

## The ctenophore *Mnemiopsis leidyi* A. Agassiz 1865 in coastal waters of the Netherlands: an unrecognized invasion?

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### Abstract

The introduction of the American ctenophore *Mnemiopsis leidyi* to the Black Sea was one of the most dramatic of all marine bioinvasions and, in combination with eutrophication and overfishing, resulted in a total reorganization of the pelagic food web and significant economic losses. Given the impacts this animal has exhibited in its invaded habitats, the spread of this ctenophore to additional regions has been a topic of much consternation. Here, we show the presence of this invader in estuaries along the Netherlands coast, based both on morphological observation and molecular evidence (nuclear internal transcribed spacer region 1 [ITS-1] sequence). Furthermore, we suggest the possibility that this ctenophore may have been present in Dutch waters for several years, having been misidentified as the morphologically similar *Bolinopsis infundibulum*. Given the level of shipping activity in nearby ports (e.g. Antwerp and Rotterdam), we find it likely that *M. leidyi* found its way to the Dutch coast in the ballast water of cargo ships, as is thought for *Mnemiopsis* in the Black and Caspian Seas. Given the magnitudes of the impacts this ctenophore has shown in its native and introduced ranges, the animal's potential effects on the North Sea pelagic ecosystem and fisheries warrant close observation in the coming years. The development of large ctenophore aggregations during recent years was probably driven by the higher than average sea surface temperatures in the North Sea, and we hypothesize that populations from the southern North Sea may have been the source of the invaders reported in the Baltic Sea and the Skagerrak. If these northern populations (Baltic, etc.) are not able to over-winter, it is possible that established populations in southern North Sea estuaries may serve as a yearly supply of invaders to the colder waters to the north.

**Key words:** ctenophore, invasive species, *Mnemiopsis leidyi*, ballast water, *Bolinopsis infundibulum*, DNA, nuclear

### Introduction

Ballast-water mediated biological invasions are on the rise around the world (Carlton 1985, Carlton and Geller 1993) and one of the premier examples is the invasion of the Black Sea ecosystem by the zooplanktivorous comb jelly *Mnemiopsis leidyi* (A. Agassiz 1865), an animal native to the eastern seaboard of the Americas (from Massachusetts [U.S.A.] to southern

Argentina) (GESAMP 1997, Mianzan 1999). The chronological history of the *Mnemiopsis* invasion has been covered well elsewhere (GESAMP 1997, Kideys 2002, Bilio and Niermann 2004), but we will present a short summary here. *M. leidyi* appeared in the Black Sea in the early 1980's and, by 1988, exhibited a massive population explosion, reaching concentrations of 1.5 kg/m<sup>2</sup> (Vinogradov et al. 1989). Coinciding with the ctenophore's increase was a drastic

reduction in the stocks of zooplankton species fed on by *Mnemiopsis* (Vinogradov and Shushkina 1992) and a subsequent crash in the landings of the Black Sea's major fishery, that of the zooplanktivorous anchovy *Engraulis encrasicolus* (GFCM 1993 as referenced in Kideys 1994), with estimated losses in the hundreds of millions of U.S. dollars (Knowler 2005). While there is some discussion about the absolute role of *Mnemiopsis* in the anchovy collapse (Bilio and Niermann 2004), it was believed that the ctenophore affected fish stocks by feeding on fish eggs and larvae and by diminishing their food supplies (Kideys 1994). *Mnemiopsis* later spread from the Black Sea into the Sea of Azov (Vinogradov et al. 1989), the Sea of Marmara (Shiganova 1993), the Aegean Sea (Kideys and Niermann 1994), the Eastern Mediterranean Sea (Kideys and Niermann 1993) and finally the Caspian Sea (Ivanov et al. 2000). Similar ecological and economic impacts have followed the ctenophore's invasion of the Caspian Sea (summarized in Kideys 2002 and Stone 2005). Given the vast ecological and economic impacts the *M. leidyi* invasion had in its invaded habitats, the possible appearance of this ctenophore elsewhere in the world has been a topic of major concern.

