

SUPPORTING INFORMATION

for

**A national scale “BioBlitz” using citizen science and eDNA metabarcoding for
monitoring coastal marine fish**

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15, DK-2100 Copenhagen Ø, Denmark*
- 3) *Norwegian College of Fishery Science, UiT - The Arctic University of Norway,
Tromsø, Norway*
- 4) *The Danish Society for Nature Conservation, Masnedøgade 20, DK-2100
Copenhagen Ø, Denmark*
- 5) *NIVA Denmark Water Research, Njalsgade 76, DK-2300 Copenhagen, Denmark*
- 6) *World Maritime University, Fiskehamnsgatan 1, SE-211 18 Malmö, Sweden*

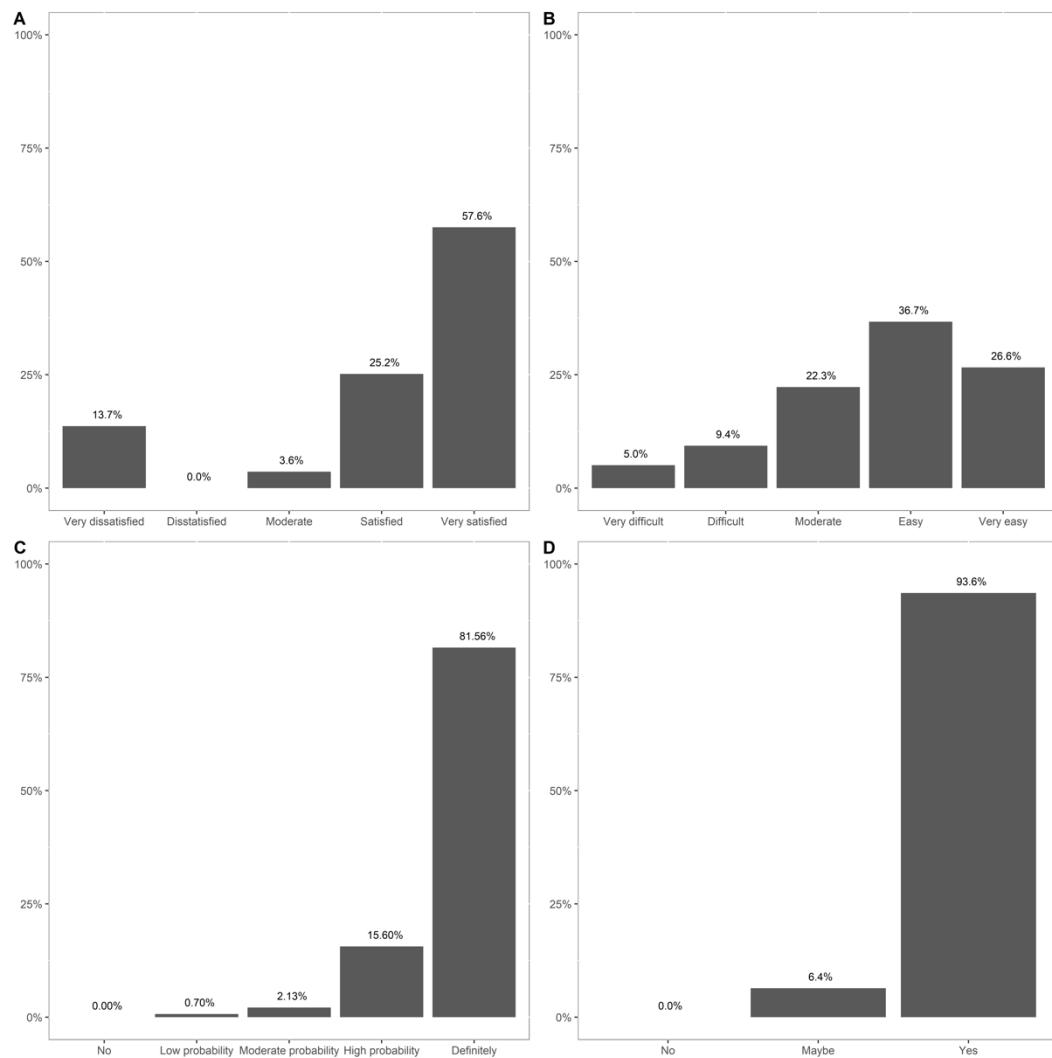


Figure S1. Distribution of answers given by the volunteers to the following questions: A) “How satisfied have you been with your education/preparations for your sampling?”, B) “How would you evaluate environmental DNA sampling as a volunteer?”, C) “Would you participate in a similar citizen science project with the Danish Society for Nature Conservation another time?”, D) “Would you recommend other people to participate in a similar citizen science project with the Danish Society for Nature Conservation?”.

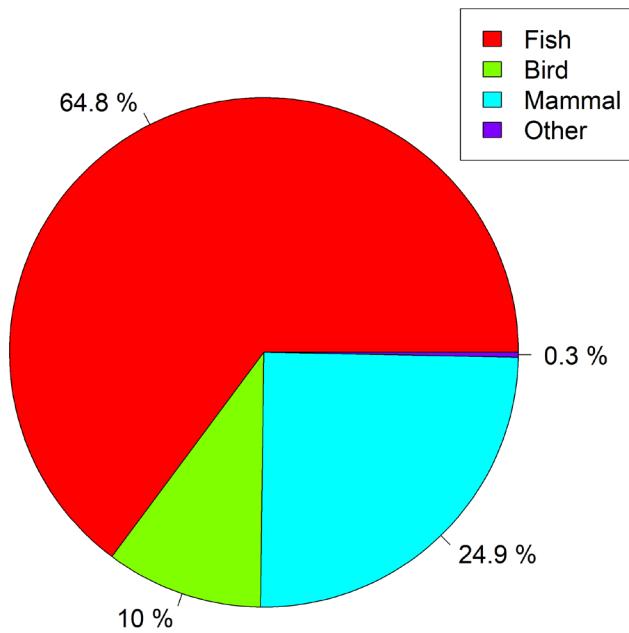


Figure S2. The proportional distribution of total reads between the taxonomic classes found by eDNA, after rarefying to the median read count of alle eDNA samples. Fish are represented by 64.8 % of the reads, mammals by 24.9 % and birds by 10 % of the reads. The last 0.3 % of the reads are shared among bacteria, amphibians, and sea stars.

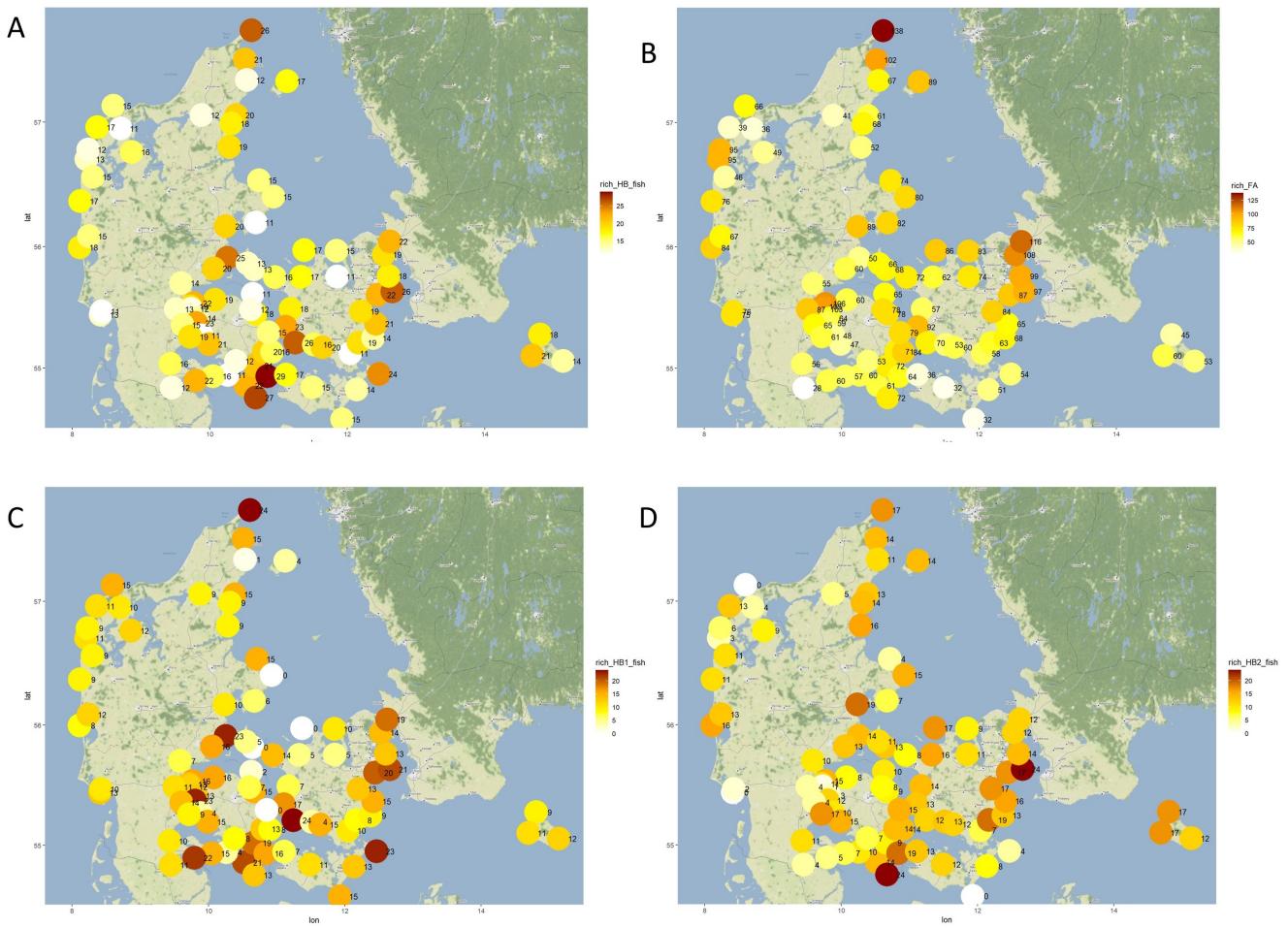


Figure S3. Species richness on each location: A) total richness based on eDNA, B) total richness registered in the Fish Atlas, C) richness based on eDNA from autumn sampling, D) richness based on eDNA from spring sampling. Sixteen locations with a total eDNA-based richness of <11 were removed from all datasets.

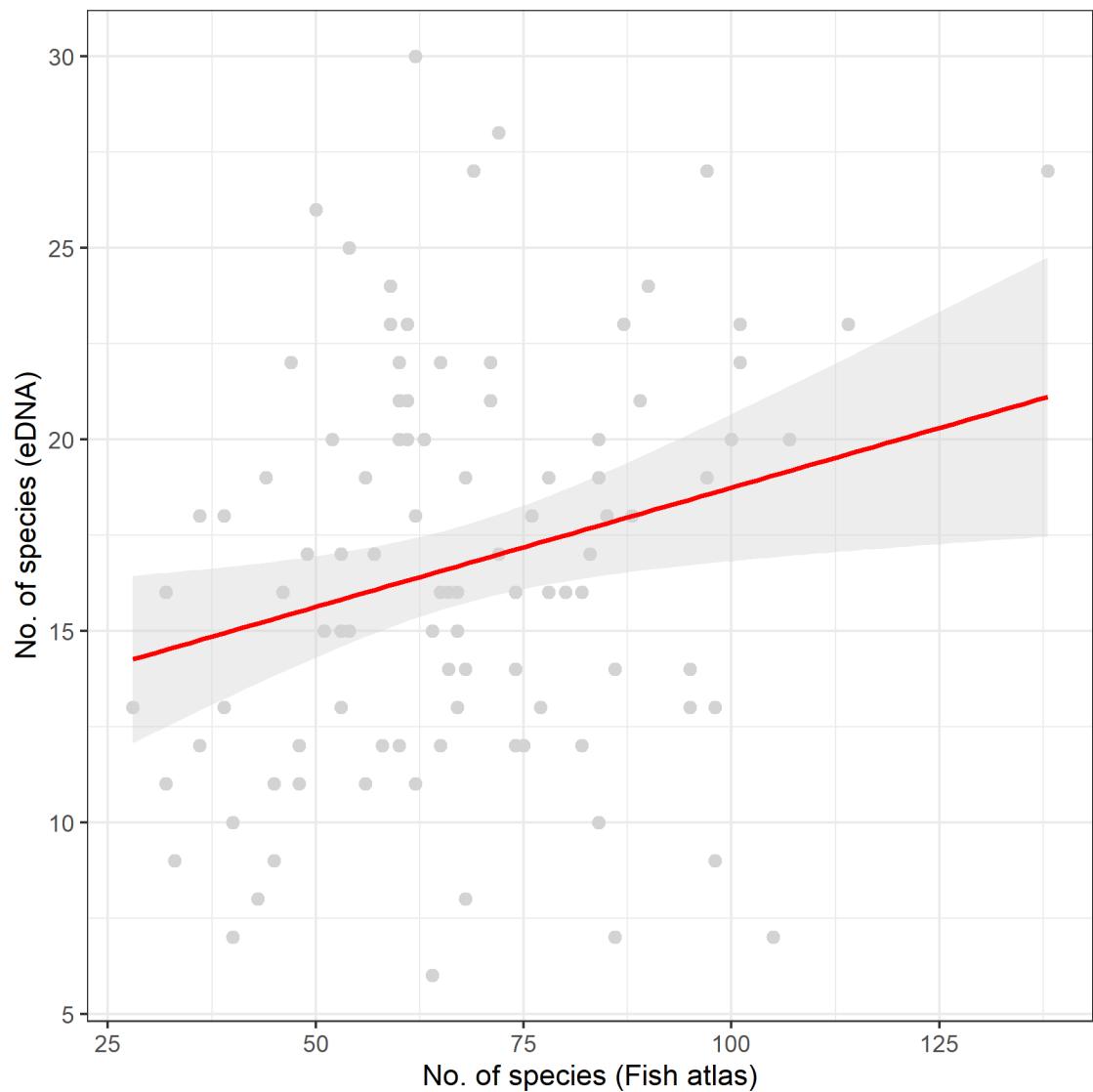
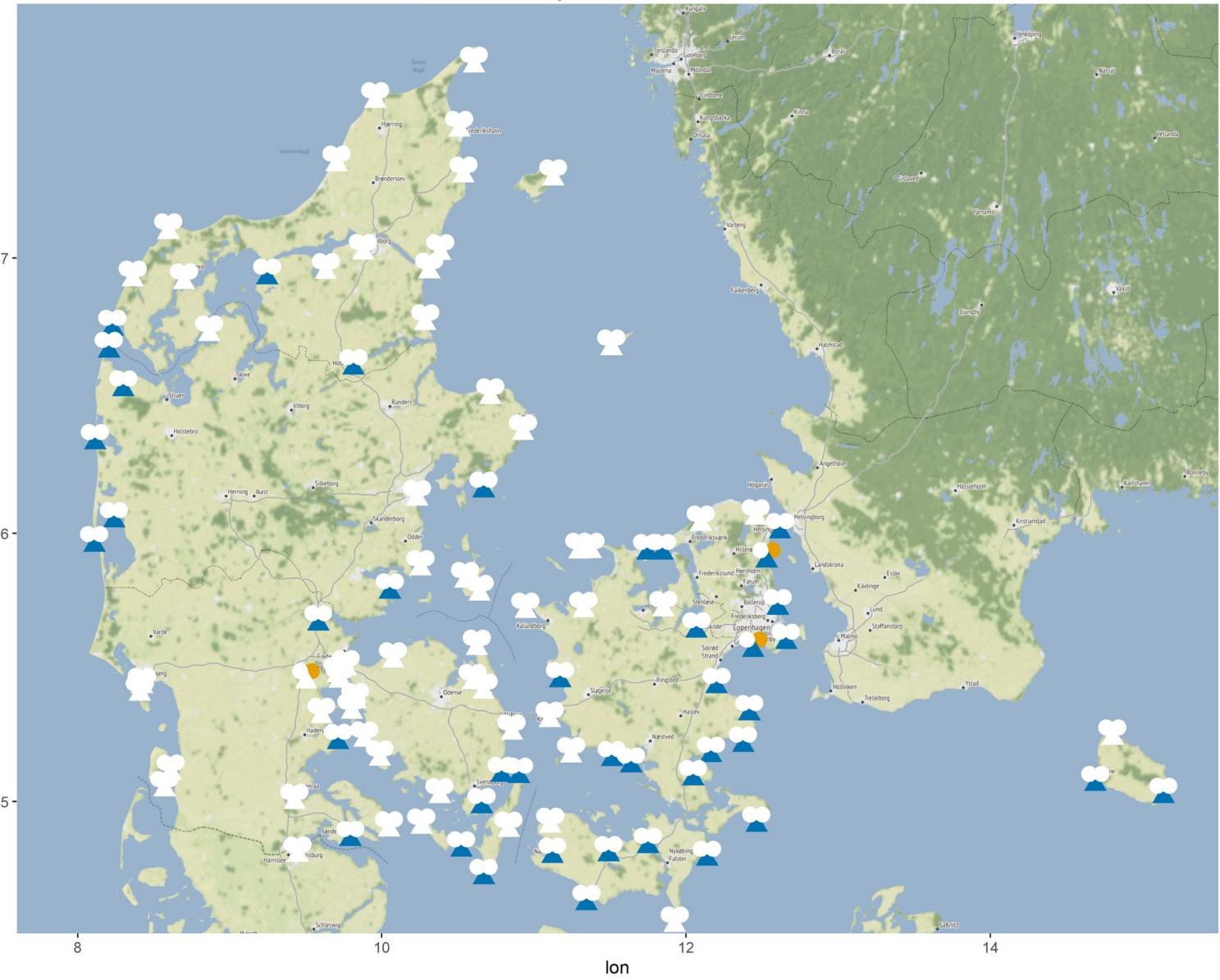
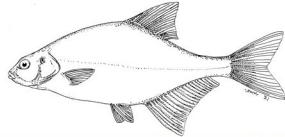


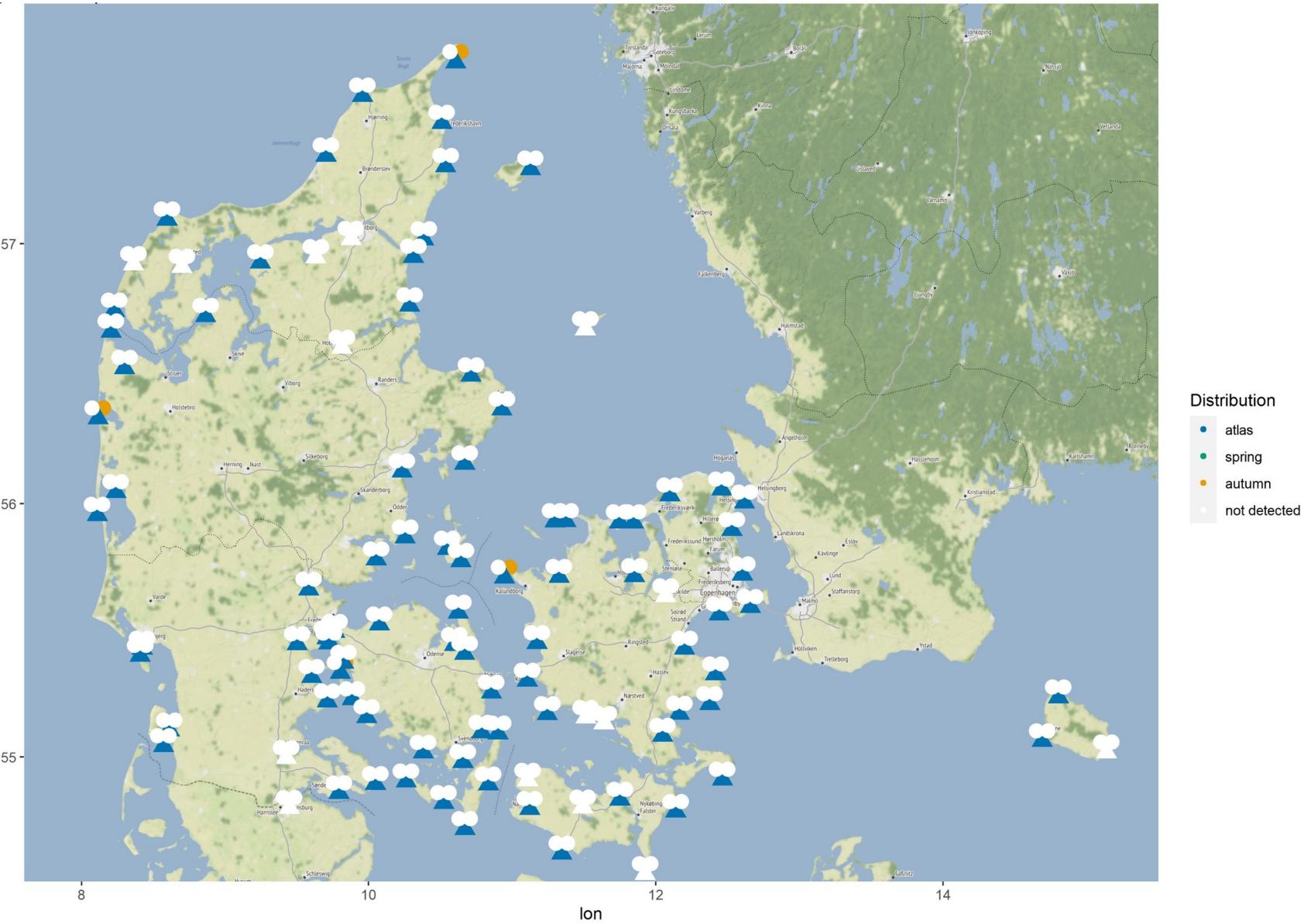
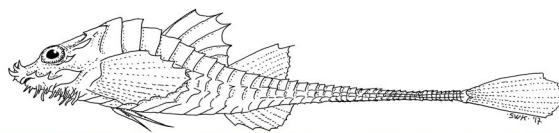
Figure S4. A Pearson test of the correlation between species richness based on eDNA and based on Fish Atlas data, respectively. There is a small but significant and positive correlation ($R^2 = 0.06$, $p = 0.014$) between the two datasets ($n=100$).

Figure S5. (Below) Species distribution maps for 47 selected species. Detections with eDNA during spring (bluish green) or autumn (vermilion) are represented by the left and right circle, respectively. Registrations from the Fish Atlas (blue) are represented by triangles. White triangles or circles indicate that the species was not detected on the location for the dataset in question. All fish drawings were made by Steen Wilhelm Knudsen.

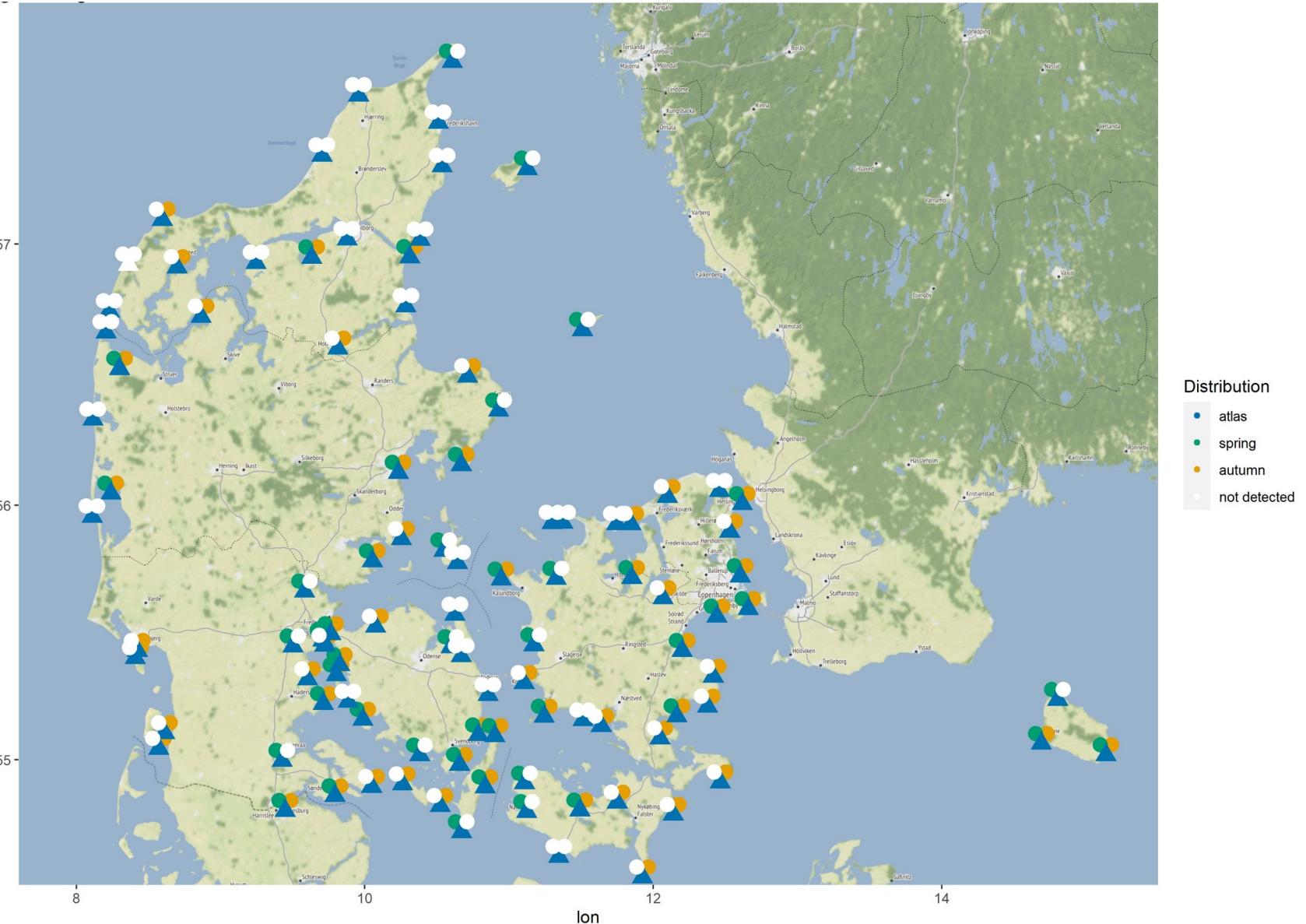
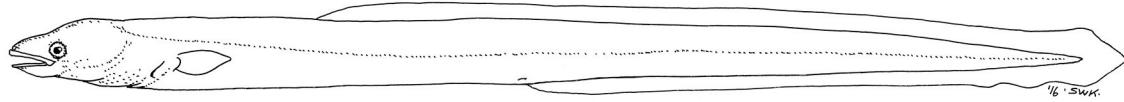
Abramis brama



