# Ecology of gelatious plankton

With emphasis on feeding interactions, distribution pattern and reproduction biology of Mnemiopsis leidyi in the Baltic Sea

**Jaspers, Cornelia; Kiørboe, Thomas**

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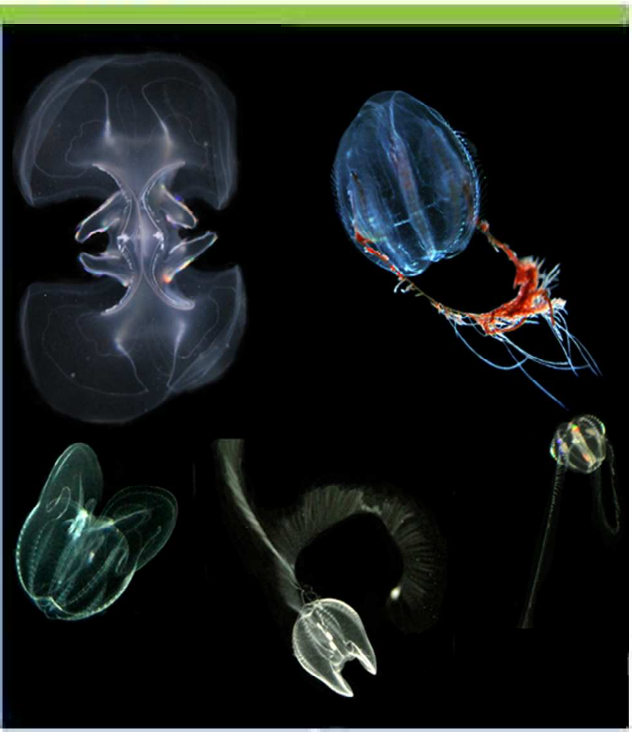
PhD Thesis



**Written by Cornelia Jaspers**
**Defended 14 May 2012**

DTU Aqua
National Institute of Aquatic Resources

Technical University of Denmark

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**PhD thesis by**
Cornelia Jaspers

# Ecology of gelatinous plankton

## With emphasis on feeding interactions, distribution pattern and reproduction biology of *Mnemiopsis leidyi* in the Baltic Sea

**Defended: 14 May 2012**
**3 year PhD Project**
**Supervisor: Prof. Thomas Kiørboe**
**Co-supervisor: Prof. Torkel G. Nielsen**

## Summary

Comb jellies were a relatively obscure group of zooplankton, until *Mnemiopsis leidyi* invaded the Black Sea in the 1980's with cascading effects on several ecosystem levels including commercial fisheries. 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. leidyi* was sighted in Northern Europe for the first time, similar consequences were feared. The aim of my PhD project was to understand the potential impact of *M. leidyi* on the Baltic Sea ecosystem and constrains on its dispersal. Specifically, the project investigated (i) direct and indirect effects of *M. leidyi* on the Baltic cod population in its most important spawning ground, (ii) factors governing the spatial and temporal distribution of *M. leidyi* eggs, larvae and adults in the Baltic and, (iii) *M. leidyi* reproduction and its effect on population development. 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 modeling.

The low feeding rates and passive negative selection of cod eggs in experiments demonstrate that *M. leidyi* does not pose a direct threat to the Baltic cod population at the environmental conditions characteristic for its spawning ground. Furthermore, the drastically reduced reproduction rates observed under low salinities suggest *M. leidyi* is not likely to compete with cod recruits prey. Spatial and temporal surveys show highest abundances during October, with a consistent absence of adult and larval *M. leidyi* in the central Baltic. Abundances in the Kattegat were 60 times higher than in the central Baltic, suggesting that the *M. leidyi* population in the central Baltic is dependent on advection from high saline areas. This interpretation is consistent with the low reproduction rates measured and a low fraction of up-growing animals in the central Baltic. 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. Maximum reproduction rates are shown to be attained at low food concentrations, helping reconcile the high population densities observed in localized areas despite low food concentrations.

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 central Baltic the *M. ovum* population consists exclusively of larval-sized animals that are actively reproducing and maintaining a self-sustained population. Natural selection can favor early maturation at small size when mortality rates are high, and our results are consistent with this hypothesis.

Currently, *M. leidyi* has established itself permanently in high and intermediate saline areas in Northern Europe. While the ecological impact of *M. leidyi* in the central Baltic appears to be limited concern, the environment in other European waters should be more favourable to their populations. In these areas, it is suggested that *M. leidyi* constitutes a potential threat to fisheries through resource competition with fishes.

# Dansk resumé

Ribbegopler var en forholdsvis ubeskrevet gruppe af zooplankton, indtil dræbergoplen (*Mnemiopsis leidyi)* invaderede Sortehavet i 1980'erne og medvirkede til sammenbrud af økosystemet inklusiv det kommercielle fiskeri. *M. leidyi,* som stammer fra den nordamerikanske østkyst, fik stor offentlig og videnskabelig bevågenhed pga. denne invasion og de økologiske og økonomiske konsekvenser. Da den i 2005 blev fundet i Skagerrak og senere i den centrale Østersø, opstod der frygt for lignende effekter.

Det overordnede formål med mit ph.d.-projekt var at undersøge effekten af *M. leidyi* på Østersøens økosystem og mulige begrænsende faktorer for dens udbredelse. Mere specifikt undersøgtes (i) direkte og indirekte effekter af *M. leidyi* på Østersøens bestand af torsk på dens gydeplads ved Bornholm, (ii) faktorer som bestemmer udbredelsen af æg, larver og voksne *M. leidyi* i Østersøen og (iii) artens reproduktion og populationsdynamik. Der gennemførtes 13 månedlige overvågningstogter, som strakte sig fra Skagerrak med høj saltholdighed til den brakke nordlige Østersø, kombineret med felt- og laboratorieforsøg af reproduktion og fødesøgning, molekylære analyser til artsbestemmelse samt statistisk modellering.

Forsøgene viste at pga. lave græsningsrater og fravalg af torskeæg udgør *M. leidyi* ikke en direkte trussel mod Østersøens torsk. Andre forsøg dokumenterede at artens reproduktion er kraftigt reduceret ved lave saltholdigheder og at *M. leidyi* derfor næppe vil opbygge en bestand i den centrale Østersø og blive en fødekonkurrent til torskeyngel.

De højeste tætheder af *M. leidyi* fandtes i oktober, men voksne og larver blev aldrig fundet i den nordlige Østersø. Tæthederne var 60 gange højere i Kattegat sammenlignet med den centrale Østersø, hvilket antyder at *M. leidyi* bestanden i den centrale Østersø er afhængig af at dyr driver ind med havstrømme fra høj-saline områder. Dette understøttes af de lave reproduktionsrater og en lille andel af unge stadier af dyr i den centrale Østersø. Mens der ikke fandtes voksne dyr i de høj-saline områder fra april til juni, fandtes larver hele året rundt. Det er endnu ukendt hvor *M. leidyi* overvintrer, men resultaterne peger på at de høj-saline områder er vigtige som kilde til den årlige start på bestandsudviklingen.

Det var en uventet opdagelse at den arktiske relikt ribbegople *Mertensia ovum,* som mentes begrænset til den nordlige Østersø, også forekommer i det høj-saline Kattegat/Skagerrak om vinteren og foråret. Det var endvidere overraskende, at bestanden af *M. ovum* i den nordlige Østersø udelukkende består af larvestadier, der opretholder en selv-reproducerende bestand. Naturlig udvælgelse forudsiges at favorisere tidlig kønsmodning hvis dødeligheden er høj, og det observerede fænomen stemmer overens med denne hypotese.

Forsøg i laboratoriet og felten bekræftede at reproduktion er en nøglefaktor til forklaring af *M. leidyi*'s succes som invasiv art, omend saltholdighed regulerer og sandsynligvis begrænser dens udbredelse i nordeuropæiske farvande. De højeste reproduktionsrater blev målt ved lave fødekoncentrationer, hvilket kan forklare at høje bestandstætheder og opblomstring finder sted i områder til trods for lave fødetætheder.

*M. leidyi* har i dag etableret sig i nordeuropæiske farvande med høj og intermediær saltholdighed. Mens den økologiske effekt af *M. leidyi* i den centrale Østersø er begrænset, kan den i andre mere marine områder potentielt udgøre en trussel mod fiskeriet pga. fødekonkurrence med fisk.

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**Paper II**: Salinity gradient of the Baltic Sea limits the reproduction and population expansion of the newly invaded comb jelly *Mnemiopsis leidyi*. Jaspers et al. (2011) PLoS ONE 6(8): e24065. doi:10.1371/journal.pone.0024065

**Paper III:** Ctenophore population recruits entirely through larval reproduction in the central Baltic Sea. Jaspers et al. (2012) Biology Letters doi:10.1098/rsbl.2012.0163

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# 1 Gelatinous zooplankton

## 1.1 Who are they and why should we care?

Gelatinous zooplankton is an overarching synonym for functionally different groups sharing the trait of a soft body texture with dilute carbon content such as ctenophores, jellyfish and pelagic tunicates, as compared to crustacean zooplankton which have a hard exoskeleton containing chitin (e.g. copepods). Gelatinous zooplankton includes some of the fastest growing metazoans on Earth (Hopcroft and Roff 1995). Certain groups can directly feed on the smallest components of the oceans (Deibel and Lee 1992) leading to predator prey ratios similar to Baleen whale and Krill. Though jellyfish belong to the zooplankton they reach sizes of 1 m and weights of up to 150 kg (Omori and Kitamura 2004), and it has been shown that ctenophores and jellyfish have similar clearance rates as fish if compared on a carbon basis (Acuña et al. 2011). Despite these astonishing attributes, gelatinous zooplankton remain understudied and disregarded in most food web investigations (Hopcroft and Roff 1998) and our knowledge about their population dynamics is fragmentary (Condon et al. 2012). This lack of knowledge can partly be explained by their patchy distribution (Costello and Mianzan 2003) and the difficulty to quantitatively catch and preserve gelatinous organisms in traditional plankton samples (Hamner et al. 1975).

Lately, reports have shown that gelatinous plankton blooms are increasing in magnitude and frequency (e.g. Brodeur et al. 1999; Lynam et al. 2006; Hay 2006; Daskalov et al. 2007; Richardson et al. 2009; Purcell 2012). These blooms have been attributed to human activity such as eutrophication, over-fishing, establishment of artificial hard substrate, climate change and species translocations (Mills 2001; Arai 2001; Richardson et al. 2009; Lynam et al. 2011; Uye 2011; Purcell 2012; Costello et al. 2012). This has led to the paradigm that gelatinous plankton will increase under future climate and global change. Although evidence is accumulating for this paradigm (e.g. as reviewed in Richardson et al. 2009; Uye 2011; Purcell 2012), it has recently been questioned since systematic, long time monitoring data are sparse or non-existing (Condon et al. 2012). The predicted increasing role of gelatinous plankton therefore calls for a systematic research and monitoring effort.

**Fig. 1.** Examples of gelatinous zooplankton from tropical, temperate and arctic ecosystems. Note that pictures are not scaled. Photographs taken by Prof. R.R. Hopcroft and C. Jaspers during 2006-2012.

## 1.2 Climate change and biological invasions

Human induced climate change due to rising atmospheric $CO_2$ levels has manifold effects on the oceans of the world, such as altered sea surface temperatures, stratification and ocean circulation, nutrient dynamics, oxygen concentrations and pH levels. These changes will potentially have large and widespread cascading effects on current marine ecosystems, their structure, functioning and biodiversity (Doney et al. 2012). At the same time, due to increased globalization and ship-traffic activity, the rate of marine invasions is increasing (Carlton and Geller 1993; Molnar et al. 2008), leading to a homogenization of the worlds biota (Lodge 1993; Stachowicz et al. 2002).

The combined effect of climate change and biological invasions have been highlighted as challenges and severe stressors to marine biodiversity (Stachowicz et al. 2002; Butchart et al. 2010; Sorte et al. 2010a). As a consequence of increased ocean temperatures, species have been shown to expand their current distribution ranges, e.g., pole ward shift of species (Burrows et al. 2011). The mechanisms and effects of climate-induced species expansions and direct human mediated invasions are in many ways similar (Sorte et al. 2010b; Canning-Clode et al. 2011). Both have been shown to have consequences for the ecosystem and its biodiversity including alterations of food web structures (Carlton and Geller 1993; Kideys 2002; Sorte et al. 2010a).

With respect to climate change, gelatinous zooplankton share physiological attributes making it likely that they take advantage of changing environmental conditions (with anticipated changes outlined in Doney et al. 2012) compared to most other zooplankton groups (Richardson 2008). This includes their ability to tolerate low oxygen concentrations (Condon et al. 2001; Decker et al. 2004; Rutherford and Thuesen 2005), to starve and shrink during low food conditions (Hamner and Jenssen 1974; Anninsky et al. 2005) and their broad physiological tolerance to increased temperatures which is additionally likely to foster their reproduction and growth rates (e.g. Purcell 2005; Purcell et al. 2007; Richardson 2008).

Even though invasions have gained large attention and seem to be an overall threat to biodiversity, the actual percentage of successfully establishing animals in a new, recipient environment are low (Williamson and Fitter 1996). The likelihood of an invasive species to establish a population in the recipient ecosystem, can be generalized by the “tens rule” which is linked to the survival of several invasion stages. In general, only 10% of the arriving species are found in the new environment, of which only 10% successfully reproduce and establish. The percentage of invaders forming blooms or achieving pest status are very low and only c. 0.1% of the originally arriving species (Williamson and Fitter 1996). However, some exceptions exist to this general “tens rule” for example if the biogeography is very similar between native and recipient habitat (e.g. voles in Ireland coming from the UK) or if release rates are extremely high (e.g. insects in biological control campaigns), where establishment success can reach 100% (Williamson and Fitter 1996).

The processes involved in invasion success are complex, but generally, invasive species are characterized by tolerating a broad set of environmental and anthropogenic stressors (Crooks et al. 2011; Lenz et al. 2011). The success of an invader will depend on its life history traits, the recipient ecosystem and the resident or native species community (Facon et al. 2006). Intrinsic attributes such as fast growth, high reproduction rates (r-strategists) and phenotypic plasticity have been shown to make invaders competitive especially under circumstances where natural predators are lacking or the ecological niche of the invader is not occupied leading to high resource availability (Sax and Brown 2000; Facon et al. 2006; Sorte et al. 2010b). If combined with asexual reproduction or self-fertilizing hermaphroditism, an invader has an additional advantage by offsetting the Allee effect which may be encountered by sexually reproducing species during early colonization (Berec et al. 2007; Tobin et al. 2011).

On the other hand, in situations with occupied niches in the recipient environment, invasive K-strategists with low reproduction rates can be favoured (Facon et al. 2006). The latter life-history trait will lead to a competitive-colonization trade off where the better competitor excludes the other (Byers 2000; Facon et al. 2006). Many exceptions exists to these general characteristics of successful invaders making generalizations difficult (Lodge 1993), but it has been shown and predicted that disturbed aquatic systems are especially vulnerable to invasions (Daskalov et al. 2007; Richardson et al. 2009; Crooks et al. 2011; Purcell 2012). Further, for European brackish water systems, e.g. the Baltic Sea, the Black Sea and adjacent waters, areas of intermediate salinity and lowest species richness have been shown to be especially vulnerable to invasions (Paavola et al. 2005).

### 1.3 *Mnemiopsis leidyi* in European waters

The comb jelly *Mnemiopsis leidyi,* native to the east coast of America (Purcell et al. 2001), is an example of an invasive species which has reached a globally wide expansion range (as reviewed in Costello et al. 2012). It is listed among the 100 most notorious invaders world wide (Lowe et al. 2000) and it shares many characteristics of a successful invasive r-strategy species (Fig. 2). In the 1980’s, large scientific and public attention was devoted to *M. leidyi* after its introduction into the Black Sea and the observed ecosystem alterations, especially the collapse of the commercial anchovy fisheries which was attributed to direct predation on anchovy eggs and larvae by *M. leidyi* (e.g. Kideys 2002; Oguz et al. 2008). Although the collapse of the anchovy stocks coincided with *M. leidyi* blooms, the ecosystem was already heavily disturbed by human activity including eutrophication, oxygen depletion and over-fishing, and the combination of these effects opened a niche allowing *M. leidyi* to achieve blooms with 2 kg wet weight $m^{-2}$ (as reviewed and cited in Bilio and Niermann 2004).

Currently, *M. leidyi* has successfully established populations in many European waters. After its introduction into the Black Sea in the 1980’s it quickly spread over all adjacent waters such as the Sea of Azov, the Sea of Marmara, the

Aegean Sea and the Caspian Sea (Shiganova et al. 2001). A habitat model predicted that *M. leidyi* is likely to be established in many regions of the Mediterranean Sea especially in areas where terrestrial nutrient input supports higher zooplankton biomass and regions which are strongly disturbance by human activity (Siapatis et al. 2008). *M. leidyi* was later found in many of the predicted areas including western parts of the Mediterranean Sea (e.g. Fuentes et al. 2010).

The first sighting of *M. leidyi* in northern Europe (Oslofjord, Skagerrak) dates back to 2005 (Oliveira 2007) and it was subsequently discovered that the invader was widely distributed in all Danish waters (Tendal et al. 2007), the south western Baltic Sea (i.e. Kiel Bight see Javidpour et al. 2006), the southern central Baltic (i.e. Bornholm Basin see Haslob et al. 2007; Huwer et al. 2008) as well as the North Sea (Faasse and Bayha 2006; Boersma et al. 2007; Van Ginderdeuren et al. 2012) (Fig. 3). Reports of *M. leidyi* occurring in the northern central Baltic Sea including the Gulf of Bothnia and Finland (Lehtiniemi et al. 2007; Lehtiniemi et al. 2008; Viitasalo et al. 2008) turned out to be a misidentifications and it was later confirmed to be an arctic relict species, namely *Mertensia ovum* (Gorokhova et al. 2009; Gorokhova and Lehtiniemi 2010). This highlighted the need for incorporating molecular tools in species verification especially for larval ctenophore stages (Gorokhova et al. 2009; Gorokhova and Lehtiniemi 2010).

Further, molecular analysis revealed that the *M. leidyi* populations in northern Europe originate from different regions along the east coats of the USA than the *M. leidyi* found in southern Europe (Black Sea including adjacent waters and the Mediterranean) (Reusch et al. 2010; Ghabooli et al. 2011). Animals found in northern Europe stem from the north east coast of the USA (Reusch et al. 2010; Ghabooli et al. 2011), where populations have been shown to survive cold winter temperatures (Costello et al. 2006). In contrast, *M. leidyi* present in southern Europe, originate from the Gulf of Mexico area (Reusch et al. 2010; Ghabooli et al. 2011), and a strong temperature dependency has been shown with water temperatures <4˚C being lethal for *M. leidyi* populations (Shiganova et al. 2001). This indicates that different invasion development and population dynamics are to be expected and that the two primary invasion events in northern and southern Europe can not directly be compared. This highlights the need for research effort to investigate the effect on local ecosystem scale in Northern Europe.

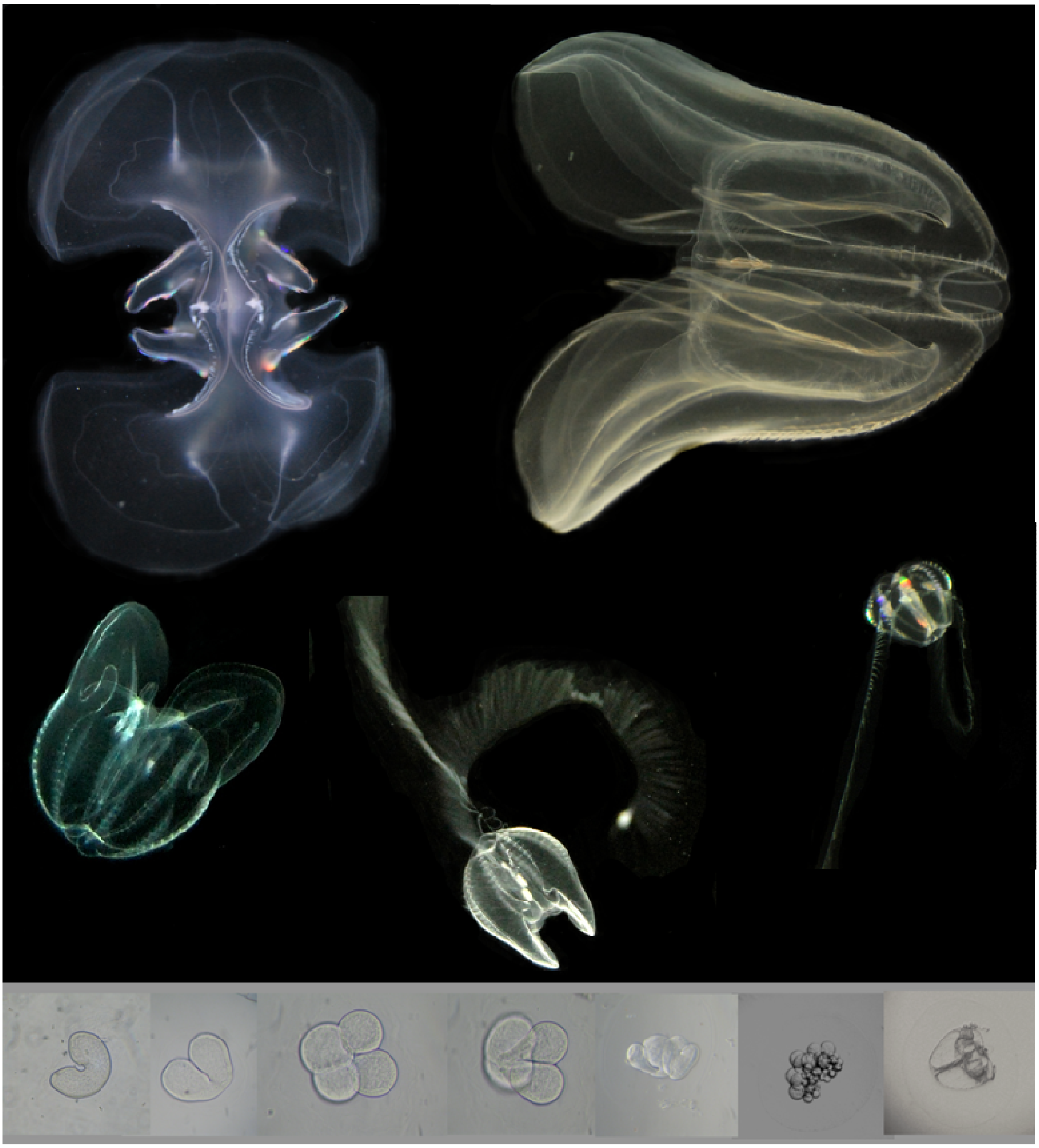

**Fig. 2.** Life stages of the comb jelly (Ctenophore) *Mnemiopsis leidyi* from eggs (development sequence, lower panel) which are produced from simultaneous hermaphrodites on a daily basis over larvae, which feed by use of tentacles to transitional stages (middle row) which are metamorphosing stages where tentacles get reduced and lobes and auricles develop until fully attained in the adult stage (upper row). Photographs taken from several generations of cultured *M. leidyi* kept at DTU Aqua, Charlottenlund, Denmark, C. Jaspers.

## 1.4 Baltic Zooplankton Cascades (BAZOOCA) Project

The invasion of *M. leidyi* into the Baltic Sea raised public and scientific concerns since the Baltic Sea is confronted with a similar cocktail of human stressors like eutrophication, over-fishing and climate change, which contributed to the severe regime shift coinciding with the invasion of *M. leidyi* in the Black Sea in the 1980's (Bilio and Niermann 2004). To understand the impact and detect possible cascading effects of *M. leidyi* on the food web structure and functioning of the Baltic Sea, the EU Bonus project BAZOOCA was funded and conducted during a 3 year period starting December 2008. The present thesis is one out of three PhD projects funded by BAZOOCA, which included 13 monitoring cruises in the Baltic Sea 2009/2010 (Fig. 3).

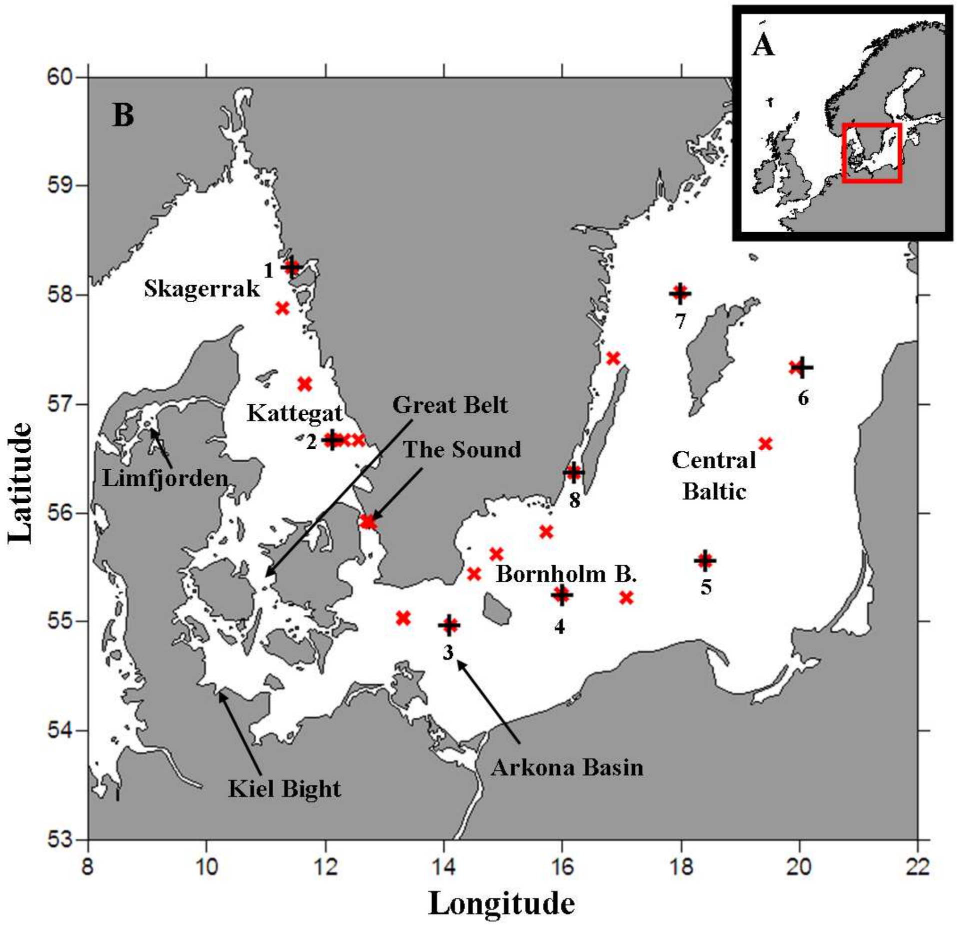

**Fig. 3.** Northern Europe (A) and the Baltic Sea (B) with locality names where *M. leidyi* has been sampled as part of the present PhD– monitoring program during 2009/2010 (monitoring stations 1-8 indicated by black cross and number, red stars indicate process cruise stations sampled once during peak abundance season in Oct. 2009).

## 2 Objectives of my PhD project

The aim of my PhD project was to understand the potential impact of the newly invaded *Mnemiopsis leidyi* on the Baltic Sea ecosystem and what factors might regulate its distribution. The approach was organized around answering the following questions:

**I. Does *Mnemiopsis leidyi* constitute a direct and/or indirect threat to the Baltic cod population?**

The introduction of *M. leidyi* in the Black Sea is regarded as having led to the collapse of the local Anchovy fish stocks. Therefore, we investigated direct and indirect effects of *M. leidyi* on the most important commercial Baltic fish species, namely the Eastern Baltic Cod. To address this, we first studied direct predation impact of *M. leidyi* on Baltic cod eggs and larvae by laboratory feeding experiments. Secondly, we considered competitive interactions from *M. leidyi* populations in the most important cod spawning ground by *in situ* and laboratory reproduction experiments.

**II. What factors govern the distribution range of *Mnemiopsis leidyi* and the recently discovered arctic relict *Mertensia ovum* ctenophore species in the Baltic Sea?**

To unravel possible source-sink relationships and constrains on the distribution of *M. leidyi* as well as the arctic relict ctenophore *M. ovum* in the Baltic Sea, we investigated environmental factors as well as life history traits affecting their seasonal ranges. To address this we analyzed spatial and temporal distributions of ctenophore adults, eggs and larvae in relation to the hydrography, backed-up by molecular tools for species identification for larval stages.

**III. What is the effect of *Mnemiopsis leidyi* reproduction on seasonal population development?**

Although *M. leidyi* is characterized by high feeding rates and large reproduction capacity, the contribution of fecundity to their range expansion in northern Europe is unknown. To address this, cohort experiments in the laboratory investigated the age at onset of reproduction, and resource allocation to growth versus egg production. The effect of food concentrations on reproduction rates was examined using food dependent egg production and starvation experiments *in situ* and in the laboratory.

# 3 Findings of my PhD

## 3.1 Does *Mnemiopsis leidyi* constitute a direct and/or indirect threat to the Baltic cod?

## (Paper I+II)

Since its invasion into the Baltic Sea in 2006, *Mnemiopsis leidyi* has been suspected of serious predation on the early life stages of eastern Baltic cod due to a temporal and spatial overlap (Haslob et al. 2007; Huwer et al. 2008) in the most important cod spawning ground, the Bornholm Basin (Köster et al. 2005). We conducted laboratory incubation experiments and video observations to quantify feeding rates on Baltic cod eggs and larvae (Paper I). Further, to assess indirect effects due to food competition, *in situ* egg production experiments were performed to assess the likely population size of *M. leidyi* in the low saline Bornholm Basin (Paper II).

**Results:** We show that *M. leidyi* preyed on cod larvae at low rates while feeding rates on cod egg were not significantly different from zero. When offered *Artemia salina* and cod eggs simultaneously, *M. leidyi* passively selected against cod eggs. Video recordings confirmed that eggs were not retained on the tentillae and if ingested, eggs were often regurgitated (88 %). Applying our clearance rates to *in situ* abundances of cod eggs, larvae and *M. leidyi* during the peak of the eastern Baltic Cod spawning season, we demonstrate that the predation pressure of the invasive ctenophore is negligible (Paper I).

We show by *in situ* reproduction experiments in different areas of the Baltic Sea and by laboratory controlled experiments, that reproduction rates are drastically reduced under low salinities characteristic for the Baltic Sea and the Bornholm Basin (Paper II).

