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► **To cite this version:**

A. Larivain, C. Zatylny-Gaudin, E. Gonzalez, G. Pierce, A. Power, et al.. Progress in the analysis of English Channel loliginid squid diets using DNA-metabarcoding techniques. *Marine Biology*, 2024, 171 (7), pp.135. 10.1007/s00227-024-04448-9 . hal-04601649

**HAL Id: hal-04601649**

**<https://hal.science/hal-04601649v1>**

Submitted on 5 Jun 2024

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**Title:**

Progress in the analysis of English Channel loliginid squid diets using DNA-metabarcoding techniques

**Authors:**

Larivain A.<sup>1</sup>, Zatylny-Gaudin C.<sup>1</sup>, Gonzalez E<sup>2</sup>, Pierce G.J.<sup>3</sup>, Power A.M.<sup>4</sup>, Robin J.P.<sup>1</sup>

**Affiliations:**

<sup>1</sup> Université de Caen Normandie, Laboratoire de Biologie des ORganismes et Ecosystèmes Aquatiques UMR 8067 MNHN, SU, UCN, CNRS, IRD-207, UA, Caen, France.

<sup>2</sup>Metagenomics team, Canadian Centre for Computational Genomics (C3G), McGill University, Canada

<sup>3</sup>Departamento de Ecología y Recursos Marinos, Instituto de Investigaciones Marinas (Consejo Superior de Investigaciones Científicas), Vigo, Spain

<sup>4</sup> Ryan Institute, School of Natural Sciences, University of Galway, Ireland.

**Abstract:**

Study of the diet of squid is hampered by the fact that these animals masticate their prey prior to ingestion and do not necessarily ingest the hard parts that allow the identification of prey remains present in the stomach. The result is a large percentage of prey in stomach contents that can be identified only according to broad categories (e.g., "teleost fish"). This is widely described in the literature and has also been found in samples from the English Channel of the two loliginid squid fished in this area (*Loligo forbesii* and *Loligo vulgaris*). New biological samples of both species were collected at the Port-en-Bessin fish market during the 2019-2020 fishing season. Stomach contents were studied through DNA metabarcoding targeting a small fragment of the COI mitochondrial gene selected by PCR and sequenced using high-throughput next generation sequencing. The DNA metabarcoding results were compared with databases available online. These preliminary results validated a protocol based on commercial samples kept frozen (-20°C) before analysis. Sequences allowed 34 different types of prey to be identified to the species level, including 17 teleost fish species. Results revealed the high occurrence of cuttlefish eaten by both species of squid. Food composition was analysed in order to make comparisons between species, between seasons (in *L. vulgaris*) and according to the size of the predator. Differences in diet of *L. vulgaris* between November and March samples were greater than the difference between *L. vulgaris* and *L. forbesii*, which is consistent with opportunistic behaviour in these squids. Differences in diet related to predator size do not suggest a change in trophic level during the growth of recruited stages.

**Keywords:**

stomach contents, Next Generation Sequencing, prey identification, *Loligo* (Mollusca: Cephalopoda), discriminant analysis.

**Contact Author:** Jean-Paul Robin [jean-paul.robin@unicaen.fr](mailto:jean-paul.robin@unicaen.fr)

Université de Caen Normandie, Biologie des ORganismes et Ecosystèmes Aquatiques BOREA (MNHN, UPMC, UCBN, CNRS-2030, IRD-207) CS 14032, 14032 Caen, France.

Telephone: (+33) (0) 2 31 56 55 95 / (+33) (0) 6 52 15 01 66

**Introduction:**

Visual analysis of prey remains in loliginid squid stomachs indicates that these squid, especially larger individuals, eat predominantly teleost fish (e.g., Rocha et al. 1994; Wangvoralak et al. 2011, Büring et al. 2021). However, a significant proportion of these prey cannot be identified to the species level and

are recorded as "(unidentified) teleost fish". The occurrence of this category of prey in diet studies was higher in *Loligo vulgaris* (45%) than in *Loligo forbesii*: (34 %) according to Rocha et al. (1994). Among those teleost fish that can be further identified, it is often only to family level: 14% in *L. forbesii*, according to Wangvoralak et al. (2011), and the same difficulty applies to cephalopod prey, making it difficult to distinguish cannibalism from predation on other cephalopod species. Difficulties with prey identification are common in cephalopods and are most likely related to the fact that these predators manipulate and masticate their prey, tearing them into small (often unidentifiable) pieces (Ibañez et al. 2021), which is essential since the oesophagus of cephalopods passes through the brain. The determination of food regime is important to determine the role of squid in the food web. Where detailed studies have been performed, squids display wide trophic width and versatility, emphasising their complex and important role in marine foodwebs (e.g., Gasalla et al. 2010; Coll et al. 2013). English Channel populations of these two *Loligo* species represent an important resource for French and British fishermen and English Channel catches dominate in Northeast Atlantic squid production (ICES 2020).

The application of molecular markers to the identification of aquatic organisms is rapidly increasing, in particular to identify partially digested food remains from stomach contents (Rosel and Kocher 2002; Bucklin et al. 2011, Barbato et al. 2019, Siegenthaler et al. 2019). Other potential applications of stomach contents analysis with molecular approaches include the use of fish stomachs as 'samplers' of cephalopod diversity in under-sampled regions such as the Amazon reef system (Sales et al. 2019). The field has rapidly advanced from identification of single prey types to identification of DNA from multiple prey organisms. According to Taberlet et al. (2012) "DNA metabarcoding refers to the automated identification of multiple species from a single bulk sample containing entire organisms or from a single environmental sample containing degraded DNA (soil, water, faeces, etc.)".

