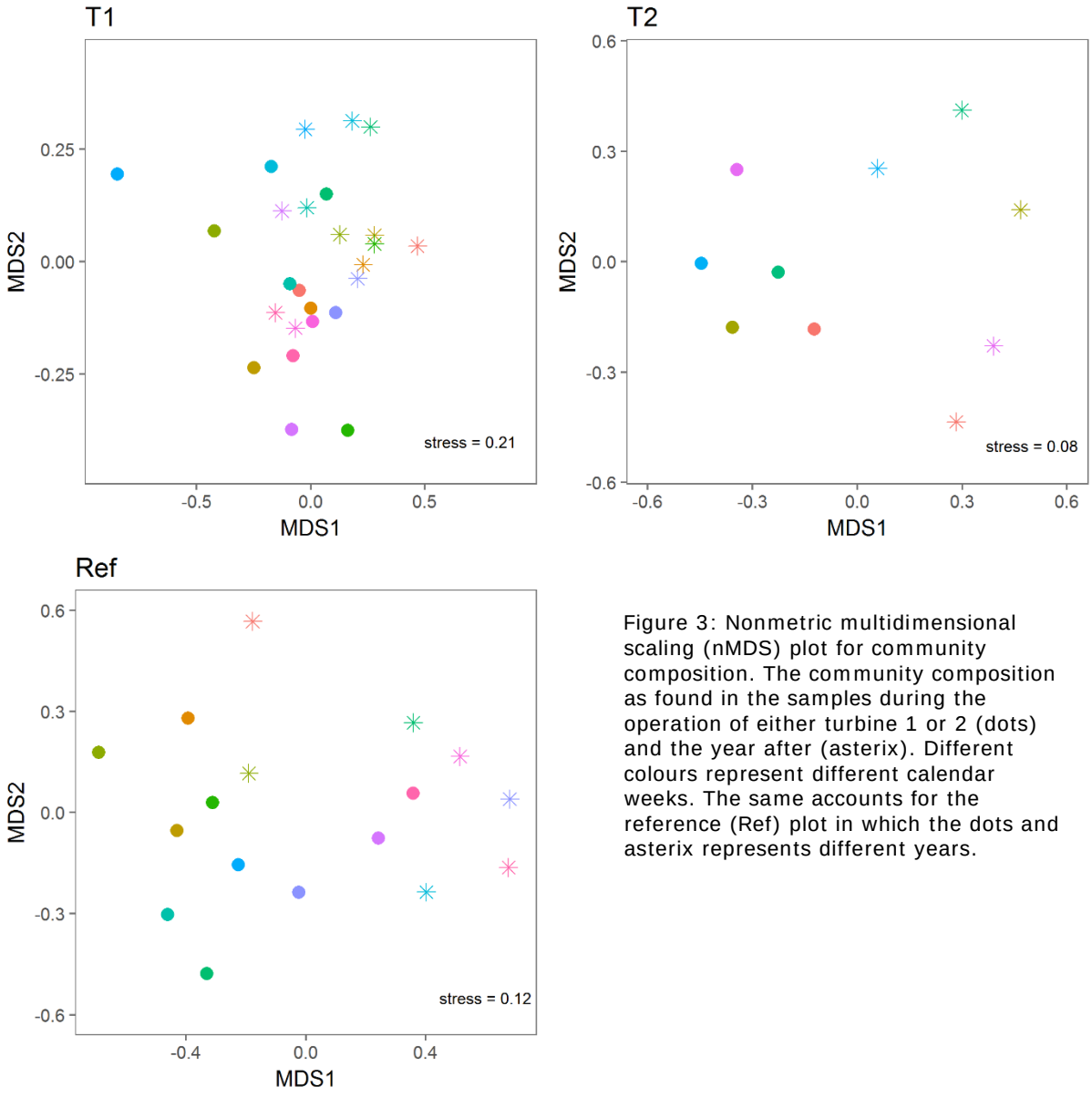


Figure 3: Nonmetric multidimensional scaling (nMDS) plot for community composition. The community composition as found in the samples during the operation of either turbine 1 or 2 (dots) and the year after (asterix). Different colours represent different calendar weeks. The same accounts for the reference (Ref) plot in which the dots and asterix represents different years.

4.2. Lake IJssel

Of the three selected species, only the European eel (A. Anguilla) was detected in Lake IJssel based on eDNA presence (Figure 4). The relative abundance of reads for the European eel did not differ significantly between the two sample locations (t-test, t12 = 0,76, p = 0.462).