*Bolinopsis infundibulum* (O. F. Müller, 1776), the dominant lobate ctenophore in the North Sea, is a typically cold-water species that was not commonly recorded in the warmer waters of the Dutch coast prior to 1992 (Greve 1975, Hayward and Ryland 1995). However, recent mass occurrences of *B. infundibulum* in the late-summer coastal waters of the Netherlands have raised concerns that a new invasion of the morphologically similar *Mnemiopsis leidyi* might have been involved. Beginning in 1992, *B. infundibulum* was reported in increasing numbers during the summer months in several estuaries along the Netherlands coast, including the Wadden Sea in the north and various estuaries in the southwest (Grevelingen and Oosterschelde) (Holsteijn 2002). There were two records in 1992 (Holsteijn 2002, M.A. Faasse pers. obs.), two in 2000, one in 2001 and numerous reports from 2002 onwards (Holsteijn 2002). In 2002, *B. infundibulum* was found to be so common by late June onwards, that Holsteijn (2002) stopped listing records, a fact not recorded in other years. These findings were puzzling, since one would not expect to find blooms of a typically cold-water ctenophore in the productive late summer waters of the Dutch estuaries. Earlier this year,

between August and November of 2006, lobate ctenophores were found in visibly larger aggregations than ever before in several Netherlands estuarine regions (the Grevelingen, Oosterschelde, Westerschelde and the Wadden Sea).

In order to definitively identify these ubiquitous lobate ctenophores, we collected animals from two different estuarine regions along the Dutch coast for species-level analyses based both on morphology and genetics. Here we confirm that the large blooms observed this summer in Dutch estuarine regions were, in fact, formed by the ctenophore *Mnemiopsis leidyi* and indicate that it is possible that this ctenophore may have been present in Dutch waters for several years.