To our knowledge, the first application of DNA metabarcoding to describe a cephalopod diet from field samples concerned arrow squid *Nototodarus gouldi* (Braley et al. 2010). Subsequently there were similar studies to juveniles and/or adults with barcoding in *Idioteuthis cordiformis* (Braid and Bolstad 2014), metabarcoding in *Octopus minor* (Bo et al. 2020), and subadults of *Doryteuthis sanpaulensis* (Ribas et al. 2021). There have also been several studies on the diet of cephalopod paralarvae, in which visual identification of prey remains is especially difficult, e.g. in *Octopus vulgaris* (Olmos-Perez et al. 2017; Roura et al. 2023), *Alloteuthis media* (Olmos-Perez et al. 2017), *Loligo vulgaris* (García-Mayoral et al. 2022), ommastrephids (Fernández-Álvarez et al. 2018), and subadults of. The present study is the first application of this approach to all fished stages of two European squids, *L. forbesii* and *L. vulgaris*, sampled in the main fishing ground for these resources.

## **Material and Methods:**

### Squid sampling

Squid samples were collected from commercial trawlers landings at the Port-en-Bessin fish market (figure 1) on three occasions (in September and November 2019 and March 2020, see table 1) and kept frozen (-20° C) at the Caen laboratory until the analysis was carried out in July 2020. Before extracting stomach contents, the usual biometric data was recorded on each specimen (species identification according to Holme (1974), dorsal mantle length (DML), body weight, sex, maturity stage (according to the macroscopic maturity scale (Boyle and Ngoile, 1993) and weight of the reproductive organs.

Only specimens with non-empty stomachs were further analysed (see sample size in table 1, and supplementary data Annex1).

### DNA Extraction and Amplification

Stomach contents sampling and DNA extraction were carried out in such a way as to limit contamination. The forceps and scissors used to dissect the stomachs were washed with soap and

rinsed thoroughly between each stomach. The contents of each stomach were frozen in liquid nitrogen and ground in a mortar with a pestle for DNA extraction. Total genomic DNA was extracted from 10 mg of each powdered sample using a Qiagen DNeasy blood and tissue kit (Qiagen, Germany) following the manufacturer's instructions adapted for tissue. DNA concentration and purity were controlled using a NanoDrop™ Spectrophotometer.

A plate with 96 samples (93 extractions from stomach contents, 2 controls with only squid DNA, and 1 control with ultrapure water) was shipped to Genome Quebec CES (Canada) who prepared PCR amplification of the COI gene using mlCOIintF (5' GGWACWGGWTGAACWGTWTAYCCYCC-3') and jgHCO2198 primers (5'-TAAACTTCAGGGTGACCAAARAAYCA-3') for a 313 bp amplicon as described by Leray et al. (2013). The PCR amplification was performed with the following conditions: 1X QIAGEN buffer (QIAGEN), 5 % of DMSO, 0.2 mM of dNTP mix (New England Biolab), 0.6 µM of each primer, 2–3 ng of template DNA and 0.02 U/µL of Qiagen HotStarTaq (QIAGEN) in a final volume of 20 µL. Thermocycler conditions followed an initial denaturation step of 96 °C for 15 min; 45 cycles of 94 °C for 30 s, 48 °C for 90 s, 72 °C for 90 s; and a final extension step of 72 °C for 10 min. The PCR product was visualized on 2 % agarose to confirm amplicon size.

#### Library Preparation for Illumina sequencing

A unique combination of Illumina indices (Illumina, San Diego, CA, USA) was annealed to each sample using an enrichment PCR. The PCR amplification was performed with the following conditions: 1X ROCHE buffer (ROCHE), 5 % of DMSO, 1.8 mM of MgCl<sub>2</sub> 0.2 mM of dNTP mix (New England Biolab), 0.6 µM of each index (CS1: 5'ACACTGACGACATGGTTCTACA 3' CS2: 5'TACGGTAGCAGAGACTTGGTCT 3', 1µL of template of primary PCR product and 0.025 U/µL of ROCHE FastStart High Fi (ROCHE) in a final volume of 17 µL. Thermocycler conditions followed an initial denaturation step of 95 °C for 10 min; 15 cycles of 95 °C for 15 s, 60 °C for 30 s, 72 °C for 60 s; and a final extension step of 72 °C for 3 min. To ensure successful incorporation of indexes, these PCR products was visualized on 2 % agarose.

Quantification of each amplicon was performed with a Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies). The library is then generated by pooling the same quantity (1 ng) of each amplicon.

The final pooled library also contained 93 samples and 3 controls, 1 without DNA and 2 with *Loligo* DNA (1 of each predator species). The libraries were first normalized at 2 nM, then pooled and denatured in 0.05 M NaOH. The pool was diluted to 8 pM using HT1 buffer and were loaded on a MiSeq and sequenced for 2X250 cycles according to the manufacturer's instructions. A phiX library was used as a control and mixed with libraries at 12 % level. The MiSeq control Software (MCS) version was 2.5.0.5 and RTA version was 1.18.54. Program bcl2fastq v1.8.4 was then used to demultiplex samples and generate fastq reads.

