UNESCO environmental DNA (eDNA) Expeditions in marine World Heritage sites

Biodiversity survey for Gulf of Porto: Calanche of Piana, Gulf of Girolata, Scandola Reserve

Interim Results
Not for public distribution

Credit: Christelle Alix

About eDNA Expeditions

UNESCO's eDNA Expeditions is a global, citizen science initiative that is conducted with the intention to measure marine biodiversity and predict the impact climate change on marine community composition across UNESCO World Heritage marine sites.

eDNA is a cost effective and minimally invasive method to measure biodiversity in any given area. Marine species continuously shed DNA into the water around them in the form of waste, mucus, or cells. By extracting this DNA from water samples, and subsequently multiplying and sequencing specific regions within these DNA strands, a wide variety of species can be detected without removing any organisms from their environment. The regions in the DNA targeted for sequencing are selected depending on the scope of the study: while some markers are suitable to obtain a broad overview of biodiversity across many groups of species, other markers are particularly suited to get high resolution insight for a more specific taxonomic group such as fish. The UNESCO eDNA Expeditions initiative combines a number of markers tailored for marine vertebrates (fish, mammals, and turtles). A general marker was added to also allow detection of species from other groups such as invertebrates, which can give a broader insight in the marine species diversity in a given area.

Between September 2022 and May 2023, UNESCO organized eDNA sampling campaigns in 21 marine World Heritage sites across 17 countries around the world. At every World Heritage site, about 20 samples were collected from different habitats. Over 250 young people participated in the local sampling expeditions. They were guided by local experts and park management staff, using protocols and eDNA sampling equipment provided by UNESCO.

Biodiversity inventories generated from the samples are being combined with existing species distribution data from public biodiversity databases such as the Ocean Biodiversity Information System (OBIS) in view of obtaining a comprehensive overview of marine life across marine World Heritage sites. Through the use of climate scenarios and species distribution models, an estimate will be made of the impact of climate change, in particular rising temperatures, on local biodiversity and its potential future distribution patterns.

More information about the initiative and a press kit are available on the UNESCO website: https://www.unesco.org/en/edna-expeditions.

Results

Sampling and sample processing

eDNA sampling was conducted in Gulf of Porto: Calanche of Piana, Gulf of Girolata, Scandola Reserve in March and April 2023. A total of 20 samples were collected at 5 locations in the park covering different habitats: elbu, ficaccia, ficaghjola, foccocara, and punta_a_scopa (Figure 1). Up to 1,500 mL of seawater was filtered through filter cartridges containing a filter with a 0.8 µm pore size. After sampling, the filter cartridges were flushed with preservation liquid and shipped to UNESCO. DNA from the samples was extracted and amplified, and subsequently sent to the sequencing facility at KU Leuven in Belgium, a specialized university eDNA lab contracted by UNESCO for this work. PCR amplification was done using five primer pairs selected to capture as much biodiversity as possible in the target groups of the study: fish, mammals, and turtles.