**Conclusion:** The regurgitation of cod eggs and the observation that they were not retained on the tentillae demonstrate negligible predation on fish eggs for the environmental conditions characteristic for the Bornholm Basin. We conclude that *M. leidyi* constitutes no direct threat to the Baltic cod population under the current environmental conditions. We caution that our results should not be extrapolated to other regions of higher temperatures or with different fish species without supporting experimental studies. Due to the combined effect of temperature and salinity on reproduction rate, large abundances of *M. leidyi* are not expected for the Bornholm Basin, hence *M. leidyi* is not likely to constitute a threat, neither direct nor indirect to the Baltic Cod population.

**Fig. 4.** Development sequence of eastern Baltic Cod eggs and larvae at 7˚C. Photograph by C. Jaspers

## 3.2 What factors govern the distribution range of *Mnemiopsis leidyi* and the recently discovered arctic relict *Mertensia ovum* ctenophore species in the Baltic Sea?

**(Paper III+IV+V)**

After the *Mnemiopsis leidyi* invasion into northern Europe, much research was devoted to its range expansion. To date, monitoring activities have been regionally restricted, i.e. to the Bornholm Basin (Haslob et al. 2007; Huwer et al. 2008, Schaber et al. 2011a,b) and the SW Baltic Sea (Javidpour et al. 2006; Kube et al. 2007; Javidpour et al. 2009a,b), or included only a limited investigation period in for example the Danish Straits (Riisgård et al. 2010). No published data from the Skagerrak and Kattegat area are available and it is unknown how far *M. leidyi* has expanded into the northern central Baltic Sea. The aim of our monitoring investigation was to describe the seasonal distribution pattern of the invasive *M. leidyi* in its new habitat, to map the range expansion and couple the observed abundances with biotic and abiotic variables to investigate possible constrains on its dispersal. To further investigate active recruitment and understand the population dynamics with possible source-sink mechanisms of *M. leidyi* in the Baltic Sea, egg and larval stages were monitored (paper IV) along with adult abundances (paper V). Our investigation is the first to investigate a gradient from high saline Skagerrak/Kattegat areas to the low saline northern central Baltic Sea on a monthly basis (Fig. 3). This investigation was supplemented by a process oriented cruise during *M. leidyi* peak abundance season (October 2009) where the monitoring stations were supplemented by additional stations to achieve a higher resolution.

Due to the lack of incorporating molecular tools in earlier studies, the geographic range expansion of *M. leidyi* was mischaracterized due to confusion and misidentification with *Mertensia ovum* which has so far only been confirmed to be present in the northern most Baltic Sea regions (Gorokhova et al. 2009; Gorokhova and Lehtiniemi 2010). Here we address the range expansion and population dynamics of both ctenophore species covering the entire salinity range in the Baltic Sea through monitoring, molecular species identification and *in situ* reproduction experiments (paper III, IV).

**Results:** Adult *M. leidyi* showed highest abundances in high saline areas of the Kattegat and Skagerrak with population peak during October 2009. Though animals were sporadically observed in the southern Baltic Sea (Bornholm Basin) with a 3 month delay to the first observations in the Kattegat, their abundances were nearly two orders of magnitude lower compared to high saline areas. *M. leidyi* was never found in the low saline central Baltic Sea. The depth distribution and delay in its occurrence in the southern Baltic suggest drift recruitment from high saline areas as population source for the Bornholm Basin (Paper V). This has previously been hypothesized and substantiated by drift models for other jellyfish in the same area (Barz et al. 2006). Further, low juvenile to adult ratios of *M. leidyi* along with low larvae abundances suggest that active recruitment in the low saline areas such as the Bornholm Basin is low (Paper IV). Lower reproduction was substantiated by *in situ* reproduction rates in different areas of the Baltic, where reproduction rates in the southern Baltic Sea were nearly a factor of 10 lower compared to high saline areas of the Kattegat (Paper II). Temperature and food abundance could not explain the observed differences. Laboratory investigations confirmed a strong effect of salinity on reproduction rates (Paper II). Though adults were not found from April until June, *M. leidyi* larvae were present throughout the year in high saline areas as confirmed by molecular analysis (Paper IV).

We show for the first time that *Mertensia ovum* was not restricted to the northern and central Baltic Sea but also occurred in high saline areas during winter and spring (Paper IV). It was discovered that in the central Baltic Sea, the population exclusively consists of larval sized animals which are actively reproducing and maintaining a self-sustained population (Paper III).

**Conclusion:** Our investigation suggests that the annual range expansion of *M. leidyi* is seeded in high saline Skagerrak/Kattegat areas. Whether the population is sourced from recruiting larvae, adults overwintering close to the bottom, or drift recruitment from the warmer North Sea remains unclear. However, the population peak observed in October 2009 was seeded 2 month earlier, coinciding with the occurrence of largest sized adults and largest amount of observed ctenophore eggs. We conclude that salinity has a strong effect on the range expansion and population size structure of *M. leidyi* in the newly invaded Baltic Sea. Under the current environmental conditions and predation regime, a self-sustained population in the central Baltic Sea is not to be expected (unless *M. leidyi* adapts to the low salinity regime). Hence, source sink mechanisms as described for other native and invaded habitats seem to be important in population maintenance and dynamics of *M. leidyi* in the Baltic Sea. Another closely related ctenophores, *M. ovum*, has adapted to the regime of the Baltic Sea and recruits solely through larval reproduction (paedogenesis). Natural selection favors early maturation at a small size when mortality rates are high. Our observations are consistent with this and confirm the vital importance to include all life stages into assessments of population structure and dynamics; otherwise entire ctenophore populations may remain unrecognized or potential source regions and onset of population blooms remain unnoticed.

Additionally, the low abundances of *M. leidyi* in the southern Baltic Sea and their drift recruitment supports our conclusion from paper II that they are not a severe food competitor with fish recruits in the southern Baltic Sea.

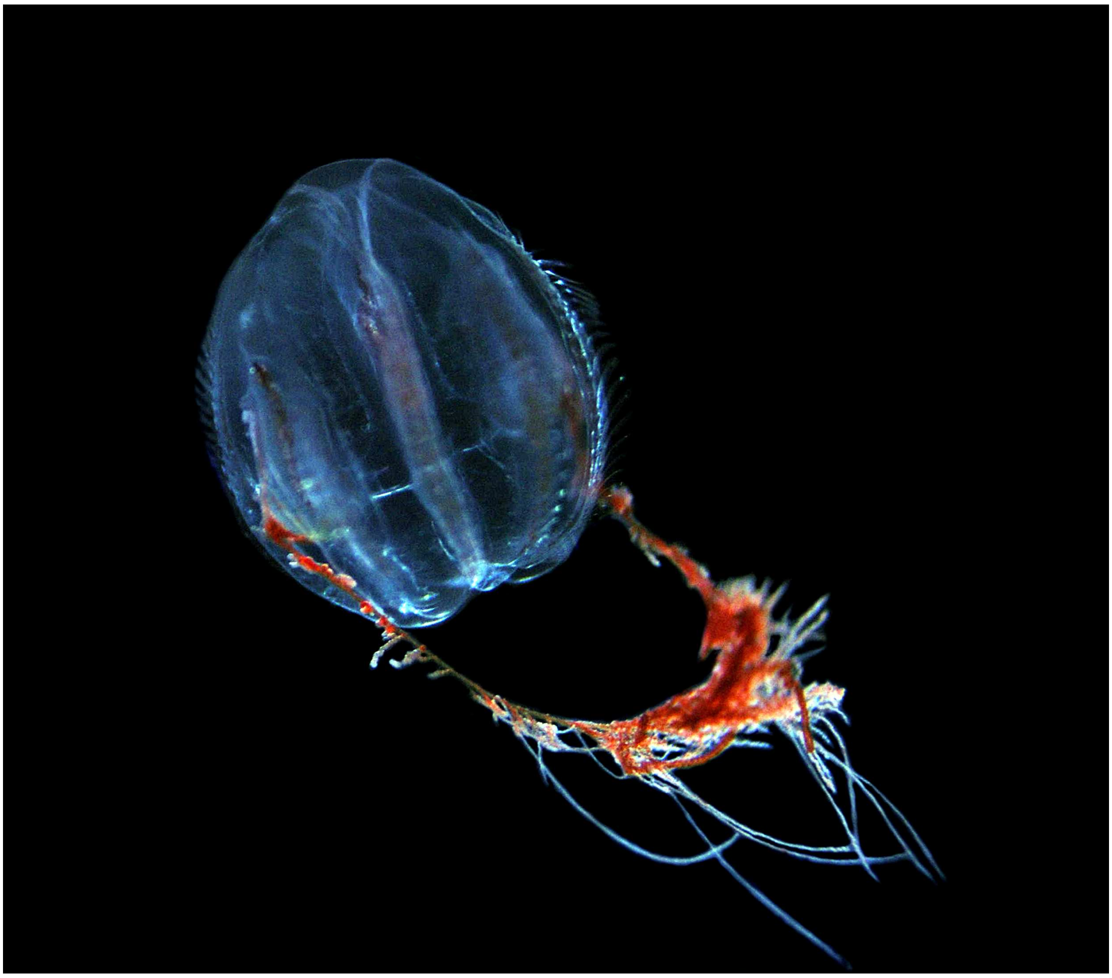

**Fig. 4.** *Mertensia ovum* from the Arctic Ocean. Photograph C. Jaspers.

## 3.3 What is the effect of *Mnemiopsis leidyi* reproduction on seasonal population development?

## (Paper VI)

Although *M. leidyi* is a simultaneous hermaphrodite, characterized by high feeding rates and large reproduction capacity, the contribution of fecundity to their range expansion in northern Europe has not yet been studied. Laboratory investigations regarding *M. leidyi* egg production experiments are sparse and so far it has been shown that egg production is very dependent on food conditions (Reeve et al. 1989). However, large ctenophore blooms with active recruitment (as estimated by the contribution of larval size classes to the total population) are commonly observed in semi-enclosed systems where food resources quickly get depleted (Riisgård et al. 2007; Javidpour et al. 2009a,b; Riisgård et al. 2010; Riisgård, Jaspers et al. submitted). Here we address the effect of food concentration on reproduction rates in *M. leidyi* in the laboratory and investigate the effect of starvation on reproduction and their size development. Additionally, cohort experiments in the laboratory were conducted to investigate the energy allocation of growth versus egg production.

**Results:** Laboratory investigations and fecundity experiments suggest that a key to the invasion success of *M. leidyi* is its high reproduction capacity (paper VI). Animals were shown to produce eggs on a daily basis with maximum egg production rates of field caught animals of 11,250 eggs *M. leidyi* $d^{-1}$ (paper VI). Further, half-saturation concentration for egg production was reached at low food concentrations of 10 and 23 µgC $L^{-1}$ for small- and large-sized *M. leidyi*, respectively. Cohort experiments show that energy allocation changes during growth with higher growth rates before lobe formation of 0.41 $d^{-1}$ and slower growth ca. 14 days after hatch with 0.08 $d^{-1}$ coinciding with metamorphosis and onset of permanent egg production. Similarly, *in situ* egg production rates from field caught animals during the onset of their population bloom suggest that egg production and size scale with 2 different slopes. Hence, while small animals allocate more energy to growth compared to egg production, adults allocate more energy to egg production than to growth. The change in this resource allocation is observed around 22 mm oral aboral length. Similarly, if we compare with size dependent egg production from our cohort experiment the same relationship is shown, but shifted to smaller size ranges. Hatching time at 16˚C is ca.

1-2 days with a hatching success of 60-90%. When comparing night to day time egg production, > 97.8 % of the eggs were produced during night (n=33942), i.e. between 7pm and 7am during natural light conditions. Though starved, egg production rates remained high for the first 24 hours and subsequently decreased. After 11 days of starvation, oral aboral length was decreased by ca. 30% and no egg production was detected.

**Conclusion:** Our results show that a key to the success of *M. leidyi* is its high egg production capacity which can remain high even though food resources become limited. Furthermore, in situations with food shortage, adults shrink and channel energy into egg production, which can be interpreted as a strategy to maximize fitness. Although egg production is not highly sensitive to food concentrations, food has a significant impact on reproduction rates and ceases after e.g. 11 days of starvation. The results reconcile why large population blooms are observed and maintained in localized areas where water exchange is limited and food resources get quickly depleted by the high grazing impact exhibited by *M. leidyi*. Another attribute which can explain the success of *M. leidyi* is that as a self-fertilizing hermaphrodite, it can offset the Allee effect which might limit sexually reproducing species during early phases of an invasion.

# 4 Perspectives of my work

Hydrographic regime, in particular salinity, plays an important role in explaining the invasion and observed range expansion of *M. leidyi* in the Baltic Sea. Salinity and its repercussions on reproduction appear to restrict the penetration of *M. leidyi* into the southern-central Baltic, where its existence is dependent on advective recruitment from higher saline source areas. This, in combination with low feeding rates on cod eggs and larvae, indicates that under the current regime, *M. leidyi* poses neither a direct nor an indirect threat to the Baltic cod population within its most important spawning ground, the Bornholm Basin. Apart from its large predation potential (Colin et al. 2010), a key to *M. leidyi*'s invasion success is its high egg production capacity with maximum egg production rates reached at relatively low food concentrations. This might explain why large population densities and active recruitment are observed especially in fjord systems or semi-enclosed embayments, even though food conditions are low (Riisgård et al. 2007; Javidpour et al. 2009a,b; Riisgård et al. 2010).

Although the overwintering biology of *M. leidyi* remains unknown, we show that larvae were present in high saline Baltic Sea areas year round. Thus overwintering larvae are a potential source, seeding the population of large sized adults which were first observed to occur in high saline areas during summer. These large sized adults have a high reproduction capacity which was confirmed by large numbers of ctenophore eggs observed in the high saline Skagerrak during August. Although egg mortality rates were estimated to be high in these areas, it is suggested that the annual population expansion spreads from high to low saline regions in the Baltic Sea. We can not exclude that local self-sustained populations of *M. leidyi* exist in the SW Baltic Sea where salinities are relatively high, compared to the southern central Baltic, allowing for lower but active recruitment, e.g. Kiel Bight (Javidpour et al. 2009; Javidpour et al. 2009). However, the disappearance of *M. leidyi* from the Baltic Sea as witnessed in spring, summer and autumn of 2011 and its first re-occurrence in December 2011 (Riisgård, Friis-Møller, Jaspers pers. comm.) hints to the local extinction of these sub-populations in the Baltic Sea and subsequent re-colonization. Such a re-introduction may stem from Limfjorden, which connects the

North Sea to the Kattegat and where high abundances (180 *M. leidyi* $m^{-3}$) were recorded in November 2011 (Riisgård, Jaspers et al. in review).

The North Sea is generally warmer and therefore could be the source of a *M. leidyi* re-colonization into the Baltic Sea, especially after several severe ice winters in a row as was observed in 2009, 2010 and 2011. This has recently been substantiated by the year round observation of *M. leidyi* occurrence in coastal North Sea waters, irrespective of overall cold winter conditions in northern Europe (Van Ginderdeuren et al. 2012, van Walraven pers. comm.). However, molecular analysis suggests that the invasion of the North Sea originates from the Baltic Sea (Reusch et al. 2010) and not *vice versa*. This apparent contradiction suggests that further research effort should be devoted to the winter biology of *M. leidyi* since the winter survival appears to be a bottleneck for population establishment in Northern Europe – especially so during large climate variability as recently observed. Irregardless, we can conclude that *M. leidyi* has established permanently in Northern Europe.

Like most studies, enough has been learned to make suggestions for improvement. Future investigations should include video plankton recording systems since *M. leidyi* has been shown to accumulate in dense layers close to the see floor (Costello and Mianzan 2003) and at such times would be undersampled or even missed by conventional methods. In particular, population estimates during low-abundance winter seasons may be heavily biased if these bottom accumulations are not assessed. Furthermore, monitoring activities in the Baltic Sea should include high saline areas such as the Kattegat and Skagerrak. Due to the documented salinity-dependent reproduction capacity of *M. leidyi*, these areas are likely to support high abundances which can lead to a potential food competition with fish.

So far, the actual contribution and quantitative linkage of ctenophores in the food web is largely unknown. Ctenophores can constitute an essential part of the diet of certain fish species (Mianzan et al. 1996) and might constitute a carbon link to higher trophic levels rather than a carbon sink. This is exemplified by the study of *Mertensia ovum* in the central Baltic which suggests a heavy predation control of the population, although the predators remain unknown. I suggest predator-prey investigations with fish in the laboratory in combination with gut content and quantitative PCR methods applied in the field in order to illuminate the position and linkages of ctenophores in the food web. This is essential for incorporating this new invader in particular, and ctenophores and jellyfish in general, into food web models. Ecosystem models of the Baltic Sea remain incomplete if we fail to incorporate these gelatinous plankton groups.

On a more general note, the paradigm of a more gelatinous future due to global change remains speculative, but several factors hint towards this scenario due to the generally better adaptability of gelatinous organisms to the anticipated changes (e.g. Richardson et al. 2009; Purcell 2012). Thus far, such observations have emphasized mostly the large scyphozoans routinely sampled during fisheries surveys. Ironically, if our current ignorance of other gelatinous plankton is maintained, we may not even notice that these changes are taking place as systematic long term data are sparse.

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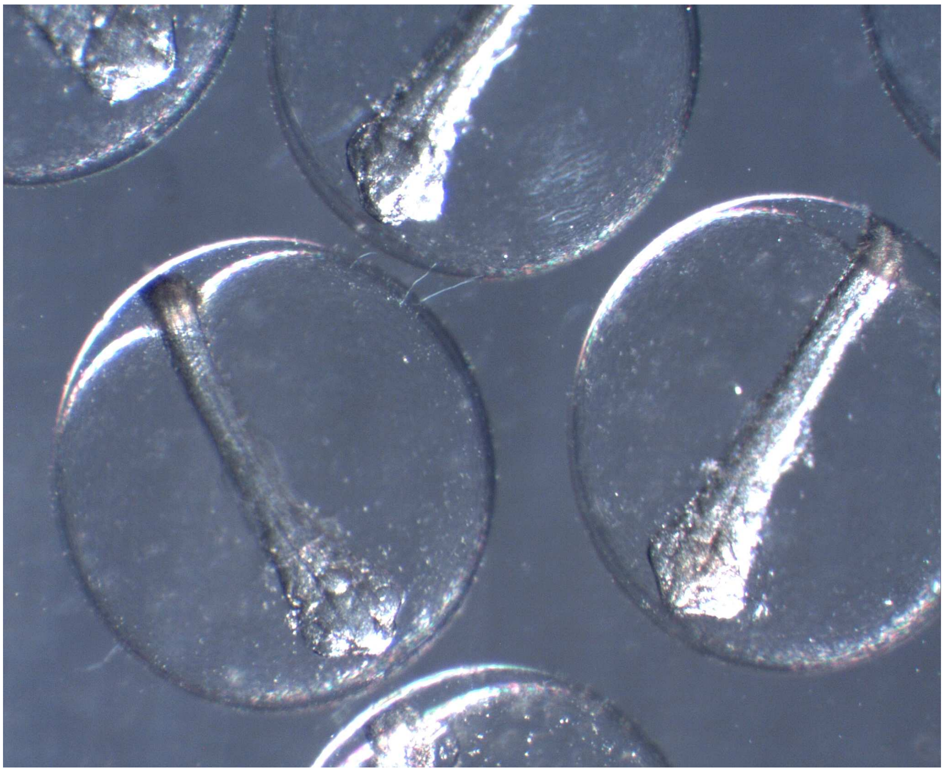
# Paper I



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# The invasive ctenophore *Mnemiopsis leidyi* poses no direct threat to Baltic cod eggs and larvae

Cornelia Jaspers,[a,*] Josefin Titelman,[b] Lars Johan Hansson,[c] Matilda Haraldsson,[d] and Christine Røllike Ditlefsen[d]

[a] National Institute of Aquatic Resources, Technical University of Denmark, Charlottenlund, Denmark
[b] University of Oslo, Department of Biology, Blindern, Oslo, Norway
[c] Department of Marine Ecology–Kristineberg, University of Gothenburg, Fiskebäckskil, Sweden
[d] Department of Marine Ecology–Göteborg, University of Gothenburg, Gothenburg, Sweden

## *Abstract*

Since its invasion in to the Baltic Sea in 2006, the ctenophore *Mnemiopsis leidyi* has been suspected of serious predation on the early life stages of Baltic cod (*Gadus morhua callarias* L.) due to a temporal and spatial overlap in the most important cod spawning ground, the Bornholm Basin. We conducted laboratory incubation experiments and video observations to quantify feeding rates on Baltic cod eggs and larvae. Ingestion rates increased with cod larvae concentrations up to 8 prey $L^{-1}$, beyond which ingestion remained constant. Neither *Mnemiopsis* size nor egg concentration (1–16 prey $L^{-1}$) affected feeding rates on cod eggs. Observed feeding rates pooled from all experiments conducted at nonsaturating prey concentrations were low, with the highest volume-specific clearance on $<$ 4.5-d-old yolk-sac larvae ($0.05 \pm 0.02$ L (mL *Mnemiopsis*)$^{-1}$ $h^{-1}$), and lower rates on 4.5–8-d-old larvae ($0.02 \pm 0.02$ L (mL *Mnemiopsis*)$^{-1}$ $h^{-1}$) and eggs ($0.02 \pm 0.03$ L (mL *Mnemiopsis*)$^{-1}$ $h^{-1}$). When offered *Artemia salina* and cod eggs simultaneously, *Mnemiopsis* passively selected against cod eggs. Video recordings showed that eggs did not trigger the capture response that *Mnemiopsis* shows toward motile prey, and ingested eggs were often ejected (88%, $n = 8$). Applying our clearance rates to in situ abundances of cod eggs, larvae, and *Mnemiopsis* for the peak of the spawning season, we demonstrate that the predation pressure of the invasive ctenophore is negligible. We conclude that *Mnemiopsis* constitutes no direct threat to the Baltic cod population.

During recent decades invasive species have become a major concern due to their direct and cascading effects on marine ecosystems and biodiversity (Carlton and Geller 1993; Graham and Bayha 2007; Molnar et al. 2008). Particularly, the collapse of fisheries that coincided with the invasion of the ctenophore *Mnemiopsis leidyi* in the Black Sea raised major scientific and public attention (Shiganova and Bulgakova 2000; Kideys 2002; Oguz et al. 2008). Deleterious effects of *Mnemiopsis* include predation on eggs and early life stages of fish (Monteleone and Duguay 1988; Cowan et al. 1992; Purcell et al. 1994) as well as competition for food (Purcell 1985; Mills 1995; Bilio and Niermann 2004).

*Mnemiopsis leidyi* (A. Agassiz, 1865) was first sighted in Northern Europe in 2005 (Oliveira 2007) and has since spread rapidly into the Baltic Sea (Javidpour et al. 2006; Huwer et al. 2008) and the southern North Sea (Boersma et al. 2007). In the Baltic Sea, *Mnemiopsis* overlaps spatially and temporally with Baltic cod (*Gadus morhua callarias* L., 1758), especially in the most important nursery and spawning ground, the Bornholm Basin (Haslob et al. 2007; Huwer et al. 2008). Qualitative observations from the Bornholm Basin have shown *Mnemiopsis* with fish eggs in their guts (Haslob et al. 2007). This has raised serious concern that the invader may decrease local fish stocks and fishery revenues, especially those of Baltic cod (Haslob et al. 2007; Huwer et al. 2008; Storr-Paulsen and Huwer 2008). However, the direct predation rates of *Mnemiopsis* on Baltic cod have not yet been quantified.

Here we measured the predation rate of *Mnemiopsis* on the early life stages of Baltic cod at the low salinities and temperatures representative of the most important cod spawning area in the Baltic. We applied detailed video observations of the predator–prey interactions to explain observed feeding rates.

## Methods

All experiments were conducted at the accredited fish hatchery 'Fonden Bornholm Lakseklækkeri' in Nexø on the island of Bornholm, central Baltic Sea, during late April 2009, matching the physical environment of the central Bornholm Basin spawning area of Baltic cod (7°C, 1013 g $L^{-1}$; Köster et al. 2005).

*Experimental animals*—*Mnemiopsis leidyi* were raised from laboratory-cultured eggs (20°C, 1023 g $L^{-1}$) from ctenophores originating from the Eastern Skagerrak, southwestern Swedish coast (58°15′N, 11°24′E). Two weeks prior to the experiments the ctenophores were gradually acclimatized to the less saline experimental conditions by successive additions of distilled water to the cultures. *Mnemiopsis* were fed with 1-d-old *Artemia* nauplii reared from cysts, and starved 24 h before the start of experiments and used only once.

Brood stock cod at the fish hatchery 'Fonden Bornholm Lakseklækkeri' originated from the Eastern Baltic spawning grounds. Cod eggs and yolk-sac larvae from several different spawning events were supplied by the hatchery. Eggs were provided within 6 h of spawning, and kept in

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[*] Corresponding author: coja@aqua.dtu.dk

Table 1. Summary of experimental conditions of *Mnemiopsis leidyi* feeding on Baltic cod eggs and larvae (7°C, 1013 g L$^{-1}$). Data are mean ± SD. Prey ages are in days from spawning for eggs and days posthatch for larvae, corrected for average incubation times. $L_{\text{o–a}}$ is oral–aboral length. Four experiments were conducted: (A) functional response (FR), (B) prey selection of cod eggs vs. *Artemia salina* (select), (C) clearance as a function of prey age (age), and (D) size-dependent clearance rate (size). Data were pooled and included in subsequent analyses as indicated by superscripts A–D in the replicates column. Controls without predators were performed in parallel with experiments A–D (overall prey loss due to handling: eggs 0.00%, $n$ = 14; larvae 0.14%, $n$ = 13).

| Experiment | Prey | Prey conc. (L$^{-1}$) | Prey age (d) | Prey length (mm) | Predator $L_{\text{o–a}}$ (mm) | Replicates ($n$) | Duration (h) |
|---|---|---|---|---|---|---|---|
| A-FR | eggs | 1 | 1.7±0.6 | 1.58±0.04($n$=53) | 12±1.7 | $3^{C,D}$ | 7.8±1.8 |
| | | 2 | 1.7±0.6 | 1.58±0.04($n$=53) | 12±1.7 | $2^{C,D}$ | 7.8±1.8 |
| | | 4 | 1.7±0.6 | 1.58±0.04($n$=53) | 12±1.7 | $2^{C,D}$ | 7.8±1.8 |
| | | 8 | 1.7±0.6 | 1.58±0.04($n$=53) | 12±1.7 | $3^{C,D}$ | 7.8±1.8 |
| | | 16 | 1.7±0.6 | 1.58±0.04($n$=53) | 12±1.7 | 5 | 7.8±1.8 |
| | larvae | 2 | 4.3 | 4.3±0.4($n$=37) | 12.5±0.7* | $2^{C,D}$ | 5.2±0.3 |
| | | 4 | 4.3 | 4.3±0.4($n$=37) | 12.5±0.7* | $2^{C,D}$ | 5.2±0.3 |
| | | 8 | 4.3 | 4.3±0.4($n$=37) | 12.5±0.7* | $2^{C,D}$ | 5.2±0.3 |
| | | 16 | 4.3 | 4.3±0.4($n$=37) | 12.5±0.7* | 2 | 5.2±0.3 |
| B-select | eggs and | 4 | 0.9 | 1.54±0.03($n$=36) | 13.5±1.7 | $9^{C,D}$ | 16.8±0.5 |
| | *Artemia* | 4 | 1 | 0.92±0.09($n$=34) | 13.5±1.7 | $9^{C,D}$ | 16.8±0.5 |
| C-age | eggs | 4 | 3 | 1.56±0.02($n$=34) | 12±1.0 | $5^{D}$ | 16±1.2 |
| | | 4 | 4.8 | 1.54±0.02($n$=23) | 12.4±1.1 | $5^{D}$ | 16.8±0.2 |
| | | 4 | 5.9 | 1.58±0.01($n$=16) | 11.7±1.8 | $5^{D}$ | 17.8±0.3 |
| | | 4 | 7.5 | 1.55±0.02($n$=22) | 11.5±2.6 | $5^{D}$ | 9.3±0.4 |
| | | 4 | 8.5 | 1.56±0.03($n$=26) | 11.1±0.6 | $4^{D}$ | 11.7±0.2 |
| | | 4 | 11.5 | 1.57±0.06($n$=63) | 10.3±0.7 | $4^{D}$ | 11.9±0.2 |
| | larvae | 4 | 0–0.5 | 3.7($n$=25)† | 10.5±1.0 | $4^{D}$ | 10.3±0.1 |
| | | 4 | 3.3 | 4.2±0.4($n$=17) | 11.5±1.5 | $5^{D,‡}$ | 16.9±0.2 |
| | | 4 | 6.5 | 4.6±0.3($n$=37) | 16.8±5.5 | $10^{D}$ | 8.7±0.3 |
| | | 4 | 8 | 4.8±0.4($n$=35) | 11.5±2.0 | $5^{D}$ | 11.5±0.3 |
| D-size | eggs | 4 | 2.4 | 1.54±0.01($n$=12) | 15.2±6.1 | $15^{C}$ | 5.6±0.5 |
| | larvae | 4 | 5.9 | 4.3±0.3($n$=38) | 7.2±2.5* | $7^{C}$ | 15.2±0.5 |
| | | 4 | 5.4 | No data | 20.9±3.8 | $10^{C}$ | 7.8±0.6 |

* 2–8 predators bottle$^{-1}$.

† Data from Petereit (2004), no SD given.

‡ Four clearance rates included in FR analysis A (4 prey L$^{-1}$; size 12.4±0.8 mm; 9-mm observation excluded).

separate flow-through systems (7°C, 1013 g L$^{-1}$) in artificial seawater (Instant Ocean®) from the hatchery circulation system (10-$\mu$m–filtered and ultraviolet-light–treated). Cod larvae were not fed, and cohorts were kept separately.

*Incubation experiments*—We conducted four series of experiments targeting (A) the functional response to prey concentrations (conc.) for *Mnemiopsis* feeding on cod eggs and larvae, (B) potential prey selection, (C) the effect of prey age and, (D) the effects of predator size on the predation rate. The details of the experimental conditions are given in Table 1, while the general experimental protocol is outlined below. When applicable, data from the various experiments were also used in other analyses (Table 1).

Incubations were conducted in wide-mouthed 13.5-liter Nalgene® polycarbonate bottles filled with water from the hatchery system. *Mnemiopsis* were added to each bottle, and allowed to acclimatize until they had fully expanded their lobes. *Mnemiopsis* were incubated individually (in 101 out of 116 bottles) unless otherwise indicated (Table 1).