#### ESV inference and annotation

A high-resolution ANCHOR pipeline (Gonzalez et al. 2019) was used to process and annotate raw sequence reads. Briefly, primers were removed with cutadapt (Martin 2009) version 2.10, sequences from all samples were pooled, aligned and dereplicated using Mothur (Schloss et al. 2009) before selection of Exact Sequence Variants (ESVs) using a count threshold of 48 across samples corresponding to 3 times the lower number of replicates (*Loligo vulgaris* harvested in March; n=16). The ANCHOR annotation process used two sequence repositories with strict BLASTn criteria (99 % identity and coverage): NCBI nt and GenBank COI (Porter and Hajibabaei 2018). Multiple, equally good (highest identity/coverage), annotation was retained and reported. Amplicons with low counts (< 48) were binned to high-count sequences in a second BLASTn, using a lower threshold of 98 % identity/coverage. All annotation should be considered as putative and interpreted with caution as databases contain errors and are subject to change. All data for each sample was deposited in GenBank "GenBank OR334492-OR334579"

#### Differential abundance

Differential abundance analysis on ESVs was performed using DESeq2 (Love et al., 2014), which can perform well with uneven library sizes and sparsity common to 16S rRNA gene data (Brereton et al.

2021; Thorsen et al. 2016). A false discovery rate (FDR; Benjamini-Hochberg procedure) < 0.05 was applied. Normalized counts were estimated by applying a regularized log (rlog) transformation across samples in Phyloseq R library (McMurdie and Holmes 2013). Sparsity and occurrence cut-offs were applied whereby an ESV count in a single sample is < 90 % of the count in all samples, and ESV counts must occur in at least 3 samples.

## Data analysis

The table of Exact Sequence Variants (ESV) was cleaned by deleting stomachs which contained only the DNA of the predator (15 stomachs), and also DNA that could not belong to a prey, like human contamination or a microalga Chlorophyta (*Pycnococcus provasolii*). Only ESV (exact sequence variants) recorded more than 100 times were taken into account, a threshold close to what Deagle et al. (2018) suggested with a high total number of reads per sample (>10000), and the different sequences corresponding to the same prey species were merged so as to describe presence/ absence of prey. Prey occurrence is defined as its presence in a predator's stomach. The sum of prey occurrences divided by the number of sampled stomachs, expressed as a percentage, gives the frequency of occurrence, as defined by Roura et al. (2023).

Comparisons of sample dorsal mantle length (DML) distributions were carried out after testing for normality with a Shapiro-Wilks test and (since the distribution was not normal) using a Kruskal-Wallis test. Sex ratios were compared using a Chi-squared test. All these tests were carried out using R statistical software (R Core Team 2021).

Multivariate comparisons of diet composition were carried out using Correspondence Analysis and Discriminant Analysis, applied using the R package ade4 (v1.7-22; Dray and Dufour 2007) to the occurrence matrix (Annex3). Differences between the three samples were tested with a permutation test whose output is a graph showing the observed differences compared to the distribution of simulated (permutation based) differences.

## **Results:**

### Biological characteristics of sampled predators

It is worth noting that almost half of the stomachs were empty (107/ 218). Of the 93 stomachs whose DNA was analysed, 15 contained only the DNA of the predator species and 78 contained the DNA of prey species (table 2). Length distributions (DML) are significantly skewed to the right (Shapiro-Wilk test,  $p = 0.0028$ ) so the Kruskal-Wallis test was applied to the 3 samples but did not reveal significant differences ( $p = 0.0594$ ). Nevertheless, the largest *L. vulgaris* were observed in the March sample (table 2). The three samples did not reveal differences in sex-ratio or departures from a 1:1 sex-ratio (Chi-square test with  $df = 2$ ,  $p = 0.22$ ).

### Prey determination and diet analyses

In total, 220 different sequences were obtained from 93 stomach content samples. With a stringent similarity threshold of 99 %, 45 % of the sequences were identified to species (Annex2).

Molecular determination of squid diet enabled identification of 32 different prey species: 16 Teleost fish, one Elasmobranch and 15 invertebrate species (table 2 and figure 2).

The average number of prey species identified in one squid stomach content was between 1.7 and 1.9 (range 1 to 6). No significant differences in the number of prey species per stomach were seen between the two *Loligo* species (Wilcoxon test,  $p = 0.29$ ) and the number of prey species was not correlated with predator size, DML ( $r = 0.15$ ,  $p = 0.19$ ).

Although Teleost fish dominated the diet it is worth noting that other cephalopods (mainly *Sepia officinalis* but also *Alloteuthis* sp., *Eledone cirrhosa* and *Loligo forbesii*) were eaten by squid. The list of prey indicates that loliginid squid can feed on benthopelagic species like the gurnard *Chelidonichthys cuculus* or the crabs *Liocarcinus* spp., but also on pelagic species (such as sprat, mackerel, sardine or the chaetognath *Parasagitta setosa*).

Multivariate analysis combined a Correspondence Analysis applied to the presence/ absence matrix (78 stomach contents and 32 prey species coded as 1 or 0) and a Discriminant Analysis which used the three samples as groups (figure 3) and thus allowed projections on two axes. The first axis opposed cuttlefish (positively) to sprat and mackerel whereas the second axis opposed gurnard and poor cod (*Trisopterus minutus*) to cuttlefish, dragonet and sprat.

The three regimes appeared significantly different according to ade4 permutation test (Annex4). Along the first axis of the DA plot, *L. forbesii* stomach contents appeared in between the two *L. vulgaris* samples (fig. 3) which suggested that seasonal variations in *L. vulgaris* diet were more important than differences between the diet of the two *Loligo* species.