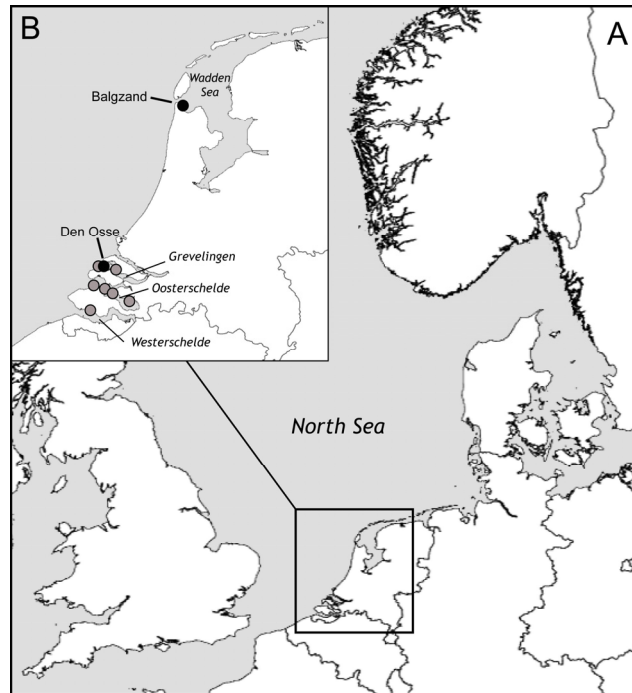
## Materials and Methods

### *Ctenophore sampling*

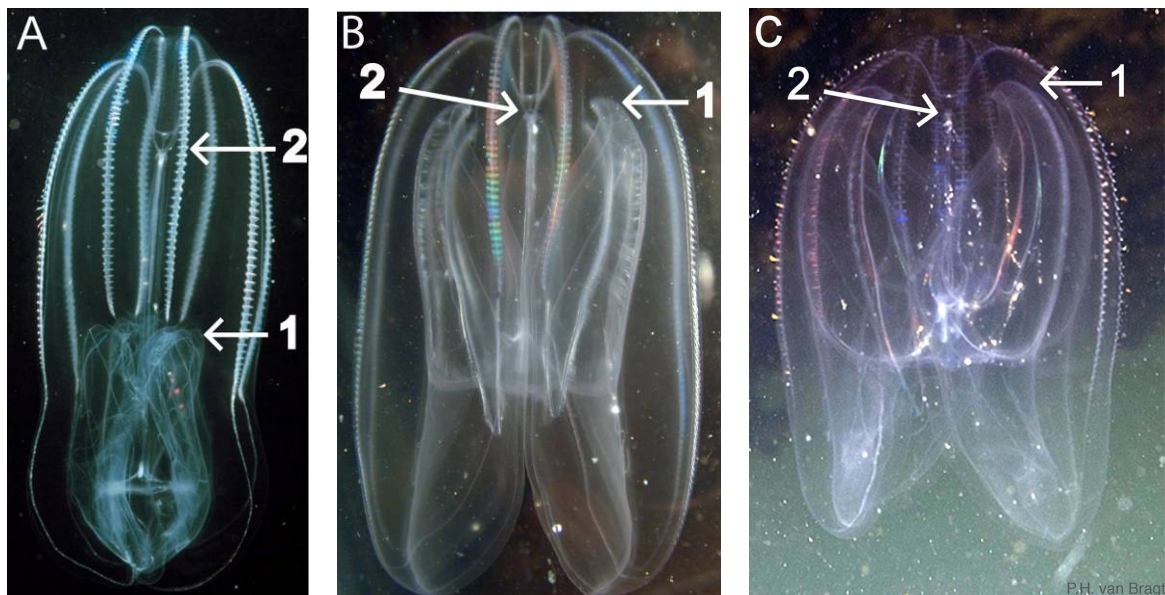
A total of 86 lobate ctenophores were collected between August and November of 2006 from two different estuarine regions along the Dutch coast: the Wadden Sea in the north and three different regions within the southwestern estuaries (the Grevelingen, the Oosterschelde and the Westerschelde) (Figure 1; Annex). Animals were collected in glass jars by SCUBA divers at depths of 5 – 15 meters and at the surface near Borssele and the Balgzand. Animals were then transferred to the lab for morphological identification. In some cases, ctenophores were preserved in 75–100% ethanol for later molecular identification.

### *Morphological species identification*

In the lab, animals were observed in order to identify morphological characters delineating *Bolinopsis infundibulum* from *Mnemiopsis leidyi*, with small ctenophores observed under a dissecting microscope. The main morphological character delineating *B. infundibulum* from *M. leidyi* is the extent of the animal's oral lobes. In *B. infundibulum*, the oral lobes terminate between the mouth and apical sense organ (Figure 2A), while in *M. leidyi*, the oral lobes terminate near the apical sense organ (statocyst) and span nearly the entire length of the body (Mayer 1912, Mianzan 1999) (Figure 2B). The extent of the oral lobes was recorded for each animal.



**Figure 1.** A) Map of the North Sea coastal region B) Map of the Netherlands coast showing areas sampled for lobate ctenophores in this study, including sampling sites in the Wadden Sea (Balgzand), the North Sea (Spuisluis Brouwersdam and Vluchthaven Neeltje Jans), the Grevelingen (Scharendijke and Den Osse), the Oosterschelde (Anna Frisopolder, Goesse Sas, Bergsedieppluis and Zierikzee) and the Westerschelde (Borssele). Sites where ctenophores were collected and identified morphologically are labeled with grey circles and both morphologically and genetically are labeled with black circles



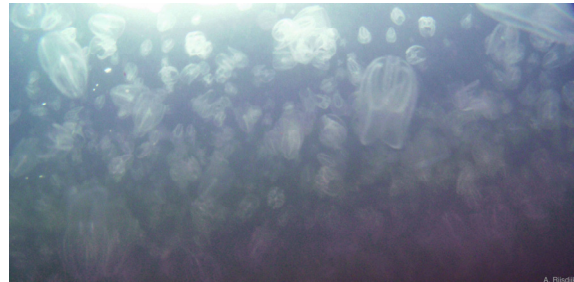
**Figure 2.** Photographs of A) *Bolinopsis infundibulum* B) *Mnemiopsis leidyi* and C) lobate ctenophore (*M. leidyi*) taken from Bommeneede in the Grevelingen (02.09.2006). Note the termination of the oral lobes (1) and the placement of the statocyst (2). In *M. leidyi*, the oral lobes extend all the way to the statocyst, while they terminate near the mouth in *B. infundibulum* (*B. infundibulum* photograph (A) courtesy of G. R. Harbison [Woods Hole Oceanographic Institute], *M. leidyi* photograph (B) by K.M. Bayha and Bommeneede *M. leidyi* photograph (C) by P.H. van Bragt)

