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## Report of the Joint CIESM/ICES Workshop on *Mnemiopsis* Science (JWMS)

18–20 September 2014

A Coruña, Spain



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## Executive summary

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The ICES/CIESM Joint Workshop on *Mnemiopsis* Science (JWMS) chaired by Sophie Pitois (UK) and Tamara Shiganova (Russia), met in A Coruña, Spain from 18 to 20 September 2014. Scientists affiliated with the International Council for the Exploration of the Sea (ICES) and the Mediterranean Science Commission (CIESM) participated in the Joint Workshop to discuss the latest advances regarding the biogeography, ecology, impact and economic aspects of the invasive ctenophore *Mnemiopsis leidyi* and its *Beroe* predators (both native and invasive). The JWMS was attended by 20 scientists from 14 countries. The main objective of the workshop was to provide a forum for scientists to present and discuss the results of ongoing relevant research projects in the North Atlantic and Mediterranean. The 13 presentations stimulated wide ranging discussions relevant to *Mnemiopsis* science.

### Highlights

- The latest knowledge regarding the biogeography and ecology of *Mnemiopsis leidyi* and of its *Beroe* predators were reviewed, as well as the spatial- and temporal occurrences across the ICES-CIESM sea basins. It was recognized that *Beroe* spp. are not the only predators of *M. leidyi* and that the various interactions of *M. leidyi* with the different components of the ecosystem, as well as mechanisms involved, are too complex to be comprehensively understood.
- *Mnemiopsis leidyi* is most likely exhibiting source-sink population dynamics within its European range.
- European seas are warming at present, and temperature is likely to affect the timing and distribution of both *M. leidyi* and *Beroe* spp. in those areas. However, the link with temperature was questioned, and the hypothesis was put forward, that food web perturbations from overfishing combined with eutrophication in coastal areas and major influx of invasive aliens, have permitted the establishment of *Mnemiopsis* propagules in some areas.
- *M. leidyi* has a wide environmental tolerance and phenotypic variability. Environmental conditions influence the development of *M. leidyi*, however it can establish in highly contrasting ecosystems. Its morphological features, growth rate, metabolism, size of maturity, fecundity differ according to environmental conditions particularly considering salinity, temperature, productivity and prey concentration.
- Modelling tools, based on experimental physiological knowledge, help to predict/understand the aforementioned processes and interactions. Several approaches were presented and discussed.
- At the same time it was acknowledged that there is not enough information on *M. leidyi* occurrence, seasonal and interannual variability in some areas, particularly in northern Europe and specific areas of the Mediterranean Sea. Improved monitoring and more comprehensive coverage of investigated areas are therefore deemed necessary, in particular field investigations of overwintering areas are needed.
- A key parameter to understand population dynamics is the winter biology of *M. leidyi*. Therefore more research effort should be devoted (i) to understand the low temperature/low food environmental interactions with *M. leidyi* and its survival under these conditions; (ii) to identify sites of overwintering populations.

- Citizen science may provide a valuable tool to improve our knowledge on poorly monitored areas, and should be encouraged.

## 1 Introduction and Terms of Reference

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*Mnemiopsis leidyi* invaded the Black Sea in the 1980s with cascading effects on several ecosystem levels including commercial fisheries. From the Black Sea it spread with currents to the Sea of Azov via Kerch Strat and via Bosphorus to the Sea of Marmara from where it regularly appeared in the northern Aegean Sea (Shiganova *et al.*, 2001). This ctenophore, native to the east coasts of America, triggered large public and scientific attention as a result of this invasion and its ecological and economic impacts. In 2005, when *M. leidyi* was sighted in northern Europe for the first time (e.g. Faasse and Bayha, 2006; Javidpour *et al.*, 2006), similar consequences were feared.

The idea to hold the Joint Workshop on *Mnemiopsis* Science (JWMS) was originally proposed at the Consultation meeting on non-native species cooperation between ICES and CIESM (Copenhagen, Denmark, 19. December 2013). Participants at the Consultation meeting agreed that invasions of alien species are a continuous process which can hardly be controlled, but perhaps only minimized in terms of ecological and economical damage. The common issues between both organizations in the field of alien (incl. invasive) species were discussed. A currently spreading pelagic invasive alien species in both CIESM and ICES waters is the comb jelly *Mnemiopsis leidyi*. It was identified as a target species with potential for cooperation. It was felt that such a workshop was important in addressing and discussing issues of common interest and, for comparative purposes, to explore similarities and differences between the ecosystems in the ICES and CIESM areas.

The Workshop was divided into six theme sessions following the Term of Reference adopted by the ICES Science Committee (SCICOM):

- a) Review and discuss the latest knowledge regarding the biogeography and ecology of *Mnemiopsis leidyi* and of its main predators *Beroe* spp.
- b) Compile existing knowledge on spatio-temporal occurrence in areas where *M. leidyi* is already established across the ICES-CIESM sea basins. By identifying and geo-locating the likely vectors of introduction, establish which areas are the most at risk and where *M. leidyi* is likely to appear and probably be established next.
- c) Compare environment-specific parameters used for modelling potential habitat and population dynamics of *M. leidyi* across different sea basins. Discuss the existence of potential sea-basin specific ecological and physiological adaptations.
- d) Review the molecular genetic techniques that have been or could potentially be used to study this species. Discuss the sampling techniques currently being used and the need for a standard protocol.
- e) Review the environmental and socio-economic consequences of *M. leidyi* in areas that have already been affected.
- f) Outline future research needs/next steps and identify management measures.

It is our hope that the interaction between the ICES and CIESM scientific communities during the workshop as well as the present proceedings will mutually enrich the research activity on *Mnemiopsis leidyi* expansion and blooms of other native and non-native gelatinous species within marine systems in general.

## 2 Opening of the meeting

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### 2.1 Opening and Welcome

Co-chairs Dr. Sophie Pitois, marine scientist at the Centre for Environment, Fisheries & Environment, UK, and Prof. Tamara Shiganova, marine scientist at the P.P. Shirshov Institute of Oceanology, Russia, opened the meeting and welcomed the participants. ICES Head of Science Adi Kellerman also welcomed the participants to the workshop at A Coruña, following the ICES Annual Science Meeting. General Director of CIESM Prof. Frederic Briand could not participate and Tamara Shiganova as a co-president of CIESM session on behalf of Prof. Frederic Briand welcomed participants.

The list of participants appears in Annex 1.

## 3 Presentations

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### 3.1 Adaptive strategy of the invader *Mnemiopsis leidyi* and its predators *Beroe* spp. in the Eurasian seas

**Shiganova T. A.<sup>1</sup>, Molinero J.<sup>2</sup>, Sommer U.<sup>2</sup>, Javidpour J.<sup>2</sup>, Malej A.<sup>3</sup>, Christou E.<sup>4</sup>, Marambio M.<sup>5</sup>, Fuentes V.<sup>5</sup>, Mirsoyan Z.A.<sup>6</sup>, Gulsahin N.,<sup>7</sup> Lombard Fabien<sup>8</sup> Lilley M.K.S.,<sup>9</sup> and Angel D. L.<sup>10</sup>**

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**Keywords:** *Mnemiopsis leidyi*, *Beroe ovata*, *Beroe* spp, Eurasian seas

#### Introduction

Invasive species are widely accepted now as one of the leading threats to global biodiversity. Although numerous non-native species are transported and expanded around the world daily, the harm is caused by only a few of them, the invasive species. The need to understand the causes and impacts of similar invasions has never been more urgent, and the development of effective responses to existing and threatened invasions is a critical issue. Among them the most aggressive “super” invader is a ctenophore *Mnemiopsis leidyi* (A. Agassiz) that has colonized and continues to colonize new areas around the World Ocean. Cross-oceanic transport within ballast waters of intercontinental shipping vessels has contributed to the range of its expansion and initiated a rapid global spread of *M.leidyi*. Now the biogeographical distribution of *M.leidyi* in the Eurasian seas covers a wide range of habitats, from the brackish closed and semi-closed seas to the Mediterranean Sea with very high salinity and Atlantic coastal areas with oceanic salinity, from temperate to subtropical, from high productive to oligotrophic environments.



The goal of the review is a comparative analysis of variability of *Mnemiopsis leidyi* morphological, ecophysiological features, pattern of spatial and temporal population dynamics in the Black, Azov, Caspian, several areas of the eastern and western Mediterranean and the North and Baltic seas as a response on different environmental conditions (salinity, temperature, productivity, prey concentration, predators availability) of these seas and climate shift effect; determination sensitive of ecosystem for invasion of *M.leidyi* and for invaders in general.

### **Material and methods**

Authors of this review have available field and experimental data from the initial population creation in study seas till the present time. Data have been selected for analyses: from the northeastern Black Sea (Shiganova T.A.), the total Sea of Azov (Mirsoyan Z.A.), the Caspian Sea (Shiganova T.A.),the Aegean Sea (Turkish area, Gülsahin N.), Christou E. (the northern Aegean Sea), Levantine Sea (Angel D. L.), Northern Adriatic Sea (Malej A.), Western Mediterranean(Liguria Sea), Berre Lagoon (Lombard F., Lilley M.), North Western Mediterranean (Marambio M., Fuentes V.), Baltic Sea, western Baltic Sea (Kiel Fjord); (Javidpour J., Molinero J., Sommer U.). Other data were selected from published sources.

### **Results and discussion**

On the base of *M.leidyi* collaborative comprehensive synthesis of field and experimental data and published sources we have analysed:

Current spatial distribution with special attention of first finding (seeding area) to show chronology of *Mnemiopsis leidyi* invasions (Figure 1).

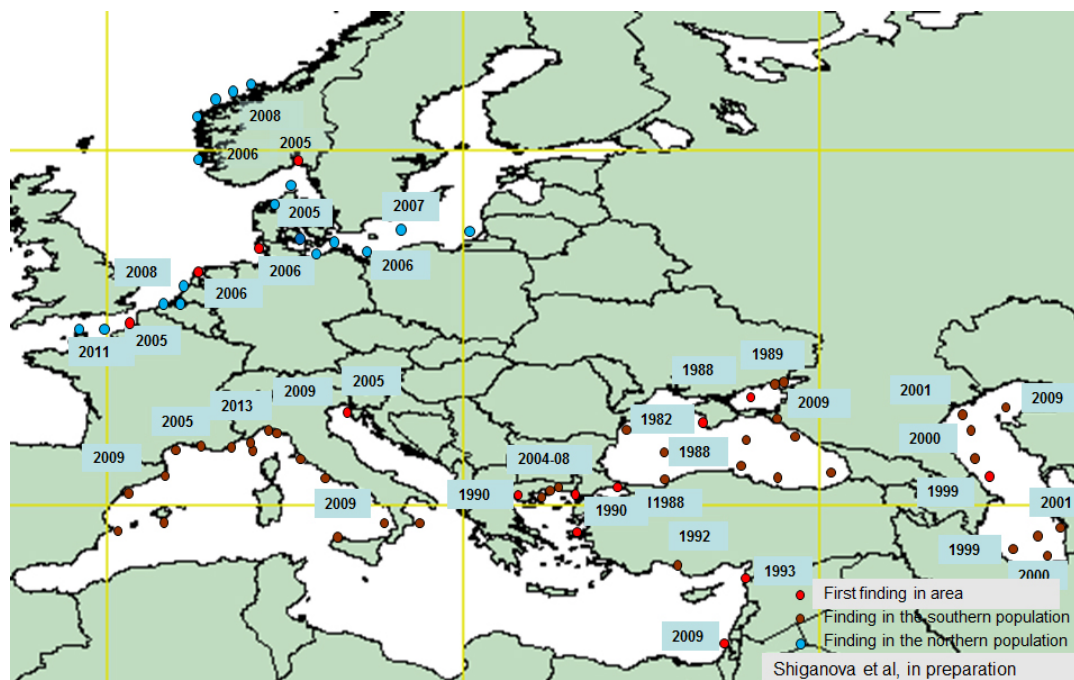


Figure 1. Chronology of *Mnemiopsis leidyi* invasion and dispersal in the seas of Eurasia (sources: Pereladov, 1988; Vinogradov *et al.*, 1989; Mutlu, 1999; Studenikina *et al.*, 1991; Mirsoyan *et al.*, 2006; Shiganova *et al.*, 2001; Shiganova, 1993; Shiganova & Malej, 2009; Shiganova, 2011; Galil *et al.*, 2009; Boero *et al.*, 2009; Lilley *et al.*, 2014; Fuentes *et al.*, 2010; Marambio *et al.*, 2013; Javidpour *et al.*, 2006; Boersma *et al.*, 2007; Faasse & Bayha, 2006; Hansson, 2006; Oliveira, 2007; Tendal *et al.*, 2007; Van Ginderdeuren *et al.*, 2012; Antajan *et al.*, 2014; Hosia & Falkenhuag, 2013; Delpy *et al.*, 2012).

- Interannual variability individual and population *M.leidy* size, spatial distribution and seasonal dispersal in adjacent areas and seas;
- Difference phenology in contrasting conditions of ecosystems (first of all temperature);
- Change morphology depending on environment (first of all salinity), which includes: body size, weight, length of lobes, change of body proportions, presents or absence warts, central oral tentaculars. Earlier three species were determined based on these differences (Mayer, 1912), now we may conclude that these changes are result of phenotypic plasticity of *M.leidy* in different environment (Figure 2); (Shiganova, 2007).

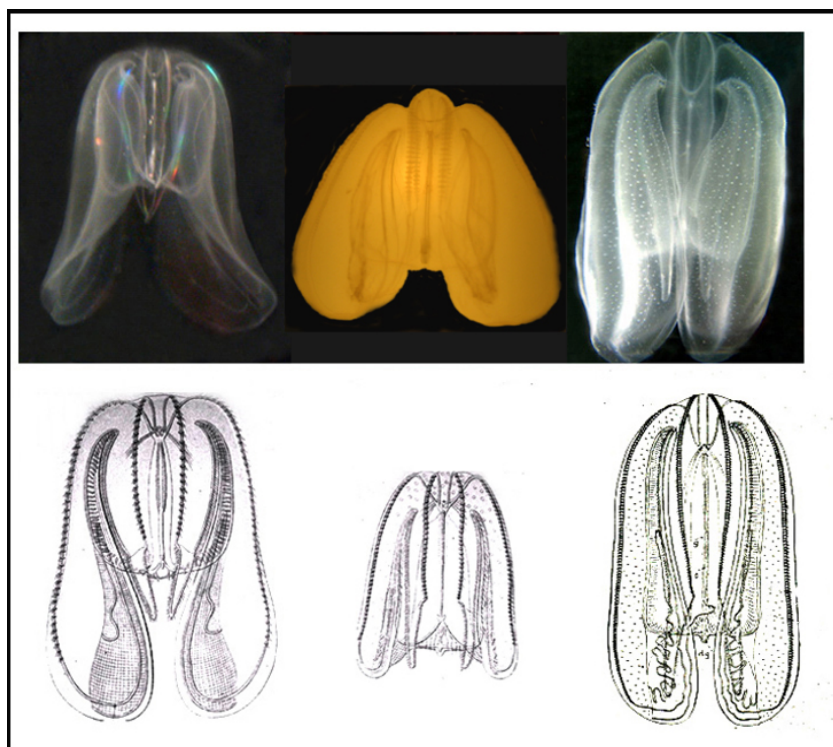


Figure 2. Morphological differences of *M. leidyi* individuals from the Black, Azov, Caspian and Aegean seas. Upper row: our data; below after Mayer (1912) (Shiganova *et al.*, 2009).

Variability of *M. leidyi* physiological features in different environments that determine life cycle, which includes metabolic rate, reproduction time and duration, fecundity, growth rate and size of maturity, pattern of distribution and finally predation rate on zooplankton. As it was observed fecundity is lower in lower salinity conditions but if we estimate weight specific fecundity it is higher in lower salinity conditions of the Caspian and Sea of Azov than in the Black and Aegean seas with higher salinity (Shiganova *et al.*, 2004). Effect of decrease fecundity in lower salinity was observed in the Baltic Sea in detailed experimental study (Jaspers *et al.*, 2011)

We made niche assessment. We depicted environmental windows relative to temperature, salinity and chlorophyll "a" favouring the presence of *Mnemiopsis leidyi* in different European Seas. Favourable environmental windows were computed by means of a non-parametric test of association and Monte-Carlo randomization. As a result it was concluded that according to these parameters there are two different origin populations: Southern population (which includes the Ponto Caspian basin and Mediterranean Sea) and Northern population (which includes the Baltic and North Sea) (Shiganova *et al.*, review, in preparation).

We use data of genetic study to determine the sources of *M. leidyi* populations using both mitochondrial (COI) and nuclear ribosomal Internal Transcribed Spacer (ITS) markers of the ctenophore *Mnemiopsis leidyi* that has provided understanding of its invasion history including dynamics of new areas' colonization and population source, which support our previous conclusion (Ghabooli *et al.*, 2011, 20013). These results are supported our previous conclusion.

The analysis of all observations has shown that *M. leidyi* has a wide environmental tolerance and phenotypic variability. Environmental conditions of contrasting ecosystems in recipient regions (salinity, temperature and productivity) facilitate the devel-

opment of *M. leidy* in wide range of variations in morphological features, growth rate and size, metabolism level, size of maturity, fecundity.

*M. leidy* effects on the European seas ecosystems are also different. The strongest impact was recorded in the productive and disturbed Black, Azov and Caspian Seas, during last years some effect was observed in the Baltic Sea and coastal areas of the eastern and western Mediterranean. But effect depends on population size, which control by current environmental and biotic conditions and climate forcing.

We observed availability of predators mainly another invader ctenophore *Beroe ovata* and other native *Beroe* species for definite area. *B. Ovata* follows for *M. leidy* in the areas of its expansion where *M. leidy* established and control *M. leidy* population size. *B. ovata* changes phenology follow changes phenology of *M. leidy* in new area. Native *Beroe spp.* also arrived in the areas where *M. leidy* available and feed on it (Figure 3). In the southern seas among native *Beroe* who hunt on *M. leidy* should be mentioned *B. cucumis* and *B. forskalii*; in the Baltic Sea *B. cucumis* and *B. gracilis*. *B. gracilis* is a species from the south-east North Sea (Greve *et al.*, 1976) who penetrates in the Baltic to feed on *M. leidy*. There is one more predator on *M. leidy* in the Mediterranean *Pelagia noctiluca* which consumes mainly small individuals. But any way it is very important for decreasing *M. leidy* population (Tilves *et al.*, 2013; Lombard and Lilley. in press). In addition *P. Noctiluca* is widely distributed in the Mediterranean.

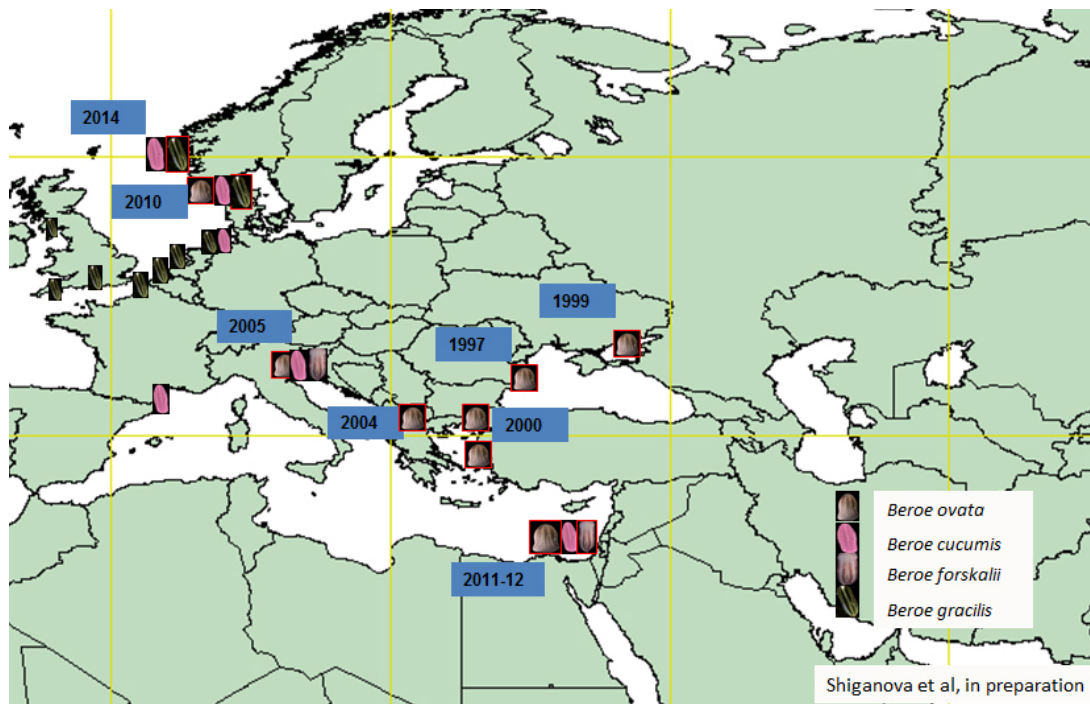


Figure 3. Chronology of *Beroe ovata* dispersal in new basins, expansion of *Beroe cucumis*, *Beroe forskalii* in the Mediterranean Sea and *Beroe gracilis* in the Baltic Sea from the North Sea Red frame showed that species invaded in area (Konsulov & Kamburska, 1997; Shiganova *et al.*, 2001; Tarkan, 2000; Shiganova *et al.*, 2007; Shiganova & Malej, 2009, Shiganova *et al.*, 2014; Greve *et al.*, 1976; Ringvold *et al.*, 2014, Vansteenbrugge *et al.*; in review).

Thus, representatives of order Beroida currently play an important role in trophic food webs of the changed coastal environments where *M. leidy* was introduced and established. Before *M. leidy* expansion in new areas where native *Beroe* species occur, *Beroe sp.* had only definite prey among zooplanktivorous ctenophores occurring in that area and in some cases additionally salps if they available. Now they are capable

also to consume *M.leidy*. Thus these predators control *M. leidy* and other zooplanktivorous ctenophore populations reducing the negative impact of *M.leidy* on zooplankton. In some cases when a newly introduced population of *M. leidy* vanishes in presence of *Beroe spp.*, it will not recover unless a new introduction event occurs. That has happened in the Northern Adriatic (Shiganova & Malej, 2009). Therefore distribution and taxonomy of representative of Beroidea should be especially studied.

In Kerteminde harbor one more observation by H. U. Riisgård (identification by T. Shiganova) was done. It was observed that in the case of overlap of occurrence of two lobate ctenophores like *M.leidy* and any of *Bolinopsis infundibulum* probable their hybridization. Among other ctenophores some individuals were found showed a mixture of characters that could result from hybridization between *M. leidy* and *B. infundibulum*. They had oral lobes originating at the level of infundibulum as in *M. leidy*, but the lowest part of the lobes looked as is characteristic for *B. infundibulum*. Auriculae were long and originated at the level of origin of oral lobes as it is typical for *M. leidy*, but the spherostome (body) had a shape intermediate between *Mnemiopsis* and *Bolinopsis*. Unfortunately, no genetic analyses were performed for these individuals. Because these hermaphrodite species *M.leidy* and *B. infundibulum* release both ova and sperm directly into the water cross fertilization may have been possible under the unusual conditions of occurrence described here.

This phenomenon is in need of further studies.