In addition to identification of prey, it is worth noting that nematode parasites that infest the guts of the squid were detected in 30 % of the samples. This concerned 10 out of 38 *L. vulgaris* stomachs and 18 out of 28 *L. forbesii* specimens; these numbers are too small to detect differences in infection rate (Chi square test,  $P = 0.087$ ). Parasite identification remained ambiguous and sequences could belong to the families Anisakidae (*Contracaecum muraenesoxi* and/or *Anisakis brevispiculata*) or Raphidascaridae (genus *Raphidascaris* and/or *Hysterothylacium*).

### Discussion:

This study is the first trial of meta-barcoding tools on the stomach contents of squid samples collected from the commercial fishery landings in Europe. The identification of a large proportion of the sequences indicates that the protocol is valid (i.e., that commercial samples can be used). In addition, despite prey remains being in various stages of digestion, DNA extraction was efficiently carried out with the Qiagen DNeasy kit (Schwerer et al. 2015). The number of prey species identified in stomach contents (32) might have been slightly greater by inhibiting amplification of the host DNA. To design such blocking primers in the two predator species can be difficult (Homma et al. 2022) but it is an interesting challenge for future diet studies.

The COI sequence that was analysed in this study is a commonly-used gene in barcoding studies and is documented in databases for a large number of marine species. There are other molecular markers that have been applied to gut contents, for example the 18S rRNA (Blankenship and Yayanos 2005), 18S v9 (Fernández-Álvarez et al. 2018), 16S rRNA (Fernández-Álvarez et al. 2018) or cyt b (Paquin et al. 2014). However, the objective of the study was not to compare the efficiency of different markers, which can vary according to the prey taxa, but rather to apply a proven protocol, similar to the one applied to Adriatic hake (Riccioni et al. 2018).

Metabarcoding of stomach contents cannot provide information about cannibalism because it is not possible to indicate if a detection of DNA of the predator species comes from self-contamination or from a congener (Traugott et al. 2020). However, this study shows that accurate identification of cephalopod prey is useful because the latter is possible to a much lower degree with morphological analysis, where prey animals are generally torn into small bits of muscle and presumably other soft tissues too (Carreño Castilla et al. 2020). This study sheds a new light on the predation by squid on cuttlefish. *Sepia officinalis* was not recorded in the stomachs of *Loligo* squids from Galician waters (Rocha et al. 1994) but can be a common prey item in the English Channel. The striking difference between these two results is likely related to the abundance of cuttlefish in the two areas and the fact that the English Channel cuttlefish stock is by far the largest in the Northeast Atlantic (ICES 2020).

Teleost fish are the most diverse group of prey, which is consistent with previous analyses of loliginid squid diets (Pierce et al. 1994; Rocha et al. 1994 ; Collins et al. 1994 ; Wangvoralak et al. 2011).

Trophic relationships between English Channel finfish and squid are likely complex since some prey like bib (*Trisopterus luscus*) or red gurnard (*Chelidonichthys cuculus*) have also been described as eating cephalopods (Daly et al. 2001). On the other hand, analysis of a large stomach contents database

(DAPSTOM <https://www.cefas.co.uk/data-and-publications/fish-stomach-records/>) provided no evidence that many of the fish species which appear in the *Loligo* diet in the present study, including bib, poor cod (*Trisopterus minutus*), horse mackerel (*Trachurus trachurus*), spratt (*Sprattus sprattus*) and pilchard (*Sardina pilchardus*), are predators of cephalopods in the Greater North Sea / English Channel (Barrett et al. 2022). It is worth mentioning that DAPSTOM database contains only a few records of *Loligo vulgaris* stomach contents (6), most of them empty (5), and the only identified prey item a decapod *Brachyura* crab.

The multivariate analysis of the prey occurrence matrix highlights the broad and variable nature of the diet of loliginid squid in the English Channel. While such results are typically interpreted as indicating opportunistic diet selection this is generally impossible to test, since doing so would require detailed information on the availability of each prey type as experienced by the squid. Seasonal changes in *L. vulgaris* regime are likely related to food availability. Because of the small size of the samples and uncertainty about the fishing ground, certain hypotheses cannot be tested but some differences between *L. forbesii* and *L. vulgaris* might be related to differences in habitat (with different species of dragonet or gurnard eaten by each predator).

Potential applications of the results include foodweb analysis (e.g., Cherel et al. 2009) and ecosystem-based fisheries management, particularly since the knowledge of cephalopod diets within foodwebs is quite poor, especially in the north east Atlantic, and this hampers proper implementation of ecosystem models (de la Chesnais et al. 2019). Nevertheless, loliginid squid score highly on indices of 'keystoneness' in south Atlantic ecosystems (Gasalla et al. 2010) and evidence has been provided of strong interrelationships between neritic squid and their predators and prey (Coll et al. 2013).

Stable isotope or fatty acid analysis can complement the presence/absence data provided by metabarcoding, by providing information on relative dependence on different prey types (see Braid and Bolstad 2014), also giving a time-integrated view of the diet (whereas metabarcoding provides more of a snapshot). In a large study screening isotopic signatures of a list of demersal species (Jennings and Van der Molen 2015) underlined the rather high trophic level of *L. forbesii* (4.7) from the Celtic Sea and English Channel.