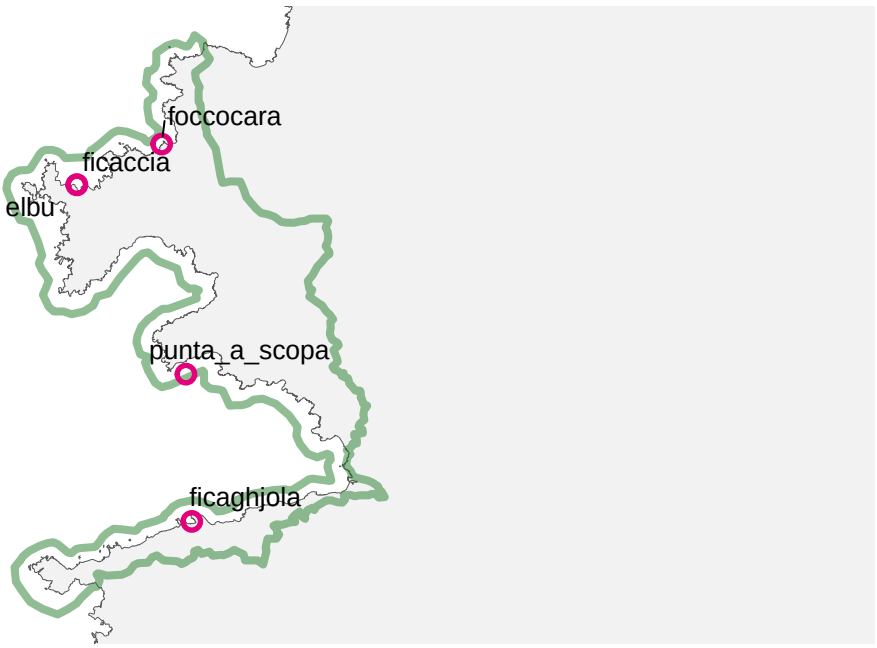

Figure 1: Map of the sampling locations.

DNA sequencing

Sequencing of the DNA from 20 samples resulted in over 28 million sequence reads, from which we collected 19,869 unique sequences or ASVs (Tables 1 and 2).

reads	asvs
28354649	19869

Table 1: Reads, ASVs, and species across all samples.

Species identification

The marker sequences obtained from sequencing were matched with sequence reference databases built using public data available from the National Center for Biotechnology Information (NCBI). This resulted in the identification of 208 species, including 65 fish species, 1 mammalian species, and 0 turtle species (Table 3). Species from other groups, such as algae, worms, molluscs, cnidarians, and copepods, were also detected (Figures 2 and 3). This number of species detected represents about 17% of the 1,294 species known from Gulf of Porto:

locality	materialSampleID	sampleSize	reads	asvs	species
Elbu	EE0307	1500	2172050	7001	78
Elbu	EE0315	1500	1440348	7944	59
Elbu	EE0317	100	1637083	5090	18
Elbu	EE0318	1500	1503952	6003	28
Ficaccia	EE0309	1500	1331133	5276	73
Ficaccia	EE0312	1500	1375063	7220	52
Ficaccia	EE0320	1500	2238269	9012	82
Ficaccia	EE0321	1500	2329575	7311	36
Ficaghjola	EE0301	1500	1581537	3441	48
Ficaghjola	EE0303	1500	29401	110	6
Ficaghjola	EE0305	1500	132	12	3
Ficaghjola	EE0306	1500	1989022	981	51
Ficaghjola	EE0313	1500	1073751	6426	72
Ficaghjola	EE0319	1500	1789366	6177	72
Foccocara	EE0265	1500	1704735	4672	26
Foccocara	EE0308	1500	1497293	3784	23
Foccocara	EE0310	1500	840529	4155	26
Foccocara	EE0311	1500	390372	687	8
Foccocara	EE0316	1500	1768600	7282	22
	EE0322	1010	1662438	6149	44

Table 2: Reads, ASVs, and species by sample.

Calanche of Piana, Gulf of Girolata, Scandola Reserve in the OBIS database. Of the 208 species detected, 123 are not among the species previously reported from Gulf of Porto: Calanche of Piana, Gulf of Girolata, Scandola Reserve to the UNESCO OBIS database. 3 of the detected species are listed as threatened on the IUCN Red List (Table 4). A full list of species is added at the end of this report.

group	eDNA species	total species	fraction
fish	65	368	0.18
mammals	1	9	0.11
turtles	0	1	

Table 3: Number of species in the three target groups, from the OBIS database and from eDNA sampling.

category	eDNA species	total species	fraction
CR	1	14	0.07
EN	0	11	
VU	2	25	0.08

Table 4: Number of species by IUCN Red List category, from the OBIS database and from eDNA sampling.

