Online Resource 1. List of primers and PCR conditions



Online Resource 2:

Total number of reads/sample or control in original MiSeq output and number of reads/sample after bioinformatic processing. Number of reads/sample for each taxa by sample by markers.

Online Resource 3:

In the 16S dataset, the control samples are largely contaminated by human DNA (more than 99% of reads in some control samples). The metabarcoding workflow is very sensitive and when the sample does not contain target DNA, there is a higher probability to detect very small external contamination. Besides human DNA, we also found a considerable number of reads assigned to the fish genus *Cottus* in control 3 and assigned to the cattle (genus *Bos*) in control 5. The fish contamination might originate from the lab, as the species was present only in one of field samples and in very limited number of reads. The cattle DNA probably originated from contamination present in water samples, similarly to the perch, pig, dog and cat DNA found in our 16S dataset.

For the COI dataset, most of the sequences present in the control samples could not be assigned and their origins are difficult to establish. Within the assigned COI sequences present in control samples, the majority corresponds to human contamination (more than 99% of the reads). Interestingly, some control samples contain also a low number of reads assigned to copepods. As the same species of copepods are abundant in the field samples, we consider that their presence in control samples could be caused by a cross contamination between samples on the sequencing flow cell.

The control samples of MiDeca dataset are also dominated by unassigned sequences that are possibly artefacts of reamplification. This is particularly the case of control 3. The other sequences detected in the control samples, correspond to the species abundant in the field samples, which might suggest that as in other datasets, they are resulting of a cross contamination between samples on the sequencing flow cell.

Online Resource 4. Coverage of reference database

We started by comparing the list of species collected by trawling and identified morphologically (survey from 1987 to 2016; http://atlasbenthal.ifremer.fr/) with the public reference database (NCBI GenBank, May 2021). The morphological list comprises 430 species and higher taxa. About 30% of them (131) are represented by less than 10 specimens and can be considered as rare. The list is dominated by fish (over 200 species) and to the lesser degree by decapods and mollusks. These 3 groups count for almost 75% of all taxa on the list. The majority of morphologically identified macrofauna are present in the genetic database. The COI sequences are available for 384 morphospecies (89%), while the 16S sequences are available for 326 taxa (76%).

Online Resource 5. Detailed taxa identified at genus level by either a single, two or three methods. S = number of genus

|  |  |  |  |
| --- | --- | --- | --- |
|  | eDNA (S = 17) | Scientific trawling (S = 27) | Underwater video (S = 1) |
| Genus identified by a single method | *Ammodytes**Boops**Centrolabrus**Ciliata**Crystallogobius**Dipturus**Euthynnus**Gobiusculus**Gymnammodytes* | *Lepidotrigla**Lesueurigobius**Lithognathus**Maurolicus**Mola**Parablennius**Platichthys**Pomatoschistus* | *Alloteuthis**Alopias**Cancer**Echiichthys**Gadiculus**Gadus**Glyptocephalus**Helicolenus**Homarus* | *Hyperoplus**Illex**Maja**Molva**Octopus**Palinurus**Petromyzon**Phrynorhombus**Rossia* | *Scyllarus**Symphodus**Todaropsis**Torpedo**Trigla**Trigloporus**Umbrina**Zeugopterus**Zeus* | *Echiodon* |
|  | **eDNA + scientific trawling** (S = 14) | **Scientific trawling + underwater video** (S = 9) |
| Genus identified by two methods | *Arnoglossus**Belone**Buglossidium**Ctenolabrus**Engraulis**Gaidropsarus**Labrus* | *Microstomus**Mustelus**Necora**Pagellus**Sardina**Scomber**Sprattus* | *Eledone**Eutrigla**Leucoraja**Loligo**Melanogrammus* | *Pecten**Phycis**Raja**Sepia* |
|  | **eDNA+ scientific trawling + underwater video** (S = 23) |
| Genus identified by all three methods | *Argentina**Callionymus**Capros**Cepola**Chelidonichthys**Conger* | *Dicologlossa**Enchelyopus**Lepidorhombus**Lophius**Merlangius**Merluccius* | *Microchirus**Micromesistius**Mullus**Munida**Nephrops**Pollachius* | *Scyliorhinus**Solea**Trachinus**Trachurus**Trisopterus* |