### Acknowledgement

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### 3.2 *Mnemiopsis leidyi* in the Baltic Sea region: Origin, transport and seasonality

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**Keywords:** comb jelly, drift model, invasive species, northern Europe

#### Introduction

The comb jelly *Mnemiopsis leidyi*, native to the east coast of America, has a long invasion history in European waters. Its first sighting dates back to the early 1980s from the Black Sea (Purcell *et al.*, 2001). *Mnemiopsis leidyi* invaded the Black Sea in the 1980s

with cascading effects on several ecosystem levels including commercial fisheries (Kideys, 2002). This native to the east coasts of America triggered large public and scientific attention as a result of this invasion and its ecological and economic impacts. In 2005, when *M. leidy* was sighted in Northern Europe for the first time (Faasse and Bayha, 2006), similar consequences were feared. *M. leidy* introduced to northern Europe around originates from a different North American sub-population. Hence, microsatellite markers revealed that the south European *M. leidy* population stems from the southeast coast of the US, namely the Gulf of Mexico, while the north European population could be traced back to the northeast coast (Reusch *et al.*, 2010). To date it is unknown if pre-adaptation in its native range has set the invasion corridor of *M. leidy* in European waters. However, so far it has been shown that the south European *M. leidy* population cannot tolerate water temperatures  $<4^{\circ}\text{C}$  (Shiganova *et al.*, 2001), while the northern population has been shown to overwinter under the ice in its native range (Costello *et al.*, 2006). Due to difficulties of morphological identification of its larval stages, there has been some ambiguity about *M. leidy*'s distribution range in northern Europe, especially the Baltic Sea. Here we synthesize data on the range expansion of *M. leidy* in the entire Baltic Sea region, including its larval stages as confirmed by DNA analyses (Jaspers *et al.*, 2013) and we present preliminary results of a drift model to predict possible seeding areas.

### Materials and methods

We outline the potential impact of *M. leidy* on the Baltic Sea ecosystem and constraints on its dispersal. Specifically, we present results from (i) direct (Jaspers *et al.*, 2011b) and indirect (Jaspers *et al.*, 2011a; Haraldsson *et al.*, 2013) effects of *M. leidy* on the Baltic cod population in its most important spawning ground, (ii) factors governing the spatial and temporal distribution of *M. leidy* eggs, larvae and adults in the Baltic (Haraldsson *et al.*, 2013; Jaspers *et al.*, 2013) and, (iii) *M. leidy* reproduction and its effect on population development (Jaspers *et al.*, 2011a). The approach involved 13 monthly monitoring cruises from high saline Skagerrak to low saline northern Baltic regions, in situ and laboratory controlled reproduction and feeding experiments, molecular analysis for species verification, and statistical modelling.

### Results and discussion

Along with reproduction rates in different regions of the Baltic Sea and salinity dependent egg production experiments in the laboratory, we have shown that reproduction rates in low saline central Baltic Sea regions are drastically reduced (Jaspers *et al.*, 2011), while maximum reproduction rates in high saline regions of the Skagerrak reach  $>11\,000$  eggs produced  $\text{ind.}^{-1}\text{d}^{-1}$  suggesting that the Baltic Sea is a sink dependent on *M. leidy* drifting in from high saline areas. From 2006 to 2010, *M. leidy* was widely distributed in northern Europe including the Baltic Sea. Though animals were present in the North Sea and Danish fjord systems up to now (Riisgård *et al.*, 2012; Antajan *et al.*, 2014), *M. leidy* disappeared from the southwest and central Baltic Sea in spring 2011. For example, no animals were observed during weekly (April – July) and monthly (August – March) ichthyoplankton samplings in the SW Baltic Sea from spring 2011 until summer 2014 (Christian Hesse, GEOMAR, personal communication). In the higher saline Kattegat/Skagerrak region, *M. leidy* was only sporadically observed during late autumn/winter 2011, while no animals were sighted at all during 2012, despite of an intensive monitoring program. On the other hand, since its first record in 2007 *M. leidy* has been present every year in Limfjorden, a highly eutrophicated, high saline Danish fjord system connecting the North Sea and the Kattegat/Baltic Sea region (Riisgård *et al.*, 2007; Riisgård *et al.*, 2012). However, abundances



fluctuate by > 2 orders of magnitude between sampling years along with different timing of bloom abundances between years.

Our results show that in 2012, when only very few *M. leidyi* were observed in Limfjorden late in the season (November, max. 0.5 ind. m<sup>-3</sup>), no animals were recorded in the Skagerrak, while one year later, when Limfjorden densities were high and maximum abundances measured again early in the season, *M. leidyi* was observed c. 1 month later in Gullmar Fjord, Skagerrak. Conversely, only sporadic sightings were recorded in Gullmar Fjord, Skagerrak during winter 2011/2012, when an exceptionally late *M. leidyi* bloom occurred in Limfjorden during November 2011 (Riisgård *et al.*, 2012). Our preliminary drift model results show that 10 to 20% of the particles released from Limfjorden reach the other side of the Kattegat/Skagerrak within 10 days. Considering extraordinary high abundances in Limfjorden (Riisgård *et al.*, 2007), along with high reproduction rates of *M. leidyi*, and local extinction of *M. leidyi* in the southern Kattegat and SW Baltic Sea after 2011, Limfjorden is an important refuge, hosting yearly *M. leidyi* populations in northern Europe. Our drift model results combined with empirical observations suggest that Limfjorden has the potential to act as source region seeding *M. leidyi* into the Baltic Sea, hence assuring reinvasions after extinction of local *M. leidyi* populations following harsh winter conditions for consecutive years.

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### 3.3 Looking for *Mnemiopsis leidyi* in Dunkirk Harbour (Southern North Sea, France)

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#### Background

Dedicated efforts to investigate the presence of *Mnemiopsis leidyi* in the French harbours of the Eastern English Channel and Southern North Sea were initiated during the 2010–2013 MEMO Project funded by the EU Interreg-2seas- IVa programme. During this time period the presence of *M. leidyi* was confirmed in several locations (see Antajan *et al.*, 2014, and contribution in the present volume). In Dunkirk, the only French industrial harbour of the North Sea, the presence of the species was confirmed from September to November 2012 by weekly sampling efforts in a basin (Forme 4) from the eastern harbour. The presence of *M. leidyi* in this area and more specifically the perspective of possible bloom events, led local coastal industries to fund a dedicated environmental monitoring programme to assess the risk of water intake clogging by gelatinous plankton in Dunkirk industrial harbour (i.e. in the western harbour).

#### Methods

Since April 2013, three stations located within Dunkirk Western Harbour have been sampled twice a month from April to October and once a month from November to March. Station 1 is located at the entrance of the harbour (outer harbour) and allowed to consider species tidal introduction from the North Sea, station 2 is located at the limit between the outer and inner harbour so as to integrate possible mixing between inner and outer populations, and station 3 is located in the inner port, at the entrance of the canal linking the western and eastern harbours (Figure 1). Since May 2014, a

fourth station has been added to the sampling programme to investigate the existence of possible retention zones in the most sheltered part of the harbour.

Gelatinous plankton and other macro-plankton are sampled using a WP3 plankton net (1m<sup>2</sup> opening, 1 mm mesh size) towed for 10 min from surface to near bottom depth in undulating movements. A WP2 plankton net (0.25 m<sup>2</sup> opening, 200 µm mesh size) towed from surface to near bottom depth in oblique tow is used to sample mesozooplankton and thus, to consider potential preys of gelatinous plankton.

WP3 samples are brought back alive to the lab 2–3 hours after sampling. At laboratory, gelatinous plankton is sorted, identified, counted and measured alive (umbrella diameter for cnidarians and oral-aboral length for ctenophores) under a stereomicroscope. The rest of the WP3 catch is fixed in 4% formalin and stored until analysed (i.e. sorting and counting of other macroplankton components). WP2 samples are visually inspected on board for the presence of ctenophores (especially *Mnemiopsis leidyi*) before being preserved in 4% formalin. All fixed samples are analysed within 9–12 months after collection.

A multiparametric probes (YSI 6600V2) is used to measure temperature, salinity, pH, turbidity, concentration of dissolved O<sub>2</sub>, and *in-situ* fluorescence profiles at each sampling station. 2L of water are sampled within 2 m depth with a Niskin bottle for further chlorophyll *a* analyses.



Figure 1. Location of sampling stations in Dunkirk (France) western harbour and of the visually prospected basins in Dunkirk eastern harbour.

## Results

In 2013, *Mnemiopsis leidyi* has only been observed in Dunkirk Western Harbour from September to December. The first individuals observed already measured 32 mm (oral-aboral length). So far, only small abundances have been recorded (Table 1), with a maximum of 0.02 ind. m<sup>-3</sup> corresponding to 6 individuals caught in a 10 min WP3 haul in mid-October 2013. The same day, 4 *Mnemiopsis leidyi* were caught in a single oblique WP2 haul, which led to an estimate abundance of 0.32 ind. m<sup>-3</sup>, i.e. one order of magnitude higher, highlighting the difficulty to get reliable abundance estimates for low abundances.

Two other ctenophore species were present in Dunkirk Western Harbour: *Pleurobrachia pileus*, which is by far the most abundant one, and *Beroe* sp. which was infrequently present in samples, always at low abundance.

The environmental conditions between year 2013 and 2014 differed quite a lot, with a noticeably cold late winter-early spring in 2013 compared to 2014, leading to 5.6°C higher temperatures in late April 2014 compared to the same date in 2013. The presence of *Mnemiopsis* in autumn and early winter coincided with water temperature from 17.2 to 9.8°C and salinity from 34.5–33.5 (Figure 2).

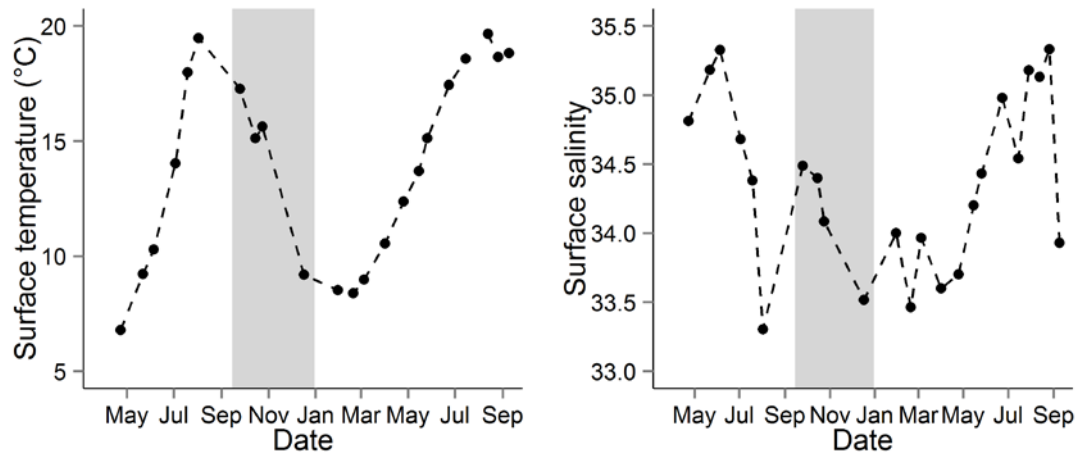


Figure 2. Seawater surface temperature and salinity in Dunkirk Western Harbour (Station 1) from April 2013 to September 2014. The shaded area indicates the period of *Mnemiopsis leidyi* presence.

Table 1. Abundance of *Mnemiopsis leidyi* and other ctenophore species in Dunkirk Western harbour in 2013 and 2014.

	<i>Mnemiopsis leidyi</i>			<i>Beroe sp.</i>			<i>Pleurobrachia pileus</i>		
	St.1	St.2	St.3	St.1	St.2	St.3	St.1	St.2	St.3
23-apr.-13	0.00	0.00	0.00	0.00	0.00	0.003	0.13	0.36	0.43
6-may-13	0.00	0.00	0.00	0.01	0.02	0.13	0.24	<b>0.46</b>	<b>1.15</b>
22-may-13	0.00	0.00	0.00	0.01	0.00	0.00	0.49	0.24	0.80
5-jun.-13	0.00	0.00	0.00	0.00	0.00	0.00	<b>0.88</b>	0.29	0.89
20-jun.-13	0.00	0.00	0.00	0.00	0.03	0.04	0.03	0.07	0.47
3-jul.-13	0.00	0.00	0.00	0.00	0.01	0.01	0.09	0.39	0.32
19-jul.-13	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.03
2-aug.-13	0.00	0.00	0.00	0.01	0.04	0.01	0.01	0.01	0.01
29-aug.-13	0.00	0.00	0.00	0.00	0.002	0.01	0.00	0.05	0.03
25-sep.-13	0.01	*	0.00	0.00	0.00	0.00	0.04	0.01	0.01
15-oct.-13	*	*	*	0.00	0.00	0.00	0.03	0.03	0.01
24-oct.-13	0.01	0.02	0.02	0.00	0.00	0.00	0.07	0.08	0.02
17-dec.-13	0.00	*	0.003	0.004	0.002	0.004	0.07	0.03	0.06
30-jan.-14	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.001	0.001
19-feb.-14	0.00	0.00	0.00	0.00	0.00	0.00	0.003	0.04	0.03
5-mar.-14	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.02
18-mar.-14	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.002	0.00
1-apr.-14	0.00	0.00	0.00	0.00	0.004	0.002	0.02	0.06	0.09
25-apr.-14	0.00	0.00	0.00	0.002	0.01	0.03	0.86	0.22	6.51
13-may-14	0.00	0.00	0.00	0.00	0.00	0.00	<b>10.67</b>	<b>53.30</b>	<b>12.88</b>

26-may-14	0.00	0.00	0.00	0.00	0.00	0.00	4.14	1.40	11.58
23-jun.-14	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.67	0.95
15-jul.-14	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.23	0.02
29-jul.14	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.23	0.36
13-aug.-14	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.23	0.55
26-aug.-14	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.16	0.21
9-sep.-14	0.00	0.00	0.00	0.00	0.00	0.00	0.62	0.13	0.17

\* *M. leidyi* not present in WP3 net (used to estimate abundances) but caught in WP2 net.

Post-meeting addendum: *Mnemiopsis* was observed in Dunkirk West at the end of September 2014 by E. Antajan (0.04 to 0.09 ind. m<sup>-3</sup>, corresponding to 9 and 2 individuals caught in a Bongo net and a WP2 net respectively), confirming the presence of the species in the area for the year 2014. The 30<sup>th</sup> September 2014, a visual prospection for *M. leidyi* was conducted in the basins of Dunkirk eastern harbour. No *M. leidyi* were seen despite good visibility conditions. Two weeks later, in early October, divers reported having observed the species in large numbers in the same area (Dunkirk eastern harbour).

## Discussion

The temporary autumnal presence and the small abundances recorded in Western Dunkirk Harbour in 2013 and so far in 2014 contrasted with what was observed the same years in neighbouring regions of the North Sea (e.g. Spuikom at Oostende, Belgium) as well as in some regions of the Eastern English Channel (Le Havre Harbour and Bay of Seine, France) where the species was present throughout summertime and sometimes in very high numbers (L. Vanstenbrugge, *pers. comm.*). This logically raises questions on the origin of the animals observed in Dunkirk Harbour. As previously discussed by Antajan *et al.*, (2014) it is thought that Dunkirk Harbour *Mnemiopsis leidyi* population behaves as a sink population depending on seasonal re-inoculation. This is supported by the absence of organisms in the samples collected the rest of the year, including in areas of the inner harbour which were *a priori* identified as potential sheltered zones where *M. leidyi* could reproduce and maintain a permanent population. The hypothesis of re-inoculation may be further supported by the fact that the first recorded individuals measured >30 mm and >50 mm in 2013 and 2014 respectively (i.e. relatively big individuals). However, what is known from local current patterns would suggest that a spread of *M. leidyi* into Dunkirk Harbour from neighbouring areas is unlikely (Antajan *et al.*, 2014).

A serious limitation in the sampling protocol is the fact that plankton tows would not sample individuals that may form near-bottom discrete aggregations (Costello and Mianzan 2003). This question should be investigated with direct observations by divers or using imaging systems (video or camera).

A last point that deserves to be considered is the difficulty to reliably measure the abundance of the species when occurring in low numbers. As highlighted in results, sampling with Bongo, WP3 and WP2 nets lead to very different abundance estimates.

## Conclusion

*Mnemiopsis* has been present for at least 3 consecutive years in Dunkirk harbour, but only during the autumn and early winter. Analysis of the rest of the data collected during the environmental monitoring program of the harbour (i.e. zooplankton

abundance, chlorophyll *a* biomass, and abiotic parameters) should provide further insights into the environmental conditions favourable to the presence of this species.

### Acknowledgement

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## 3.4 Recurrent winter observations of *Mnemiopsis leidyi* swarms in the Southern North Sea

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**Keywords:** ctenophore, invasive species, *Mnemiopsis leidyi*, North Sea

### Introduction

Since the first records of the lobate ctenophore *Mnemiopsis leidyi* (A. Agassiz 1865) in the North Sea in summer 2005 (in Grevelingen, The Netherlands, reported by Faasse and Bahya 2006 and in Nissum Fjord, Denmark, as reported by Tendal *et al.*, 2007), large blooms were regularly observed in estuaries and along coastal regions of the eastern and southern North Sea. Most of these *M. leidyi* records occurred during summer and autumn when temperature was comprised between 10 to 23°C. Some authors pointed out that winter temperature conditions in the North Sea may not favour *M. leidyi* survival (Boersma *et al.*, 2007), even in a shallow seaway (Riisgård *et al.*, 2011). Whereas *M. leidyi* is found in an extremely wide range of environmental conditions in its native habitat of the Atlantic coast of North and South America (temperature of 2–32°C; Purcell 2001), the species does not survive at temperatures lower than 4°C in the Black Sea (Shiganova *et al.*, 2001) and lower than 2°C in the subarctic Baltic Sea (Viitsalo *et al.*, 2008). Faasse and Bayha (2006) suggested that estuaries may serve as temperature refuge areas allowing *M. leidyi* to over-winter in the North Sea. Here we report on the recurrent observations of *M. leidyi* swarms surviving off shore in the southern North Sea during winter.

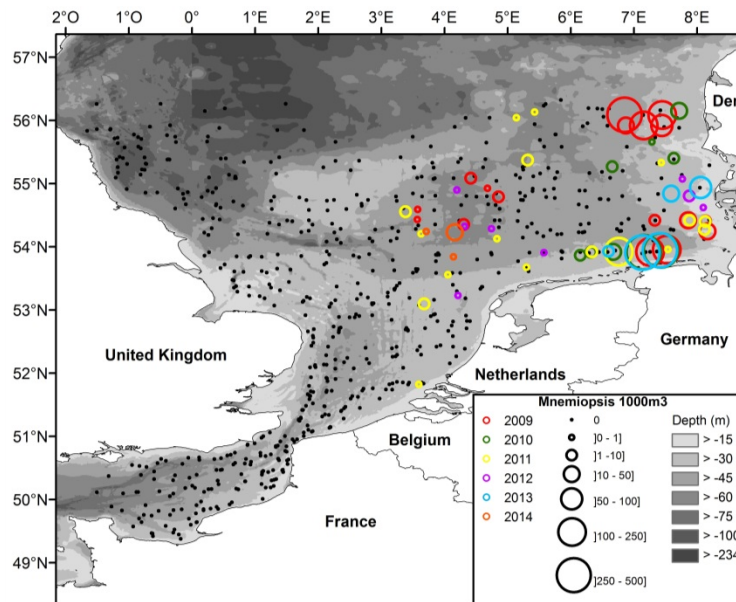
### Methods

The study was carried out on regular International Bottom Trawl Surveys (IBTS; ICES 2010) on board the R/V *Thalassa* in January and February from 2009 to 2014. Sampling effort extended from the eastern English Channel to latitudes up to 56°N. *Mnemiopsis leidyi* were identified, counted and measured alive from samples collected at night with a Midwater Ring Net designed for fish larvae sampling (black conical

net of 13 m in length, opening diameter 2 m, mesh size 1.6 mm except the last meter and the cod-end for which the mesh size is 500  $\mu\text{m}$ ). The haul profile is oblique, and the fishing speed of 3 knots, for at least 10 minutes. *Mnemiopsis leidyi* were measured for length (aboral-oral dimension) and assigned to size classes to the closest 0.5 cm. Morphological identification of *M. leidyi* was based on the position of the oral lobes extending to the apical statocyst (sense organ) over nearly the entire body length (Antajan *et al.*, 2014). To confirm morphological identifications some ctenophore specimens were preserved in 99% alcohol every year since 2011 for further DNA analysis according to the method describe in Van Ginderdeuren *et al.*, (2012; see superscript in Annex). Temperature and salinity profiles were recorded at each sampling station using an SBE-19plus.

**Results**

In 2009, we had the surprise to discover numerous individuals (up to 782 in one trawl) of *M. leidyi* in Midwater Ring Net samples that were sorted alive on board immediately after sampling for fish larvae study. Since then, recurrent patches of *M. leidyi* were observed off the Danish and the Netherlands coasts and in the German bight during winter IBTS surveys (Figure 2). Few individuals were also recorded in the central region of the southern North Sea, but never off the English coast and in the eastern English Channel. Genetic analysis carried out since 2011 on some stations confirmed the morphological identification of the ctenophores as *M. leidyi*. The abundance of *M. leidyi* ranged from 0.12 to 456.13 ind. $\cdot$ 1000m<sup>-3</sup> over the six years. At stations where *M. leidyi* occurred, temperature ranged between 1.2 and 7.5°C and salinity between 27.1 and 35.1 (Figure 2).



**Figure 1.** Distribution of *Mnemiopsis leidyi* (ind. 1000 m<sup>-3</sup>) overwintering in the southern North Sea from 2009 to 2014 and bathymetry of the studied area.

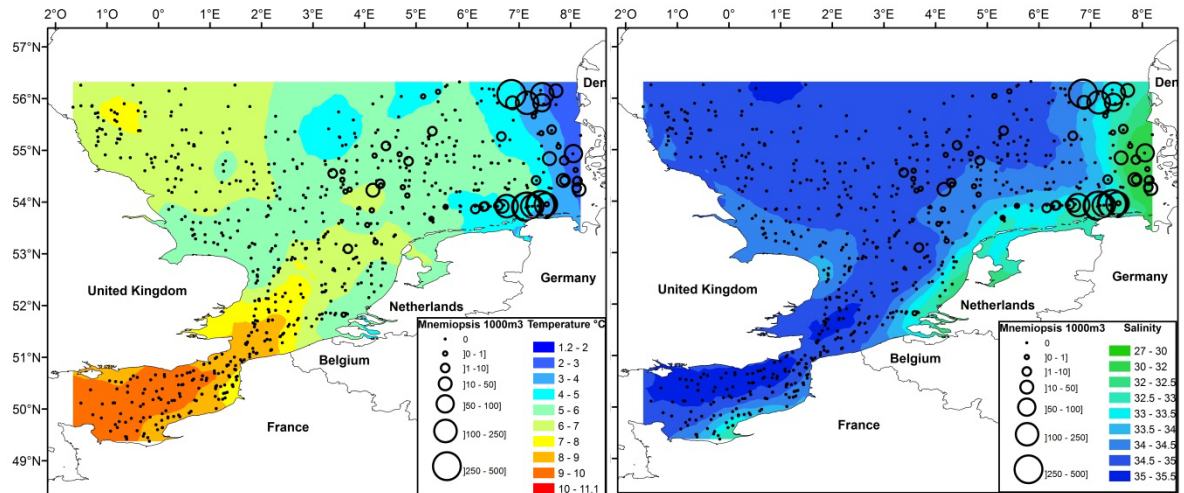


Figure 2. Interpolated temperature (left) and salinity (right) during January-February from 2009 to 2014 in the southern North Sea with distribution of *Mnemiopsis leidyi* (ind. 1000 m<sup>-3</sup>).

All *M. leidyi* collected were lobate individuals, and their oral-aboral length was most often of 2 or 3 cm, even though some individuals could reach 7 cm (figure 3).

### Discussion

The present study demonstrated that *M. leidyi* is capable of surviving off shore in the North Sea during winter even at temperatures less than 2°C. Highest densities (> 100 ind.·1000m<sup>3</sup>) were clearly observed in the colder and desalinated waters off the Wadden Sea and induced mainly by the Rivers Rhine and Elbe run-off which are the most relevant sources of freshwater for the German bight. However the densities observed were much lower than what could be observed in the Wadden Sea (up to 500 ind. m<sup>-3</sup> in summer, van Walraven *et al.*, 2013). In 2012 and 2014, few individuals were observed, and many seemed much degraded (comb-rows of cilia and lobes damaged), while other years individuals were in good state with sometime visible prey in their gut. Additional studies are needed to understand in what physiological state are individuals observed in winter, if they are food-limited and if they are able to survive winter conditions in the North Sea.