Cod egg incubations were started with the subsequent addition of prey. In experiments with larvae, the prey was added first and experiments started with the addition of *Mnemiopsis*. They fully expanded their lobes within 2–5 min, corresponding to < 1.7% of the total incubation time. All prey were picked individually; eggs under a stereomicroscope and larvae by eye. Animals were kept submerged at all times to ensure that both predators and prey were in good condition.

Bottles were topped, sealed with household film and lids and incubated on a rolling table. The bottles rotated around their longitudinal axis at 0.9 rounds min$^{-1}$. Based on previous, unpublished feeding measurements, incubation time (5–18 h) and number of predators were set to an expected clearance of 30% of the bottle volume. Experiments were run in darkness to avoid confounding effects of light on larval behavior (Grønkjær and Wienand 1997; Skajaa et al. 2003; Titelman and Hansson 2006). Incubations were terminated by removing the predators; thereafter, remaining prey were enumerated. Cod eggs were concentrated by reverse filtration using a 20-$\mu$m Nitex® plankton gauze filter before enumeration. Larvae were not concentrated, but individually counted by eye and removed from the total volume with a white spoon, which offered good contrast to the larvae. Simultaneously incubated controls without predators showed negligible prey losses for both eggs (0.00%, $n$ = 14) and larvae (0.14%, $n$ = 13) and were, therefore, not corrected for.

After each incubation the *Mnemiopsis* were measured (oral–aboral length, $L_{\text{o–a}}$) to the nearest mm and checked for number of ingested prey. Developmental stages and sizes of cod eggs and larvae in the cohorts were assessed on a daily basis. Images at 6–50× magnification were captured by a Leica DFC290 camera and sizes determined using the software ImageJ (version 1.43n; Rasband 1997–2009).

Ingestion rates ($I$, prey individual$^{-1}$ h$^{-1}$) were calculated from differences between initial ($C_i$) and final ($C_f$) prey conc. (L$^{-1}$) as a function of time. Clearance rate ($F$, L ind.$^{-1}$ h$^{-1}$) was estimated from experimental observations of prey disappearance over time,

$$F = \left(\frac{V}{n \times t}\right) \times \ln\left(\frac{C_i}{C_f}\right) \tag{1}$$

where $V$ is bottle volume (L), $n$ is number of predators, and $t$ is duration of the incubation (h).

*Functional response (A)*—To ensure that subsequent experiments were conducted at nonsaturating prey concentrations (securing maximum clearance rates and no handling limitation) we first conducted functional response experiments on both eggs and larvae using predators of similar size (12 ± 1.7 mm and 12.5 ± 0.7 mm, respectively). Prey concentrations ranged from 1 L$^{-1}$ and 2 L$^{-1}$ to 16 L$^{-1}$ for eggs and larvae, respectively (Table 1).

*Prey selection (B)*—In prey selection experiments we offered 1-d-old *Artemia salina* in combination with freshly spawned cod eggs at the same concentrations (Table 1). Because the swimming and escape abilities of both these prey types are minimal, we expect similar encounter rates. Thus, for no postencounter prey selection we expected $F_{\text{cod egg}} = F_{Artemia}$.

*Effect of prey age (C)*—Clearance as a function of prey age was examined using prey from several cohorts, as well as utilizing clearance measurements conducted at nonsaturating food concentrations from other experiments (Table 1). To ensure comparable data, all clearance rates were standardized to *Mnemiopsis* volume (L [mL *Mnemiopsis*]$^{-1}$ h$^{-1}$). $L_{\text{o–a}}$ (mm) was converted to volume ($V$, mL) using the empirically determined relation for *Mnemiopsis* in Limfjorden, Denmark (Riisgaard et al. 2007):

$$V = 0.0226 \times L_{\text{o–a}}^{1.72} \tag{2}$$

*Effect of predator size (D)*—Size-dependent clearance experiments on both eggs and larvae were conducted by using different-sized predators, and different prey cohorts, at a prey concentration of 4 L$^{-1}$. The data set was supplemented with clearance rates from other experiments for nonsaturated prey concentrations (1–8 L$^{-1}$), such that the size range in the entire analysis became 4.5 mm to 26 mm $L_{\text{o–a}}$ ($n$ = 62 for eggs and $n$ = 47 for larvae; Table 1).

*Behavioral observations*—To qualitatively assess predator behavior upon prey encounter, silhouette video recordings in darkness were conducted with a black and white analog camera in 1-liter or 8-liter aquaria. Collimated light was provided by an infrared diode shining through a condenser lens and pointing toward the camera. The behavior of free-swimming *Mnemiopsis* with cod eggs at very high prey concentrations or with cod larvae were monitored. *Mnemiopsis* generally captures nonmotile prey that have been entrained in the feeding current on the tentillae. In contrast, motile prey, such as copepods, elicit a rapid closure of the lobes and such prey are primarily captured on the inner surfaces of the lobes (Waggett and Costello 1999). Both the fraction of lobe closing responses by *Mnemiopsis* and the fraction of prey retained on the lobes or tentillae after prey touch were measured.

Three *Mnemiopsis* that had fed on cod eggs during the incubation experiments were subsequently followed for several days to investigate the fate of ingested cod eggs and to estimate digestion times. The animals were placed individually in 2-liter aquaria at 7°C and observed during the digestion or ejection process for up to 3 d. To investigate whether cod eggs could be digested at higher temperatures, similar to those at which experiments on anchovies have been conducted (i.e., ~ 22°C; Cowan and Houde 1993), one *Mnemiopsis* was monitored in a 2-liter aquarium at 22°C for several hours subsequent to feeding, and photographed at regular intervals.

*Statistical analyses*—Statistical analyses were conducted in GraphPad Prism 4.0 and Table Curve 2 dimensional 5.0 with all curve fits and associated significance tests. Plots were generated in Sigma plot 10.0. Functional response experiments (A) were analyzed using linear regressions on nonaveraged raw data in the nonsaturating part of the functional response curves, where the slope proxies the maximum clearance rate. A separate slopes model was used to test for differences between slopes. We used a paired 2-tailed $t$-test to test for differences in clearance rates on *Artemia* and cod eggs in the selection experiment (B). The effect of larval age on volume-specific clearance rate in experiment C was tested with an ANOVA, and associated Newman–Keuls multiple-comparison post hoc test. Size-dependent clearance (experiment D) was analyzed with power regression analyses on raw data. Differences between regression parameters were tested using separate slopes models (covariance analyses) on log (x + 1)-transformed data. Control treatments without predators were performed and showed negligible prey loss for both cod eggs (0.00%, $n$ = 14) and cod larvae (0.14%, $n$ = 13).

## Results

Overall, our results showed that *Mnemiopsis* fed on cod larvae at low rates, while feeding rates on eggs were extremely low and often zero. Across all incubation experiments, *Mnemiopsis* ingested no prey at all in 40% of the incubations with eggs, compared to 15% when larvae were offered. *Artemia* were ingested in 100% of the incubations in which they were offered.

*Functional response (A)*—The functional response experiment revealed significant differences between ingestion

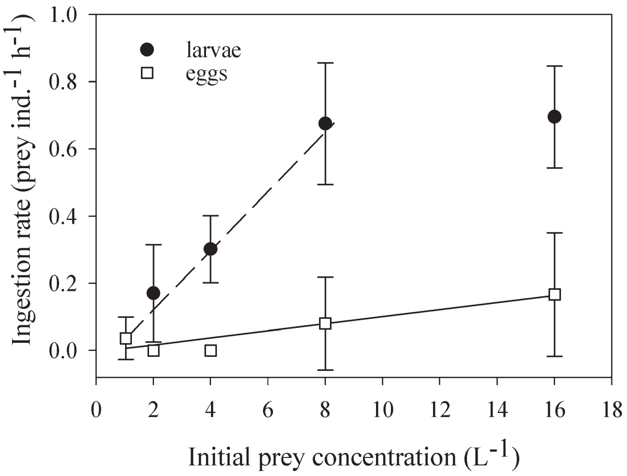

Fig. 1. Functional response of *Mnemiopsis* feeding on Baltic cod eggs and larvae (experiment A; Table 1). Each data point is the mean ± standard deviation for $n = 2–6$. The regressions for nonsaturating food concentrations using nonaveraged data are $I = 0.088c - 0.056$ ($r^2 = 0.68$, $p = 0.0035$, $n = 10$) for larvae and $I = 0.01c - 0.004$ ($r^2 = 0.25$, $p = 0.058$, $n = 15$) for eggs as prey. The slopes differ from one another (separate slopes model, $F_{21} = 13.78$, $p = 0.0013$).

of eggs and larvae (Fig. 1). Ingestion rates of cod eggs were very low at all prey concentrations with no signs of saturation (Fig. 1), generating an average clearance rate of 0.01 ± 0.03 L ind.$^{-1}$ h$^{-1}$. The slope of the linear regression of cod egg ingestion rate vs. concentration was not significantly different from 0 ($F_{13} = 4.317$, $p = 0.06$). Feeding rates on larvae were higher, and increased with increasing prey concentrations up to 8 L$^{-1}$, with no further increase at the highest prey concentration tested (16 L$^{-1}$), yielding an average maximum clearance rate of 0.088 ± 0.02 L ind.$^{-1}$ h$^{-1}$ at nonsaturating prey concentrations (Fig. 1).

*Prey selection (B)*—In the prey selection experiments all *Mnemiopsis* were actively filtering as indicated by their feeding on *Artemia* (0.114 ± 0.03 L ind.$^{-1}$ h$^{-1}$; Fig. 2). Simultaneous clearance on eggs was 16 times lower than clearance on *Artemia*, but similar to rates obtained in the functional response experiment for cod eggs (Figs. 1, 2). The differing clearance rates on the two prey types clearly demonstrated that *Mnemiopsis* passively selected *Artemia* nauplii over cod eggs (Fig. 2).

*Effect of prey age (C)*—There was no significant effect of egg age on the rate at which they were cleared (ANOVA: $F_{8,53} = 1.74$, $p = 0.11$) and data were, therefore, pooled. The pooled overall average volume-specific clearance on cod eggs was 0.02 ± 0.03 L (mL *Mnemiopsis*)$^{-1}$ h$^{-1}$ (Fig. 3). Larval age affected clearance rates, with higher clearance on < 4.5-d-old yolk-sac larvae (0.05 ± 0.02 L [mL *Mnemiopsis*]$^{-1}$ h$^{-1}$) compared to 4.5–8-d-old larvae (0.02 ± 0.02 L [mL *Mnemiopsis*]$^{-1}$ h$^{-1}$; Fig. 3). Feeding rates on cod eggs and 4.5–8-d-old larvae were similar (ANOVA: $F_{4,89} = 0.75$, $p = 0.56$; Fig. 3). Generally, mean clearance rates on cod larvae were lower than those on *Artemia* (Fig. 3).

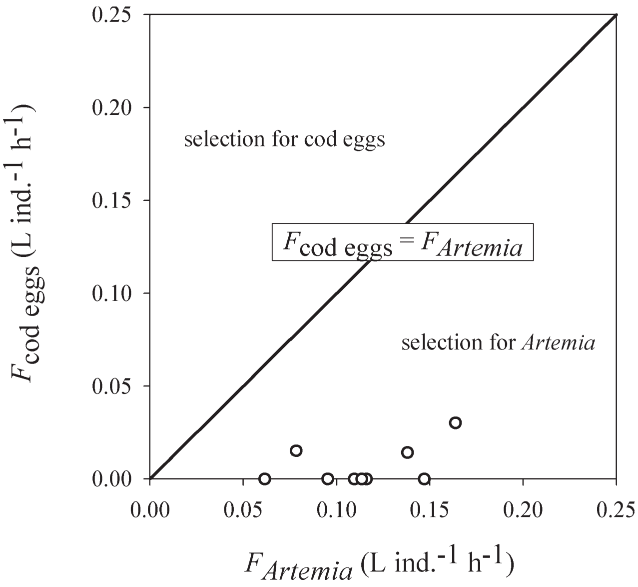

Fig. 2. Clearance rate of individual *Mnemiopsis* (13.5 ± 1.7 mm) on cod eggs and *Artemia* offered simultaneously (experiment B; Table 1). The straight line indicates the predicted equal clearance rates on cod eggs and *Artemia*; data above the line indicate selection for cod eggs, while data below the line indicate selection for *Artemia*. Mean clearance on *Artemia* was 0.114 ± 0.03 L ind.$^{-1}$ h$^{-1}$ while 0.007 ± 0.01 L ind.$^{-1}$ h$^{-1}$ on cod eggs. A 2-tailed paired *t*-test confirmed the different clearance rates on the two prey types (i.e., negative selection for cod eggs [$t = 10.99$, $p < 0.00001$, df = 8]).

*Effect of predator size (D)*—Because larval age affects clearance rates (Fig. 3) we separated the predator-size–dependent clearance-rate observations into clearance on young (< 4.5 d posthatching) and older (4.5–8 d posthatching) cod larvae (Fig. 4A). The narrow predator size range (9–13.3 mm, $n = 15$) in incubations with the younger larvae did not allow for testing for size dependency. For older larvae, however, clearance rates increased with predator size to a power of 1.74 ± 0.51 (Fig. 4A). Given that predator volume scales with length to a power of 1.72 (Riisgaard et al. 2007), clearance rates on cod larvae scale almost perfectly isometrically with predator volume. Thus, the use of predator volume-normalized clearance rates removes any effect of predator size and is adequate for comparison of clearance rates among differently sized *Mnemiopsis*. When feeding on cod eggs the size scaling was only to a power of 0.6, but not significant (Fig. 4B).

*Behavioral observations*—Nonmotile cod eggs rarely triggered a lobe-closing response in *Mnemiopsis*, even when clearly touching the capture sites (*see* Web Appendix, www.aslo.org/lo/toc/vol_56/issue_2/0431a.wmv). *Mnemiopsis* did not react to cod egg encounters in > 95% of the video observations ($n = 63$ for 7 *Mnemiopsis* $L_{\text{o–a}} = 12.0$–16.2 mm). *Mnemiopsis* reacted more often with lobe closing to fish larvae (20.0%, $n = 70$ for six *Mnemiopsis* $L_{\text{o–a}} =$

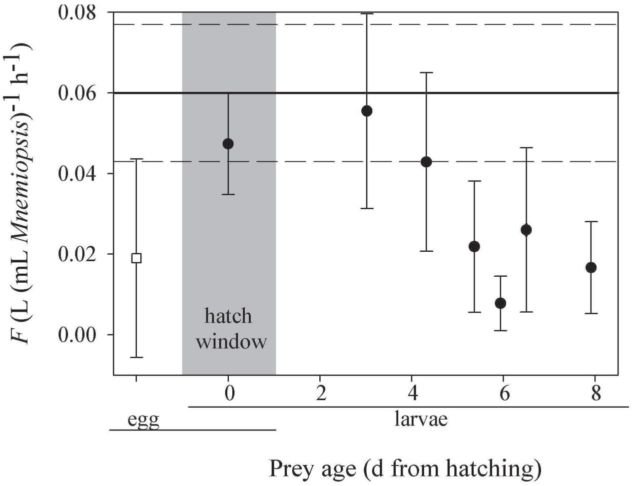

Fig. 3. *Mnemiopsis* volume-specific clearance rate as a function of prey age for data obtained at nonsaturating food concentrations (experiment C; Table 1). Rates on eggs were pooled from experiments A, B, and D ($n$ = 62). Clearance on larvae varied with larval age (ANOVA: $F_{6,40} = 5.752$, $p = 0.0002$), forming two clusters of < 4.5-d-old and 4.5–8-d-old larvae (Newman–Keuls post hoc test, $p > 0.05$). At 7°C Baltic cod eggs hatch within 12 ± 1 d postfertilization and the grey box indicates this hatch window. The solid (mean) and dashed (± SD) lines indicate volume-specific clearance on *Artemia* from experiment B.

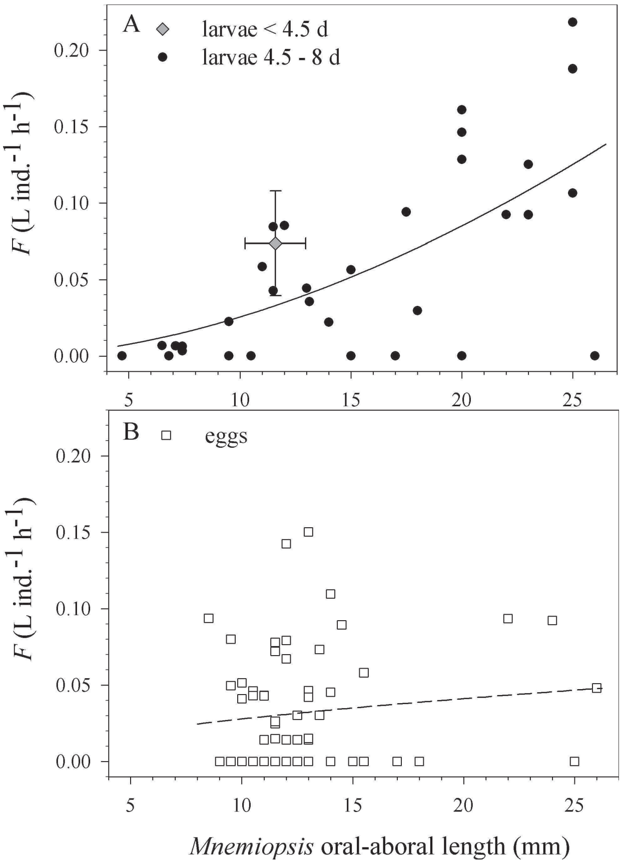

Fig. 4. *Mnemiopsis* clearance rate on (A) cod larvae, and (B) eggs as a function of size (experiment D; Table 1). (A) $F_{\text{larvae}} = 0.0005 \times L_{\text{o–a}}^{1.74}$ for $L_{\text{o–a}}$ of 4.5–26 mm ($r^2 = 0.43$, $p < 0.0001$, $n = 32$). The exponent (1.74 ± 0.51) differs from 0 ($p < 0.0001$). Data for young larvae (< 4.5 d) are mean ± SD ($n$ = 15) and excluded from the regression. (B) $F_{\text{egg}} = 0.008 \times L_{\text{o–a}}^{0.6}$ ($r^2 = 0.01$, $p = 0.389$, $n = 62$), the exponent 0.6 ± 0.6 did not differ from 0 ($p = 0.42$).

12.7–17.7 mm). More surprisingly, the cod eggs were not retained on the inner lobes or the tentillae after contact (0%, $n = 63$). We observed the ingestion of eight fish larvae out of 70 contacts (11.4%) for six *Mnemiopsis*. Three *Mnemiopsis* that had fed on cod eggs during the incubation experiments actively ejected seven out of eight eggs and the regurgitation process lasted on the order of 3 d at 7°C.

To investigate whether cod eggs could be digested at higher temperatures, *Mnemiopsis* were followed for several hours subsequent to feeding at 22°C (Fig. 5). The ejection of five out of six ingested eggs took around 2 h for a 25-mm $L_{\text{o–a}}$ animal. In contrast, cod larvae were successfully digested within minutes and no ejection was observed.

## Discussion

We demonstrate experimentally that feeding rates of the invasive ctenophore *Mnemiopsis* on cod eggs and larvae are extremely low and often zero, at environmental conditions relevant to the major spawning grounds of the Baltic Sea. *Mnemiopsis leidyi* has been present in northern European waters since 2005 (Javidpour et al. 2006; Boersma et al. 2007; Oliveira 2007). Our results sharply contrast with those obtained from other native and invaded habitats, outside northern Europe, where predation rates and predation potential of *Mnemiopsis* on ichthyoplankton are reported to be high (Cowan and Houde 1993; Rilling and Houde 1999; Purcell and Arai 2001). For example, extrapolations from laboratory experiments reveal that *Mnemiopsis* was the major source of ichthyoplankton mortality in the coastal Cape Cod area, USA, where it accounted for 10–65% and 3–65% of the daily Bay Anchovy egg and larvae mortality, respectively (Table 2; Monteleone and Duguay 1988). Due to such findings, and based on an observed spatial and temporal overlap of *Mnemiopsis* and cod eggs and larvae in its major spawning area, it has been speculated that *Mnemiopsis* poses a serious mortality threat to recruits of the commercially most important fish species in the Baltic Sea (Haslob et al. 2007; Huwer et al. 2008; Storr-Paulsen and Huwer 2008).

*Feeding on fish larvae*—Previous studies of *Mnemiopsis* predatory interactions with fish eggs and larvae have demonstrated higher clearance rates than in our study, but those studies have exclusively been conducted at much higher temperatures (Table 2). Respiration rates and energetic demand increase dramatically with temperature for *Mnemiopsis* ($Q_{10}$ = 4, for 10.3–24.5°C; Kremer 1977), and similar differences in predation rates are to be expected. One low-temperature study of *Mnemiopsis* reports similar feeding rates on copepods at 8°C (Miller 1970) as those that we observed on 4.5–8-d-old cod larvae at 7°C (Table 2). However, low temperature effects on

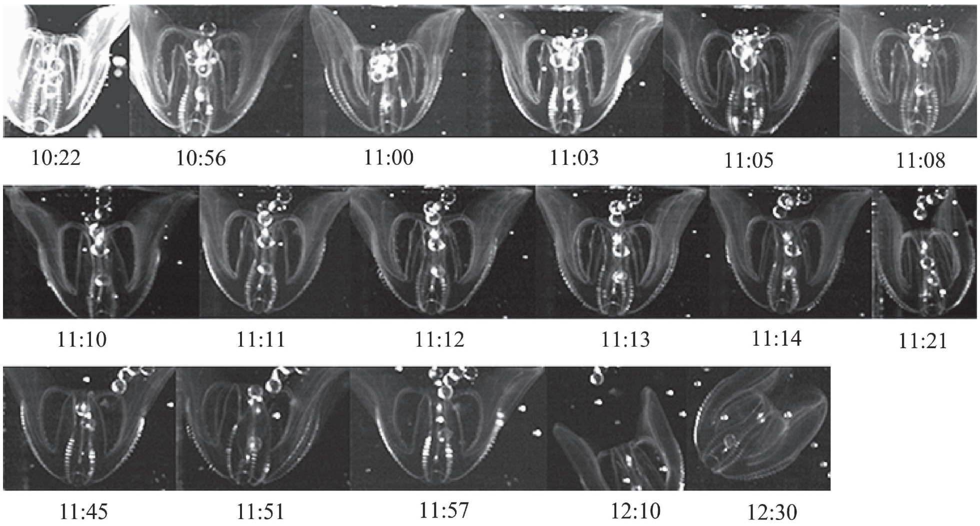

Fig. 5. Sequence of pictures following a *Mnemiopsis* (25-mm $L_{o-a}$) ejecting cod eggs at 22°C, 1013g $L^{-1}$ over $\sim$ 2 h. Time of the day is indicated below each picture, sequence from upper left (six eggs ingested) to lower right (one egg left in stomach). The *Mnemiopsis* had been incubated under extremely high prey concentrations.

feeding rates have been neglected in the evaluation of the potential implications of *Mnemiopsis* so far.

In general, differences in clearance rates, such as those observed here between young and older larvae, can usually be explained by differing predator or prey behaviors. Predators like *Mnemiopsis* detect the hydrodynamic signals produced by actively swimming prey items, and normally react with a capture response upon perception in their encounter zone (Costello et al. 1999). The hydrodynamic signal of the prey depends on prey speed, size, and behavior (Kiørboe et al. 1999). On the other hand, prey may perceive the flow field produced by the predator and attempt to escape. However, escape ability differs with prey types (Kiørboe et al. 1999; Waggett and Costello 1999), and also with different development stages of fish larvae, including cod, whose survival in interactions with other gelatinous predators increases during their development (Bailey and Batty 1984; Titelman and Hansson 2006). For example, old yolk-sac anchovy larvae have lower mortality rates than younger ones in interactions with *Mnemiopsis* (Table 2; Monteleone and Duguay 1988), maybe due to increasing predator perception and escape abilities of the larvae. Similarly, we found that clearance on young yolk-sac cod larvae (0–4.5 d posthatch) was 2.5× higher compared to that on older larvae. In general, cod larvae are quite passive and yolk-sac larvae do not engage in active search behavior (Skajaa et al. 2003). However, older cod larvae are generally better escapers, probably because they are able to sustain relatively higher swimming speeds about 4 d after hatching (Yin and Blaxter 1987). Therefore, decreasing feeding rates of *Mnemiopsis* on cod larvae during their early development may be explained by differing stage-specific abilities of the prey.

*Feeding on fish eggs*—*Mnemiopsis* fed on cod eggs only at very low rates, if at all (Fig. 1). Nonmotile cod eggs produce negligible hydrodynamic signals and have no ability to escape. If all contacted prey were captured, clearance rates on cod eggs should theoretically equal the volume encounter rate produced by the flow field of the *Mnemiopsis*. However, our experiments demonstrated a clear negative, passive selection of cod eggs when offered in combinations with poorly escaping *Artemia*. In accordance with the rate measurements, our video recordings revealed that the capture response in *Mnemiopsis* was not triggered by encounters with nonmotile cod eggs (*see* Web Appendix). We repeatedly observed *Mnemiopsis* swimming through dense patches of eggs without responding. This may suggest that encountered cod eggs were not perceived by *Mnemiopsis*, but in some cases entrained in the feeding current and accidentally ingested without the normally observed capture response (Costello et al. 1999). In corroboration with our experimental results an experiment conducted at extreme prey densities (50 $L^{-1}$) each of copepods and a mix of fish eggs (0.8–1.5 mm) at 8°C demonstrated almost no feeding on fish eggs (0.003 ± 0.5 eggs $h^{-1}$; Hamer et al. 2011).

In contrast, several authors have demonstrated feeding of *Mnemiopsis* on anchovy eggs (0.7 mm) at higher temperatures (21–27°C; Table 2, references therein). The reason for the different predation rates on eggs of anchovy and cod is not clear, but cannot be explained solely by temperature as *Mnemiopsis* is clearly capable of feeding at these very low temperatures on larvae and *Artemia* (Table 2), as well as on copepods (Miller 1970). Egg size or surface properties may possibly matter, but more detailed studies are necessary to enlighten this.

Eggs were digested at a much lower rate than larvae and the majority of ingested eggs were regurgitated. For cod eggs the ejection process took $\sim$ 2 h at 22°C, while it was on the order of 3 d at 7°C. Similar observations of inhibited digestibility and regurgitation of fish eggs has been described for the ctenophore *Bolinopsis infundibulum*, which ejected undigested plaice eggs after several hours (Gamble 1977). Because of the long duration of the ejection process (days) compared to that of our incubations (hours), we are confident that the egg clearance rates are reliable

Table 2. Published *Mnemiopsis* feeding rates on copepods, fish eggs or larvae, and *Artemia* at different temperatures, recalculated to volume-specific rates (*) if not presented in the original source; predator size shown as oral–aboral length (mm), anchovy (*Anchoa mitchilli*), cod (*Gadus morhua callarias*), and goby (*Gobiosoma bosci*). Only rates from experiments conducted at limited prey concentrations are included.

| Prey | Temp. (°C) | Specific clearance ($L\ mL^{-1}\ h^{-1}$) | Predator length (mm) | Volume (L) | Reference |
|---|---|---|---|---|---|
| Copepods | 8 | 0.024 | 20–50 mm | 6 | Miller (1970) |
| Copepods | 16.5 | 0.09 | 20–50 mm | 6 | Miller (1970) |
| Copepods | 24.5 | 0.18 | 20–50 mm | 6 | Miller (1970) |
| Fish eggs | | | | | |
| Anchovy | 21–22.7 | 0.4 | 37.5–41.7 mm† | 750 | Cowan and Houde (1990) |
| Anchovy | 23–23.5 | 0.9 | 41.7–46.7 mm† | 3000 | Cowan and Houde (1990) |
| Anchovy with alternative prey | 19.5–22 | 0.2 | 41.7 mm† | 750 | Cowan and Houde (1990) |
| Anchovy | 21–24 | 0.26 | 20–25 mm | 15 | Monteleone and Duguay (1988) |
| Anchovy | 26 | 0.13±0.06* | 66.4 mm† | field‡ | Purcell et al. (1994) |
| Fish | 20 | 0.76 | Average: 17.6 mm† | 2200 | Cowan et al. (1992) |
| Cod | 7 | 0.02±0.03 | 8.5–26 mm | 13.5 | This study |
| Fish larvae | | | | | |
| Anchovy yolk sac | 21–24 | 0.44 | 20–25 mm | 15 | Monteleone and Duguay (1988) |
| Anchovy 3 d starved | 21–24 | 0.18 | 20–25 mm | 15 | Monteleone and Duguay (1988) |
| Anchovy 5 d starved | 21–24 | 0.53 | 20–25 mm | 15 | Monteleone and Duguay (1988) |
| Anchovy 5 d fed | 21–24 | 0.23 | 20–25 mm | 15 | Monteleone and Duguay (1988) |
| Goby | 25 | 0.3* | 49 mm† | 3200 | Cowan and Houde (1992) |
| Cod <4.5 d | 7 | 0.05±0.02 | 4.5–26 mm | 13.5 | This study |
| Cod 4.5–8 d | 7 | 0.02±0.02 | 4.5–26 mm | 13.5 | This study |
| *Artemia* | | | | | |
| *A. salina* | 7 | 0.06±0.02 | 13.5±1.7 mm | 13.5 | This study |
| *A. salina* | 17.5±1 | 0.5 | 13.5 mm | 13.5 | L. J. Hansson unpubl. |

† Length (without lobes) conversion from volume based on equations in Kremer and Nixon (1976).
‡ From gut content analyses.

and not underestimated. The fate of regurgitated eggs remains uncertain, because we did not follow the developmental success of regurgitated eggs. However, while dead eggs tend to become white as proteins denature, the ejected eggs followed by picture sequence remained clear.