Online Resource 6. Occurrence and frequency [ = (occurrence\*100)/number of samples] of each genus per sampling method. N = number of samples. Highlighted in bold are the genera detected by the three sampling methods. In yellow, the 15 most occurrent genera for each method

|  |  |  |  |
| --- | --- | --- | --- |
|  | eDNA | Scientific trawling | Underwater video |
|  | **occurrence** | **frequency** | **occurrence** | **frequency** | **occurrence** | **frequency** |
| Genus  | **N = 37** | **N = 141** | **N = 218** |
| *Alloteuthis* | 0 | 0 | 67 | 47.5 | 0 | 0 |
| *Alopias* | 0 | 0 | 1 | 0.7 | 0 | 0 |
| *Ammodytes* | 2 | 5.4 | 0 | 0 | 0 | 0 |
| *Argentina* | **6** | **16.2** | **118** | **83.7** | **7** | **3.2** |
| *Arnoglossus* | 4 | 10.8 | 141 | 100 | 0 | 0 |
| *Belone* | 5 | 13.5 | 1 | 0.7 | 0 | 0 |
| *Boops* | 2 | 5.4 | 0 | 0 | 0 | 0 |
| *Buglossidium* | 4 | 10.8 | 3 | 2.1 | 0 | 0 |
| *Callionymus* | **21** | **56.8** | **141** | **100** | **111** | **50.9** |
| *Cancer* | 0 | 0 | 95 | 67.4 | 0 | 0 |
| *Capros* | **4** | **10.8** | **120** | **85.1** | **9** | **4.1** |
| *Centrolabrus* | 1 | 2.7 | 0 | 0 | 0 | 0 |
| *Cepola* | **6** | **16.2** | **94** | **66.7** | **15** | **6.9** |
| *Chelidonichthys* | **7** | **18.9** | **113** | **80.1** | **3** | **1.4** |
| *Ciliata* | 5 | 13.5 | 0 | 0 | 0 | 0 |
| *Conger* | **4** | **10.8** | **93** | **66.0** | **9** | **4.1** |
| *Crystallogobius* | 6 | 16.2 | 0 | 0 | 0 | 0 |
| *Ctenolabrus* | 2 | 5.4 | 2 | 1.4 | 0 | 0 |
| *Dicologlossa* | **2** | **5.4** | **1** | **0.7** | **2** | **0.9** |
| *Dipturus* | 1 | 2.7 | 0 | 0 | 0 | 0 |
| *Echiichthys* | 0 | 0 | 3 | 2.1 | 0 | 0 |
| *Echiodon* | 0 | 0 | 0 | 0 | 6 | 2.8 |
| *Eledone* | 0 | 0 | 131 | 92.9 | 72 | 33.0 |
| *Enchelyopus* | **1** | **2.7** | **42** | **29.8** | **22** | **10.1** |
| *Engraulis* | 35 | 94.6 | 4 | 2.8 | 0 | 0 |
| *Euthynnus* | 1 | 2.7 | 0 | 0 | 0 | 0 |
| *Eutrigla* | 0 | 0 | 114 | 80.9 | 3 | 1.4 |
| *Gadiculus* | 0 | 0 | 4 | 2.8 | 0 | 0 |
| *Gadus* | 0 | 0 | 1 | 0.7 | 0 | 0 |
| *Gaidropsarus* | 1 | 2.7 | 20 | 14.2 | 0 | 0 |
| *Glyptocephalus* | 0 | 0 | 1 | 0.7 | 0 | 0 |
| *Gobiusculus* | 2 | 5.4 | 0 | 0 | 0 | 0 |
| *Gymnammodytes* | 3 | 8.1 | 0 | 0 | 0 | 0 |
| *Helicolenus* | 0 | 0 | 2 | 1.4 | 0 | 0 |
| *Homarus* | 0 | 0 | 1 | 0.7 | 0 | 0 |
| *Hyperoplus* | 0 | 0 | 3 | 2.1 | 0 | 0 |
| *Illex* | 0 | 0 | 116 | 82.3 | 0 | 0 |
| *Labrus* | 2 | 5.4 | 2 | 1.4 | 0 | 0 |
| *Lepidorhombus* | **9** | **24.3** | **107** | **75.9** | **116** | **53.2** |
| *Lepidotrigla* | 1 | 2.7 | 0 | 0 | 0 | 0 |
| *Lesueurigobius* | 4 | 10.8 | 0 | 0 | 0 | 0 |