Finally, information obtained in this study that was complementary to that obtained on diet, e.g., that of sequences belonging to nematode parasites in the squid gut, remains preliminary, but suggests that in addition to *Anisakis simplex* observed in *Loligo vulgaris* in Galician waters (Gonzalez et al. 2003) other species are likely infesting (or are at least ingested by) both *L. vulgaris* and *L. forbesii*. Parasites in squid stomachs are expected to have been ingested secondarily, i.e., because they were present in the prey and some of the species detected are known to infest many fin fish species, including sardine, mackerel, gurnard or horse mackerel (Duran et al. 1989; Levsen et al. 2018).

## **Conclusion**

The application of meta-barcoding techniques to stomach contents of commercial samples of loliginid squid shows that a wide range of prey can be identified to the species level of taxonomy. Meta-barcoding permits more accurate determination than is possible by morphological analysis of prey remains, illuminates the predation of squid on cuttlefish, confirms the wide variety of preys eaten by of English Channel loliginids and the similarities in the diet of all exploited sizes and sex and of both *Loligo* species.

## **Acknowledgements:**

This study is based on work undertaken under the auspices of ICES WGCEPH and the Ceph & Chefs project (EAPA\_282/2016, Atlantic INTERREG). We are also grateful to Genome Quebec for the quality of the sequencing.

### **Declaration:**

Financial support for this study was received within the Ceph's & Chefs project (EAPA\_282/2016, Atlantic INTERREG).

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: this is not relevant in this study.

Data and/or Code availability: all raw data are included into the supplementary data.

Authors' contribution:

- + A. Larivain: sample collection, biometric data, stomach dissection and DNA extraction and preliminary sample comparisons.
- + C. Zatylny-Gaudin: literature review and design of DNABarcoding analysis, contacts with Genome Quebec and C3G and analysis of raw sequence outputs, and related sections of the paper.
- + E. Gonzalez: Bioinformatic analysis of Genome Quebec sequence data and related sections of the manuscript.
- + G. J. Pierce: review of existing literature, advice in statistical analysis and manuscript checking.
- + A. M. Power: review of existing literature and additional data sources (DAPSTOM) thorough manuscript editing and improvements in references list, tables and figures.
- + J.-P. Robin: co-ordination of the study, samples collection, statistical analysis of results, first draft and revised versions of manuscript.

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Supplementary data: list of annexes

Annex 1: Biometric data of all samples collected and analysed in this study.

Annex 2: ESV table of stomach content sequencing

Annex 3: Presence/absence table used in Discriminant Analysis

Annex 4: Ade4 graphical output of permutation test post Discriminant Analysis

Table 1: Number of squid collected at the Port-en-Bessin fish market and analysed for diet composition by date and species. Samples selected for DNA sequencing are the non-empty stomachs with highest DNA content and purest DNA according to A260/280 ratio.

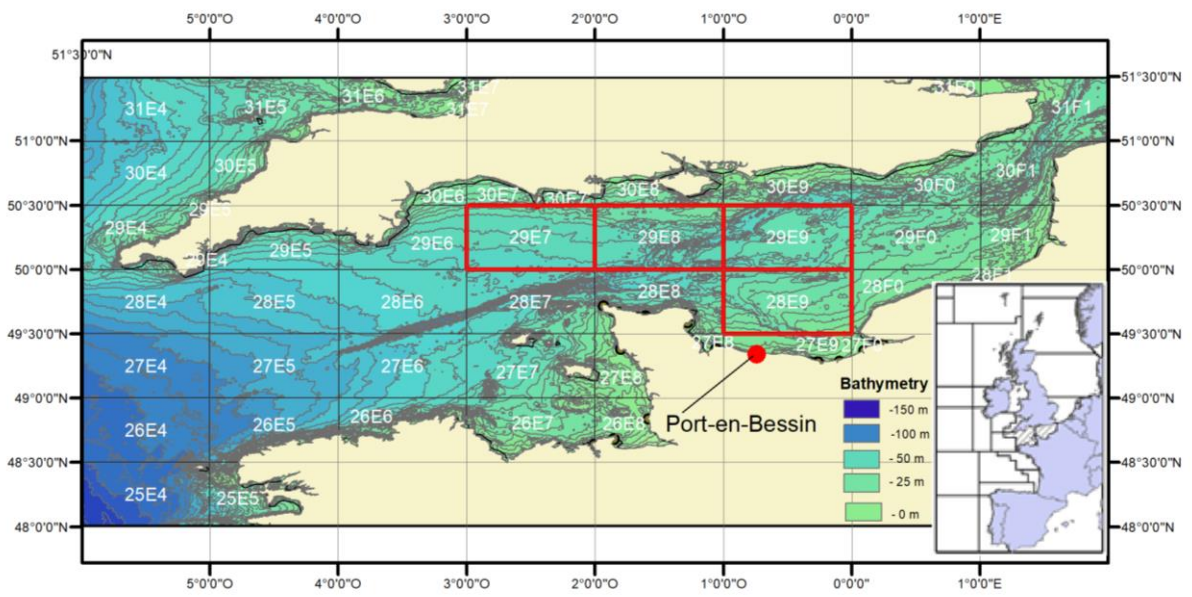
Time	<i>Loligo forbesii</i>	<i>Loligo vulgaris</i>	Total	Samples prepared for DNA sequencing
23/09/2019	113		113	46
25/11/2019		58	58	31
16/03/2020		46	46	16
Total	113	104	217	93

1 Table 2: Main characteristics of samples containing prey DNA and species identified using COI  
 2 sequences. DML is Dorsal Mantle Length, occurrence is the number of stomachs in which a prey species  
 3 is observed. Taxonomic details according to WORMS (<https://www.marinespecies.org/>)