### Molecular species identification

Molecular species identification was performed using sequence data from the nuclear internal transcribed spacer region 1 (ITS-1). This region has been shown to be extremely useful in delineating species in a variety of organisms (Anderson and Adlard 1994, Dawson and Jacobs 2001, Schroth et al. 2002), including ctenophores (Podar et al. 2001, Bayha et al. 2004) and is easily amplified in comb jellies. For lobate ctenophores collected in Dutch waters, 3 animals were sequenced (two ctenophores from Den Osse in the Grevelingen and one from the Balgzand in the Wadden Sea [Figure 1; Annex]). DNA extraction, PCR amplification and DNA sequencing of the ITS-1 region was performed as in Bayha et al. (2004), with PCR amplification performed using the primers ITS1A (5'-GGTTTCTGTAGGTGAACCTGC-3') and N73 (5'-GGCTCAGACAGACATGCTCC-3') (Gaffney, unpublished). In addition, two *Bolinopsis infundibulum* individuals collected from the Northwest Atlantic (near N 38.53 W 70.60) were sequenced for ITS-1 in an identical manner. All DNA sequences were assembled using SeqMan II (DNASTar, Inc.) and electropherograms were checked by eye for poor base calls and sequence quality. Sequence identity was evaluated by performing BLASTN searches against GenBank (Altschul et al. 1997). In addition, DNA sequences were aligned against *B. infundibulum* sequence generated for this study and U.S. Atlantic *Mnemiopsis leidyi* (NCBI Accession # AF293700) ITS-1 sequence from NCBI GenBank using CLUSTALW (Thompson et al. 1994) in MegAlign (DNASTar, Inc.). Sequence divergence values (uncorrected p) were calculated in PAUP 4.0b10 (Swofford 2002).

### Results

Between August and November 2006, lobate ctenophores were seen in the two different estuarine regions along the Dutch coast, in the Wadden Sea in the north and the southwestern estuaries (the Grevelingen, Oosterschelde and Westerschelde) (Figure 1; Annex), oftentimes in extremely large aggregations (Figure 3).



**Figure 3:** Underwater photo of a *Mnemiopsis leidyi* bloom near Bommenede in the Grevelingen (26.08.2006). Photo courtesy of A. Rijdsdijk

All 86 ctenophores collected from the four different water bodies (Annex) morphologically resembled *Mnemiopsis leidyi*. Ctenophores collected in August and September were found in 19 - 23°C water, while those collected in October and November were found at temperatures down to 10°C. Collected animals were between 1.5 and 8 cm in length, with the majority falling within 4 - 6 cm. A typical picture of one of the invasive ctenophores can be seen in Figure 2C. In all ctenophores, the animals' oral lobes terminated even with the animal's apical sense organ, as in *M. leidyi* (Figure 2B), the main character that differentiates it from *B. infundibulum* (Figure 2A).

ITS-1 sequences for all animals sequenced (two ctenophores from Den Osse in the Grevelingen and one from the Balgzand in the Wadden Sea [Figure 1; Annex]) were 233 base pairs long and identical to one another. Both *Bolinopsis infundibulum* ITS-1 sequences were 228 base pairs long and differed from each other by a single base at a degenerate site (T in one individual and Y [C and T] in the other). All ITS-1 sequences from Netherlands ctenophores were nearly indistinguishable from published *Mnemiopsis leidyi* sequence, differing by a single base (T in our specimens and Y [C and T] in the published sequence). In contrast, the Netherlands ctenophore ITS-1 sequences differed from those of *Bolinopsis infundibulum* by 11.5%. The identical *M. leidyi* sequences were deposited in the NCBI Genbank database under the accession number EF175463, while the two *B. infundibulum* sequences were deposited under the accession numbers EF175464 and EF175465.