| *Leucoraja* | 0 | 0 | 12 | 8.5 | 1 | 0.5 |
| *Lithognathus* | 12 | 32.4 | 0 | 0 | 0 | 0 |
| *Loligo* | 0 | 0 | 56 | 39.7 | 5 | 2.3 |
| *Lophius* | **7** | **18.9** | **135** | **95.7** | **13** | **6.0** |
| *Maja* | 0 | 0 | 11 | 7.8 | 0 | 0 |
| *Maurolicus* | 1 | 2.7 | 0 | 0 | 0 | 0 |
| *Melanogrammus* | 0 | 0 | 21 | 14.9 | 2 | 0.9 |
| *Merlangius* | **3** | **8.1** | **19** | **13.5** | **1** | **0.5** |
| *Merluccius* | **7** | **18.9** | **141** | **100** | **40** | **18.3** |
| *Microchirus* | **13** | **35.1** | **132** | **93.6** | **105** | **48.2** |
| *Micromesistius* | **13** | **35.1** | **137** | **97.2** | **2** | **0.9** |
| *Microstomus* | 1 | 2.7 | 20 | 14.2 | 0 | 0 |
| *Mola* | 2 | 5.4 | 0 | 0 | 0 | 0 |
| *Molva* | 0 | 0 | 6 | 4.3 | 0 | 0 |
| *Mullus* | **3** | **8.1** | **93** | **66.0** | **1** | **0.5** |
| *Munida* | **2** | **5.4** | **118** | **83.7** | **90** | **41.3** |
| *Mustelus* | 1 | 2.7 | 33 | 23.4 | 0 | 0 |
| *Necora* | 1 | 2.7 | 7 | 5.0 | 0 | 0 |
| *Nephrops* | **13** | **35.1** | **134** | **95.0** | **138** | **63.3** |
| *Octopus* | 0 | 0 | 1 | 0.7 | 0 | 0 |
| *Pagellus* | 3 | 8.1 | 2 | 1.4 | 0 | 0 |
| *Palinurus* | 0 | 0 | 1 | 0.7 | 0 | 0 |
| *Parablennius* | 1 | 2.7 | 0 | 0 | 0 | 0 |
| *Pecten* | 0 | 0 | 4 | 2.8 | 3 | 1.4 |
| *Petromyzon* | 0 | 0 | 2 | 1.4 | 0 | 0 |
| *Phrynorhombus* | 0 | 0 | 18 | 12.8 | 0 | 0 |
| *Phycis* | 0 | 0 | 116 | 82.3 | 4 | 1.8 |
| *Platichthys* | 1 | 2.7 | 0 | 0 | 0 | 0 |
| *Pollachius* | **1** | **2.7** | **3** | **2.1** | **1** | **0.5** |
| *Pomatoschistus* | 6 | 16.2 | 0 | 0 | 0 | 0 |
| *Raja* | 0 | 0 | 16 | 11.3 | 1 | 0.5 |
| *Rossia* | 0 | 0 | 14 | 9.9 | 0 | 0 |
| *Sardina* | 18 | 48.6 | 2 | 1.4 | 0 | 0 |
| *Scomber* | 21 | 56.8 | 8 | 5.7 | 0 | 0 |
| *Scyliorhinus* | **1** | **2.7** | **137** | **97.2** | **26** | **11.9** |
| *Scyllarus* | 0 | 0 | 2 | 1.4 | 0 | 0 |
| *Sepia* | 0 | 0 | 141 | 100 | 28 | 12.8 |
| *Solea* | **2** | **5.4** | **117** | **83.0** | **12** | **5.5** |
| *Sprattus* | 4 | 10.8 | 3 | 2.1 | 0 | 0 |
| *Symphodus* | 0 | 0 | 1 | 0.7 | 0 | 0 |
| *Todaropsis* | 0 | 0 | 103 | 73.0 | 0 | 0 |
| *Torpedo* | 0 | 0 | 1 | 0.7 | 0 | 0 |
| *Trachinus* | **1** | **2.7** | **5** | **3.5** | **1** | **0.5** |
| *Trachurus* | **19** | **51.4** | **48** | **34.0** | **5** | **2.3** |
| *Trigla* | 0 | 0 | 1 | 0.7 | 0 | 0 |
| *Trigloporus* | 0 | 0 | 2 | 1.4 | 0 | 0 |
| *Trisopterus* | **11** | **29.7** | **134** | **95.0** | **40** | **18.3** |
| *Umbrina* | 0 | 0 | 1 | 0.7 | 0 | 0 |
| *Zeugopterus* | 0 | 0 | 2 | 1.4 | 0 | 0 |
| *Zeus* | 0 | 0 | 116 | 82.3 | 0 | 0 |