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Research Article

Preferential presence in harbours confirms the non-indigenous species status of *Ammonia confertitesta* (Foraminifera) in the English Channel

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Abstract

Though the morphological discrimination of the three pseudo-cryptic *Ammonia* species, *A. aberdoveyensis*, *A. confertitesta* and *A. veneta*, has been recently established, information on their ecology and habitats are still relatively scarce. This study aims to define distribution patterns of these species at eight sites scattered along the French coasts of the English Channel, over a total of 39 stations. These sites were classified into two habitats, either harbours (heavily modified sites) or less impacted (moderately influenced sites). The use of *IndVal* index (an index based on how a species is statistically specific to a habitat) clearly indicates that *A. confertitesta* is recorded preferentially in or close to harbours. Considering its non-indigenous species (NIS) status in Europe, we investigated its reported occurrences in Europe in the literature. It almost always showed a proximity to major European harbours. Sometimes, this species occurred relatively far away from these harbours, suggesting a secondary spread. Finally, this work interprets *A. confertitesta* being a NIS in the eastern English Channel with assumptions of being invasive regarding its dominance over the indigenous species *A. aberdoveyensis* and *A. veneta*. Complementary works such as retrospective core studies of fossil faunas are needed to quantitatively assess when and where *A. confertitesta* was introduced in Europe and potentially started to replace its congenerics *A. veneta* and *A. aberdoveyensis*.

Key words: benthic foraminifera, *Ammonia* species, exotic species, Northeast Atlantic, International commercial harbours

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Introduction

Several studies reported the presence of various non-indigenous species (NIS) along the French coasts of the English Channel, mostly in harbours such as Le Havre (review in Pezy et al. 2021), Dunkerque (Brylinski 1981; Gothland et al. 2014; Antajan 2014; Dauvin et al. 2019), Calais (Brylinski et al. 2012) and Caen-Ouistreham (Charles et al. 2018; Faillettaz et al. 2020). Most of these NIS originate from the North Pacific Ocean and were first reported in anthropised areas such as harbours and marinas (Minchin et al. 2013). Worldwide, harbours are acknowledged as one of the main gateways for the introduction of NIS. Specifically, NIS are transported *via* ballast waters, sediments and hull fouling from commercial shipping (Goulletquer et al. 2002; Oliveira 2007; Minchin et al. 2013). The English Channel noticeably hosts at least 152 NIS belonging to different groups of organisms (Goulletquer et al. 2002; Dewarumez et al. 2011; Pezy et al. 2021) such as ctenophores (Antajan 2014), copepods (Seuront 2005; Brylinski et al. 2012), sea squirts (Dupont et al. 2007), isopods (Raoux et al. 2020), bivalves (Faillettaz et al. 2020; Dauvin et al. 2022), polychaetes and amphipods (Spilmont et al. 2018). To the best of our knowledge, the only NIS foraminifera recorded in the English Channel is *Trochammina hadai*, Uchio 1962 (Bouchet et al. 2023). The number of exotic foraminifera may be largely underestimated considering the numerous NIS macro-invertebrates recorded in the English Channel so far (see above references). While foraminifera are often overlooked in NIS surveys due to their minute size, they occupy a pivotal position in the benthic food web by linking lower and higher trophic levels (Lipps and Valentine 1970; Nomaki et al. 2008; Haynert et al. 2020). Because NIS proliferation may impact ecosystem functioning (McKinney and Lockwood 1999; Sousa et al. 2011; Mayer-Pinto et al. 2015), it is important to track these inconspicuous invaders.

Ammonia is one of the most common foraminiferal genera in intertidal area and shallow waters of the English Channel coast (Alve 2001; Hart et al. 2020; Richirt et al. 2021). In the English Channel, records of *Ammonia* species are mostly lumped under the morphogroup *Ammonia tepida* (Swallow 2000; Armynot du Châtelet et al. 2009, 2018; Francescangeli et al. 2020). Nevertheless, *A. tepida* actually represents a complex of three pseudo-cryptic species, first discriminated by molecular studies (Hayward et al. 2004), and only recently using morphological criteria (Richirt et al. 2019; Pavard et al. 2021). Among these three species, i.e. *A. veneta*, Schultze 1854, *A. aberdoveyensis* Haynes 1973 and *A. confertitesta*, Zheng 1978 (respectively phylotypes T1, T2 and T6; Hayward et al. 2021), *A. confertitesta* is regarded as a NIS originating from Asia and introduced in Europe through ballast waters (Hayward et al. 2004; Pawlowski and Holzmann 2008), a hypothesis supported by the remarkable disjunct distribution of *Ammonia confertitesta* between Europe and Asia (Hayward et al. 2021). The complete absence of *Ammonia* in the Baltic Sea (Hermelin 1987; Murray 2006) until the first record of *A. confertitesta* in sediment dated from 2000 (Schweizer et al. 2011) further suggests that this species is a NIS. In Europe, it has been reported in Hanö Bay (Sweden; Bird et al. 2020), the Elbe Estuary (Germany; Francescangeli et al. 2021), Lake Grevelingen (the Netherlands; Richirt et al. 2020), Great Britain coasts of the North Sea, the English Channel, the Celtic Sea and the Irish Sea (Saad and Wade 2016), along the French coasts of the eastern English Channel (Richirt et al. 2021) and further south along the French Atlantic coasts, in the 'Baie de l'Aiguillon' (Bird et al. 2020), in the Gironde Estuary (Pavard et al. in press) and also in the Mediterranean Sea (Camargue; Richirt et al. 2019).

In this context, the objectives of this study are (i) to document the distribution of the three *Ammonia* species along the French coasts of the eastern English Channel, (ii) to compare their distribution between several water bodies and (iii) to further confirm the NIS status of *Ammonia confertitesta* in Europe.