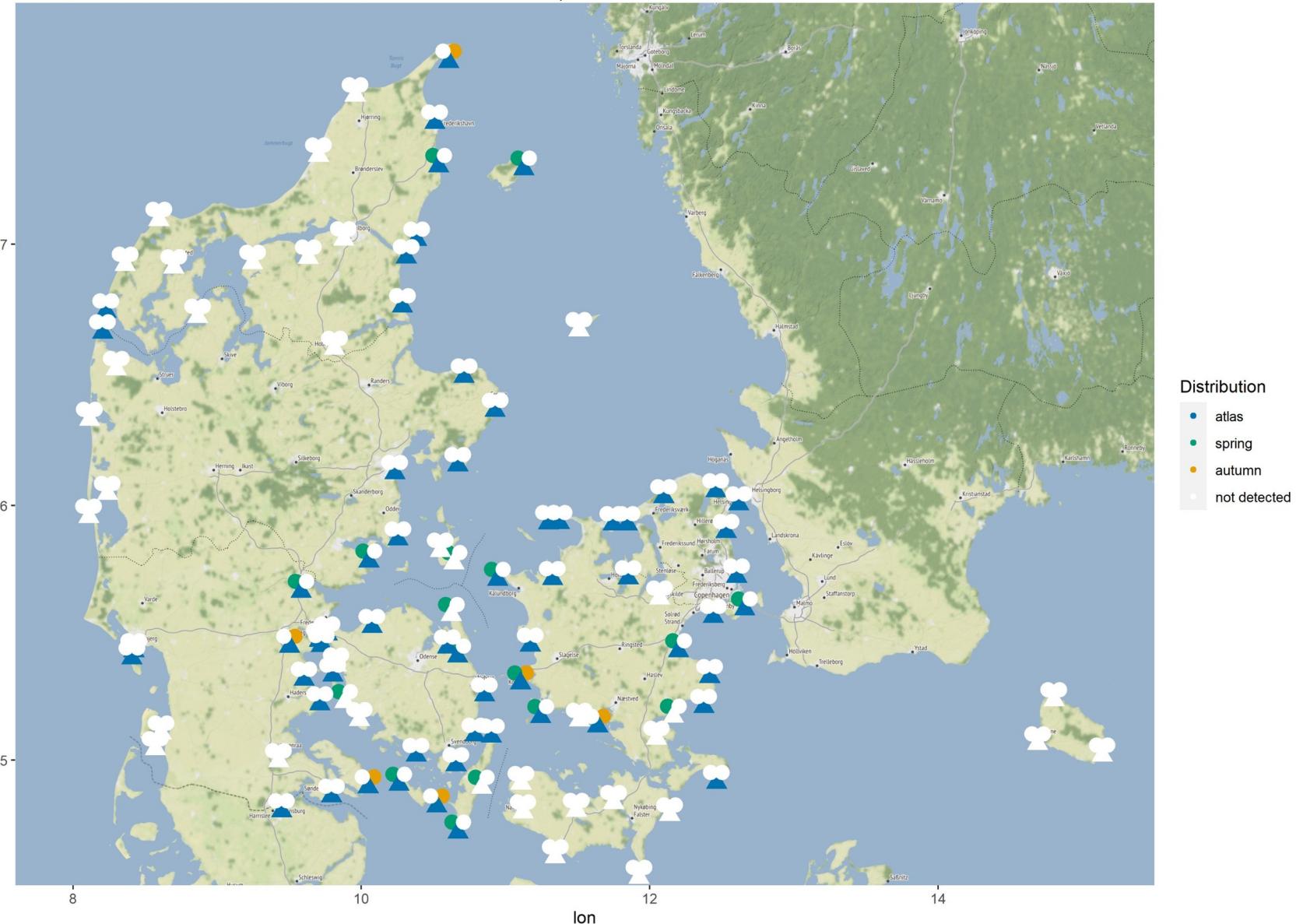
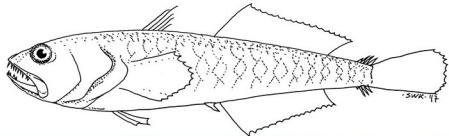
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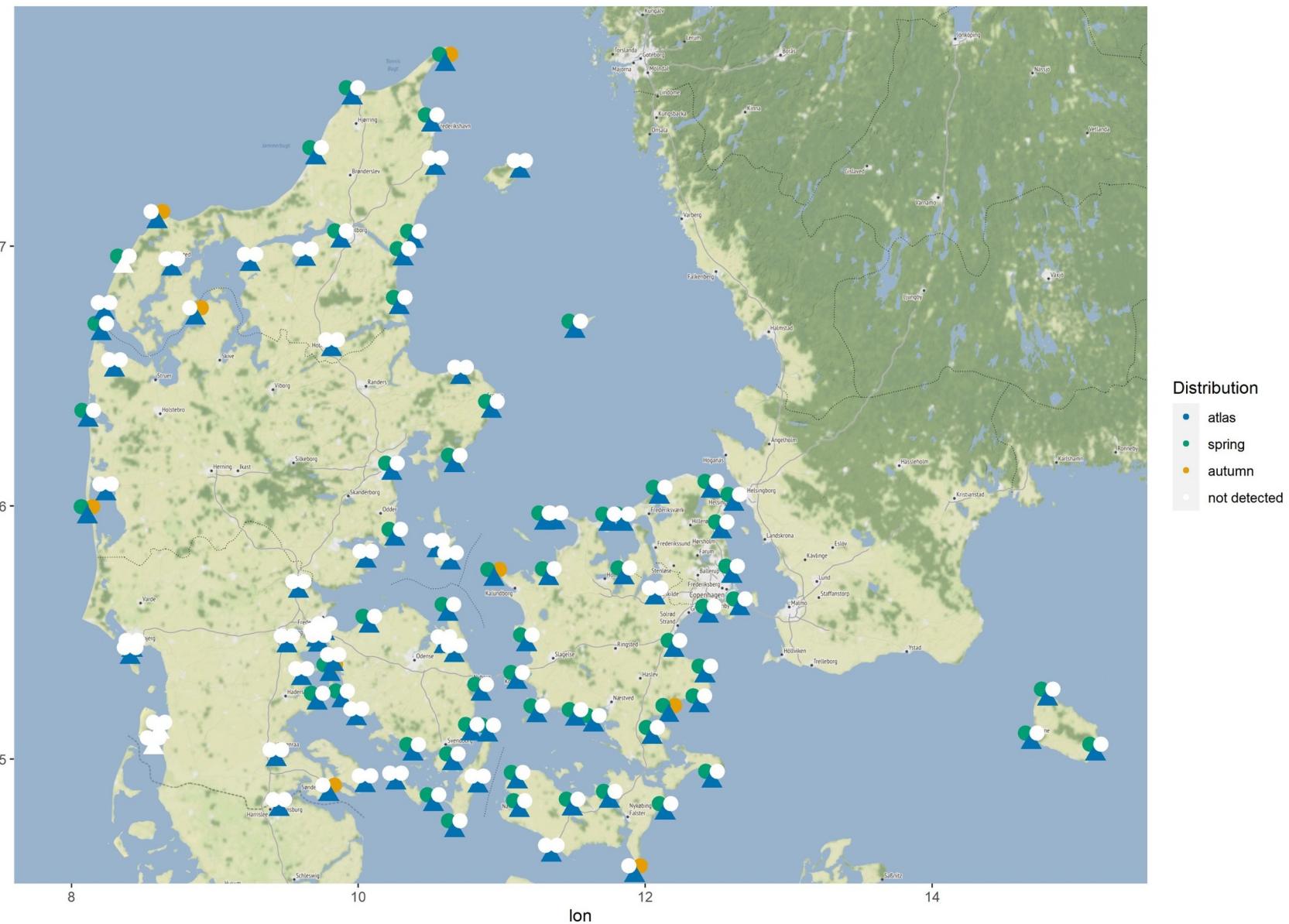
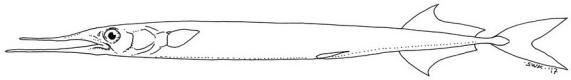
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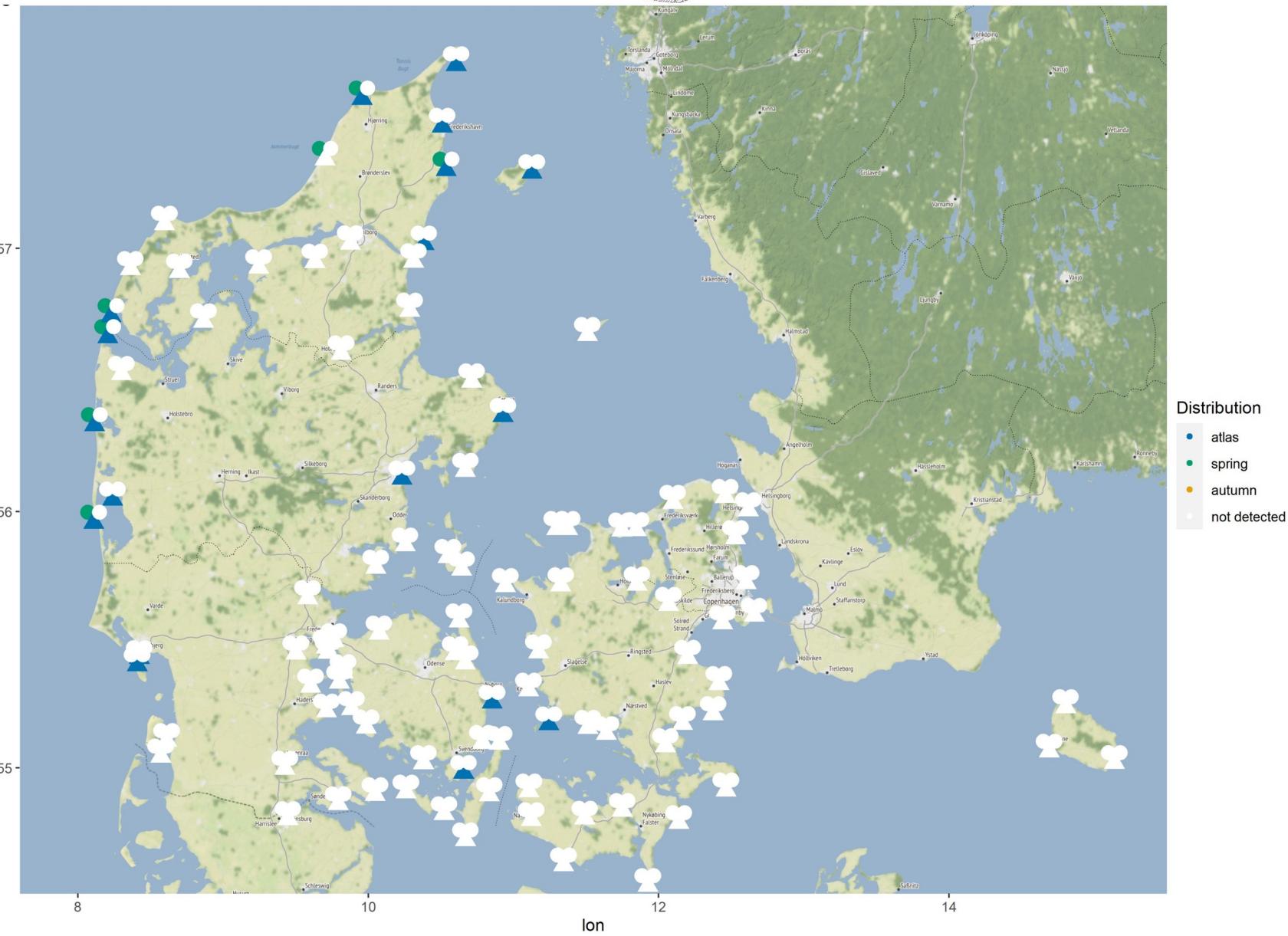
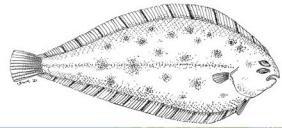
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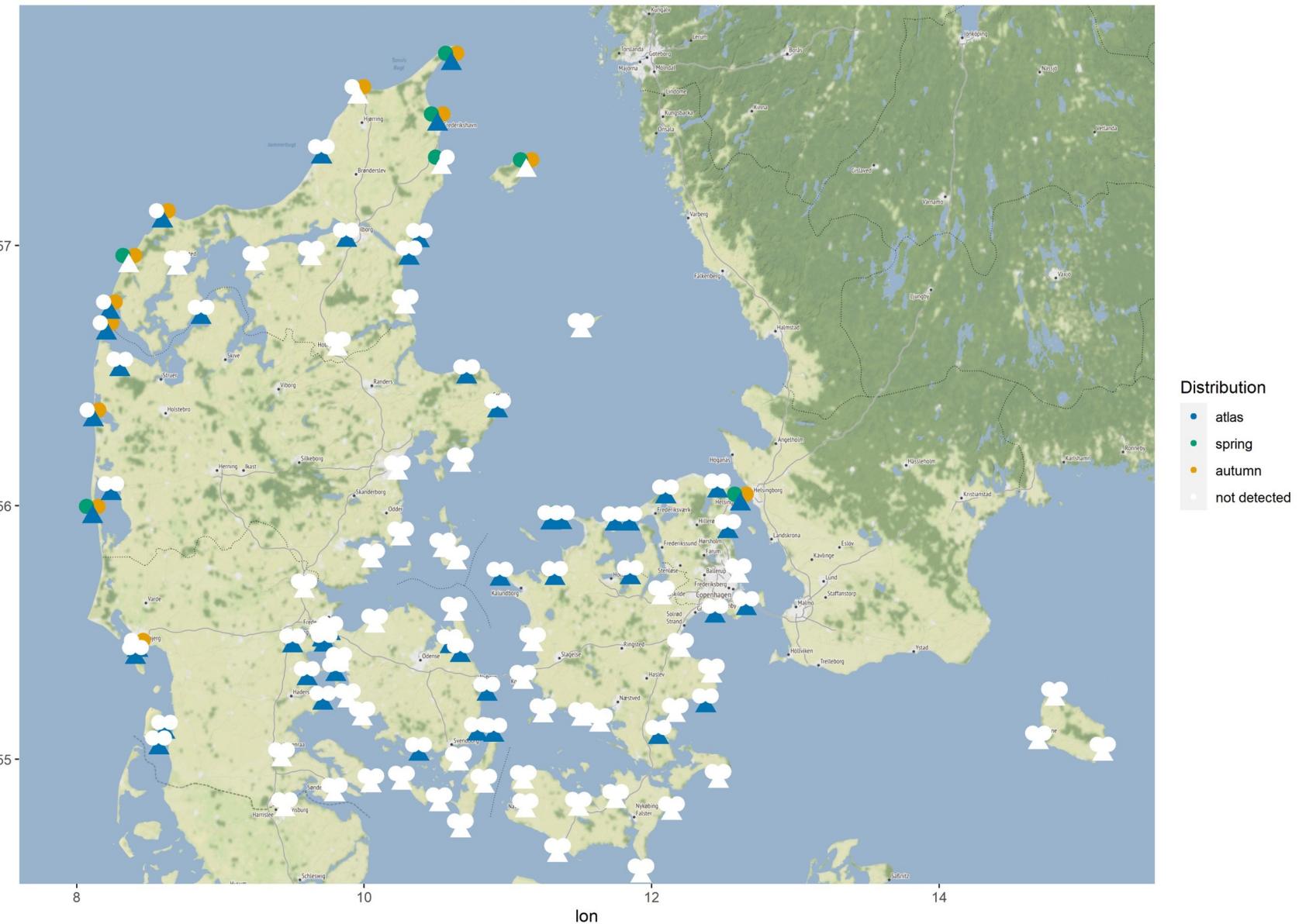
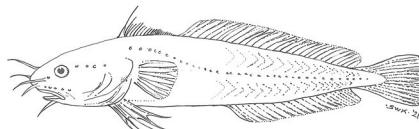
Belone belone



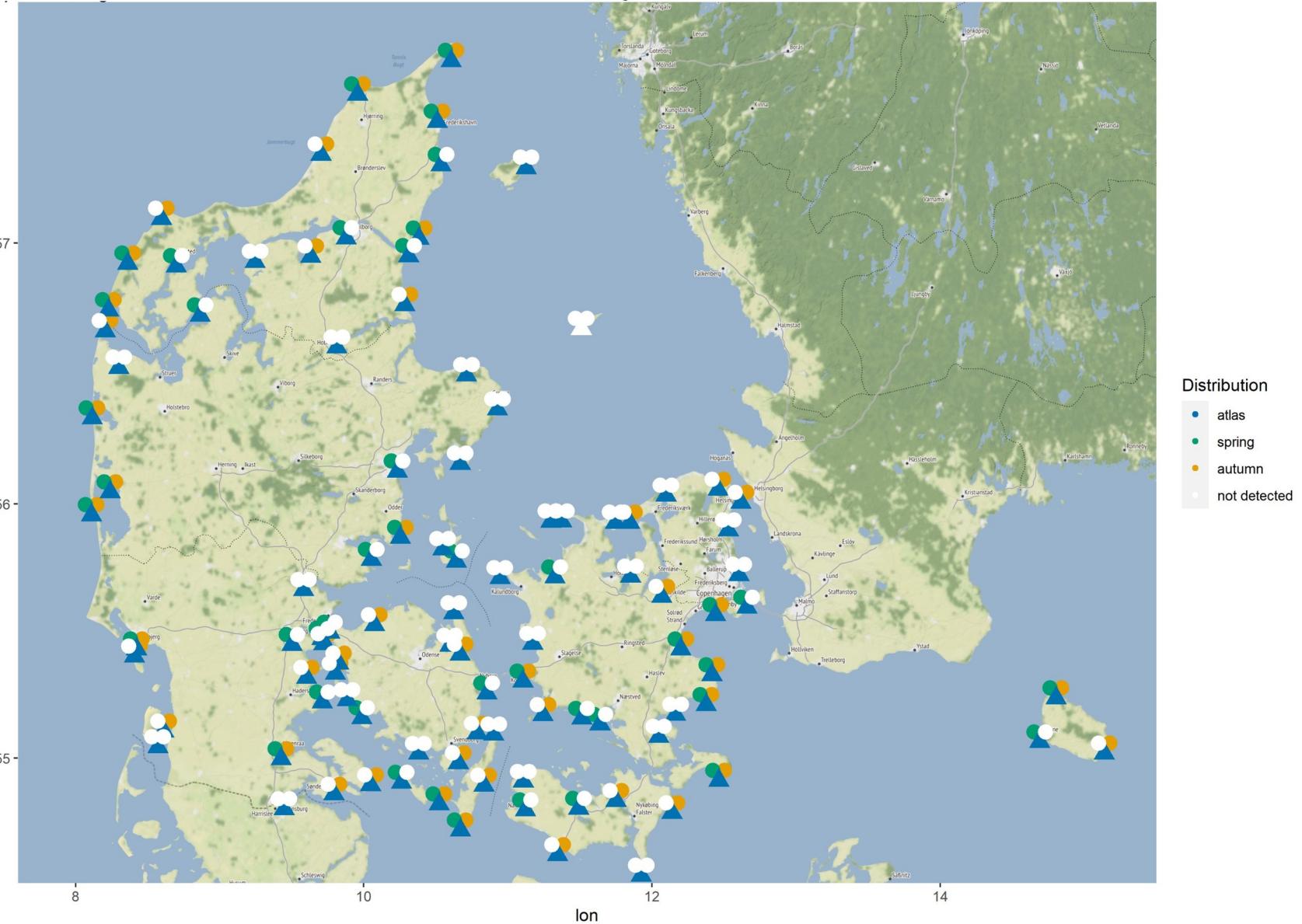
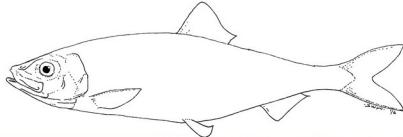
Buglossidium luteum



