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Rapid Communication

Among us: first record of the non-indigenous amphipod *Incisocalliope aestuarius* (Watling and Maurer, 1973) in Germany

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Abstract

The pleustid amphipod *Incisocalliope aestuarius* (Watling and Maurer, 1973) was described from the North American Atlantic coast in the 1970s. In this study, the species is reported from the German North Sea for the first time. An established population was found in four different harbour locations along the coast of Lower Saxony for five consecutive years since 2016. In Europe, *I. aestuarius* has already been reported from France, the Netherlands and Belgium. As a member of fouling communities, the species tolerates reduced salinities and is often associated with hydroids growing on artificial hard substrates. Due to its cryptic lifestyle, as well as its challenging identification, the species has been overlooked for years. Therefore, it seems likely that *I. aestuarius* has already been established in more estuarine or harbour locations in Europe.

Key words: North Sea, non-indigenous species, estuaries, brackish water, pleustids, fouling communities

Introduction

The establishment and spread of non-indigenous species are often unnoticed processes in marine and coastal ecosystems. Assessment programs are fundamental for an effective management strategy to approach and document these processes. In the German Bight and German coastal waters, an annual national monitoring was established in the year 2009 with the aim of recording the species inventory of all macrobenthic organisms, new introduction events and spreading of already known non-indigenous species (Lackschewitz et al. 2010; Buschbaum et al. 2012; HELCOM 2013).

During several of these investigations, the amphipod *Incisocalliope aestuarius* (Watling and Maurer, 1973) was detected. The species was first described by Watling and Maurer (1973) from the Delaware Bay region in North America and placed in the genus *Parapleustes* Buchholz, 1874. In Europe, *I. aestuarius* is known from the Netherlands, Belgium and France. In the Netherlands, the species was found in the saline Lake Grevelingen in 2016 (Verduin et al. 2018), the brackish Noordzeekanaal (VLIZ 2020) and

the Dutch part of the Eems Dollard estuary in 2017 (Ton van Haaren, Eurofins Amsterdam, *pers. comm.*), but it was identified for the first time by Faasse and Van Moorsel (2003) from the Schelde estuary. Prior to their study, the species was already detected in this region in 1991, but it was misidentified as *Pleusymtes glaber* (Boeck, 1861) (Ysebaert et al. 2000; Faasse and Van Moorsel 2003). In the same year, the species was also found in French waters of the English Channel and the French North Sea (Curd 2019a, b). In 2015, the species was reported even further South in the Gironde estuary (Curd 2021). Marchini and Cardeccia (2017) listed *I. aestuarius* as a valid alien species in an overview of alien amphipods.

Incisocalliope aestuarius is most probably a commensal of hydrozoan species as it is also known from *Incisocalliope derzhavini* (Gurjanova, 1938) from the Pacific (Chapman 1988). The non-triturative mandibular process of both species indicates this lifestyle, and *I. aestuarius* has been recorded among hydroids both in its distribution area in the NW Atlantic (Maurer and Watling 1973; Watling and Maurer 1973) and in Europe (Faasse and Van Moorsel 2003). The species has been found on different kinds of hard substrates, in brackish water with fluctuating salinities ranging from 10 to 33 (Faasse and Van Moorsel 2003) and from 9.1 to 30.6 (IfAÖ 2019, 2020a). European indigenous pleustid species like *Parapleustes assimilis* (G.O. Sars, 1883) are not known from estuary fouling communities with low salinity conditions so far (Lincoln 1979); their presence therefore seems very unlikely.

In this study, we report the first finds of *I. aestuarius* in Germany. Great numbers of this non-indigenous amphipod species were found in four different locations in the years 2016 to 2020. This indicates an established population in several estuaries along the German North Sea coast. Since *I. aestuarius* has been misidentified several times, we provide a summary of its main identification characters in comparison to four closely related or morphologically similar indigenous pleustid species.