	<b>Predator</b>	<i>L. forbesii</i>	<i>L. vulgaris</i>	<i>L. vulgaris</i>	<i>L. vulgaris</i>
	<b>Time</b>	23/09/2019	25/11/2019	16/03/2020	Nov+March
	<b>stomachs with prey DNA</b>	36	29	13	42
	<b>number of occurrences</b>	68	54	22	76
	<b>Mean prey species/stomach</b>	1.89	1.86	1.69	1.81
	<b>min DML</b>	11	11	17	11
	<b>mean DML</b>	21	19	25	21
	<b>max DML</b>	36	29	44	44
	<b>Diet species richness (S)</b>	18	12	12	19
<b>Phylum (Subphylum)</b>	<b>Prey species</b>	<b>occurrences</b>	<b>occurrences</b>	<b>occurrences</b>	<b>occurrences</b>
Chordata (Vertebrata)	<i>Blennius ocellaris</i>	1	0	0	0
Chordata (Vertebrata)	<i>Callionymus lyra</i>	0	7	0	7
Chordata (Vertebrata)	<i>Callionymus reticulatus</i>	2	0	0	0
Chordata (Vertebrata)	<i>Chelidonichthys cuculus</i>	13	4	1	5
Chordata (Vertebrata)	<i>Chelidonichthys lucernus</i>	0	0	1	1
Chordata (Vertebrata)	<i>Gobius gasteveni</i>	0	1	0	1
Chordata (Vertebrata)	<i>Mullus surmuletus</i>	0	0	1	1
Chordata (Vertebrata)	<i>Pomatoschistus pictus</i>	3	0	0	0
Chordata (Vertebrata)	<i>Sardina pilchardus</i>	4	0	0	0
Chordata (Vertebrata)	<i>Scomber scombrus</i>	2	0	5	5
Chordata (Vertebrata)	<i>Sparus aurata</i>	2	1	0	1
Chordata (Vertebrata)	<i>Spondyliosoma cantharus</i>	0	1	0	1
Chordata (Vertebrata)	<i>Sprattus sprattus</i>	8	1	6	7
Chordata (Vertebrata)	<i>Trachurus trachurus</i>	1	1	0	1
Chordata (Vertebrata)	<i>Trisopterus luscus</i>	0	3	1	4
Chordata (Vertebrata)	<i>Trisopterus minutus</i>	7	0	0	0
Chordata (Vertebrata)	<i>Mustelus asterias</i>	0	0	1	1
Chaetognatha	<i>Parasagitta setosa</i>	1	0	0	0
Mollusca	<i>Alloteuthis sp.</i>	3	0	0	0
Mollusca	<i>Eledone cirrhosa</i>	1	0	0	0
Mollusca	<i>Loligo forbesii</i>	NA	8	1	9
Mollusca	<i>Sepia officinalis</i>	11	25	1	26
Mollusca	<i>Tritonia hombergii</i>	0	0	1	1
Echinodermata	<i>Asterias rubens</i>	0	0	2	2
Echinodermata	<i>Crossaster papposus</i>	0	0	1	1
(Crustacea)	<i>Liocarcinus depurator</i>	1	0	0	0
(Crustacea)	<i>Liocarcinus holsatus</i>	5	0	0	0
(Crustacea)	<i>Liocarcinus pusillus</i>	0	0	0	0
(Crustacea)	<i>Pisidia longicornis</i>	1	0	0	0
(Crustacea)	<i>Processa modica</i>	0	1	0	1
(Crustacea)	<i>Upogebia deltaura</i>	0	1	0	1
Cnidaria	<i>Chrysaora hysoscella</i>	2	0	0	0