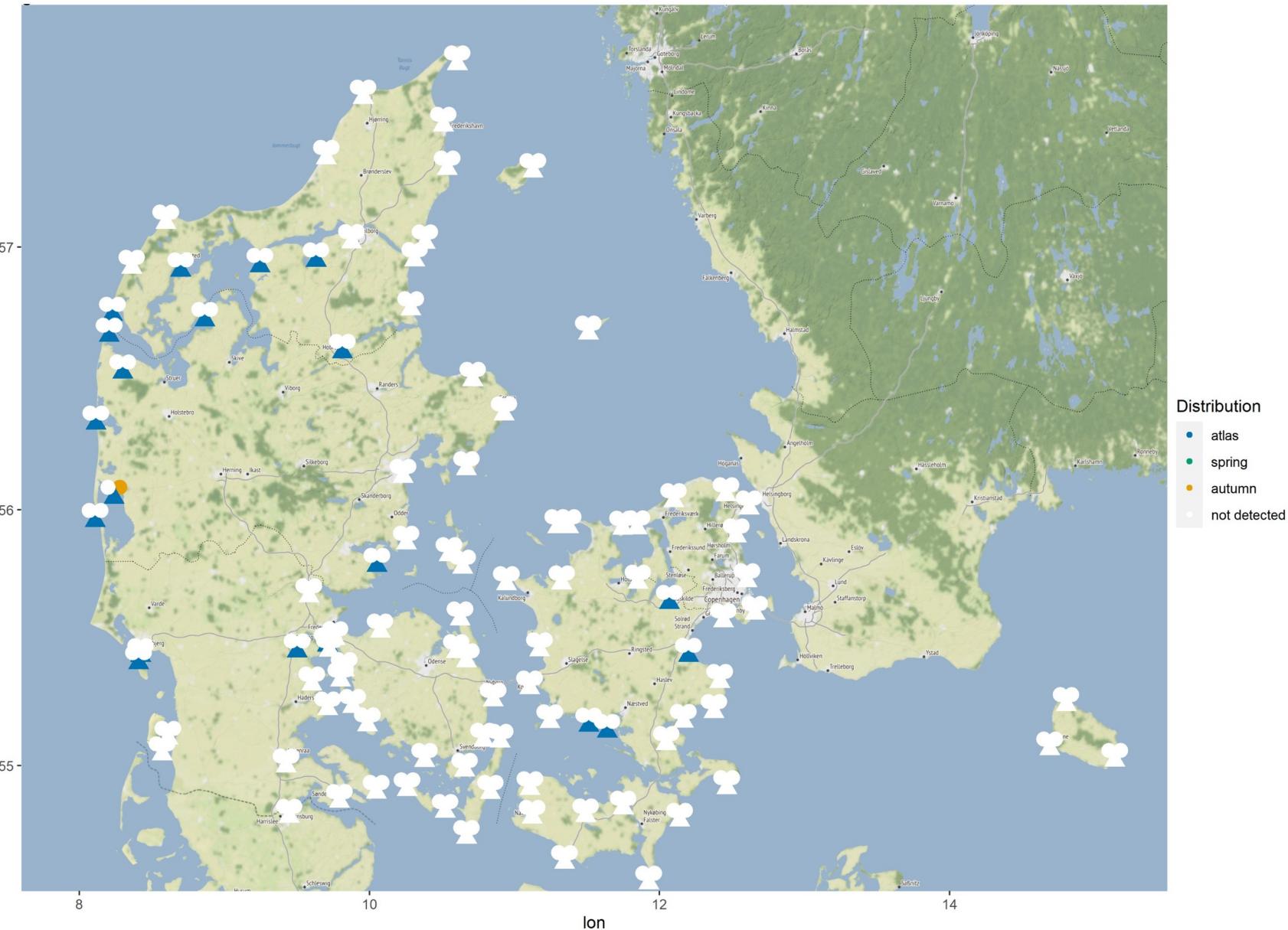
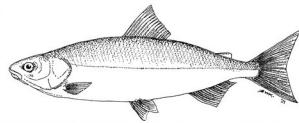
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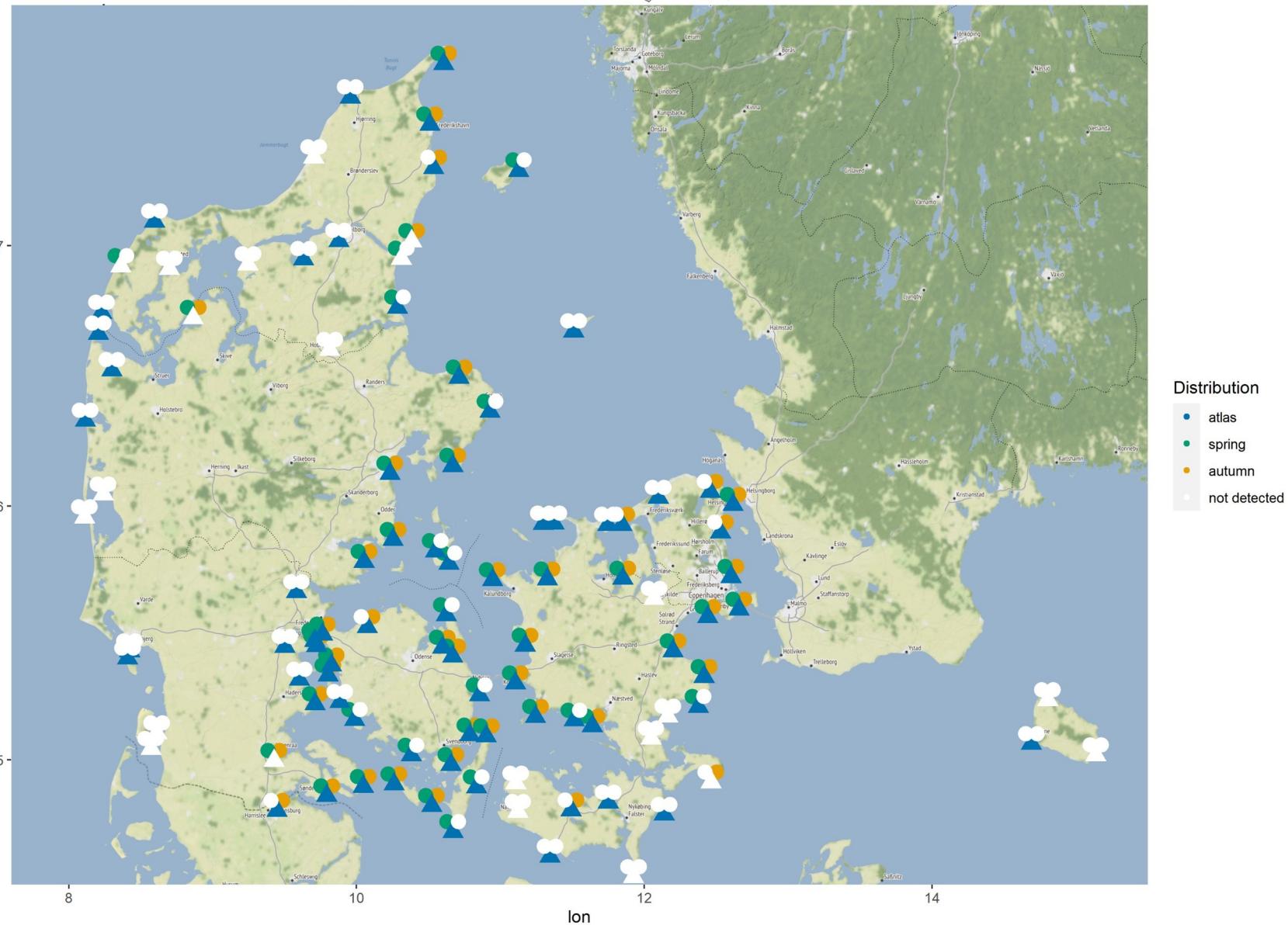
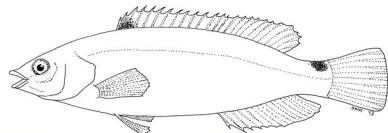
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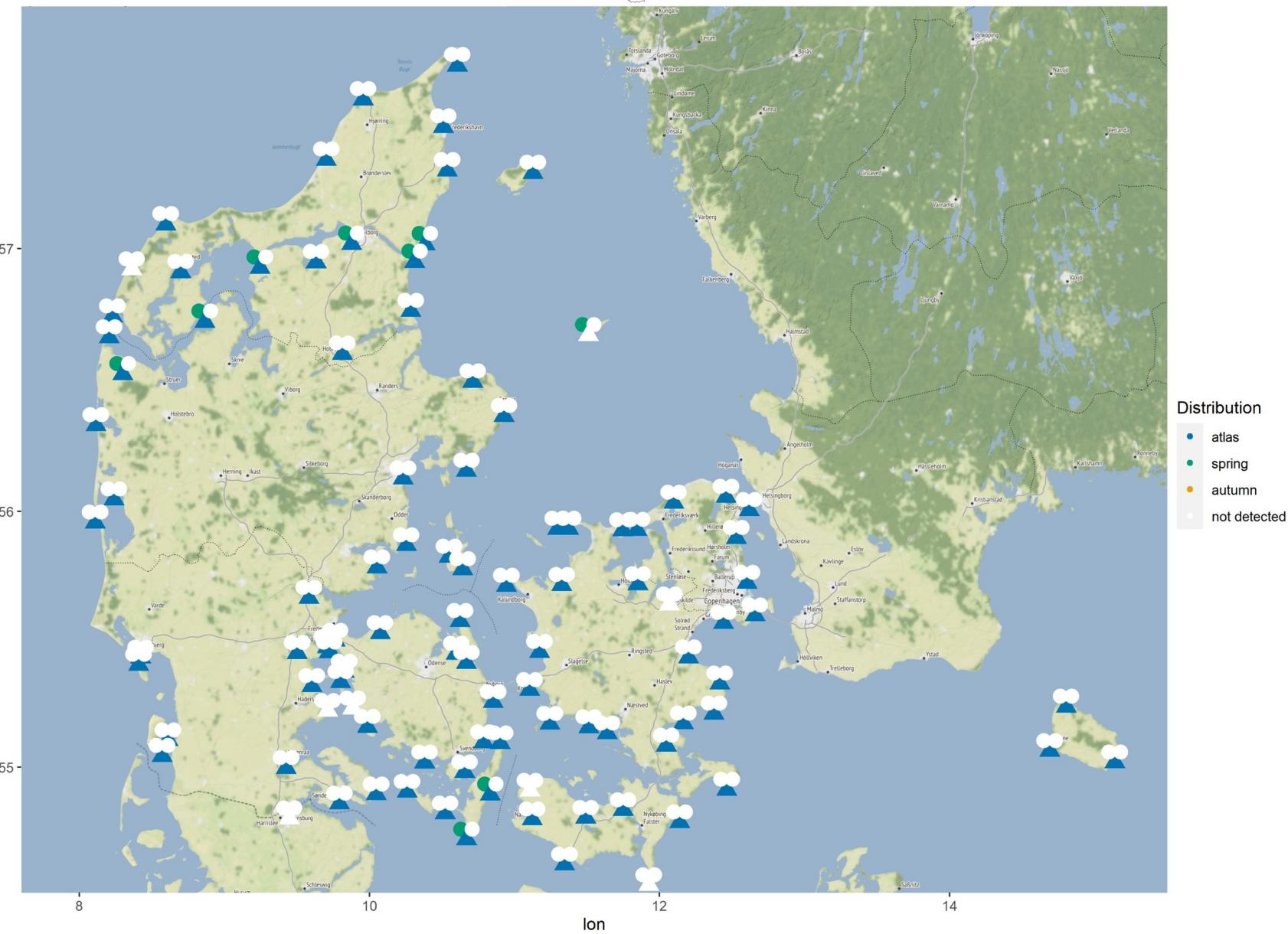
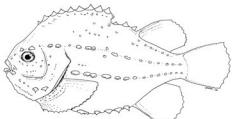
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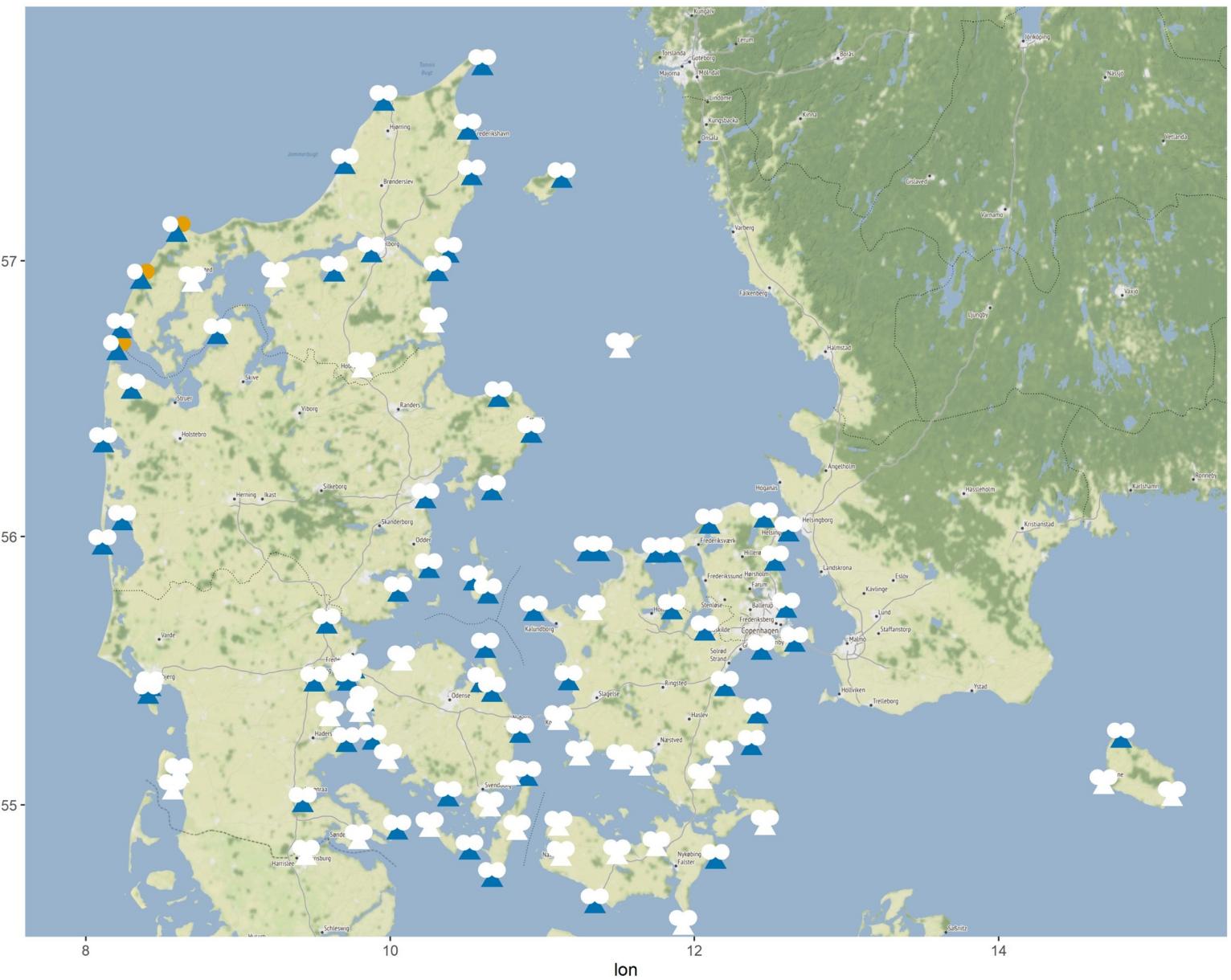
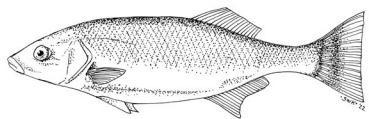
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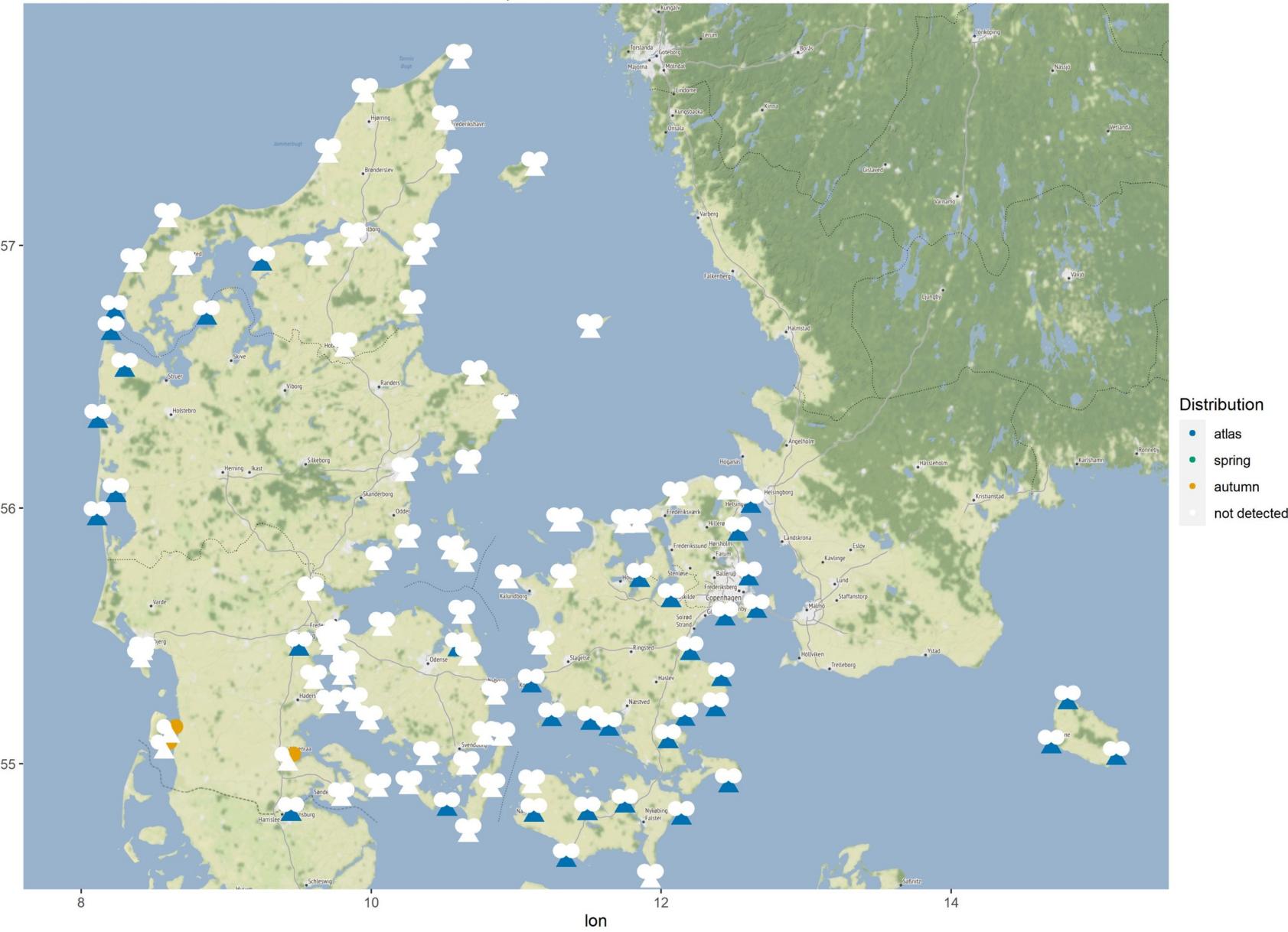
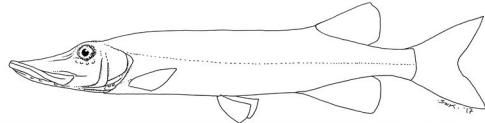
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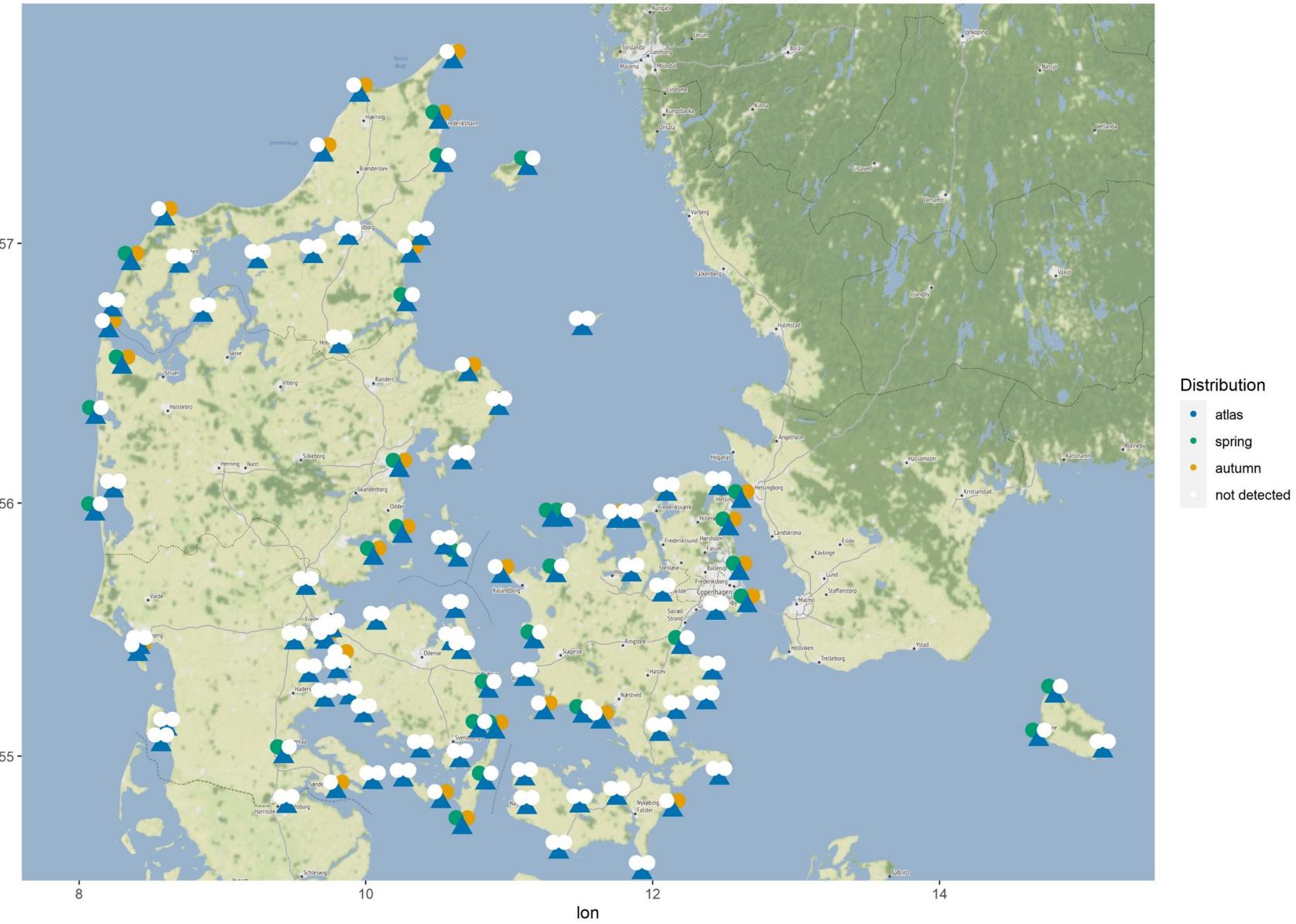
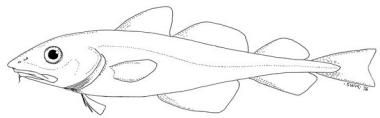
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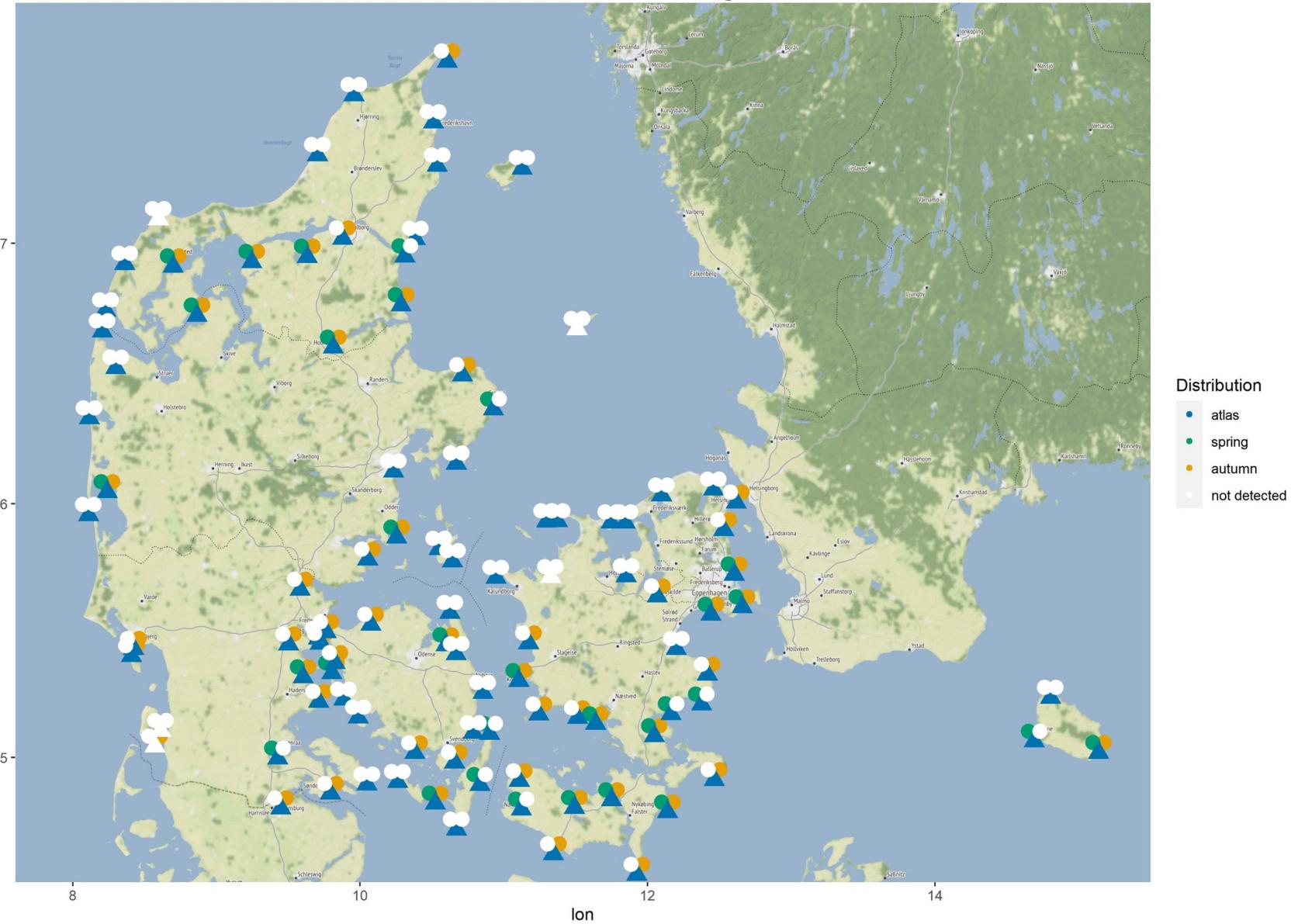
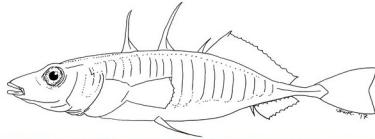
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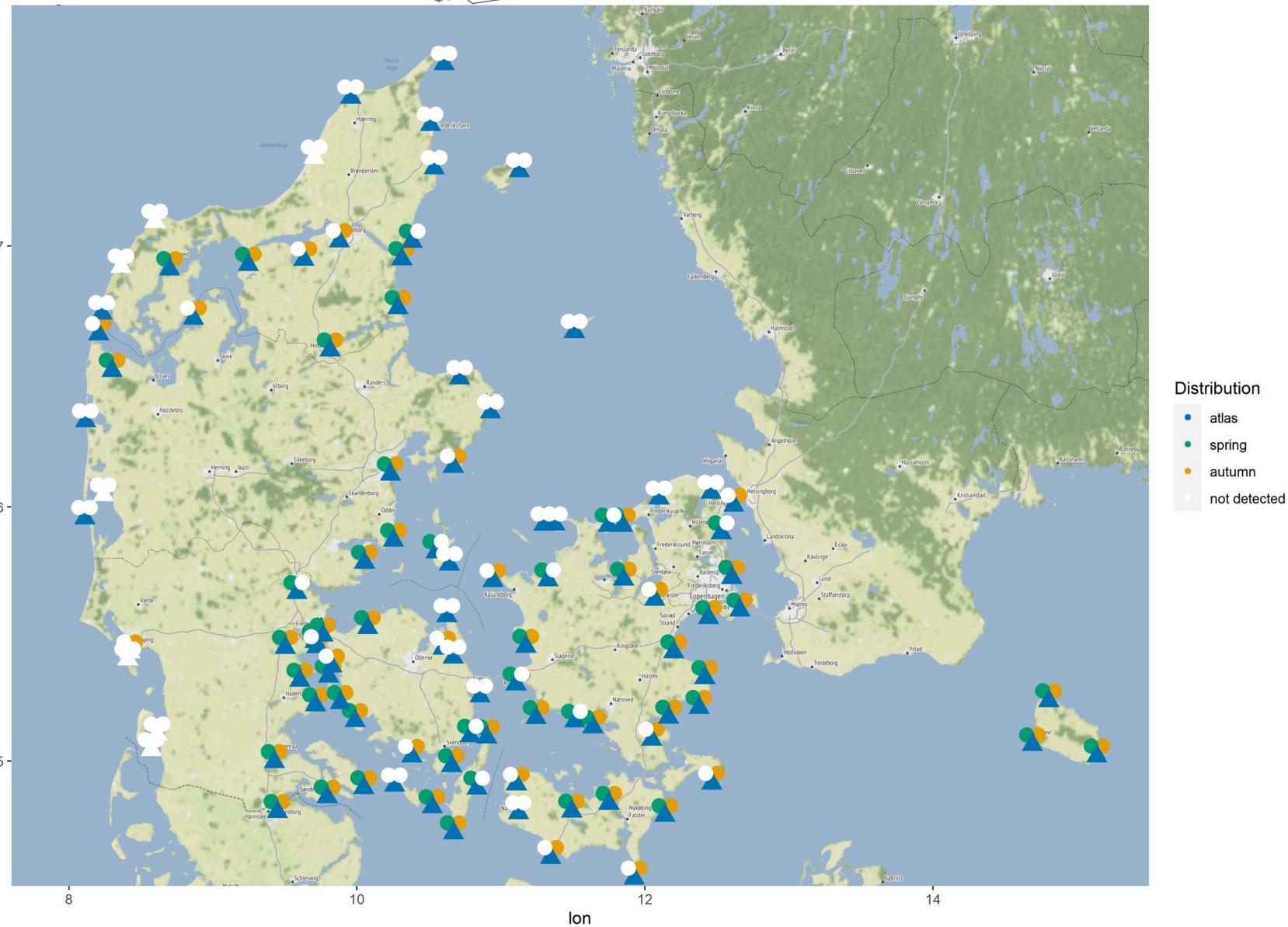
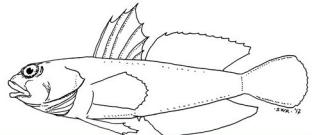
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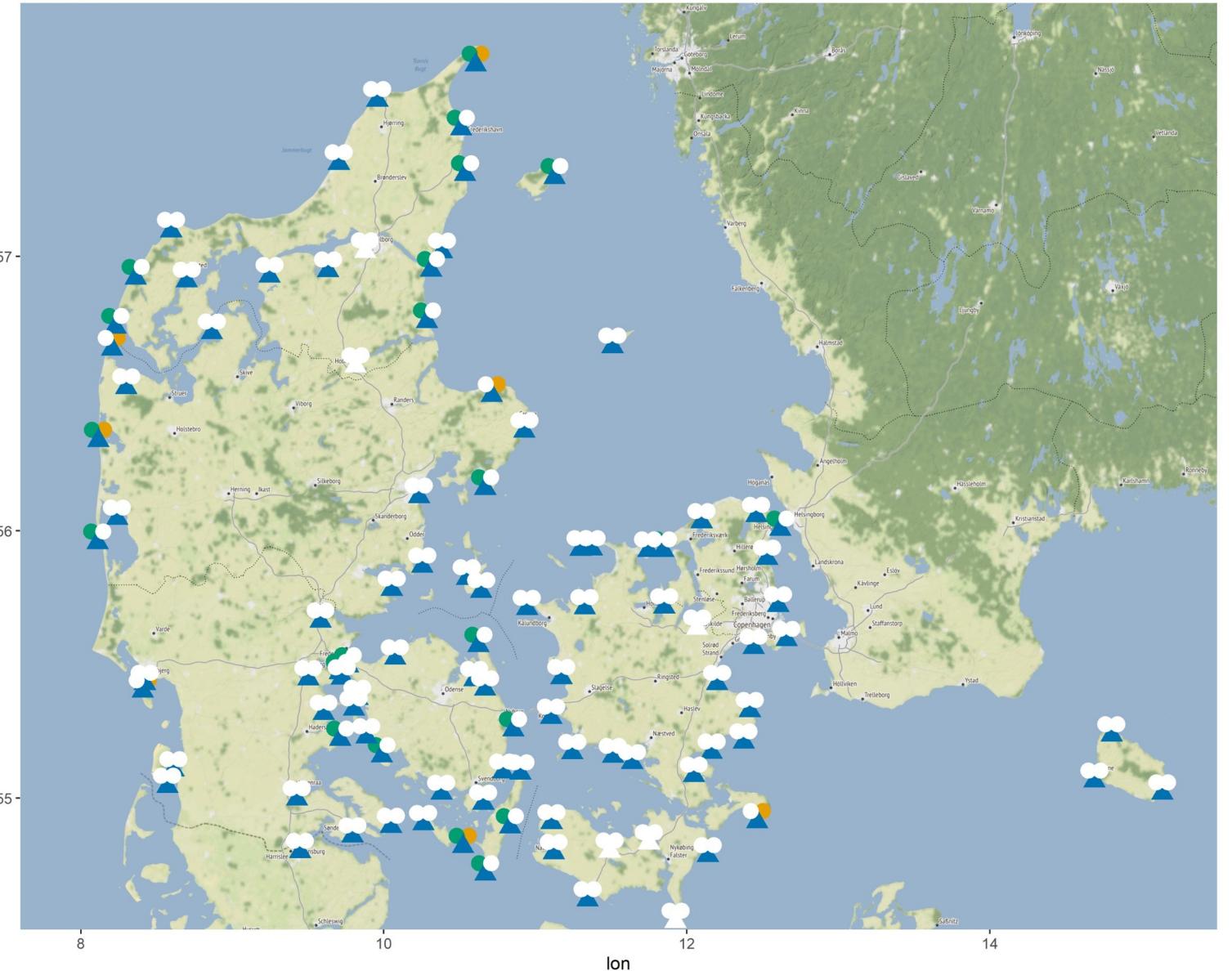
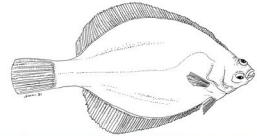
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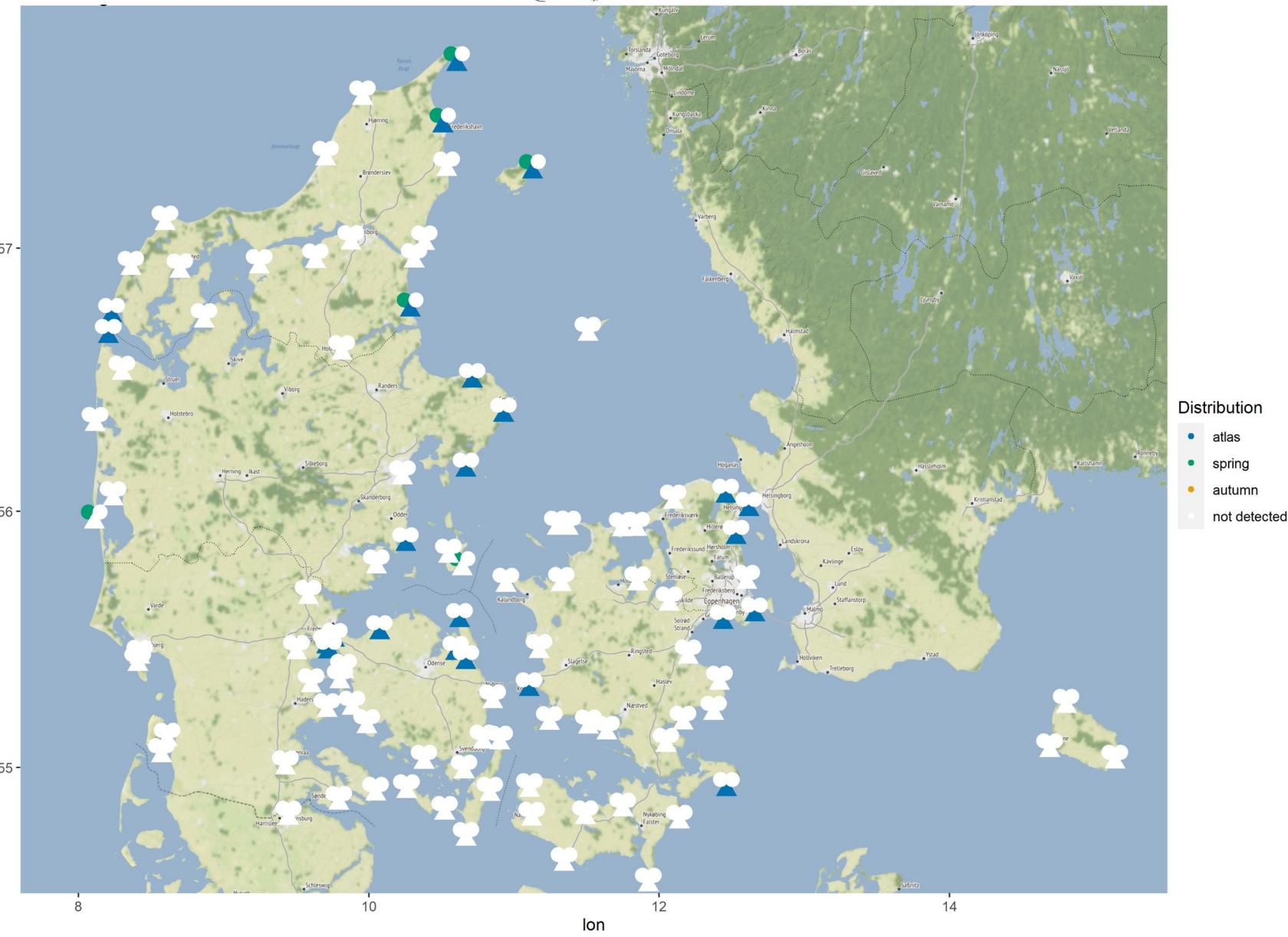
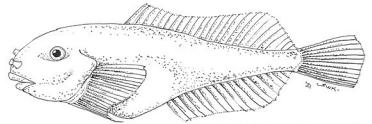
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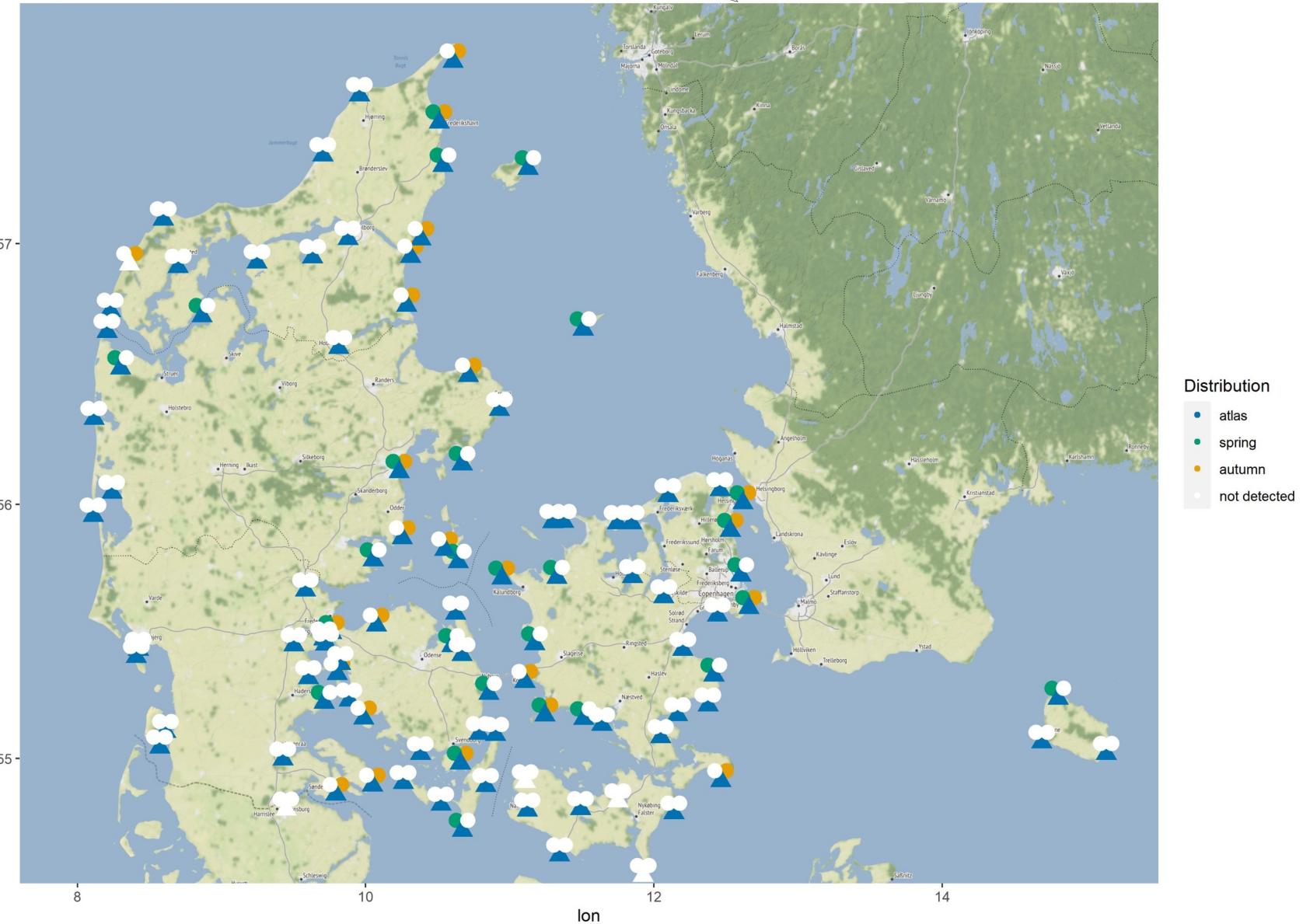
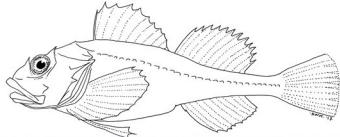
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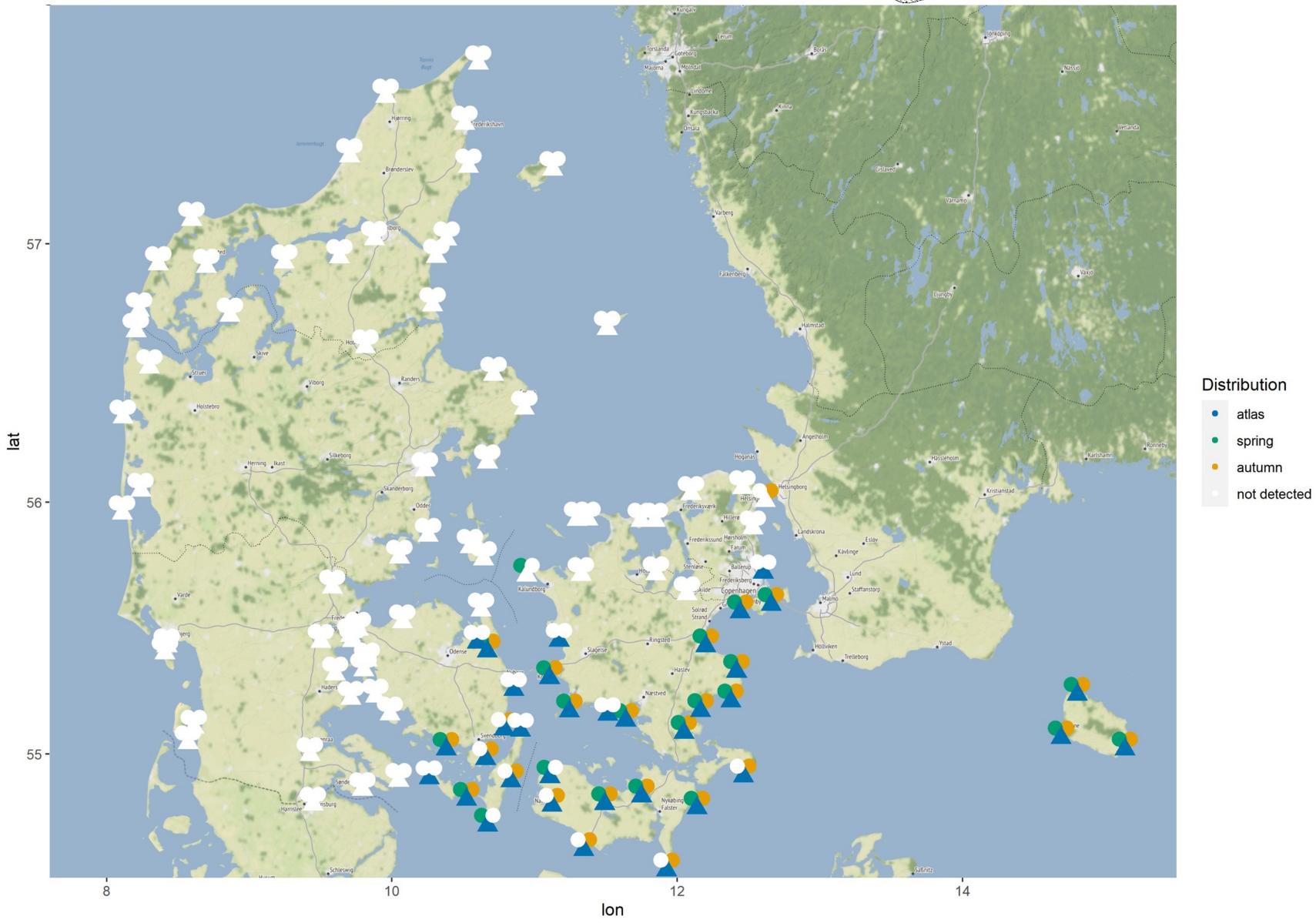
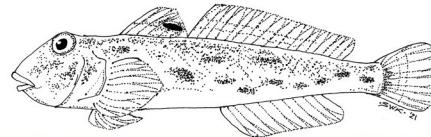
Liparis montagui