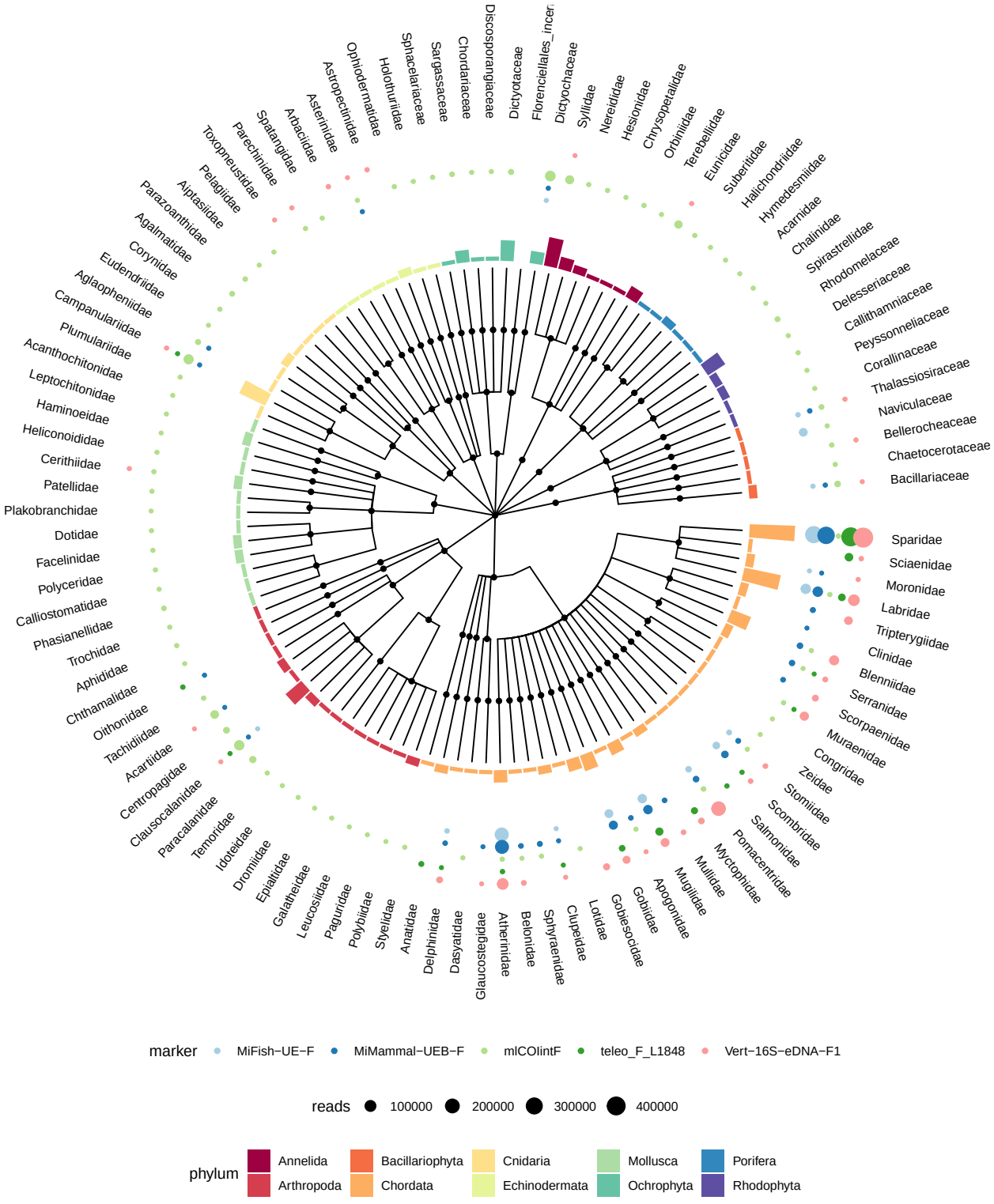

Figure 2: Number of DNA reads (bubbles) and species detected (bars) by family.