Material and methods

Sampling sites and stations

A total of 39 stations were sampled at eight sites of transitional environments (*sensu* McLusky and Elliott 2007) in the eastern English Channel along French coasts (Fig. 1). Five sites were harbours and were considered as water bodies heavily modified by human activities (WFD 2000/60/EC), i.e. Caen-Ouistreham (CO), Le Havre (LH), Calais (CL), Boulogne-sur-Mer (BL) and Dunkerque (DK). The three other sites were considered as less impacted sites moderately influenced by human activities (Nasseh and Texier 2000; Poirier et al. 2006; Caplat et al. 2006), i.e. the Bay of Veys (BV), the Orne River (O) and the Authie Estuary (AE). For all sites, the sampling date and a brief description of each station is reported in Table 1. All stations from harbours were sampled in shallow subtidal environment (maximum depth: 18 m) except for all stations in Boulogne-sur-Mer and the station LH1 in Le Havre that were sampled in intertidal environment. All stations of sites considered as less impacted outside harbours were sampled in intertidal environment.

Environmental parameters

Environmental parameters were assessed from four replicated sediment cores at each station. Three replicates were dedicated to measurement of Total Organic Carbone (TOC) and one to grain size characterisation. The three replicates for TOC analysis were first frozen, freeze-dried, crushed and pre-acidified. Then, TOC content was determined by high-temperature combustion of dry samples (60 °C, 48 h). Measurements of CO_2 were finally done by thermal conductometry using an elemental analyser (FlashEA, Thermo Electron Corporation). Sediment grain size was obtained by diffraction and diffusion of a monochromatic laser beam on suspended particles (Trentesaux et al. 2001).

Foraminiferal sampling, *Ammonia* species identification and descriptors of species assemblages

Foraminiferal community compositions were assessed from three replicate cores, except in Boulogne-sur-Mer where only one replicate was sampled. The surface sediment (0–1 cm) was sampled from three different deployments with a Reineck corer (160 cm^2) for subtidal stations or a handcorer (56 cm^2) for intertidal stations. Sediment samples were preserved in ethanol and Rose Bengal solution (2 g L^{-1}). A total of 107 samples from the 39 stations were used for foraminiferal analysis in this study.

In the laboratory, samples were sieved through a 63μm-mesh and the fraction >63μm was dried at 50 °C. Foraminifera were then concentrated by flotation using trichloroethylene (density = 1.46). For each sample, at least 300 living (stained) benthic foraminiferal individuals were collected and identified to the species level when possible, for both statistical validity (Fatela and Taborda 2002) and representativity

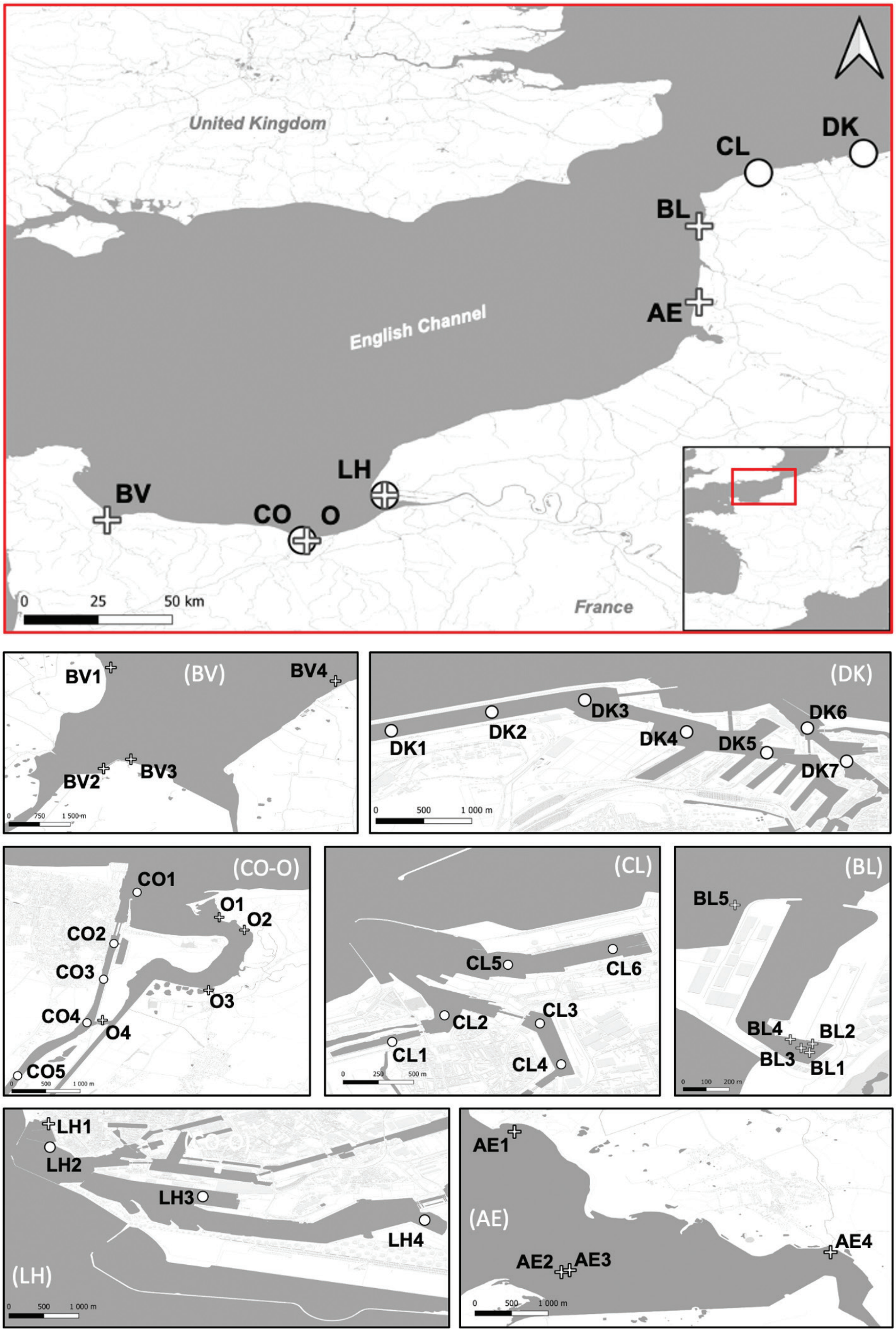

Figure 1. Location of the sampling sites (red outline) and position of the sampling stations (black outline) on French coasts of the English Channel. White crosses and circles respectively represent intertidal and subtidal sites. Names of sites are abbreviated such as BV: Bay of Veys, CO (Caen-Ouistreham harbour) O (Orne estuary), LH (Le Havre harbour), AE (Authie estuary), BL (Boulogne-sur-Mer harbour), CL (Calais harbour), DK (Dunkerque harbour).