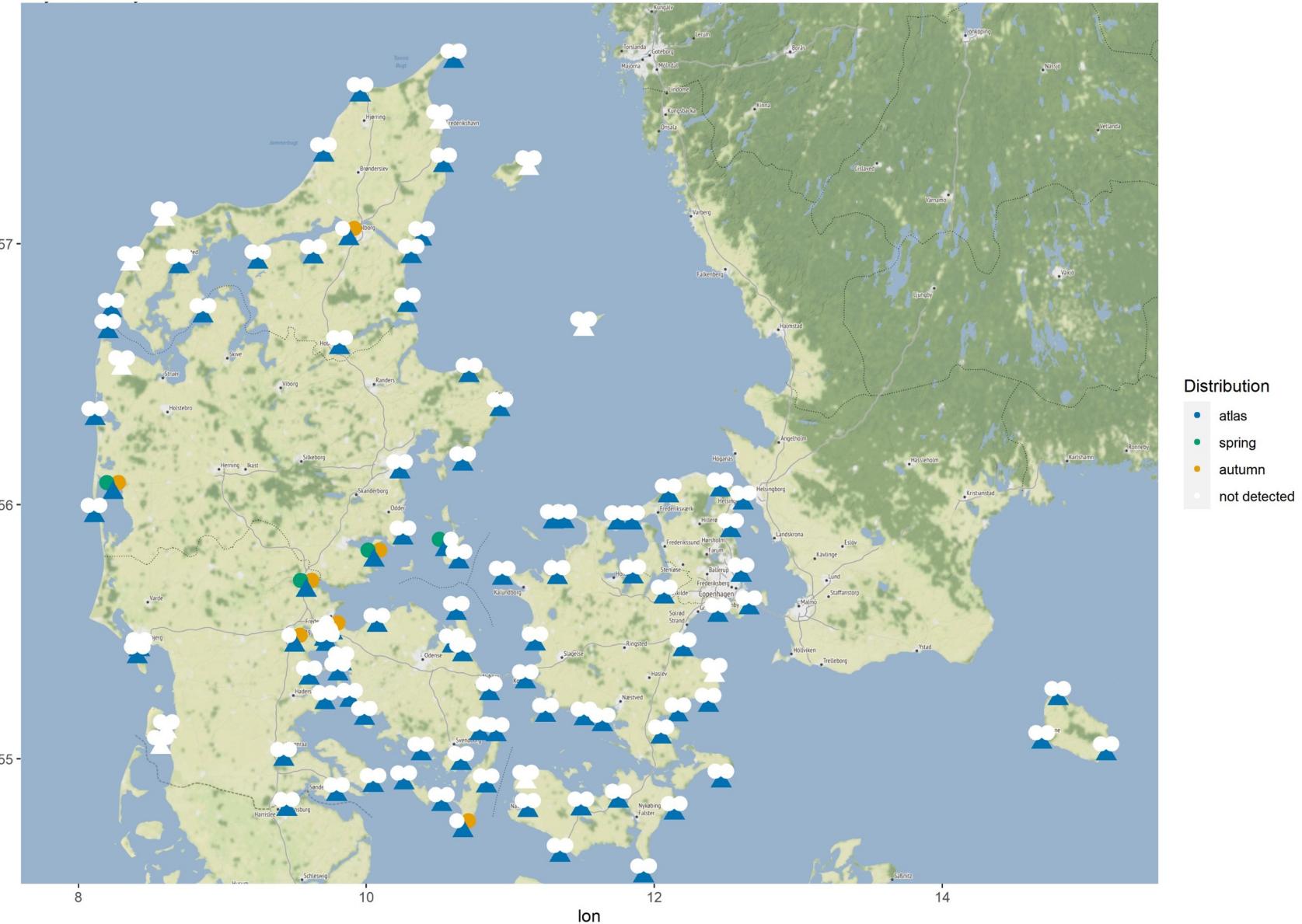
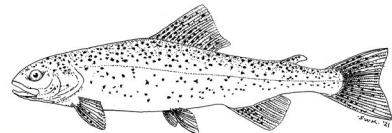
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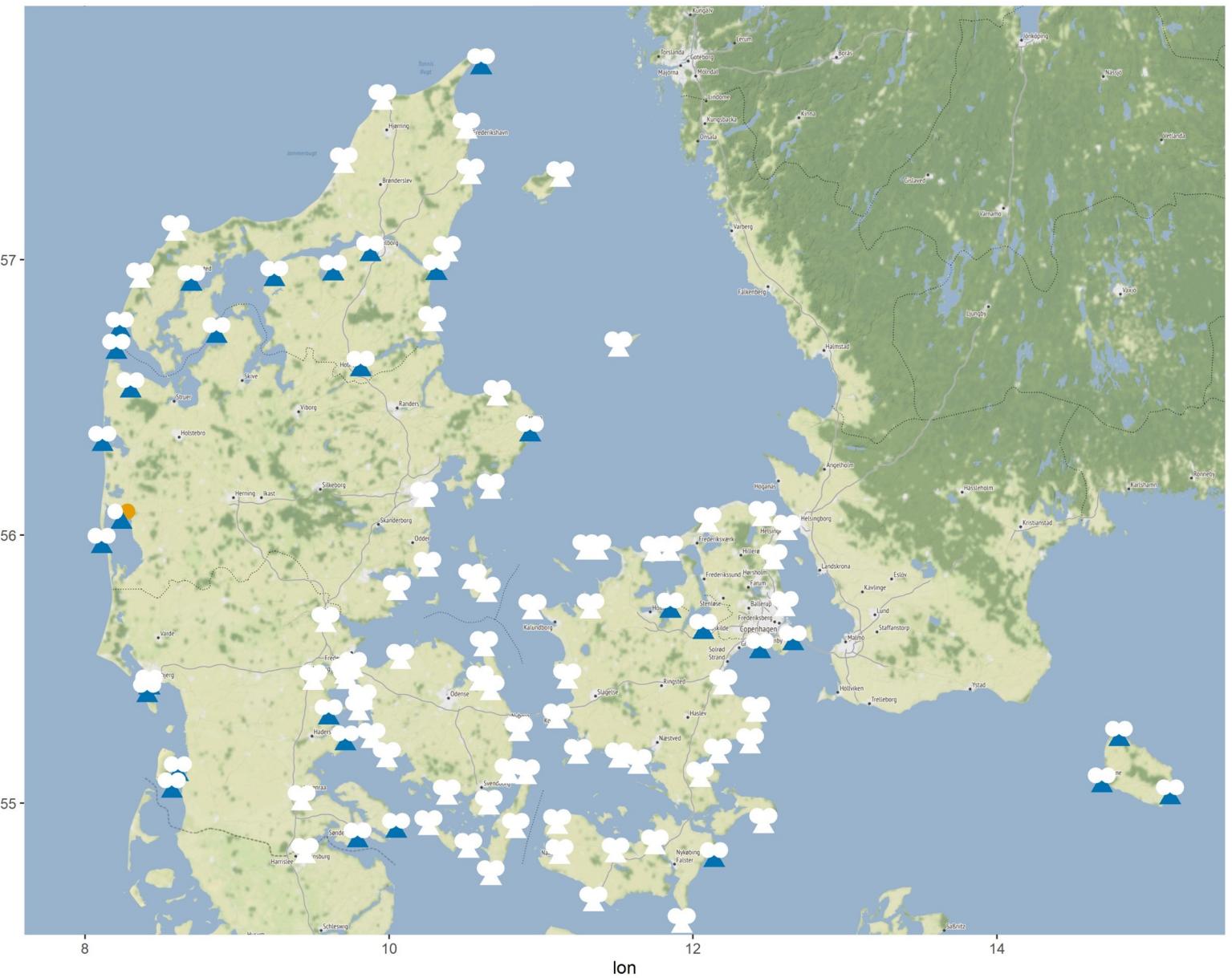
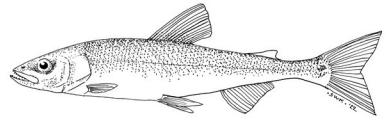
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Oncorhynchus mykiss



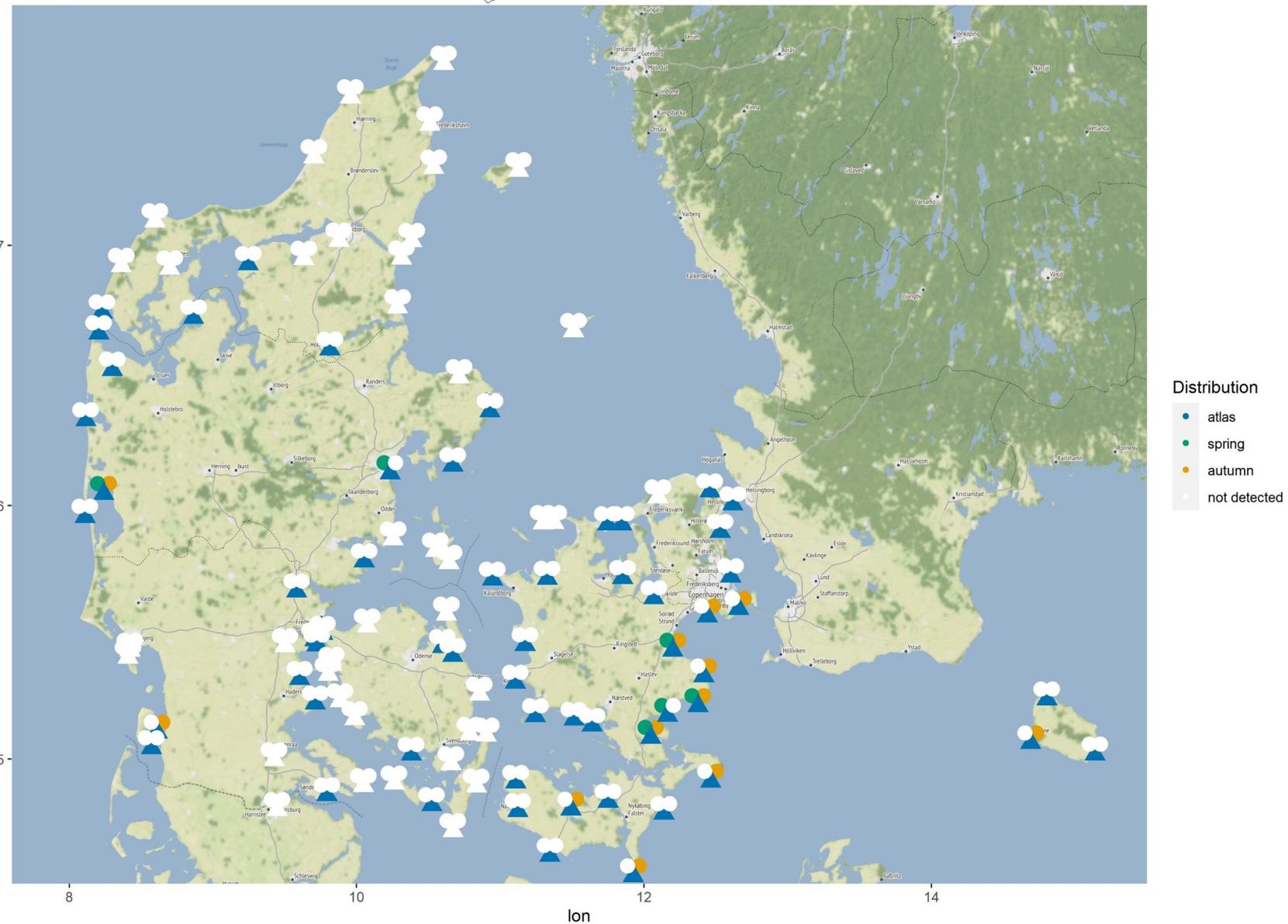
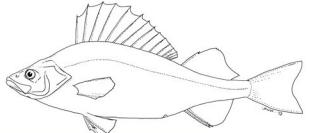
Osmerus eperlanus



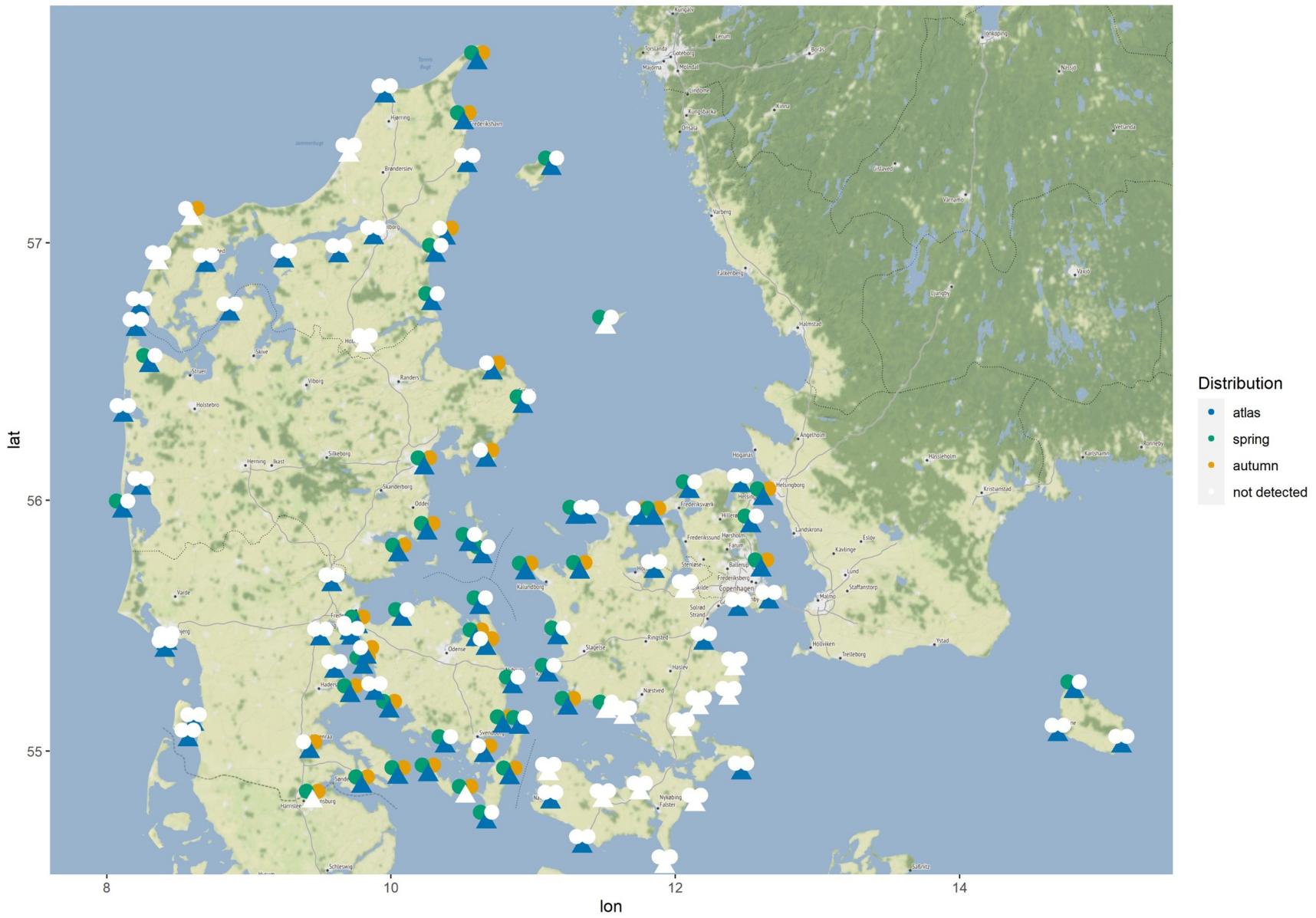
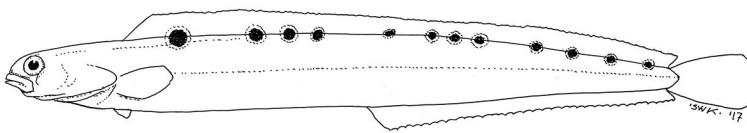
Distribution