Materials and methods

All investigated ports are situated on the coast of Lower Saxony from the port of Emden in the West to the port of Cuxhaven in the East (Figure 1) and are influenced by North Sea tides. Salinities in the different ports range from mesohaline conditions in the inner harbour of Emden to fully marine in the outer harbours of Wilhelmshaven.

On behalf of the Wadden Sea National Park Administration of Lower Saxony (NLPV), we carried out an annual monitoring of non-indigenous species between 2015 and 2020 in the ports of Emden, Wilhelmshaven (JadeWeserPort and southern ports) and Cuxhaven and on the oyster bed "Nordland" south of Juist, as well as the ports of Bensersiel (only 2016), and Norddeich (since 2017) (IfAÖ 2016, 2017, 2018, 2019, 2020a, IfAÖ *unpublished*). The monitoring was assigned within the scope of the European Marine Strategy Framework Directive (MSFD). Additionally, we investigated

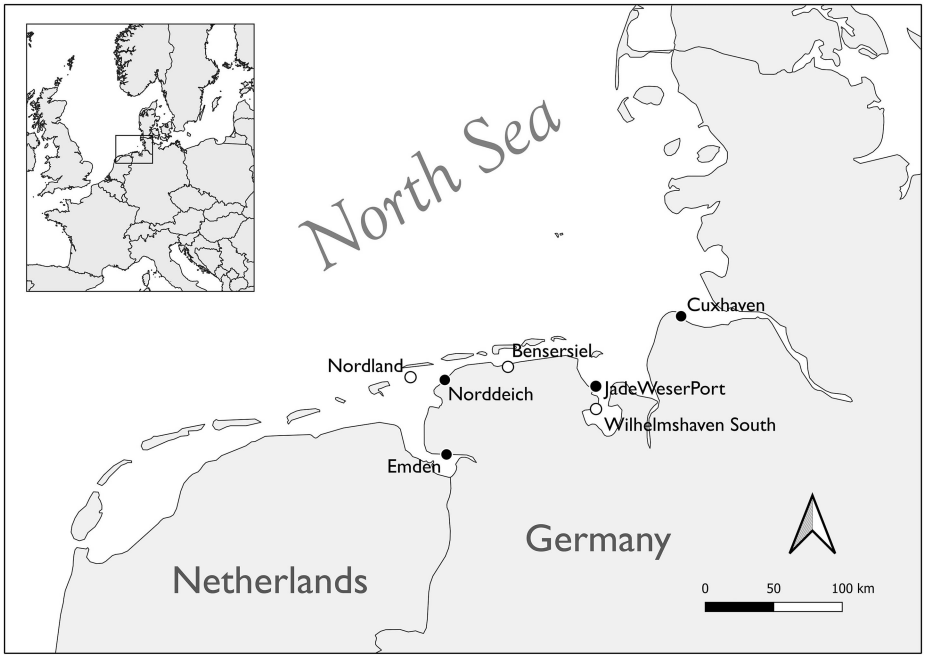

Figure 1. Sampling locations of *Incisocalliope aestuarius* along the German North Sea coast from 2016 to 2020. Small map displays the general geographical location of the sampling area in the European North Sea. Large detail map shows the seven sampling locations. Black circles indicate the four locations Emden, Norddeich, JadeWeserPort, and Cuxhaven where *I. aestuarius* was found. White circles are locations without *I. aestuarius* record.

the occurrences of non-indigenous species in 2018 during the harbour assessment in the JadeWeserPort and Cuxhaven on behalf of the Federal Maritime and Hydrographic Agency of Germany, Hamburg (BSH) (IfAÖ 2020b). The applied survey methods within this project followed the "HELCOM/OSPAR Joint Harmonized Procedure on the granting of BWM convention exemptions" (JHP). At each port, we chose three different sites for the surveys.