Table 1. Sites, dates of sampling, anthropogenic impact level, station ID, GPS coordinates and typological description.

Site-Date-Impact level	Station	Coordinates (Latitude, Longitude	Description
Foram-INDIC project			
Bay of Veys (BV)	BV1	49°23'35"N, 1°9'27"W	Intertidal, located at the limit of the bay, sandy mud sediment
09/02/2019	BV2	49°21'33"N, 1°9'36"W	Intertidal, located at the mouth of a river, slightly sandy mud sediment
Less impacted	BV3	49°21'44"N, 1°9'3"W	Intertidal, located at the mouth of a river, sandy mud sediment
	BV4	49°23'19"N, 1°4'55"W	Intertidal, located at the limit of the bay, sandy mud sediment
Caen-Ouistreham (CO)	CO1	49°17'11"N, 0°14'42"W	Subtidal, located before the sluice and direct link to the sea, sandy mud sediment
09/10/2019	CO2	49°16'34"N, 0°14'59"W	Subtidal, located after the sluice in the canal, slightly sandy mud sediment
Harbour	CO3	49°16'8"N, 0°15'6"W	Subtidal, located after the sluice in the canal, slightly sandy mud sediment
	CO4	49°15'36"N, 0°15'18"W	Subtidal, located after the sluice in the canal, slightly sandy mud sediment
	CO5	49°14'58"N, 0°16'8"W	Subtidal, located after the sluice in the canal, slightly sandy mud sediment
Orne River (O)	O1	49°16'53"N, 0°13'43"W	Intertidal, sandy mud sediment
09/03/2019	O2	49°16'44"N, 0°13'25"W	Intertidal, slightly sandy mud sediment
Less impacted	O3	49°16'0"N, 0°13'51"W	Intertidal, slightly sandy mud sediment
	O4	49°15'38"N, 0°15'7"W	Intertidal, slightly sandy mud sediment
Le Havre (LH)	LH1	49°29'26"N, 0°5'48"E	Intertidal, in a marina, mud sediment
09/11/2019	LH2	49°29'9"N, 0°5'49"E	Subtidal, in the industrial part, mud sediment
Harbour	LH3	49°28'34"N, 0°7'38"E	Subtidal, in the industrial part, mud sediment
	LH4	49°28'17"N, 0°10'15"E	Subtidal, in the industrial part, mud sediment
Authie estuary (A)	AE1	50°23'38"N, 1°33'53"E	Intertidal, located in the bay part of the estuary, slightly sandy mud sediment
10/16/2019	AE2	50°22'1"N, 1°34'25"E	Intertidal, located at the mouth of the estuary, slightly sandy mud sediment
Less impacted	AE3	50°22'2"N, 1°34'31"E	Intertidal, located at the mouth of the estuary, sandy mud sediment
	AE4	50°22'14"N, 1°37'31"E	Intertidal, located in the river of Authie, slightly sandy mud sediment
Calais (CL)	CL1	50°57'39"N, 1°50'39"E	Subtidal, in a marina separated by a sluice, mud sediment
10/29/2019	CL2	50°57'48"N, 1°50'58"E	Intertidal, sampled at high tide, located after the sluice with a direct link to the sea, mud sediment
Harbour	CL3	50°57'45"N, 1°51'33"E	Subtidal, in the industrial basin of the harbour, separated from the sea by a sluice, mud sediment
	CL4	50°57'31"N, 1°51'40"E	Subtidal, in the industrial basin of the harbour, separated from the sea by a sluice, slightly sandy mud sediment
	CL5	50°58'7"N, 1°51'21"E	Subtidal, located in the ferries part of the harbour, slightly sandy mud sediment
	CL6	50°58'12"N, 1°51'59"E	Subtidal, located in the ferries part of the harbour, slightly sandy mud sediment
Dunkerque (DK)	DK1	51°2'58"N, 2°18'14"E	Subtidal, located in the canal of the main industrial basin, slightly sandy mud sediment
09/08/2020	DK2	51°3'8"N, 2°19'8"E	Subtidal, located in the canal of the main industrial basin, slightly sandy mud sediment
Harbour	DK3	51°3'14"N, 2°19'58"E	Subtidal, located in the canal of the main industrial basin, slightly sandy mud sediment
	DK4	51°2'57"N, 2°20'53"E	Subtidal, in the main industrial basin, slightly sandy mud sediment
	DK5	51°2'46"N, 2°21'36"E	Subtidal, in the main industrial basin, slightly sandy mud sediment
	DK6	51°2'59"N, 2°21'57"E	Subtidal, located in the marina, slightly sandy mud sediment
	DK7	51°2'41"N, 2°22'18"E	Subtidal, located in the marina, mud sediment
SURICATES project			
Boulogne-sur-Mer (BL)	BL1	50°43'3"N, 1°34'32"E	Intertidal, industrial basin of the harbour, muddy sand sediment
18/03/2019	BL2	50°43'5"N, 1°34'32"E	Intertidal, industrial basin of the harbour, slightly muddy sand sediment
Harbour	BL3	50°43'4"N, 1°34'30"E	Intertidal, industrial basin of the harbour, muddy sand sediment
	BL4	50°43'6"N, 1°34'27"E	Intertidal, industrial basin of the harbour, slightly muddy sand sediment
	BL5	50°43'38"N, 1°34'15"E	Intertidal, outside of the harbour, in front of dike, slightly sandy mud sediment

of foraminiferal species assemblages (Schönfeld et al. 2012). Among these 300 individuals, *Ammonia* species were identified morphologically under a stereomicroscope following Pavard et al. (2021). Specifically, *Ammonia aberdoveyensis*, *A. confertitesta* and *A. veneta* were morphologically discriminated following a dichotomous procedure of discrimination based on the pore diameter and the elevation of sutures on the central part of the spiral side (Richirt et al. 2019; Richirt et al. 2021).