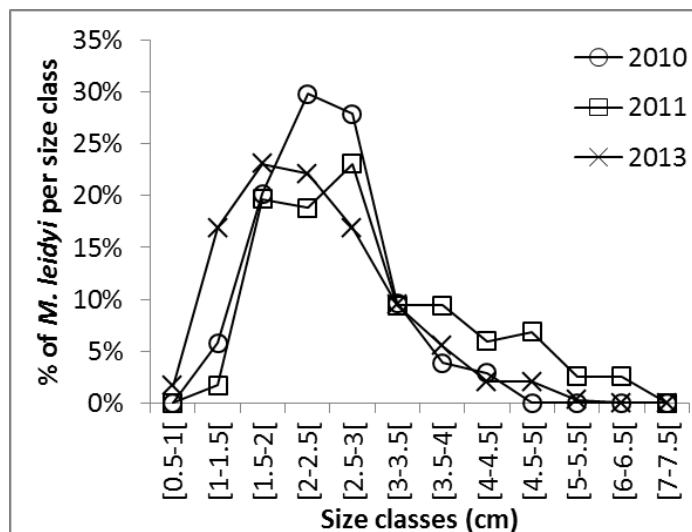


Figure 3. Percentage of *Mnemiopsis leidyi* per size classes (cm) and per year in the southern North Sea in winter.

The Midwater Ring Net was particularly useful as it allows filtering a large amount of water (about 4000 m<sup>3</sup>) to observe the presence of isolated ctenophores. The downside is that *M. leidyi* can be badly damaged after a tow of 10 minutes or more, and it is not always easy (even impossible) to rinse a net of 13 m length once on board. The result is an underestimation of the *M. leidyi* abundance that it must be corrected by using other type of plankton nets, such as WP2 or WP3 nets. Preliminary comparisons on few stations during IBTS 2014 have shown that the Midwater Ring Net may underestimate *M. leidyi* abundances by 30% compare to a WP3 net that was also towed for ten minutes (1 m. s<sup>-1</sup>).

### Acknowledgements

We gratefully acknowledge the crew of the R/V *Thalassa* for help with ctenophore collection. We wish to thank Dr Otto Oliveira for his help and advice during the initial morphological identification of *Mnemiopsis leidyi*. This work was part-funded by the EU under the Interreg IVa (CHARM phase III project) and Interreg IVa-2seas (MEMO project) programs.

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### 3.5 Distribution and size structure of comb jellyfish, *Mnemiopsis leidyi* (Ctenophora) in Izmit Bay during mucilage event

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**Keywords:** *Mnemiopsis leidyi*, mucilage, Izmit Bay, the northeastern Marmara Sea

#### **Abstract**

The Marmara Sea has a rather complex hydrological system, in a zone of transition between dense (salinity 37–38.5 ‰) and warmer waters originating in the Mediterranean Sea, and cold, lower-salinity water (20–22 ‰) coming from the Black Sea and plays significant role on biodiversity of adjacent seas. The Marmara Sea, in the last twenty years, went through a series of massive disruptions that greatly impaired local economies and ecosystem functions due to especially eutrophication caused by industrial and urban activities. The last years characterized by jellyfish blooms, red tides and mucilages. The Ctenophore *Mnemiopsis leidyi* was introduced into the Marmara Sea in early 1990s and effected all the ecosystem (Isinibilir *et al.*, 2004; Isinibilir 2012), and first mucilage aggregates were observed in the Marmara Sea in October 2007 throughout all water column of the Marmara Sea (Aktan *et al.*, 2008). But, these were denser and longer duration in Izmit Bay, which is affected by intense industrial activity, and which has a weaker circulation compared to the Marmara Sea. Izmit Bay located in the northeastern part of the Marmara Sea, is one of the most polluted areas in the Marmara Sea. In this study, we focused on spatial and temporal distributions of the invaders *Mnemiopsis leidyi* in Izmit Bay during mucilage period in 2008 and the findings of the present study were also compared with those of previous years.

Jellyfish samples were collected using a WP2 closing net (157 mm mesh, 0.5 m diameter) from bottom to the surface in Izmit Bay at 3 stations from April–December 2008.

Samples were identified and measured immediately on board. The wet weight was calculated by formula:

$$W \text{ (g)} = 1.4061 \times L \text{ (cm)}^{1.6161}$$

where W is the wet weight, and L is total length of *M. leidyi* (Isinibilir 2012).

*M. leidyi* was available from May to September 2008. While mean abundance (31 ind.m<sup>-3</sup>) and biomass (144 g.m<sup>-3</sup>) was highest in August (Figure 1), the maximum abundance (58 ind.m<sup>-3</sup>) and biomass (353 g.m<sup>-3</sup>) of *M. leidyi* was observed in western part of the bay in August 2008. Maximum length of the jellyfish was 70 mm that was found in June (Table 1). *Beroe ovata* was found only in September. After September, all jellyfishes disappeared and mucilage aggregation was observed in October. Compared to 2001-2002, mean abundances of *M. leidyi* were higher in summer 2008 (Isinibilir 2012) and *Noctiluca scintillans* density decreased more than ten-fold in October 2008 (Table 1). The most important differences in total zooplankton abundance were observed in October when abundance of some significant species deteriorated from 2001 to 2008. The most severe decrease was observed in *Penilia avirostris*, dominant species in the summer-autumn periods in the Marmara Sea.

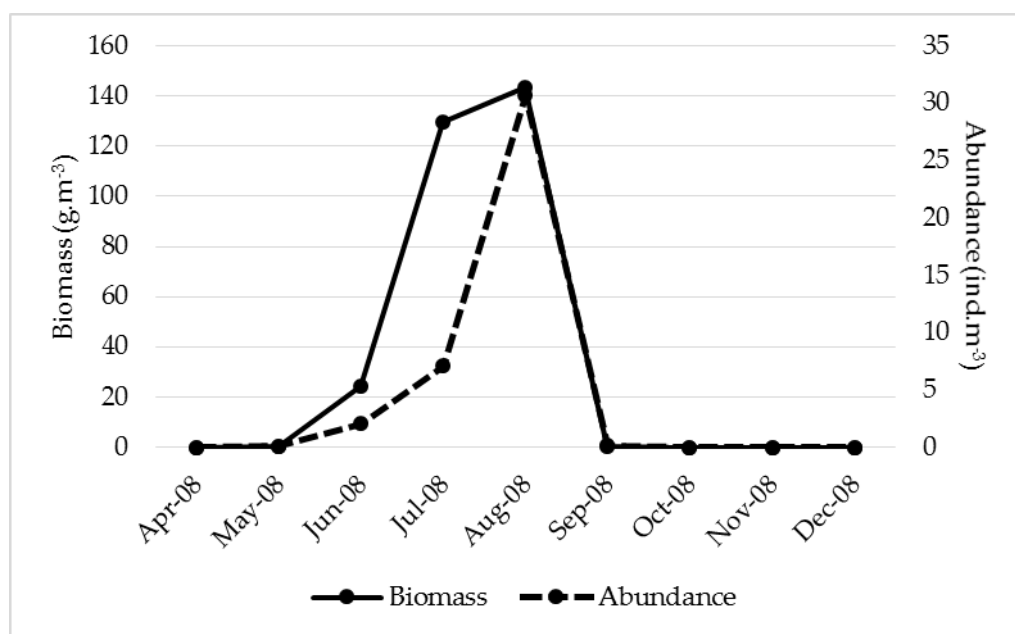


Figure 1. Seasonal fluctuations of abundance and biomass of *Mnemiopsis leidyi*, in Izmit Bay during 2008 mucilage period.

Table 1. Summer composition (June, July, August) of some parameters at the surface during the mucilage period in 2008 and the without mucilage periods in 2001-2002 in Izmit Bay.

	2001	2002	2008
Water temperature (°C)	26	24	18
Surface salinity (ppm)	-	21	6
Ch a (µg.L <sup>-1</sup> )	-	5	4
Secchi disc (m)	4	3	4
Mean number of <i>M. leidyi</i> (m <sup>-3</sup> )	6	0.5	13
Maximum number of <i>M. leidyi</i> (m <sup>-3</sup> )	51	4	58
Mean biomass of <i>M. leidyi</i> (m <sup>-3</sup> )	35	6	99
Maximum biomass of <i>M. leidyi</i> (m <sup>-3</sup> )	245	0.7	353

Maximum length of <i>M. leidyi</i> (cm)	17	3	7
Mean number of Copepoda (m <sup>-3</sup> )	12464	2323	243
Mean number of <i>A. clausi</i> (m <sup>-3</sup> )	2631	747	202
Mean number of Cladocera (m <sup>-3</sup> )	23532	1605	1443
Mean number of <i>P. avirostris</i> (m <sup>-3</sup> )	17607	603	1418
Mean number of <i>N. scintillans</i> (m <sup>-3</sup> )	1352	5627	117023

In 2007/2008 mucilage periods, mucilage aggregates was characterized by high values of diatoms and dinoflagellates density and bacterial activity (Tüfekçi *et al.*, 2010). It is known that inorganic nutrients excreted by jellyfish populations provide a small but significant proportion of the N and P required for primary production by phytoplankton and excretion of dissolved organic matter may also support bacterioplankton production (Pitt *et al.*, 2009). After bloom of *M. leidyi* in the summer period, its decomposition may result in a large release of inorganic and organic nutrients in Izmit Bay.

In conclusion, the Marmara Sea in recent years has been undergoing profound changes, mostly due to excessive fishing, increasing eutrophication, introduction and bloom of jellyfish species that strongly impact the zooplankton communities (Zengin and Mutlu, 2000; Isinibilir *et al.*, 2010, Isinibilir 2012). Furthermore, massive mucilage event occurred in 2007/2008 has caused significant shifts in zooplankton abundance and community structure. Finally, long term studies on jellyfish species in the Marmara Sea should be investigated in order to predict potentially their effects on this delicate ecosystem.

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### 3.6 *Mnemiopsis* in the Berre Lagoon, what are the main triggers for its expansion?

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#### **Introduction**

Thousands of marine species are moved around yearly due to human activities either deliberately or not into areas where they are not indigenous. Among these introduced or alien species only a few, then called invasive species, thrive in their new environment, impacting biodiversity, ecosystem functioning and human activities (i.e. fisheries, industrial complex, and tourism). These invasive species see their population rapidly increase in their new habitat, where no or very little natural controls exist (i.e. predators, parasites), reaching densities out of control. Among invasive jelly species, the invasive success history of the ctenophore *Mnemiopsis leidyi* in most European waters since the 1980s offers a unique opportunity to study population dynamics and dispersal. Two main invasion pathways have been identified for *M. leidyi* introduction into Eurasia: the first via the Gulf of Mexico into the Black Sea; the second via the East Coast of the USA into the North and Baltic Seas. This particularly voracious predator reduces the amount of food available for other predators such as commercially important fish species, thus creating modifications in prey abundance, specific diversity and ecosystem functioning all the way down to bacteria and virus. Understanding the population dynamics of gelatinous zooplankton is thus mandatory to establish their actual role and potential threat in marine ecosystem.

Since 2005, this species has been a resident feature of several coastal lagoons along the French shore of the Mediterranean Sea. An extensive study in the Berre Lagoon has been conducted for the past 4 years on the impact of the environmental conditions on the success of this species. This lagoon is under strict regulation of freshwater and silt inputs going thru the hydro-electrical power plant in the North since 2006. Other limitations in the inputs of chemicals are in place.

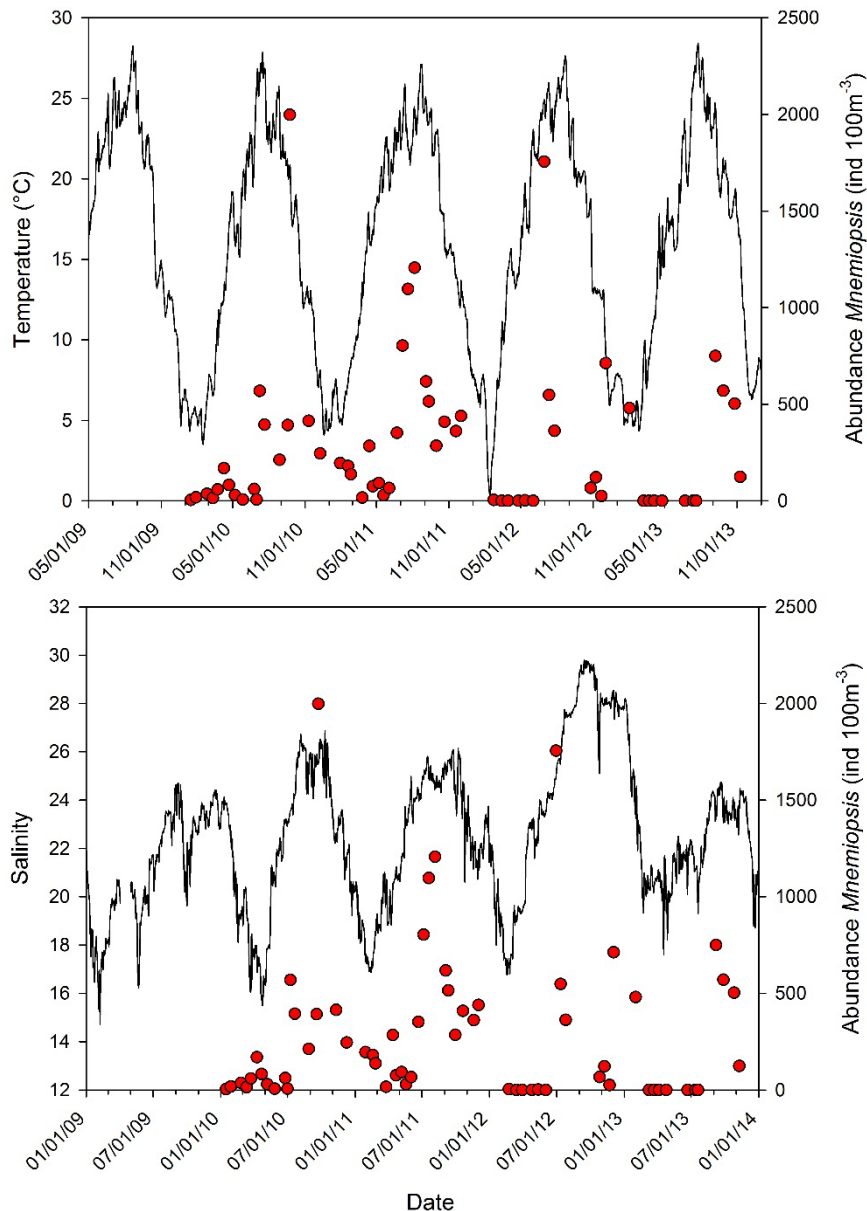
#### **Material and methods**

Environmental conditions were recorded in continuous at several depths at three stations located in the upper middle and lower part of the main lagoon. Bi-weekly samplings were conducted, using a modified Nansen nets (700 µm mesh size, 2 m long and 75 cm opening area). Nets were towed horizontally for 2 to 5 min. Life stages, size (oral-aboral length) and total weight was recorded.

#### **Results and discussion**

Temperatures displayed a clear temperate pattern, with summer values around 26–28°C and winter ones down to 5°C (Figure 1). Regulation in the amount of freshwater inputs applied in 2006 showed a real impact on the salinity minimum of the lagoon, with an increase of about 4 units of salinity between 2009 and 2013. Maximum values varied between 26 and 30 (Figure 1). Winter conditions in 2011/12 and 2012/13 were harsher with lower temperature reaching 0°C and ice developing on the surface of the lagoon in 2011/12 and winter conditions lasting longer than usual in 2012/2013.

*Mnemiopsis leidyi* appeared in September 2005, but the monitoring started only in January 2010. The invasive species was present year around from 2010 to the beginning of 2012. Even if the lagoon is still showing large variations in salinity, temperatures seem to have a clear impact on the population dynamics. Inter-annual variability in the timing of the bloom of this species appeared strongly impacted by the length and strength of the winter conditions. Very cold winter conditions in early 2012 and 2013 clearly affected the population of *Mnemiopsis* (Figure 1) with no organisms recorded for several months.



**Figure 1.** Temperature and salinity variations at Station SA2 from 2009 to 2013. In red circles, abundance (ind. 100m<sup>-3</sup>) of *Mnemiopsis leidyi* (Thibault *et al.*, in prep).

Maximal abundance (up to 23 ind m<sup>-3</sup>) of adults are observed at the end of summer (August/September) While several studies have shown that egg production is strongly correlated to temperature, salinity and food availability (Baker and Reeve 1974; Reeve *et al.*, 1989; Jaspers *et al.*, 2011) with optimal conditions: temperature >12°C (Purcell *et al.*, 2001, Costello *et al.*, 2006, van Walraven *et al.*, 2013), salinity 10–30 (Le-

thiniemi *et al.*, 2012) and food  $> 3 \text{ mg C m}^{-3}$  (Kremer 1994; Purcell *et al.*, 2001). But high reproduction is taking place in Berre thru winter as highest numbers of juvenile have been recorded in January-February (Figure 2), when salinity and food availability are within the optimal ranges (Delpy *et al.*, submitted).

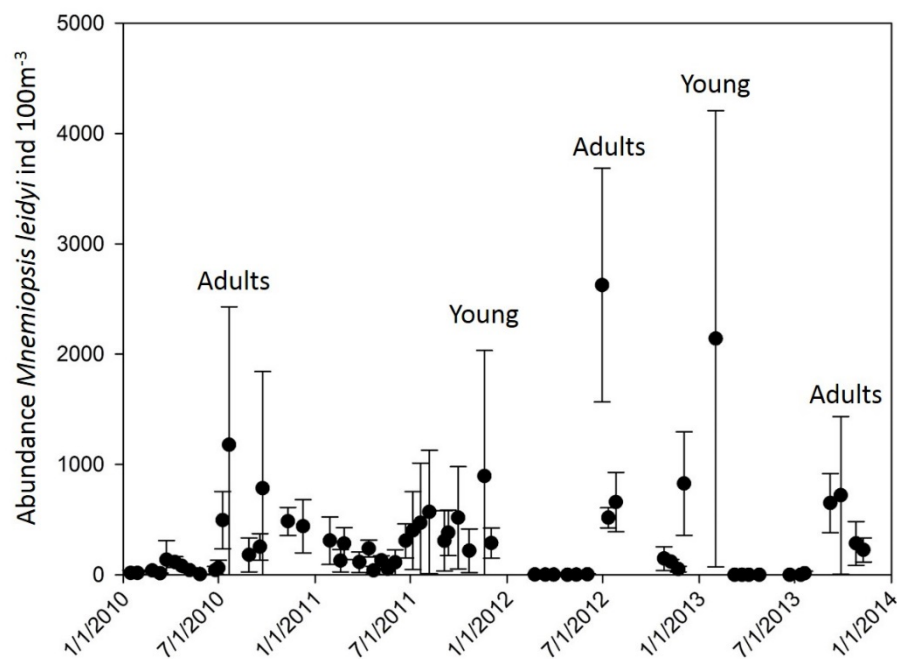


Figure 2. Distribution of *Mnemiopsis* (average +/- stdev) in the Berre Lagoon from 2010 to 2013 (Thibault *et al.*, in prep). Development of continuous massive gelatinous populations, like in Berre which presents a perennial *M. leidyi* population, might in the future transform the pelagic ecosystems functioning and benthic-pelagic interactions.

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### 3.7 The invasive ctenophore *Mnemiopsis leidyi* in the Spanish Mediterranean coast

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**Keywords:** comb jelly, invasive species, NW Mediterranean

#### Introduction

The American comb jelly *Mnemiopsis leidyi* is native to estuaries and coastal regions of the Western Atlantic Ocean, from the United States to southern Argentina (Mianzan, 1999). Since the early 1980s, when it was first introduced into the Black Sea, and during the last three decades, it has invaded and expanded to almost all European waters, including the western basin of the Mediterranean Sea. The occurrence of the invasive ctenophore *Mnemiopsis leidyi* in the Spanish Mediterranean coast was reported and genetically confirmed in 2009 at several locations (Catalonia, Alicante, Valencia, Balearic Islands) but disappeared after that summer (Fuentes *et al.*, 2010). One year after, in 2010, the presence of this ctenophore was more evident, mainly in the southern limit of the Catalan coast in the Ebro River Delta, specifically in the Fangar and Alfacs bays, an area with high anthropogenic disturbance but also, with high socio-economic importance due to aquaculture and fishing, and ecological relevance as Natural Park, RAMSAR wetland and European site Natura 2000. The presence and abundance of *M. leidyi* in the Alfacs bay has been a constant since then, becoming more than just an isolated or seasonal fact. Additionally, in 2012, blooms of *M. leidyi* were reported in the Mar Menor lagoon, a hypersaline environment (42.3–45.6 psu). Only adults were registered and the abundance was significantly related to temperature (Marambio *et al.*, 2013a).

#### Materials and Methods

A monthly monitoring programme was established in the Alfacs Bay at the end of 2010 in order to determine the eco-physiology and population dynamics of the species. Field sampling includes abundance and distribution assessment of *M. leidyi*, analysis of the zooplankton community together with environmental parameters (i.e.: temperature, salinity, chlorophyll-a, dissolved oxygen, nitrate and phosphate). Environmental variables were correlated to the abundances of the species using GAM models. The data available of the occurrence in the Mediterranean Sea was used to predict potential establishment areas for *M. leidyi* in the whole Mediterranean basin, based on an Ensemble modelling approach.



## Results and Discussion

Results suggest a successful establishment of *M. leidyi* in the study area with apparently no restrictions on the population. All life-stages (i.e. tentaculate larvae, transitional stage and adult stage) were observed and collected during sampling. The largest collected individuals achieved 100–120 mm of total length (oral-aboral with lobes). Reproduction events occurred all-year round. Seasonal dynamics showed that adults dominate the population structure during summer and autumn reaching maximum densities of 281 ctenophores  $m^{-3}$ , while larvae reach densities higher than 50 individuals  $m^{-3}$ .

Adult and larvae densities showed a positive relationship with temperature, salinity and phosphate, a humped-shaped relationship with chlorophyll-a and dissolved oxygen and a negative relationship with nitrate (Figure 1), revealing the capacity of this species to adapt and develop in the study area with the current characteristics of an ecologically disrupted ecosystem (Marambio *et al.*, 2013b). Moreover, the elasticity analysis from the matrix population study showed that the most important proportional contributions to the population growth rate focus in the larvae stage (Marambio *et al.*, 2013b), enhancing the importance of the positive correlation of *M. leidyi*, specially the larval stage with environmental parameters in such a degraded habitat.