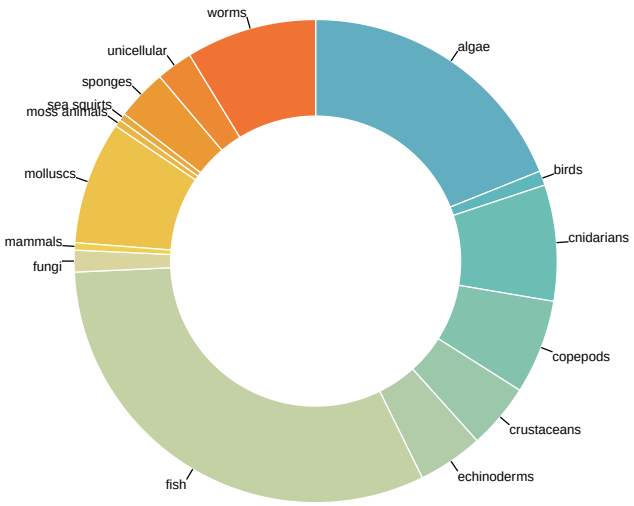

Figure 3: Distribution of detected species across groups.

phylum	class	species	group	category	new	vernacular
Amoebozoa	Discosea	Neoparamoeba pemaquidensis	unicellular		yes	
Annelida	Polychaeta	Chrysopetalum debile	worms		yes	
Annelida	Polychaeta	Eunice vittata	worms		yes	
Annelida	Polychaeta	Gyptis propinqua	worms		yes	
Annelida	Polychaeta	Haplosyllis spongicola	worms		yes	
Annelida	Polychaeta	Hesiospina aurantiaca	worms		yes	
Annelida	Polychaeta	Leodice harassii	worms		yes	
Annelida	Polychaeta	Lysidice unicornis	worms		yes	
Annelida	Polychaeta	Myrianida edwarsi	worms		yes	
Annelida	Polychaeta	Odontosyllis gibba	worms		yes	
Annelida	Polychaeta	Phylo foetida	worms		yes	
Annelida	Polychaeta	Platynereis agilis	worms		yes	
Annelida	Polychaeta	Platynereis dumerilii	worms		yes	comb-toothed nereid
Annelida	Polychaeta	Platynereis jourdei	worms		yes	
Annelida	Polychaeta	Polycirrus aurantiacus	worms		yes	
Annelida	Polychaeta	Proceraea aurantiaca	worms		yes	
Annelida	Polychaeta	Syllis compacta	worms		yes	
Annelida	Polychaeta	Syllis gerlachi	worms		yes	
Annelida	Polychaeta	Trypanosyllis zebra	worms		yes	
Arthropoda	Copepoda	Acartia (Acartiura) clausi	copepods		yes	
Arthropoda	Copepoda	Clausocalanus furcatus	copepods		yes	
Arthropoda	Copepoda	Clausocalanus lividus	copepods		yes	
Arthropoda	Copepoda	Clausocalanus mastigophorus	copepods		yes	
Arthropoda	Copepoda	Clausocalanus paululus	copepods		yes	
Arthropoda	Copepoda	Clausocalanus pergens	copepods		yes	
Arthropoda	Copepoda	Euterpina acutifrons	copepods		yes	