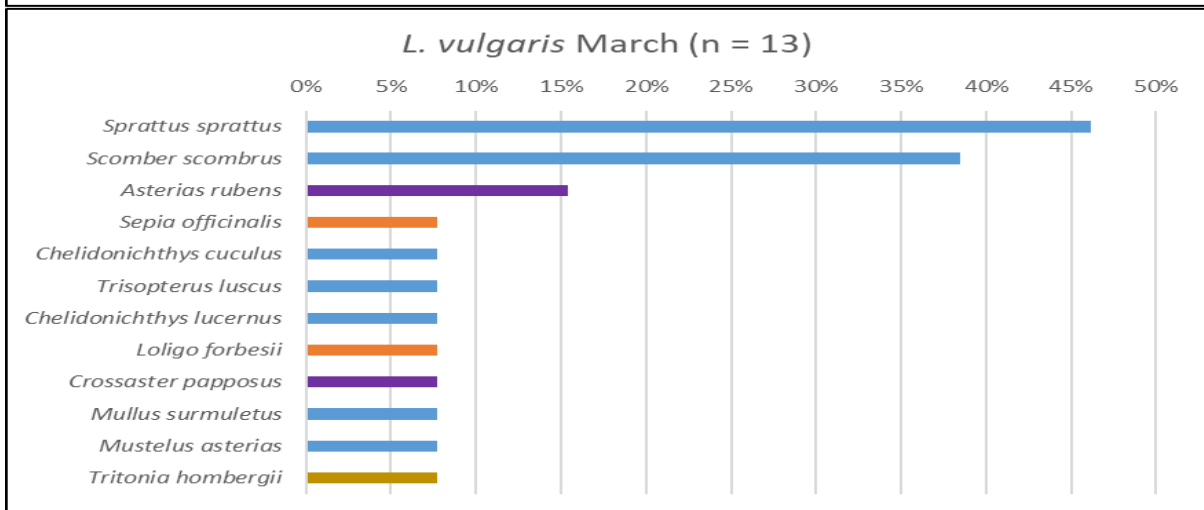
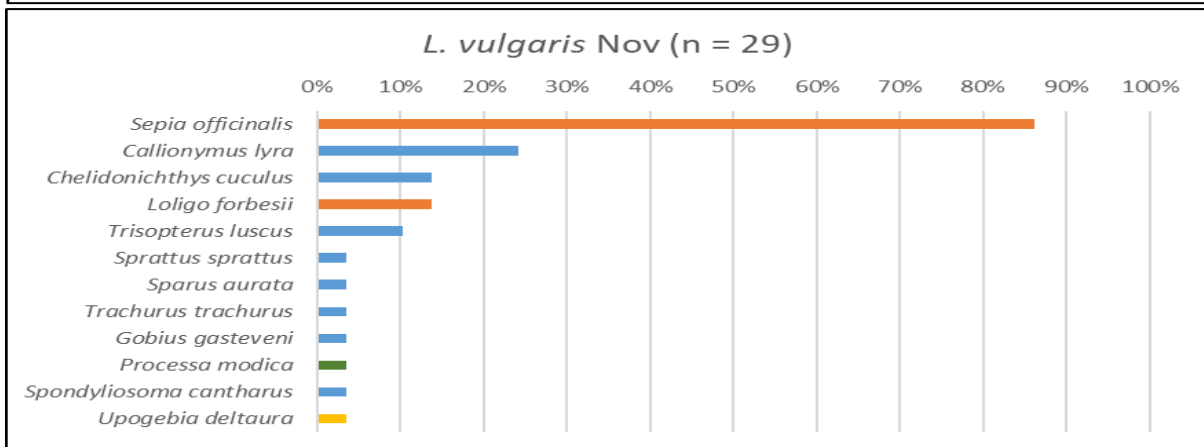
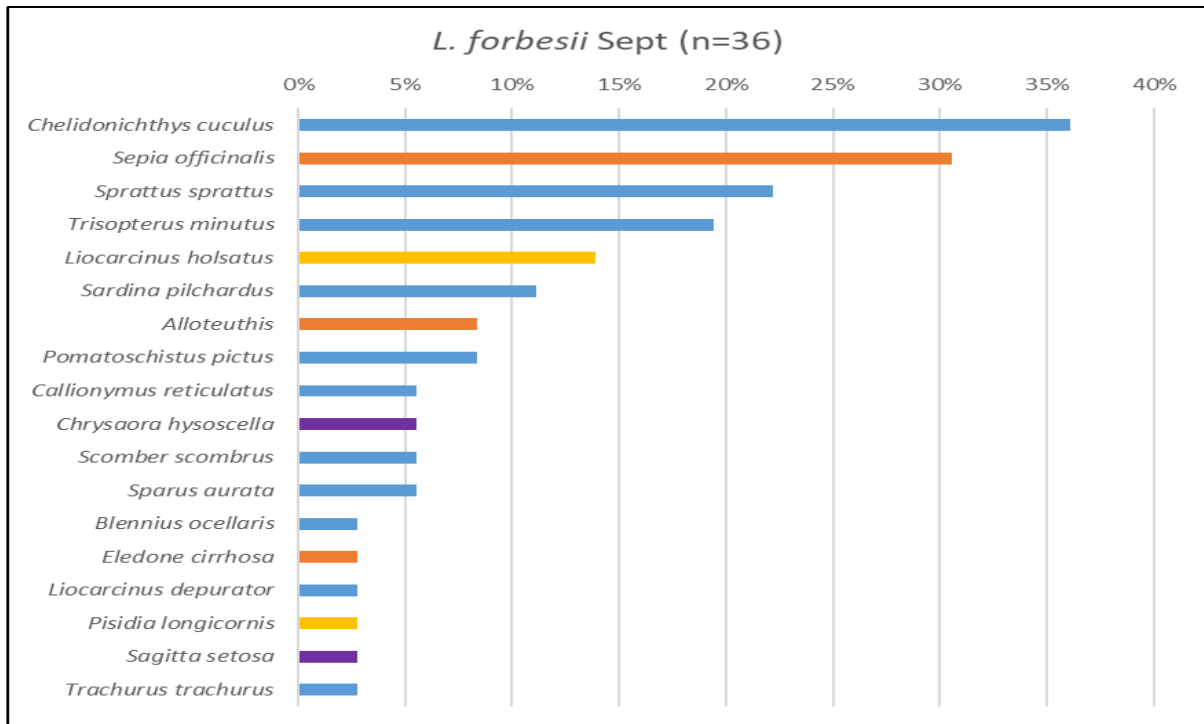