For each station, relative abundances (mean ± standard deviation) and absolute abundances (ind 50cm^{-2}, mean ± standard deviation) were calculated (excluding for the site of Boulogne-sur-Mer, where only one sample was taken). Relative abundances were also calculated at the scale of sites (containing several stations). As number of stations sampled was not constant between sites (e.g. four in the Authie Estuary and seven in Dunkerque harbour) and sampling was not done at the same time (e.g. Boulogne-sur-Mer and Dunkerque harbours), relative abundances data were used instead of absolute abundances to have comparable data. Count, normalised and relative abundance data are available as Suppl. materials 1–3.

Indicator Value calculation

The Indicator Value index, *IndVal*, allows to determine if a species is indicative of a specific habitat (Dufrêne and Legendre 1997). We applied the calculation of IndVal on the normalised abundance dataset to assess if the three *Ammonia* species investigated here could be typical of stations in harbours or of stations located in less impacted sites outside harbours. IndVal is calculated as:

$$IndVal_{ij} = A_{ij} \times B_{ij} \times 100$$

where IndVal is the Indicator Value (%) of a species i at stations of group j (i.e. either harbour or less impacted), with A_{ij} = $Nindividuals_{ij}$ /$Nindividuals_i$, and B_{ij} = $Nstations_{ij}$ / $Nstations_j$. A_{ij} is a measure of specificity of species i where $Nindividuals_{ij}$ is the mean number of individuals of species i across sites of group j. $Nindividuals_i$ is the sum of mean numbers of individuals of species i over all groups. B_{ij} is a measure of fidelity. $Nstations_{ij}$ is the number of stations of j group where species i is present and $Nstations_j$ is the total number of stations in that group j. Normalised numbers of individuals (for 50 cm^2) were used to calculate A_{ij}. The IndVal index calculation (iterations: n = 999; package *labdsv* 2.0-1, Roberts and Roberts 2016) was performed using the software R 4.2-1 (R Core Team, 2022).

Investigation of occurrences of *A. confertitesta* next to international commercial harbours in Europe

To investigate the causal link between the presence of commercial harbours and the occurrence of *Ammonia confertitesta*, we compiled datasets on its distribution from this study and from previously published distribution of this species in Europe (Holzmann 2000; Langer and Leppig 2000; Hayward et al. 2004; Ertan et al. 2004; Schweizer et al. 2011; Saad and Wade 2016; Chronopoulou et al. 2019; Richirt et al. 2019, 2021; Bird et al. 2020; Francescangeli et al. 2021). We also indicated the distance between the species occurrences and close commercial harbours and their equivalent volume of tonnage of freight (Suppl. material 4).

Results

Environmental parameters

Total Organic Carbon values ranged from 0.81 ± 0.09% (BV3) to 10.69 ± 0.40% (D1; Table 2). Stations in the Bay of Veys exhibited the lowest values of TOC and ranged between 0.81 ± 0.09% (BV3) and 0.79 ± 0.04% (BV2). Conversely, stations in Dunkerque showed the highest TOC values, ranging from 1.45 ± 0.18% (DK4) to 10.69 ± 0.40% (DK1). On average, TOC values were higher in

Table 2. TOC content (%), proportions of silt and sand in sediment (%) in all sampled stations. Mean ± sd values by sites for each parameter are italicised.

Site	Station	TOC (%)	Silt (%)	Sand (%)
Dunkerque	DK1	10.69 ± 0.40	90.0	9.8
	DK2	4.16 ± 1.15	84.3	15.6
	DK3	4.06 ± 0.05	89.5	10.4
	DK4	3.38 ± 0.42	88.8	11.1
	DK5	3.40 ± 0.10	85.3	14.6
	DK6	3.79 ± 0.09	95.0	4.7
	DK7	4.21 ± 0.08	94.1	5.8
	Mean	**4.81 ± 2.61**	**89.6 ± 4**	**10.3 ± 4.1**
Calais	CL1	3.70 ± 0.04	95.7	4.1
	CL2	4.22 ± 0.57	95.3	4.6
	CL3	3.85 ± 0.16	95.9	3.9
	CL4	2.32 ± 0.27	84.0	15.9
	CL5	1.47 ± 0.16	78.7	21.1
	CL6	1.07 ± 0.22	84.5	14.1
	Mean	**2.77 ± 1.33**	**89 ± 7.5**	**10.6 ± 7.4**
Boulogne-sur-Mer	BL1	1.4	33.4	66.4
	BL2	2.4	19.8	80.1
	BL3	2.2	32.6	67.2
	BL4	1.8	18.6	81.3
	BL5	1.9	85.9	13.8
	Mean	**1.9 ± 0.4**	**38.1 ± 27.6**	**61.8 ± 27.7**
Authie	AE1	1.19 ± 0.35	81.6	18.3
	AE2	1.61 ± 1.61	75.4	24.4
	AE3	1.1 ± 0.21	64.9	34.9
	AE4	1.88 ± 0.29	82.6	16.6
	Mean	**1.44 ± 0.37**	**76.1 ± 8.2**	**23.5 ± 8.3**
Le Havre	LH1	3.18 ± 0.07	94.4	5.2
	LH2	2.1 ± 0.14	93.6	6.2
	LH3	2.6 ± 0.03	95.6	4.1
	LH4	2.96 ± 0.14	95.2	4.2
	Mean	**1.16 ± 0.2**	**94.7 ± 0.9**	**4.9 ± 1**
Orne	O1	1.57 ± 0.1	73.6	26.2
	O2	1.36 ± 0.15	77.6	22.1
	O3	2.27 ± 0.08	88.2	11.7
	O4	2.54 ± 0.15	89.6	10.3
	Mean	**1.93 ± 0.56**	**82.3 ± 7.9**	**17.6 ± 7.8**
Caen-Ouistreham	CO1	0.83 ± 0.36	63.4	36.2
	CO2	3.03 ± 0.23	91.4	8.5
	CO3	1.95 ± 0.91	84.1	15.8
	CO4	1.87 ± 0.21	82.4	17.4
	CO5	3.83 ± 0.2	78.4	21.6
	Mean	**2.30 ± 1.16**	**79.9 ± 10.4**	**19.9 ± 10.3**
Bay of Veys	BV1	0.93 ± 0.02	72.9	26.9
	BV2	1.84 ± 0.09	84.0	15.9
	BV3	0.81 ± 0.09	61.4	38.4
	BV4	1.43 ± 0.46	52.9	46.9
	Mean	**1.25 ± 0.48**	**67.8 ± 13.5**	**32 ± 13.5**