Arthropoda	Copepoda	Isias clavipes	copepods		yes	
Arthropoda	Copepoda	Oithona similis	copepods		yes	
Arthropoda	Copepoda	Paracalanus nanus	copepods		yes	
Arthropoda	Copepoda	Paracalanus quasimodo	copepods		yes	
Arthropoda	Copepoda	Pteriacartia josephinae	copepods		yes	
Arthropoda	Copepoda	Temora stylifera	copepods		yes	
Arthropoda	Hexapoda	Aulacorthum solani			yes	
Arthropoda	Malacostraca	Dromia personata	crustaceans			sponge crab, crabe dormeur
Arthropoda	Malacostraca	Ebalia edwardsii	crustaceans		yes	
Arthropoda	Malacostraca	Galathea intermedia	crustaceans			
Arthropoda	Malacostraca	Idotea balthica	crustaceans		yes	Baltic isopod
Arthropoda	Malacostraca	Liocarcinus corrugatus	crustaceans			wrinkled swimming crab
Arthropoda	Malacostraca	Liocarcinus marmoreus	crustaceans		yes	marbled swimming crab
Arthropoda	Malacostraca	Pagurus forbesii	crustaceans			rough-clawed hermit crab
Arthropoda	Malacostraca	Pisa armata	crustaceans		yes	Gibb's sea spider, araignée à rostre pointu
Arthropoda	Thecostraca	Chthamalus stellatus	crustaceans		yes	Poli's stellate barnacle
Ascomycota	Dothideomycetes	Cladosporium herbarum	fungi		yes	
Ascomycota	Eurotiomycetes	Penicillium chrysogenum	fungi		yes	
Bacillariophyta	Bacillariophyceae	Bellerochea polymorpha	algae		yes	
Bacillariophyta	Bacillariophyceae	Chaetoceros socialis	algae		yes	
Bacillariophyta	Bacillariophyceae	Cylindrotheca closterium	algae		yes	
Bacillariophyta	Bacillariophyceae	Navicula minima	algae		yes	
Bacillariophyta	Bacillariophyceae	Pseudo-nitzschia delicatissima	algae		yes	
Bacillariophyta	Bacillariophyceae	Thalassiosira profunda	algae		yes	