To record benthic non-indigenous species, we applied three different methods:

First, we deployed settlement collectors on the three different sites of each port for a period of three months. Each settlement collector consisted of three horizontal PVC plates. The settlement collectors were installed in a way to ensure the following position of the three plates during low tide: One 0.5 m below the water surface, one 0.5 m above the bottom and one in between. Upon retrieval, plates were separated carefully from the connecting rope and preserved separately. In the course of the non-indigenous species monitoring (NLPV), the settlement collectors hung between July and October in the years 2016 to 2020. Due to very shallow water during low tide in Bensersiel, since 2017, investigations were shifted to the port of Norddeich. During the harbour assessment in 2018 (BSH), settlement collectors were deployed from April to July.

Secondly, we conducted the standardized Rapid Assessment Survey (RAS) in accordance with the RAS sampling protocol as described by Gittenberger et al. (2010) and Buschbaum et al. (2012). At each site of a location, we

inspected predominant and accessible artificial hard substrates visually for benthic organisms. In addition to the protocol, we collected scratch samples from two different hard substrate types at each site. This method was applied annually during autumn in the JadeWeserPort from 2015 to 2020 and on the oyster bed "Nordland" between 2015 and 2018 as well as in in the ports of Emden, Norddeich (also 2017 and 2018), Wilhelmshaven South and Cuxhaven in 2020 (non-indigenous species monitoring, NLPV). It was also realized during the harbour assessment (BSH) in July 2018 in Cuxhaven and the JadeWeserPort.

In addition, to assess mobile epifauna, we deployed traps in the port of Cuxhaven and the JadeWesertPort in 2018 within the scope of the harbour assessment (BSH). We attached two kinds of baited light weight traps to existing harbour structures and deployed them for up to 48 h in July. Likewise, we used two different artificial habitat collectors and retrieved them about three months later in October (Hewitt and McDonald 2013; IfAÖ 2020b). All four kinds of traps were deployed at each of the three sites in the two ports.

Samples from the RAS as well as all specimens on or inside the traps were carefully rinsed and collected above a sieve with 1 mm mesh size. All collected samples and plates were preserved in a 70% ethanol solution until further analysis at the IfAÖ laboratory.

Specimens were identified using a stereomicroscope (Olympus SZX10), and a light microscope (Olympus BX51). Species identification was performed with the characters given in Watling and Maurer (1973), Lincoln (1979), Barnard and Karaman (1991), Bousfield and Hendrycks (1995), and Faasse and Van Moorsel (2003). *Incisocalliope aestuarius* specimens were stored in 70% ethanol at the species collection of the Institute for Applied Ecosystem Research in Neu Broderstorf.

Photos were taken with an Olympus microscope camera (UC 30). Measurements were made with the Olympus CellSens Dimension program, version 1.4.1. The figures and map were arranged and labelled using Gimp 2.10.8 and QGIS version 3.18.0.

For salinity determination, practical salinity scale was used.

Results and discussion

The first individuals of *I. aestuarius* were recorded in the year 2016 in the harbours of Cuxhaven and Emden, and the species was present in these two locations in the five following years until 2020 (Supplementary material Table S1). In 2018, *I. aestuarius* was also found at the JadeWeserPort, and in the years 2019 and 2020 in Norddeich. In many cases we recorded single specimens, sometimes masses up to 50 individuals per site. Therefore, our estimated minimum total number is 150 individuals within 69 samples. Among the specimens, females with eggs and juveniles occurred. Therefore, we suspect an established population.

At 36 sampling sites, salinity was measured, the average being 19.3 with a range from 9.1 to 30.6 (Table S1). The harbour locations of Emden and Cuxhaven with the highest frequency of *I. aestuarius* (64 samples), are influenced by tides and freshwater inflow, and therefore characterised by fluctuating salinities. In contrast, *I. aestuarius* was rarely found at sites with higher salinities and almost marine conditions like Norddeich and JadeWeserPort. It seems that the amphipod prefers middle salinities around 20, or it may be supplanted at higher salinities by indigenous species. This agrees with the observations of Watling and Maurer (1973) and Faasse and Van Moorsel (2003), and it may explain why we did not find the species near Nordland, an oyster bed far from the coast, with high salinity waters (Figure 1). Its euryhalinity makes *I. aestuarius* a successful invader. In areas of brackish waters, non-indigenous species are more likely to establish themselves due to the naturally low species richness (Wolff 1999).