*In situ predation effect*—Our feeding-rate measurements demonstrated that young yolk-sac larvae (< 4.5 d posthatch) represent the most susceptible life stage for predation from *Mnemiopsis*. Cod spawning in the Bornholm Basin takes place from March to September, peaking in May and June around the halocline (Grønkjaer and Wieland 1997). At the depth of the highest densities of cod eggs and yolk-sac larvae, the maximum observed in situ abundance of *Mnemiopsis* in May 2007 was 0.5 ctenophores m$^{-3}$ (Haslob et al. 2007), with a $L_{\text{o–a}}$ of 14.4 ± 0.5 mm and cod egg and larvae densities of 4.5 m$^{-3}$ and 0.02 m$^{-3}$, respectively (H. Haslob pers. comm.). If we apply our measured clearance rates, *Mnemiopsis* would clear at maximum 0.13% of the cod larvae and 0.05% of the cod eggs d$^{-1}$, respectively. After the yolk-sac stage, cod larvae migrate upwards in the water column for first feeding (Grønkjaer and Wieland 1997), whereby they virtually escape the potential predation by *Mnemiopsis* on later life stages, because *Mnemiopsis* are rarely found at those depths in the Bornholm Basin (own data, unpubl.). Thus, despite the temporal and spatial overlap of *Mnemiopsis* and cod recruits in the most important spawning and nursery area of the Baltic, the direct predation effect of the alien, invasive ctenophore on cod eggs and larvae is negligible.

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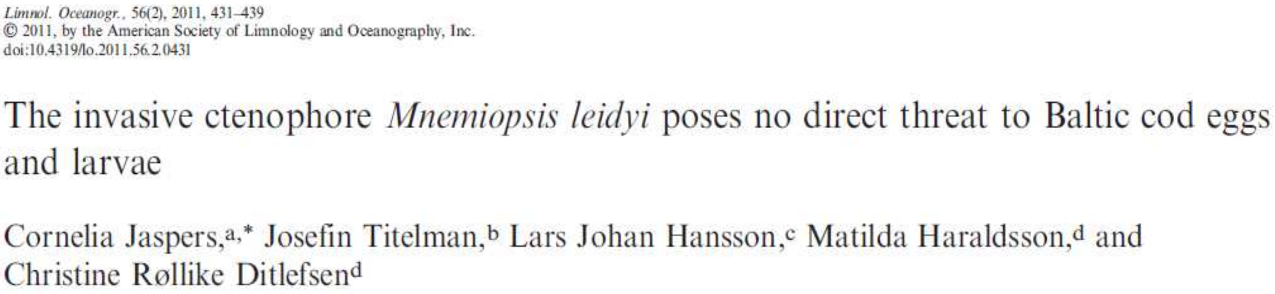
# Supplementary video

## Web Appendix found at:

**www.aslo.org/lo/toc/vol_56/issue_2/0431a.wmv**



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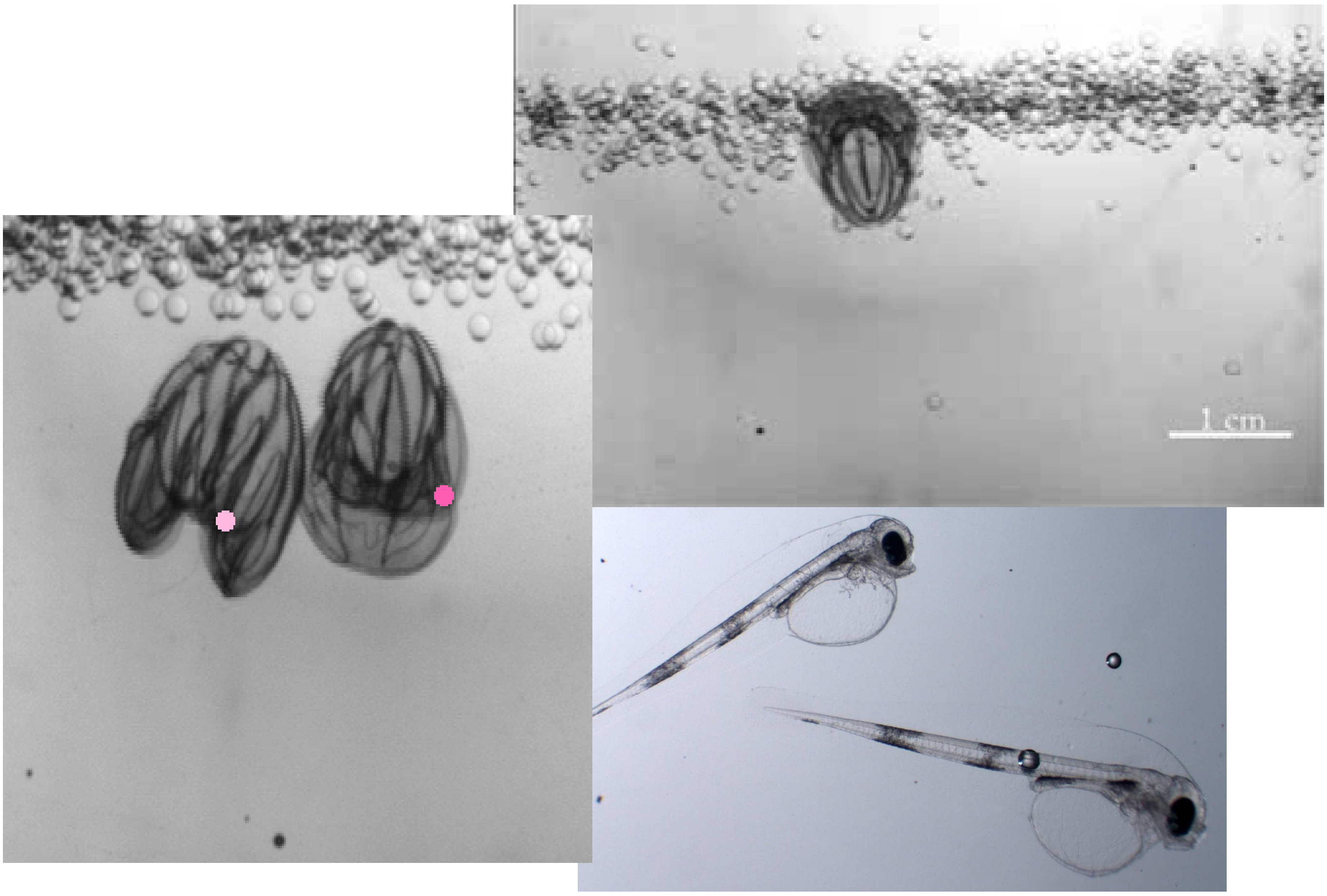
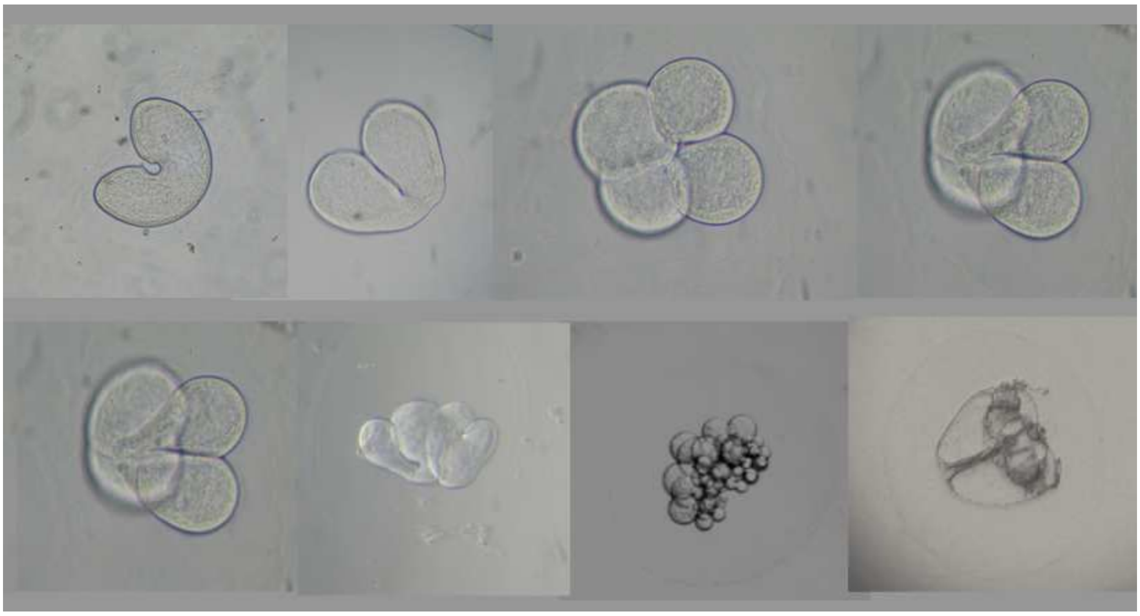
The invasive ctenophore *Mnemiopsis leidyi* poses no direct threat to Baltic cod eggs and larvae

Cornelia Jaspers,[a,*] Josefin Titelman,[b] Lars Johan Hansson,[c] Matilda Haraldsson,[d] and Christine Røllike Ditlefsen[d]



# Paper II



# Salinity Gradient of the Baltic Sea Limits the Reproduction and Population Expansion of the Newly Invaded Comb Jelly *Mnemiopsis leidyi*

**Cornelia Jaspers**[1]*, **Lene Friis Møller**[2], **Thomas Kiørboe**[1]

1 National Institute of Aquatic Resources, Technical University of Denmark, Charlottenlund, Denmark, 2 Department of Marine Ecology – Kristineberg, University of Gothenburg, Fiskebäckskil, Sweden

## Abstract

The recent invasion of the comb jelly *Mnemiopsis leidyi* into northern European waters is of major public and scientific concern. One of the key features making *M. leidyi* a successful invader is its high fecundity combined with fast growth rates. However, little is known about physiological limitations to its reproduction and consequent possible abiotic restrictions to its dispersal. To evaluate the invasion potential of *M. leidyi* into the brackish Baltic Sea we studied in situ egg production rates in different regions and at different salinities in the laboratory, representing the salinity gradient of the Baltic Sea. During October 2009 *M. leidyi* actively reproduced over large areas of the Baltic Sea. Egg production rates scaled with animal size but decreased significantly with decreasing salinity, both in the field (7–29) and in laboratory experiments (6–33). Temperature and zooplankton, i.e. food abundance, could not explain the observed differences. Reproduction rates at conditions representing the Kattegat, south western and central Baltic Sea, respectively, were 2.8 fold higher at the highest salinities (33 and 25) than at intermediate salinities (10 and 15) and 21 times higher compared from intermediate to the lowest salinity tested (6). Higher salinity areas such as the Kattegat, and to a lower extent the south western Baltic, seem to act as source regions for the *M. leidyi* population in the central Baltic Sea where a self-sustaining population, due to the low salinity, cannot be maintained.

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* E-mail: coja@aqua.dtu.dk

## Introduction

Invasive species in marine environments have gained public and scientific attention due to their documented direct and cascading effects, once successfully established, on ecosystems as well as biodiversity [1]. Ecosystems disturbed by, for example, eutrophication or overfishing are especially vulnerable to invasions [2]. The ctenophore *Mnemiopsis leidyi*, native to the east coast of the Americas, is such a successful invasive species as documented after its introduction into the Black Sea in the 1980's [3]. In 2005, *M. leidyi* was first sighted in northern European waters [4] and has since spread over large areas such as the North Sea [5], all Danish waters [6], as well as the western [7] and central Baltic Sea [8,9]. The invasion success of *M. leidyi* is partly due to its high reproduction capacity, being a hermaphrodite with a daily production of up to 14,000 eggs ind$^{-1}$ [10].

To better predict how *M. leidyi* will expand into, and potentially impact, the Baltic Sea it is important to understand how environmental characteristics affect its reproduction capacity. Generally, the distribution of marine organisms are governed by their physiological tolerance to biotic and abiotic factors and the demographics will be restricted by their tolerance limits [11]. In the Baltic Sea, the salinity gradient has been shown to influence invasive mesozooplankton species with higher species number in higher salinity waters [12] whereas in the western Mediterranean Sea mass occurrences of invasive jellyfish have been documented to be directly correlated to temperature [13]. In *M. leidyi*, reproduction has been shown to vary with temperature and food availability [14–19], but the dependency on salinity is unknown. The Baltic Sea is one of the largest brackish water bodies in the world and characterized by strong vertical and horizontal salinity gradients. The surface salinity ranges from around 4 in the north-east, to 6–8 in the central Baltic, 15–25 in the south-west [20], and increases to >18–33 in the connecting Danish Straits and the Kattegat (Fig. 1). The question arises whether blooms, as observed in other invaded and native habitats [3,15], can be expected for the entire Baltic Sea or if salinity will restrict the population expansion.

The aim of this work was to evaluate effects of salinity on egg production by *M. leidyi* in the newly invaded Baltic Sea. We examined in situ egg production rates of *M. leidyi* during its seasonal abundance peak in high and low salinity areas of the Baltic Sea and carried out laboratory experiments to test the effect of salinity on egg production rates. We can show that salinity has a strong impact on reproduction rates and seems to limit the establishment of a year round, self-sustained *M. leidyi* population in the central Baltic Sea.

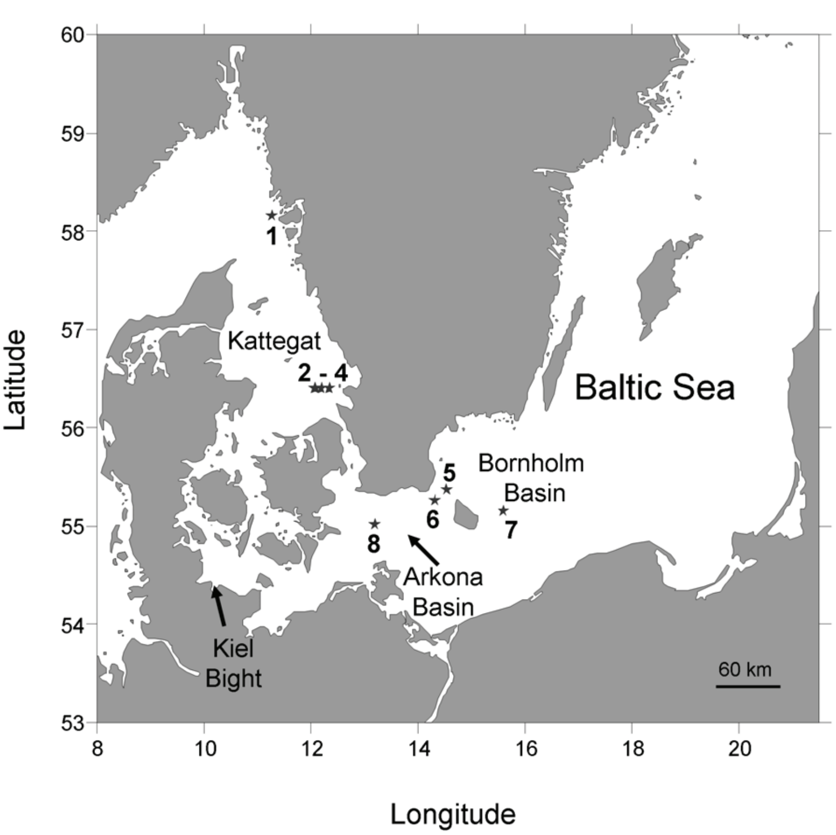

**Figure 1. Investigation area.** In situ *Mnemiopsis leidyi* egg production experiments were performed in the Kattegat (Stations 1–4, high salinity) and central Baltic Sea (Stations 5–8, low salinity) in October 2009. Locality or area names are specified as used throughout the manuscript. Station 2 to 4 comprise a short transect.
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## Materials and Methods

### Ethics statement

No specific permits were required for the described field and laboratory studies. The locations visited are not privately-owned or protected and the field studies did not involve endangered or protected species.

### Study area

The overall hydrography of the Baltic Sea is governed by large fresh water input in the north eastern parts that is compensated by surface water outflow through the Danish Straits and subsequent deep water inflow, with detailed dynamics governed by meteorological forces [20]. Our investigation covered the Bornholm and north Arkona Basin (hereafter referred to as central Baltic) which has a low surface salinity (7–9) and the Swedish west coast (Kattegat) which is characterized by a much higher surface salinity (21–29).

### Field investigation

In situ egg production rates of *M. leidyi* were measured at 8 stations in the Kattegat and central Baltic Sea (Fig. 1) during 12–21 October 2009 onboard R/V Skagerak (University of Gothenburg). Vertical profiles of salinity and temperature were measured using a Seabird 9/11 CTD. Assessment of food availability for *M. leidyi* was based on zooplankton abundances. Zooplankton was collected in 10 m depth strata with a HYDRO-BIOS© MultiNet sampler (0.25 $m^2$ net opening) equipped with 90-µm nets and preserved in 2% acidified Lugol solution. Zooplankton sizes were corrected for shrinkage using a correction factor of 1.17 for chitineous and 1.22 for gelatinous zooplankton [21]. Their biomass was estimated from the average sizes of developmental stages for all copepod species and the average sizes for nauplii, cladocerans and other zooplankton groups, applying species specific regressions if applicable [following 22]. Dry weight or ash free dry weight were converted to carbon using conversion factors of 0.4 [23] and 0.45 [24], respectively.

Egg production experiments were performed at 4 stations (St.) in each of the Kattegat (St. 1–4) and the central Baltic Sea (St. 5–8), with 52 and 47 individual experiments respectively (Fig. 1). Station 2 egg production rates were measured twice at noon for 2 subsequent days; at all other stations experiments were performed only once. Station 2 to 4 comprised a short transect. *M. leidyi* were caught with a black, 2 mm mesh size, 1.7 $m^2$ and 5 m long ring net with a 10 L non filtering cod end. Animals were collected in the upper 10 m of the water column. The cod end was opened into a 20 L bucket pre-filled with incubation water (from 5 m depth) and immediately transferred into a temperature controlled room ($12.5 \pm 1.3$°C). Incubation container volumes ranged from 1 to 13.5 L. No effect of container size on egg production was observed, neither for 2.8 cm animals in 1 and 13.5 L ($t = 0.31$, $p = 0.765$, $df = 6$), nor for size-specific egg production rates in 1, 2 and 13.5 L containers in the Kattegat ($F = 1.067$, $p = 0.352$, $df = 2$). Experiments started within 1 hour after collection by gently transferring, into individual containers, actively swimming *M. leidyi* that showed no signs of damage. Egg production was

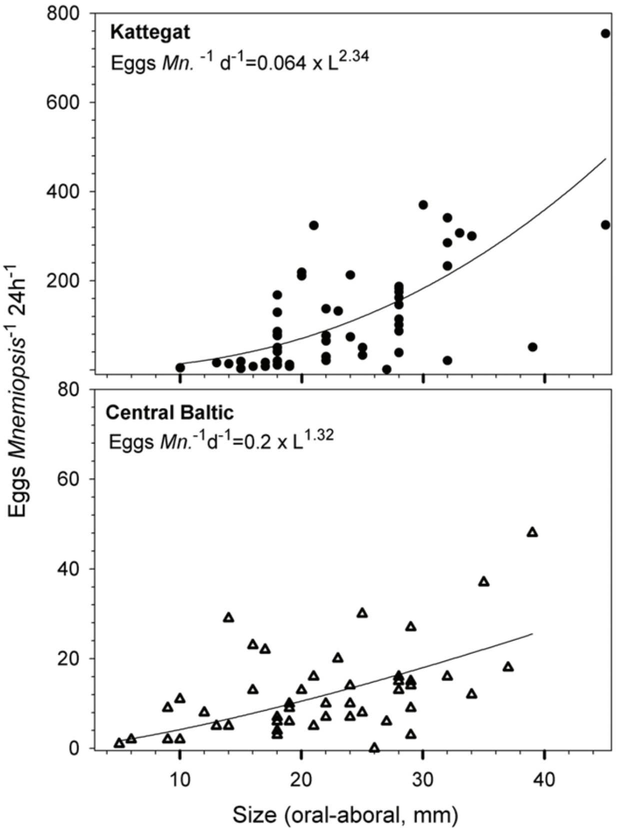

**Figure 2. In situ size dependent reproduction rates of the invasive comb jelly *Mnemiopsis leidyi*, northern Europe.** The egg production differed significantly between the two investigation areas ($F = 12.28$, $p = 0.0007$, $df = 95$) with higher rates in the Kattegat ($n = 52$) than in the central Baltic Sea ($n = 47$), $12.5\pm1.3^\circ C$, October 2009. Per capita egg production scaled significantly with length (oral aboral, mm). The observed production for a 15.1 mm standard animal used during salinity-dependent laboratory experiments is 37 versus 7 eggs *Mnemiopsis*$^{-1}$ d$^{-1}$ in the Kattegat and central Baltic, respectively.
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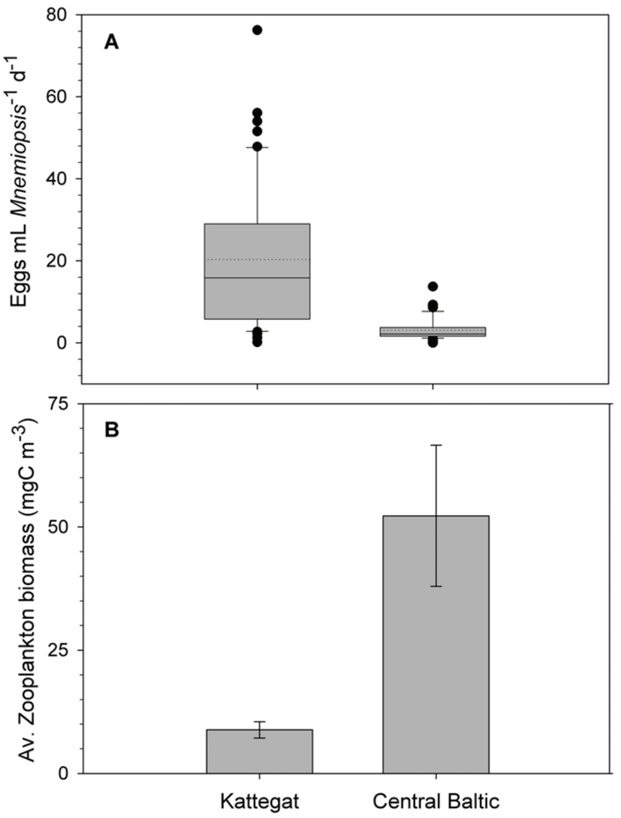

**Figure 3. Field observations of *Mnemiopsis leidyi* standardized in situ egg production and average zooplankton food concentrations. A**) Box-whisker plot of standardized egg production rates of *M. leidyi* in the Kattegat ($n = 52$) and central Baltic Sea ($n = 47$) with median indicated by solid line, mean presented as dotted line, 75 and 25 percentiles stated in the box; 90 and 10 percentiles indicated by error bars. Standardized egg production was significantly different between the two regions ($t = 6.77$, $p < 0.0001$, $df = 97$) with $20\pm17$ versus $3\pm2.5$ eggs mL *Mnemiopsis*$^{-1}$ d$^{-1}$ in the Kattegat and central Baltic, respectively. **B**) Zooplankton biomass as a measure of potential *M. leidyi* food availability. Zooplankton biomass was 6 times higher in the central Baltic compared to the Kattegat ($t = 3.012$, $p = 0.024$, $df = 6$). Bars present average biomasses ($\pm$SE) for the same stations where egg production was investigated.
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measured over 24 h including one full night and following the natural light regime, at constant temperature ($12.5\pm1.3^\circ C$) and irrespective of collection temperature (8 to 12°C). At termination, *M. leidyi* were removed and measured to the nearest mm (oral-aboral length). Eggs were concentrated by reverse filtration (45 µm), preserved in 2% acidified Lugol solution and enumerated within two weeks. Sizes were converted by use of the oral-aboral length (L, mm) to volume (V, ml) regression: $V = 0.0226 \times L^{1.72}$ [25], and rates presented as volume-specific egg production (eggs mL *Mnemiopsis*$^{-1}$ day$^{-1}$).

## Laboratory experiments

To confirm in situ observations, laboratory controlled salinity-dependent egg production experiments were conducted at the Sven Lovén Centre for Marine Sciences, Sweden in June 2011. *M. leidyi* originated from laboratory cultures kept at a salinity of 33 that were originally caught in the Kattegat (58°15′N 11°24′E). Experimental animals were raised to a standard size of 15 mm from a cohort spawned in April 2011. Animals were acclimated over 5 days via step wise dilution to the target salinities (6, 10 15, 25 and 33) and kept at those conditions for at least 7 days before the start of the experiments. Animals were fed ad libitum with *Acartia tonsa* from laboratory cultures and their carbon content was estimated from literature regressions [following 22]. Copepods were acclimated to the different salinities for at least 24 h before the start of the experiments. Individual egg production was measured in filtered seawater during 24 h in 7.5 L incubation container after 12 h food acclimatization at a mean copepod prey concentration of 75 µg Carbon L$^{-1}$. Prey concentrations at the end of preconditioning did not vary between treatments ($p = 0.23$). Eggs were counted and animals shifted to fresh water every 12 h to prevent egg cannibalism, even though *M. leidyi* does not cannibalize their eggs [26] but have been shown to intensively prey on their larvae >5 mm [27]. Egg production rates are presented as individual rates 24 h$^{-1}$. Egg production experiments always started at 7 pm and the overall experimental temperature was $18.8\pm0.4^\circ C$.

## Statistical analyses

Statistical analyses were conducted in R 2.13.0 (www.r-project.org/) using a significant level of 0.05. Size dependent egg production experiments were analyzed using power regression

**Table 1.** Average *Mnemiopsis leidyi* sizes and reproduction rates from laboratory salinity-dependent production experiments in Sweden, June 2011.

| Salinity | Oral aboral length (mm) | | Egg production ($Ind^{-1}$ $d^{-1}$) | | Replicate |
|---|---|---|---|---|---|
| | *Average* | *SD* | *Average* | *SD* | *n* |
| **6** | 13.7 | 1.5 | 2 | 2.2 | 4 |
| **10** | 14.8 | 1.8 | 46 | 31.2 | 5 |
| **15** | 15.6 | 2.0 | 37 | 37.5 | 5 |
| **25** | 16.3 | 1.1 | 103 | 40.2 | 5 |
| **33** | 14.6 | 2.1 | 129 | 28.3 | 5 |

The overall animal sizes used was 15.1±1.8 mm and did not differ between salinity treatments (F = 0.855, p = 0.365, df = 22).
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analyses on raw data. A separate slopes model was used to test for differences between slopes on log (x+1) transformed data. Laboratory egg production rates were square root transformed to meet normality assumptions before 1-way ANOVA and subsequent equality of variance and homogeneity tests were performed. Student Newman Keuls post hoc test was used to detect significant groupings.

## Results

### Hydrography

In October 2009 we observed a typical situation with marked surface salinity differences between high salinity stations in the Kattegat (25±3, average in upper 10 m) compared to low salinity waters in the central Baltic (7.8±0.3). Surface temperatures in the central Baltic ranged from 8.4°C at station 5 to 10.7°C at station 7 and 8 and 11.3±0.8°C at all Kattegat stations. Across all stations the surface temperature averaged 11±1.2°C. The mixing depth of the surface waters differed between the two areas with a shallower upper mixed layer in the Kattegat between 10 m and 20 m, compared to 35 m for all central Baltic stations.

### Egg production in the field

We found no difference in volume-specific egg production rates between low and high in situ temperatures in the central Baltic (F = 2.28, p = 0.116, df = 2). Per capita egg production was a power function of body length, with exponents of 2.3±0.3 for the high salinity Kattegat compared to 1.3±0.4 for the low salinity central Baltic (Fig. 2). Animals that originated from the Kattegat showed a factor 10 higher reproduction rate than *M. leidyi* from the central Baltic (Fig. 2).

Volume-specific production rates also differed significantly between the two regions. The Kattegat showed a nearly 7 times higher average specific production of 20±17 compared to 3±2.5 eggs mL $Mnemiopsis^{-1}$ $d^{-1}$ in the central Baltic (Fig. 3A).

In both areas, the major part of the zooplankton biomass was comprised of copepods. The potential food quality for *M. leidyi* was therefore similar between the two regions, whereas the zooplank-

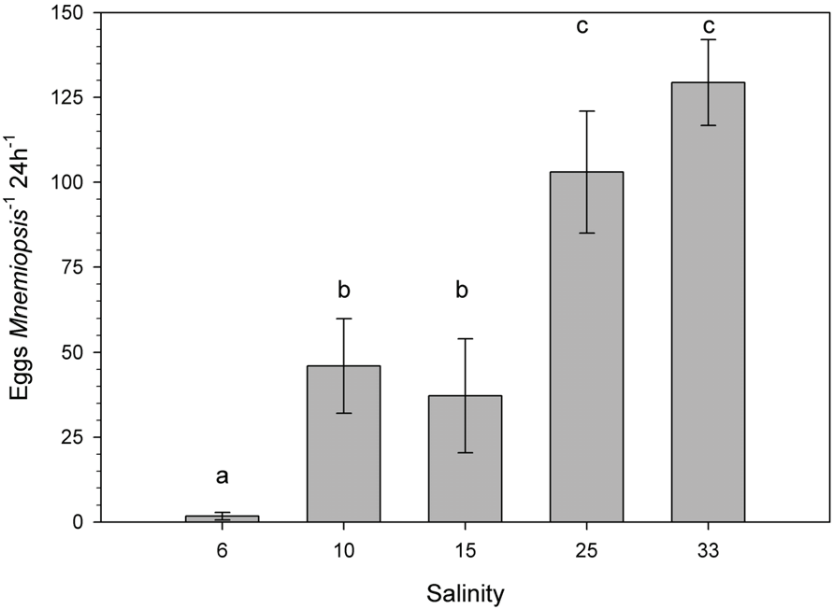

**Figure 4. *Mnemiopsis leidyi* egg production at salinities representative for different hydrographic regimes of the Baltic Sea.** Laboratory reared animals (mean oral aboral length 15.1±1.8 mm, n = 4–5) were individually incubated and mean egg production per salinity is stated 24 $h^{-1}$ as bars ±SE. Egg production rates differed significantly (F = 41.33, p<0.0001, df = 22) with salinity forming 3 clusters of 33/25, 15/10 and 6 (Student-Newman-Keuls post hoc test with significant groups indicated by letters a–c).
doi:10.1371/journal.pone.0024065.g004

ton biomass in the central Baltic was 6 times higher than in the Kattegat (Fig. 3B).

### Laboratory salinity-dependent egg production rates

The size of individuals used in the 5 salinity treatments did not differ significantly and the overall average size was 15.1±1.8 mm (oral aboral length) (Table 1). Egg production was significantly affected by salinity ($F = 41.33$, $p < 0.0001$, $df = 22$ see Table 1, Fig. 4). Highest egg production rates were obtained at the highest salinities tested (25 and 33) with an average of 40 eggs mL $Mnemiopsis^{-1}\ d^{-1}$. At intermediate salinities of 15 and 10, egg production was significantly lower and the lowest egg production rates were observed at the lowest salinity of 6 with 0.9 eggs mL $Mnemiopsis^{-1}\ d^{-1}$.