- atlas
- spring
- autumn
- not detected

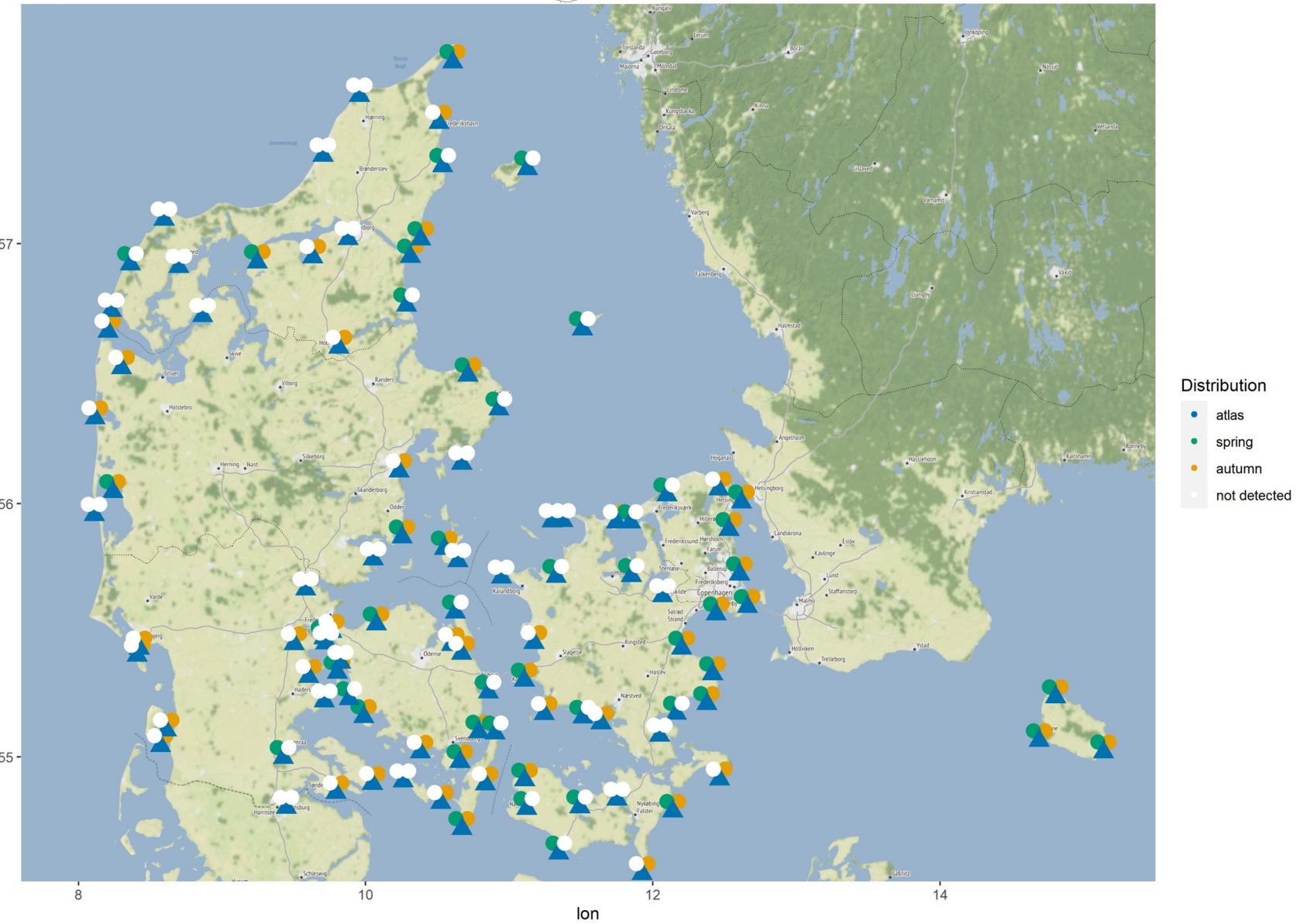
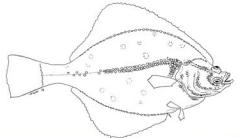
Perca fluviatilis



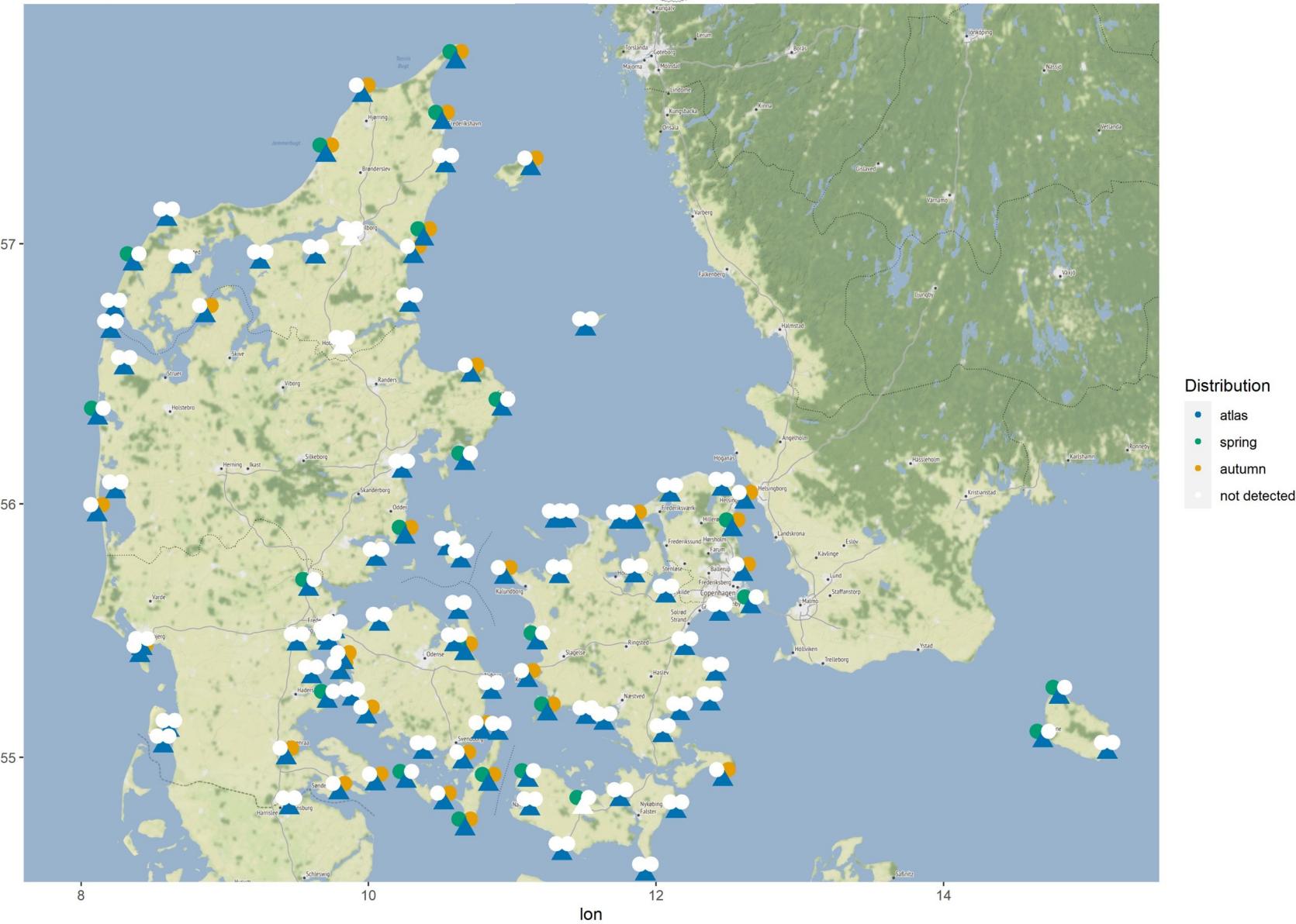
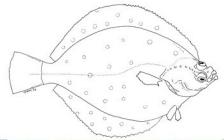
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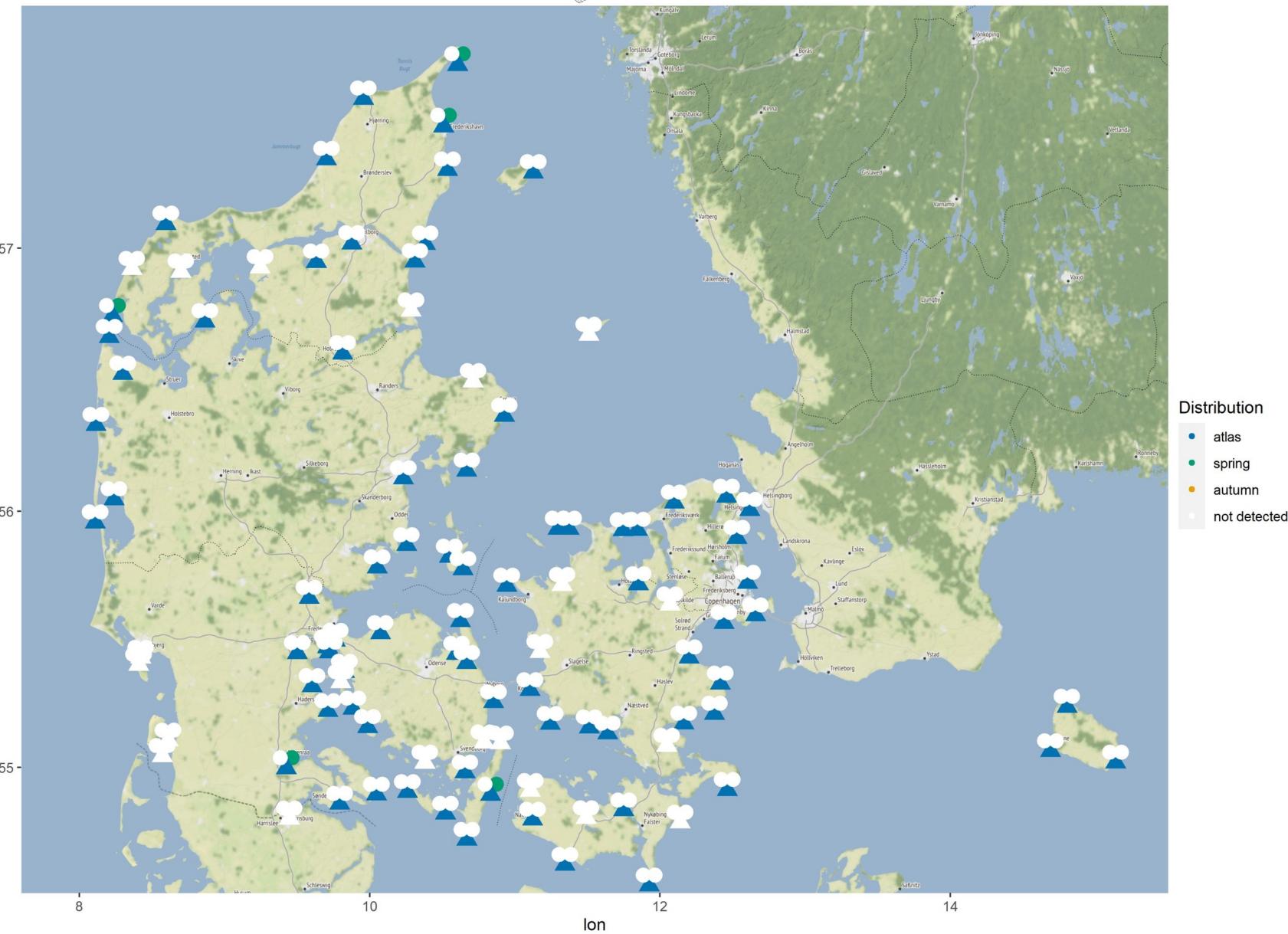
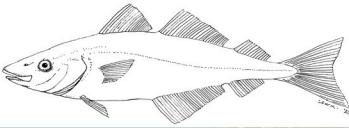
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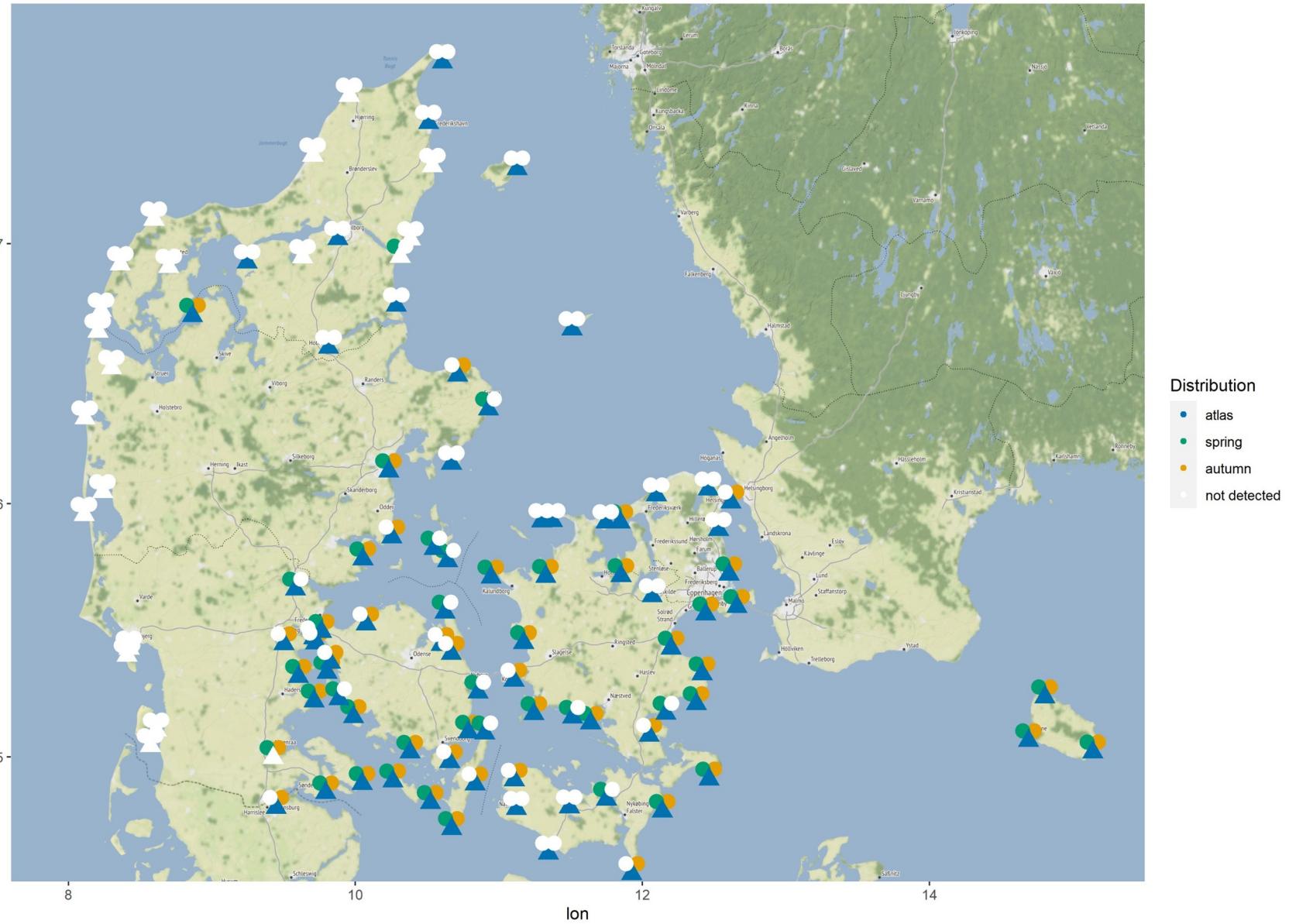
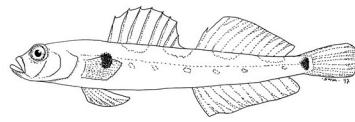
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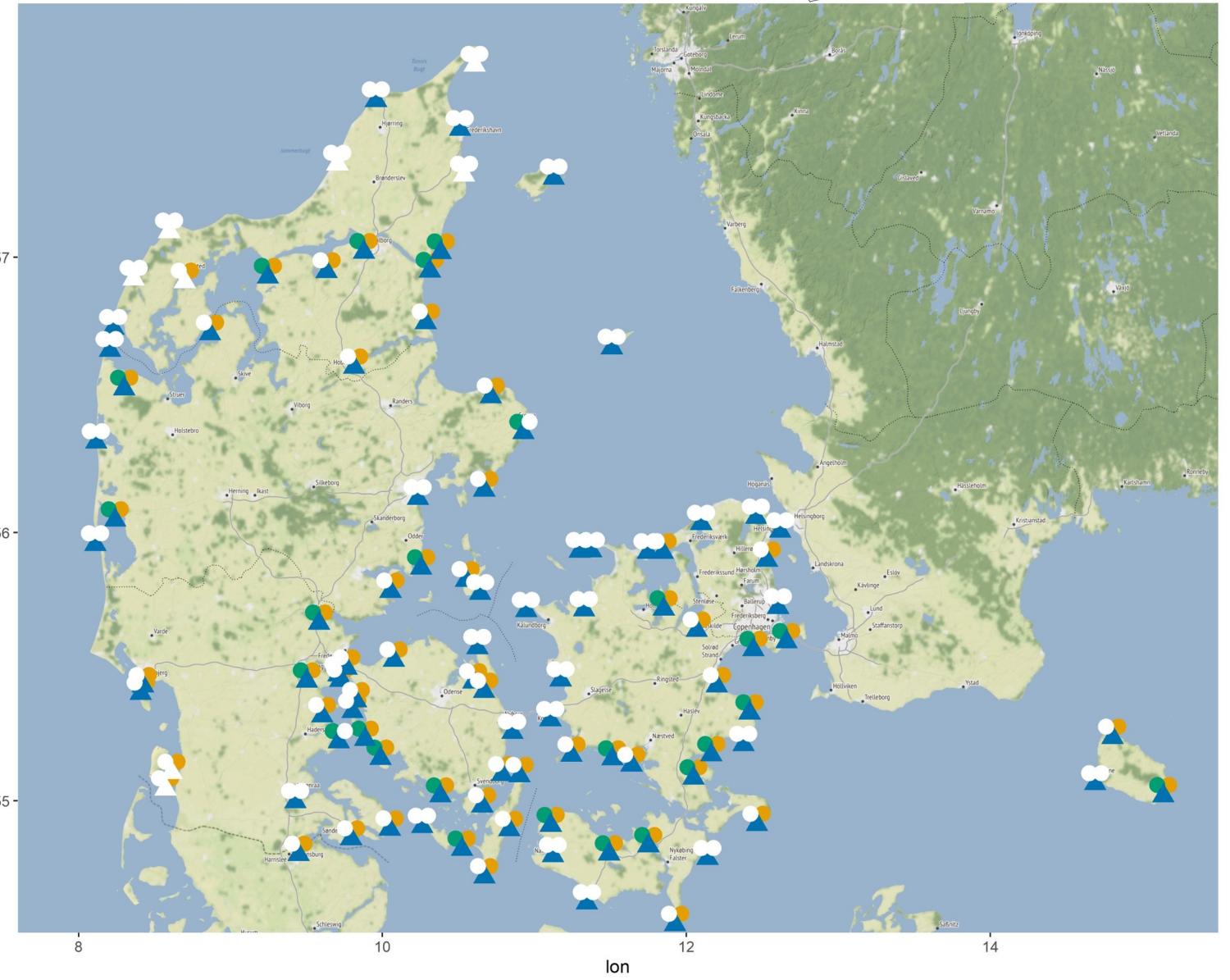
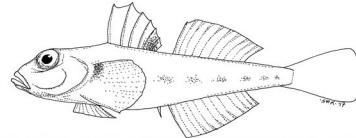
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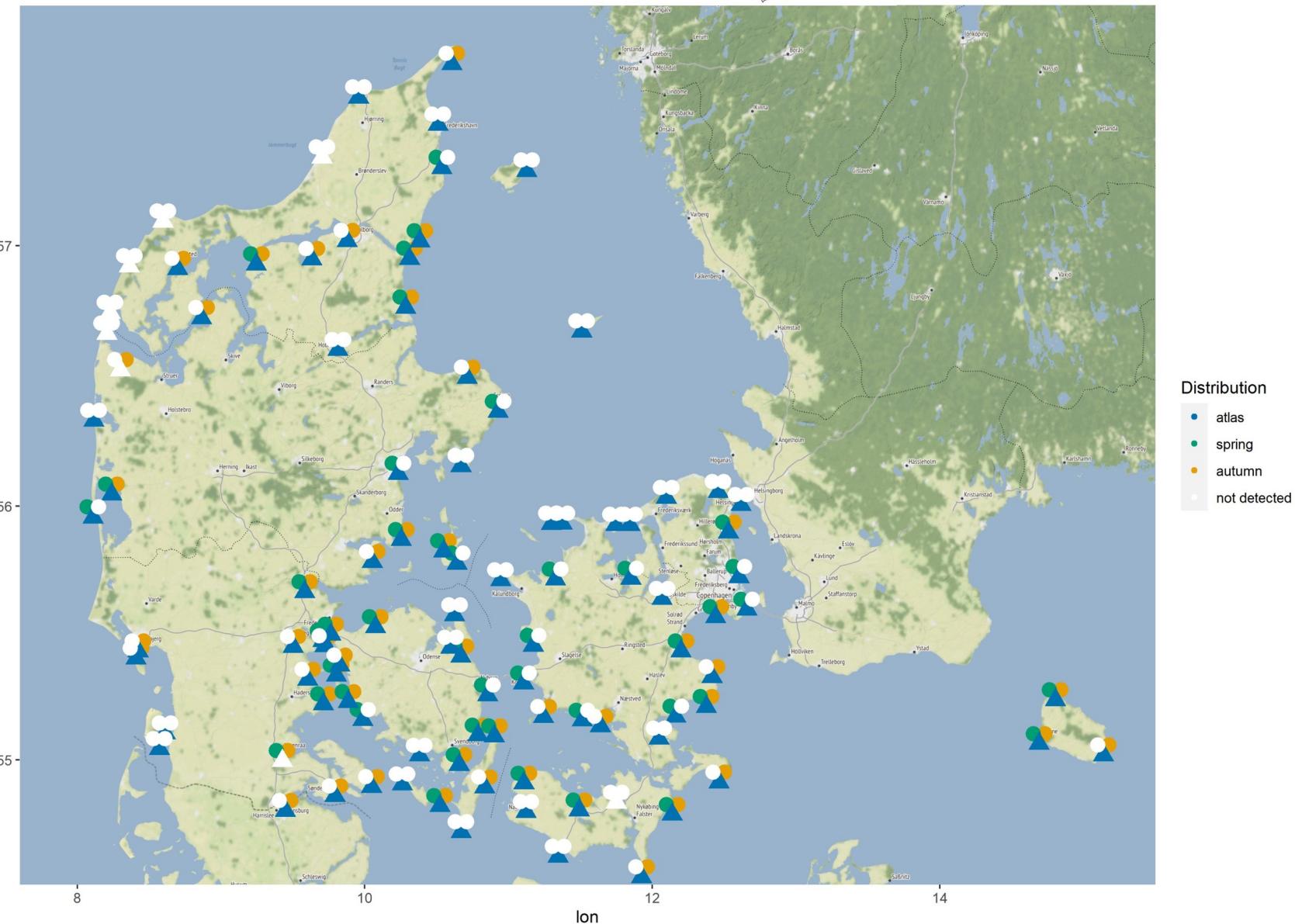
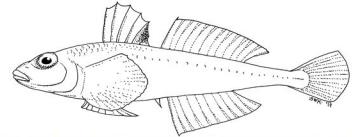
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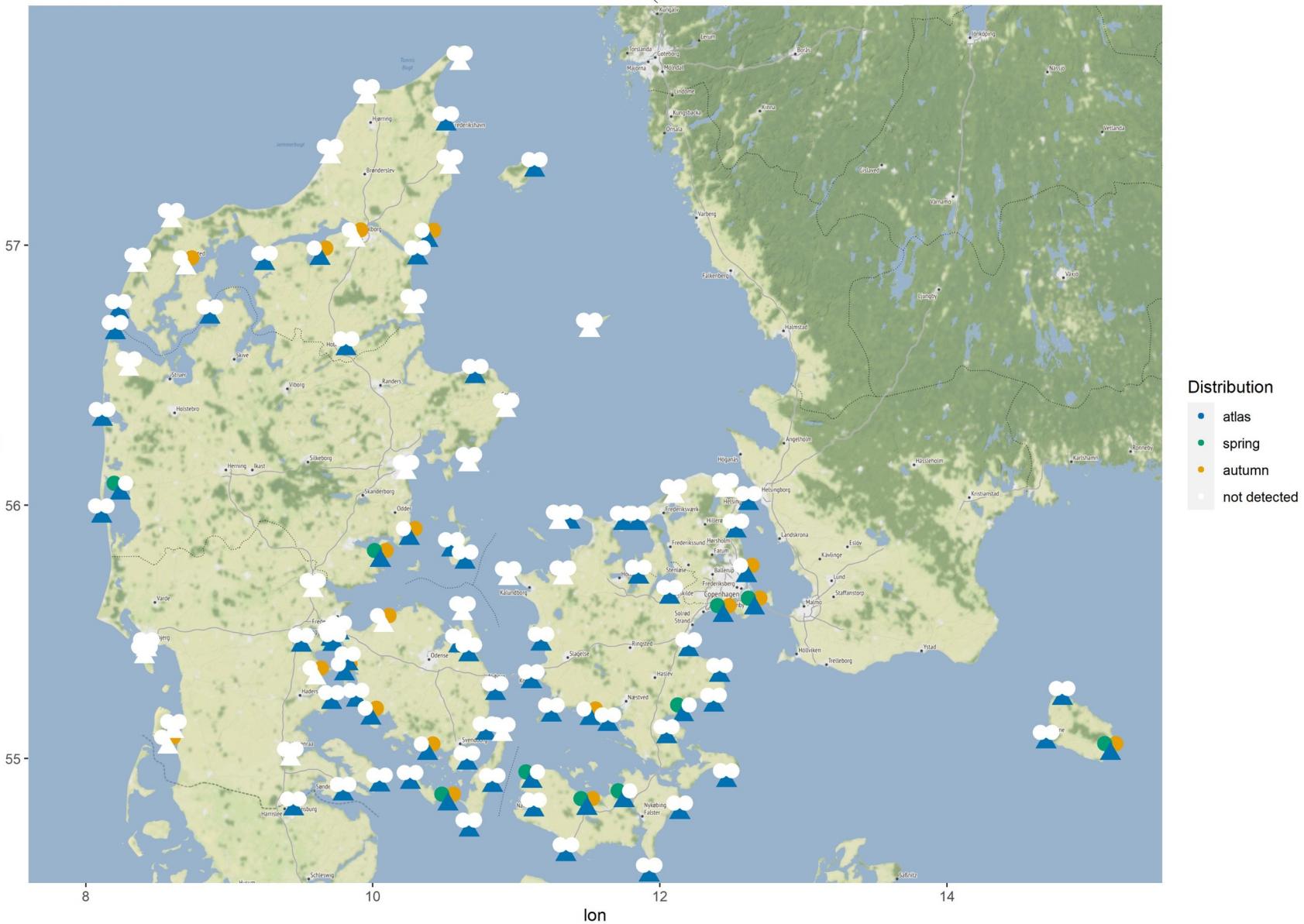
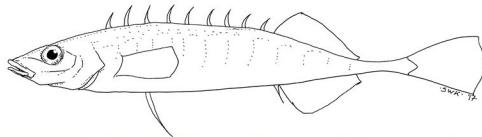
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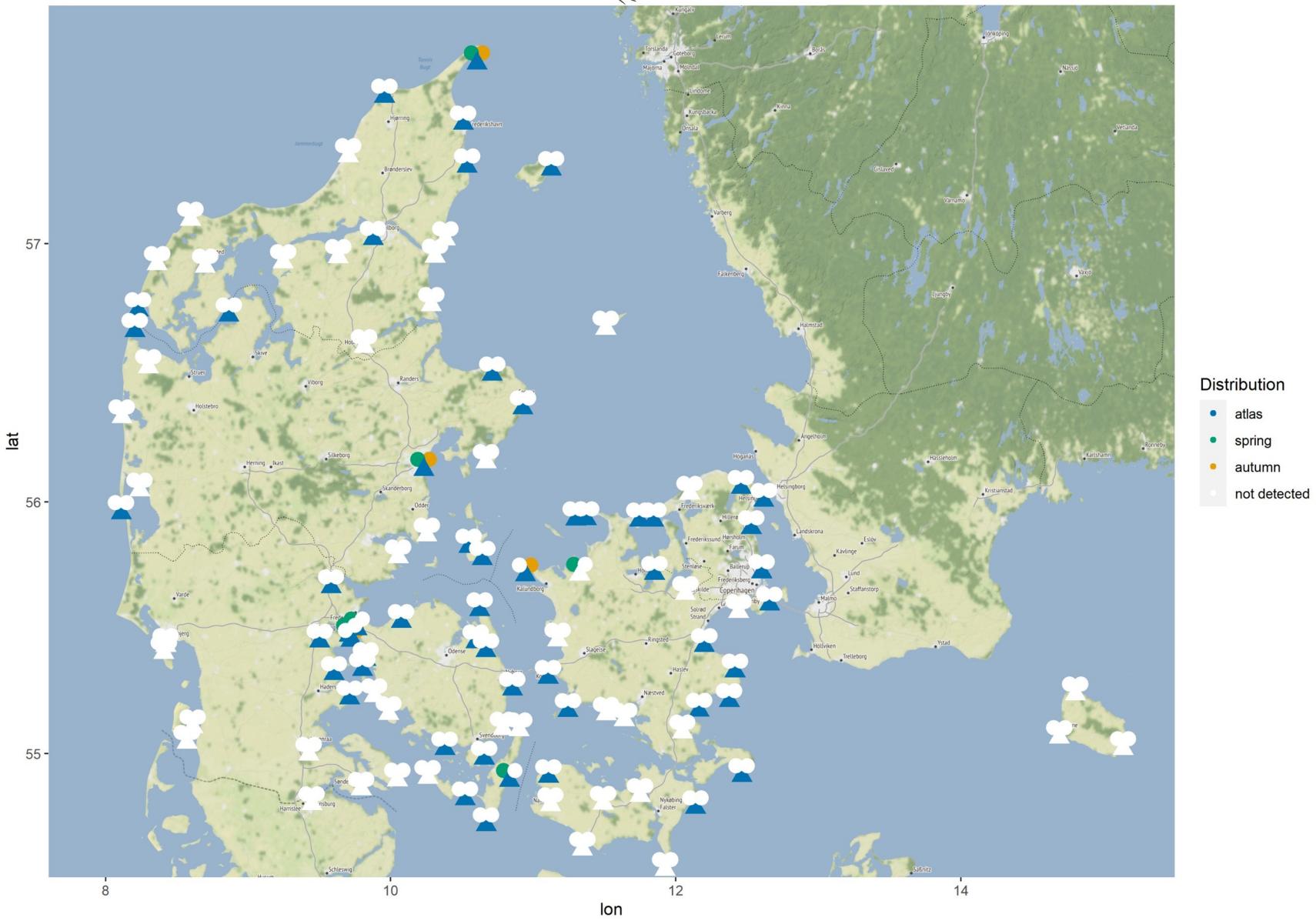
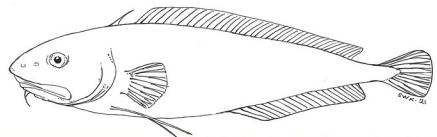
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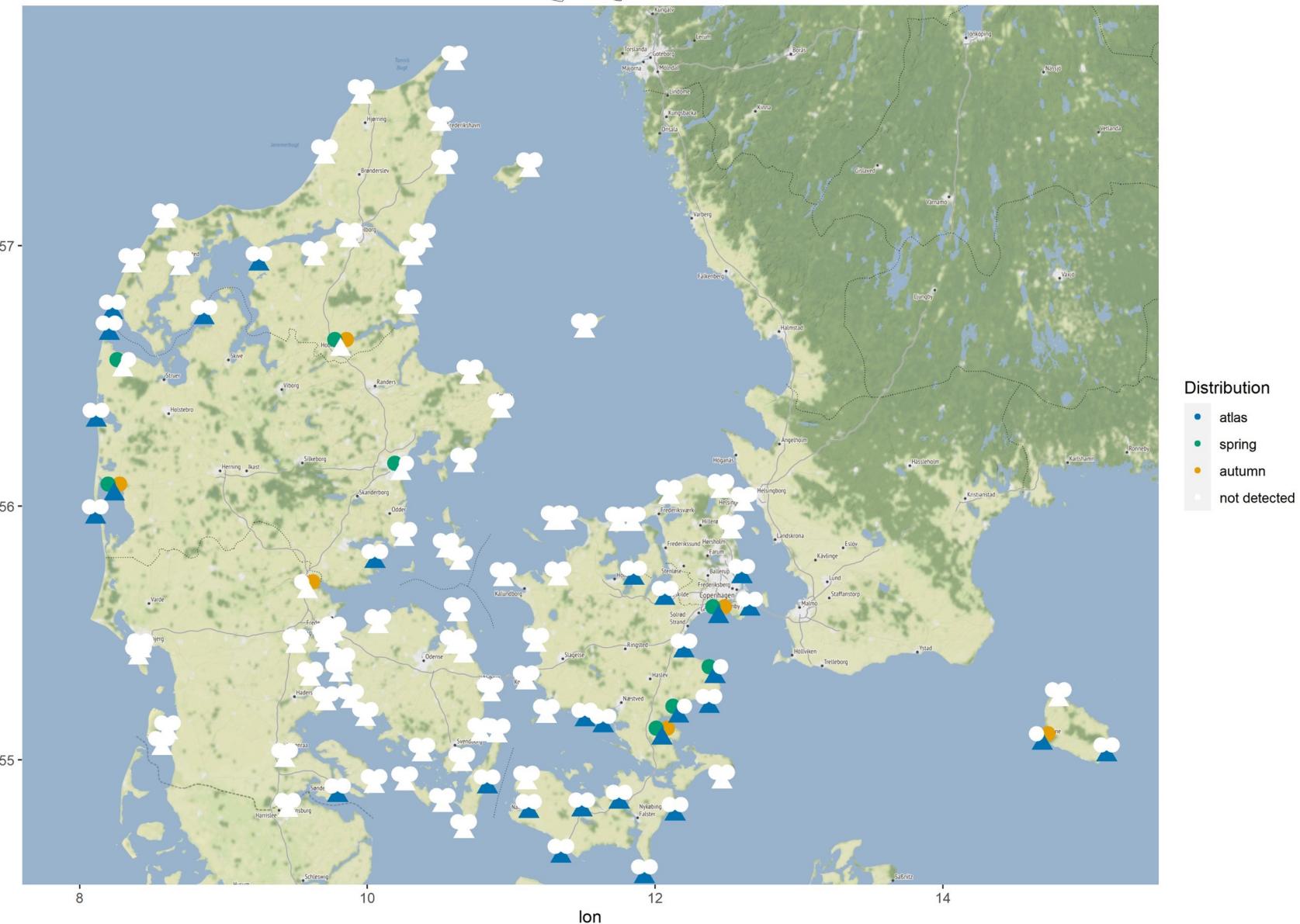
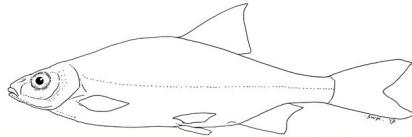
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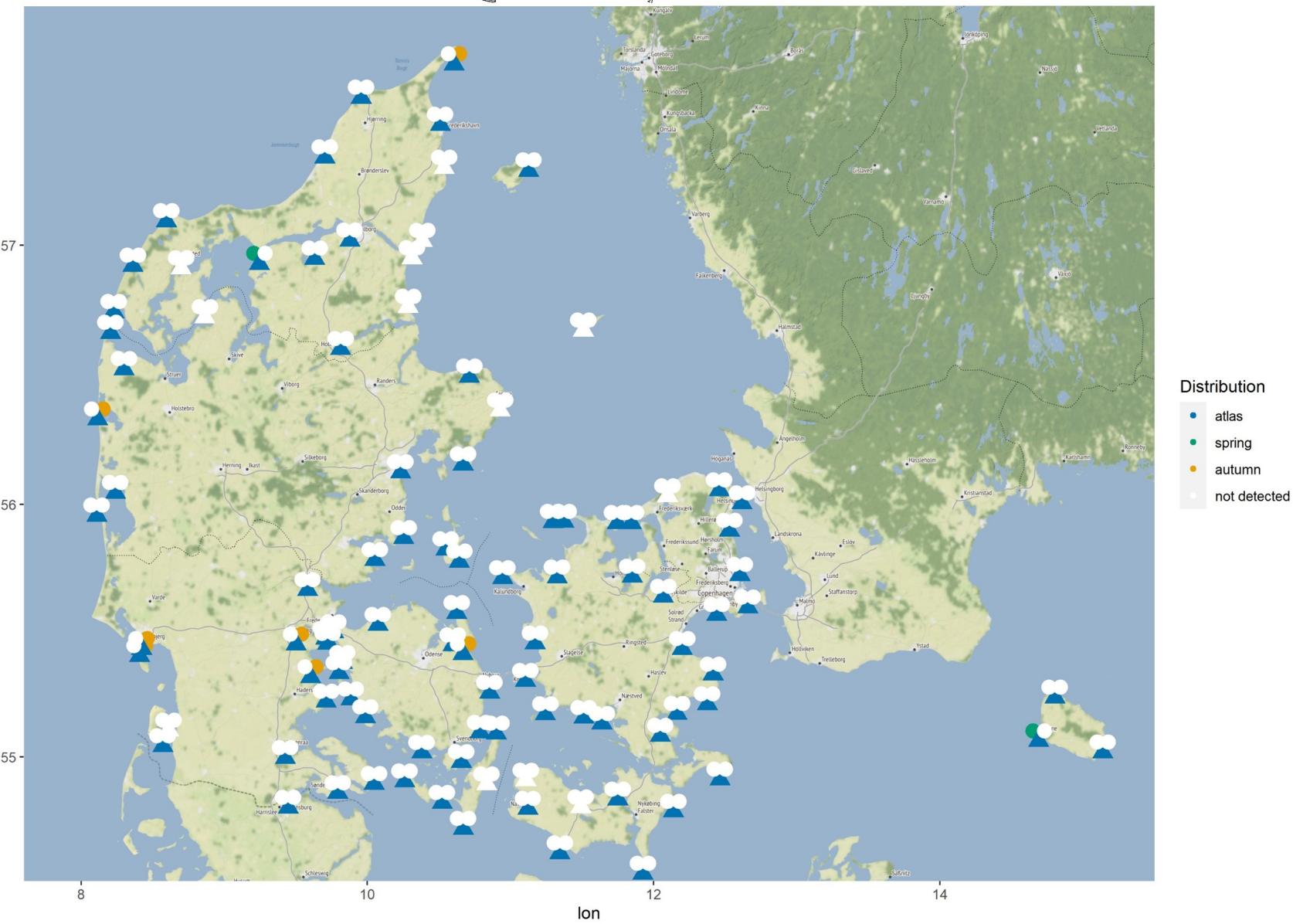
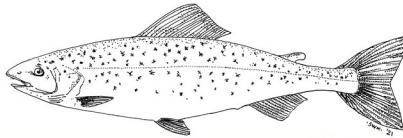
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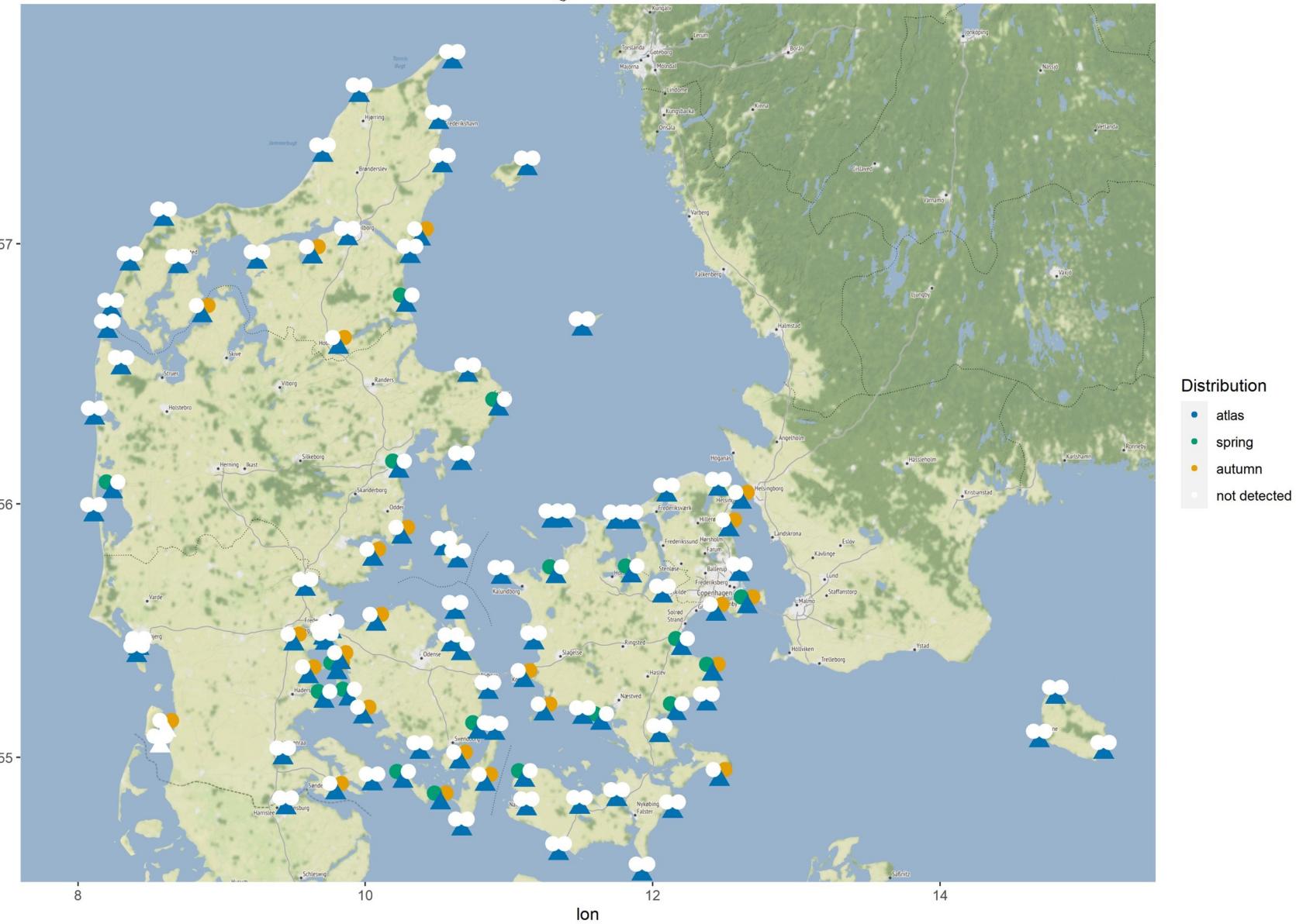
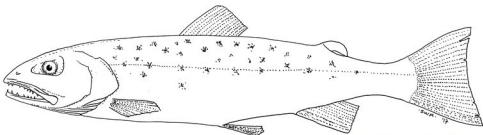
Rutilus rutilus