At 64 out of the 69 sampling sites, *I. aestuarius* was found on settlement plates. On these plates, a variety of hydrozoan species could be identified: three taxa of athecate hydroids and four different species of Campanulariidae (Table S1). Watling and Maurer (1973) described *I. aestuarius* in the context of investigations on the oyster-associated fauna of the North American Delaware region, where it was found on *Hartlaubella gelatinosa* (Pallas, 1766) and *Ectopleura crocea* (Agassiz, 1862) (Maurer and Watling 1973). This combination of hard substrate and hydrozoans was also mentioned by Faasse and Van Moorsel (2003) who found *I. aestuarius* often together with *H. gelatinosa* growing on Pacific oysters *Magallana gigas* (Thunberg, 1793). In our samples, the amphipod was found within 30 samples together with *Obelia bidentata* Clark, 1875, within 19 samples with the non-indigenous *Calyptospadix cerulea* Clarke, 1882 and within 17 samples with *Gonothyraea loveni* (Allman, 1859). These species build erect colonies and often grow in dense tufts providing *I. aestuarius* with numerous possibilities to hide and maybe feed. In summary, *I. aestuarius* does not seem to be strictly bound to oysters or other biogenic hard substrate. As a matter of fact, artificial hard substrate together with a variety of hydrozoan species will be settled frequently and fast (within 3 months). In the choice of its hydrozoan species, *I. aestuarius* does not seem to be very specific for it can be associated with at least seven species of both thecate and athecate hydroids.

Incisocalliope aestuarius was misidentified several times, including in our own reports, as the indigenous pleustids *Pleusymtes glaber* or *Parapleustes assimilis* (Cattrijsse et al. 1993; Brummelhuis et al. 1997; Faasse and Van Moorsel 2000; Ysebaert et al. 2000; IfAÖ 2017, 2018, 2019, 2020a, b). In 2020, we managed to identify *I. aestuarius* correctly using the key from Bousfield and Hendrycks (1995). The small and conical, non-triturative process of the mandible distinguishes it from the indigenous *P. glaber*; from *P. assimilis* it differs by the stronger setation of the anterior basis of

Table 1. Characters for the identification of *I. aestuarius* in comparison to the closely related *I. filialis* and *I. derzhavini*, as well as two morphologically similar pleustid species from Europe, and their known geographical distribution. After (Boeck 1861; Sars 1883; Gurjanova 1938; Watling and Maurer 1973; Lincoln 1979; Ishimaru 1984; Hirayama 1988; Bousfield and Hendrycks 1995).

	Incisocalliope aestuarius	*Incisocalliope filialis*	*Incisocalliope derzhavini*	*Parapleustes assimilis*	*Pleusymtes glaber*
	NW-Atlantic, NE-Atlantic	*Japan*	*NE-Pacific, NW-Pacific, Hawaii*	*NW-Atlantic, NE-Atlantic*	*NW-Atlantic, NE-Atlantic, Arctic*
antenna 1 peduncle segment 1 length	basic, = segments 2+3	basic, = segments 2+3	prolonged, > segments 2+3	basic, = segments 2+3	basic, = segments 2+3
triturating process on mandible	no	no	no	no	yes
coxa 1, hind cusps	**2-3**	1	1	1	1
pereopod 1 basis	> 20 setae	> 20 setae	< 15 setae	< 15 setae	< 15 setae
pereopod 4 basis	anterior row of short setae	anterior row of short setae	anterior weakly setose	n.a.	n.a.
pereopod 7 hind loop	ordinary	deep	deep	ordinary	deep
epimeral plate 3	acuminate, produced	squared	acuminate, produced	produced, not acuminate	with hook
uropod 2	rami subequal	outer ramus 2/3 inner ramus	rami subequal	outer ramus significantly shorter than inner	n.a.