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phylum	class	species	group	category	new	vernacular
Bryozoa	Gymnolaemata	Amathia lendigera	moss animals		yes	
Chlorophyta	Mamiellophyceae	Bathycoccus prasinos	algae		yes	
Chlorophyta	Mamiellophyceae	Dolichomastix tenuilepis	algae		yes	
Chlorophyta	Mamiellophyceae	Mantoniella squamata	algae		yes	
Chlorophyta	Mamiellophyceae	Micromonas commoda	algae		yes	
Chlorophyta	Mamiellophyceae	Micromonas pusilla	algae		yes	
Chordata	Ascidiacea	Botryllus schlosseri	sea squirts		yes	golden star tunicate, botrylle étoilé
Chordata	Aves	Anas poecilorhyncha	birds	LC	yes	
Chordata	Aves	Cairina moschata	birds	LC	yes	
Chordata	Elasmobranchii	Dasyatis pastinaca	fish	VU		stingray, pastenague commune
Chordata	Elasmobranchii	Glaucostegus cemiculus	fish	CR	yes	
Chordata	Mammalia	Tursiops truncatus	mammals	LC		bottlenose dolphin, grand dauphin
Chordata	Teleostei	Aidablennius sphynx	fish	LC		sphynx blenny, blennie sphinx
Chordata	Teleostei	Apogon imberbis	fish	LC		
Chordata	Teleostei	Atherina boyeri	fish	LC		big-scale sand-smelt, joel
Chordata	Teleostei	Atherina hepsetus	fish			Mediterranean sand smelt, siouclet
Chordata	Teleostei	Atherinomorus lacunosus	fish	LC	yes	robust silverside
Chordata	Teleostei	Belone belone	fish	LC		sea needle, aiquille
Chordata	Teleostei	Boops boops	fish	LC		bogue, bogue
Chordata	Teleostei	Borostomias antarcticus	fish	LC	yes	straightline dragonfish
Chordata	Teleostei	Chelon labrosus	fish	LC		thick-lipped grey mullet, muge lippu
Chordata	Teleostei	Chromis chromis	fish	LC		damselfish
Chordata	Teleostei	Clinitrachus argentatus	fish	LC		
Chordata	Teleostei	Clupea harengus	fish	LC	yes	herring, hareng de l'Atlantique
Chordata	Teleostei	Conger conger	fish	LC		conger, congre
Chordata	Teleostei	Coris julis	fish	LC		rainbow wrasse, girelle
Chordata	Teleostei	Dentex dentex	fish	VU		dentex, denté commun
Chordata	Teleostei	Dicentrarchus labrax	fish	LC		seabass, bar
Chordata	Teleostei	Dicentrarchus punctatus	fish	LC		spotted sea-bass, bar moucheté
Chordata	Teleostei	Diplodus annularis	fish	LC		annular sea bream, sparaillon
Chordata	Teleostei	Diplodus sargus	fish	LC		white seabream, sar commun du Maroc
Chordata	Teleostei	Diplodus vulgaris	fish	LC		two banded sea bream, sar à tête noire
Chordata	Teleostei	Gaidropsarus mediterraneus	fish			shore rockling, motelle
Chordata	Teleostei	Gobius cruentatus	fish	LC		red-mouthed goby
Chordata	Teleostei	Gobius paganellus	fish	LC		rock goby, gobie paganel
Chordata	Teleostei	Hygophum benoiti	fish	LC	yes	Benoit's lanternfish
Chordata	Teleostei	Labrus bergylta	fish	LC	yes	wrasse, labre
Chordata	Teleostei	Labrus merula	fish	LC		brown wrasse, merle
Chordata	Teleostei	Labrus mixtus	fish	LC		cuckoo wrasse, vieille coquette
Chordata	Teleostei	Lepadogaster candolii	fish			
Chordata	Teleostei	Lepadogaster purpurea	fish	LC	yes	
Chordata	Teleostei	Lipophrys trigloides	fish	LC		
Chordata	Teleostei	Mugil capurrii	fish	LC	yes	
Chordata	Teleostei	Mullus surmuletus	fish	LC		striped red mullet, rouget-barbet de roche
Chordata	Teleostei	Muraena helena	fish	LC		Mediterranean moray, murène commune
Chordata	Teleostei	Myctophum punctatum	fish	LC		spotted lanternfish, lanterne ponctuée
Chordata	Teleostei	Oblada melanurus	fish			
Chordata	Teleostei	Oedalechilus labeo	fish			boxlip mullet, ange-sabon
Chordata	Teleostei	Opeatogenys gracilis	fish	LC		
Chordata	Teleostei	Pagellus bogaraveo	fish	NT		blackspot seabream, daurade rose