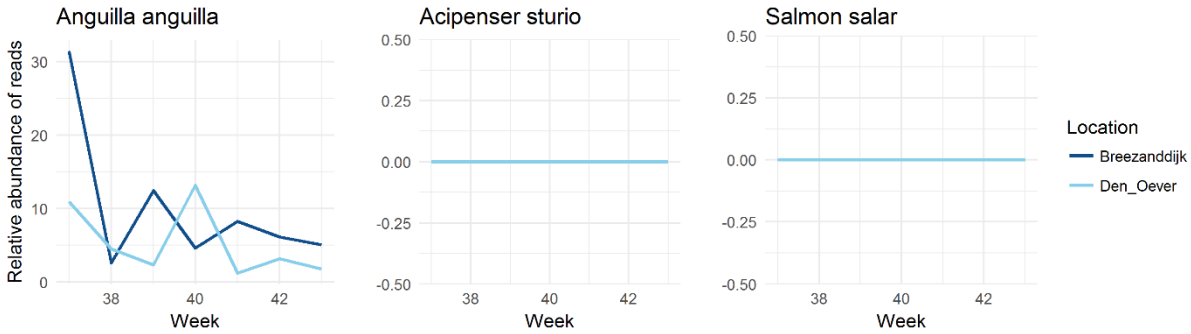


Figure 4: Relative abundance of reads for a selection of three species at two sample location in Lake IJssel.

5. Discussion

The foremost aim of this report was to detect changes in fish species community composition related to the operation of the tidal turbine located at the Marsdiep tidal inlet near Texel. In order to detect effects related to the turbine operation solely, the exclusion of seasonal and year-to-year trends as well as spatial effects is required. The original research plan included these requirements; however, the turbine was decommissioned within a month after the projects' kick-off. This report now shows results from a stripped-down version of the original research design, and hence, results should be interpreted with caution.

False positives and false negatives are a constant concern within ecological research, also for eDNA methods (Pochon et al., 2013, Barnes et al., 2016, Sassoubre et al, 2016). False positives can occur from cross-contamination or misidentification and for commercial fish even through the sewage of humans. During the data-analysis a minimum threshold for read percentages has been set to countervail cross-contaminations. False negatives can originate from again misidentification or from target eDNA not being captured. Although this report is not the platform for a full review of eDNA limitations, stressing the need for caution is unavoidable. For the majority of the fish species in the Marsdiep, the detection efficiency of eDNA is similar to 7 daily fyke catches pooled together (van Bleijswijk et al, in prep). Therefore, changes in abundance of eDNA in our study are considered to reflect actual changes in relative fish abundance.

The species from the Clupeidae family within this analysis showed a decrease in abundancy during the operation period of the turbine. The European sprat/herring had a lower abundance during the operation of the turbine in winter whereas the European pilchard showed a lower abundance during the operation of the turbine in summer. All of these species live in the pelagic zone, the zone in which the turbine was active, and are more often preyed upon rather than being predators. The Sea trout on the other hand, is a predator species in the pelagic zone. Abundancies found for this species were overall low but showed an increase in abundance during the operation of the turbine both in winter and summer. The species lesser sand eel/great sand eel and the sand goby are both prey species who are mostly found in the demersal zone during the day and in the pelagic zone during night. Although they share ecological features, their abundance according to the eDNA method is affected differently by the operation of the turbine. Not only a difference in abundance was found related to the operation of the turbine, also the reference samples (i.e., two sets of samples taken in the same seasonal period for two consecutive years) showed a high variation in abundance for most of the species. For instance, the thick lip grey mullet was completely absent in one year and had a mean relative abundance of 10% of all fish species the other year. Moreover, the species composition was shown to differ significantly between the reference subsets. Hence, year to year differences cannot be assumed to be subordinate to the turbine effect.

The second aim of the report was to test for the presence and abundance of three sensitive fish species in Lake IJssel near the discharge turbine in Den Oever. Of the three species, Atlantic salmon, European sea sturgeon and the European eel, only the European eel was detected within the eDNA samples. Although species of European sea sturgeon have been re-introduced in European fresh waters, the presence of Lake IJssel was not expected yet. The abundance of the European eel did not differ between Den Oever and the reference location, thereby showing no avoidance effect of the turbine at Den Oever.

6. Concluding remarks

The concentration of environmental DNA of the fish species in the Wadden Sea is an indicator of actual fish biomass. The effects of the energy turbine on fish abundancy are therefore in theory measurable. However, year-to-year differences were found to significantly contribute to eDNA presence and are not separable from the turbine effects. Hence, the real effects of the energy turbine on the fish community remain unclear.

Nevertheless, trends in increasing or decreasing abundance for certain species were still distinguishable. The abundance of prey species living in the pelagic zone seemed to decrease during the operation of the turbine whereas the abundance of predator species living in the pelagic zone increased.

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Protecting and using our blue planet responsibly starts with understanding our changing seas. NIOZ conducts excellent marine research for society, from the deltas to the deepest oceans. Our science and national marine facilities help scientific communities businesses, ngo's and policy makers to address some of the biggest challenges ahead.

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