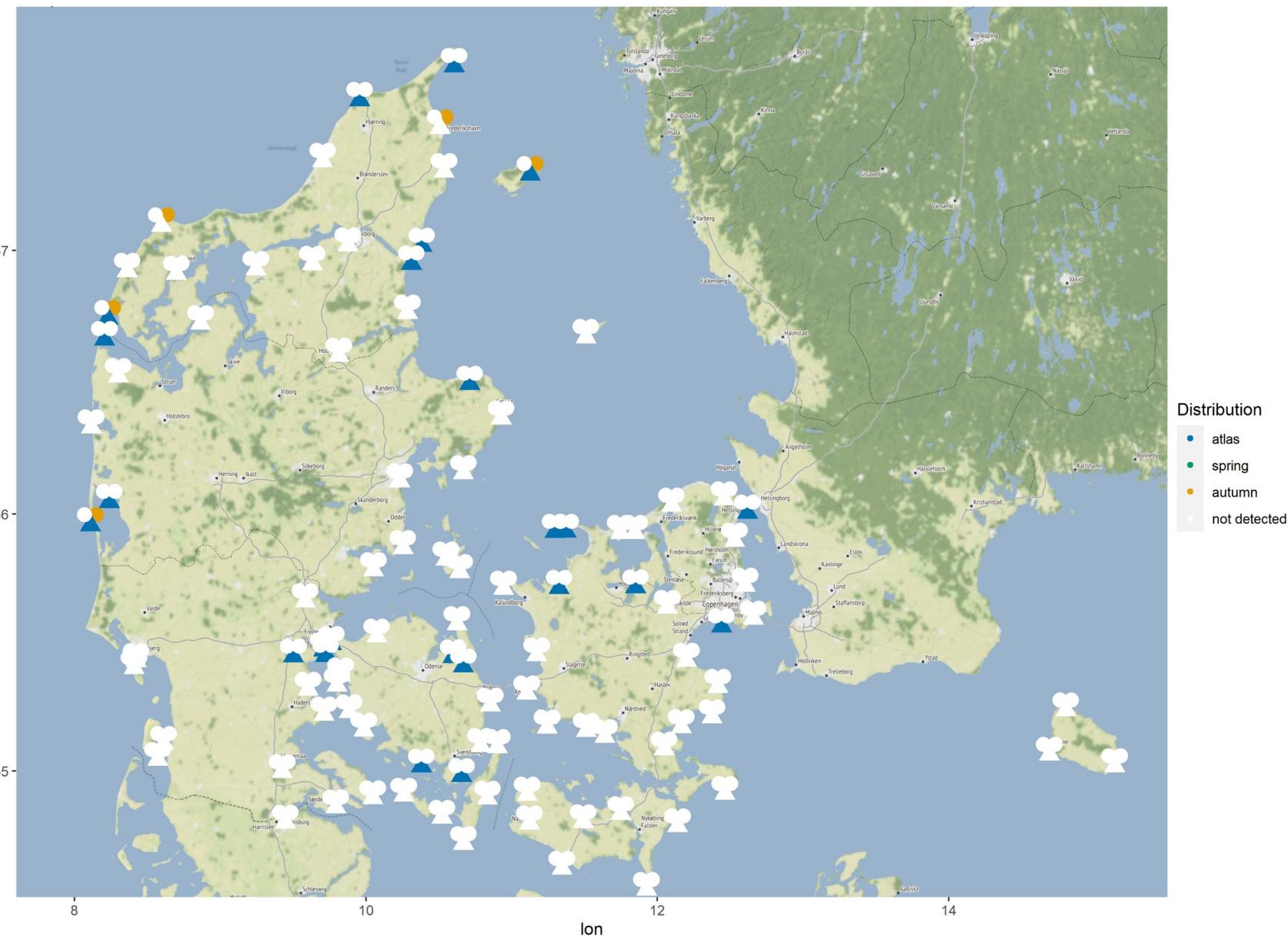
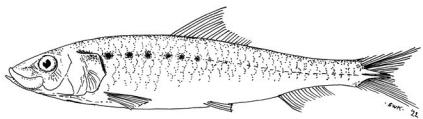
Salmo salar



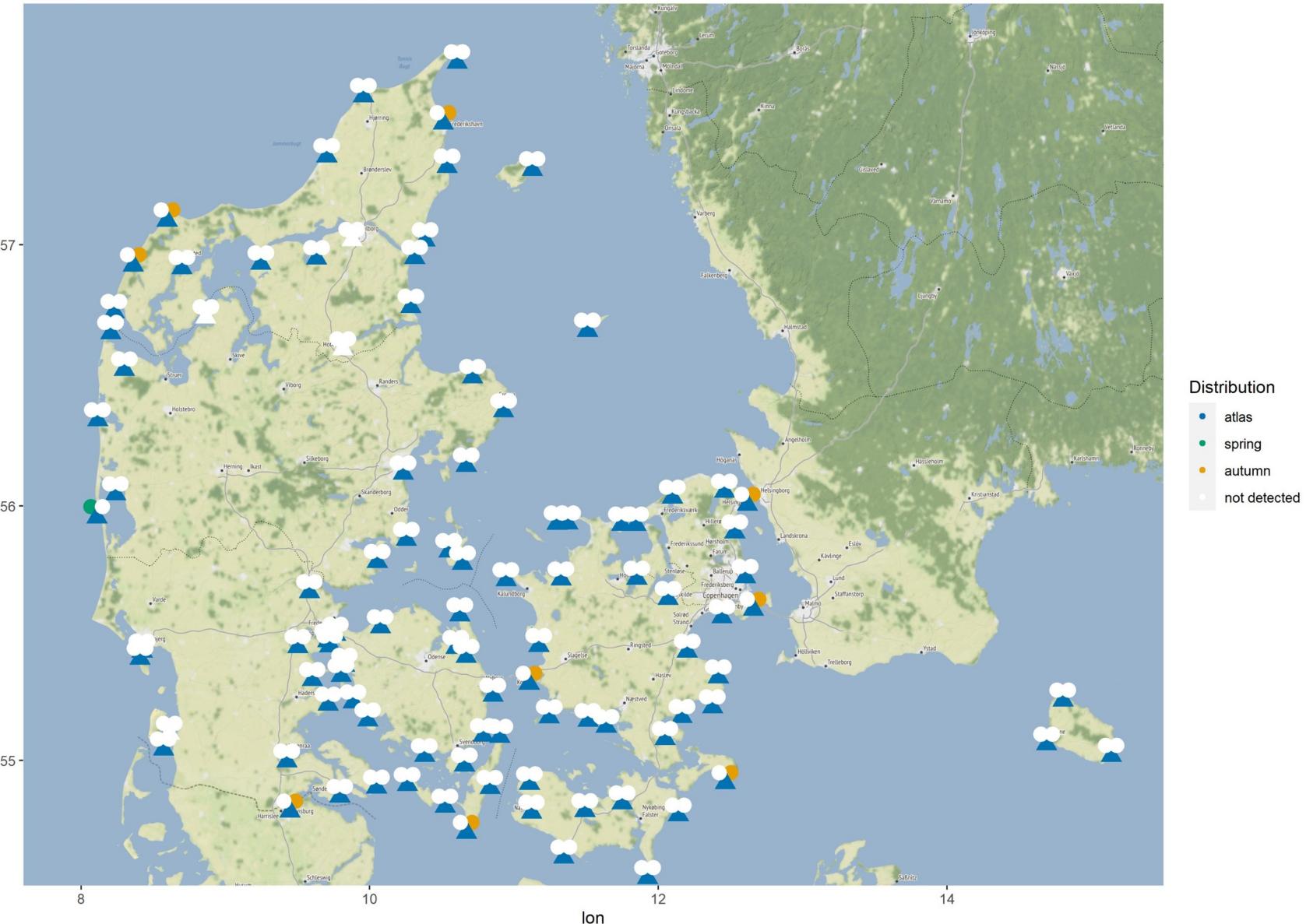
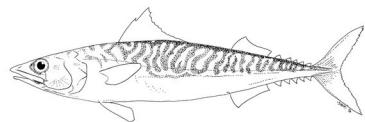
Salmo trutta



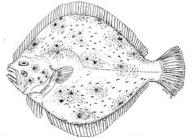
Sardin pilchardus



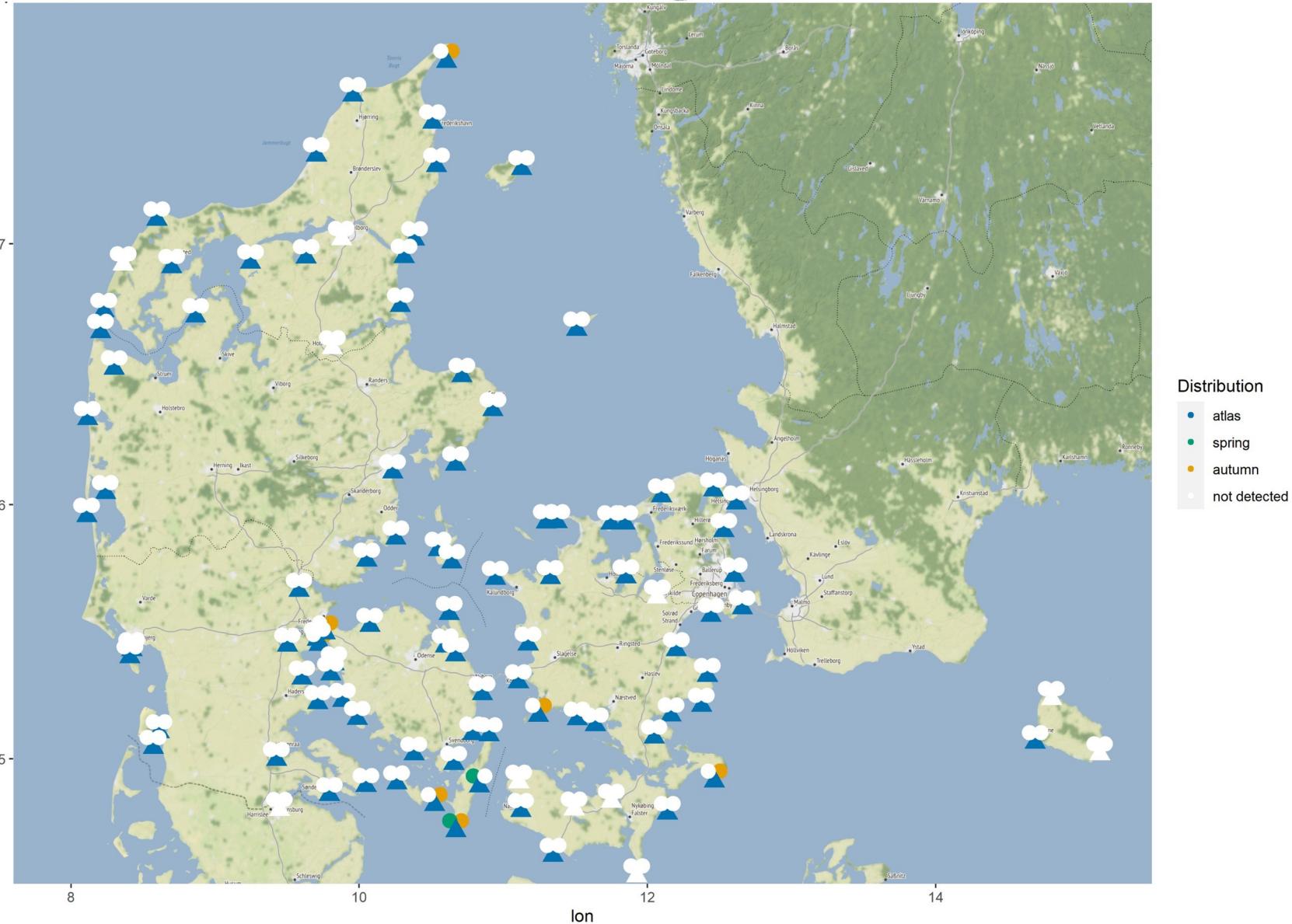
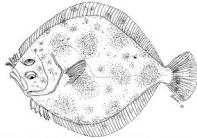
Scomber scombrus