## Discussion

Earlier reviews [e.g. 14,15] emphasized the importance of temperature, zooplankton and predators in determining the *Mnemiopsis leidyi* population size without considering the effect of salinity. Here we have demonstrated both from in situ observations and in laboratory experiments that the reproduction rate of the invasive ctenophore *M. leidyi* is highly sensitive to the range of salinities found in the newly invaded Baltic Sea area.

*M. leidyi* had tenfold higher egg production rates in the high salinity Kattegat area compared to the low salinity central Baltic Sea. Food availability has been reported to strongly influence egg production in *M. leidyi* [e.g. 19,28] but in situ reproduction rates were highest in areas with relatively lower food availability (Kattegat) and hence the observed effect of salinity in the field data is conservative.

Salinity-dependent egg production rates in the laboratory, where food, temperature and sizes were kept constant, confirmed our field observations. We found almost no reproduction at a salinity of 6, representative for the conditions in the central Baltic, intermediate reproduction at salinities of 10 to 15 characteristic for the south western Baltic Sea, and highest rates at salinities of 25 to 33 as observed in the Kattegat. Our results suggest that salinity should be considered as an important explanatory variable in determining in situ reproduction rates. If we compare our results with published size-specific production rates from native habitats in the US, a large scatter within the same temperature ranges is obvious [e.g.15]. However, the average egg production rate at 12 to 16.5°C in the US is similar to high salinity in situ reproduction rates measured in the Kattegat (12.5±1.3°C), viz. 20 eggs mL $Mnemiopsis^{-1}\ d^{-1}$.

### Distribution

After the first sighting of *M. leidyi* in 2005 [4], it has rapidly become abundant in northern European waters with the highest densities in the Kattegat and south western Baltic especially during summer [6,7,29]. So far, *M. leidyi* is generally absent from the central Baltic Sea during summer, e.g., the Bornholm Basin, but appears in these low salinity areas in low abundances from autumn to spring [8]. In the south western Baltic the *M. leidyi* abundances peak in August, corresponding to the reproduction peak as shown from analysis of population size structures [7]. The lack of *M. leidyi* in the central Baltic during summer may therefore indicate poor reproduction and survival. Our study shows that *M. leidyi* is hardly reproducing at the low salinities found in the central Baltic which might explanation their low abundances. It seems that the appearance of *M. leidyi* in this area must be due to drift of individuals from higher salinity source areas [8].

Drift model studies and abundance observations have suggested that the southern Kattegat and south western Baltic Sea are source areas for the population of the jellyfish *Aurelia aurita* in the central Baltic Sea, e.g., the Bornholm Basin [30]. Drift models estimate that it takes about 2 months for animals recruited in the Danish Straits and the Kiel Bight to reach the Bornholm Basin [30]. Consistently, the *M. leidyi* in the Bornholm Basin appears ca. 2 months [8] after its peak occurrence in the south western Baltic [7]. Further evidence for drift recruitment is that both species early in the season are found mainly in the deeper, higher salinity, depth strata in water of more western origin [8,30]. Even though the salinity at depth is higher, ranging between 7–14 at the maximum abundance position of *M. leidyi* around the halocline [9,31], the year round low temperatures [8] probably constrain their reproduction at this depth due to very low feeding rates [32].

Our laboratory results suggest that the south western Baltic Sea may be a source area for the central Baltic *M. leidyi* autumn population. In the Kiel Bight area the reproduction peaks at salinities above 15 and high summer temperatures (>14°C) as concluded from population size structure analysis [7]. In this area, animals are present the whole year, which indicates that a self-sustained subpopulation has established in the intermediate saline waters of the south western Baltic Sea [7].

### Conclusion

The documented predatory impact of *M. leidyi* has lead to the concern of its range expansion and local population establishment especially in the central Baltic. The Bornholm Basin is of special interest since this is the most important cod spawning ground in the central Baltic [33]. Previous work has shown that *M. leidyi* does not constitute a direct threat to cod eggs and larvae in this area as a predator [32], although it may compete with larval cod for zooplankton prey. Here we demonstrate that the reproduction rates of the invasive ctenophore *M. leidyi* are considerably reduced under the low salinities that are characteristic of the central Baltic Sea. Hence, salinity acts as a bottle neck for the population expansions in this newly invaded area. Higher salinity areas such as the Kattegat, and to a lower extent the south western Baltic, seem to act as source regions for the *M. leidyi* population in the central Baltic Sea where a self-sustaining population, due to the low salinity, cannot be maintained. Hence, both in terms of direct and indirect effects *M. leidyi* is unlikely to become a threat to early life stages of cod in the central Baltic.

## Acknowledgments

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## Author Contributions

Conceived and designed the experiments: CJ TK LFM. Performed the experiments: CJ LFM. Analyzed the data: CJ LFM TK. Contributed reagents/materials/analysis tools: CJ LFM. Wrote the paper: CJ TK LFM.

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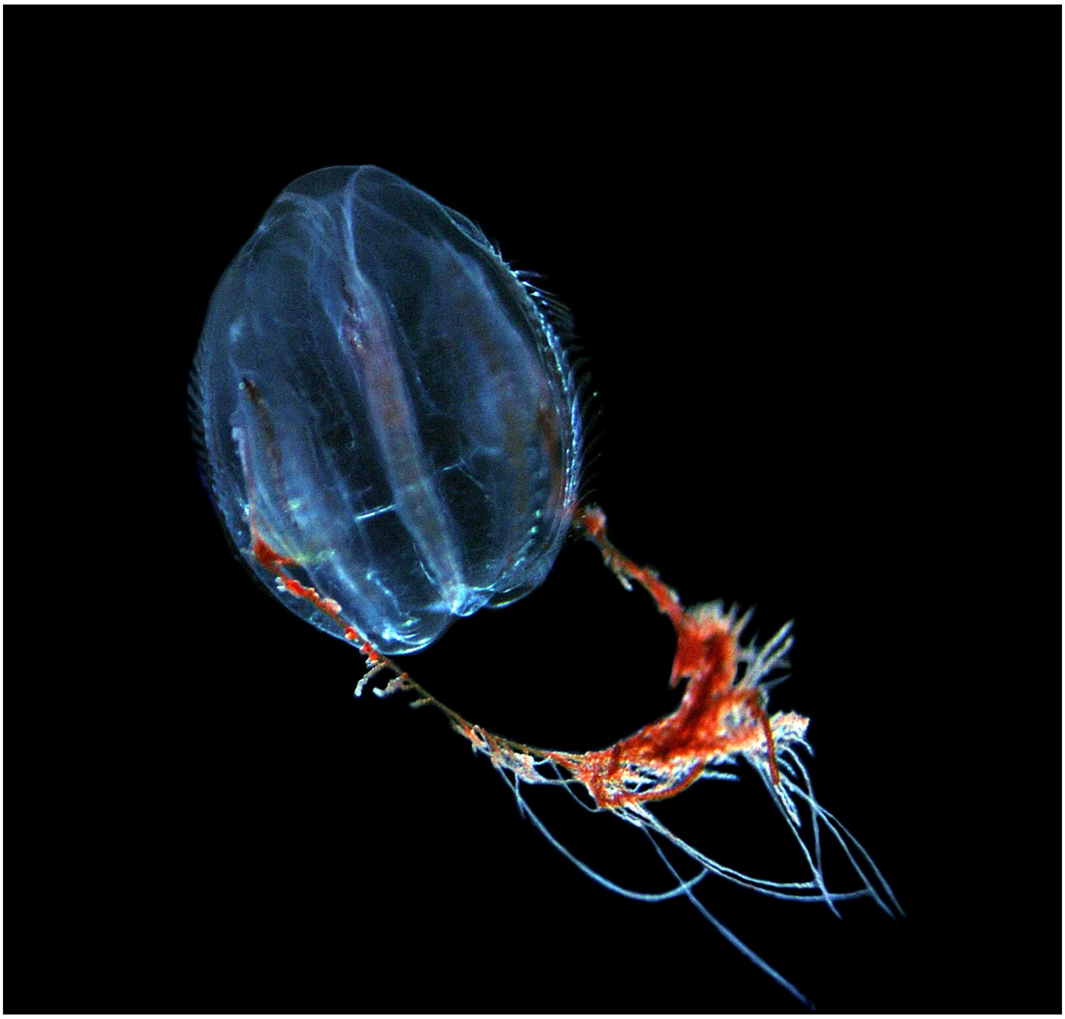
# Paper III



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# Ctenophore population recruits entirely through larval reproduction in the central Baltic Sea

Cornelia Jaspers[1,*], Matilda Haraldsson[2],
Sören Bolte[3], Thorsten B. H. Reusch[3],
Uffe H. Thygesen[1] and Thomas Kiørboe[1]

[1]Centre for Ocean Life, DTU-Aqua, Technical University of Denmark, Kavalergården 6, 2920 Charlottenlund, Denmark
[2]Biological and Environmental Sciences, Gothenburg University, Kristineberg 566, 45178 Fiskebäckskil, Sweden
[3]Helmholtz-Centre for Ocean Research (GEOMAR), Düsternbrooker Weg 20, 24105 Kiel, Germany

[*]Author for correspondence (coja@aqua.dtu.dk)

**The comb jelly *Mertensia ovum*, widely distributed in Arctic regions, has recently been discovered in the northern Baltic Sea. We show that *M. ovum* also exists in the central Baltic but that the population consists solely of small-sized larvae (less than 1.6 mm). Despite the absence of adults, eggs were abundant. Experiments revealed that the larvae were reproductively active. Egg production and anticipated mortality rates suggest a self-sustaining population. This is the first account of a ctenophore population entirely recruiting through larval reproduction (paedogenesis). We hypothesize that early reproduction is favoured over growth to compensate for high predation pressure.**

**Keywords:** *Mertensia ovum*; comb jelly; reproduction; Paedogenesis

## 1. INTRODUCTION

Reproduction before metamorphosis in the larval stage owing to delayed somatic growth (neoteny) or precocious maturation (paedogenesis) is known among amphibians and parthenogenetic insects [1]. In some extreme cases, natural populations consist exclusively of larvae [1].

Marine ctenophores are similarly capable of reproduction in the larval stage [2–5]. While larvae are normally defined as a non-reproductive developmental stage before metamorphosis, larval reproduction in ctenophores has been shown for both metamorphosing (Lobata) and non-metamorphosing (Cydippida) orders in their early life stage less than ca 2.7 mm [3–5]. In the cydippid *Pleurobrachia* spp., larval gonad structures are different from adult gonads and first reproduction has been described at a minimum size of 0.4 mm [3]. After larvae of both major orders reach a threshold size, reproduction ceases, animals rapidly grow and then become reproductive again as adults (6–10 mm) [4,5]. While metamorphosis in amphibians generally involves a habitat shift [6], ctenophore paedogenesis is linked to early age reproduction being favoured over growth [4,5] and has been hypothesized to compensate for high mortality [2–5]. However, this hypothesis has never been confirmed at population level in nature. Recently, the Arctic cydippid ctenophore *Mertensia ovum* was discovered in the Baltic Sea possibly as a relict population form the former ice age [7]. While Arctic specimens measure up to 90 mm, *M. ovum* in the northern Baltic remain small (less than 6.5 mm) [7,8]. Here, we describe for the first time that a ctenophore population is recruiting solely through larval reproduction (paedogenesis). The lack of larger sized specimens is hypothesized to be due to high predation pressure.

## 2. MATERIAL AND METHODS

Zooplankton sampling was conducted (13 monthly cruises) during 2009/2010 in the central Baltic Sea (figure 1), with vertical (90 µm HYDRO-BIOS Kiel, Germany, 0.25 m22 midi-MultiNet) hauls in five depth-strata from 70, 200 and 180 m for stations 1 to 3. At station 4, the total water column (17 m) was sampled without depth-resolution. Ctenophore eggs/larvae were measured, either live or after 2% acidified Lugol preservation. Live versus preserved animal sizes (n=1114) were compared. Preserved sizes were 75±12.2% of unpreserved ones and were multiplied by 1.33 to correct for this shrinkage. Ctenophore DNA from dried samples (n=121) was verified using species-specific primers for the ITS-1 region of the ribosoma-RNA genes for *M. ovum* and invasive *Mnemiopsis leidyi*, respectively [9]. Analyses used standard PCR protocols (electronic supplementary material). All eggs are assumed to be *M. ovum* since no other ctenophore larvae [7, this study] have been confirmed for this area. For reproduction experiments (October 2009, station 2), larvae were collected with vertical 300 µm (MultiNet) tows (90–50 m) and incubated individually in 20 ml tissue-culture trays (Nunc Roskilde, Denmark) with 20 µm filtered water at 7±18˚C, Salinity of 7. For 24 animals (0.54–1.33 mm), eggs were counted after 48 h with sizes based on averages (before/after the experiment). Regression analysis was performed for estimating size-dependent egg production. Expected egg abundance at each station was estimated from the observed ctenophore size distribution and the size-dependent egg production, multiplied by the hatching time (T, days), excluding station 4 where eggs were never observed. Hatching time for *M. leidyi* eggs is 7 days (7˚C, C. Jaspers 2010, unpublished data), and we assume the same for *M. ovum*. From this, expected egg abundance (squared metre) at each station was computed assuming no mortality. With no egg mortality, this computation should yield a larger observed egg abundance than expected at half the stations, on average. To test the hypothesis of zero mortality, the fraction of such stations was compared with 50 per cent using binomial statistics (electronic supplementary material).

## 3. RESULTS

Ctenophores were present throughout the year (figure 1). Molecular analysis confirmed that the individuals sampled were *M. ovum*. Population sizes peaked during winter with maximum abundances recorded at the deep-water, northernmost station 3. Ctenophores were not observed at the shallow-water station 4 during summer when water temperatures were high (ca 17˚C). *Mertensia ovum* showed a difference in depth distribution with season, residing deeper during warm seasons, and distributed throughout the water column or in surface waters during cold seasons (electronic supplementary material, figure S1). Hence, the temperature range where larvae were observed was low (-0.3˚C to 11.6˚C). Size distributions differed significantly between seasons with the lar-

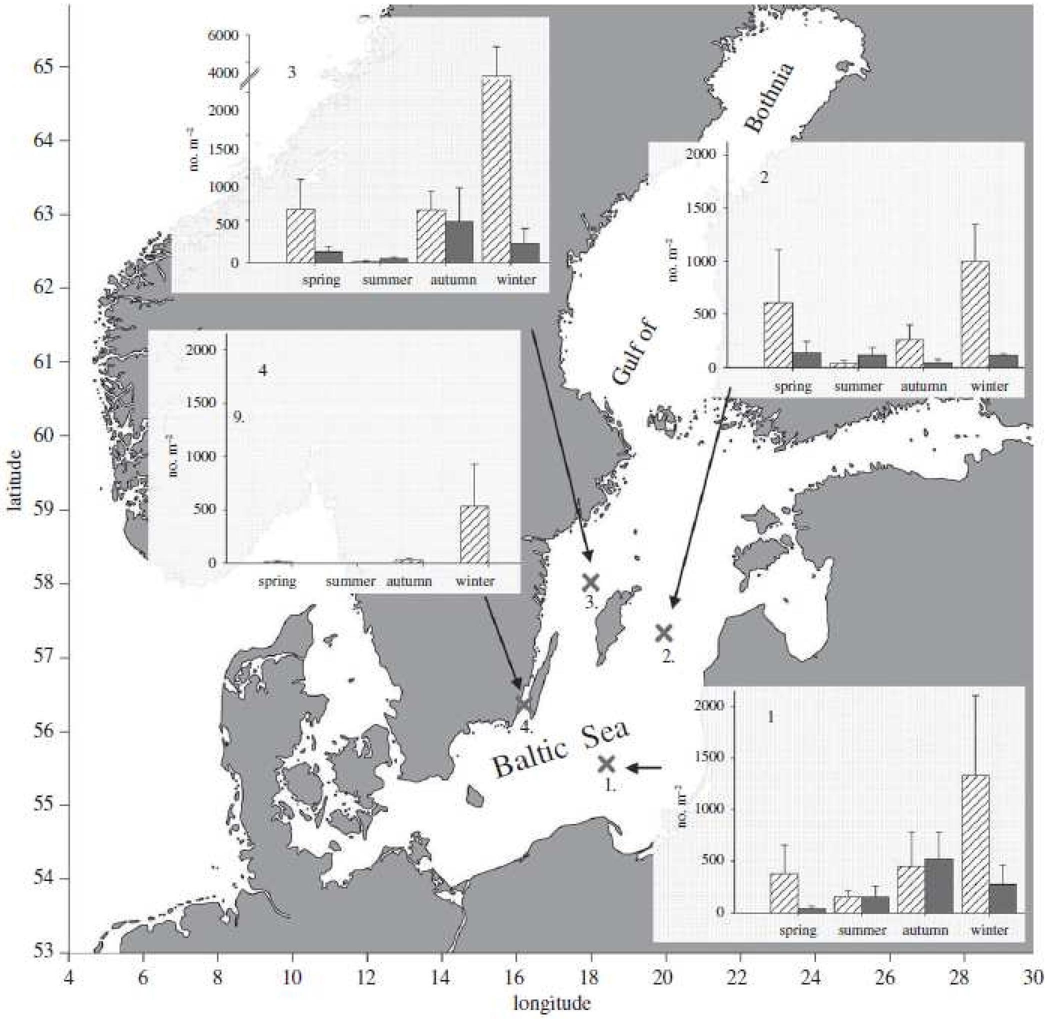

**Figure 1**. Integrated seasonal *Mertensia ovum* larvae (bars with stripes) and egg (grey bars) distribution in the central Baltic Sea, 2009/2010 (pooled for season ± s.d.).

gest sizes during summer (figure. 2a). However, through-out the year and area, ctenophores were very small: 95% of the population were less than 1.1 mm and the largest specimen found was 1.6 mm (n=7192).

High numbers of ctenophore eggs were observed (figure1).While larvae abundances were highest in winter, most eggs were present in autumn. Highest egg density was observed for the southernmost station 1, while at the shallow-water station 4 eggs were never found. Reproduction experiments revealed that *M. ovum* greater than 0.75 mm produced eggs and production increased significantly with size (figure 2b). Overall, nine animals produced 22 eggs in 48 $h^{-1}$, while 14 animals (0.54 – 1.04 mm) did not. The fraction of stations with lower egg abundance than expected did not deviate significantly from 50 per cent, either for the entire sampling period or within seasons; hence egg mortality can be hypothesized to be zero (figure 2c).

## 4. DISCUSSION

Our data show for the first time that a ctenophore population in nature consists entirely of small, larval size classes (less than 1.6 mm) throughout the year. We argue that our observations are consistent with a self-sustaining population maintained entirely through larval reproduction.

First, it is unlikely that the population in the central Baltic is supplied by advection from the Gulf of Bothnia in the north. In the north, *M. ovum* individuals are 4 times larger but their densities are only 1–8% compared with the central Baltic and they occur primarily at depth greater than 50 m [7]. Since the average residence time of water in the Gulf of Bothnia is 6 years and the southward advection mainly consists of surface water [10], drift recruitment is unlikely to be important.

Second, *M. ovum* larvae greater than 0.75 mm produce eggs at significant rates. Minimum size for larval reproduction in *Pleurobrachia* spp. is 0.4 mm [3], and 1.2mm *P. bachei* produces eight eggs per day at 15˚C [5]. The lobate *M. leidyi* (2 mm) produces up to 14 eggs per day at 22˚C at high food concentrations [4].

*Mertensia ovum* (less than 1.3 mm) produces up to two eggs $day^{-1}$. Considering the variation in temperature [11] and sizes, larval reproductive rates are similar for the different species and confirm our observation of reproducing larvae in the Baltic.

Finally, the observed egg production is sufficient to

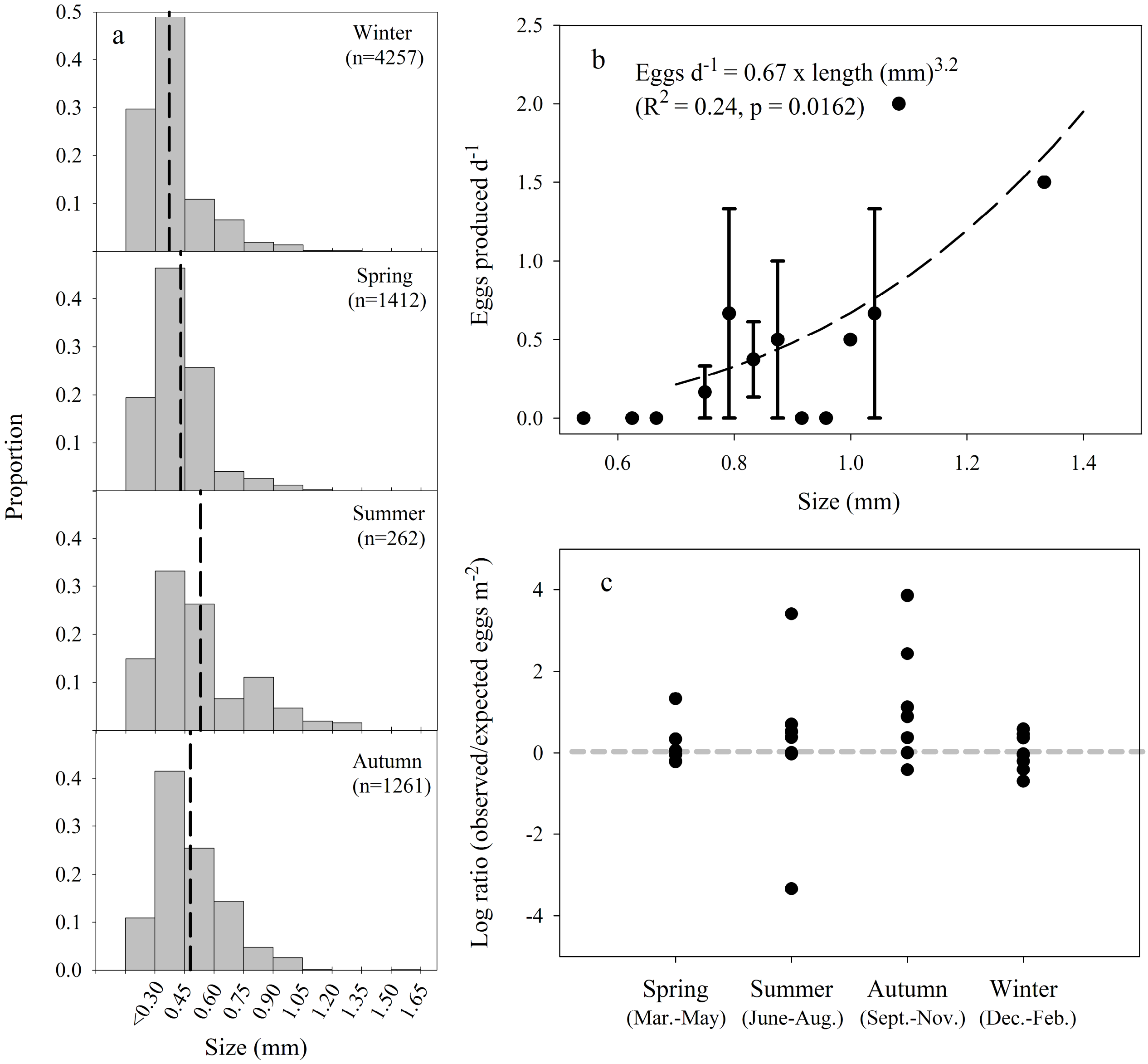

**Figure 2**. (a) Seasonal size distribution of *Mertensia ovum* in the central Baltic (0.15 mm size bins, averages (dashed lines), log-transformed lengths significantly differed with season (one-way ANOVA $F_{3,7845} = 821$, $p < 0.0001$) (b) size-dependent egg production at 7˚C, October 2009 (average sizes ± s.e.) (c) log ratio of observed to expected egg abundance with zero observations substituted by 0.1 (log ratio = 0, no egg mortality). The fraction of stations where expected egg abundance exceeds observed egg abundance does not differ significantly from 50% ($p = 0.36$).

maintain the population in the face of likely mortality rates. The requirement for population maintenance is that the net reproductive rate $R_0 \geq 1$ for simultaneous hermaphrodites. Assuming an egg hatching time of $T_1$, that it takes time $T_2$ to grow to reproductive size whereupon growth is replaced by egg production at a constant rate (m), and that mortalities in the egg ($\delta_1$) and post-hatch ($\delta_2$) stages are constant, then $R_0=\exp(-\delta_1 T_1)(m/\delta_2)\exp(-\delta_2 T_2)$ [12]. Using temperature corrected maximum growth rates reported for *M. leidyi* larvae [11,13], it would take *M. ovum* about 8 days to reach 1 mm size at 7˚C. If we insert the numbers relevant to our October reproduction experiments ($\delta_1=0$, $T_2=8$ d, $m=0.7$ $d^{-1}$), then $R_0 > 1$, as long as the mortality is less than 0.174 $d^{-1}$. Faster growth and reproduction during summer allows for higher mortality, and conversely during winter. In general,

mortality rates of similarly sized pelagic organisms are similar or less [14] and the observed larval fecundity is thus consistent with a self-sustaining population. Why does *M. ovum* population in the central Baltic consists of only larvae that are 1–2 orders of magnitude smalller in length (3–6 orders in mass) than in the Arctic [7,8]? The temperature–size rule predicts that individual sizes within a species increase with decreasing temperatures, but a temperature difference as observed here of 10˚C predicts only a 30 per cent difference in individual masses [15]. Similarly, marine species are often smaller in brackish water systems like the Baltic [16], but not to the degree observed here.

Also, the largest *M. ovum* were observed in the least saline Gulf of Bothnia [7]. Therefore, neither temperature nor salinity can explain the observed differences.

Life-history theory predicts that the optimum age of maturation decreases with increasing juvenile mortality[17]. The trade-off is between maturing early at a small size and low fecundity but high chance of surviving to maturity versus maturing late at a large size and high reproduction but lower chance of reaching maturity. Our observation of early reproduction in *M. ovum* is consistent with the suggestion of high juvenile mortality. We substantiate this by a simple calculation. If juvenile growth in mass is a power function of time with an exponent of c=2 as in the initial phase of the von Bertalanffy growth model [14], egg-production rate is proportional to individual mass (or length$^3$, figure 2b), and growth ceases sub-sequent to start of egg production [4,5], then the maturation time yielding the highest $R_0$ is $c/\delta_2$ [12]. Assuming $\delta_2$=0.17 $d^{-1}$ as has been shown for less than 19 day larvae of the ctenophore *Pleurobrachia bachei* [18] and as calculated above, implies a development time of 12 days, close to the estimated time required to reach reproductive size. Higher temperatures may imply shorter maturation time but probably also higher mortality, and vice versa for lower temperatures.

Why would mortality be higher in the Baltic than in the Arctic where *M. ovum* are much bigger? We have no strongly substantiated explanation of this but note that planktivorous fish, potential predators on *M. ovum*, are abundant in the central Baltic Sea [19], and, more gene-rally, that the relative significance of pelagic versus demersal fish increases from Arctic to temperate and tropical ecosystems [20]. The Baltic relict population of *M. ovum* [7] has had several thousand years to adapt to the local predation pressure.

In amphibians, a wide range of life-history strategies exist including facultative and obligate metamorphosis [1], and neoteny has been shown to be a response to density-dependent processes in newts with adult and larval reproduction occurring at the same age [6]. In extreme cases, natural populations are 'trapped' in the larval stage [1]. In insects, paedogenesis has been linked to optimum utilization of food patches, leading to short generation times in response to food availability [1]. In contrast, *M. ovum* paedogenesis is suggested to be caused by high predation pressure. However, very little is known about the potential predators on ctenophores in the Baltic, and this suggestion should be tested, e.g. through diet analyses and experiments with and without the presence of predators.

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## Supporting Online Material, Jaspers *et al*. (2012)

1. Supporting materials and methods
    a. Molecular analysis
    b. Statistical analysis of zero egg-mortality
2. Supporting figure S1

### 1a) Supporting materials and methods

Ctenophore larvae (n = 121) were individually dried on cellulose filter paper for 48 hours at c. 60˚C. For DNA extraction, dried samples were extracted with the Invisorb® spin tissue kit (Invitek, Germany) at 52°C for 30min as described earlier (Reusch et al. 2010).

Species verification was conducted using species-specific diagnostic primers designed for the internally transcribed spacer 1 (ITS 1)-region of the ribosomal-RNA genes based on an alignment of published sequences for the following ctenophore species: *Bolinopsis infundibulum*, *Beroe cucumis*, *Pleurobrachia bachei*, *Mnemiopsis leidyi* and *Mertensia ovum*. Two primer pairs were specific for *M. leidyi* and *M. ovum*, showed no cross amplification and had product sizes of 150 and 160bp, respectively. For *M. ovum* (1) and *M. leidyi* (2), forward (a) and backward/reverse (b) primers are as follows:

**1a) Mert_ITS_diag fw:** cct gcg gaa gga tca tta ac
**1b) Mert_ITS_diag rev:** agc aag ctc acc acg cta ct
**2a) Mnem_ITS_diag02fw:** ttc acg ggc gga gct cga
**2b) Mnem_ITS_diag02rev:** cgc ttt tag gca act ctc gtt c

PCR was conducted using Phire-Hot-Start DNA Polymerase® (Finnzymes®, Finland), always including negative and positive control templates for *M. leidyi* and *M. ovum.* PCR solution mix (10 µl) contained 2 µl 5x Phire-reaction-buffer, 0.2 µl dNTP's, 1 µl forward primer, 1 µl reverse primer, 0.2 µl Phire-Polymerase, 4.6 µl HPLC-$H_2O$ and 1µl template. The PCR-cycling conditions consisted of a denaturation and enzyme activation period of 5 min at 98°C, followed by 33 cycles of denaturation 98°C (8sec), annealing 58.5°C (15sec) and elongation 72°C (15sec) with a final extension at 72°C for 2 min.

Species specific PCR-products were separated by electrophoresis on 1,5% agarose gels stained with ethidium bromide and scored by eye.

Reusch, T., Bolte, S., Sparwel, M., Moss, A. & Javidpour, J. 2010 Microsatellites reveal origin and genetic diversity of Eurasian invasions by one of the world's most notorious marine invader, *Mnemiopsis leidyi*. *Mol. Ecol.* **19**, 2690-2699.

## 1b) Statistical analysis of zero egg-mortality

To assess egg mortality, the observed egg abundance at each station was compared with the expected egg abundance at the same station. Due to the large scatter in data, a direct estimation of egg mortality, based on a comparison between expected and observed abundance, does not yield reliable estimates.