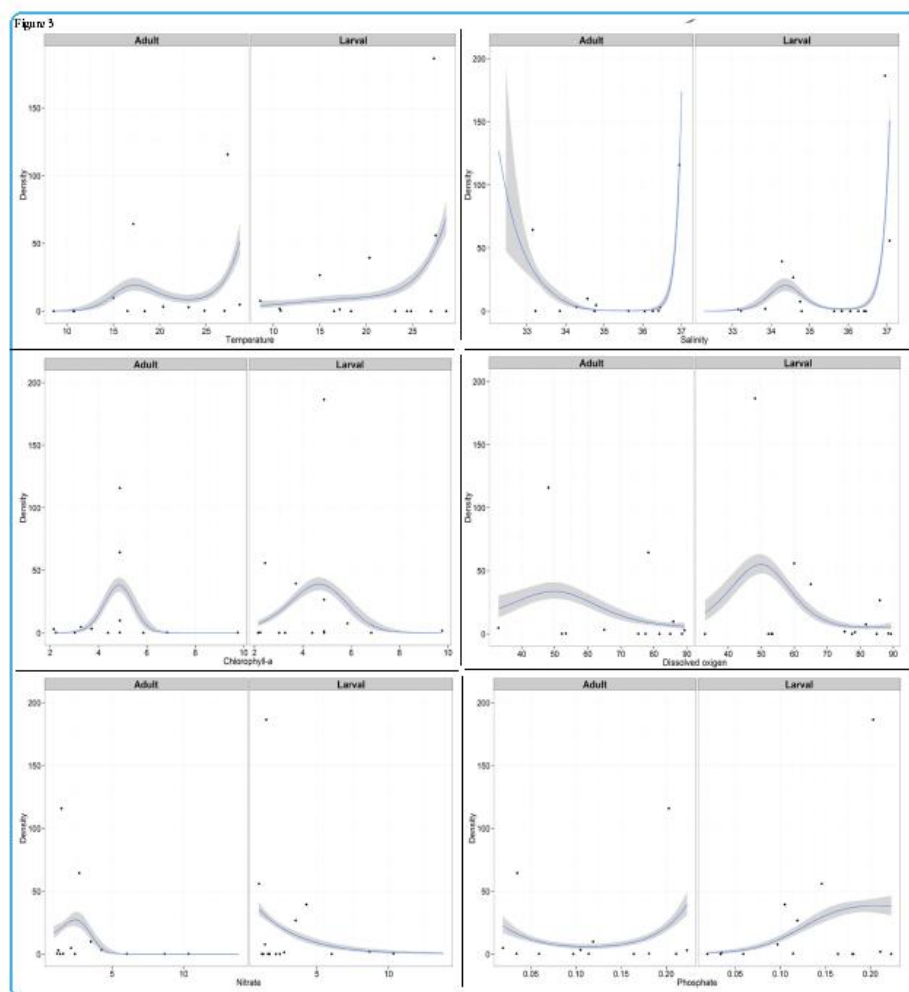


Figure 1. Association between adult and larvae individuals (densities, ind. m<sup>-3</sup>) with environmental variables: Temperature (upper-left panel), Salinity (upper-right panel), Chlorophyll-a (middle-left panel), Dissolved oxygen (middle-right panel), Nitrate (bottom-left panel) and Phosphate (bottom-right panel) measured in the Alfacs Bay, Spanish Mediterranean coast (Marambio *et al.*, 2013).

The Ensemble forecasting showed that along the Spanish Mediterranean coast *M. leidyi* find high suitable areas all along the north and central Spain, with less suitable areas in the south (Figure 2). Conclusions revealed that this species may inhabit a wide range of habitats in the western Mediterranean waters, but establishment seems to occur in particular places with high degradation conditions (Canepa *et al.*, 2013). The established population with reproduction all-year round may be an important threat to an already vulnerable ecosystem. The ecologic and socio-economic importance of the area is undeniable, therefore, the impact of this invasive species over the ecosystem and the associated aquaculture production need to be determined in order to adopt control, mitigation and conservation measures.

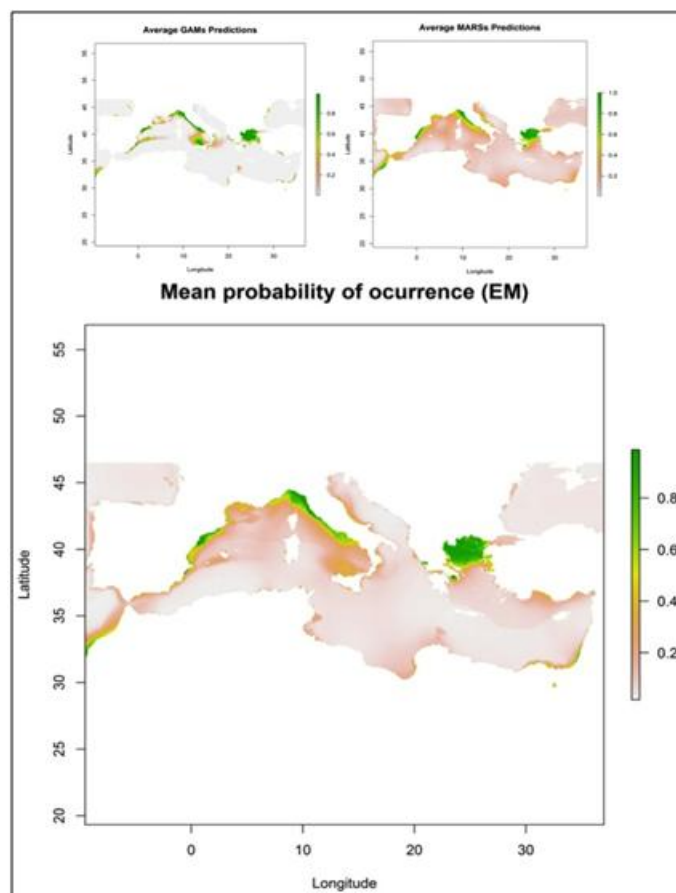


Figure 2. Suitable habitats for *Mnemiopsis leidyi*. Average prediction from GAM (upper-left panel) and MARS models (upper-right panel). Lower panel shows the ensemble forecasting for the potential distribution of *M. leidyi* in the Mediterranean Sea (Canepa *et al.*, 2013).

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### 3.8 Jellyfish, jellypress and jellyperception

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#### Introduction

During the last decades, invasions and blooms of jellyfish have increasingly been reported, both in scientific literature and in the general media (Condon *et al.*, 2012), although due to the scarcity of extended time series, the global jellification paradigm remains unsolved. In this study we tried to figure out to what extent the main messages about jellyfish (increase, causes, threats, solutions, etc.) in the Flemish media correspond with the knowledge and perception in the tourism sector at the Belgian coast. The results of the study were discussed in the framework of integrated coastal zone management. The study was triggered by the occurrence of the non-indigenous ctenophore *Mnemiopsis leidyi* in Belgian waters (Van Ginderdeuren *et al.*, 2012), and the subsequent threats to commercial activities such as fisheries and tourism.

#### Material and Methods

The study was carried out at the Belgian coast and was based on two data sources. First, we conducted a media search for articles featuring jellyfish. All results were entered in a database listing title, date, source, species (if specified), region (if specified), category (health, science, consequences of blooms, and drama) and key words (words that convey the content of the article, selected by the author of this study). Every article was scanned for mentions of causes and threats of, and solutions for jellyfish blooms. Second, we conducted a questionnaire survey for tourists and the tourism industry, that was adapted from the one developed within the GELAMED project (Bonnet, 2013). The questions targeted (1) personal information (gender, age, relation to the coast), (2) personal perception on jellyfish (experiences, emotions, observations) and (3) a personal opinion on the importance of jellyfish increases and their consequences, on their causes and on policy measures. The questionnaire survey was done in summer 2012. Questionnaires were distributed both physically (field survey at the beach and dike of Oostende) and digitally (e-mail survey).

#### Results and Discussion

The number of articles in the Flemish media (in total 140) increased from <5 in 2000 to 27 in 2010, half of them reporting on jellyfish from the Belgian part of the North Sea. Almost 75 % of these articles reported on the causes (overfishing mentioned as the main cause) and economic consequences of jellyfish blooms, and articles about the dramatic consequences of stinging, poisonous and non-indigenous species were well-represented. The questionnaire survey indicated that jelly perception is only partly driven by the general media, while personal experience seemed at least equally important as driver. Information on causes, threats, consequences and solutions for jel-

lyfish problems corresponded to a large extent with the answers of the tourist respondents (Table 1). There was also agreement that all underlying causes of a potential jellification problem should be addressed and tackled at an international level. With key words like pain, smell and slime, jellyfish don't receive much sympathy, and most recreants are extremely careful with any type of jellyfish, especially when children are involved. Species-specific knowledge (names, ecology, stinging vs. harmless species) provided by the media is not assimilated by most tourists or local officials, except for divers who have quite a different perception of jellyfish. This lack of knowledge turned out to be a key issue in perception among tourists. As public perception is a key driver in policy decisions, integrated coastal zone management and measures should provide good and easily digestible information, for example by distributing leaflets and putting up warning boards on the beach. This will result in a better acceptance of jellyfish (Baumann & Schernewski, 2012), and high quality data in citizen science programs (Boero, 2013), covering all people and sectors that may be affected by jellyfish increases.

**Table 1. Comparison between information derived from newspaper and the questionnaire survey concerning jellyfish and their blooms.**

	newspapers	questionnaire
species	species specific in about 50% of articles	general, limited species knowledge
perception on jellification	increase	only 10% of respondents perceive increase
key words top 10	washed ashore, overfishing, poison, beach, wind, global warming, warmth, swim, infestation, tourist	sting, foul, slime, nuisance, danger, sea, tentacles, pain, transparent, fear
most important causes	Natural causes + global change & overfishing	global change & overfishing
most important threats	fisheries & tourism	fisheries & tourism
best solutions	jellyfish removal and consumption	stop overfishing and pollution, jellyfish fishing

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### 3.9 Defining public opinion about *M. leidyi* invasion and jellyfish proliferation along the French coast of the English Channel

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## Background

The first observation of the American comb jelly *Mnemiopsis leidyi* in the North Sea ecosystem in 2009 (Antajan *et al.*, 2014) has raised scientists awareness on its potential impact on the marine ecosystem and led to the development of the INTERREG IV a "2 seas" MEMO project (2010–2013). One part of the project was to evaluate the potential socio-economic cost related to the species. The present work considers public feeling about gelatinous plankton (cnidarians and ctenophores) with a special emphasis on *M. leidyi* along the French Opale coast (Eastern English Channel).

## Methods

A questionnaire initially developed by CEFAS to investigate public experience with and feeling about gelatinous organisms in the UK, was adapted and translated in French to be submitted to people on the Côte d'Opale (French coastline of the Eastern English Channel and southern North Sea, spanning from the town of Montreuil-sur-Mer to Dunkirk city).

The questionnaire was short (11 questions) to increase the chance to get answers from vacationers. It included 11 questions distributed in three sections. Section 1 was about the interviewees' habits as regards going to the beach (which beach do they go to, how long they usually stay in the area, since when have they been going there, what time of year do they swim/dive). Section 2 was to collect information about the experience of interviewees with jellyfish: whether or not they have ever been in the presence of a swarm (where, when?), whether this had negative consequences for them, what the nature of the experienced disturbance was, and whether there are specific areas they avoided because they would expect to encounter jellyfish swarms there. This section also included a specific question about *Mnemiopsis leidyi*. The interviewees were shown a picture (Figure 1) of *M. leidyi* and asked whether they "have ever seen the so-called 'sea-walnuts' (*M. leidyi*)?". Section 2 ended with a question asking whether the interviewees have experienced any changes in the occurrence of jellyfish over the last decade(s). Finally, questions of Section 3 investigated the perception of the interviewees on the "potential jellyfish problems", asking them what they think the main causes of jellyfish blooms are (multiple choices possible) and what would be the main effects of jellyfish invasions (who will encounter most problems) (again, multiple choices possible).

The questionnaire was submitted to 107 people (beachgoers, surfers, recreational fishermen and people walking on the seafront) in Wimereux (Pas de Calais, France) between the 23<sup>rd</sup> of July and the 26<sup>th</sup> of August 2013.



Figure 1. Picture of *Mnemiopsis leidyi* shown to interviewees. Picture by F. Chevallier.

## Results

72% of responders have at least once “been in the presence of” jellyfish, but this had direct negative consequences only for 10.2% of interviewees: 3.7% have been (or one of their relative has been) stung and 6.5% would not enter the sea because of the presence of jellyfish. When considering only the people who encountered jellyfish in the Côte d’Opale ( $n=57$ , 53% of total responders), only one has been stung and two would not enter the water to swim. None of the interviewee cited a place that they would avoid because of jellyfish presence.

When asked whether they noticed a change in the abundance of jellyfish over the years, 44% of responders declared they observe more jellyfish now than before, 19% stated the opposite (i.e. fewer jellyfish than before) and 37% said there has been no change in jellyfish abundance over the years. If considering only the answers from people who previously stated they have actually been in the presence of jellyfish along the Opale Coast, these figures change to 32.5%, 21% and 35%, respectively.

About *Mnemiopsis leidyi*, 14% of responders said that they have previously seen this species, precising that this was either on TV or in aquaria. However, none of them was aware of the presence of the species in the area.

Finally, 60% of responders think that jellyfish proliferations are due to natural causes, with increase in water temperatures, climate change, and water pollution also identified as “main causes of jellyfish blooms” by 50%, 47%, and 37% of responders, respectively. 84% of responders believe that tourists are the most likely to be negatively impacted by jellyfish proliferations; fishermen and the natural environment are also mentioned by 38% and 34% of responders, respectively.

## Discussion

Such interview may be useful to investigate public feeling about jellyfish in general, and what the consequences of a jellyfish bloom might be. The conclusions that can be

drawn from the present study are obviously limited since all interviews were conducted at a single location. An additional limit may be the lack of data on the identity of the responders (sex, age, education, occupation). Despite this, the inconsistencies between answers clearly highlighted that asking people whether they observed a change in jellyfish abundance over the past decade(s) is unlikely to lead to useful information. Answers to such question will be subjective, influenced by partial memories and potentially biased media reports from other places than the one investigated (e.g. reports from the French coasts of the Mediterranean Sea where summer swarm alerts are frequent).

As regards to *Mnemiopsis leidyi*, >10% of responders declared that they know the species. This may partially be the result of the extensive media campaign conducted locally during the MEMO project, and the fact that *M. leidyi* is now on display at the nearby Aquarium in Boulogne-sur-Mer (Nausicaa). However, none of the responders was aware of the introduction of the species in the area. Because of its relatively small size and body transparency, *M. leidyi* clearly is not as easy to notice as medusae. Furthermore, *Mnemiopsis* has never been observed in very high abundances along the Opale Coast (except perhaps in some basins of Dunkirk harbour, Antajan *et al.*, 2014). More importantly, it can be expected that non-specialists would have great difficulty to tell the species apart from other ctenophore species present in the area (even *Beroe* spp. and *Pleurobrachia pileus*). Finally, even if someone declared he/she saw *Mnemiopsis leidyi* in the area, the identification of the species would probably remain doubtful without visual proof (pictures).

### Acknowledgements

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## 3.10 *Mnemiopsis leidyi* distribution and biomass index as an indicator of Good Environmental Status (GEnS) in Bulgarian waters

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### Abstract

An issue for the non-indigenous species management is that, once a marine organism has been introduced to its new environment, it is nearly impossible to eradicate the invader, if it has established in the area. The presence of NIS in itself indicates some degree of deviation from the pristine ecological status. The consequence is that assessing a status of an area as “bad” means that the area will stay in the bad status without the possibility to return to the past condition (HELCOM, 2012).

As a key factor for the development of mesozooplankton, *M. leidyi* becomes a reliable indicator of the pelagic Black Sea ecosystem dynamic, functioning of the pelagic food web, ecosystem stability and health. *M. leidyi* mass development induces trophic cas-



cedes, as it affects (direct impact) the population size and composition of the zooplankton and indirectly the primary producers in the food chains (Daskalov, 2002; Daskalov *et al.*, 2007).

### Material and Methods

The long term (1998–2013) zooplankton data set of IO-BAS was used in the study.

Abundance or biomass, trend in population, temporal occurrence and spatial distribution of alien species identified as invasive are an important indicator to determine the status of the species and respectively the opportunity to achieve good status in respect of MSFD requirements, particularly descriptor 2 (D2). Proposed indicators to criteria 2.1.1 in D2 is *Mnemiopsis leidyi* biomass [ $\text{g}\cdot\text{m}^{-3}$ ] or [ $\text{g}\cdot\text{m}^{-2}$ ]. The indicator is relevant for Descriptors 1 (Biodiversity) and D3 (Food web) as well.

The Water Frame Directive did not require the zooplankton as a biological quality element. National monitoring programme of coastal marine waters in Bulgaria started from 2012 and zooplankton there was included as a complementary biological element. We tried to implement WTD approach for zooplankton. *M. leidyi* biomass was proposed as an indicator of the ecological status of coastal waters with built up classification system on the base of percentile approach (table 1).

**Table 1. Classification and EQR (Ecological Quality Ratio) based on *M. leidyi* biomass [ $\text{g}\cdot\text{m}^{-3}$ ] index**

<i>M.leidyi</i> [ $\text{g}\cdot\text{m}^{-3}$ ]	High	Good	Moderate	Poor	Bad
<b>Spring-summer</b>	0	1-4	4-20	20-50	>50
<b>EQR</b>	1	0.96	0.75	0.38	

### Results and Discussion

Data on abundance/biomass and distribution of NIS present in the system is a prerequisite for the assessment. The major issue for the descriptor and criteria respectively is reference points or baseline/reference conditions provision. *Mnemiopsis leidyi* biomass indicator was proposed in the Bulgarian Initial Assessment Report (Moncheva, Todorova *et al.*, 2013) as a pressure indicator and  $4\text{g}\cdot\text{m}^{-3}$  or  $120\text{g}\cdot\text{m}^{-2}$  (Vinogradov *et al.*, 2005) were used as thresholds for good ecological state.

In the present decade, *Mnemiopsis* population was controlled by *Beroe ovata*. The *Mnemiopsis leidyi* impact on trophic zooplankton structure was reduced to summer months of the year instead of 6–8 months before *B. ovata* arrival (Kamburska *et al.*, 2006, Mihneva 2011). But the ecosystem functioning still manipulated by *Mnemiopsis*, although *Beroe* modulates its impact during the summer-autumn season (Figure 1).

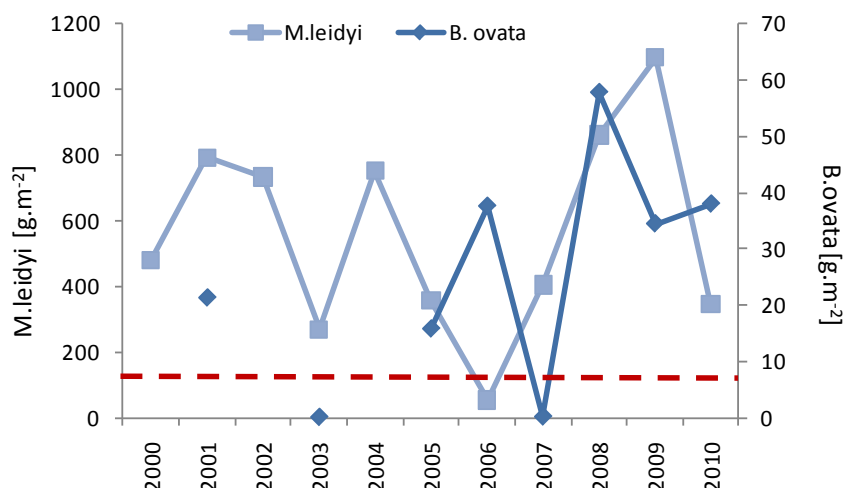


Figure 1. *M. leidyi* and *B. ovata* biomass dynamic (2000–2010). The red line presents 120 g.m<sup>-2</sup> – threshold for GEnS along the Bulgarian coast.

The summer population during the period 1998–2013 has never reached a size as elevated as in the end of 1980s – mid-1990s. Nevertheless, coastal and shelf habitats manifested larger fluctuations and higher biomass in comparison with the open sea (figure 2). The magnitude of the summer pulse of the population correlated strongly with the physical forcing, the plankton fauna components and grazing by *B. ovata* (Kamburska, 2004).

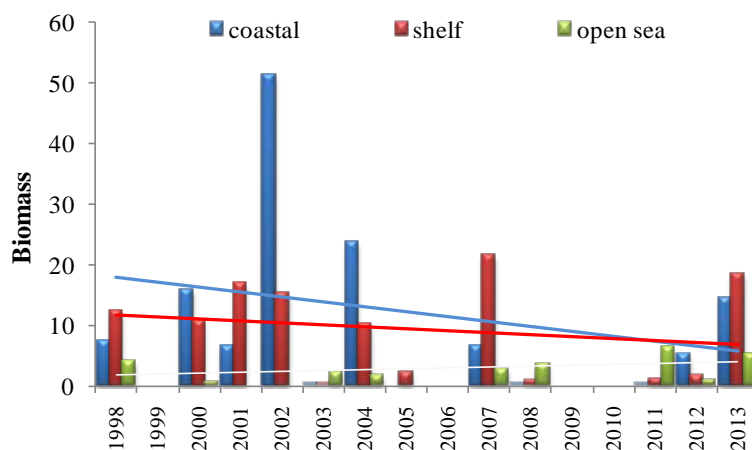


Figure 2. *M. leidyi* biomass [g.m<sup>-3</sup>] and trends at three habitats in c. Galata transect during 1998–2013.

Monitoring investigations (2012) of coastal marine waters also demonstrated large fluctuations and patchiness of *M. leidyi* biomass distribution (mean 12 g.m<sup>-3</sup> ±34.5, maximum – 209 g.m<sup>-3</sup>). Large aggregates of *M. leidyi* formed “hot spots” along the coast during the summer and ecological state according to the biomass of *M. leidyi* varied from bad (to the north) and good/high to the south (Table 2).

Table 2. *M. leidyi* biomass index [g.m<sup>-3</sup>] in summer 2012 and ecological state of the Bulgarian Black Sea coast according to *M. leidyi* biomass index.

Water body	July	August	September/October	Average	Ecological state
BG2BS000C001	209.94	0.201	0	70.04±121.1	bad
	21.03	0.000	0	7.01±12.1	
BG2BS000C002	6.25	0.000	0.234	2.16±3.5	good
BG2BS000C003	18.28	0.037	0.001	6.11±10.5	moderate
BG2BS000C004	16.10	0.064	0	5.39±9.3	moderate
	2.10	0.000	0.037	0.71±1.2	
BG2BS000C013	0.02	0.000	0	0.01	good
	5.61	0.026	0	1.88±3.2	
BG2BS000C005	8.16	0.000	0.178	2.78±4.7	moderate
	17.76	0.220	0	5.99±10.2	
BG2BS000C006	6.33	0.234	0.47	2.34±3.5	good
BG2BS000C007	0.00	0.119	0	0.034±0.1	high
BG2BS000C008	0.00	0.000	0	0.00	moderate
	0.05	20.812	0.481	7.11±11.9	
	0.37	19.166	0	6.51±11	
BG2BS000C009	0.08	5.580	0	1.89±3.2	good
BG2BS000C010	0.00	0.000	0	0.00	high
	0.58	0.000	0.099	0.23±0.3	
BG2BS000C011	0.03	0.036	0	0.021±0.02	high
BG2BS000C012	0.00	0.000	0.093	0.031±0.1	high
	0.01	0.045	0	0.02±0.023	

### Gaps and Uncertainties

- Gaps in data, regular and specific monitoring strategy for NIS with relevant frequency and the degree of deficiency of scientific knowledge
- Validation of the indicators and thresholds adequacy
- Regular monitoring of abundance, biomass, temporal occurrence, impact of *M. leidyi* and *B. ovata*

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### 3.11 Jellyfish invaders and the impact of the blooms on the Easternmost Mediterranean

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#### Introductions

Periodic outbreaks of indigenous scyphozoan jellyfish have long been noted in the Mediterranean (Boero 2013). Various anthropogenic perturbations including eutrophication, overfishing, global warming and the increase of littoral man-made hard substrates have been suggested as contributing to the proliferation of their populations. But whereas most jellyfish outbreaks in the western and central Mediterranean consist of native species, non-indigenous species (NIS) have taken over in the eastern part of the sea (Galil 2012); (Figure 1). The SE Levant is unique in hosting four non-indigenous scyphozoan jellyfish concurrently, in addition to two non-indigenous ctenophores.

#### Materials and methods

The coastal waters of the southern Levantine Sea are ultraoligotrophic (Berman *et al.*, 1984, 1986), sea surface salinity in the summer months tops 39.5 psu (I. Gertman, pers. comm.), and the temperature – 31.5°C (L. Raskin, pers. comm.). The upper layer, to depth of 100 m, is mixed in most winters, whereas during the remainder of the year a sharp halocline and thermocline begins at 10 m depth.

Though the ‘National Monitoring’ program along the Mediterranean coast of Israel does not include gelatinous zooplankton – despite the immense annual swarms of the invasive scyphozoan *Rhopilema nomadica* – since the 1990s an ‘informal’, largely opportunistic, network has been recording and collating information on gelatinous macrozooplankton off the Mediterranean coast of Israel.