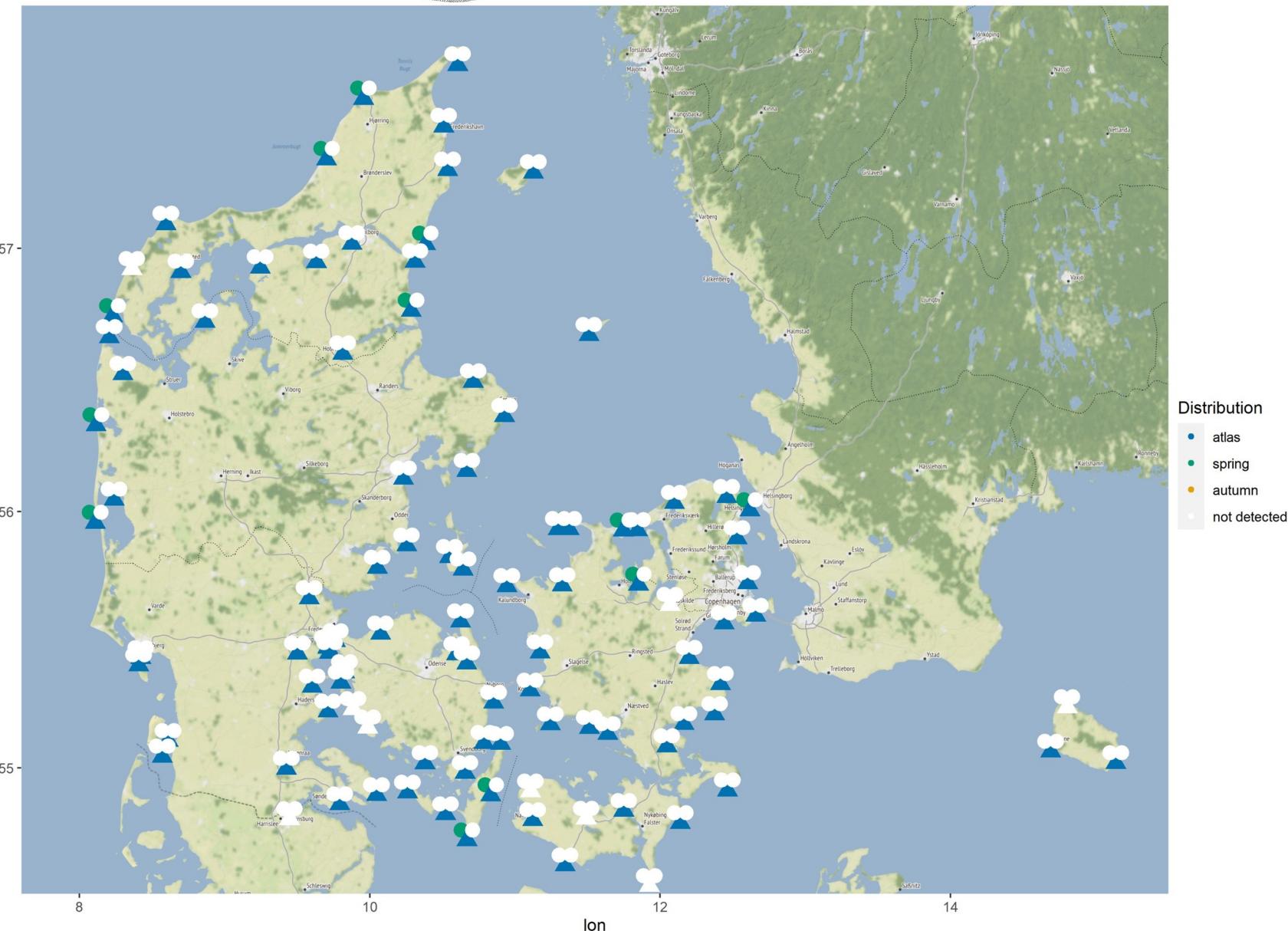
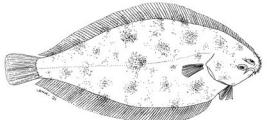
Scophthalmus maximus



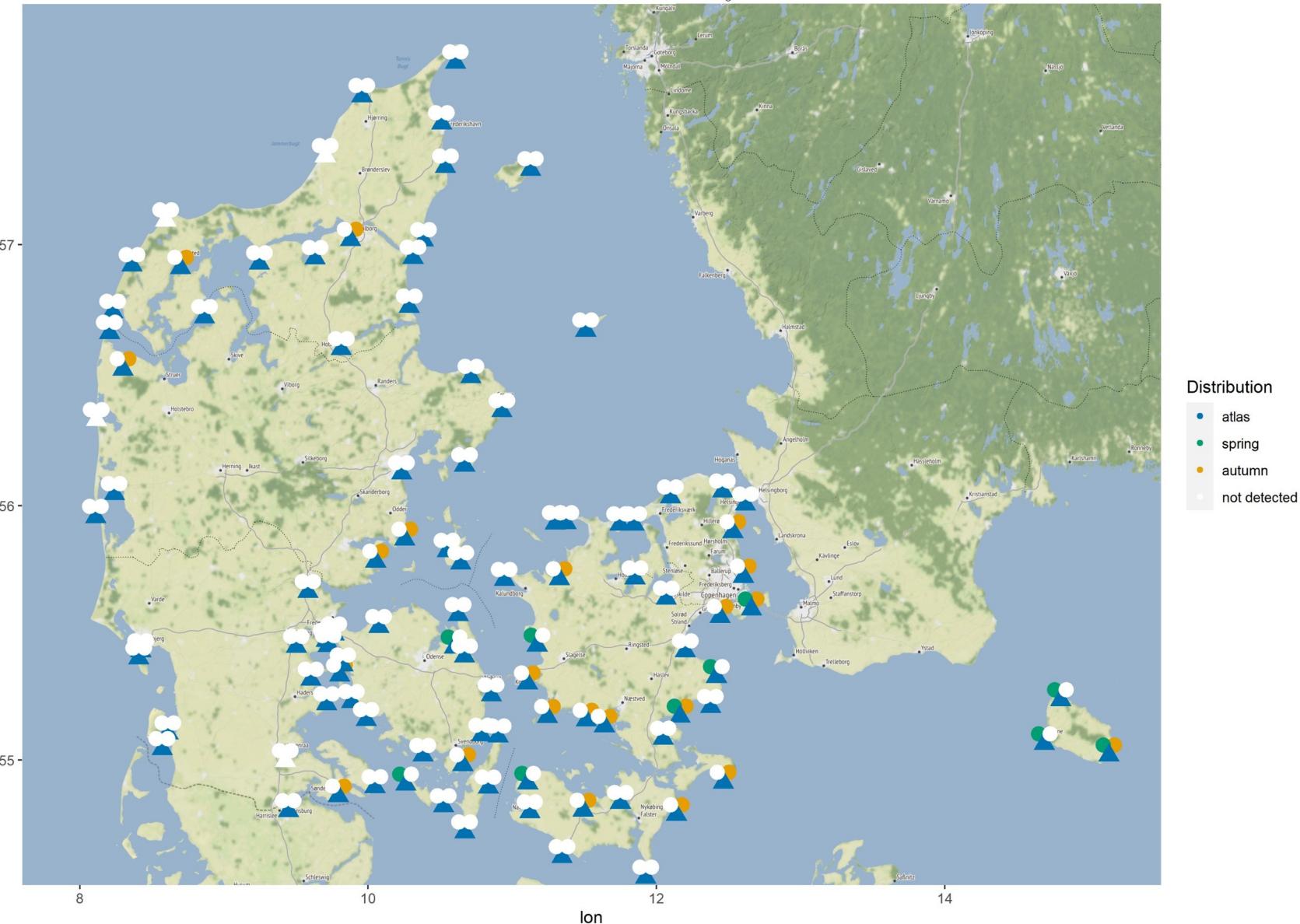
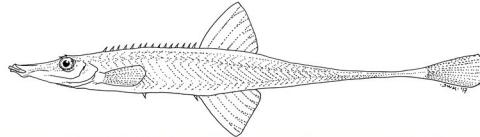
Scophthalmus rhombus



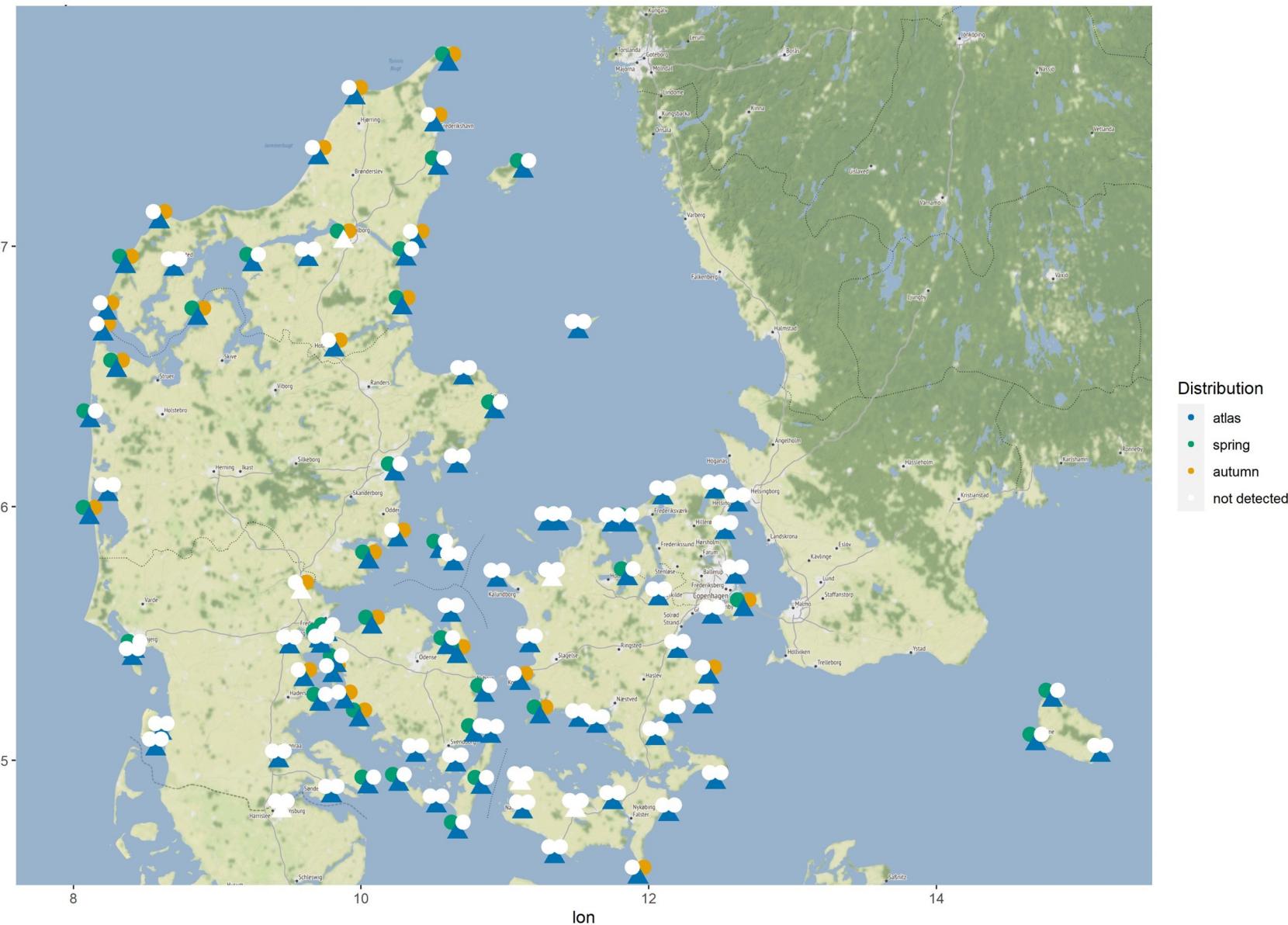
Solea solea



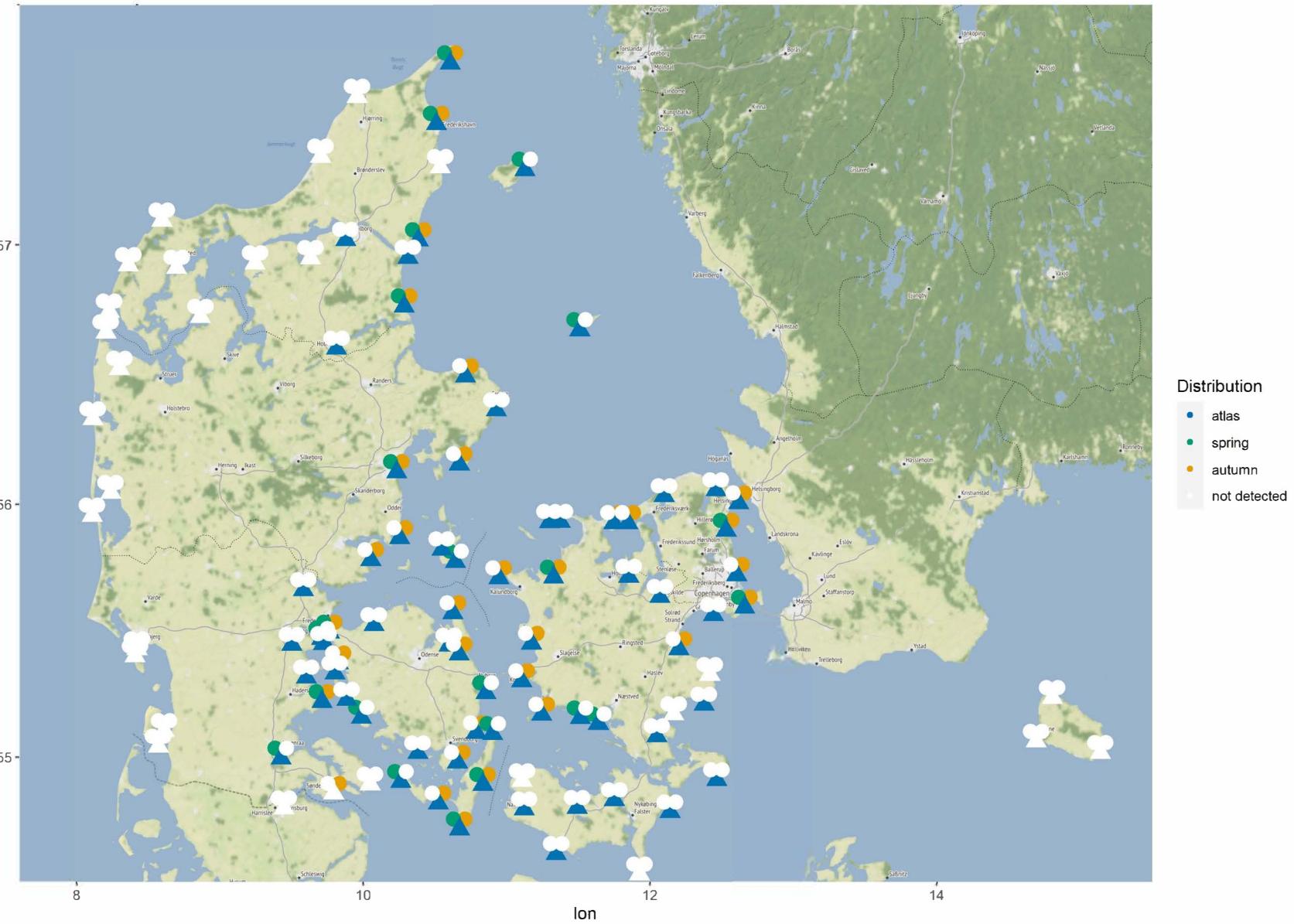
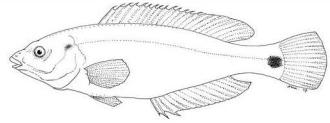
Spinacia spinacia



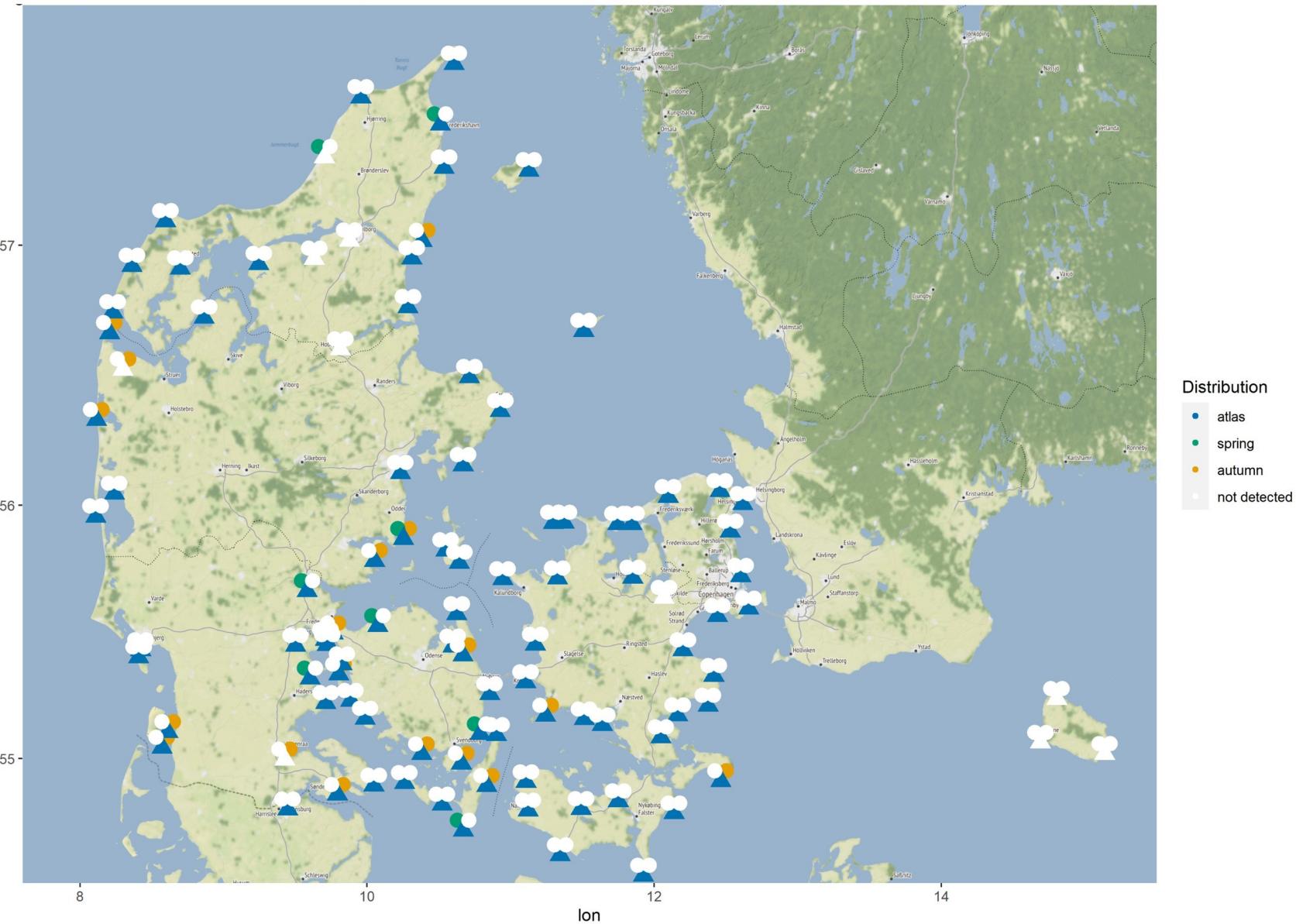
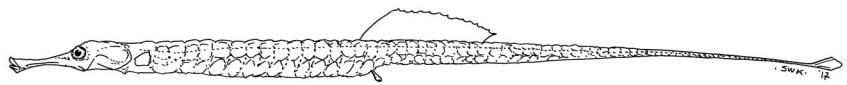
Sprattus sprattus



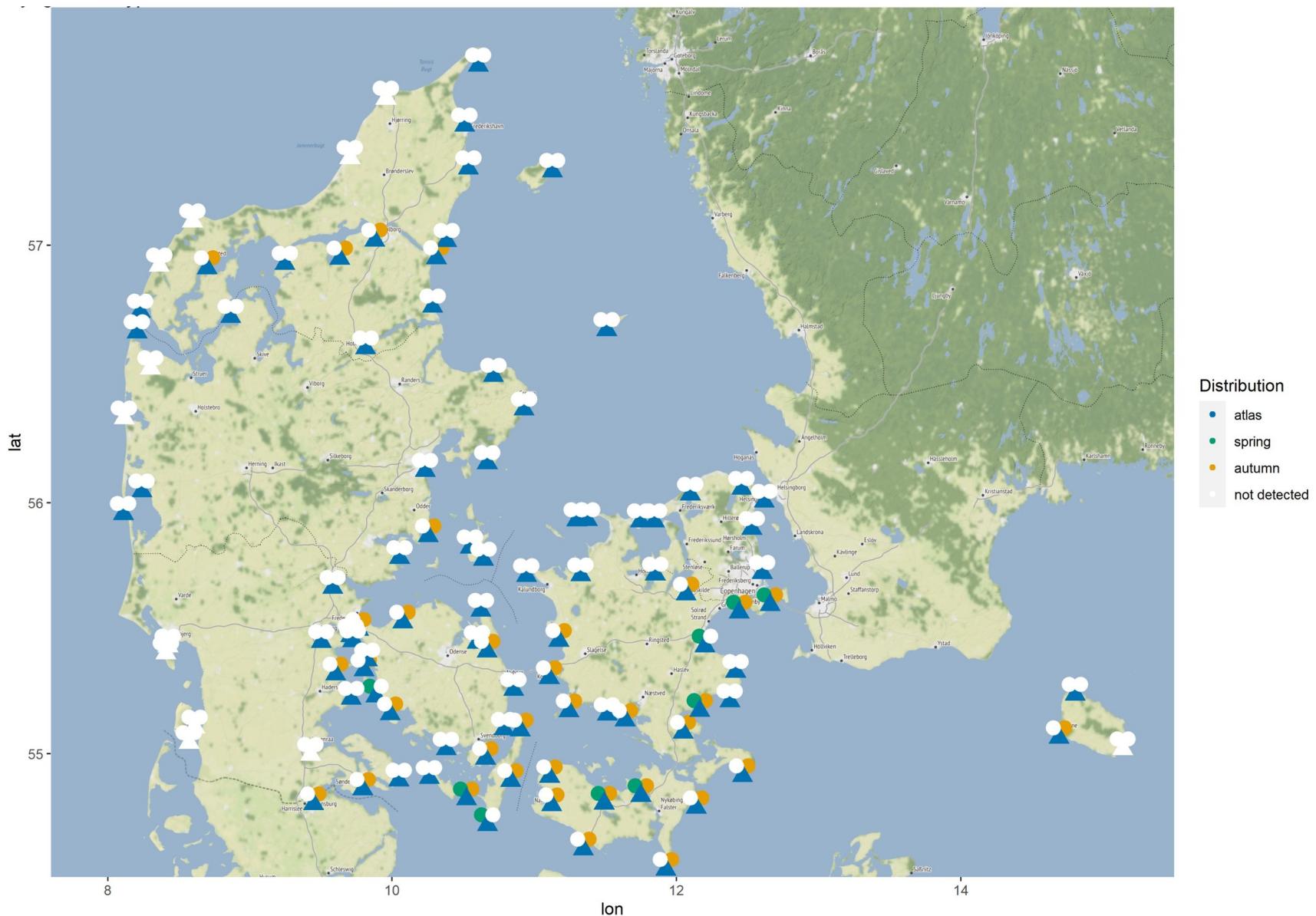
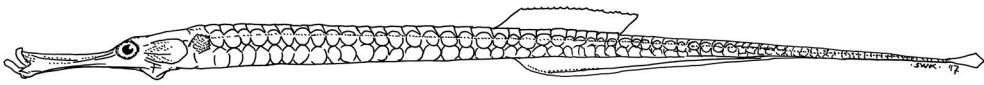
Syphodus melops



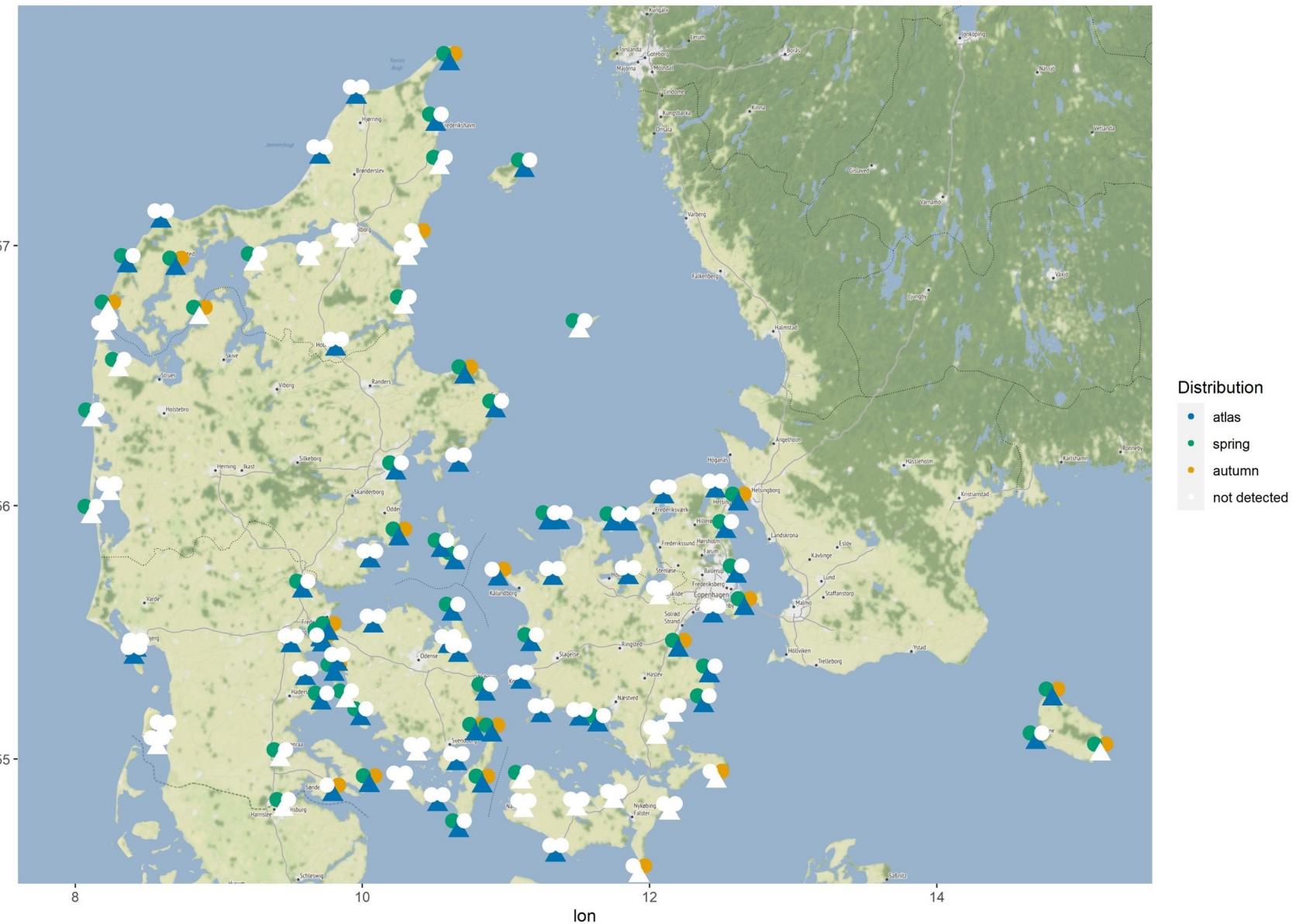
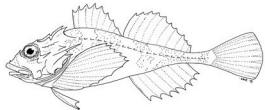
Syngnathus rostellatus