We therefore tested statistically whether the mortality is significantly different from zero. The test proceeded as follows: At each station, it was recorded if the observed egg abundance exceeded the expected egg abundance. Under the hypothesis of zero egg mortality, this should be the case at half the stations. The hypothesis of zero egg mortality was therefore accepted if the fraction of such stations was not significantly different from 0.5. Specifically, the hypothesis was rejected if:

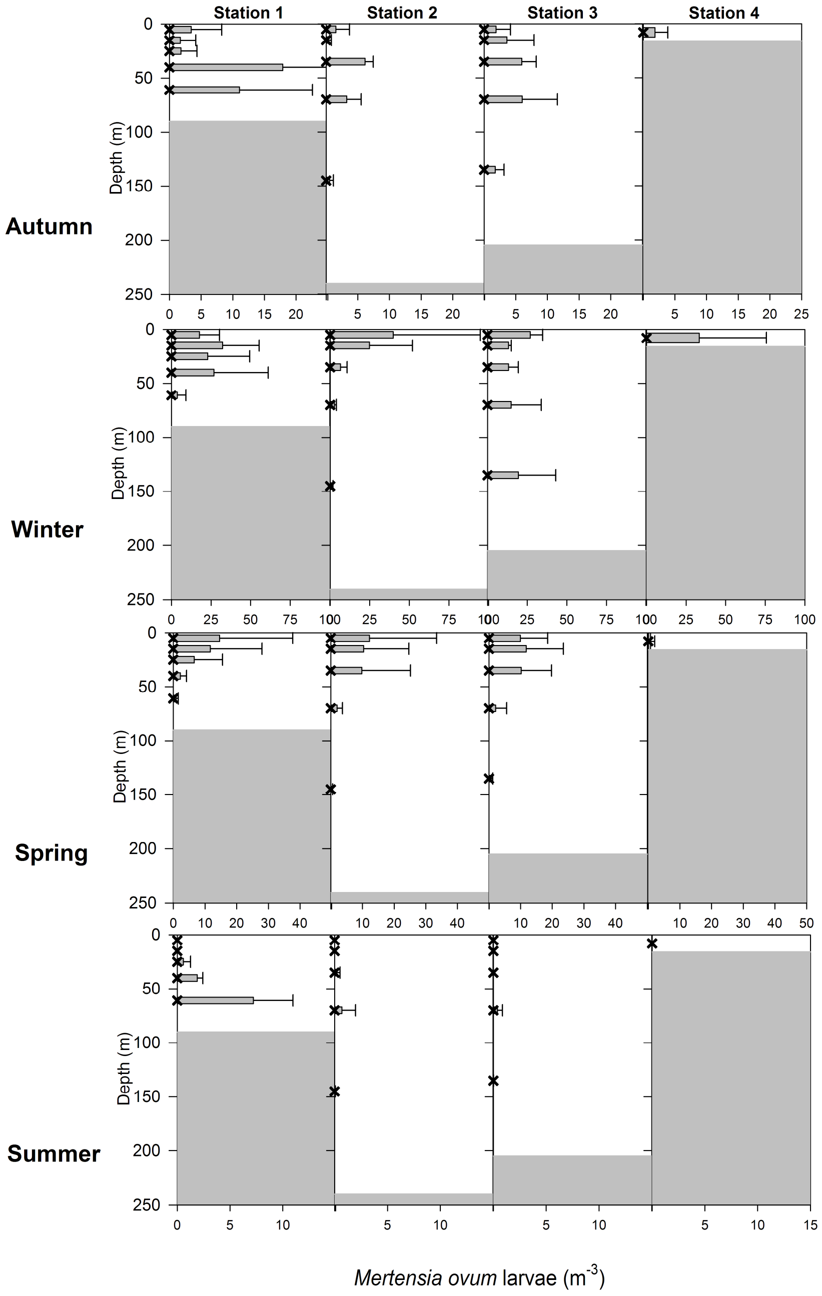
$P(X > x) < \alpha$

where $\alpha$ is the level of significance, $x$ is the number of stations with more eggs observed than expected, and $X \sim B(n,0.5)$ is a binomially distributed random variable where $n$ is the number of stations. The critical level of significance was computed using the R (version 2.10.1) command pbinom.

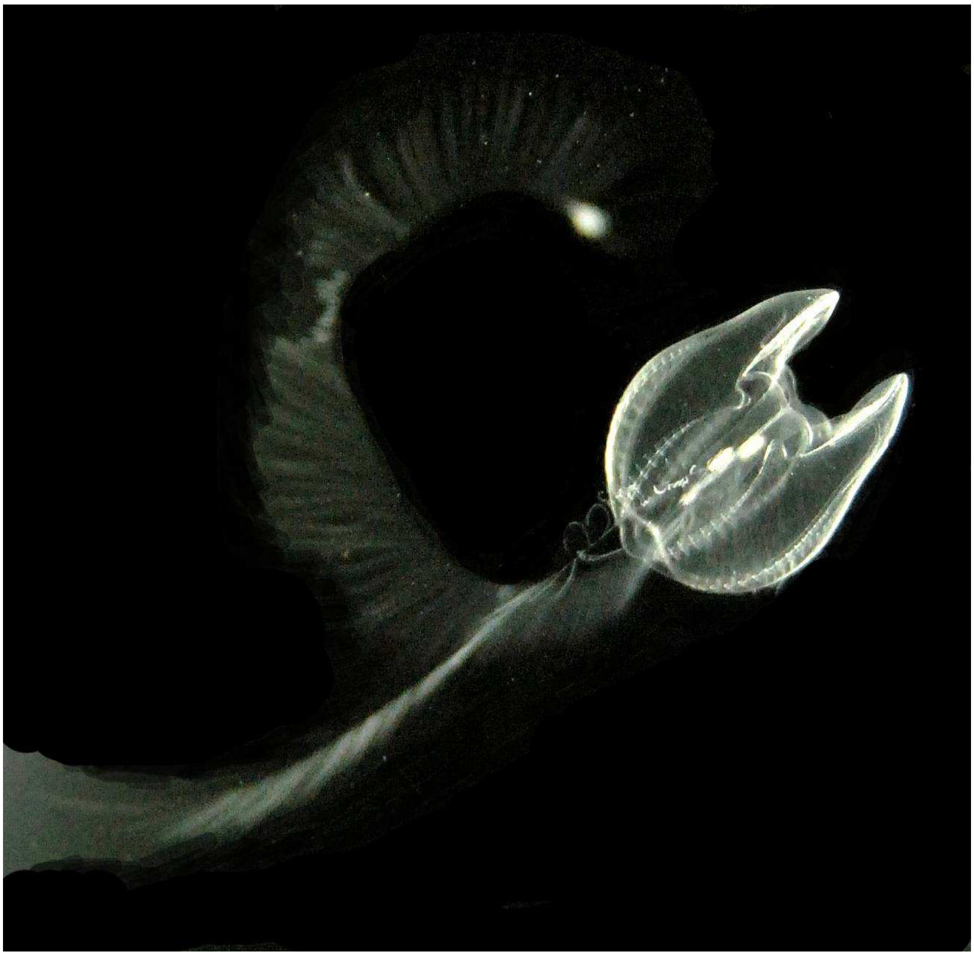
When pooling all stations, the critical level of significance was p=0.36. When testing each season separately, the p-values were 0.66 for spring, 0.62 for summer, 0.06 for autumn, and 0.86 for winter. The hypothesis of zero egg mortality was therefore accepted.

## 2. Supporting Figure S1:

Vertical distribution of ctenophore larvae (ind. m$^{-3}$) per depth strata (±SD, seasonal samplings) from 90 µm HYDRO-BIOS MultiNet© (0.25 m$^2$) samples in the central Baltic Sea in 2009/2010. The bottom depth at each station is indicated with grey fillings. Note, scale for abundances changes between seasons, and each station was visited three to four times per season. One to five depth strata are presented per station. The depth resolution for the stations is as follows: station 1 from 0 - 10 m, 10 – 20 m, 20 – 30 m, 30 – 50 m and 50 – 70 m; station 2, of 0 – 10 m, 10 – 20 m, 20 – 50 m, 50 – 90 m and 90 – 200 m; station 3 is the same as station 2 but the last net was only down to 180 m; station 4 without depth resolution from 0 – 17 m.



# Paper IV



# Seasonal dynamics of invasive and native ctenophore larvae in the Baltic Sea: clues to invasion and bloom potential

Jaspers, Cornelia[1*], Haraldsson, Matilda[2], Lombard, Fabien[3], Bolte, Sören[4] and Kiørboe, Thomas[1]

[1] Centre for Ocean Life, DTU Aqua, Technical University of Denmark, Kavalergården 6, 2920 Charlottenlund, Denmark.

[2]University of Gothenburg, Department of Biological and Environmental Sciences – Kristineberg, 45178 Fiskebäckskil, Sweden.

[3]Université Pierre et Marie Curie, Observatoire Océanologique, Laboratoire d'Océanographie de Villefranche sur mer BP 28, 06234 Villefranche sur mer, France

[4]Helmholtz-Centre for Ocean Research (GEOMAR), Düsternbrooker Weg 20, 24105 Kiel, Germany

*Corresponding author: coja@aqua.dtu.dk

**Running head: Seasonality of ctenophore larvae in the Baltic Sea**

**Keywords**: Invasive species, *Mnemiopsis leidyi*, *Mertensia ovum*, ctenophore egg sinking rates, egg mortality rates

## ABSTRACT

Recently both the invasive ctenophore *Mnemiopsis leidyi* and the arctic *Mertensia ovum* were discovered in the Baltic Sea but their ranges remain unclear due to misidentification of their larval stages. Supported by molecular species verification we describe seasonal abundance and distribution of larvae and eggs of the two species covering low to high salinity regimes. We show that their occurrence is significantly but oppositely related to salinity. *M. ovum* was present year round throughout the brackish Baltic Sea but also occurred in high saline areas during cold seasons. Larvae of *M. leidyi* occurred throughout all seasons in high saline areas but never extended further than into the southern Baltic Sea. Highest ctenophore egg abundances were observed in high saline areas during summer along with the appearance of large sized *M. leidyi* adults. Both larvae and adult *M. leidyi* abundances peaked two months later, suggesting that population expansion was seeded from high saline areas. Even though egg mortality rates were high in the suggested source region, *M. leidyi* compensated this by high reproduction rates. We conclude that quantification of larvae and eggs is essential to understand population dynamics of ctenophores. If not, whole populations and source areas may remain unnoticed.

## INTRODUCTION

Biological invasions are hypothesized to homogenize the world's biota as a consequence of increased globalization (Lodge, 1993). Ecosystem alterations and consequences for biodiversity have been documented (Carlton and Geller, 1993; Kideys, 2002) with disturbed aquatic systems being especially vulnerable for invasions (Richardson *et al*., 2009; Crooks *et al*., 2011). However, only a minor fraction of invasive species survive in their alien environment, with c. 1% of the released species establishing and c. 0.1% of the arriving ones achieving high abundances (Williamson and Fitter, 1996). Successful invasions have been attributed to several factors, such as broad tolerance to environmental conditions and anthropogenic stressors (Crooks *et al*., 2011; Lenz *et al*., 2011) or intrinsic attributes like fast growth, high reproduction and phenotypic plasticity (Sax and Brown, 2000; Facon *et al*., 2006; Sorte *et al*., 2010), although many exceptions exist to these general characteristics (Lodge, 1993). European brackish water systems, e.g., the Baltic Sea and the Black Sea, are areas of intermediate salinity and low species richness and are especially vulnerable to invasions (Paavola *et al*., 2005). In these brackish water systems, the invasive comb jelly *Mnemiopsis leidyi* has appeared and reaches seasonally high abundances (Shiganova *et al*., 2001; Oguz *et al*., 2008; Javidpour *et al*., 2009; Riisgård *et al*., 2010). *M. leidyi* shares many of the characteristics of a successful invasive species; especially its high reproduction and growth rates and ability to self fertilize may explain its recently documented global invasion dispersal (Costello *et al*., 2012). In its native habitat *M. leidyi* is known to have a large distribution range along the east coast of America (Purcell *et al.*, 2001), including estuaries and open water regions (Costello *et al*., 2006). For the southern-central Baltic Sea its distribution has been shown to be regulated by salinity which restricts its range expansion due to drastically reduced reproduction rates under low salinities (Jaspers *et al.*, 2011) and drift model studies suggest that the Bornholm Basin is a sink for *M. leidyi* with no substantial recruitment into the northern part of the central Baltic Sea (Lehtiniemi *et al.*, 2011).

In 2009 it was discovered by means of molecular analyses that another ctenophore species, *Mertensia ovum*, exists in the Baltic Sea, and previous records of *Pleurobrachia pileus* and *M. leidyi* in the northern Baltic Sea were clarified to be *M. ovum* (Gorokhova *et al*., 2009). *M. ovum* is distributed in the arctic and the population in the Baltic is likely a relic from the Ice age. The Baltic population of *M. ovum* does not grow to adult size but consists of reproducing larvae (Jaspers *et al*., 2012) that are morphologically difficult to identify to species level, and the current status of the range of the two species is confusing (Lehtiniemi *et al.*, 2007; Viitasalo *et al.*, 2008; Javidpour *et al.*, 2009) and needs clarification (Gorokhova *et al.*, 2009; Gorokhova and Lehtiniemi, 2010). Furthermore, it is difficult to assess the distribution range of both species since animals may drift far away from where they are recruited (Schaber *et al*., 2011) as has been shown for jelly fish populations in the southern-central Baltic Sea (Barz *et al*., 2006). Therefore, understanding the population dynamics, including the contribution of eggs and larval stages to the composition of a given population, is important to evaluate if the observed distribution is fuelled by reproduction or drift.

Supported by molecular species verification we here describe the seasonal abundance and distribution range of larvae and eggs of the two prominent ctenophore species in the Baltic Sea covering low to high salinity regimes. Combining data on adult and larvae abundances of *M. leidyi* with egg production and mortality rates, we point to potential source areas and the time of onset of the invasive *M. leidyi* population expansion in the Baltic Sea.

## MATERIALS AND METHOD

***Study area –*** The hydrography of the Baltic Sea is governed by large salinity differences with fresh water input in the north-eastern parts, compensated by surface water outflow into the Kattegat through the Danish straits. Detailed dynamics are governed by meteorological forces including Kattegat water inflow into the western Baltic as well as high saline deep water inflow events into the southern and central Baltic Sea basins (Krauss, 2001). These dynamics lead to strong horizontal and vertical salinity gradients in the Baltic Sea.

***Field sampling -*** Sampling was conducted during 13 monthly cruises investigating 8 stations (st.) in the Baltic Sea during 2009/2010 (Fig. 1). The stations were located along the Swedish west coast (Skagerrak and Kattegat) which is characterized by a higher surface salinity of 21-31 (st. 1-2), the lower saline Arkona and Bornholm Basins (st. 3-4, surface salinity of 7-9) and low saline central Baltic stations (st. 5-8) that have surface salinities of 5-6 (Fig. 1). For each station, vertical profiles of salinity, temperature and fluorescence were measured with a Seabird SBE9+ CTD.

In October 2009, the regular monitoring stations were supplemented by 13 stations, including two transects and two 24 hours stations in the southern Kattegat (n = 6) and the Sound (n = 6), respectively. This lead to a total of 32 samplings which were grouped into the areas Skagerrak (including northern Kattegat, n = 4), Kattegat (n = 8), the Sound (n = 6), Arkona Basin (n = 2), Bornholm Basin (n = 6) and the central Baltic (n = 6) (Fig. 1).

Ctenophore eggs and larvae (≤ 2 mm) were sampled with a 90 µm MultiNet-midi© (HYDRO-BIOS, Kiel, Germany; net opening: 0.25 $m^2$) with vertical hauls in up to five depth strata. Additionally 300 µm oblique samples at 1 - 2 knots towing speed were attained at the same stations and depths (target water volume: $100m^{-3}$ $net^{-1}$) to assess > 2 mm ctenophore abundances (Haraldsson *et al*., in revision). Based on oral aboral lengths, we differentiate *M. leidyi* into adults > 6.5 mm, corresponding to the start of continuous egg production (Jaspers, 2012), and juvenile animals between 2 - 6.5 mm, here referred to as transitional animals. We assess active recruitment by the proportion of up-growing (transitional animals) to adult *M. leidyi* abundances.

***Sample preparation*** – Ctenophore larvae and eggs were individually counted and measured to the nearest 40 µm either live or after 2 % acidified Lugol preservation. Preserved specimens were corrected for shrinkage by applying a factor of 1.33 (Jaspers *et al*., 2012). For DNA analysis, live ctenophore larvae were individually dried on cellulose filters at c. 60˚C for 48 hours. For species identification, diagnostic primers were developed for the internally transcribed spacer 1 (ITS-1) region of the ribosomal RNA, based on an alignment of published sequences for the following ctenophore species: *Bolinopsis infundibulum*, *Beroe cucumis*, *Pleurobrachia bachei*, *Mnemiopsis leidyi* and *Mertensia ovum* following published protocols (Jaspers *et al*., 2012). The monthly cruises were grouped into season 1 to 4 for spring to winter, respectively. Stations of similar surface salinity were grouped into 3 clusters: Skagerrak/Kattegat (st. 1-2), southern Baltic (st. 3-4) and central Baltic (st. 5-8).

Subsamples of animals ≤ 2 mm (n = 348 for 12 monitoring cruises and n = 99 for the October process cruise) were identified with molecular analysis and used to estimate the relative contribution of *M. ovum* and *M. leidyi* in the different seasons and regions (n = 9494). In the Skagerrak/Kattegat other ctenophores (*Bolinopsis infundibulum*, *Beroe* spp and *Pleurobrachia pileus*) were present but primarily *P. pileus* occurred simultaneously with *M. leidyi* adults although at very low abundances.

***Statistical analysis*** - To investigate the relation between environmental variables and the occurrence as well as abundance of ctenophore larvae ≤ 2 mm in the Baltic Sea, a zero-inflated, mixed logistic-poisson generalized linear model analysis was conducted for *M. leidyi* and *M. ovum* larvae using R 2.13.1 (www.r-project.org). Continuous explanatory variables consisted of temperature, salinity and fluorescence (a proxy for phytoplankton biomass) and nominal explanatory variables included season, station and depth strata from where the animals originated. A binominal model on presence/absence of *M. leidyi* and *M. ovum* was conducted first and subsequently the logarithmic transformed abundance data (to meet normal distribution) were analyzed. Model selection was conducted by step-wise reduction of least significant explanatory variables based on significance level and Akaike information criteria with subsequent comparison of the full versus the nested model using anova function with $\chi^2$ specification. Models were cross validated by comparison with a generalized additive model and residual inspection for the abundance models.

***Mortality rates*** – For regions and seasons where only *M. ovum* or predominantly *M. leidyi* were found we used the log-ratio of observed egg abundance (# $m^{-2}$) to that expected on the assumption of no egg mortality as a proxy of egg mortality (log-ratio of 0 = no egg mortality). The expected egg abundance was computed as the estimated population egg production rate multiplied by the egg hatching time. Egg production rates for the two species were based on observed abundance and size distributions of ctenophores and size-dependent egg production rates (Jaspers *et al.*, 2011; Jaspers *et al.*, 2012 and unpublished data, see figure legend 6 for details). Hatching times of *M. leidyi* is 7 days at 7˚C (own unpublished observation) and 1 day at 18˚C (Jaspers, 2012) and assumed to apply to both species. Rates were corrected to *in situ* temperature assuming a $Q_{10}$ of 2.8 (Hansen *et al.*, 1997) for high and low temperature ranges, respectively.

***Sinking rate experiments*** **-** To examine whether sedimentation is a significant loss factor of eggs, we measured settling velocities in a steady water column at two different salinities (33 and 20) and two different egg ages. Eggs were spawned at the same salinities and individually transferred into a 5 x 5 x 20 cm plexi-glass column with a glass-pipette and deposited with as little water as possible at >10 egg diameters away from the walls. Eggs were illuminated with a laser sheet (650 nm wave length) fixed to a b/w CCD camera oriented perpendicular to the laser. The camera could be moved to allow for following the sinking eggs. Egg buoyancy was estimated from settling speed of 23 eggs with 3-5 video sequences analysed per egg. Analyzes were conducted as change of position over time correcting for turbulence by subtracting ambient particle velocity from settling velocity of the egg following (Lombard and Kiørboe 2010). Excess density ($p_{excess}$) of the spherical eggs was calculated from settling velocities (v, cm $s^{-1}$) using Stoke's law (Reynolds number <0.2) with the viscosity of seawater µ (g $cm^{-1}$ $s^{-1}$), the egg radius (r, cm) and the gravity (g, cm $s^{-2}$) as:

$$p_{excess} = \frac{9}{2}\frac{v\mu}{r^2 g} \qquad (1)$$

## RESULTS

***Ctenophore larvae distribution*** - Ctenophore larvae of *M. leidyi* and *M. ovum* ≤ 2 mm were present throughout all seasons in 2009/2010 as confirmed by molecular analysis (Fig. 2).

*M. leidyi* larvae were found mainly in the high saline areas, and their abundances decreased drastically along the surface salinity gradient into the Baltic Sea (Figs. 2,3). They did not extend further into the Baltic than the southern part (Bornholm Basin, Fig. 2). In the Bornholm Basin, *M. leidyi* larvae were absent only during summer (Fig. 2); in the Skagerrak/Kattegat area larvae were present in all seasons, even though we did not find adults between April and June (Haraldsson et al. in revision). Maximum densities of *M. leidyi* larvae were recorded in autumn at high saline Skagerrak station 1. Thus, generally, larvae of *M. leidyi* were distributed in surface waters along with adults in high saline areas during autumn and winter (Haraldsson *et al*., in revision, Fig. 2).

*M. ovum* larvae were present year round in the Bornholm Basin (Figs. 2,3). They are not restricted to the low saline central Baltic Sea but occurred also in the high saline Kattegat and Skagerrak although only during cold seasons (Fig. 2). *M. ovum* reached highest densities during winter and was absent from the shallowest, low saline station 8 during summer, when water temperatures were high (16 - 18˚C). The depth distribution changed with season: *M. ovum* was distributed in surface waters or throughout the water column in spring and winter, but resided at depth during summer and autumn (Fig. 2). In October 2009, abundances increased towards low salinity, deep stations in the central Baltic Sea (Fig. 3).

Results of binominal generalized linear model analysis of larval *M. leidyi* (Table Ia) and *M. ovum* (Table IIa) showed that the presence/absence of both species was significantly related to station, season and salinity. The occurrence of *M. ovum* was also significantly explained by temperature (Table IIa). On basis of abundance data, salinity remained significant together with station and season for invasive ctenophore larvae (Table Ib). For *M. ovum* the abundance of larvae was significantly explained by station, season, temperature and depth while salinity was insignificant (Table IIb). Models were cross-validated by comparison with generalized additive models and further inspection of residuals for abundance models showed no pattern and normal distribution with no indication of extreme values.

Throughout the 13 monitoring cruises, highest abundance and largest geographic extend of adult *M. leidyi* was observed in October 2009 (Haraldsson *et al*., in revision). Based on our high resolution investigation during October 2009, integrated *M. leidyi* adult abundances (> 6.5 mm) showed the same trend of decreasing densities with decreasing salinity (Fig. 4a) as observed for *M. leidyi* larvae (Fig. 3). Highest ratio of transitional (2 to 6.5 mm) to adult *M. leidyi* was observed in the Kattegat with a factor 10 lower ratio in the Bornholm Basin (Fig. 4b), suggesting that the fecundity and active recruitment decreases along the decreasing salinity gradient.

During October 2009, other ctenophore species (*Pleurobrachia pileus*, *Beroe* spp. and *Bolinopsis infundibulum)* were only present in the Skagerrak and Kattegat area. *M. leidyi* constituted on average 98 ± 3 % of the total adult ctenophore abundance based on our 300 µm MultiNet samples.

***Ctenophore eggs and mortality rates*** **-** The highest ctenophore egg abundances were observed at the high saline Skagerrak station 1 during summer (Fig. 5) along with first occurrence of largest sized *M. leidyi* adults (Haraldsson *et al*., in revision). On a monthly basis, high egg densities started to build up during the end of July in the Skagerrak and maximum observed egg abundance in that area was 6,000 eggs m$^{-2}$ during August 2009. In contrast, low egg densities were observed in the southern Baltic Sea (station 3) and at the shallow, low saline station 8, eggs were never found (Fig. 5).

Ctenophore eggs were not differentiated to species level since molecular analysis did not succeed from dried nor from 2% acid Lugol preserved egg samples. However, during the season when adult *M. leidyi* were present in large numbers (summer to autumn), only few other ctenophore species were present at very low densities in the high saline Skagerrak/Kattegat area only. Therefore, in these high saline areas, ctenophore eggs can be assumed to be predominantly *M. leidyi*. In the low saline central Baltic Sea on the other hand, only *M. ovum* was found; hence the eggs were assumed to be solely *M. ovum.* This allows for estimating species-specific mortality proxies in the two regions by use of their size distribution and published egg production rates. Expected egg production was very high in the Skagerrak, leading to an estimated abundance of 60,000 *M. leidyi* eggs m$^{-2}$ during August, while maximum observed ctenophore egg abundance was only 10% of that. Hence; mortality rates for *M. leidyi* eggs (expressed as log-ratios of observed to expected egg abundances) were substantial, ranging between -1 to -3 from the Skagerrak to Arkona. In contrast, calculated ratios for *M. ovum* in the central Baltic Sea during October 2009 showed an average log ratio of 0.5. This indicates that ctenophore egg mortality was substantial at intermediate (Arkona) to high (Skagerrak) salinity stations, but low in the central Baltic at low salinities (Fig. 6).

Egg sinking rates did not differ with regard to tested salinity or egg age, and the average overall egg sinking rate was 25 ± 5 m d$^{-1}$ (n = 23, Table 3).

## DISCUSSION

In both native and invaded habitats, small size classes or larval stages of the ctenophore *M. leidyi* often dominate populations (Deason, 1982; Costello *et al*., 2006; Javidpour *et al*., 2009), and larvae may have a seasonally substantial grazing impact (Deason, 1982; Waggett and Sullivan, 2006; Sullivan and Gifford, 2007; Riisgård *et al*., 2010). Other ctenophore populations like *M. ovum* can be dominated by small size classes as well (Gorokhova *et al*., 2009) and in regions may even exclusively consist of reproducing larvae (Jaspers *et al*., 2012). Despite their ecological importance, larvae and egg stages of ctenophores have been largely ignored in previous studies especially in the Baltic Sea, which has partly been due to identification problems or under-sampling (Gorokhova *et al*., 2009; Gorokhova and Lehtiniemi, 2010). In this study we demonstrate that quantitative sampling of ctenophore larvae and eggs is essential to understand the population dynamics of the invasive *M. leidyi* and the arctic relict species *M. ovum,* which are the most prominent ctenophores in the Baltic Sea (this study, Gorokhova *et al*., 2009).

Statistical analyses of the distribution of *M. leidyi* larvae based on occurrence and abundance data consistently showed a significant relationship with salinity, station and season. This is in accordance with the documented drastic effect of salinity on reproduction rates (Jaspers *et al*., 2011). For *M. ovum*, the occurrence model suggests that temperature is additionally an important factor, consistent with the Arctic distribution of the species outside the Baltic.

While *M. leidyi* larvae were present in the high saline Skagerrak/Kattegat area throughout the year, it is uncertain where or if adult *M. leidyi* overwinter in these regions. Hence, the origin of observed large sized adults in high saline areas during summer is not clear. In native habitats in northeastern USA, from where the Baltic population originates (Reusch *et al*., 2010), adult animals can overwinter under the ice in shallow embayments and generally re-establish from shoreward areas (Costello *et al*., 2006). In the Black Sea, on the other hand, where invaded *M. leidyi* originates from the southeastern USA (Reusch *et al*., 2010), temperatures < 4˚C have been shown to be lethal and re-establishment occurs from core areas with higher winter temperatures (Shiganova *et al*., 2001). In extreme cases as shown for the Sea of Azov, *M. leidyi* is dependent on reintroduction every year due to low winter temperatures (Shiganova *et al*., 2001).

While adult *M. leidyi* have been found in coastal waters during winter in the North Sea (Van Ginderdeuren *et al*., 2012), *M. leidyi* adults were not observed in the Baltic Sea between April to June 2010 after the Kattegat had been partly frozen (Haraldsson *et al*., in revision). We can not exclude that adults were present but missed by our sampling since *M. leidyi* may accumulate in dense layers close to the sea floor (Costello and Mianzan, 2003). However, due to the starvation capacity and high growth rates of *M. leidyi* larvae (Stanlaw *et al*., 1981), overwintering larvae may be a source of large sized adults as observed during summer in the Skagerrak.

Although we did not differentiate ctenophore eggs to species level, eggs could anyway be referred to species with high likelihood in areas or seasons where one or the other of the two species dominated entirely. Also, it is unlikely that other ctenophore species contributed significantly to observed abundances. During the peak in egg abundance, only *P. pileus* was present but at a factor 10 (end of July) to 100 (August) lower densities than *M. leidyi* in the Skagerrak/Kattegat. Furthermore, maximum egg production in *P. bachei* is 2 orders of magnitude lower (Hirota, 1972) than for *M. leidyi* (Jaspers, 2012). We show that peak ctenophore egg abundances occurred in the Skagerrak during August 2009 with c. 2 months delayed adult *M. leidyi* abundance peak in the southern Kattegat including the Danish straits (this study, Haraldsson *et al*., in revision). This suggests that even though a self-sustained subpopulation may exist in the western Baltic Sea (Javidpour *et al*., 2009), invasions from high saline areas can be important to maintain populations in such pockets in the longer term. *M. leidyi* has a very high egg production capacity with up to 11,500 eggs ind.$^{-1}$ d$^{-1}$ (Jaspers, 2012). Nevertheless, observed densities of ctenophore eggs in high saline areas was only 0.1-10% of that expected from *M. leidyi* egg production rates and hatching times, indicating that egg mortality rates were high in these regions. In contrast, egg mortality rates of *M. ovum* in the central Baltic were low. High egg mortality can partly be due to loss by sedimentation, partly due to predation. During August at 16˚C water temperature, hatching time is on the order of 1-2 days (Jaspers, 2012). Therefore, eggs will sink approximately 25 to 50 m before they hatch and a significant fraction may reach the sea floor prior to hatching in shallow areas. Egg mortality due to sinking is a potential contribution to the high mortality rates especially in the Kattegat/Skagerrak and into the southern Baltic Sea where the water depth at our investigated stations ranged between 14 to 65 m. In the central Baltic, ctenophore eggs were only observed at deep stations (up to 240 m), and not at the only shallow water station (st. 8, 20 m). The contribution of fish predation to high mortality rates in European waters is unknown but for coastal waters of Argentina, it has been shown that 15 to 23 % of the fish species prey on ctenophores (Mianzan *et al*., 1996). Investigations are needed to qualify and quantify potential predators and their impact in order to appropriately link ctenophores in the food web not only of the Baltic Sea.