Members of this network recorded large swarms of *Mnemiopsis leidyi* as early as March 2009 along the entire Israeli coast from Ashkelon (34°33'E, 31°41'N) to Rosh HaNikra, from the intertidal to depth of 20 m, inside ports and along the open shore. From October 2011 to January 2012, and again from December 2012 to May 2013, in-

dividuals and dispersed swarms of *M. leidy* were sighted along the Israeli coast from Maagan Michael (34°53'E, 32°33'N) to Ashkelon.

Specimens of *Beroe ovata* were recorded and photographed outside the main breakwater of the Port of Ashdod (31°49'00"N, 34°39'00"E).

Confirmed sightings of *Beroe cucumis* took place off Ashdod (34°38'E, 31°48'N), on December 2011 and January 2012, and again in December 2012, and February, April and May 2013. On 21 February 2013, a swarm was photographed off Ashdod at 20 m depth. The swarm was composed of large-sized individuals (50–100 mm TL). On 8 May 2013, another swarm was photographed off Ashkelon (34°34'E, 31°39'N), at depth of 15–20 m. Individual *B. cucumis* were noted and photographed as engulfing *M. leidy* whole and undigested *M. leidy* were clearly visible in the gut of *B. cucumis*.

*Leucothea multicornis* was first sighted 3 km west of Shavei Zion (32.982828N 35.0662148E), water depth 26 m, 6 May 2014 and 16 May 2014. Specimens were observed and photographed, though not collected. Additional specimens were sighted near Ashdod Port breakwater (31.8425668N 34.6343998E), water depth 17–20 m, 30 May 2014 and 11 June 2014.

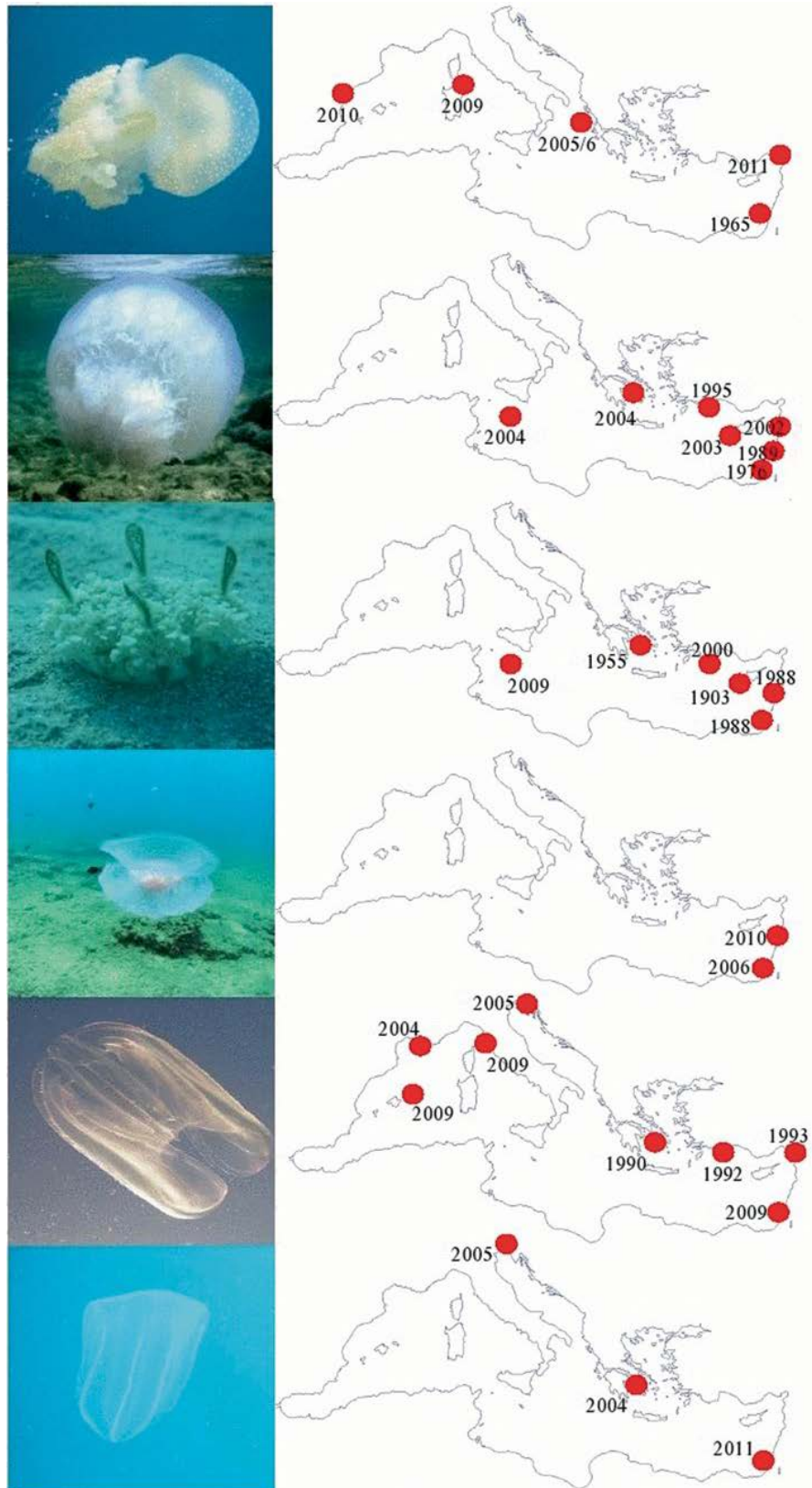


Figure 1. Non-indigenous gelatinous species in the Mediterranean Sea, with distribution maps and date of first record per country. From top: *Phyllorhiza punctata* Lendenfeld, 1884, *Rhopilema nomadica* Galil, 1990, *Cassiopea andromeda* (Forsskål, 1775), *Marivagia stellata* Galil & Gershwin, 2010, *Mnemiopsis leidyi* A. Agassiz, 1865, *Beroe ovata* sensu Mayer 1912 (Photos by IOLR, R. Gevili, G. Paz, G. Rilov, S. Shafir); (After Galil 2011).

## Results

Given the severe ecological and economical harm elsewhere, the introduction of *M. leidyi* into the Mediterranean is of major concern. It was first recorded in the Aegean Sea, presumably swept with the outflow of the Black Sea, thereafter off Turkey, Syria and the Bay of Piran (Galil *et al.*, 2009). As all those locales are in the vicinity of ports, and no population persisted long, it was suggested that it had been introduced with ballast water. Suddenly, in 2009, large swarms appeared along the Israeli coast as well as the Ligurian, Tyrrhenian, and Ionian shores of Italy, and Spain (Boero *et al.*, 2009; Fuentes *et al.*, 2009; Galil *et al.*, 2009). *Mnemiopsis*-blocked water intake pipes threaten desalination plants and force plant engineers to modify their operation by increasing the frequency of backwash cycles in the pre-treatment stage and consequently raising the discharge of coagulants such as ferric sulphate into the sea, and ultimately reducing output.

The ctenophore *Beroe ovata*, a specialized predator of *M. leidyi*, was first noted in the Mediterranean in Greece, in 2004, and since 2011 off Israel (Galil *et al.*, 2011). Though tolerant of a wide range of salinity and temperature over a broad range of inshore habitats, both invasive predator and prey have flourished when introduced to bodies of water of low salinities and temperatures and high productivity – the Black Sea, Azov, Caspian and the Marmara, northern Great Belt in the North Sea, and in the Mediterranean – in enclosed, eutrophic gulfs along the northern reaches of the sea. Yet, the high seawater temperatures and salinity in the SE Levant are far from the values deemed optimal for these species.

Since October 2011 aggregations of the native comb jelly *Beroe cucumis* were observed and photographed along the Israeli coast preying on the invasive American comb jelly *M. leidyi* (Galil and Gevili 2013). The Mediterranean records of *B. cucumis* are few, and spatially and temporally scattered. It had not been previously recorded off the Israeli coast though special attention has been paid in recent years to scyphozoans and ctenophorans. Its recently documented occurrences along the Israeli coast provide the first record in the region, as well as evidence for predation on the invasive *M. leidyi*. The spatial and temporal occurrences of the two beroids in the SE Levant overlap to some degree with that of *M. leidyi*. Exploiting their high feeding and growth rate potentials, *B. ovata* and *B. cucumis* may be capable of controlling the populations of *M. leidyi*.

The native warty comb jelly, *Leucothea multicornis*, was noted off the Mediterranean coast of Israel in May and June 2014. Though recorded multiple times in the western and central Mediterranean Sea, it is the first record of the species from the eastern basin (Galil *et al.*, 2014).

## Discussion

The succession of records of ctenophores, non-indigenous and native, seems to have been triggered by the appearance of *Mnemiopsis* swarms off the SE Levant. Invasive non indigenous scyphozoan and ctenophorans may impact the ecosystem in ways we neither expect nor understand, and which are more significant than their obvious impacts in economic or human health terms. As gelatinous plankton plays a pivotal role in marine food webs and elemental fluxes, invasive non indigenous populations may affect production cycles in plankton and benthos. Exploiting their high feeding and growth rate potentials, their massive consumption of plankton, including eggs and larvae of fish and invertebrates, may directly impact recruitment, to the point it may be impossible for the pre-invasion ecosystem to recover. The transient localized

blooms and longer-term persistent increases in native and non indigenous ctenophore abundance indicate changes in the structure and function of the Levantine ecosystem.

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### 3.12 Modelling aspects of *Mnemiopsis leidyi* in the North Sea

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## Introduction

*Mnemiopsis leidyi*'s presence has been regularly recorded in the North Sea since 2005 (Antajan *et al.*, 2014). It has been seen along the continental coast from France to Denmark but so far hasn't been recorded along the UK coasts. The North Sea is the home of commercially important fish stocks and spawning and nursery grounds (Ellis *et al.*, 2011), and also shares the depleted state of fish stocks that characterized the Black Sea when *M. leidyi* was introduced (Boersma *et al.*, 2007; Fuentes *et al.*, 2010). Considering the reported ecological and economical consequences following the establishment of *Mnemiopsis* in the Black Sea, it is important to evaluate the risk posed by *M. leidyi* in the North Sea. We describe 2 modelling approaches. The first one (Collingridge *et al.*, 2014) attempts to evaluate the suitability of the North Sea for survival and reproduction of *M. leidyi* and predict whether and where in the North Sea it is likely to become established by using the best available knowledge of the species' environmental requirements in combination with modelled temperature, salinity, and food fields. The second approach (van der Molen *et al.*, in review at OS) aims to provide insight into the potential spreading and population dynamics of *M. leidyi*, by applying two different models to simulate aspects of transport, survival and reproduction of *M. leidyi* in the Scheldt estuaries and the North Sea.

## Materials and methods

### First approach (Collingridge *et al.*, 2014)

Data were obtained from a 3D hydrodynamic model (GETM: General Estuarine-Ocean Transport Model) coupled with an ecosystem model (ERSEMBFM: European Regional Seas Ecosystem Model-Biogeochemical Flux Model) for the area from 40°27'N to 60°27'N and 5°44'W to 16°17'E, covering the North Sea. For the years where *M. leidyi* was found in the North Sea (2005–2011), daily data were extracted from GETM-ERSEM-BFM for the variables temperature, salinity, and biomass of the main food groups of *M. leidyi* larvae and adults (in mg C m<sup>-3</sup>). The adult food groups extracted were omnivorous mesozooplankton and carnivorous mesozooplankton (0.2–20 mm), and the larval food groups were microzooplankton and microphytoplankton (diatoms, flagellates and dinoflagellates, < 0.2 mm) (Sullivan and Gifford 2004; Salihoglu *et al.*, 2011). Daily concentrations for adult food types were summed and multiplied by a factor of 10 to account for underestimation of the model, found during validation for three locations in the North Sea (van der Molen *et al.*, 2013).

For each day in each year, binary daily layers were created by selecting cells with temperature, salinity, and mesozooplankton concentrations greater than the respective survival and reproduction thresholds (Table 1). The daily layers were then summed across each year to find the number of days suitable for survival of *M. leidyi* in the year. To give a better idea of the risk of *M. leidyi* blooms, the reproduction map was classified according to the number of life cycles that could be completed while the conditions were suitable for reproduction, using a life cycle length of 40 days, as at 15°C (Salihoglu *et al.*, 2011).

To incorporate the increase in egg production and *M. leidyi* biomass with temperature (exponential), and food (linear), found both in the field and in the laboratory (Salihoglu *et al.*, 2011; Purcell *et al.*, 2001; Lehtiniemi *et al.*, 2012), daily temperature and food values were reassigned to a reproductive suitability index of 0–10 (0 = unsuitable, 1 = fulfils minimum thresholds, to 10 = non-limiting). The suitability indices for temperature and food and the binary index for salinity (below 10 = 0, above 10 = 1) were then multiplied for each day and the daily layers summed to obtain overall

suitability for the year. The above processes were repeated for each year from 2005–2011, and the average and standard deviations calculated. All maps show the average for the time series.

**Table 1. Constraining *M. leidy* survival and reproduction (Purcell *et al.*, 2001; Sarpe *et al.*, 2007; Kremer 1994; Fuentes *et al.*, 2010; Lehtiniemi *et al.*, 2012; Jaspers *et al.*, 2011; Sullivan and Gifford 2007; Salihoglu *et al.*, 2011).**

	Parameter	Threshold
Survival	Temperature (°C)	2
	Salinity (PSU)	4.5
	Adult food (mg C/m <sup>3</sup> )	3
Reproduction (minimum)	Temperature (°C)	12
	Salinity (PSU)	10
	Adult food (mg C/m <sup>3</sup> )	3
	Larval food (mg C/m <sup>3</sup> )	40
Reproduction (non-limiting)	Temperature (°C)	18
	Salinity (PSU)	10
	Adult food (mg C/m <sup>3</sup> )	25
	Larval food (mg C/m <sup>3</sup> )	90

#### Second approach (van der Molen *et al.*, in review at OS)

Two existing models were used (Delft 3D and GETM-ERSEM-BFM model with particle tracking (GITM)), with limited adaptations, to simulate aspects of transport and reproduction of *M. leidy* in the Scheldt estuaries and the North Sea. The Delft3D model implementation at high spatial resolution in the estuaries, and with tracking of passive particles provided insight into the potential role of the Scheldt estuaries as a nursery and source of *M. leidy*, and in the role of estuarine-marine exchange processes. The GETM-ERSEM-BFM model with particle tracking (GITM) was developed to include a simple reproduction model (Salihoglu *et al.*, 2011), and was used to study transport, connectivity and population dynamics at the scale of the North Sea. This reproduction mechanism was implemented to affect the number of individuals represented by a super-individual (particle). The main simplifications were: (i) each super-individual was assumed to represent a number of adults of average mass; (ii) egg and juvenile stages were assumed to be infinitely short to allow for (i); (iii) food stocks were assumed not to be impacted upon by *M. leidy*. The reproduction mechanism was implemented as follows; all values and constants were taken from Salihoglu *et al.*, (2011) unless specified otherwise. Eggs were only produced if temperature and salinity were above the thresholds of 12°C and 10 PSU, respectively (Lehtiniemi *et al.*, 2012). The number of eggs produced per time step depended on food availability. GETM-ERSEM-BFM was used to produce hydrodynamics and food fields for the particle tracking model.

The particle tracking model was run from the 1 June 2008 to the 31 January 2009, releasing 3 particles per day from the 1 June until the 30 October in each of 6 grid cells just seaward of the Dutch estuaries, corresponding with expected bloom times (e.g. Collingridge *et al.*, 2014). The particles were assumed to be passive tracers. Upon release, each particle was assumed to represent 1000 *M. leidy* individuals. Daily particle positions, particle characteristics and environmental conditions were stored. The results were processed into density contour maps, and into time series of properties aggregated over all the particles (i.e. the standard run). To illustrate the effect of temperature on reproduction, and to compare with the response in warmer waters, an

additional scenario run was carried out in which the particles experienced 10% higher temperatures. The sensitivity to juvenile mortality was assessed by a model run with two thirds of juvenile mortality at normal temperatures, and a run with four thirds of juvenile mortality at the 10% higher temperatures. To study interconnectivity between ports and estuaries along the French Channel coast and areas in the southern North Sea, a model run was carried out releasing 20 particles per day at one grid cell in the mouth of the river Seine, and one grid cell in the mouth of the river Somme during the same period as in the previous simulations.

### Results and discussion

Most areas were found to be suitable for *M. leidyi* survival for between 200 and 300 days per year, with, on average, a maximum of 350 suitable days per year (Figure 1). The number of days per year suitable for *M. leidyi* reproduction ranged from 0 to a maximum of 212 (in 2011), with a mean of 55 (Figure 1). Large parts of the North Sea were found to be suitable for *Mnemiopsis leidyi* reproduction in summer months, although in most areas the suitable time window would not allow completion of more than two life cycles.

When taking into account the relationships between food and reproduction, and temperature and reproduction, the highest average suitability for *M. leidyi* reproduction for 2005–2011 were in southern coastal and estuarine regions and in the Skagerrak and Kattegat, due to a combination of high temperatures and high food concentrations (Figure 2). Importantly, food was found to limit winter survival and so may restrict the overwintering population. Reproductive suitability peaked in mid to late summer in most areas. Coastal Dutch, German and Danish waters had early peaks and the central North Sea had the latest peak – in early autumn.

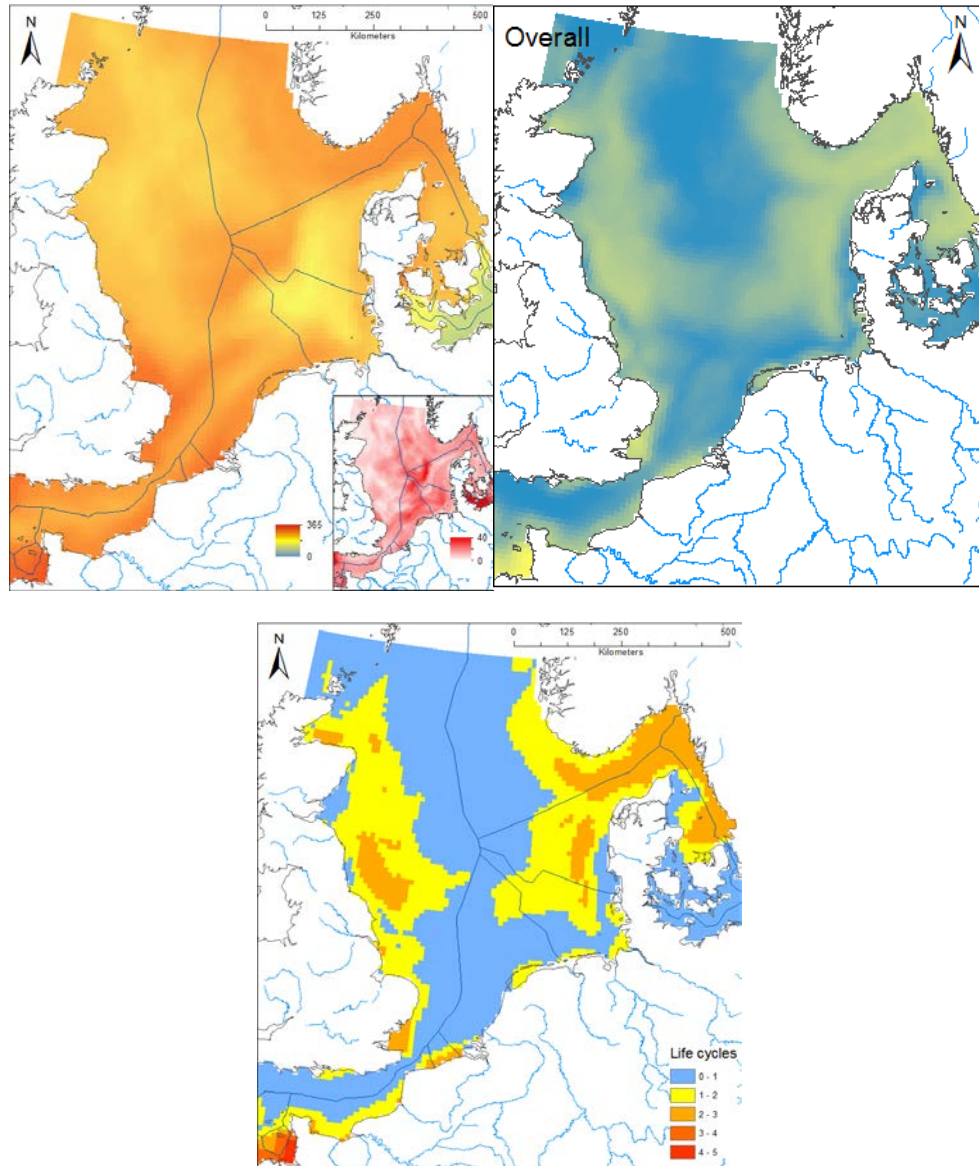
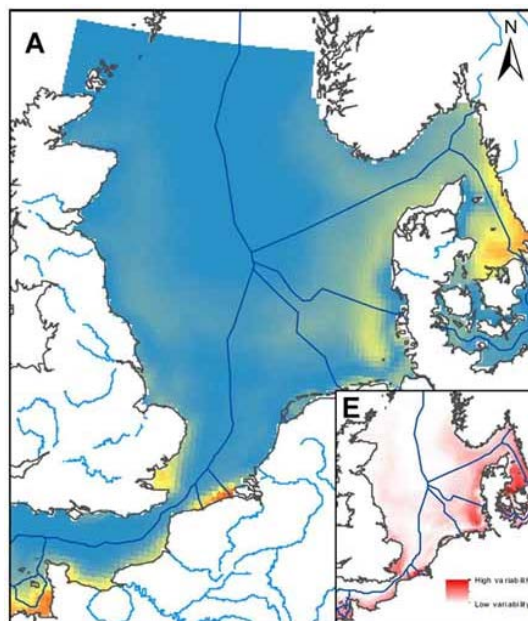
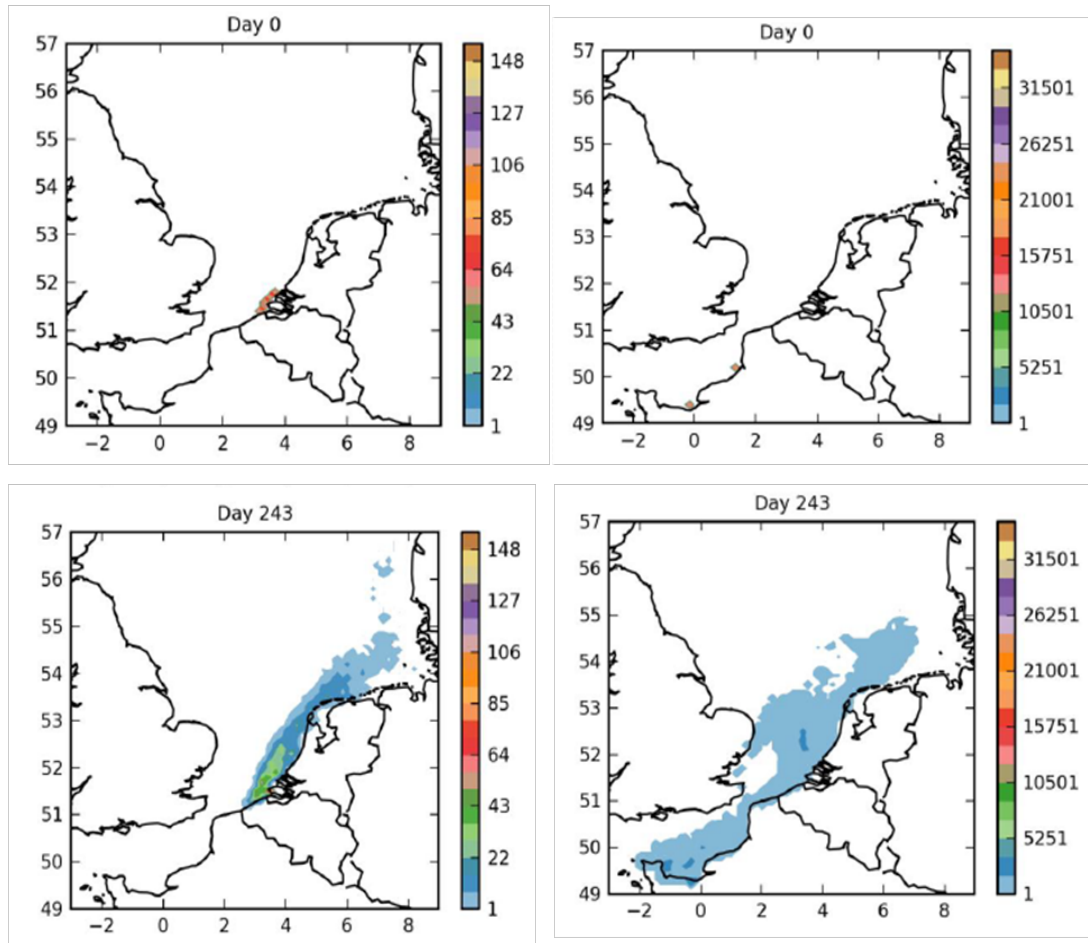


Figure 1. Maps showing the average (for 2005–2011) number of days per year suitable for survival of *Mnemiopsis leidyi* (top left) and reproduction (top right) as determined by temperature, salinity and adult food (mesozooplankton); and the number of reproductive cycles (bottom left) possible within the time window when conditions are suitable for *Mnemiopsis leidyi* reproduction, assuming a life cycle length of 40 days, as at 15°C.