the first gnathopod, the acuminate third epimeral plate and the additional cusps on the first coxa (Table 1, Figure 2). *Incisocalliope derzhavini* is a species of the genus known to be invasive (Chapman 1988; Marchini and Cardeccia 2017), although not yet reported from Europe. In contrast to *I. aestuarius*, it shows a weaker setation on gnathopod 1 basis and pereopod 3 and 4 bases. *Incisocalliope filialis* (Hirayama, 1988) is closely related to *I. aestuarius* and most similar morphologically. It can be separated by the deeper extended basis of pereopod 7 reaching to the merus, squared epimeral plates and only one cusp on coxa 1.

The morphological features of our specimens fit the description by Watling and Maurer (1973) very well. There are some minor deviations to the key of Bousfield and Hendrycks (1995) according to the length of uropod 2 rami and the length of pereopod 7 dactyl in relation to the propodus. We observed that the uropod 2 rami lengths vary between specimens, the outer ramus being 18–30% shorter than the inner ramus. The pereopod 7 dactylus was always longer than described by Bousfield and Hendrycks (1995), but it concurs with the illustration in Watling and Maurer (1973).

Its cryptic way of life as a commensal of hydrozoans, its life colour pattern and behaviour (crawling into hydroids) make *I. aestuarius* hard to find in the field, and could explain why we did not find the species more often during visual assessments, but very often on settlement plates. The possibility to misidentify *I. aestuarius* as an indigenous pleustid species may be the reason why it has not been reported more often and earlier. Its distribution area in the German Bight may therefore already be wider. A potentially suitable location where we measured similarly low salinity conditions but did not find *I. aestuarius,* is for example, Bensersiel. We only investigated this site in 2016 and data for the years 2017 to 2020 are missing. Also, Bensersiel is a small harbour with more local and less international