Dunkerque, Calais, Boulogne-sur-Mer and Le Havre harbours, indicating an enrichment in organic matter (Table 2). It is more contrasted in Caen-Ouistreham harbour where only two stations over six showed values of TOC > 2.0%. In these harbours, the poorest stations in TOC were CO1, CL5 and CL6, all situated at the most opened parts (seaward). Symmetrically, highest concentrations were measured in the most enclosed parts of the harbours, most of the time separated from the open sea by a sluice (e.g. DK1, CL3 and CO5). Sediment samples from harbour sites (i.e. Dunkerque, Calais, Le Havre) generally had a smaller grain size than samples in less impacted ones (Authie, Orne, Bay of Veys; Table 2). Silt content in Dunkerque harbour ranged from 84.3% (DK2) to 95.0 (DK7) and from 93.6% (LH2) to 95.6% (LH3) in Le Havre harbour. Conversely, stations of the Bay of Veys, the Orne and the Authie estuaries exhibited in general a higher sand content, often reaching from 20% to more than 40% in BV4 for example (Table 2). It is more contrasted in stations more exposed to open sea (i.e. CO1 and CL5) which are less muddy than other stations from their sampling sites. Finally, highest content in sand were measured in the Boulogne-sur-Mer harbour with values ranging from 66.4% to 81.3% except for the most external station BL5, which showed a substantially lower value (13.8%).

Distribution of *Ammonia* species and specific habitat associated

Among *Ammonia* species, *A. aberdoveyensis* dominated in less impacted sites (Fig. 2A), especially in both the Bay of Veys (56.2 ± 27.9%) and the Orne Estuary (68.7 ± 12.0%). In contrast, *A. confertitesta* dominated *Ammonia* assemblages in almost all harbours, especially in Le Havre and Caen-Ouistreham where it dominated all stations (Fig. 2). It was more contrasted in Dunkerque, where only three out of seven stations were dominated by *A. confertitesta* (i.e. DK3, DK4, DK5) and *A. aberdoveyensis* dominated elsewhere (Fig. 2B). Only few *Ammonia* individuals were found in DK6 and DK7, and as such those results should be considered with caution (i.e. < 20 individuals per sample; Suppl. material 1). *Ammonia veneta* was barely found in harbours, with relative abundances ranging from 1.4 ± 2.4% in Dunkerque to 9.4 ± 13.7% in Caen-Ouistreham but showed greater proportions in the less impacted sites of the Bay of Veys (28.5 ± 36.2%) and the Orne Estuary (27.5 ± 11.6%). No *Ammonia* individuals were found in the Authie Estuary. Finally, over the 35 stations of sites investigated where *Ammonia* was present, only four stations (i.e. 11.4%) did not contain any *Ammonia* individuals, three (8.6%) contained only one of the three species, two species co-occurred in four stations (11.4%) and the three *Ammonia* species co-occurred in 24 stations (68.6%).

Only *A. confertitesta* in harbours (i.e. highly impacted habitats) showed *IndVal* value greater than 60.0% (86.6%, $p < 0.001$; Table 3). Concerning the other two species, none of the *IndVal* values were significant for any habitat.

Discussion

Co-occurrences and distribution of the three *Ammonia* species in French transitional waters of the English Channel

In the present work, living individuals of the three *Ammonia* species were present at all sites, except in the Authie Estuary. More specifically, the three species of *Ammonia* co-occurred in most stations. These results are sharply contrasting with molecular studies that found at most one or two *Ammonia* species co-occurring in a same station (Hayward et al. 2004; Saad and Wade 2016; Bird et al. 2020; Richirt et al. 2021). The three *Ammonia* species were recorded occurring together once at