## Discussion

These morphological and molecular analyses mark the first confirmed report of *Mnemiopsis leidyi* in the North Sea and among the first reports outside of the animal's native range or the Mediterranean-Black-Caspian Seas (see Sai Sastry and Chandramohan 1989). Furthermore, it is possible that *M. leidyi* has actually been present in Dutch estuaries several years, as some of the late summer 2002 reports of *B. infundibulum* by Holsteijn (2002) may actually have been misidentifications of *M. leidyi*. The superficial morphological similarity of the two species (Figure 2) could easily result in misidentification, especially by those not familiar with *M. leidyi* or ctenophores in general. In addition, it is curious that Holsteijn (2002) recorded large numbers of the boreal *B. infundibulum* in warm, productive late-summer estuarine waters, waters that would be much more amenable to *M. leidyi* (Kremer 1994, Purcell et al. 2001). So, while this is far from definitive, it is possible that *Mnemiopsis* has been present in Dutch estuaries for some time, slowly increasing its population size over the years, much like the ctenophore did in the Black Sea between its initial sighting in the early 1980's and its population explosion in 1988 (Vinogradov et al. 1989).

Ballast water transfer is well known as a major vector for the worldwide transport of invasive species (Carlton 1985, Carlton and Geller 1993). Two of the largest European ports, Antwerp and Rotterdam, are very geographically close to the southwestern estuarine region of the Netherlands (Grevelingen, Oosterschelde and Westerschelde). The amount of ballast water discharge in Dutch ports alone is estimated at 7.5 million tonnes/year (Aquasense 1998 as referenced in Wetsteyn and Vink 2001). The North Sea has received a large number of invading organisms (Reise et al. 1999, Wolff 2005) and it is estimated that the North Sea is the second-most invaded water body in Europe, behind the Mediterranean Sea, based both on total number of invaders, as well as those distributed by shipping (Gollasch 2006). Given the amount of ballast water transported to Dutch ports, as well as the proximity to the invaded areas, it is likely that *Mnemiopsis leidyi* arrived in Dutch waters as a result of ballast water transfer, much as it has been assumed of its invasions of the Black and Caspian Seas

(Vinogradov et al. 1989, Ivanov et al. 2000), though the specific source region is unclear at this time.

The presence of *M. leidyi* in northern Europe is an alarming development and it is essential that effects on the environment be closely monitored. Patterns seen in the Black Sea might be replicated in northern Europe, with impacts on zooplankton species and their predators. Two of the most important fisheries in North Sea waters are for the plaice (*Pleuronectes platessa*) and sole (*Solea solea*), whose annual combined catch amounts to EUR 300 million (European Commission 2006). *Mnemiopsis* might impact fish stocks by preying on eggs and larvae, as the ctenophore *Pleurobrachia pileus* does on both species (Lebour 1923, Greve 1975) and by competing with larvae for food, as there is dietary overlap between *M. leidyi* and both fish species (Last 1978). This is especially important for the Dutch coast, since Dutch estuaries and coastal waters serve as nursery habitats for both sole and plaice (van Beek et al. 1989). Furthermore, it is unclear if any species will feed heavily on *Mnemiopsis* within Dutch waters. Typically, only *Beroe gracilis*, a ctenophore predator that feeds on *Pleurobrachia pileus*, occurs in Dutch estuaries in great numbers (Gmelig Meyling et al. 1999), and given its small size in relation to *Mnemiopsis*, it remains to be seen whether or not it will efficiently feed on *M. leidyi*. Perhaps in the future, the area may experience an invasion of the *Mnemiopsis* predator *Beroe ovata* sensu Mayer from the western Atlantic, which may help control *M. leidyi* populations, as it did in parts of the Black Sea (Finenko et al. 2003).