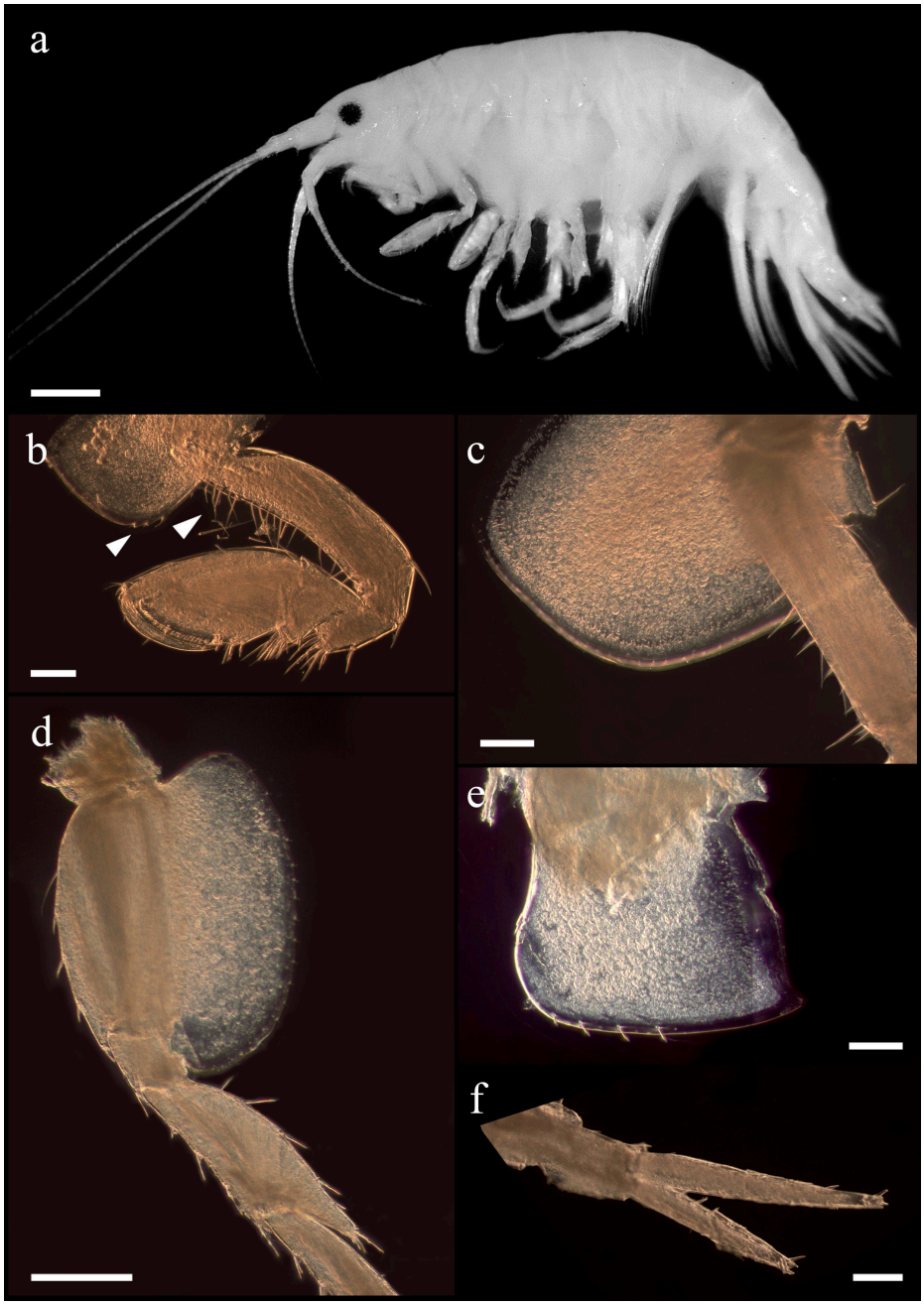

Figure 2. *Incisocalliope aestuarius* from the German North Sea coast. a) habitus, lateral view; b) Gnathopod 1 with hind cusps on coxa and setation on basis visible (arrows); c) Pereopod 4 basis; d) Pereopod 7 basis; e) Epimeral plate 3; f) Uropod 2. Scale bars: a) = 500 µm; b), c), e), f) = 100 µm, d) = 200 µm. Photograph a) by JL; photographs b) – f) by LS.

shipping traffic, therefore it is possible that *I. aestuarius* is not established there, yet. Another potentially suitable location is the Weser estuary. There, Wetzel et al. (2014) investigated the species composition of artificial hard substrates and found *P. assimilis* among other invertebrate species. As mentioned above, in this location and brackish salinity conditions the occurrence of *P. assimilis* is highly unlikely. Consequently, *I. aestuarius* could have arrived in German waters before 2014.

For *I. aestuarius*, the vector of introduction to the German Bight may be either hull fouling on ships or ballast water. Both are listed in the literature

for this species (Gollasch et al. 2009; Noël 2011). Amphipods are generally likely to be displaced by ballast water when getting into the water column (Marchini and Cardeccia 2017) and due to its lifestyle attached to hydroids, *I. aestuarius* can be carried on vessels' biofouling as well. Nevertheless, a "natural" spread by water currents from their invaded distribution area in the Netherlands into German harbours is also possible, since Faasse and Van Moorsel (2003) observed a preference for strong currents.

Its property as euryhaline fouler makes a further spread and even invasiveness very likely. Through the Kiel Canal, *I. aestuarius* is able to reach the Baltic Sea, which is predestined for an introduction with its mesohaline conditions. As a matter of fact, *I. aestuarius* is already listed as a possible target species with a risk of spreading to the Baltic by ship transfer (Gollasch et al. 2011).

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Authors' contribution

JL and LS contributed equally to the writing of the manuscript with major input from SN. All authors contributed revisions and approved the final version of the manuscript.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Details of sampling sites of *Incisocalliope aestuarius* in four different locations in the years 2016 to 2020.

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2021/Supplements/BIR_2021_Leitinger_etal_SupplementaryMaterial.xlsx