The *M. ovum* population in the central Baltic is self-sustained by larval reproduction, but reproductive rates are several orders of magnitude lower than in *M. leidyi*. This is compensated for by short generation times (Jaspers *et al*., 2012) and low egg mortality rates (Fig.6). We found larvae of *M. ovum* in Skagerrak/Kattegat, but only during winter and spring and presumably advected here with surface water outflow from the source population in the Baltic proper. *M. ovum* is an arctic species and the Baltic population likely a relict adapted to the local conditions. High mortality rates of ctenophore eggs in the Kattegat/Skagerrak area may restrict the species to the central Baltic Sea.

We conclude that assessing early life stages including eggs is essential to understanding the range expansion and population dynamics of ctenophores. Without considering all age classes, recruitment processes, the onset of population expansion and possible source and sink regions of especially invasive species remain unnoticed. Our data support that *M. leidyi* in the southern Baltic Sea is dependent on drift recruitment from its preferred habitat ranges, namely higher saline areas such as the Kattegat and Skagerrak. These high saline areas are important for population recruitment due to the year round presence of larvae and early occurrence of large sized adults with high reproduction potential. Despite substantial egg mortality rates in high saline areas, the Skagerrak seems to seeds the new *M. leidyi* generation in the southern Kattegat and gives rise to the population peak during autumn. *M. ovum* on the other hand sustains through larval reproduction in low saline areas where egg mortality rates are low.

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**Table I.** Summary table for invasive *Mnemiopsis leidyi* larvae in relation to environmental explanatory variables in the Baltic Sea from generalized linear model analysis. Depth corresponds to depth strata sampled and season resulted from grouping of 13 monthly monitoring cruises into 4 seasons. Step-wise model reduction of least significant explanatory variables was performed based on significance level and Akaike information criteria (AIC) with afterwards comparison of full versus nested model using anova function with chi square specification (see *p*-value). **A)** Binominal model for presence/absence data with the most parsimonious model including salinity, station and season (AIC: 231.2). **B)** Gaussian model for ln transformed abundance data following the same procedure with the final model including the same variables (AIC: 269.8).

| | **variable** (to be dropped) | **df** | **Resid. deviance** | **Resid. df** | **AIC** | ***P*** ($\chi^2$) |
|---|---|---|---|---|---|---|
| **a** | full model | - | 197.0 | 359 | 233.0 | |
| | -temperature | 1 | 198.5 | 360 | 232.5 | 0.230 |
| | -fluorescence | 1 | 199.3 | 361 | 233.3 | 0.130 |
| | -salinity | 1 | 201.8 | 360 | 235.8 | **0.028**$^{*}$ |
| | -depth | 4 | 203.2 | 365 | 231.2 | 0.188 |
| | -station | 7 | 319.5 | 372 | 343.5 | **<0.0001**$^{***}$ |
| | -season | 3 | 241.9 | 368 | 272.9 | **<0.0001**$^{***}$ |
| **b** | full model | - | 109.6 | 63 | 275.7 | |
| | -temperature | 1 | 110 | 64 | 274 | 0.63 |
| | -fluorescence | 1 | 122.1 | 69 | 272 | 0.051 |
| | -salinity | 1 | 133.4 | 69 | 278.8 | **0.0012**$^{**}$ |
| | -depth | 4 | 115.6 | 68 | 269.8 | 0.509 |
| | -station | 3 | 140.3 | 71 | 278.7 | **0.0023**$^{**}$ |
| | -season | 3 | 130.7 | 71 | 273.3 | **0.0310**$^{**}$ |

**Table II.** Summary table for *Mertensia ovum* larvae in relation to environmental explanatory variables in the Baltic Sea from generalized linear model analysis with depth corresponding to depth strata sampled and season resulted from grouping of 13 monthly monitoring cruises. Step-wise model reduction of least significant explanatory variables was performed based on significance level and Akaike information criteria (AIC) with afterwards comparison of full versus nested model using anova function with chi square specification (see *p*-value). **A)** Binominal model for presence/absence data with the most parsimonious model including salinity, temperature, station and season (AIC: 264.4). **B)** Gaussian model for ln transformed abundance data following the same procedure with the final model including depth, station and season, while temperature being border-line significant and remained in the final model (AIC: 665).

| | **variable** (to be dropped) | **df** | **Resid. deviance** | **Resid. df** | **AIC** | ***P*** ($\chi^2$) |
|---|---|---|---|---|---|---|
| **a** | full model | - | 237.7 | 359 | 273.7 | |
| | -fluorescence | 1 | 237.7 | 360 | 271.7 | 0.969 |
| | -salinity | 1 | 242.9 | 361 | 274.9 | **0.023**$^{*}$ |
| | -temperature | 1 | 320.7 | 361 | 350.7 | **<0.0001**$^{***}$ |
| | -depth | 4 | 238.4 | 364 | 264.4 | 0.950 |
| | -station | 6 | 261.0 | 366 | 282.0 | **<0.0007**$^{***}$ |
| | -season | 3 | 309.7 | 367 | 329.7 | **<0.0001**$^{***}$ |
| **b** | full model | - | 296.6 | 175 | 668.6 | |
| | -salinity | 1 | 296.7 | 176 | 666.7 | 0.791 |
| | -fluorescence | 1 | 297.2 | 177 | 665.0 | 0.590 |
| | -temperature | 1 | 303.4 | 178 | 667 | 0.055$^{(*)}$ |
| | -depth | 4 | 355.4 | 181 | 691.5 | **<0.0001**$^{***}$ |
| | -station | 6 | 366.7 | 183 | 693.6 | **<0.0001**$^{***}$ |
| | -season | 3 | 361.8 | 180 | 699 | **<0.0001**$^{***}$ |

**Table III.** Sinking rates of *Mnemiopsis leidyi* eggs for salinities of 33 (A) and 20 (B), testing two age groups of young (<8) and old (<32 hours after spawn) eggs, respectively. Sinking velocities did not significantly different between salinity and egg age (1-way ANOVA on log transformed data: $F_{3,19}$=0.30, p=0.828, n=23) leading to an average sinking rate of 25 ± 5 m $d^{-1}$.

| | **Age** | **Eggs (n)** | **Total obs. (n)** | **Diameter (µm)** | **Sink speed (m $d^{-1}$)** | **Reynolds number** | **Water density (g $cm^{-3}$)** | **Dynamic viscosity (g $cm^{-1}$ $s^{-1}$)** | **Excess density (g $cm^{-3}$)** |
|---|---|---|---|---|---|---|---|---|---|
| **a** | young | 8 | 18 | 600 | 24.34±4.81 | 0.16±0.03 | 1.023 | 0.0107 | 0.0015 |
| | old | 6 | 13 | 630 | 25.86±5.09 | 0.18±0.04 | 1.024 | 0.0106 | 0.0015 |
| **b** | young | 5 | 15 | 480 | 23.38±5.79 | 0.12±0.03 | 1.015 | 0.0111 | 0.0024 |
| | old | 4 | 11 | 550 | 25.60±5.68 | 0.15±0.04 | 1.014 | 0.0106 | 0.0019 |

## FIGURE LEGENDS

**Fig. 1.** Investigation area in the Baltic Sea with identification of stations sampled monthly (crosses and numbers) and more intensively during October (stars). The stations were grouped into regions with n = number of stations sampled (some stations sampled twice) in the respective regions (Skagerrak included one station in the northern Kattegat, indicated by $^{z}$) during October.

**Fig. 2.** Seasonal depth distribution of ≤ 2 mm ctenophore larvae from 13 monthly monitoring cruises with 8 stations in 2009/2010. Average sampling depth is indicated by cross and average ctenophore abundances $m^{-3}$ for each discrete depth strata by bar (±SD, grouped per season), with bottom depth at the respective stations indicated by grey filling. Sampling depth for station 1 to 8: 37, 37, 29, 70, 70, 200, 180 and 17 m, respectively. DNA ratios with contribution of *Mnemiopsis leidyi* (black) and *Mertensia ovum* (white) (≤ 2 mm larvae, n = 1340 *M. leidyi*, n = 8154 *M. ovum*) are shown as pie charts for the different seasons and pooled for surface salinity regions Skagerrak/Kattegat (stations 1,2; DNA samples n = 116), Arkona/Bornholm Basin (stations 3,4; DNA samples n = 111) and central Baltic (stations 5-8; DNA samples n = 121) as indicated by black bar in each row. In total 13 *M. ovum* between 2.1 to 3.3 mm were found (average, SD: 2.5 ± 0.43 mm) plus one 6.5 mm, corresponding to 0.17 % of all *M. ovum* caught.

**Fig. 3.** Abundances ($m^{-2}$) of larvae (≤ 2 mm) of *Mnemiopsis leidyi* (black) and *Mertensia ovum* (hatched) and surface salinity (average, upper 10 m) for 32 samplings in the Baltic Sea, October 2009. Species differentiation was based on DNA ratios (n = 99). The Sound has been added as additional region connecting the southern Baltic with the Kattegat and is separated from the two Arkona stations by dotted line.

**Fig. 4.** *Mnemiopsis leidyi* abundances during peak season October 2009 in 4 regions: a) integrated adult abundances $m^{-2}$ (> 6.5 mm oral aboral length, bar ±SD) and average salinity for the upper 10 m (black points ±SD) and, b) transitional (2-6.5 mm) to adult ratios based on raw counts per net including 95 % confidence intervals.

**Fig. 5.** Average seasonal ctenophore egg abundances $m^{-2}$ (±SD) for station 1 to 8 from 13 monthly cruises in 2009/2010. Bottom depth (m) indicated by hatched area, right y-axis.

**Fig. 6.** Log-ratio of observed to expected ctenophore eggs for regions with predominantly *M. leidyi* or *M. ovum* based on DNA analysis (log-ratio = 0, no mortality). Regression for expected egg production for *M. leidyi* (oral aboral length) were adjusted to a standard temperature of 15˚C (Hansen *et al.*, 1997) for Skagerrak/Kattegat: $\log(\text{eggs ind}^{-1}\text{d}^{-1})=4.77\text{x}\log(\text{length, mm})-4.44$ ($R^2 = 0.69$, $p < 0.0001$) and for the Sound $\log(\text{eggs ind}^{-1}\text{d}^{-1})=2.44\text{x}\log(\text{length, mm})-1.40$ ($R^2 = 0.63$, $p < 0.0001$) and for *M. ovum* is $\text{eggs ind}^{-1}\text{d}^{-1}=0.67 \text{ x length(mm)}^{3.2}$ ($R^2 = 0.24$, $p = 0.016$) for 0.75-1.33 mm size range (Jaspers *et al.*, 2012).

**Fig. 1**

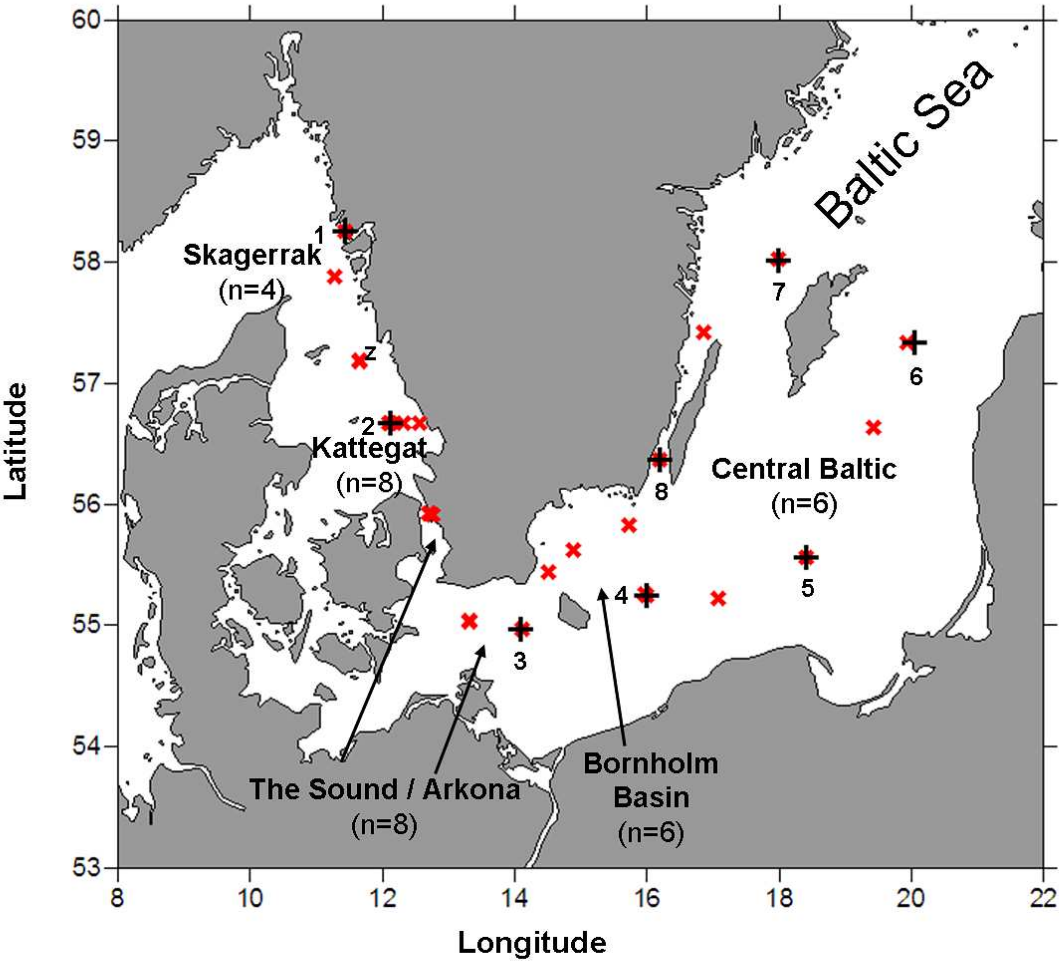

Baltic Sea
Skagerrak
(n=4)
Kattegat
(n=8)
Central Baltic
(n=6)
The Sound / Arkona
(n=8)
Bornholm
Basin
(n=6)
1
2
3
4
5
6
7
8
z
60
59
58
57
56
55
54
53
8
10
12
14
16
18
20
22
Latitude
Longitude

**Fig. 2**

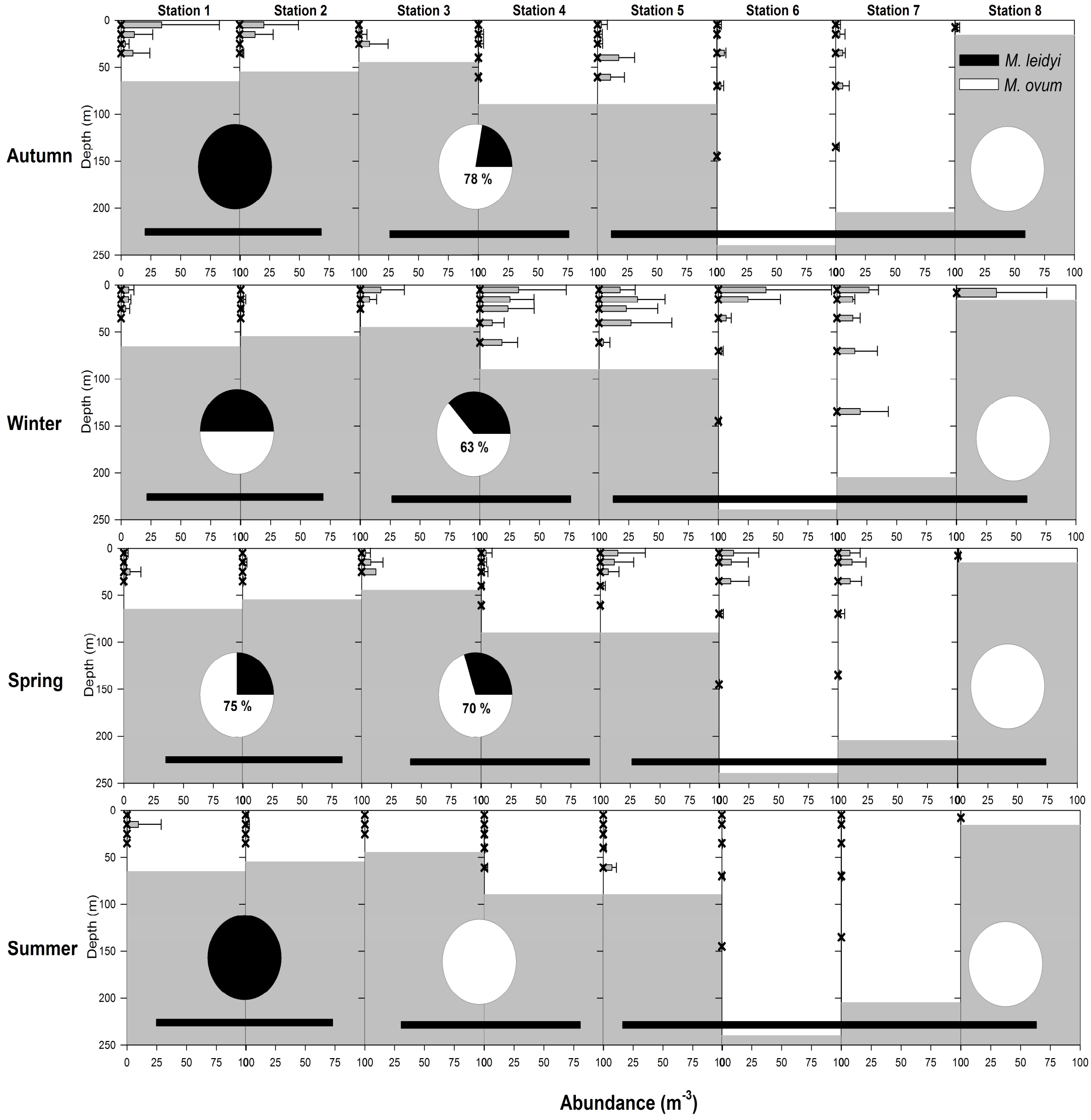

Station 1
Station 2
Station 3
Station 4
Station 5
Station 6
Station 7
Station 8
M. leidyi
M. ovum
Autumn
Winter
Spring
Summer
Depth (m)
0
50
100
150
200
250
0
25
50
75
100
78 %
63 %
75 %
70 %
Abundance (m-3)

**Fig. 3**

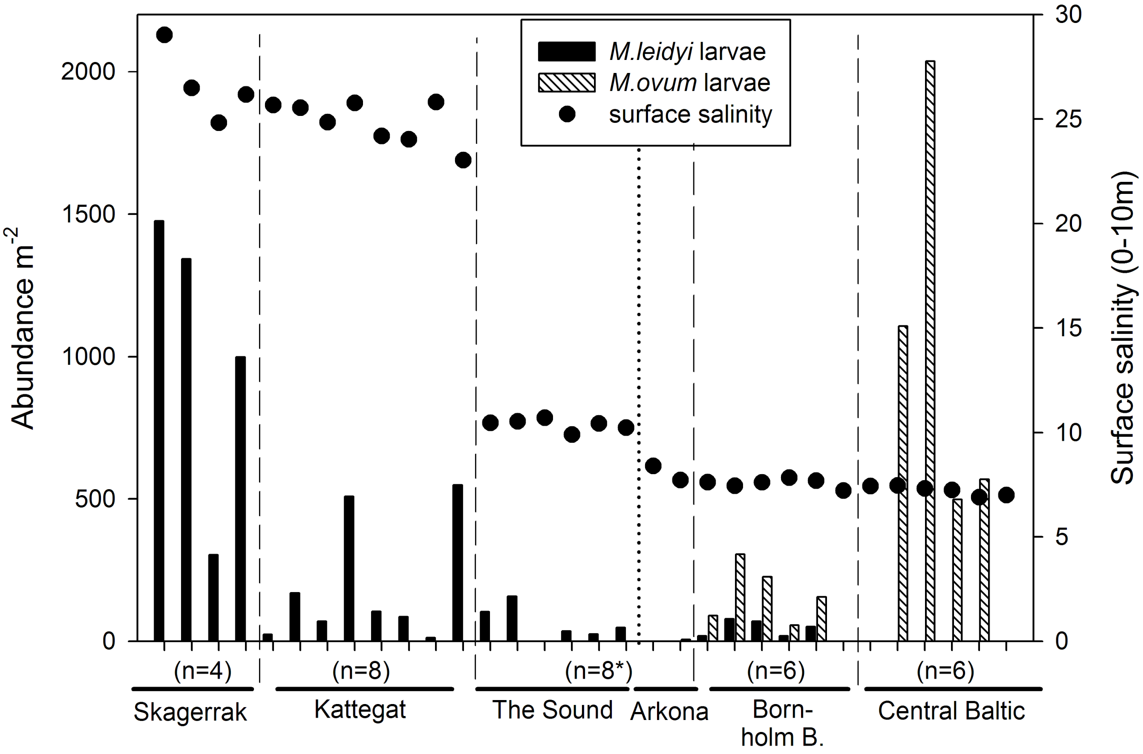

*M.leidyi* larvae
*M.ovum* larvae
surface salinity
Abundance m$^{-2}$
0
500
1000
1500
2000
Surface salinity (0-10m)
0
5
10
15
20
25
30
(n=4)
(n=8)
(n=8*)
(n=6)
(n=6)
Skagerrak
Kattegat
The Sound
Arkona
Born-
holm B.
Central Baltic

**Fig. 4**

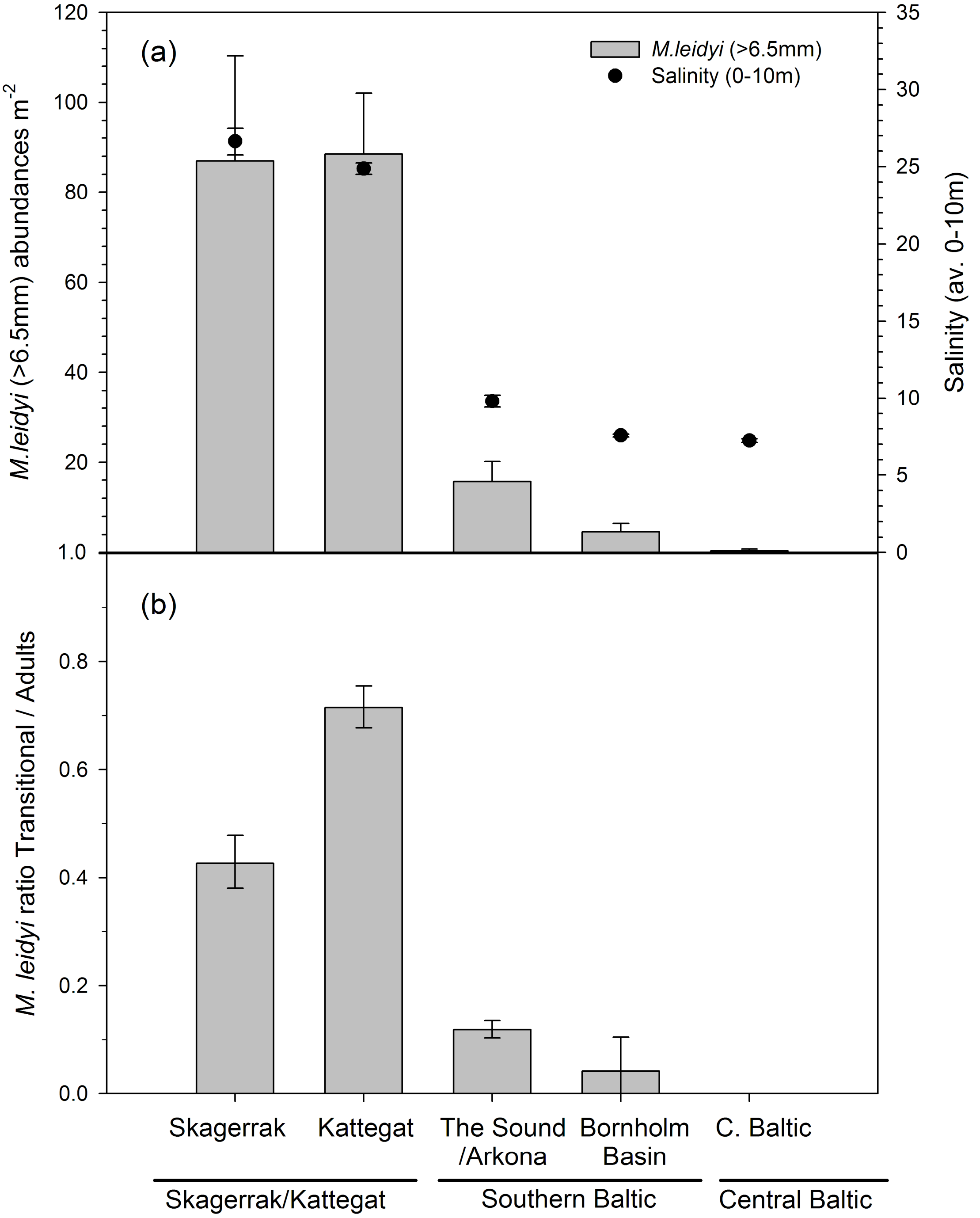

(a)
(b)
*M.leidyi* (>6.5mm)
Salinity (0-10m)
*M.leidyi* (>6.5mm) abundances m$^{-2}$
Salinity (av. 0-10m)
*M. leidyi* ratio Transitional / Adults
120
100
80
60
40
20
1.0
0.8
0.6
0.4
0.2
0.0
35
30
25
20
15
10
5
0
Skagerrak
Kattegat
The Sound /Arkona
Bornholm Basin
C. Baltic
Skagerrak/Kattegat
Southern Baltic
Central Baltic

**Fig. 5**

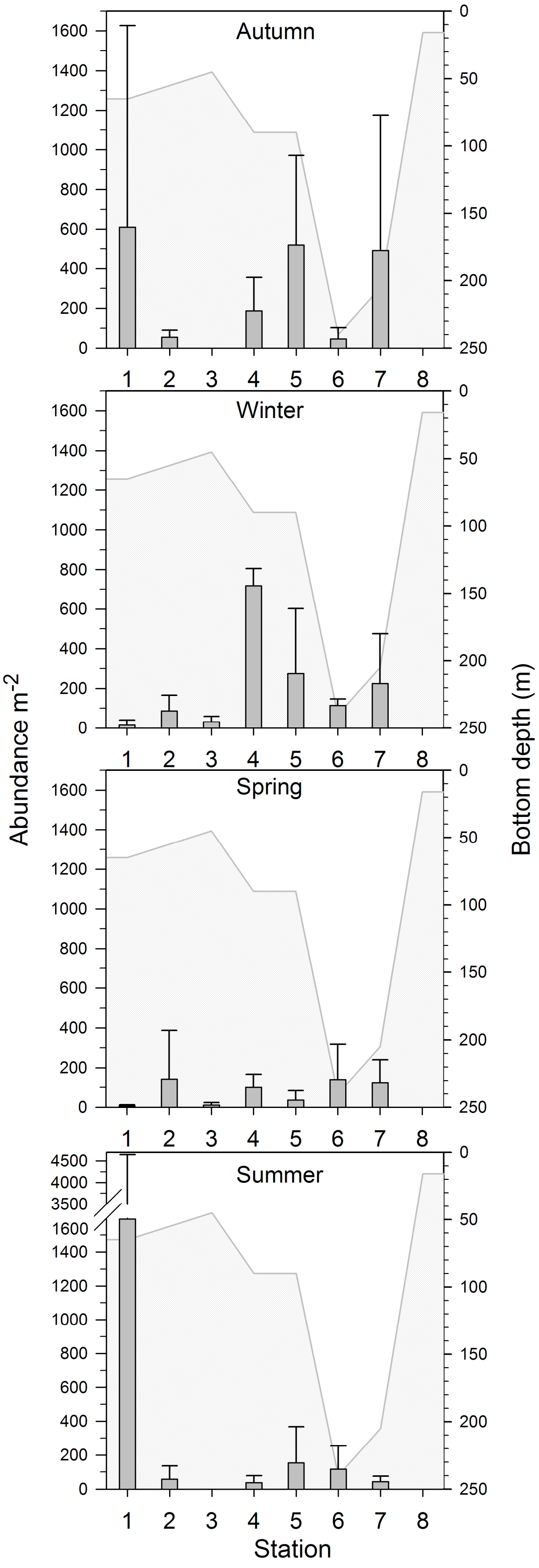
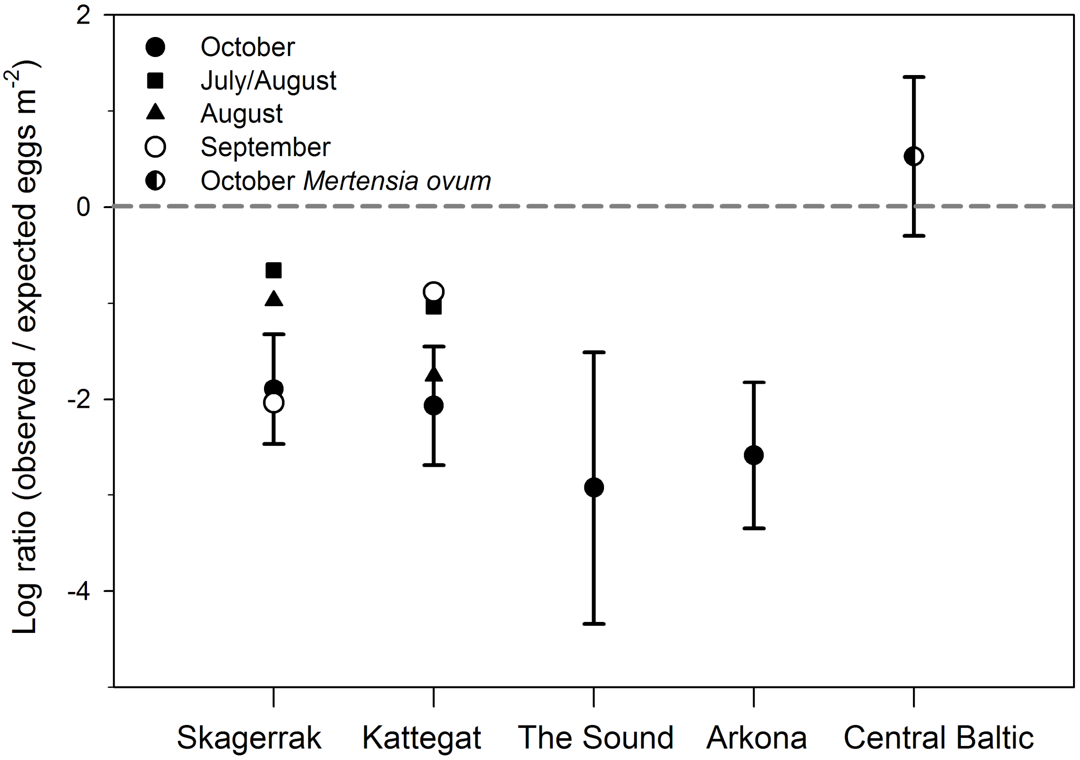

Autumn
Winter
Spring
Summer
Abundance m$^{-2}$
Bottom depth (m)
Station
0
200
400
600
800
1000
1200
1400
1600
3500
4000
4500
0
50
100
150
200
250
1
2
3
4
5
6
7
8

**Fig. 6**


Log ratio (observed / expected eggs m$^{-2}$)
2
0
-2
-4
● October
■ July/August
▲ August
○ September
◐ October *Mertensia ovum*
Skagerrak
Kattegat
The Sound
Arkona
Central Baltic

# Paper V

# Environmental constraints of the invasive *Mnemiopsis leidyi* in Scandinavian waters

Matilda Haraldsson [1*], Cornelia Jaspers [2], Peter Tiselius [1], Dag L. Aksnes[3], Tom Andersen [4], Josefin Titelman [4]

[1] University of Gothenburg, Department of Marine Ecology – Kristineberg, SE-45178 Fiskebäckskil, Sweden

[2] Technical University of Denmark, National Institute of Aquatic Recourses, Charlottenlund Slot, DK-2920 Charlottenlund, Denmark

[3] University of Bergen, Department of Biology, N-5020 Bergen, Norway

[4] University of Oslo, Department of Biology, POB 1066 Blindern, N-0316 Oslo, Norway

* Corresponding Author.