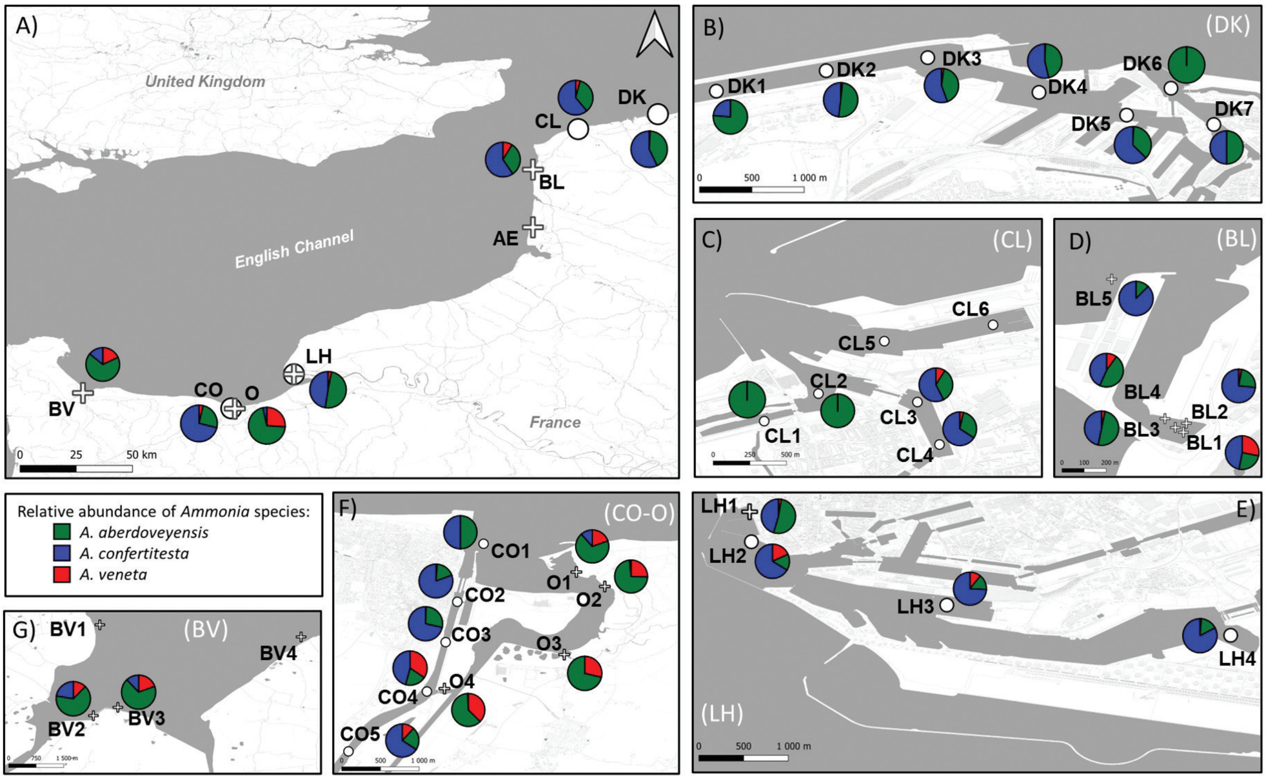

Figure 2. Maps representing pie charts of proportions of *Ammonia veneta* (red), *Ammonia aberdoveyensis* (green) and *Ammonia confertitesta* (blue) in: **A** sampled sites **B** Dunkerque harbour (DK) **C** Calais harbour (CL) **D** Boulogne-sur-Mer harbour (BL) **E** Le Havre harbour (LH) **F** Caen-Ouistreham harbour (CO) and Orne river (O) and **G** Bay of Veys (BV). AE: Authie estuary. White crosses represent sites and stations sampled in intertidal environments. White circles represent those which were sampled in subtidal environments.

Table 3. IndVal values for each species for less impacted and harbour habitats with the corresponding p-value and the preferential habitat.

Species	IndVal for less impacted habitat (%)	IndVal for harbour habitat (%)	p-value	Habitat Indicator
A. veneta	31.3	26.2	0.99	None
A. aberdoveyensis	20.7	56.5	0.13	None
A. confertitesta	1.1	86.6	0.001*	Harbours

Ouistreham (Richirt et al. 2021). Noticeably, when *A. confertitesta* occurred, it barely co-occurred with other *Ammonia* species (five out of 29 locations; Bird et al. 2020), which sharply contrasts with our observations. These differences may be related to differences in the method of species identification, i.e. molecular and morphometrical (Scanning Electron Microscope, SEM) *vs.* morphological (stereomicroscope). Indeed, single cell molecular identification is often based on few individuals, conversely to morphological methods that allow to identify a much larger number of individuals, i.e. tens to thousands. Moreover, the stereomicroscope method, compared to the SEM one, is faster to identify *Ammonia* spp. individuals and then, could more quickly generate data. In the context of globalisation, where worldwide exchanges increase and ecosystems are rapidly altered, the investigation of more sites and more individuals quickly allows to build distribution maps way easier (i.e. cheaper and less-time consuming) to respectively track NIS and potential global changes associated to anthropogenic impact. However, note that if the proportion of *Ammonia* species in an assemblage is represented by low count data (i.e. 0 to 20 individuals), the user should be aware of the inherent error rate of the morphological method (Pavard et al. 2021).Therefore, results for DK1, DK6, DK7, CL1, CL2, CL5, CL6, BL5 and BV2 should be taken with caution.

The distribution pattern of *A. veneta*, which is present at almost all sites over the French coasts of the eastern part of the English Channel, is congruent with its cosmopolitan distribution characteristic (Holzmann and Pawlowski 2000; Hayward et al. 2021). It should nevertheless be stressed that this species was generally present in low abundances and proportions compared to others *Ammonia* species, with the exception of the intertidal stations in both the Orne River and the Bay of Veys. This intertidal preference is consistent with previous observations reporting this species in intertidal ecosystems both in mud and muddy sand soft-sediments (Saad and Wade 2016).

To date, *A. aberdoveyensis* has only been encountered in the North Atlantic and the Mediterranean Sea (see review in Hayward et al. 2021). In the present study, *A. aberdoveyensis* was the major species in the less impacted habitats of the Orne Estuary and the Bay of Veys, but also in a few stations in harbours (DK1, DK2, DK6, CL1, CL2 and LH1). Results in DK6, CL1 and CL2 should, however, be taken with care as this species was represented by only one individual at each of those stations and could be the result of events of passive transport, i.e. wave action and turbulence (Alve 1999). This species was also observed at some subtidal stations, such as in a core sampled at 34 m depth in the Grevelingen lake (Richirt et al. 2020). However, Bird et al. (2020) described *A. aberdoveyensis* as a species restricted to intertidal environment. In the harbour of Le Havre, while it is dominant in the intertidal station LH1 of the recreational part of the harbour, it was also recorded in the subtidal stations in the international shipping area, suggesting that the species can dwell in both intertidal and subtidal zones.

Ammonia confertitesta is known to have a disjunct geographical distribution between Asia and Europe (Hayward et al. 2004, 2021). In this study, it was the dominant *Ammonia* species in the subtidal studied harbours. It was only present at low abundances in the intertidal stations of the Bay of Veys and in the Orne Estuary (O1) but with high abundances in all stations of Boulogne-sur-Mer and in the sole intertidal station of Le Havre harbour (LH1). This observation contrasts with previous studies that considered *A. confertitesta* as an intertidal species (Saad and Wade 2016; Bird et al. 2020), but is consistent with observations of this species from both subtidal and intertidal brackish waters (Bird et al. 2020; Holzmann 2000; Pavard et al. in press; Saad and Wade 2016; Schweizer et al. 2011) to marine subtidal waters (Petersen et al. 2016; Bird et al. 2020; Richirt et al. 2020), confirming the statement of the species being an euryhaline species (Bird et al. 2020; Hayward et al. 2021). This tolerance to a large spectrum of salinity may offer an advantage to colonise different sites as shown in this study. Considering that *A. confertitesta* is a NIS that may have the potential to become invasive species and replace its congeneric (see Richirt et al. 2022; Pavard et al. in press and the discussion below), *A. aberdoveyensis* could have found a refuge higher on shores or in the inner part of an estuary, such as in the Orne Estuary, where *A. confertitesta* is not established yet or do not favour these kinds of environments (Richirt et al. 2021). A similar pattern was previously reported in the Brancaster Staithe high marsh (England; Richirt et al. 2021), in the Verse Meer (The Netherlands; Richirt et al. 2021) and in the Gironde Estuary (France; Pavard et al. in press) where *A. aberdoveyensis* was the main *Ammonia* species in higher parts of these systems instead of *A. confertitesta*, which was more abundant in lower parts. In the Elbe Estuary, *A. confertitesta* was reported as the dominant foraminiferal species in living assemblages, though it co-occurred with *A. veneta* instead of *A. aberdoveyensis*, which was only present in dead assemblages (Francescangeli et al. 2021). In the same study, the authors showed that *A. confertitesta* replaced the species *Elphidium selseyense*, in the living assemblages over the last 40 years. This could demonstrate the ability of *A. confertitesta* to replace other species at a decadal scale.