**Figure 2. Average suitability for *M. leidyi* reproduction (model for 2005–2011) from reproductive indices based on temperature and available food (Collingridge *et al.*, (2014)).**

The results of the particle tracking coupled with the reproduction model show that when released from the Scheldt estuaries, *M. leidyi* dispersed as a plume along the continental coast to the north and to a limited extent to the south. The concentration of particles decreased steadily along the plume, in response to both the temporal distribution of release and dispersion; densities were strongly reduced in winter in response to adult mortality (Figure 3). The model run releasing particles in the rivers Seine and Somme resulted in moderate transport to the West up to Cap de la Hague, and substantial transport along the continental coast to the North through the Strait of Dover, along the Dutch coast and into the German Bight. Low numbers crossed the North Sea to the UK and were found in the Thames estuary and off the coast of East Anglia. None of the particles crossed the English Channel south of the Strait of Dover.



**Figure 3.** The spread of *Mnemiopsis* from the Sheldt estuary (top) and from the Somme and Seine estuaries (bottom). The maps show the density of the particles on day 0 of the simulation prior to release in the model (left), and on day 243 (25 January 2009).

For the standard run, the total number of *M. leidyi* individuals increased steadily as particles were released, levelling out in response to the background adult mortality, and declined when starvation set in December (Figure 4a, dark blue line). Food abundance for juveniles and adults was high until the beginning of October, and declined to reach low winter values by December (Figure 4b and c). Average temperature peaked at 20°C, declining to winter values of 4°C (Figure 4h). Average salinity increased until the beginning of October, consistent with reduced precipitation in summer and their transport away from the fresh-water source of the river Rhine, and decreased subsequently as river runoff increased in the autumn (Figure 4i). Over a million eggs were produced per hour by the population in July, August and September (Figure 4d, dark blue line). However, due to primarily juvenile mortality hardly any new adults were added to the population (Figure 4e–g). An important factor for juvenile mortality as implemented here is the prolonging of juvenile duration for lower temperatures, leading to strongly reduced overall survival. The scenario run with two thirds of juvenile mortality showed some bloom potential, with new individuals contributing to population growth (Figure 4, red lines). The model run in which the particles were made to experience 10% increased temperatures produced significantly different results. The maximum average temperature experienced by the particles was now approximately 22°C, with winter temperatures nearly the same as in the reference scenario (Figure 4h, green line). Over 10 million eggs were produced per hour between the beginning of August and the end of September (Figure 4d,

green line). This caused a bloom that increased the adult population at a rate far greater than the number of the additional particles that were released (Figure 4a). Increasing the juvenile mortality by one third for this experiment, however, prevented the bloom, and the associated model run thus yielded results very much like those of the standard run (Figure 4, light blue lines). For the model run releasing particles in the Seine and the Somme (Figure 4, magenta lines), the mean concentration of food encountered was slightly lower. Average salinity was higher, indicating a more seaward trajectory of the particles. Egg production and survival was comparable with the standard run, considering that approximately twice as many particles were released. As for the standard run, hardly any adults were added to the population through reproduction.

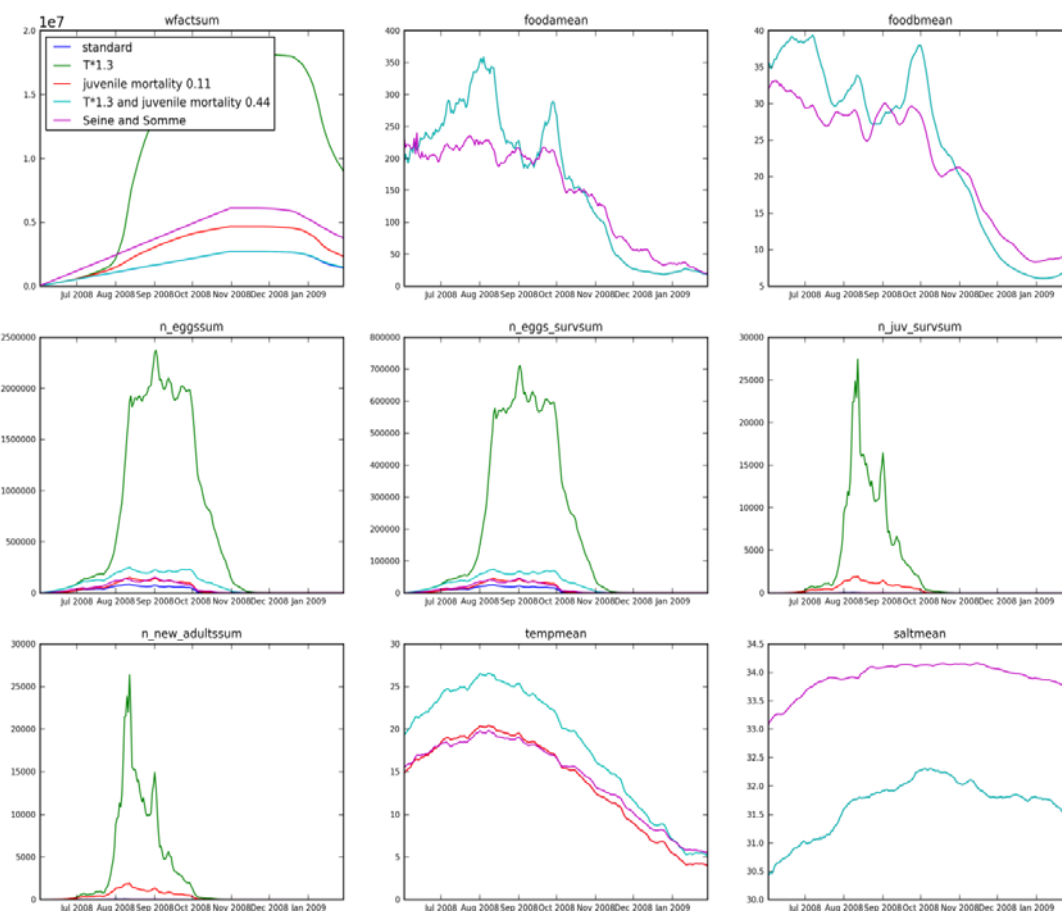


Figure 4. Cumulative results over all particles as a function of time for hindcast temperatures (dark blue), 1.1 times hindcast temperatures (red), two-thirds of juvenile mortality (green), combined 1.1 times hindcast temperatures and four-thirds of juvenile mortality (light blue), and release from the Seine and Somme (magenta). (a) Simulated number of *M. leidy* individuals; (b) average juvenile food concentration available to particles [mg C m<sup>-3</sup>]; (c) average adult food concentration available to particles [mg C m<sup>-3</sup>]; (d) total number of eggs released per hour; (e) total number of surviving eggs per hour; (f) total number of surviving juveniles per hour; (g) total number of adults added to the population through reproduction per hour; (h) average temperature experienced by the particles; (i) average salinity experienced by the particles.

The above results indicate that the North Sea is somewhat suitable for *Mnemiopsis leidy*. Food, as the most likely limiting factor for survival and reproduction, are sufficient in the North Sea coastal waters to sustain a *M. leidy* population in summer and a reduced population until mid-winter. Current offshore water temperatures were

too low in summer and autumn for *M. leidyi* to reproduce in large numbers, thus rendering the occurrence of blooms in the North Sea proper unlikely. The scenario simulation with increased summer temperatures suggested that water temperature is an important limiting condition for blooms in the North Sea. The model results suggest that blooms may occur in some years as a result of interannual variability in temperature, and that such incidences may increase in frequency in the future as a result of global warming. This result is consistent with the parameterisations in the model, and with observed reproduction behaviour in warmer seas. Moreover, blooms tend to be found in estuaries, which experience higher water temperatures and food biomass than the surrounding seas. The simulated blooms for the increased temperature scenario should be considered an upper estimate, as food concentrations are not impacted on by grazing of *M. leidyi* in the present model implementation. Other limiting conditions such as predation may exist as well, but these were not included in the model.

To our knowledge, *M. leidyi* has so far not been found in the UK. The above results suggest only minor potential for *M. leidyi* to colonise UK waters through natural transport processes from continental populations. The most likely stretch of UK coast vulnerable to colonisation appeared to be the East Anglian coastline. As the general residual coastal flow converges from north and south in this area, and then moves 10 across the North Sea towards Scandinavia, *M. leidyi* is not expected to be able to colonise further along the UK coast through natural transport processes should it be able to establish itself in East Anglia.

## Conclusion

Results from the current study suggest that: (i) the estuaries possess enough retention capability to keep an overwintering population, and enough exchange with coastal waters of the North Sea to seed offshore populations; (ii) *M. leidyi* can survive in the North Sea, and be transported over considerable distances, thus facilitating connectivity between coastal embayments; (iii) under current climatic conditions, *M. leidyi* may not be able to reproduce in large numbers in coastal and offshore waters of the North Sea, but this may change with predicted climate change scenarios.

## Acknowledgments

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### 3.13 Maximum entropy modelling of potential habitat of the invasive ctenophore *Mnemiopsis leidyi* in the Mediterranean basin

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**Keywords:** maximum entropy modeling, potential habitat, invasive species, *Mnemiopsis leidyi*

#### **Introduction**

The ctenophore *Mnemiopsis leidyi* was accidentally introduced into the Black Sea in the early 1980s (Vinogradov *et al.*, 1989) possibly with ballast water from ship coming from the northwestern Atlantic coastal region. It exhibited an explosive mass development there since 1988, expanded to the Azov, Marmara, and eastern Mediterranean Seas (Studenikina *et al.*, 1991; Shiganova, 1993; Shiganova *et al.*, 2001a). The first occurrence *M. leidyi* in Aegean Sea was recorded during late spring-summer 1990 in Saronikos Gulf (Shiganova *et al.*, 2001b). In Aegean Sea *M. leidyi* has also been found

in Lesvos island in the northeastern Aegean Sea in 1995 and in several coastal areas of the Aegean Sea (Skyros, Limnos and Alonissos islands, Halkidiki Peninsula) between 1991 and 1996 and again in 1998 (Shiganova *et al.*, 2001a). It is believed that the flow of Black Sea water mass to the northern Aegean Sea contributes to the dispersal of *M. leidyi* in the area.

A good knowledge of the geographical distribution of species and their habitats is vital in conservation biology and environmental management. Comprehensive species presence and absence records are typically not available across large geographical areas, meaning that knowledge on species distributions remains approximate and limited to well-sampled areas. Habitat suitability modelling based on predictor variables is an effective way to fill spatial information gaps on the distribution of a species. Models are able to predict the location of habitats that are likely to be suitable for a species, or group of species, to live.

Although *M. leidyi* is a rapidly expanded invader with negative impacts in different ecosystem, till 2008 there has not been any attempt to define the potential areas which could favour the species' presence. In 2008 a spatial model (Generalised Additive Model) was applied based on presence and absence of *M. leidyi* in the North Aegean Sea and satellite data of this area to determine the environmental conditions that characterize areas where *M. leidyi* is present. Based on above it was tried a first approach to identify other potential areas that could support species presence in the Hellenic and Mediterranean Seas. (Siapatis *et al.*, 2008). The model showed that many regions within the Mediterranean were potentially viable habitats for *Mnemiopsis* invasion. In 2009, these predictions came true as well as blooms of *Mnemiopsis* were reported in waters of Israel (Galil *et al.*, 2009; Fuentes *et al.*, 2010), Italy (Boero *et al.*, 2009), and Spain (Fuentes *et al.*, 2010).

In this work we used maximum entropy modelling (Maxent vers.3.3.3k) for quantifying relative risk of invasion and mapping the potential geographic distribution of *M. leidyi* for the entire Mediterranean basin.

### Materials and methods

*M. leidyi* specimens were collected during four research surveys during early summer in the northern Aegean Sea in June 2004, 2005, 2006 and June–July 2008. Sampling design was based on a grid of stations spaced on parallel transects that were approximately 10 nautical miles apart. A total of 205 stations were located on 5 nautical miles intervals on each transect. Standard vertical plankton tows were made at each station, by a WP2 sampler (mouth opening: 0.255 m<sup>2</sup>, mesh-size: 0.200-mm). *M. leidyi* specimens were identified and counted on board (Siapatis *et al.*, 2010). A dataset of 259 presences of *M. leidyi* was used in the model.

The dedicated software Maxent (Phillips *et al.*, 2006) was used to investigate the environmental factors limiting species' distributions. Maxent compares a set of samples from a distribution over a defined space, usually recorded locations of a particular species, to a set of features, such as relevant environmental variables, over that same space. The output from MaxEnt is an estimate of habitat suitability for a species that generally varies from 0 (lowest) to 1 (highest). The algorithm used in Maxent aims to find the largest spread (or maximum entropy) in a geographic dataset (i.e. the species or habitat locations), in relation to a set of "background" environmental variables (i.e. the model predictors). Maxent starts with a uniform distribution of probability values over the entire "background grid", and conducts an optimization routine that iteratively improves model fit, measured as the gain. The gain is the likelihood (deviance)

statistic that maximizes the probability of presence in relation to the background data, corrected for the case where the probabilities of all pixels are equal (uniform distribution). For the specific dataset, MAXENT was applied using the absence data available (i.e. stations with zero abundance of *Mnemiopsis*) as background points along with the respective satellite environmental and bathymetry variables at each sampling point.

Satellite environmental data deriving from international databases, can be used for modelling as proxies to infer spatial variations of environmental factors, allowing estimations in various years, periods and regions. As environmental variables were used the bottom depth (Depth), the distance from coast (Dist), the sea surface chlorophyll concentration (CHLO), the sea surface temperature distribution (SST), the sea surface salinity distribution (SAL), the sea level anomaly (SLA) and the photosynthetically active radiation (PAR). The mean monthly values for June and July 2004, 2005, 2006 and June-July 2008 of satellite imagery were estimated for all these variables (Valavanis *et al.*, 2004).

## Results and Discussion

The evaluation of the model was based on the Receiver Operating Characteristic curve (ROC) and the AUC metric, the area under the ROC (Guisan and Zimmerman, 2000) as estimated from randomly selected dataset which used 25% of the observations. The AUC of the test data averaged over the replicate runs (10 replicates) was used. The omission rate on test samples shows a good match to the predicted omission rate (straight black line) for test data drawn from the Maxent distribution itself (Figure 1). The average AUC of the test data (25% of the observations) derived from the replicate runs (10 replicates) is 0.661, and the standard deviation is 0.035, which indicates moderate model performance (Figure 2). The final fitted MAXENT model included as the major contributing factors associated with distribution of *M. leidyi* the sea surface chlorophyll concentration (CHLO) and the sea surface salinity distribution (SAL,) followed by the sea level anomaly (SLA), the bottom depth (Depth), the distance from coast (Dist) and finally the sea surface temperature distribution (SST); (Table 1). The photosynthetically active radiation (PAR) was excluded due to small contribution at the final model.

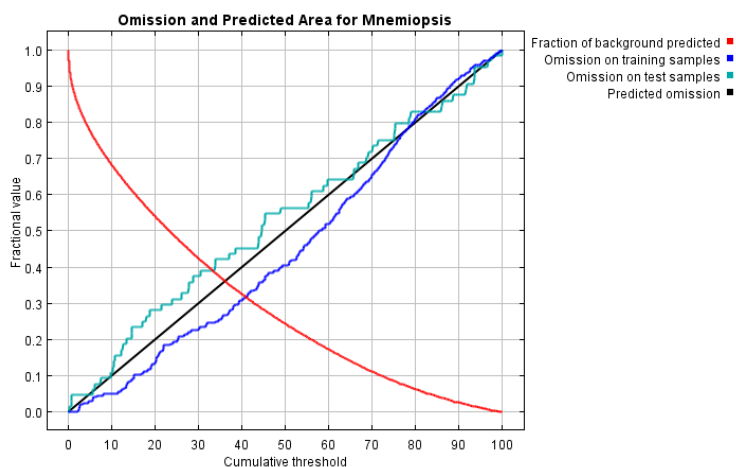


Figure 1. Omission and predicted area *M. leidyi* Maxent model output.

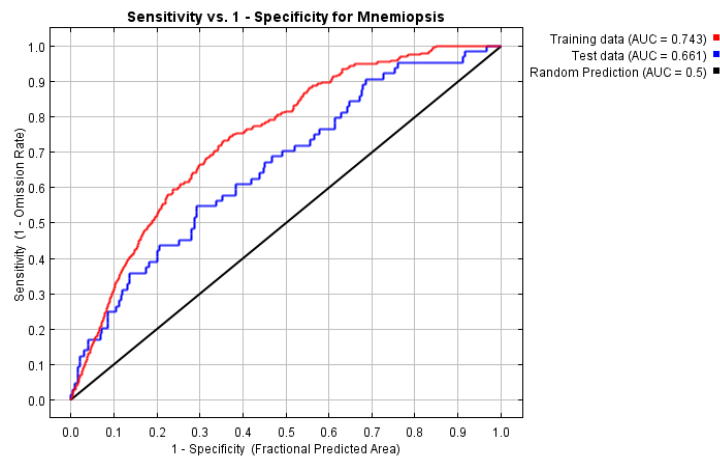


Figure 2. The receiver operating characteristic (ROC) curve for the test data (25% of the observations) averaged over the replicate runs.

Table 1. MAXENT model selection for *M. leidyi*: estimates of the relative contribution of each variables.

Variable	Percent contribution
CHL	34.1
SAL	25.3
SLA	12.4
Depth	11.4
DIST	8.9
SST	7.9

Modelling the relationship of *M. leidyi* presence with satellite environmental data from northern Aegean Sea provided maps of areas in the entire Mediterranean basin with certain environmental conditions related to certain probabilities of finding *M. leidyi* present (Figure 3). The spatial and seasonal extent of these areas is affected by the variability in climate and the environment, so estimation was restricted to early summer (June and July) for seven different years (2004–2010). These areas indicated in the Mediterranean basin varied in their spatial extent monthly and annually, depending on the environmental conditions. In the Aegean Sea, areas that have been indicated as potential habitat of *M. leidyi* characterised by Black Sea Water influence (e.g. Thracian Sea, Limnos-Imvros plateau, Saros Bay), included gulfs that are affected by river runoffs such as Thermaikos and Strymonikos gulf. In the Eastern Mediterranean basin areas have been indicated in the Levantine: the Syrian and the Lebanese coastal waters, the Turkish southeast coastal waters such as the gulf of Antalya and the gulf of Mersin and the coastal waters of Egypt. In the Western Mediterranean basin areas have been indicated in the Adriatic Sea, the coastal waters of the Ligurian and the Tyrrhenian Sea, as well as the Gulf of Lions and the Catalan Sea. These areas were similar with those predicted by the GAMs models (Sipatis *et al.*, 2008). Old and new records of the presence of *M. leidyi* in the Mediterranean basin (Uysal & Mutlu, 1993; Kideys & Niermann, 1994; Shiganova, 1997; Shiganova *et al.*, 2001; Galil *et al.*, 2009; Fuentes *et al.*, 2010; Boero *et al.*, 2009 and Turan *et al.*, 2010) generally confirm the areas indicated by the model as estimated potential species habitat.

The prediction of an invasive species potential habitat has particular interest into a closed region such as the Mediterranean basin which is characterised by strong an-

thropogenic influence and ecosystem degradation that could enhance the impact of the species into the ecosystem. *M. leidyi* has proven to be a highly successful invader and, consequently, the future of its expansion is an important issue for marine planktonic communities. Mapped predictions based on models would help identify areas of the Mediterranean basin where this species' habitats would be likely to be found, and where it would be likely to be absent. Models' output concerning *M. leidyi* presence due to limited data available should be considered only as preliminary and indicative. Additional occurrence data from additional areas and seasons are needed in order to estimate more accurate models. The model could be further improved by adding data from other areas of the Mediterranean Sea as these recorded after the bloom of 2009 and also from the Black Sea region. Additional biological data like prey availability (plankton and/or fish egg and larvae abundance), and predators' presence (*Beroe* spp.) are needed in order to estimate more accurate. Besides additional *M. leidyi* presence data, habitat suitability models also need suitable information concerning predictor variables. The latter must be available over the whole domain to be modelled, correlated to the habitat distribution, and possibly linked to it by causal relationships at an appropriate spatial scale. Unfortunately, very few, if any, predictor variables have all these properties. Although no model can fully overcome these problems and some uncertainty is to be accepted, the models we developed are state-of-the-art and as accurate as possible. Therefore, we are confident that their results will be useful to support environmental management policies at Mediterranean basin scale.

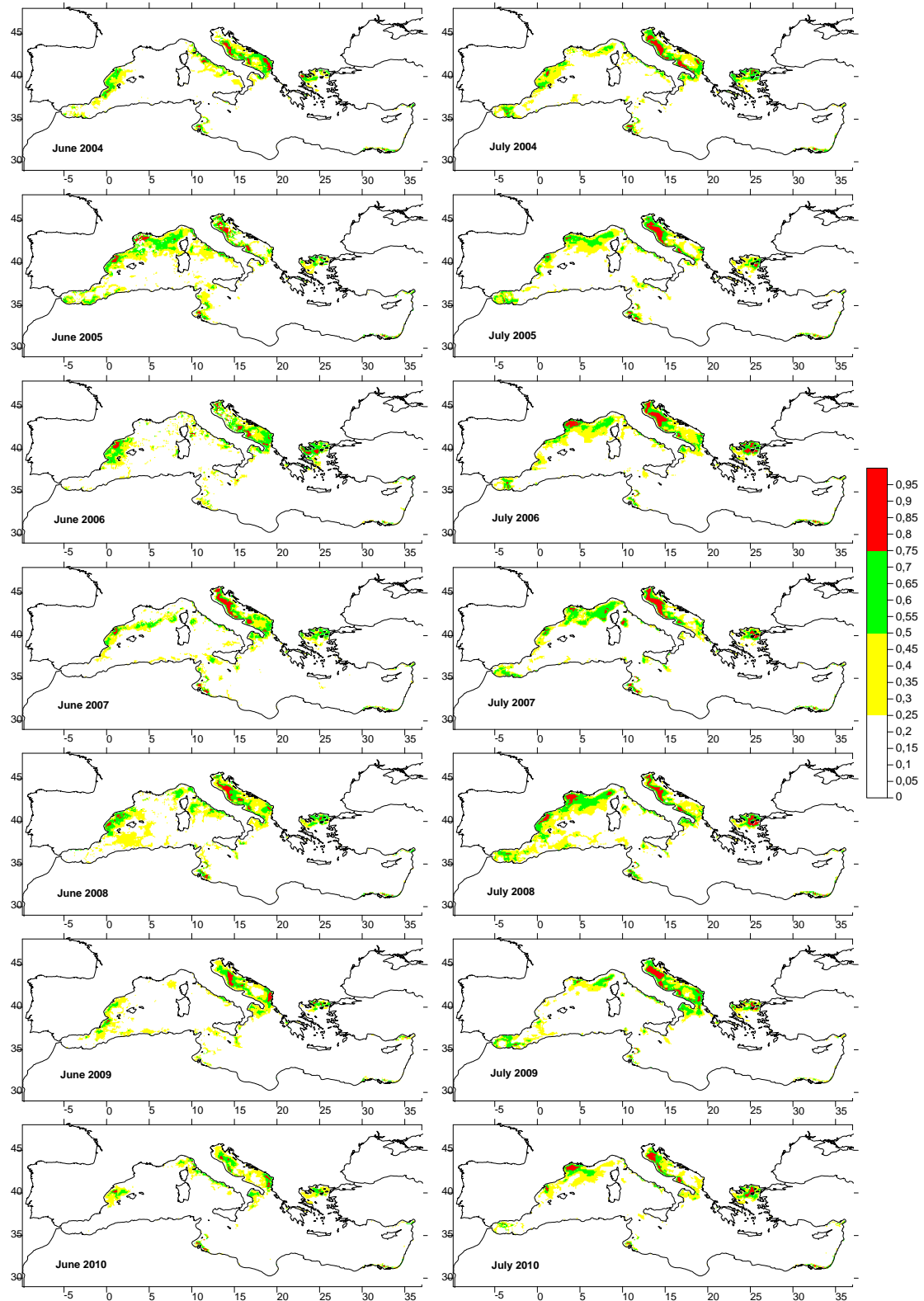


Figure 3. Maps of the probability for *M. leidyi* potential presence in the Mediterranean basin, based on maximum entropy modelling from June 2004–2010 (left) and July 2004–2010 (right).