Also worrisome is the possibility of *M. leidyi* spreading to adjacent water bodies and establishing persisting populations, especially in semi-enclosed water bodies, such as the Baltic Sea, where ctenophore impact might be more severe. North Sea surface temperatures have increased steadily in the past decades and recent temperatures (especially this past summer) have been some of the highest on record (Bundesamt für Seeschifffahrt und Hydrographie 2006). This may have been especially true in the shallower Dutch estuaries, as large parts of the Grevelingen had surface temperatures as high as 26°C during July (Hydro Meteo Centrum Zeeland 2006). In its native range, water temperature is one of the dominant factors determining the level of population increase in *Mnemiopsis* (Kremer 1994). Therefore, it is likely that increasing

temperatures aided the earlier population establishment of *M. leidyi* in Dutch estuaries, as well as formation of the large blooms seen this summer. With the high shipping traffic in Dutch ports, ballast water transport may be an important vector for ctenophores leaving the Netherlands coast, resulting in further *Mnemiopsis* invasions elsewhere, especially as *Mnemiopsis* populations get larger in Dutch waters. The large *M. leidyi* populations in the southern North Sea may be the source population for recent ctenophore sightings in Swedish waters (the Skagerrak) (H. Hansson, pers. comm., see also Hansson 2006 – this issue) and within the Baltic Sea (Kiel Bight) (J. Javidpour and U. Sommer, pers. comm., see also Javidpour et al. 2006 in this issue). It is possible that the large populations of *M. leidyi* in the southern North Sea, driven by the higher than normal sea surface temperatures of recent years, may have served as a source of invaders of the Skagerrak, the Kattegat and the westernmost part of the Baltic Sea, either via the North Sea or, more likely, via the Kiel-Canal, a canal that extends from the Wadden Sea to the Kiel Bight. It is possible that *Mnemiopsis* has survived multiple winters in the southern North Sea estuaries, where winter temperatures average about 3°C (sometimes falling to ~2°C), with higher temperature refuge areas at depth (Wolff 1973, Hydro Meteo Centrum Zeeland 2006). It remains to be seen whether or not the ctenophore will be capable of over-wintering in the more northern regions (Baltic Sea, etc.), where average winter temperatures tend to be colder (Bundesamt für Seeschifffahrt und Hydrographie 2006). If not, the southern North Sea and its estuaries may serve as a reservoir of *M. leidyi* and a yearly source of new immigrants each summer to these colder waters, similarly to the way in which the Sea of Azov must be re-invaded by *Mnemiopsis* each summer (Studenikina et al. 1991). It has been theorized that with global climate change, warm temperature organisms might be able to establish stable populations in waters that were previously too cold (Leppäkoski et al. 2002). This yearly inoculation of the Baltic Sea with new invaders could then result in persistent populations of *M. leidyi* should average sea surface temperatures continue to increase and these waters become more hospitable to *Mnemiopsis* introduction and establishment.

#### Note added to proof

A very recent article (Tulp 2006), published during our final proof preparation, morphologically identified *Mnemiopsis leidyi* in the Wadden Sea, near Den Helder (near our Balgzand station) and Lauwersoog.

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**Annex**

Sampling sites where lobate ctenophores were collected for identification.

\* indicates samples were sequenced for ITS-1 to confirm species identification molecularly. + indicates regions located just outside the dam or storm barrier of the respective water body and, therefore, just inside the North Sea

Sampling Site	Location (water body)	Location coordinates		Collection date	Number collected
		Latitude, °N	Longitude, °E		
Den Osse	Grevelingen	51.7457	3.8868	01.07.2005	1
Den Osse*	Grevelingen	51.7457	3.8868	11.08.2006	1
Den Osse	Grevelingen	51.7457	3.8868	15.08.2006	10
Den Osse	Grevelingen	51.7457	3.8868	21.08.2006	10
Den Osse*	Grevelingen	51.7457	3.8868	02.09.2006	10
Balgzand*	Wadden Sea	52.9820	4.9031	09.09.2006	4
Scharendijke	Grevelingen	51.7411	3.8464	09.09.2006	10
Spuisluis Brouwersdam	North Sea <sup>+</sup>	51.7412	3.8104	09.09.2006	10
Goesse Sas	Oosterschelde	51.5444	3.9279	30.09.2006	2
Anna Frisopolder	Oosterschelde	51.6006	3.7007	21.10.2006	3
Zierikzee	Oosterschelde	51.6292	3.9143	03.11.2006	10
Vluchthaven Neeltje Jans	North Sea <sup>+</sup>	51.6402	3.7001	11.11.2006	5
Bergsediëpsluis	Oosterschelde	51.5159	4.1730	18.11.2006	5
Borssele	Westerschelde	51.4264	3.7147	18.11.2006	5