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phylum	class	species	group	category	new	vernacular
Chordata	Teleostei	Pagrus pagrus	fish	LC		red porgy, pagre commun
Chordata	Teleostei	Parablennius gattorugine	fish	LC		tompot blenny, blennie gattorugine
Chordata	Teleostei	Parablennius incognitus	fish	LC		
Chordata	Teleostei	Parablennius zvonimiri	fish	LC		
Chordata	Teleostei	Pseudaphya ferreri	fish	LC		Ferrer's goby
Chordata	Teleostei	Salmo salar	fish	LR/lc	yes	Atlantic salmon, saumon d'Atlantique
Chordata	Teleostei	Sarpa salpa	fish	LC		Saunders's tern , saupe
Chordata	Teleostei	Sciaena umbra	fish	NT		brown meagre, maigre
Chordata	Teleostei	Scomber japonicus	fish	LC		Atlantic chub mackerel, maquereaux blanc
Chordata	Teleostei	Scorpaena porcus	fish	LC		black scorpion-fish, rascasse brune
Chordata	Teleostei	Serranus cabrilla	fish	LC		comber, serran chevrette
Chordata	Teleostei	Serranus scriba	fish	LC		painted comber, serran écriture
Chordata	Teleostei	Sphyraena viridensis	fish	LC		yellowmouth barracuda, bécune européenne
Chordata	Teleostei	Spicara maena	fish	LC		picarel, mendole commune
Chordata	Teleostei	Spicara smaris	fish	LC		picarel, picarel
Chordata	Teleostei	Sprattus sprattus	fish	LC		European sprat, sprat
Chordata	Teleostei	Symphodus ocellatus	fish	LC		
Chordata	Teleostei	Symphodus roissali	fish	LC		
Chordata	Teleostei	Symphodus rostratus	fish	LC		
Chordata	Teleostei	Symphodus tinca	fish	LC		East Atlantic peacock wrasse
Chordata	Teleostei	Thalassoma pavo	fish	LC		
Chordata	Teleostei	Tripterygion delaisi	fish	LC		black-faced blenny, tripterygion
Chordata	Teleostei	Tripterygion tripteronotum	fish	LC		
Chordata	Teleostei	Zebrus zebrus	fish	LC		
Chordata	Teleostei	Zeus faber	fish	DD		dory, jean-doré
Cnidaria	Anthozoa	Exaiptasia diaphana	cnidarians		yes	
Cnidaria	Anthozoa	Isozoanthus sulcatus	cnidarians		yes	
Cnidaria	Hydrozoa	Aglaophenia pluma	cnidarians		yes	podded hydroid
Cnidaria	Hydrozoa	Campanularia hincksii	cnidarians		yes	
Cnidaria	Hydrozoa	Clytia hemisphaerica	cnidarians		yes	
Cnidaria	Hydrozoa	Clytia linearis	cnidarians		yes	
Cnidaria	Hydrozoa	Clytia noliformis	cnidarians		yes	
Cnidaria	Hydrozoa	Coryne eximia	cnidarians		yes	
Cnidaria	Hydrozoa	Coryne muscoides	cnidarians		yes	grande coryne
Cnidaria	Hydrozoa	Eudendrium racemosum	cnidarians		yes	botryoid stickhydroid
Cnidaria	Hydrozoa	Laomedea calceolifera	cnidarians		yes	
Cnidaria	Hydrozoa	Nanomia bijuga	cnidarians		yes	
Cnidaria	Hydrozoa	Obelia geniculata	cnidarians			knotted thread hydroid, obélie
Cnidaria	Hydrozoa	Orthopyxis integra	cnidarians		yes	
Cnidaria	Hydrozoa	Plumularia setacea	cnidarians		yes	little seabristle
Cnidaria	Scyphozoa	Pelagia noctiluca	cnidarians			purplestripped jelly, méduse pélagique
Echinodermata	Asteroidea	Asterina phylactica	echinoderms		yes	
Echinodermata	Asteroidea	Astropecten aranciacus	echinoderms		yes	
Echinodermata	Asteroidea	Astropecten spinulosus	echinoderms		yes	
Echinodermata	Echinoidea	Arbacia lixula	echinoderms			Black Sea urchin, oursin mâle
Echinodermata	Echinoidea	Paracentrotus lividus	echinoderms			stony sea urchin, oursin-pierre
Echinodermata	Echinoidea	Spatangus purpureus	echinoderms			violet heart-urchin, spatangue pourpre
Echinodermata	Echinoidea	Sphaerechinus granularis	echinoderms			violet sea urchin, oursin violet
Echinodermata	Holothuroidea	Holothuria (Holothuria) mammata	echinoderms		yes	
Echinodermata	Ophiuroidea	Ophioderma longicaudum	echinoderms			