Syngnathus typhle



Taurulus bubalis



Zoarces viviparous

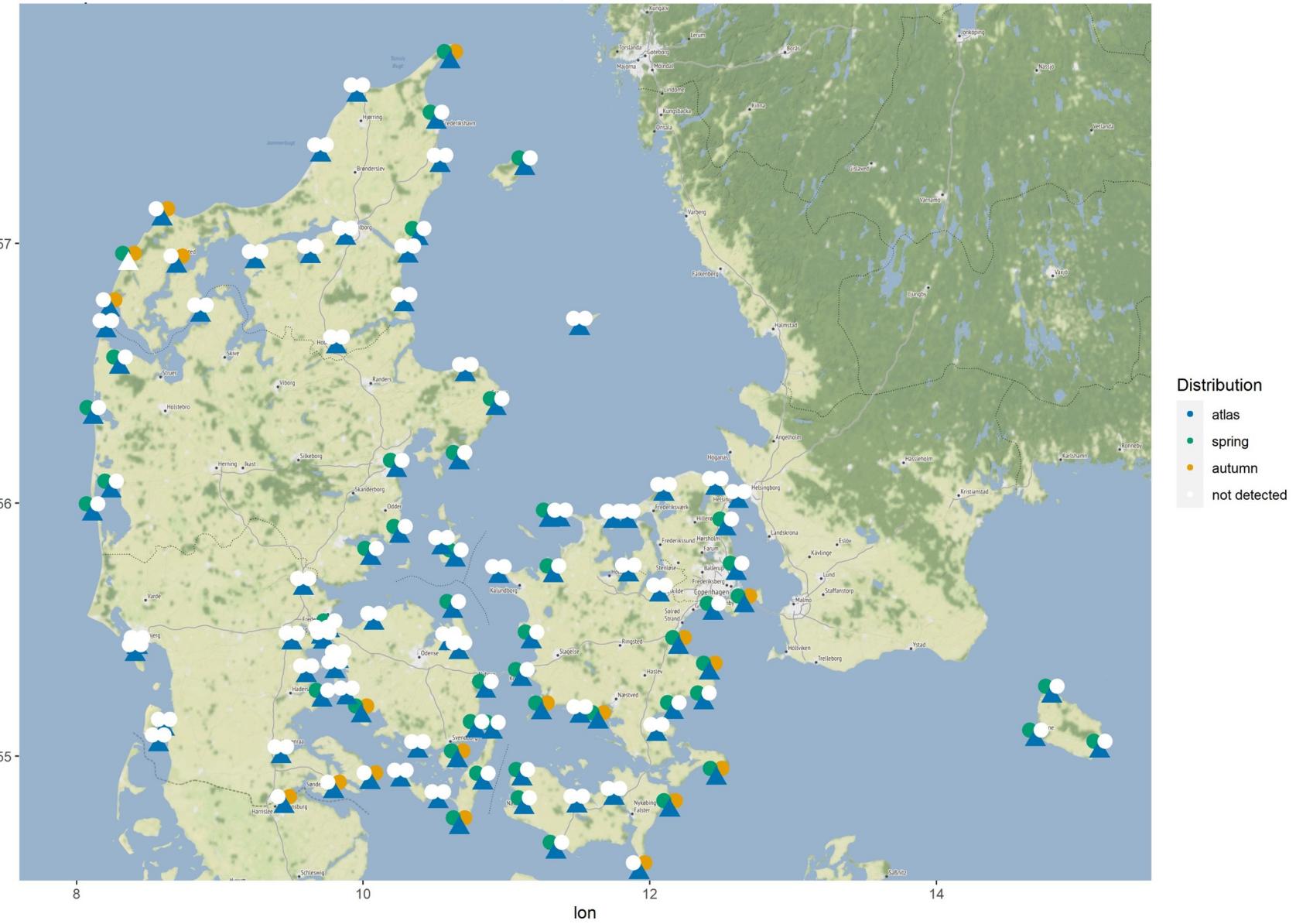
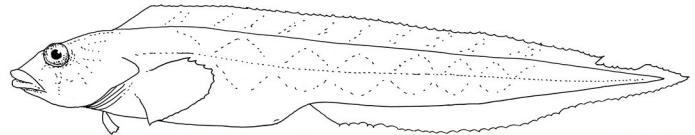
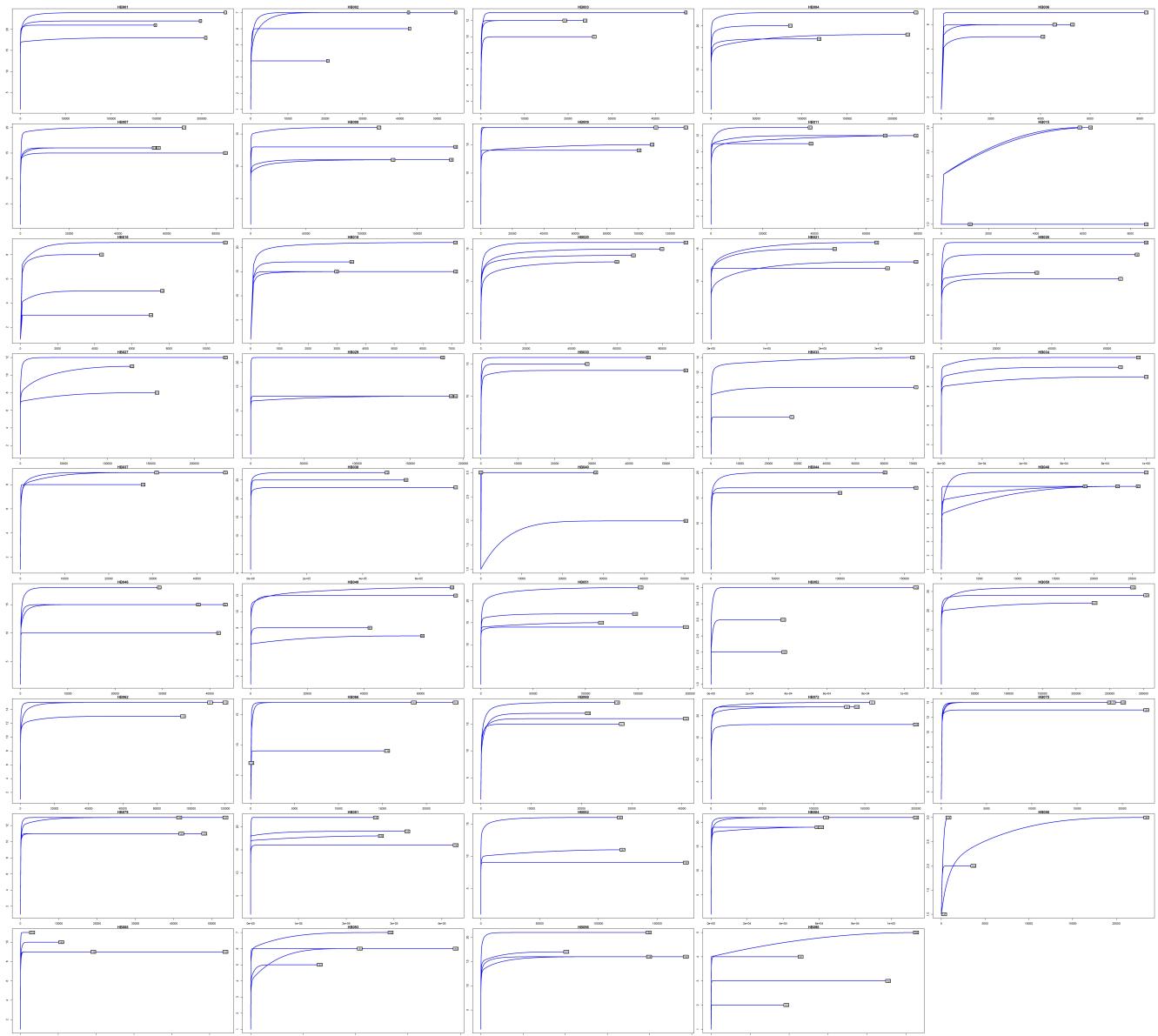


Figure S6. Rarefaction curves per PCR replicate per sample, indicating that sequencing depth was sufficient for most of the PCR replicates sequenced. The individual plots show the accumulated number of species (y-axis) recovered as a function of sequencing depth (x-axis) for each sample. The four PCR replicates are numbered with a running number. Samples named with a “S” at the end of the sample name represents spring samples, and samples without represents autumn samples.



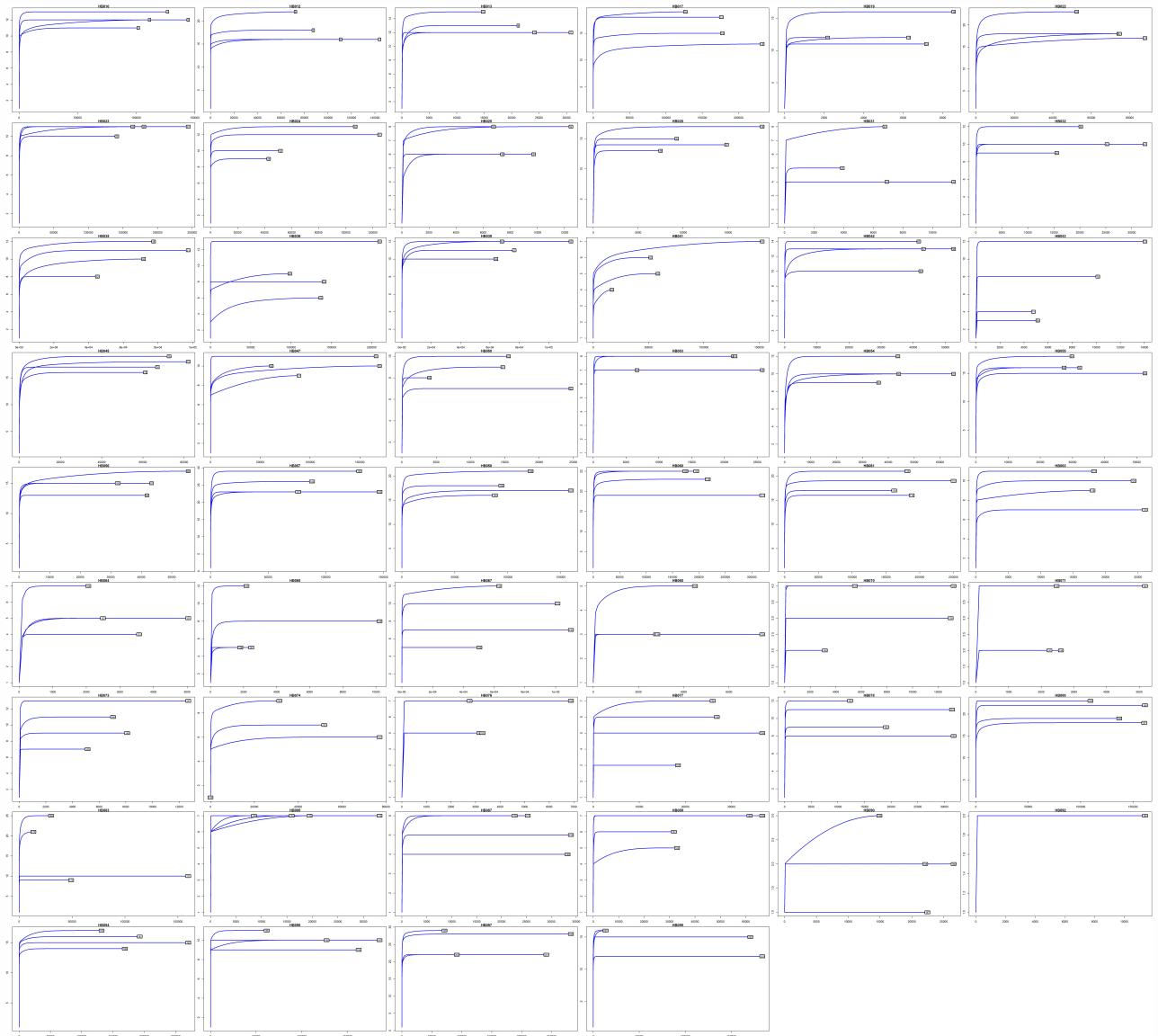






Table S1: Overview of locations with their names, geographical coordinates and the species richness found with eDNA in autumn and spring, as well as the accumulated eDNA richness for both seasons and the richness registered in the Danish Fish Atlas.

Location ID	Location name	Latitude	Longitude	eDNA richness autumn	eDNA richness spring	eDNA richness both seasons	Fish Atlas richness
HB001	Marstal Havn	54.8567737	10.52173724	21	14	22	61
HB002	Søby Havn	54.9432615	10.26025871	4	10	11	60
HB003	Mommark Havn	54.9332705	10.04696252	15	7	16	57
HB004	Sønderborg Havn	54.8967318	9.79306659	22	5	22	60
HB005	Anholt Havn	56.7137086	11.50908377	0	10	10	62
HB006	Allinge	55.278895	14.80350748	9	17	18	45
HB007	Nexø	55.0593806	15.13832896	12	12	14	53
HB008	Rønne, Nørre Kaas	55.1049827	14.69062191	11	17	21	60
HB009	Gedser Havn	54.5710605	11.92512056	15	0	15	32
HB010	Guldborg Sund	54.8707438	11.74945641	7	8	10	45
HB011	Hesnæs Havn	54.8220402	12.13824465	13	8	14	51
HB012	Valdemars Slot	55.0207428	10.65647546	19	9	21	72
HB013	Lundeborg Havn	55.1390408	10.78754486	13	14	20	71
HB014	Kerteminde Havn, Fjord og Bælt	55.4506994	10.66862904	15	9	18	78
HB015	Høfde, v. Nordskov	55.614753	10.62422219	2	10	11	65
HB016	Bregnør Havn	55.4864831	10.59760055	7	8	12	78
HB017	Bogense Havn	55.5668706	10.07375844	16	8	19	60
HB018	Strib Havn	55.5378291	9.76326871	16	15	22	106
HB019	Kongebro Havn	55.5127417	9.709431899	13	11	19	105
HB020	Middelfart Marina	55.4919093	9.723336259	12	1	12	103
HB021	Føns Vig Jollehavn	55.415903	9.825471317	13	3	14	64
HB022	Tybrind Vig stensætning	55.3758105	9.801299365	23	12	23	59
HB023	Assens Havn	55.272086	9.884813328	4	10	11	48
HB024	Agernæs Havn	55.199959	9.986035242	15	15	21	47
HB025	Fjellebro Havn	55.0591065	10.37976324	8	7	12	53
HB026	Kolund Havn	54.837513	9.446739501	11	4	12	28

HB027	Rømø Havn	55.085205	8.570312197	7	0	7	43
HB028	Rømø Dæmningen	55.1470262	8.611263154	10	0	10	32
HB029	Nordby Havn	55.4438666	8.408503236	13	0	13	75
HB030	Esbjerg Havn	55.4718398	8.422169213	10	2	11	76
HB031	Hvide Sande Mole	55.9969642	8.109057705	8	16	18	84
HB032	Ringkøbing Havn	56.0861721	8.23860813	12	13	15	67
HB033	Thorsminde Mole	56.3710266	8.114150673	9	11	17	76
HB034	Høfde, v. Thyborøn	56.7054107	8.2049171	11	3	13	95
HB035	Høfde, v. Agger	56.7845194	8.227745678	9	6	12	95
HB036	Mole, v. Nørre Vorupør	56.9619996	8.361696749	11	13	17	39
HB037	Hirtshals Havn, Nordsøen Oceanarium	57.5966419	9.956655741	5	4	8	98
HB038	Skagen Havn	57.7217956	10.60563182	24	17	26	138
HB039	Strandby Havn	57.4949581	10.50928118	15	14	21	102
HB040	Sæby Havn	57.333267	10.53604154	1	11	12	67
HB041	Løkken Mole	57.3725145	9.70244372	5	6	10	56
HB042	Hou Lystbådehavns Mole	57.0566165	10.3831568	15	13	20	61
HB043	Hals Havn	56.9900271	10.31142238	9	14	18	68
HB044	Aalborg Sejlklub	57.057866	9.876993662	9	5	12	41
HB045	Nibe Havn	56.9885409	9.631969266	10	2	10	48
HB046	Løgstør Havn	56.9686677	9.245300567	5	9	9	40
HB047	Lemvig Marina	56.5658271	8.299550336	9	11	15	46
HB048	Thisted Havn	56.9516	8.698348033	10	4	11	36
HB049	Glyngøre Havn	56.7646885	8.864293357	12	9	16	49
HB050	Øster Hurup Havn	56.8052806	10.28487274	9	16	19	52
HB051	Bønnerup Lystbådehavn	56.5370003	10.7120727	15	4	15	74
HB052	Grenå Havn, Kattegat Centeret	56.4055966	10.92762453	0	15	15	80
HB053	Ebeltoft Havn	56.1964988	10.66876952	6	7	11	82
HB054	Aarhus Havn	56.1661881	10.23171113	10	19	20	89
HB055	Hanstholm Havn	57.1313165	8.595403582	15	0	15	66
HB056	Hobro Lystbådehavn	56.6430695	9.812892531	8	3	8	33

HB057	Hou Lystbådehavn	55.9093138	10.25382275	23	14	25	50
HB058	Snaptun Havn	55.823202	10.05220415	16	13	20	60
HB059	Vejle, Høfde v. Albuen	55.7043195	9.581988459	7	10	14	55
HB060	Kolding Lystbådehavn	55.489606	9.501512681	11	4	13	87
HB061	Hejlsminde Havn	55.3600438	9.602759895	14	4	15	65
HB062	Aabenraa Havn	55.0369512	9.425765574	10	11	16	56
HB063	Årøsund Lystbådehavn	55.2626317	9.712359315	9	17	19	61
HB064	Østerby Havn	57.32281	11.12643115	4	14	17	89
HB065	Lohals Havn	55.1351532	10.90125357	8	14	16	84
HB066	SpodsbjergHhavn	54.9321749	10.83344918	16	19	29	64
HB067	Bagenkop Havn	54.7528331	10.66949082	13	24	27	72
HB068	Nakskov Havn	54.8334535	11.12125257	2	6	8	45
HB069	Bandholm Havn	54.8392666	11.49091398	11	12	15	32
HB070	Onsevig Havn	54.9475762	11.10701457	7	13	17	36
HB071	Rødby Havn	54.6535178	11.3450499	5	2	7	68
HB072	Klintholm Havn	54.9512683	12.46349218	23	4	24	54
HB073	Ballen Havn	55.8166626	10.64239154	0	13	13	68
HB074	Sælvig Havn	55.8647406	10.54814815	5	11	13	66
HB075	Præstø Havn	55.1259681	12.0465743	10	7	11	58
HB076	Faxe Ladeplads Havn	55.2133411	12.16350755	8	19	19	63
HB077	Rødvig Havn	55.2518821	12.37549021	9	13	14	68
HB078	Bøgeskov Havn	55.3704347	12.41580548	15	16	21	65
HB079	Køge Havn	55.4725221	12.20010351	13	17	19	84
HB080	Brøndby Havn	55.6068852	12.44068248	20	17	22	87
HB081	Den Blå Planet	55.6366835	12.65783143	21	24	26	97
HB082	Skovshoved Havn	55.7638668	12.60254412	13	14	18	99
HB083	Nivå Havn	55.9375451	12.52973501	14	12	19	108
HB084	Helsingør Havn, Øresundsakvariet	56.0448946	12.61728436	19	12	22	116
HB085	Hornbæk Havn	56.0961577	12.45820781	5	2	6	106
HB086	Høfde, Holløselund, Kaprifolievej	56.0726263	12.09738984	2	3	5	64

HB087	Hundested Havn	55.9676813	11.84426657	10	9	15	83
HB088	Veddelev Havn	55.6786483	12.06800243	6	0	6	40
HB089	Hammer Bakke Havn	55.7572697	11.85077095	5	11	11	74
HB090	Høfde, Dybesø, Nørrevang	55.9674587	11.74551014	5	5	9	85
HB091	Odden Havn	55.9723724	11.37188007	0	17	17	86
HB092	Sjællands Odde færgehavn, v. Molslinjen	55.9719832	11.29755724	1	6	6	87
HB093	Havnsø Havn	55.7549559	11.32551172	5	16	17	62
HB094	Røsnæs Havn	55.7521592	10.94470469	14	8	16	72
HB095	Mullerup Havn	55.4936602	11.17216513	7	14	18	57
HB096	Halskov, den gamle Færgehavn	55.344937	11.10420094	17	13	23	92
HB097	Stigsnæs Færgehavn	55.2120917	11.24334288	24	12	26	70
HB098	Høfde, v. Bisserup	55.1984145	11.51211866	4	13	16	53
HB099	Karrebæksminde Havn	55.1745591	11.63851523	15	12	20	60
HB100	Nyborg Nord Mole	55.2975596	10.85349135	0	15	15	79

Table S2: Overview of seasonal presence and read abundance in both autumn and spring of all metazoans and bacteria identified with eDNA. The columns “Autumn” and “Spring” indicate whether a species was found (“X”) or not (blank) during the sampling event for this season. The columns “No. loc. Au” and “No. loc. Sp” show the number of locations a species was present at in autumn and spring, respectively. The column “No. loc. total” is the total number of different locations a species is present. For fish detections, see Table 1.

Class	Order	Family	Species	Autumn	Spring	No. loc. Au	No. loc. Sp	No. loc. total	Reads Au	Reads Sp	Total reads
Amphibia	Caudata	Salamandridae	<i>Lissotriton vulgaris</i>	X		5	0	5	1449	0	1449
Asteroidea	Forcipulatida	Asteriidae	<i>Asterias rubens</i>	X	X	13	23	32	1715	15022	16737
Aves	Anseriformes	Anatidae	<i>Anas</i> sp.	X	X	40	22	49	91579	55730	147309
Aves	Anseriformes	Anatidae	<i>Anser</i> sp.	X	X	16	7	21	2151	7473	9624
Aves	Anseriformes	Anatidae	<i>Aythya fuligula</i>	X		4	0	4	572	0	572
Aves	Anseriformes	Anatidae	<i>Branta bernicla</i>	X	X	15	4	16	29429	47225	76654
Aves	Anseriformes	Anatidae	<i>Branta canadensis</i>	X	X	4	8	11	4249	17694	21943
Aves	Anseriformes	Anatidae	<i>Cygnus olor</i>	X	X	29	19	36	89074	16822	105896
Aves	Anseriformes	Anatidae	<i>Mareca penelope</i>	X		14	0	14	21243	0	21243
Aves	Anseriformes	Anatidae	<i>Somateria mollissima</i>	X	X	24	16	35	23863	9868	33731
Aves	Anseriformes	Anatidae	<i>Tadorna tadorna</i>	X	X	1	0	1	904	0	904
Aves	Charadriiformes	Laridae	<i>Chroicocephalus ridibundus</i>	X	X	29	2	30	14524	1031	15555
Aves	Charadriiformes	Laridae	<i>Larus</i> sp.	X	X	57	37	70	62359	44982	107341
Aves	Columbiformes	Columbidae	<i>Columba livia</i>	X		6	0	6	32755	0	32755
Aves	Columbiformes	Columbidae	<i>Columba palumbus</i>	X	X	18	9	25	7583	12390	19973
Aves	Galliformes	Phasianidae	<i>Gallus gallus</i>	X	X	40	18	54	75356	39427	114783
Aves	Galliformes	Phasianidae	<i>Meleagris gallopavo</i>	X	X	6	2	8	1493	1914	3407
Aves	Galliformes	Phasianidae	<i>Phasianus colchicus</i>	X	X	6	4	10	23954	8879	32833
Aves	Gruiformes	Rallidae	<i>Fulica atra</i>	X	X	8	2	8	21013	1305	22318
Aves	Passeriformes	Corvidae	<i>Corvus</i> sp.		X	0	2	2	0	1035	1035
Aves	Passeriformes	Passeridae	<i>Passer domesticus</i>	X	X	4	0	4	1296	0	1296
Aves	Passeriformes	Passeridae	<i>Passer montanus</i>	X	X	4	0	4	1208	0	1208
Aves	Passeriformes	Sturnidae	<i>Sturnus vulgaris</i>	X	X	5	0	5	795	0	795
Aves	Passeriformes	Turdidae	<i>Turdus merula</i>	X	X	15	0	15	4702	0	4702

Aves	Passeriformes	Turdidae	<i>Turdus philomelos</i>	X	X	1	0	1	1181	0	1181
Aves	Pelecaniformes	Ardeidae	<i>Ardea cinerea</i>	X	X	16	20	33	1485	22613	24098
Mammalia	Artiodactyla	Bovidae	<i>Bos taurus</i>	X	X	49	28	64	103303	42948	146251
Mammalia	Artiodactyla	Bovidae	<i>Ovis aries</i>	X	X	7	9	15	2287	847	3134
Mammalia	Artiodactyla	Cervidae	<i>Capreolus capreolus</i>	X	X	7	1	8	37580	1502	39082
Mammalia	Artiodactyla	Cervidae	<i>Cervus elaphus</i>		X	0	1	1	0	4137	4137
Mammalia	Artiodactyla	Phocoenidae	<i>Phocoena phocoena</i>	X	X	13	10	21	28475	2790	31265
Mammalia	Artiodactyla	Suidae	<i>Sus scrofa</i>	X	X	51	49	79	117125	76326	193451
Mammalia	Carnivora	Canidae	<i>Canis lupus</i>	X	X	52	45	78	120091	90179	210270
Mammalia	Carnivora	Canidae	<i>Vulpes vulpes</i>	X	X	8	0	8	25031	0	25031
Mammalia	Carnivora	Felidae	<i>Felis catus</i>	X	X	14	7	19	2239	1124	3363
Mammalia	Carnivora	Mustelidae	<i>Lutra lutra</i>	X		1	0	1	1033	0	1033
Mammalia	Carnivora	Mustelidae	<i>Martes foina</i>	X		2	0	2	1010	0	1010
Mammalia	Carnivora	Mustelidae	<i>Neovison vison</i>	X	X	6	0	6	4944	0	4944
Mammalia	Carnivora	Phocidae	<i>Phoca vitulina</i>	X	X	15	3	17	34346	1960	36306
Mammalia	Eulipotyphla	Soricidae	<i>Sorex araneus</i>	X	X	3	0	3	10466	0	10466
Mammalia	Lepus sp.	Lepus sp.	<i>Lepus sp.</i>		X	0	1	1	0	784	784
Mammalia	Perissodactyla	Equidae	<i>Equus caballus</i>	X	X	4	4	8	1465	2957	4422
Mammalia	Primates	Hominidae	<i>Homo sapiens</i>	X	X	95	92	100	441887	880561	1322448
Mammalia	Rodentia	Cricetidae	<i>Arvicola amphibius</i>	X		11	0	11	1033	0	1033
Mammalia	Rodentia	Cricetidae	<i>Micromys agrestis</i>	X	X	12	0	12	2886	0	2886
Mammalia	Rodentia	Cricetidae	<i>Myodes glareolus</i>	X	X	3	2	5	3536	992	4528
Mammalia	Rodentia	Muridae	<i>Apodemus flavicollis</i>	X		5	0	5	5438	0	5438
Mammalia	Rodentia	Muridae	<i>Apodemus sylvaticus</i>	X		2	0	2	2207	0	2207
Mammalia	Rodentia	Muridae	<i>Rattus norvegicus</i>	X	X	27	9	33	9085	13642	22727
Mammalia	Rodentia	Sciuridae	<i>Sciurus vulgaris</i>	X	X	0	2	2	0	5196	5196
Alphaproteobacteria	Rhodobacteraceae	Planktomarina	<i>Planktomarina temperata</i>	X	X	11	12	17	1120	1758	2878
Alphaproteobacteria	Rhodobacteraceae	Sulfitobacter	<i>Sulfitobacter sp. BSW21498</i>		X		2	2	6304		6304
Gammaproteobacteria	Alteromonadales	Alteromonadaceae	<i>Salinimonas sediminis</i>	X	X	4	0	4	1113	0	1113

Table S3: Identical barcodes in the 12S region using Tele02 primers, between Danish occurring fish species.

Species one	Species two	Species three
<i>Merlangius merlangus</i>	<i>Melanogrammus aeglefinus</i>	
<i>Leuciscus idus</i>	<i>Leuciscus leuciscus</i>	
<i>Hyperoplus lanceolatus</i>	<i>Ammodytes tobianus</i>	<i>Ammodytes marinus</i>
<i>Chelon labrosus</i>	<i>Chelon ramada</i>	
<i>Carassius auratus</i>	<i>Carassius carassius</i>	
<i>Acipenser baerii</i>	<i>Acipenser gueldenstaedtii</i>	
<i>Alosa alosa</i>	<i>Alosa fallax</i>	
<i>Anarhichas denticulatus</i>	<i>Anarhichas lupus</i>	
<i>Pseudorasbora parva</i>	<i>Cyprinus carpio</i>	
<i>Schedophilus medusophagus</i>	<i>Centrolophus niger</i>	
<i>Eutrigle gurnardus</i>	<i>Chelidonichthys lucerna</i>	<i>Chelidonichthys cuculus</i>
<i>Dipturus intermedius</i>	<i>Dipturus batis</i>	
<i>Lampetra planeri</i>	<i>Lampetra fluviatilis</i>	