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## 4 Synthesis and Discussion

### 4.1 Biogeography and ecology of *Mnemiopsis leidyi* and of its predators *Beroe* spp (ToR a)

Following Tamara Shiganova's presentation, the participants were invited to give their contributions to the map displaying occurrences of *M. leidyi* in the recipient seas of Eurasia and year of first record and pathways of dispersal (Figure 1, also see abstract 3.1).

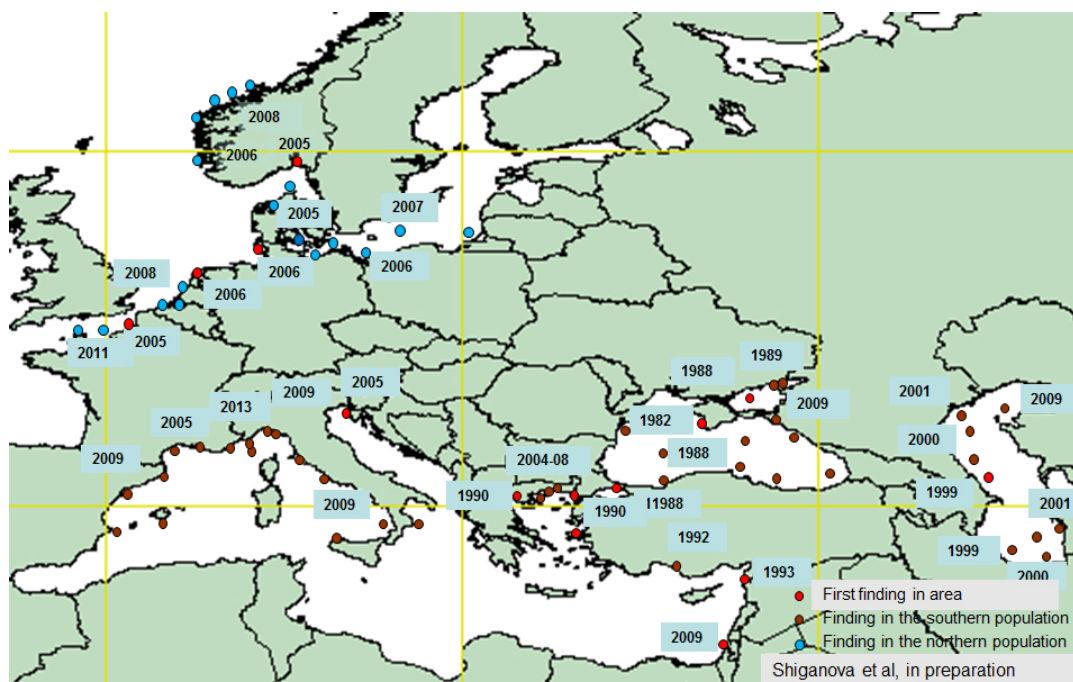


Figure 1. Chronology of *Mnemiopsis leidyi* invasion and dispersal in the seas of Eurasia (sources: Pereladov, 1988; Vinogradov *et al.*, 1989; Mutlu, 1999; Studenikina *et al.*, 1991; Mirsoyan *et al.*, 2006; Shiganova *et al.*, 2001; Shiganova, 1993; Shiganova & Malej, 2009; Shiganova, 2011; Galil *et al.*, 2009; Boero *et al.*, 2009; Lilley *et al.*, 2014; Fuentes *et al.*, 2010; Marambio *et al.*, 2013; Javidpour *et al.*, 2006; Boersma *et al.*, 2007; Faasse & Bayha, 2006; Hansson, 2006; Oliveira, 2007; Tendal *et al.*, 2007; Van Ginderdeuren *et al.*, 2012; Antajan *et al.*, 2014; Hosia & Falkenhuag, 2013; Delpy *et al.*, 2012).

*Mnemiopsis leidyi* has during the last three decades expanded in Eurasian seas. *Mnemiopsis leidyi* entered Eurasia beginning with the Black Sea in the early 1980s, followed by the Azov, Caspian, Baltic and North Seas, and, most recently, western Mediterranean Sea; and its expansion continues. The species has proven to be a highly successful invader and, the future and consequences of its expansion are of high concern for marine scientific communities and commercial fisheries, especially in view of the fisheries' collapses and ecosystem disruptions reported from areas of its introduction, in the Black, Azov and Caspian Seas (reviewed in Costello *et al.*, 2012; Shiganova *et al.*, 2004, a, b).

Population genetics analyses support the introduction of *M. leidyi* to Eurasia from at least two different pathways: the first from the Gulf of Mexico (e.g., Tampa Bay) to the Black Sea, Azov Sea and further to the Caspian Sea and to the Mediterranean; the



second from the northern part of its native distribution range (e.g., Narragansett Bay) to northern European seas (Ghabooli *et al.*, 2011, 2013; Reusch *et al.*, 2010).

*Beroe ovata*, native to northern America, was also introduced via ballast water into the Black Sea and has been shown to control *M. leidy* populations (Shiganova *et al.*, 2014). When *M. leidy* spread from the Black Sea to adjacent seas (e.g. Sea of Azov, Sea of Marmara, and the eastern followed by the western Mediterranean Sea), *B. ovata* followed. In the Mediterranean Sea, *M. leidy* may also be controlled by native Mediterranean *Beroe* species *B. cucumis* and *B. forskalii* as well as *Pelagia noctiluca* (Cnidara, Scyphozoa) which appears to consume mainly small individuals. Tamara Shiganova presented a second map showing occurrences of *B. ovata* and other *Beroe* spp (Figure 2, also see Abstract 3.1).

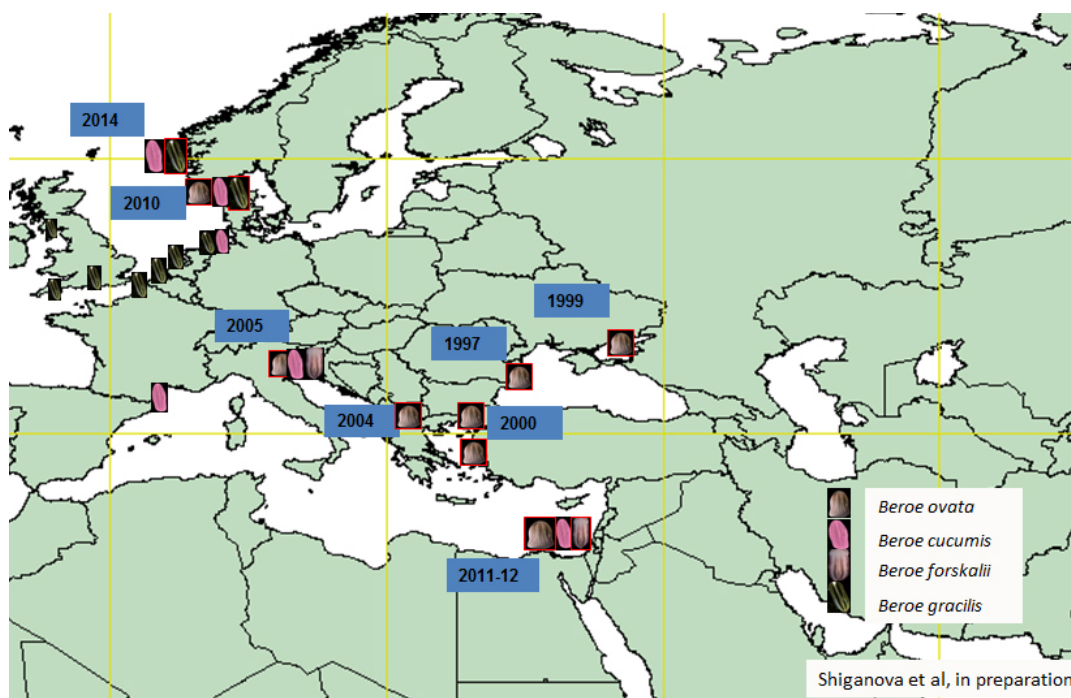


Figure 2. Chronology of *Beroe ovata* dispersal in new basins, expansion of *Beroe cucumis*, *Beroe forskalii* in the Mediterranean Sea and *Beroe gracilis* in the Baltic Sea from the North Sea. A red frame indicates that the species invaded the area (Konsulov & Kamburska, 1997; Shiganova *et al.*, 2001; Tarkan, 2000; Shiganova *et al.*, 2007; Shiganova & Malej, 2009, Shiganova *et al.*, 2014; Greve *et al.*, 1976; Ringvold *et al.*, 2014, Vansteenbrugge *et al.*; in review).

In northern European waters, the native beroids *Beroe gracilis* and *B. cucumis*, which are widely distributed in the North Sea and also observed in the Baltic Sea, have been shown to prey on *M. leidy* (Hosia *et al.*, 2011; Riisgård and Goldstein, 2014; Ringvold *et al.*, 2014). In 2012, *Beroe ovata* was recorded from Danish waters for the first time, indicating a new invasion (Shiganova *et al.*, 2014). Further native North Sea predators capable of feeding on *M. leidy* include the scyphozoans *Cyanea capillata* and *Chrysaora hysoscella* (Hosia & Titelman 2009). These gelatinous predators may thus contribute to the control of *Mnemiopsis leidy* populations in the North Sea and in some areas of the Baltic Sea. Tamara Shiganova highlighted differences between *Beroe* species and provided her publications to help for the further identification of representatives of the Beroids (Shiganova and Malej, 2009; Shiganova *et al.*, 2014). She asked participants to provide samples of *Beroe ovata* and other beroids for genetic analyses. Genetic analyses have already been conducted for some individuals from the Eastern Mediterranean and from the Danish areas of the Baltic Sea (Shiganova *et al.*, 2014). Participants

agreed to collaborate to this project. It is important now to identify and understand the factors controlling *M. leidyi* dynamics.

Before the presence of *M. leidyi* in European waters, the distribution of the two related genera of lobate ctenophores, *Mnemiopsis* and *Bolinopsis*, rarely overlapped. Now they frequently co-occur, especially in the central and northern North Sea and adjacent waters. Representative of both genera are hermaphrodites and release both ova and sperm directly into the water, and according to Tamara Shiganova, cross fertilization may have been possible under the unusual conditions of co-occurrence (individuals were found in the Danish waters by H. U. Riisgård, identified by T. Shiganova). Participants were invited to pay special attention to this possible occurrence during field observations.

Discussion on how to best present the biogeography and ecology of *M. leidyi* and of its *Beroe* spp. predators, followed Tamara Shiganova's presentation. It was stressed that the scope of ToR a) is too wide because *Mnemiopsis* has more than one identified predator and other species also interact with *Mnemiopsis*. Within the timeframe available, and given the complexity of natural ecosystems and entangling of various processes, we cannot understand fully all ecological processes related to *M. leidyi*. It is important to remember that only one little aspect at a time can be explored as ecosystems as a whole are very complex and heterogeneous. Therefore, within a given timeframe, it is better and wiser to focus on one single species or two species at best.

Exploration and discussion of the aforementioned maps followed. Several participants stressed the importance of including information on recorded abundances and phenology plots to investigate interactions between *Mnemiopsis* and *Beroe* spp. It was further noted that the morphology of *M. leidyi* varies depending on the environmental conditions in which it lives. Tamara Shiganova outlined the variations in the length of lobes, size and the occurrence of warts in differing areas. This data shows that historical definitions of three species of *Mnemiopsis* were based on morphology, while these are actually just different phenotypes of *M. leidyi*.

Northern European seas are warming at present, and temperature is possibly one of the main factors which affect the timing of both *Mnemiopsis leidyi* and *Beroe* spp occurrences. in those areas. However, in the case of the Mediterranean, Bella Galil emphasized that the temperature and salinities in the southeastern Levant are higher than elsewhere in the known range of the species (though higher salinity may seasonally occur in some lagoons). She questioned whether the sudden eruption of *Mnemiopsis* in the Mediterranean in 2009 resulted from rise in temperature, since it appeared almost simultaneously in regions that differed in their temperature, and the region experienced several temperature peaks since the 1990s - when it was already established in the Black Sea. There was no major change in shipping volume/destinations either (vector change). Furthermore, the persistence of populations throughout the year in shallow coastal waters (17–31°C) belies the temperature rise 'hypothesis' there. Bella Galil put forward the hypothesis that the Mediterranean food web was damaged, because of overfishing and of major influx of invasive aliens, and this may have permitted the establishment of these propagules. A major management issue is that although vectors and propagule pressure are fairly well known, the timing and locations of the establishment in recipient regions are still poorly understood.

Similarly to its native range, the *M. leidyi* populations in Europe most likely exhibit source-sink dynamics (Costello *et al.*, 2012). The locations of seeding areas should be identified and a map with information on established populations and their year of establishment would be useful. These seeding areas always seem to be under shel-

tered and calm conditions and new import from ballast waters might be an additional factor in some of those areas.

Henn Ojaveer presented the AquaNIS Database to the participants. This online searchable database contains information on aquatic non-indigenous and cryptogenic species and will be available soon for Europe and then North America. AquaNIS was created as part of the multidisciplinary large-scale integrated European Project VECTORS. The aim of this information system is to identify mechanisms of species outbreaks and invasions and introduction vectors. Information available will include first record and reference, location and environmental conditions, as well as species status (i.e. native or indigenous). *Mnemiopsis leidyi* will be included as well.

It was further noted that the morphology of *M. leidyi* varies depending on the environmental conditions in which it lives. Tamara Shiganova outlined the variations in the length of lobes, size and the occurrence of warts in differing areas. This data shows that historical definitions of three species of *Mnemiopsis* were based on morphology, while these are actually just different phenotypes of *M. leidyi*.

#### **4.2 Spatio-temporal occurrence in areas where *M. leidyi* is already established across the ICES-CIESM sea basins; Vectors of introduction; Areas most at risk are and where it is likely to be established next**

Genetic information has confirmed the influx of *Mnemiopsis leidyi* to Europe in both northern and southern paths with the northern European population originating from Narragansett Bay (north-east of the USA) and the southern European population from the Gulf of Mexico (Ghabooli *et al.*, 2011, 2013; Reusch *et al.*, 2010).

Cornelia Jaspers' presentation (see abstract 3.2) reminded us that the collapse of the Black Sea ecosystem in the 1980s was a result of cascading effects on several ecosystem levels including overfishing (overexploitation), eutrophication and the introduction of *Mnemiopsis leidyi* (Kideys, 2002). *Mnemiopsis leidyi* was thus able to attain high biomasses at the point when small pelagic fish stocks collapsed (Bilio and Niermann, 2004). *Mnemiopsis leidyi* was thus able to attain high biomasses at the point when small pelagic fish stocks collapsed (Bilio and Niermann, 2004). Few negative direct reports exist of the appearance of *Mnemiopsis* beside the impacts of large numbers of *Mnemiopsis* on desalination stations in the Mediterranean, and the 2008 killing of farmed fish in Norway (<http://www.nrk.no/hordaland/denne-har-tatt-livet-av-tusener-1.6241751>). However, Bella Galil noted that there are records for over 700 species introduced in the Mediterranean, and *Mnemiopsis* is listed among the world 100 worst invaders ([http://www.issg.org/worst100\\_species.html](http://www.issg.org/worst100_species.html)).

There is large debate about the actual effect of *M. leidyi* on fish populations, if it poses a negative impact through direct predation on fish eggs and larvae or if low fish stocks allow *M. leidyi* to attain high biomass due to high food availability, hence reducing competition. Though *Mnemiopsis leidyi* has been shown to feed on < 4 day old cod (*Gadus morhua*) larvae, predation on cod eggs was not significantly different from zero. Further, prey-selection experiments confirmed that cod eggs were negatively selected for (Jaspers *et al.*, 2011). If applying results from *M. leidyi* feeding experiments on cod eggs and larvae to *in situ* abundances on the most important spawning ground in the Baltic Sea, *M. leidyi* would clear at maximum 0.13% of the cod larvae and 0.05% of the cod eggs d<sup>-1</sup>, respectively (Jaspers *et al.*, 2011). This shows that direct feeding of *M. leidyi* on cod recruits in the Baltic Sea is of minor concern. Lies Vansteenbrugge concluded from her experimental work that *Mnemiopsis leidyi* ingests sea bass (*Dicentrarchus labrax*) eggs and larvae, though at much higher temperatures

(18°C compared to 7°C used by Jaspers *et al.*, 2011). However, eggs were regurgitated (Vansteenbrugge *et al.*, unpublished data). In corroboration with the results from Jaspers *et al.*, (2011), experiments revealed nearly no feeding of *M. leidyi* on fish eggs (*Limanda limanda*, *Pleuronectes platessa*, and *Ciliata mustela*) at 8°C with clearance rates of  $0.003 \pm 0.5$  eggs  $\text{h}^{-1}$  in the North Sea (Hamer *et al.*, 2010). Tamara Shiganova observed consumption and digestion of anchovy and Mediterranean horse mackerel eggs. Prey choice may be related to egg size, but also surface structure of eggs, prey motility and buoyancy, and size of *M. leidyi* individuals. Further work on predation on fish eggs is necessary. Thomas Bastian and colleagues at ULCO-LOG (France) are about to launch experiments on this. In order to make best use of research time and efforts and avoid repeating work that may already exist but is not-yet-published, they offered to liaise with others who have already conducted this type of work. Delphine Thibault's work in the Berre Lagoon showed that selectivity on natural prey is highest for nauplii, followed by calanoid copepods. Cornelia Jaspers further showed the drastically reduced reproduction rates observed under low salinities. Comparing reproduction rates at the lowest salinity (6 PSU) to the highest salinity (33 PSU), reproduction rates were nearly 2 orders of magnitude higher at the high salinity range with no significant difference between 25 and 33 PSU. Intermediate rates were observed at 10 and 15 PSU, being half the reproduction rates as observed under high salinity conditions of 25 and 33 PSU. This suggests that *M. leidyi* is not likely to build up large population sizes in the low saline Baltic Sea. Therefore, *M. leidyi* can be regarded as minor food competitor with cod recruits for the same prey. Animals present in the Baltic Sea are likely to be seeded from higher saline, high reproduction areas. Spatial and temporal surveys show highest abundances during October, with a consistent absence of adult and larval stages of *M. leidyi* in the northern Baltic Sea. Further, DNA analyses confirmed that comb jelly larvae present in the northern Baltic Sea were exclusively *Mertensia ovum* (Jaspers *et al.*, 2013) and former reports of *M. leidyi* likely a misidentification of the arctic relict species *Mertensia ovum*.

Further studies from Cornelia Jaspers showed that abundances in the Kattegat were 60 times higher than in the central Baltic during 2010, suggesting that the *M. leidyi* population in the central Baltic is dependent on advection from high saline areas (like the Kattegat with salinities > 23 psu). This interpretation is consistent with the low reproduction rates measured and a very low fraction of young animals in the central Baltic Sea. While adults were not observed from April to June in high saline areas, *M. leidyi* larvae were present throughout the year. It remains unclear where *M. leidyi* overwinters but high saline areas appear to be important in the annual establishment of the population. Laboratory and *in-situ* reproduction experiments confirmed that fecundity is a major contributor to *M. leidyi*'s invasion success, although salinity is regulating, and possibly restricting, its range expansion in Northern Europe (A minimum of 10 psu was mentioned for reproduction to occur). High reproduction rates are shown to be reached at relatively low food concentrations; this has been validated by functional response experiments investigating reproduction rates versus food concentrations in the laboratory. Therefore, high reproduction rates are attained in the field, even in localized areas despite low food concentrations (Jaspers *et al.*, in preparation). An unexpected discovery was that the arctic relict ctenophore *Mertensia ovum*, thought to be restricted to the northern Baltic, also occurs in the high saline Kattegat/Skagerrak during winter and spring. Interestingly, in the northern Baltic the *M. ovum* population consists exclusively of larval-sized animals that are actively reproducing and maintaining a self-sustained population. Natural selection can favour early maturation at small size when mortality rates are high, and results are consistent with this hypothesis (Jaspers *et al.*, 2012).

Results from Thomas Bastian and Elvire Antajan's presentations on *Mnemiopsis* in the Southern North Sea and Eastern Channel, showed that although *M. leidyi* is present in Northern France; the abundances are considerably lower than the native ctenophore *Pleurobrachia pileus*, with this species being the most common in the region. Discussions about why this species is more successful than *M. leidyi* in the region followed. It may be that the ambush predation strategy deployed by *Pleurobrachia* is more successful in mixed conditions and plankton are not aggregated in thin layers that *M. leidyi* can exploit (i.e. Turbulent conditions can interfere with *Mnemiopsis*' feeding currents (Sutherland *et al.*, 2014), while they will increase prey encounter rates for *Pleurobrachia*). Moreover, it is suggested that, the less fragile *Pleurobrachia* is more adapted to the turbulent waters of the Channel-Southern North Sea ecosystem. In 2013 *M. leidyi* was only present in the area from September to December, and at the time of the workshop, none had been observed for the year 2014, although sea water temperatures had been as high as in Autumn 2013 for one or two months already. However it was noted that *Chrysaora hysoscella* medusae (Cnidaria, Scyphozoa) were more abundant than the year before. This led to a discussion on interactions between *Mnemiopsis* and other jellyfish species; there could be competition for food between species or predation of some jellyfish species on *M. leidyi* (for example see Hosia & Titelman, 2001; Tilves *et al.*, 2013). Interactions with jellyfish species may also be a factor impacting on whether *Mnemiopsis* can settle or not in a particular area. This needs to be further investigated. Thomas Bastian also presented a small targeted study conducted by Thomas Raud in 2012 on *M. leidyi* abundance in some of the basins of Dunkirk harbour which suggested that sharp decrease of *M. leidyi* abundances are related to wind events and thus to turbulent mixing (i.e. kinetic dissipation rates induced by wind). These wind blows not only triggers strong water column mixing but could also periodically flush out some of the docks at Dunkirk thereby limiting the establishment of a longer-term population in this region. However, given the difficulty to acknowledge the vertical distribution of the species in shallow mixed areas, more effort should be dedicated to *in situ* video observation.

Another form of interaction between ctenophores and medusae has been explored by Kuplik *et al.*, (in prep.). They found that *M. leidyi* ingests planulae of *Cyanea capillata*, as do other sessile invertebrates and although there was no evidence that the ctenophores digested the planulae, the medusa larvae, engulfed in ctenophore mucus, were most probably inactivated. This evidence suggests that *M. leidyi* may impact the medusan recruitment rate reducing the chances of large bloom formation. The recent explosion in *M. leidyi* populations in Israeli coastal waters may somehow be related to the absence of major swarms of scyphomedusae in the past 2 years (Angel and Edelist, unpublished).