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phylum	class	species	group	category	new	vernacular
Haptophyta	Coccolithophyceae	Emiliania huxleyi	algae		yes	
Haptophyta	Coccolithophyceae	Phaeocystis globosa	algae		yes	
Mollusca	Gastropoda	Bittium latreillii	molluscs			
Mollusca	Gastropoda	Bittium reticulatum	molluscs			needle whelk
Mollusca	Gastropoda	Calliostoma virescens	molluscs		yes	
Mollusca	Gastropoda	Doto koenneckeri	molluscs		yes	
Mollusca	Gastropoda	Facelina rubrovittata	molluscs		yes	
Mollusca	Gastropoda	Favorinus branchialis	molluscs		yes	
Mollusca	Gastropoda	Haminoea hydatis	molluscs		yes	
Mollusca	Gastropoda	Heliconoides inflatus	molluscs		yes	
Mollusca	Gastropoda	Jujubinus gravinae	molluscs		yes	
Mollusca	Gastropoda	Limacia clavigera	molluscs		yes	orange-clubbed nudibranch
Mollusca	Gastropoda	Patella caerulea	molluscs			rayed Mediterranean limpet
Mollusca	Gastropoda	Polycera quadrilineata	molluscs		yes	four-striped polycera, limace à quatre lignes
Mollusca	Gastropoda	Thuridilla hopei	molluscs			splendid elysia
Mollusca	Gastropoda	Tricolia pullus	molluscs			pheasant shell
Mollusca	Polyplacophora	Acanthochitona crinita	molluscs		yes	bristly mail chiton, petit chiton épineux
Mollusca	Polyplacophora	Lepidopleurus cajetanus	molluscs			ribbed chiton, chiton écailleux
Mollusca	Polyplacophora	Leptochiton cancellatus	molluscs			Arctic cancellate chiton
Myzozoa	Dinophyceae	Amphidinium carterae	unicellular		yes	
Myzozoa	Dinophyceae	Heterocapsa rotundata	unicellular		yes	
Myzozoa	Dinophyceae	Kryptoperidinium triquetrum	unicellular		yes	
Myzozoa	Dinophyceae	Protoceratium reticulatum	unicellular		yes	
Ochrophyta	Dictyochophyceae	Octactis octonaria	algae		yes	
Ochrophyta	Dictyochophyceae	Octactis speculum	algae		yes	
Ochrophyta	Dictyochophyceae	Pseudochattonella farcimen	algae		yes	
Ochrophyta	Dictyochophyceae	Vicicitus globosus	algae		yes	
Ochrophyta	Phaeophyceae	Cystoseira compressa	algae		yes	
Ochrophyta	Phaeophyceae	Dictyota dichotoma	algae			
Ochrophyta	Phaeophyceae	Dictyota fasciola	algae			
Ochrophyta	Phaeophyceae	Dictyota implexa	algae			
Ochrophyta	Phaeophyceae	Dictyota mediterranea	algae			
Ochrophyta	Phaeophyceae	Dictyota spiralis	algae		yes	
Ochrophyta	Phaeophyceae	Discosporangium mesarthrocarpum	algae			
Ochrophyta	Phaeophyceae	Ericaria amentacea	algae			
Ochrophyta	Phaeophyceae	Ericaria zosteroides	algae			
Ochrophyta	Phaeophyceae	Sphacelaria cirrosa	algae		yes	
Ochrophyta	Phaeophyceae	Stilophora tenella	algae		yes	
Oomycota	Peronosporea	Eurychasma dicksonii	fungi		yes	
Platyhelminthes		Planocera ceratommata			yes	
Porifera	Demospongiae	Acarnus levii	sponges		yes	
Porifera	Demospongiae	Haliclona (Reniera) aquaeductus	sponges		yes	
Porifera	Demospongiae	Hemimycale columella	sponges			crater sponge, éponge à cratères
Porifera	Demospongiae	Hymeniacidon perlevis	sponges		yes	crumb-of-bread sponge
Porifera	Demospongiae	Phorbas topsenti	sponges		yes	
Porifera	Demospongiae	Spirastrella cunctatrix	sponges		yes	encrusting orange sponge, éponge encroûtante orange
Porifera	Demospongiae	Terpios gelatinosus	sponges			
Rhodophyta	Florideophyceae	Aglaothamnion tenuissimum	algae		yes	
Rhodophyta	Florideophyceae	Callithamnion corymbosum	algae		yes	
Rhodophyta	Florideophyceae	Chondria capillaris	algae		yes	

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phylum	class	species	group	category	new	vernacular
Rhodophyta	Florideophyceae	Dasya baillouviana	algae		yes	
Rhodophyta	Florideophyceae	Hypoglossum hypoglossoides	algae		yes	
Rhodophyta	Florideophyceae	Jania adhaerens	algae		yes	
Rhodophyta	Florideophyceae	Laurencia obtusa	algae		yes	
Rhodophyta	Florideophyceae	Leptosiphonia fibrillosa	algae		yes	
Rhodophyta	Florideophyceae	Osmundea oederi	algae		yes	
Rhodophyta	Florideophyceae	Peyssonnelia squamaria	algae			
Rhodophyta	Florideophyceae	Polysiphonia sertularioides	algae		yes	