Presence of *A. confertitesta* close to commercial harbours: is it just a coincidence?

The latest observations of *A. confertitesta* in Europe tend to confirm that it is a NIS (reported in Suppl. material 4). Its distribution pattern and its dominance over its congeneric species in international commercial harbours compared to less impacted sites highly suggests that harbours may constitute the main gateway of the introduction of this species in Europe, as previously suggested by Pawlowski and Holzmann (2008). Conversely, in Asia, *A. confertitesta* preferably occurs in natural sites in Japan (e.g. the Ramsar site of Lake Nakaumi, Toyofuku et al. 2005) and in highly anthropised site in China (e.g. Quingdao Bay, Hayward et al. 2021). Specifically, *A. confertitesta* was the major *Ammonia* species in Le Havre, Dunkerque, Calais and Caen-Ouistreham harbours, which are respectively 1st, 3rd, 4th and 13th French harbours in terms of tonnage of freight in 2019 (https://www.statistiques.developpement-durable.gouv.fr/publicationweb/326?type, https://www.caen.port.fr/le-port-de-caen-1.html) and exhibit an intense international worldwide trade. In addition, most observations of this species in France (Fig. 3, Suppl. material 4), i.e. in the Authie Estuary, the Seine Estuary, the Bay of Aiguillon (Richirt et al. 2021), the Gironde Estuary (Pavard et al. in press) and the Rhone delta (Richirt et al. 2019) were made no more than 35 km away from the international harbours of HAROPA Port, La Rochelle, Bordeaux or Marseille harbours. Likewise, in Great Britain, most

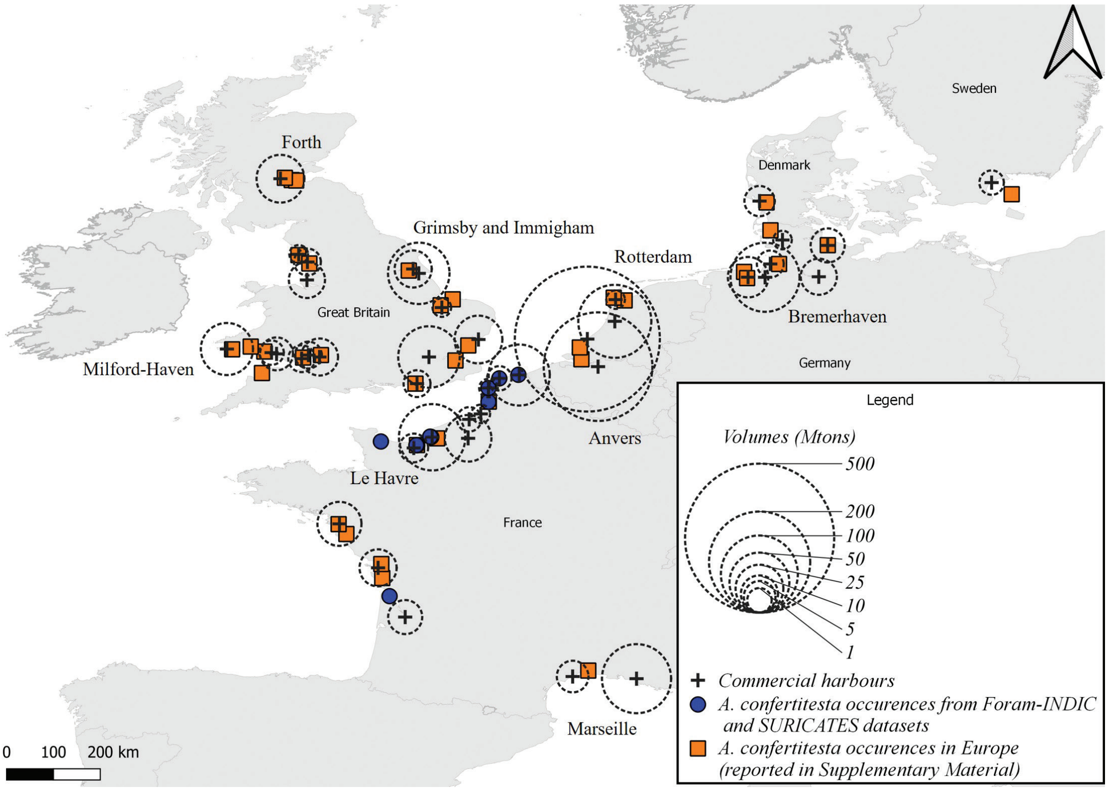

Figure 3. Map showing occurrences of *Ammonia confertitesta* in Europe (blue dots: morphological identification; orange squares: molecular identification) and nearest commercial harbours (black crosses) from occurrences. Black circles represent freight of each harbour (in Millions of tons).

observations of this species (Saad and Wade 2016; Bird et al. 2020; Richirt et al. 2021) were in the vicinity of international commercial harbours (Fig. 3). At the continental scale, from the Netherlands to Germany, Denmark or even Sweden, the distribution of *A. confertitesta* is geographically associated with harbours, which supports its NIS status in Europe. The species has likely been introduced through ballast waters or via hull fouling as already shown for other species of benthic foraminifera (Deldicq 2019; McGann et al. 2019; Bouchet et al. 2023).