Delphine Thibault presented a time series of observation from Berre Lagoon in Southern France coupled with physical data. Seasonal patterns were obvious. Prior to 2012 *M. leidyi* was present all year round, but cold winters in 2011/2012 and 2012/2013 have largely reduced the presence of this species, now absent in spring and part of the summer. Temperature appeared to have a major effect, but the improving conditions (reduction in the drop in salinity linked to higher energy demand from the surrounding cities i.e. larger inflow of freshwater through the power plant) of the lagoon may also be less conducive to the success of the species. Salinity is gradually increasing over time as the freshwater influx by industry is regulated. It is unknown how *M. leidyi* reseeds the lagoon after the winter or where individuals survive (i.e. overwintering population). There are a couple of sub-lagoons which may be refuges. This again highlights the need to study the winter biology of this invasive species to better

understand and predict its population and invasion dynamics. *M. leidyi* is also present in three other coastal lagoons along the Gulf of Lion (Berre, Bages Sigean, and Salses Leucate) but it has never been observed in Thau Lagoon located between Berre and Bages-Sigean.

Macarena Marambio showed a film of *M. leidyi* living among ropes of mussels in aquaculture along the Spanish Mediterranean coast. It is not known whether there is competition for prey between the ctenophore and the mussels, but this could have economic consequences if they share habitats. Growth scenarios suggest that slow growth would reduce the size of *M. leidyi* population and that only growing fast maintains the population densities at its observed level. Suitable open-water/semi-enclosed Mediterranean habitats have been identified in Spain, Italy and Greece through modelling.

From the results seen in the given presentations, it seems that areas with higher salinity and higher winter temperatures across the ICES-CIESM area, appear to be a refuge especially during very cold winters (i.e. dec-feb 2010, 2011, the 2012 particularly cold February, and 2013). *Mnemiopsis* is able to reproduce at 6°C (Jaspers unpublished). Many species, including *M. leidyi* display different behaviours in their marginal habitats such as the Baltic Sea compared to their regular habitats. Currently, *M. leidyi* is established in high and intermediate saline areas of Northern Europe (e.g. North Sea (Antajan pers. com.) and Limfjorden (e.g. Riisgård *et al.*, 2012). While the ecological impact of *M. leidyi* in the central Baltic appears to be of limited concern, the environment in other European waters should be more favourable to their population spreading. In these areas, it is suggested that *M. leidyi* might constitute a potential threat to fisheries through resource competition with fishes. Identifying overwintering population (location) is of major concern to acknowledge their spreading and development the following year.

Overall it is likely that *M. leidyi* has been present for longer and is more widespread than generally believed (Faasse and Bayha, 2006), but in too low abundances to be noticed. Furthermore it is probably currently underestimated because it is hard to see, misidentified, difficult to sample and preserve (but see Van Walraven *et al.*, 2013), and insufficient monitoring efforts have been conducted so far. Furthermore the public generally does not report sightings, thinking only true jellyfish (i.e. scyphomedusae) are of interest.

Melek Isnibilir's presentation on *M. leidyi* in the Marmara Sea concluded that long-term monitoring is essential for understanding interactions between oxygen depletion, formation of mucilage events and jellyfish blooms. To date it is unknown what role and contribution *M. leidyi* has, however, large blooms are regularly observed in Izmit Bay, Turkey.

#### **4.3 Environment-specific parameters used for modelling potential habitat and population dynamics of *Mnemiopsis leidyi* across different sea basins**

Laboratory work is ongoing on measuring respiration rates in Marseille (Delphine Thibault). Sophie Pitois suggested that, for model development, it would also be useful to measure growth rates. Delphine Thibault showed the participants some slides on respiration rates as a function of temperature. Because of the high plasticity of *Mnemiopsis leidyi*, it was mentioned that the influence of salinity should be emphasised more, and salinity conditions used for experiments should always be docu-

mented. Tamara Shiganova showed some slides on respiration and excretion rates in different areas normalised at 20°C.

Apostolos Siapalis's presentation on a habitat model in the Aegean Sea and predictions of where *Mnemiopsis* might occur in the Mediterranean following a GAM modelling approach showed that reality is not always in agreement with models: Some areas (for example the Adriatic Sea), are identified by the model as potentially highly suitable for *M. leidyi* in terms of the observed parameters, and outputs predict high likelihood of *Mnemiopsis* presence, where in fact *Mnemiopsis* is absent. Tamara Shiganova suggested that this could be due to the introduction of *Beroe* in these areas. Bella Galil further suggested that models should offer hypotheses as to why reality does not always agree with the model predictions, via scenario-testing. Models are an essential tool to help understanding processes, but one has to remember that they only open small windows on a set of very complex processes. Therefore detailed laboratory investigations along with long term monitoring data are important to parameterize models and are a key component for realistic model assumptions.

#### **4.4 Molecular genetics techniques that have or could potentially be used to study this species. Discuss sampling techniques used and the need for a standard protocol**

Delphine Thibault mentioned the existence of a Web portal for the genome of *Mnemiopsis leidyi*: <http://research.nhgri.nih.gov/Mnemiopsis/>. The *Mnemiopsis* Genome Project Portal (MGP Portal) is intended as a resource for investigators from a number of scientific communities to obtain genomic information on *Mnemiopsis* through an intuitive and easy-to-use interface. The scope of data available through this website goes well beyond the sequence data available through GenBank, providing annotations and other key biological information not available elsewhere. The availability of these data aims to allow investigators to advance their own research projects aimed at understanding phylogenetic diversity and the evolution of proteins that play a fundamental role in metazoan development.

Standard methods presented included:

- 1) Ethanol preservation;
  - 2) Drying of animals on filters for later DNA extraction
- Cornelia Jaspers prepared a document which is included in Annex 3.

#### **4.5 Environmental and socio-economic consequences of *Mnemiopsis leidyi* in areas that have already been affected**

Lies Vansteenbrugge's presentation showed that in Belgium, tourists would not abandon a beach as a result of jellyfish presence, if they knew that these are harmless. In general, beachgoers would like to receive more information. The importance of providing information to the public was confirmed by Bella Galil. The National Institute of Oceanography, Israel Oceanographic and Limnological Research, has been informing the Israeli public on occurrence of jellyfish swarms for over 20 years through local media outlets and a tri-lingual (Hebrew, Arabic, English) dedicated website (<http://www.ocean.org.il/meduzot/history.asp>) The website provides the forecast, historical data, information concerning the biology and ecology of the locally occurring jellyfish (Scyphozoa and Ctenophora), relevant scientific articles, video, and jellywatch poster. Bella regularly gives presentations to life guards and the wider public. Citizen science is encouraged through widely used facebook account

([www.facebook.com/jellyfishwatch](http://www.facebook.com/jellyfishwatch)), email account ([jellyfish@ocean.org.il](mailto:jellyfish@ocean.org.il)) and dedicated cellphone (050-3700337) popular for transmitting photographs. They also add extra info on the beach pamphlets. Moreover, the Israeli public regularly reports to and uses the citizen science website, <http://meduzot.co.il/> established by Dror Angel and colleagues several years ago, and the map generated by this appears on the weather page of the most popular online newspaper (<http://weather.ynet.co.il/>) during the summer months to help warn the public where high numbers of medusae or “stinging water” have been observed. On the backdrop of the stinging experience most swimmers have had after contact with the nematocysts of *Rhopilema nomadica*, the Israeli public is almost oblivious to the non-stinging ctenophores. When ctenophores or salps are abundant, swimmers and divers occasionally write to the webmaster at <http://meduzot.co.il/> to ask about this “floating gelatinous material”, but generally do not express concern (D. Angel, unpublished).

Macarena Marambio added that their lab opens doors regularly and they sometimes have aquaria with jellyfish close to the beach to give people information about these. Both Macarena and Bella stressed the importance of citizen science to inform the public about presence and absence of jellyfish. Thomas Bastian’s presentation, showed that in France, beach tourists (survey was done at Northern coast of France) have a different opinion from the Belgian tourists. A large fraction of the French tourists believes jellyfish are increasing in abundance, and often refer to the *Pelagia* spp. blooms in the Mediterranean. This is probably due to the fact that national TV reports often focus in summer on jellyfish blooms in the Mediterranean Sea, resulting in lower frequentation of beaches and hence, decrease in touristic activities. Only a small fraction of the Belgian tourists believes there is an increase in jellyfish. It therefore seems that jellyfish are perceived differently in different countries, partly as a result of varied media reports and the level of information disseminated.

Sophie Pitois noted that the presentations (3.8 and 3.9) focused on jellyfish rather than *Mnemiopsis* – probably because the public will not distinguish between them and in most cases hasn’t heard about *Mnemiopsis* (only 14% have). *Mnemiopsis* tends to be negatively viewed by the public as a jellyfish. Following correct information, they change their view. Scientists are also partly responsible for confusing the public as *Mnemiopsis* is often included within the generic term ‘jellyfish’. Henn Orjaveer mentioned that the general perception that invasive species are detrimental to human health, is also incorrect. However NIS are undesirable by definition. Communication is therefore key and care should be given to differentiate between introduced and invasive specimens.

Regarding the requirements for MSFD implementation, Cornelia Jaspers mentioned an important process: the development of indicators according to descriptors and their criteria, and the determination of thresholds for good environmental status. There is therefore a need for regular monitoring of invasive species and *M. leidyi* is an obvious choice to determine population dynamic trends. Kremena Stefanova stressed that zooplankton have not been widely used as indicators of ecosystem condition. They tried to apply the Helcom approach (2012) for D1 (Descriptor one, biodiversity). For D2 (Non-indigenous species), together with *M. leidyi* indicators they focused on the ratio non-native/native species for phytoplankton and zooplankton. But all candidate indicators need validation. Henn Orjaveer added that there are a lot of uncertainties surrounding Descriptor 2, and that proposed indicators could apply to D1 or D4 (food web) but are not appropriate for D2. However Kremena Stefanova responded that, despite many uncertainties, quantity and distribution of invasive species established in the new ecosystem could apply to D2. Tamara Shiganova added



that Russia, Ukraine and Georgia are involved in a project EMBLAS which is a mirror of MISIS project (Romania, Bulgaria, Turkey) presented from Kremena. One of the projects aims is to develop and propose indicators for good environmental status.

#### 4.6 Outline future research needs/next steps and identify management measures

- Long term monitoring including gelatinous zooplankton in general and *M. leidy* in particular to distangle climate variability and population fluctuation of *M. leidy* throughout Europe. Existing monitoring programme should at least quickly visually inspect plankton samples for the presence of ctenophores before fixation.
- Winter biology is largely unknown but a key to forecast next year's population size and understand population dynamics in different regions. Therefore more emphasis should be devoted to investigate survival under different temperatures (in the laboratory) and identify potential regions where *M. leidy* overwinters in Northern and Southern Europe e.g. via towed camera systems or scuba diving investigations.
- Predator-prey interactions between fish eggs and *M. Leidy*, including overlapping times and locations of fish spawning and *M. leidy* reproduction, effects of temperature, egg size/surface structure on feeding/ingestion/digestion/egestion rates, and predator size.
- Reliable *in situ* quantitative abundance estimates are needed to estimate the actual contribution of *M. leidy* to ecosystem processes.

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## Annex 2: Agenda

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### The Joint ICES/CIESM Workshop on *Mnemiopsis* Science (JWMS)

#### Meeting Agenda

**Meeting Place:** Palexco Congress and Exhibition Centre, A Coruña, Spain – 18-20 September 2014.

#### Thursday, 18 September

- 15:00 **Adi Kellermann; Tamara Shiganova, Sophie Pitois**  
Welcome and introduction ()
- 15.30 **ToR a) Review and discuss the latest knowledge regarding the biogeography and ecology of *Mnemiopsis leidyi* and of its predator *Beroe* spp.**  
**Tamara Shiganova:** “Adaptive strategy of *Mnemiopsis leidyi* and its predator *Beroe* spp. In the Eurasian seas”.
- 17.00 Finish

#### Friday, 19 September

- 9:00 **ToR b) Compile existing knowledge on spatio-temporal occurrence in areas where *M. leidyi* is already established across the ICES-CIESM sea basins. By identifying and geo-locating the likely vectors of introduction, establish which the areas most at risk are and where it is likely to be established next. (Lead? Rapporteur?)**  
**Cornelia Jasper:** “*Mnemiopsis leidyi* in the Baltic Sea region: Origin, transport and seasonality”  
**Elvire Antajan:** “*Mnemiopsis* in the Southern North Sea and Eastern Channel”  
**Thomas Bastian:** “Looking for *Mnemiopsis leidyi* in Dunkirk Harbour (Southern North Sea, France)”  
**Elvire Antajan:** “Recurrent winter observations of *Mnemiopsis leidyi* swarms in the Southern North Sea”  
**Delphine Thibault:** “*Mnemiopsis* in the Berre Lagoon, what are the main triggers for its expansion?”  
**Melek Isinibilir:** “Distribution and size structure of comb jellyfish, *Mnemiopsis leidyi* (Ctenophora) in Izmit Bay during mucilage event”  
**Macarena Marambio:** “The invasive ctenophore *Mnemiopsis leidyi* in the Spanish Mediterranean coast”
- 12:30 Lunch break
- 14:00 **Tor e) Review the environmental and socio-economic consequences of *M. leidyi* invasion in areas that have already been affected in ICES-CIEM area.**  
**Lies Vansteenberghe:** “Jellyfish, jellypress and jellyperception”

**Thomas Bastian:** “Defining public feeling about *M. leidyi* invasion and jellyfish proliferation along the French coast of the English Channel”

**Kremena Stefanova:** “*Mnemiopsis leidyi* distribution and biomass index as an indicator of Good Environmental Status (GENS) in Bulgarian waters of the Black Sea”

**Bella Galil:** “Jellyfish invaders and the impact of the blooms on the Eastern-most Mediterranean”

17:00 Estimated finish

### Saturday, 20 September

9:00 am **ToR c) Compare environment-specific parameters used for modelling potential habitat and population dynamics of *M. leidyi* across different sea basins. Discuss the potential sea-basin specific physiological adaptations.**

**Sophie Pitois:** “Modelling aspects of *Mnemiopsis leidyi* in the North Sea”

**Thomas Bastian:** “ Studying *M. leidyi* / fish interactions”

**Apostolos Siapatis:** “Maximum entropy modelling of potential habitat of the invasive ctenophore *Mnemiopsis leidyi* in the Mediterranean basin

11:00 **ToR d) Review the molecular genetic techniques that have or could potentially be used to study this species. Discuss sampling techniques used and the need for a standard protocol.**

11:30 **ToR f) Outline future research needs/next steps and identify management measures.**

12:30 Estimated finish

### Annex 3: DNA Sampling of *Mnemiopsis leidyi* for genotyping of populations

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Written by Cornelia Jaspers<sup>1,2\*</sup> and Thorsten Reusch<sup>1</sup> for the ICES workshop on *Mnemiopsis leidyi* held at the annual science conference in La Coruna, Spain, 2014.

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*Mnemiopsis leidyi* is a comb jelly species native to the East coast of North and South America. On a large scale, populations present in Europe can be related to two different source populations in its native habitat along with two different introduction events in European waters (Reusch *et al.*, 2010). By use of microsatellite analyses it has been shown that animals present in Northern Europe originate from the NE coast of the USA, while animals present in the Black Sea and adjacent waters are similar to *M. leidyi* populations from the Gulf of Mexico region (e. g. Reusch *et al.*, 2010). Though large scale distribution pattern and origin of populations present in northern and southern Europe are known, including the information, that the Mediterranean population is a stepping stone invasion from the Black Sea (Bolte *et al.*, 2013), details about local population extinction and re-invasion pattern are unknown.

Questions about spatial and temporal population development can be addressed by second generation sequencing using SNP's or microsatellite analyses (Reusch *et al.*, 2010). In order to assure good DNA samples, we suggest following the sampling procedures outlined below. This approach has extensively been used in current field and laboratory investigations regarding *M. leidyi* through Europe (e.g. Reusch *et al.*, 2010; Jaspers *et al.*, 2012; Bolte *et al.*, 2013; Jaspers *et al.*, 2013) but is also a standard preservation technique for museum collections.



Figure 1. *Mnemiopsis leidyi* adult shown from the oral side (left) and from the ventral side (right). Photo: Cornelia Jaspers.



### Sampling protocol

**Location:** Per hydrographically distinct sampling region, which can be defined by limited water exchange such as lagoons or semi-enclosed bays, as well as distinct areas characterized by a unique salinity/temperature signature, 50 *M. leidyi* should be collected.

**Number of samples:** We suggest to take 50 individual *Mnemiopsis leidyi* – or as many as are available with a minimum sample size of 25 individuals per distinct sampling location.

**Sampling technique:** Samples can be attained by either towed nets or by hand collection of animals with a beaker from a pier (e.g. in a harbor). Since comb jellies are easily damaged, animals for DNA analyses should be in a good shape and not totally damaged. If animals origin from a densely aggregated sample, individuals should be cleaned in filtered seawater – if available, otherwise unfiltered seawater is better than nothing - to remove attached algae and other zooplankton organisms which could lead to a false signal during molecular analyses.

**Sample preparation:** We have good experience extracting *M. leidyi* DNA from dried tissue samples. Therefore, entire animals (or parts of an animal) should be placed on a filter to suck up additional water and body fluid of the animal. Coffee filters have been proven handy, cheap and have shown to lead to good extraction results. Filters should be pre-label with date, location name, position (in decimal) by use of a pencil. Animal should be placed on the pre-labelled filters. To increase the absorption, several filters can be used. It is important to highlight (circle with a pencil) where the animal has been placed. Sometimes, it has been shown difficult to relocate individuals on filters, since parts of the animal will be cut out for DNA extraction. However, if drawing a circle with a pencil, it is important that the animal is not contaminated with the pencil. Also, it is preferred that entire animals are dried. If *M. leidyi* are cut into parts, body fluid poses a potential cross-contamination risk if the next individual is placed too close. Therefore, caution should be used not to contaminate the different *M. leidyi* samples. Also, when cutting animals, it is of uttermost importance to clean the equipment with ethanol between animal dissections. This should include tweezers, scissors and hands before handling a new specimen. Also, if the filters are too soaked and wet, potential cross-contamination can be avoided by placing only one individual per filter and place each filter into a separate bowl before placing it in the oven. New oven trays can easily be build out of cardboard covered with aluminum foil (see Figure 2).

**Drying:** The filters should be dried for 48 hours at 60°C – not more than that. Please carefully check that the filters are 100% dry. If the filters smell, then this is a sign for bacterial degradation and remaining water on the filters. The filters should then be placed in the oven again including a note that the initial drying process was not sufficient. Initial drying for 48 hours at 60°C should be sufficient and not be extended over a long period.

**Storage:** Ensure that the filters are dry before long term storage. Each filter should be followed by a blank filter and placed in a paper envelop for each distinct sampling group. Additional information such as salinity, temperature, size of the animals and exact position of the station (in decimal) should go in each envelope. The envelopes can be stored at room temperature in an office. If the environment is humid, then samples should be frozen in a plastic bag at -10°C. Avoid consecutive thawing of the material.

**Contact person:** For further information, Cornelia Jaspers will be the contact person and samples can be send to her for long term storage. It makes sense that regions are samples every year to investigate the genetic diversity of sub-populations. The results of these genetic analyses will partly deliver to the EU/ Bonus Project BIO-C3 and all partners inside and outside the consortium are welcome to contribute and join forces Please do not hesitate to contact Cornelia Jaspers: [coja@aqu.dtu.dk](mailto:coja@aqu.dtu.dk), physical addresses either Copenhagen or Kiel.

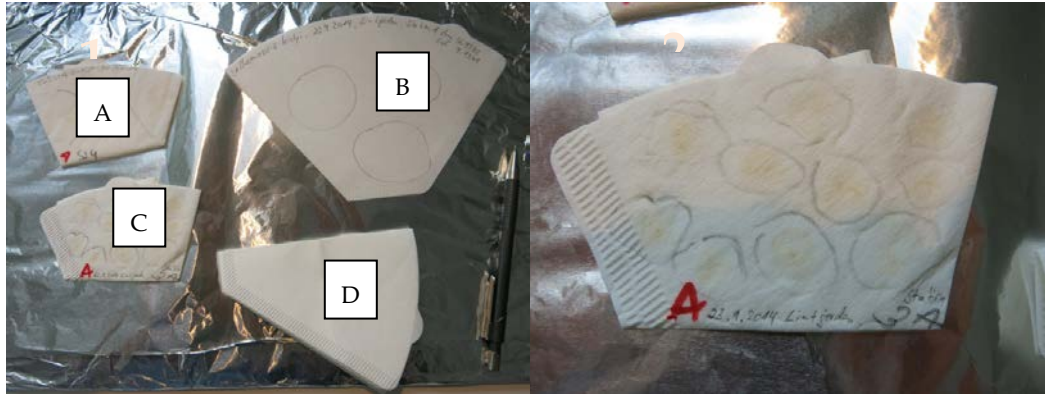


Figure 2. Example of oven tray with different coffee filters. 1 a, b two times flexed, c, once flexed and d entire filter. 2 shows an example where 10 small sized *M. leidy* have been dried.

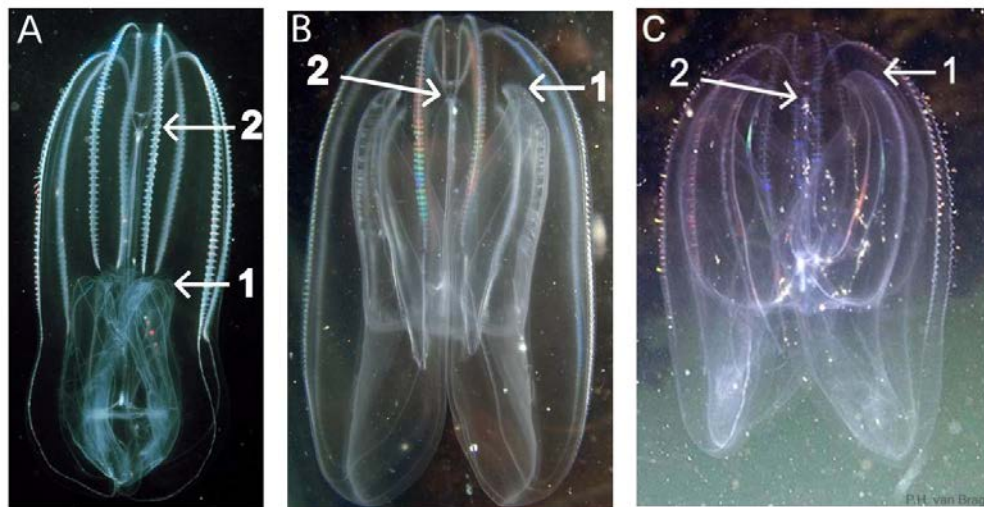


Figure 3: Photographs of A) *Bolinopsis infundibulum* B) *Mnemiopsis leidy* and C) lobate ctenophore (*M. leidy*) taken from Bommenede in the Grevelingen (02.09.2006). Note the termination of the oral lobes (1) and the placement of the statocyst (2). In *M. leidy*, the oral lobes extend all the way to 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. leidy* photograph (B) by K.M. Bayha and Bommenede *M. leidy* photograph (C) by P.H. van Bragt).

Figure 3. Quick identification key figure from Faasse and Bayha 2006.

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### Annex 3: Recommendations

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Recommendation	Adressed to
1. Suggestion to arrange a <i>Mnemiopsis</i> workshop every 5 years to evaluate the further spread or actions that have been taken to decrease the impact.	SCICOM
2. Testing the hypothesis of perturbed food web from a combination of overfishing and invasive species that is linked to <i>Mmmemiopsis</i> establishment rather than temperature?	JWMS
3. Include <i>M. leidyi</i> into current monitoring activitites: Send recommendation to existing monitoring programme to inspect plankton samples for <i>M. leidyi</i> presence before fixation.	JWMS
4. Include citizen science into observation of jellyfish bloom, particularly <i>M. leidyi</i> (scuba diving , naturalist association).	JWMS