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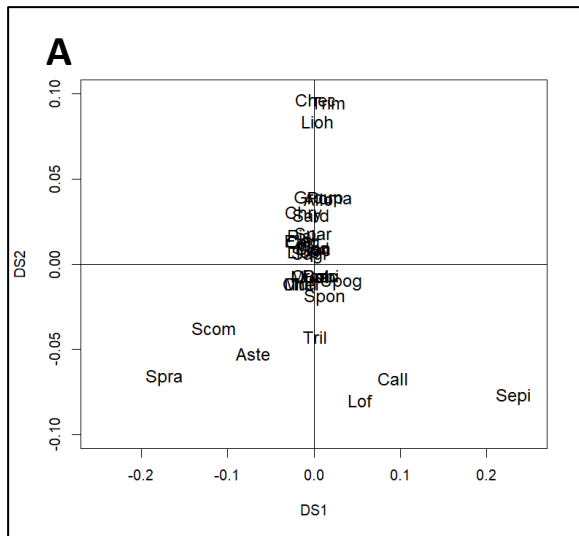
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Figure 1: map of the English Channel, with the location of the Port-en-Bessin fishmarket (●) and the ICES rectangles that were fished by Port-en-Bessin trawlers on the sampled fishing trips. Note that the rectangle 29E8 is not suitable for trawling due to hard sea bed. Bathymetry background is from EMODnet "Mean depth in multi-color style" <https://emodnet.ec.europa.eu/en/bathymetry#bathymetry-products>

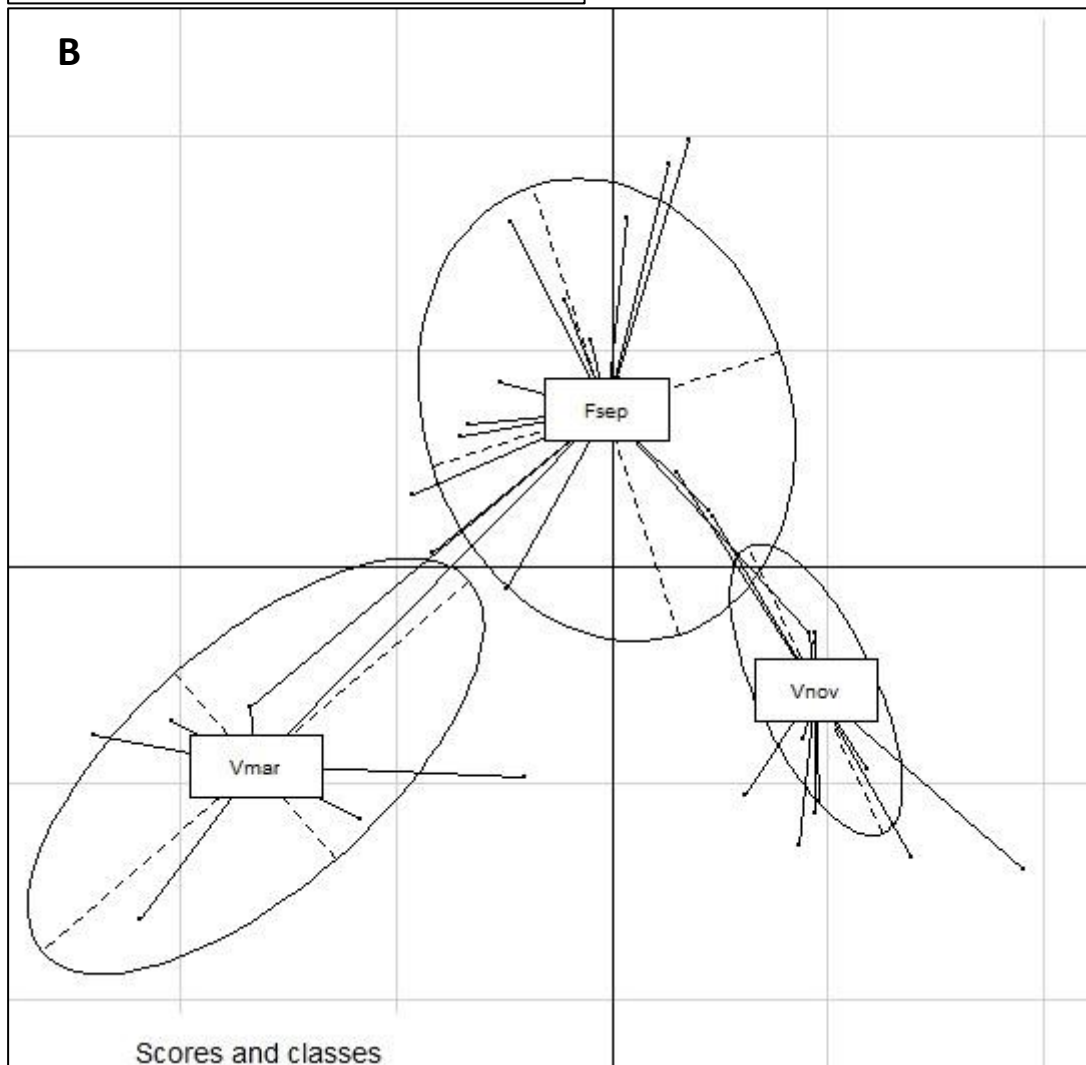


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 17 Figure 2: Frequencies of occurrence (FOO = presence of prey identified in a stomach divided by the  
 18 number of studied stomachs). In each graph (and as in table 2) n is the number of stomachs with prey  
 19 DNA and the sum of FOOs corresponds to the mean number of identified prey species per stomach.  
 20 Color codes indicate broad taxonomic grouping such as Vertebrata, Mollusca, Echinodermata, Misc.  
 21 invertebrates)





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Figure 3: Discriminant Analysis of food regimes: A projection of prey species in the plane of the two discriminant axes; B projection of stomach contents in the plane of the two discriminant axes defined to separate the three studied samples identified with ellipses and labels: (Fsep = *L. forbesii* September, Vnov = *L. vulgaris* November, Vmar = *L. vulgaris* March).