Noticeably, some smaller harbours (lower freight tonnage, i.e. Boulogne-sur-Mer, Le Tréport in France, Den Helder in The Netherlands, Husum in Germany, Ahus in Sweden) and/or *A. confertitesta* occurrences are located rather far from international commercial harbours. These smaller harbours are part of national to regional trade networks and are often connected to international harbours through shipping or smaller boats. The hypothesis of a secondary spread of a NIS (i.e. from international to national/regional harbours) has been well studied (Simkanin et al. 2009; Mineur et al. 2010; Zabin 2014; Costello et al. 2022) and could explain the occurrences of *A. confertitesta* relatively far from harbours dedicated to international trade. Over time, *A. confertitesta* could then have spread further from its arrival point following this hypothesis of a secondary spread. In this context, the restricted occurrence of *A. confertitesta* at the mouth of the Orne River (O) may been surprising given its vicinity next to the Caen-Ouistreham harbour (CO). These observations may, however, correspond to several scenarios. First, this could reflect the ongoing invasion of this species in the Orne River. Another hypothesis relates to the ability of the species to disperse according to both anthropogenic and natural structures of sites (i.e. topography and/or artificial dams). Specifically, stations from these two sites are separated by a sluice which could limit their connectivity. It is also the case in the Grevelingen lake where it is separated from the sea by a sluice (e.g Richirt et al. 2020). Moreover, the tortuosity of the estuary compared to the canal could add another obstacle to the spread of *A. confertitesta*.

Finally, large proportions of *A. confertitesta* in stations (up to 87.5%) among autochthonous *Ammonia* species in assemblages clearly question its invasiveness ability. In the Grevelingen lake, the recent analysis of a sediment core covering the period 1972–2012 indicated that *A. confertitesta* was recorded from 1986 onwards and was progressively replacing its two congeneric *A. veneta* and especially *A. aberdoveyensis* over time (Richirt et al. 2022). In addition, a monthly sampling survey sampling along a transect of the Gironde Estuary has demonstrated the dominance of *A. confertitesta* among congeneric species over both time and space (Pavard et al. in press), further confirming the hypothesis of its invasiveness. As done for other foraminiferal NIS (McGann et al. 2012; Deldicq 2019; Richirt et al. 2022), the sampling of long sediment cores in sampled sites of this study would allow us to determine when *A. confertitesta* appeared in species assemblages and eventually when it outcompeted indigenous congeneric species in stations where it was the dominant species. Nonetheless, it would be relatively difficult to sample long-cores in some dynamic systems such as estuaries (e.g. Orne). Finally, a regular monitoring of sampled sites of this study should be done to see if *A. confertitesta* outcompetes its congenerics over space and time and track its possible ongoing invasion, notably in the less impacted sites, outside harbours.

Conclusion

Thanks to recent identification method only relying on their morphology, this study documents the distribution pattern of three NE Atlantic *Ammonia* species (i.e. *A. veneta*, *A. aberdoveyensis* and *A. confertitesta*) in different sites of the English

Channel French coastline. Either considered as poorly (moderately influenced estuaries) or highly (international commercial harbours) impacted by anthropogenic activities, each site was individually investigated through several sampling stations to characterise the *Ammonia* distribution patterns at smaller spatial scale. Our results show that conversely to previous studies, the three *Ammonia* species were co-occurring in most cases (24 on 35 stations). The distribution pattern of *A. confertitesta* clearly shows its occurrence and higher relative abundances in, or relatively close to, international commercial harbours (e.g. Le Havre or Dunkerque), corroborating previous studies reporting the species in the same zones and confirming its NIS status in Europe. We hypothesise that its presence further away in smaller harbours (e.g. Boulogne-sur-Mer) is the consequence of a secondary spread due to national or regional trades. Moreover, the dominance of *A. confertitesta* over its two congeneric species in several stations of this study argues for its invasive potential. Retrospective studies investigating sediment cores could be conducted to determine the introduction date of this species and to better understand the dynamic of the shift in the community composition over time.

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Research conceptualization: J.-C. Pavard, L. Seuront and V.M.P. Bouchet. Sampling design and methodology: V.M.P. Bouchet, J.-C. Pavard, J.-C. Dauvin and J.-P. Pezy. Investigation and data collection: J.-C. Pavard, A. Courleux, E. Armynot du Châtelet, G. Duong and R. Abraham. Data analyses and interpretation: J.-C. Pavard, V.M.P Bouchet, J. Richirt and L. Seuront. Funding provision: V.M.P. Bouchet and E. Armynot du Châtelet. Writing - original draft: J.-C. Pavard, V.M.P Bouchet, J. Richirt and L. Seuront. Writing – review and editing: all co-authors.

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Supplementary material 1

Raw counts of pooled replicates of *A. aberdoveyensis*, *A. confertitesta* and *A. veneta*

Authors: Jean-Charles Pavard, Vincent M.P. Bouchet, Julien Richirt, Apolyne Courleux, Eric Armynot du Châtelet, Gwendoline Duong, Romain Abraham, Jean-Philippe Pezy, Jean-Claude Dauvin, Laurent Seuront

Data type: table (excel file)

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Supplementary material 2

Mean normalised abundances for 50cm^2 of *A. aberdoveyensis*, *A. confertitesta* and *A. veneta* by site and station

Authors: Jean-Charles Pavard, Vincent M.P. Bouchet, Julien Richirt, Apolyne Courleux, Eric Armynot du Châtelet, Gwendoline Duong, Romain Abraham, Jean-Philippe Pezy, Jean-Claude Dauvin, Laurent Seuront

Data type: table (excel file)

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Supplementary material 3

Mean relative abundances for 50cm^2 of *A. aberdoveyensis*, *A. confertitesta* and *A. veneta* by site and station

Authors: Jean-Charles Pavard, Vincent M.P. Bouchet, Julien Richirt, Apolyne Courleux, Eric Armynot du Châtelet, Gwendoline Duong, Romain Abraham, Jean-Philippe Pezy, Jean-Claude Dauvin, Laurent Seuront

Data type: table (excel file)

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Link: https://doi.org/10.3391/ai.2023.18.3.106635.suppl3

Supplementary material 4

Summarising table gathering occurrence of *Ammonia confertitesta* in Europe

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Data type: table (excel file)

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