## ABSTRACT

We studied the seasonal dynamics of the invasive ctenophore Mnemiopsis leidyi over its distribution range from Skagerrak into the Baltic Proper during one year, and related this to ambient physical and biological variables. The appearance of *M. leidyi* was sporadic in the Baltic Proper, with sixtyfold lower abundance than in the Skagerrak and Kattegat (mean 0.02 and 1.26 ind m-3 respectively). *M. leidyi* typically resided above the halocline (10-20 m depth) except in the Baltic Proper where they were found deeper. Smaller size classes were underrepresented in the Baltic Proper, indicating failed reproduction or high mortality of the younger life stages. Most (90%) of the *M. leidyi* were observed at salinities of 22-29 and 75% of the individuals in water masses warmer than 11°C, i.e. typical late summer to autumn temperatures. Results of a Generalized Additive Model (GAM) indicate that the spatio-temporal distribution of *M. leidyi* might be explained by advection of *M. leidyi* from the Skagerrak and Kattegat area to the Baltic Proper. We conclude that the low salinity (< 9) of the Baltic Proper is likely to restrict successful reproduction and establishment of *M. leidyi*, and that the advection of individuals from higher saline source areas sustained the observed Baltic Proper occurrence.

## INTRODUCTION

Rapid population growth and tolerance to a wide range of environmental conditions are typical traits of successful invasive organisms (Lee and Gelembiuk 2008). These traits characterize the ctenophore Mnemiopsis leidyi, which is considered as a notorious invasive species having invaded a wide range of novel habitats (Lowe et al. 2000). *M. leidyi* has a wide latitudinal native occurrence extending along the North American Atlantic coast (Purcell et al. 2001) and further south along the Argentinean coastline (Mianzan et al. 2010). It was first introduced, presumable via ballast water, into the Black Sea in 1982 (Shiganova et al. 2001), later into the eastern Mediterranean and Caspian Seas (Ivanov et al. 2000), and more recently into the North and Baltic seas (Javidpour et al. 2006; Boersma et al. 2007) and into the western Mediterranean Sea (Shiganova and Malej 2008; Fuentes et al. 2009). The invasion throughout Eurasian coastal waters has generated immense public, political and scientific attention due to its potential ecological and environmental effects.

In its native and exotic habitats *M. leidyi* tolerates wide ranges of temperature and salinity (Fig. 1; Table 1), and it can also withstand low oxygen levels (Kolesar et al. 2010). As a simultaneous hermaphrodite *M. leidyi* may rapidly grow and reach high numbers during favorable conditions (Purcell et al. 2001). The typical seasonal abundance pattern in temperate regions suggests a positive relation with temperature, reflecting that growth and egg production (Costello et al. 2006) increase with temperature. Salinity effects are less studied, but *M. leidyi* appears to have a wide salinity tolerance, depending on population (Fig. 1; Table 1). Nevertheless, egg production has been shown to be substantially suppressed under low salinities (<10), which may constrain *M. leidyi* in the Baltic Sea (Jaspers et al. 2011). *M. leidyi* feeds omnivorously throughout its life (Sullivan and Gifford 2004), and can tolerate long periods of starvation (Purcell et al. 2001). Tentaculate larvae capture nano- and micro-plankton (Sullivan and Gifford 2004), and lobate adults entrain mesozooplankton and ichtyoplankton in a feeding current (Purcell et al. 2001). Turbulence may directly interfere with feeding behavior and also affect vertical position through downward mixing (Mianzan et al. 2010). Also, vertically stratified waters seem to contain higher abundances than unstratified ones (Mianzan et al. 2010).

In Scandinavian waters *M. leidyi* was first observed in 2005 in the Oslo fjord (Oliveira 2007) and a year later in Skagerrak, Kattegat and the Baltic Proper (Hansson 2006; Javidpour et al. 2006) and adult *M. leidyi* has been observed as far as the Gulf of Gdańsk in the southern Baltic Proper (Janas and Zgrundo 2007). A pronounced salinity gradient characterizes this area where salty North Sea water gradually mixes with the northward flowing brackish water from the Baltic Sea (Reissmann et al. 2009). A permanent stratification is present in the area with the deepest halocline in the Baltic Sea. Previous samplings of *M. leidyi* in the Baltic Proper region have covered limited geographical areas, but reported on the local seasonal variation. A pronounced seasonal abundance pattern seems typical for *M. leidyi* in this region, with peak adult abundance during autumn or early spring (Javidpour et al. 2009; Riisgård et al. 2010; Schaber et al. 2011). Here we describe the seasonal dynamics of *M. leidyi* in Skagerrak, Kattegat and the Baltic Proper, and examine how the physical and biological environment shapes its temporal and spatial distribution.

## MATERIALS AND METHODS

***Sampling program*** - We conducted monthly sampling of gelatinous plankton and various biological and physical parameters along a transect of 8 monitoring stations (1-8) from Skagerrak into the central Baltic Proper (Fig. 2; Table 2). In total 12 cruises were carried out onboard R/V Argos from May 2009 until April 2010. An additional 13 stations (A-M) in Skagerrak, Kattegat and the Baltic Proper were sampled together with the 8 monitoring stations during October 2009 onboard R/V Skagerak (Fig. 2). Also, during December 2009, 9 additional stations were sampled in the Gulf of Bothnia (R/V Argos) (Fig. 2). Unless specifically noted only data from the 8 monitoring stations are included in the analyses. At each station, temperature, conductivity, chlorophyll fluorescence and oxygen were measured from the surface to near the bottom using a CTD Seabird SBE9+ equipped with a SBE 43 oxygen sensor. Water for chlorophyll a (chl-a) measurements were collected at 5 m intervals from the surface to 20 m followed by 10 m intervals down to 50 m depth. Mesozooplankton were sampled using a WP-2 net with 90 µm mesh size from 25-0 m in the Skagerrak and Kattegat, and from 30-0 m in the Baltic Proper. Samples were preserved with buffered formalin and organisms were identified and counted under dissecting microscope. CTD-profiles, chl-a (uncorrected for phaeopigment) and zooplankton were part of the Swedish Meteorological and Hydrological Institute’s (SMHI) national monitoring program in cooperation with the Swedish environmental protection agency. These data are publicly available at SMHI’s SHARK database (Svensk HavsARKiv).

***Sampling of Mnemiopsis leidyi*** - Gelatinous plankton were collected using a 300 µm mesh size opening and closing net (Midi MultiNet ®, Hydro-bios, Kiel), allowing for 5 vertically stratified samples within one tow. The net was towed horizontally at 1-2 knots, while slowly being retrieved from the deepest depth to surface (Table 2). To increase the sampled water volume the retrieval was regularly stopped at predetermined intervals. The sampled water volume was determined from direct flow meters recordings by the Multinet. All gelatinous plankton >2 mm were identified and sized over a transparent back lit table. Samples were stored at 4°C prior to analysis to avoid degradation of ctenophores. Analysis was conducted within 1-2 hours after collection. Oral-aboral lengths (Lo-a) were measured to the nearest mm for ctenophores, and distance between opposite rophalia for schyphomedusae. The remaining samples were preserved in 4% buffered formalin. Only *M. leidyi* >2 mm are reported here, the smaller specimens and other gelatinous plankton will be reported elsewhere (C. Jaspers and M. Haraldsson, unpubl).

***Data analysis*** - The integrated abundance (A, ind m-2), mean depth (Zm, m) and standard deviation (Zs, m) of *M. leidyi* vertical distribution were calculated as:

$$A = \sum_{i=1}^{n} \Delta Z_i D_i$$

$$Z_m = \frac{\sum_{i=1}^{n} \Delta Z_i D_i Z_i}{A}$$

$$Z_s = \sqrt{\frac{\sum_{i=1}^{n} \Delta Z_i \, D_i Z_i^{2}}{A} - Z_m^{2}}$$

where Di represents the average *M. leidyi* concentration (ind m-3) of the sampled depth layer ΔZi , Zi is the mid-depth of each layer i , and n is the number of depth layers (Table 2).

We modeled the probability of *M. leidyi* presence by Generalized Additive Models (GAMs, Hastie and Tibshirani 1986) of the binomial family, using generalized cross validation to find the optimal degrees of freedom for each spline smoother (the “mgcv” package, Wood 2006). The dependent variable in all models was presence / absence of *M. leidyi* in depth-pooled data for each station and sampling time, while the independent variables represented either spatio-temporal positions or environmental conditions. Distance from station 1 and week of the year were used as predictor variables in the spatio-temporal model (ST), and salinity, temperature and oxygen, in the full environmental model (ENV). Biotic variables like phyto- and zooplankton abundances were not used because *M. leidyi* might affect their prey rather than vice versa. The simplest model was exclusively chosen, using backwards elimination. Residual plots and plots of fitted against observed values were used for model evaluation. Since the spatio-temporal and environmental variables were highly correlated ($r > 0.6$), we partitioned the sources of variation based on a method introduced by Borcard et al. (1992). The basic principle of Borcard et al. (1992) is that the total explained variance can be partitioned into 3 components: spatio-temporal variation ([a]), environmental variation ([c]), and variance shared between spatio-temporal and environmental sources ([b]). Fitted GAM models using ST, ENV, and ST + ENV as predictors will have explained variances equal to [a] + [b], [b] + [c], and [a] + [b] + [c] respectively. From this information, the 3 variance components ([a], [b], and [c]) can be computed by simple arithmetic expressions (Borcard et al. 1992). Since our analysis uses a non-normal probability model, we used generalized variances (deviances) in the partitioning calculations, which are expressed as percentage of the total variation, represented by the deviance of a null model without any predictor variables. The resulting deviance partitioning was visualized as a Venn diagram generated by the “VennDiagram” package in R.2.11.1 (www.r-project.org).

## RESULTS

***Environmental variables*** - A strong surface salinity gradient, ranging from 25-33 in the Skagerrak to 7-8 in the Baltic Proper, was present (Fig. 3). A pronounced halocline existed at all stations. The halocline was generally shallower in Skagerrak and Kattegat (5-20 m) than in the central Baltic Proper (50-70 m). During February, March, September and December larger volumes of saltier water (i.e. salinity > annual average salinity at respective station) were observed at several stations (>60% of the stations) (Fig. 3), indicating an increased deep water inflow from the North Sea into the Baltic Sea. Density profiles generally correlated well with salinity (not shown). Temperature showed strong seasonal changes and a pronounced thermocline was observed in July-October at 10-20 m in the Skagerrak and Kattegat and at 25-30 m in the Baltic Proper. The winter 2009-2010 was extraordinary cold and larger regions were ice covered from January-March.

Oxygen and chl-a concentrations varied in a similar fashion as temperature. Oxygen concentrations were generally high (4.4-9.5 ml L-1), with the exception of a permanent hypoxic layer below 60 m in the Baltic Proper. Chl-a concentrations peaked in the surface layers in April and November at most of the stations (Fig. 3). Mesozooplankton abundances were highest during late spring and early summer and in Skagerrak and Kattegat abundances decreased drastically in August. Mesozooplankton in the Baltic Proper were slightly lower with abundances of 10-40 ind L-1 (Fig. 3).

***Seasonal and spatial variation in abundance*** - Throughout the year we found *M. leidyi* at five of the eight monitoring stations (station 1-5; Fig. 4A), and abundances varied with season. *M. leidyi* was continuously present in Skagerrak and Kattegat from July to January, while only occasionally in the Baltic Proper between September and February. In the Baltic Proper *M. leidyi* never extended further east than station 5 located at the southern Gotland basin (Fig. 2). These patterns were reflected by the spatio-temporal GAM model (ST). Space and time accounted for 69% of total deviance in the GAM for *M. leidyi* presence (Table 3), where the smoother for location indicated a linear relationship. The average abundances were sixty times larger in Skagerrak and Kattegat (mean ± SD: 1.26 ± 1.73 ind m-3) than in the Baltic Proper (0.02 ± 0.04 ind m-3). Peak abundances occurred in September-October. The highest abundance recorded at a regular monitoring station was 6.69 ± 3.7 ind m-3 at station 2 (Fig. 4A). However, during the extended October cruise abundances up to 13.4 ind m-3 were recorded in Kattegat at station C, and *M. leidyi* was also found further along the Swedish coast in the South Western Baltic Proper (station H and I; Fig. 2 and 4B). No *M. leidyi* were found in the Gulf of Bothnia during December 2009 (Fig. 2), even though *M. leidyi* were present in the southern Baltic Proper (Fig. 2, station 3-4).

***Vertical distribution and size*** - The mean depth (Zm) of *M. leidyi* was shallower and the vertical spread (Zs) was narrower in the Skagerrak, Kattegat and the Sound stations compared to the Baltic Proper stations (Fig. 4B and 5). In Skagerrak, Kattegat and southwestern Baltic Proper (station 1-3), most individuals were found between 7-20 m. In Skagerrak (station 1) individuals were located deeper during the winter months (November-January Zm: 14.2-19.0 m) than in July - October (Zm: 8.4-11.5 m). The opposite pattern was observed at the southeastern Baltic Proper (station 3), with individuals shallower during November-December (Zm: 7.8-9.4 m) compared to September-October (Zm: 16.8-20.7 m). Generally, *M. leidyi* was found above the halocline, with exception of station 4 in the Bornholm basin (Baltic Proper) in December and February (Fig. 6). Also in Skagerrak (station 1), where the halocline was generally very shallow, the mean depth of *M. leidyi* was occasionally located immediately below the halocline (Fig. 6).

The size distribution of *M. leidyi* was wider in the Skagerrak and Kattegat than in the Baltic Proper (Fig. 7). The smaller size classes (<10 mm) increased during September to December in Skagerrak and Kattegat, while these classes were generally few or lacking at the Baltic Proper stations.

***Mnemiopsis leidyi* in relation to the environmental variables** – Most *M. leidyi* were found within a narrow range of salinity with 80% of the individuals within a salinity of 22-29, salinities typical in the Skagerrak and Kattegat region (Fig. 8). The distribution was skewed towards the higher salinities of the observed range (Fig. 8). 50% of the individuals occurred within a temperature of 11-16.6°C, and only 10% of the individuals were found at temperatures <8°C (Fig. 8). Animals were abundant at a wide range of oxygen concentrations (Fig. 8). Most ctenophores were present at chl-a levels of 0.7-3.8 µg L-1, and mesozooplankton concentrations of 0.07 – 21.30 ind L-1 (Fig. 8).

Our final environmental GAM (ENV) for *M. leidyi* presence suggests that variations in temperature ($p = 0.04$) and salinity ($p = 0.0003$) accounted for 29% of the total variation (the reduced ENV, Table 3; Fig. 9A and B), with a temperature smoother indistinguishable from linear. Oxygen was not significant. The ENV GAM result indicates an optimum habitat (probability of presence >0.7) at salinities between 12-33, and temperatures above 7°C (Fig. 9B). However, the presence of *M. leidyi* was better described by the spatio-temporal model (ST) (Table 3; Fig. 9C) as indicated by the variance partitioning analysis (Fig. 9D). Location and time of the year accounted for 51% of the partitioned deviance in the probability of *M. leidyi* occurrence, while salinity and temperature explained only 12% (Fig. 9D). 18% of the total deviance was remained unexplained in the full model (ST + ENV).

## DISCUSSION

*M. leidyi* is known to tolerate a wide range of environmental conditions (Fig. 1; Table 1), which indicates that *M. leidyi* could survive in the Baltic Proper. However, egg production of Baltic Proper *M. leidyi* both *M. leidyi* and in laboratory experiments have been shown to decline with decreasing salinities (salinity interval 6-33; Jaspers et al. 2011), such that the low salinity (< 9) surface water of the Baltic Proper may limit population growth. Thus, the environmental range required for successful recruitment and establishment appears to be much narrower (Fig. 8; Fig 9B) than that tolerated by *M. leidyi* (Fig. 1; table 1). This is reflected in the narrow optimum versus the total range of the environmental variables which is particularly evident for salinity (Fig. 8), and the skewed occurrence towards higher temperatures. The delayed and sporadic presence of *M. leidyi* (Fig. 4) and the underrepresentation of the smaller sized individuals in the Baltic Proper when compared to the Skagerrak and Kattegat (Fig. 7) also indicate that the Baltic Proper environment was unfavorable for successful reproduction.

The GAM predicting *M. leidyi* presence from environmental conditions (ENV) suggests a rather narrow salinity range, which gets narrower with decreasing temperatures (Fig. 9B). However, the deviance partitioning analysis showed that almost 2/3 of the variation accounted for by temperature and salinity could just as well be accounted for by variation in space and time (ST), while the variation in the spatio-temporal variables uniquely accounted for 51% of the total variation. One interpretation is that this relatively high share of the total variation reflects transport of individuals by advection that brought *M. leidyi* outside its preferred temperature and salinity range.

Several independent indications in our data set suggest a transportation of *M. leidyi* into the Baltic Proper. Firstly, average abundances were up to sixtyfold higher in Skagerrak and Kattegat compared to in the Baltic Proper. Secondly, the appearance in the Baltic Proper was generally delayed and more sporadic (Fig. 4). Thirdly, the underrepresentation of smaller individuals in the Baltic Proper (Fig. 7), implying reduced reproduction and/or high mortality rate for the smaller size classes (C. Jaspers, M. Haraldsson et al. unpubl.), suggests unsuccessful local recruitment in the Baltic Proper. Finally, transportation of *M. leidyi* into the Baltic Proper is also suggested by the association of *M. leidyi* with deeper, high salinity, water at the Baltic Proper stations (station 3-5; Fig. 5 and 6, and Fig. 4b). Schaber et al. (2011) also reported higher densities of *M. leidyi* around the halocline (40-60 m) in the Bornholm basin. Variation in the inflow of North Sea water into the Baltic Sea depends on variation in climatic forcing (Reissmann et al. 2009), and this may affect the transportation of organisms into the Baltic Sea. In addition, the ctenophore Bolinopsis influndibulum, a typical North Sea species (Hansson 2006) was also caught in deeper water at Arkona in May 2009 (M. Haraldsson et al. unpubl.), indicating the possibility of transportation from the North Sea via Skagerrak and Kattegat. Alternatively, the association with deeper and more saline water might reflect avoidance of the shallower brackish water.

Source and sink dynamics, as indicated by our data, have also been reported for *M. leidyi* in native temperate regions where e.g. winter temperatures fall below the threshold for reproduction (Costello et al. 2006). Animals observed in the Baltic Proper may have been transported via the Skagerrak and Kattegat from the North Sea (Barz et al. 2006) or from local regions around the Kiel Bight or Kerteminde fjord where extreme abundances have been observed occasionally (Javidpour et al. 2009; Riisgård et al. 2010). It has been suggested that source populations are located in protected coastal regions during periods of low abundances (Costello et al. 2006). Costello et al. (2006) proposed that seasonal persistence throughout the year should define a source population for holopelagic species such as *M. leidyi*, because traditional demographical parameters, such as natality, mortality, immigration and emigration are difficult to estimate for such species. To our knowledge, a repeatedly year round adult population has not been reported in the region of Skagerrak, Kattegat or Baltic Proper.

Genetic analyses have revealed that the southern Ponto Caspian *M. leidyi* originate from the southern Mexican Gulf, while the North and Baltic Proper populations stem from the northern native habitat (i.e. Woods Hole and Narragansett Bay) (Reusch et al. 2010). The different genetic origins might suggest that southern and northern populations have different environmental requirements (cf. Fig, 1), and that the probability of a new establishment increases if the introduced individuals originate from similar environmental conditions (Reusch et al. 2010). However, successful invaders generally display a large genotypic flexibility, which allows them to quickly adapt to new environments (Lee 2002). If a *M. leidyi* population can be maintained in the apparent semi-favorable conditions in the Baltic Proper, they might gradually acquire tolerance to the new habitat.

In conclusion, the low salinity of the Baltic Proper appears to constrain local recruitment of *M. leidyi*, and transport of individuals by advection from source areas outside the area appears to be the main cause for the observed Baltic Proper occurrences. Thus, repeated future introductions by means of the regular advection of saline water into the Baltic Proper must be expected.

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**Table 1.** Region and representative reference to the observed salinity and temperature ranges in *Mnemiopsis leidyi*'s native and exotic habitat as illustrated in Fig. 1. Letter corresponds to letters in Fig. 1.

| Letter (Fig.1) | Region | Reference |
|---|---|---|
| a, b, c, d, e | Chesapeake Bay, Biscayne Bay, Long Island Sound, Narragansett Bay, Nueces Estuary (USA) | (Kremer 1994) |
| f | Valdés Peninsula (Argentina) | (Mianzan et al. 2010) |
| g | Pamlico River Estuary (USA) | (Miller 1974) |
| h, i | Black Sea, Sea of Azov | (Shiganova et al. 2001) |
| j | Caspian Sea | (Ivanov et al. 2000) |
| k | North Sea | (Boersma et al. 2007) |
| l | Kiel Bight | (Javidpour et al. 2006) |
| m | Bornholm basin | (Schaber et al. 2011) |
| n | Limfjorden | (Riisgård et al. 2007) |
| o | Catalan Coast | (Fuentes et al. 2009) |
| p | Gulf of Trieste | (Shiganova and Malej 2008) |
| q | Aegan Sea | (Shiganova et al. 2001) |

**Table 2.** Monitoring stations, sampling dates, and depths for the Midi Multinet. N/A = not applicable.

| **Station number** | **Position** | **Total depth (m)** | **Sampling depth strata (m)** | | | | | **Sampling dates** |
|---|---|---|---|---|---|---|---|---|
| | | | **#1** | **#2** | **#3** | **#4** | **#5** | |
| 1. | 57.52 N<br>11.18 E | 60 | 0-10 | 10-20 | 20-30 | 30-37 | N/A | 2009: 8 and 29 Jun., 27 Jul., 22 Aug., 14 Sep., 12 Oct., 9 Nov., 10 Dec., 2010: 17 Jan., 19 Mar., 12 Feb. |
| 2. | 56.40 N<br>12.07 E | 55 | 0-10 | 10-20 | 20-29 | N/A | N/A | 2009: 12 May, 9 and 30 Jun., 28 Jul., 21 Aug., 15 Sep., 15 Oct., 10 Nov., 9 Dec., 2010: 18 Jan., 18 Mar., 13 Apr. |
| 3. | 54.58 N<br>14.05 E | 47 | 0-10 | 10-20 | 20-29 | N/A | N/A | 2009: 12 May, 10 Jun., 1 and 28 Jul., 18 Aug., 16 Sep., 21 Oct., 11 Nov., 8 Dec., 2010: 19 Jan., 16 Feb., 18 Mar., 14 Apr. |
| 4. | 55.15 N<br>15.59 E | 91 | 0-10 | 10-20 | 20-30 | 30-50 | 50-73 | 2009: 13 May, 10 Jun., 1 and 29 Jul., 18 Aug., 16 Sep., 18 Oct., 11 Nov., 8 Dec., 2010: 16 Feb., 17 Mar., 14 Apr. |
| 5. | 55.33 N<br>18.24 E | 90 | 0-10 | 10-20 | 20-30 | 30-50 | 50-73 | 2009: 13 May, 10 Jun., 1 and 29 Jul., 17 Sep., 16 Oct., 08 Dec., 2010: 22 Jan., 17 Feb., 16 Mar., 14 Apr. |
| 6. | 57.20 N<br>20.03 E | 240 | 0-10 | 10-20 | 20-50 | 50-90 | 90-205 | 2009: 14 May, 11 Jun., 2 and 30 Jul., 19 Aug., 17 Sep., 17 Oct., 7 Dec., 2010: 21 Jan., 18 Feb., 16 Mar., 15 Apr. |
| 7. | 58.01 N<br>17.59 E | 205 | 0-10 | 10-20 | 20-50 | 50-90 | 90-180 | 2009: 14 May, 11 Jun., 2 and 30 Jul., 20 Aug., 18 Sep., 17 Oct., 13 and 30 Nov., 2010: 20 Jan., 15 Mar., 15 Apr. |
| 8. | 56.22 N<br>16.12 E | 21 | 0-10 | N/A | N/A | N/A | N/A | 2009: 15 May, 12 Jun., 3 and 31 Jul., 20 Aug., 16 Sep., 20 Oct., 14 and 30 Nov., 2010: 19 Jan., 21 Feb., 15 Mar., 16 Apr. |

**Table 3.** Estimated parameters for the spatio-temporal and environmental GAM model separately and combined (full model). The full model (ST + ENV) is part of the partitioning analyses, and should not be interpreted by its own due to the strong correlation between spatio-temporal and environmental variables. Dev. Exp. = Deviance explained (%), AIC = Akaike Information Criterion. All variables have a smoothing function.

| | **Overall model** | | **Model term** | | |
|---|---|---|---|---|---|
| **Model** | **Dev. Exp.** | **AIC** | **Parameter** | **df** | **p-value** |
| Spatio-temporal (ST) | 68.6 | 33 | Time | 2.89 | 0.0027 |
| | | | Location | 1.00 | 0.0001 |
| Environmental (ENV) | 28.6 | 79 | Salinity | 2.89 | 0.0037 |
| | | | Temperature | 1.00 | 0.0191 |
| | | | Oxygen | 1.00 | 0.2711 |
| Reduced Environmental (ENV) | 29.2 | 73 | Salinity | 2.47 | 0.0003 |
| | | | Temperature | 1.00 | 0.0360 |
| Full model (ST + ENV) (for partitioning analysis) | 81.1 | | Time | 2.67 | 0.0032 |
| | | | Location | 1.00 | 0.0382 |
| | | | Salinity | 2.93 | 0.1688 |
| | | | Temperature | 1.00 | 0.0986 |

## FIGURE LEGENDS

**Fig. 1.** Observed salinity and temperature ranges in *M. leidyi*'s native and exotic habitat. Salinity and temperatures as reported from the (A) native habitat, (B) Ponto-Caspian region, (C) Mediterranean, and (D) North and Baltic Sea. The letters indicates the references from Table 1.

**Fig. 2.** Stations sampled from May 2009 to April 2010. Stations 1-8 are monitoring stations sampled monthly (see Table 2), stations A-M were sampled during October 2009, and the additional 9 stations in the Gulf of Bothnia were sampled during December 2009. All stations except C, D, E and F correspond to SMHI's monitoring stations.

**Fig. 3.** Contour plots of salinity, temperature (°C), oxygen (ml $L^{-1}$), chl-*a* (µg $L^{-1}$) and zooplankton (log ind $L^{-1}$) from May 2009 to April 2010 for all monitoring stations. Zooplankton data are missing from station 5 and 7. Note the difference in scale on y-axis (depth) for station 6 and 7.

**Fig. 4**. (A) Abundance of *M. leidyi* (ind $m^{-2}$) plotted on log scale against time (month) and distance (nm) from station 1 for the regular monitoring stations, with indicated station numbers (1-8). Every station and occasion sampled are indicated with a dot, and the horizontal line represents the border between the Skagerrak / Kattegat and the Baltic Proper. (B) Abundance (± SD, ind $m^{-2}$) and mean depth ($Z_m \pm Z_s$, m) of *M. leidyi* for stations sampled during October 2009.

**Fig. 5**. Mean depth $Z_m$ ($\pm Z_s$) of *M. leidyi* and the vertical profiles of salinity for all monitoring stations and months. Note that $Z_m$ is not located according to salinity on the x-axis.

**Fig. 6.** Mean depth $Z_m$ ($\pm Z_s$) of *M. leidyi* versus halocline depth at each station and cruise. The solid line corresponds to location of $Z_m$ if it was equal to the halocline depth. Dots above this reference line indicate that the mean depth is above the halocline and vice versa.

**Fig. 7.** Length histogram of *M. leidyi* for each station and cruise.

**Fig. 8.** Distributions of environmental variables at locations where *M. leidyi* was found. Variables have been averaged over the depth interval sampled for *M. leidyi*. The box represents 50% of all observations with the solid line representing the median, the whiskers 10 and 90 percentiles, and dots outliers. Axes scales correspond to minimum and maximum average of measured variables over the sampled depth interval at all locations. The box plots are based on 3634 observations.

**Fig. 9.** Probability of *M. leidyi* presence predicted by the environmental GAM model in relation to (A) month and distance from station 1 in Fig. 2 given the observed average temperature and salinity in the region, and (B) given salinity and temperature intervals. (C) is the probability of *M. leidyi* presence predicted by the spatio-temporal model in relation to the month of the year and distance from station 1, and (D) is the partitioning of deviance explained (%) by the spatio-temporal ST, and environmental ENV model (see Table 3). The shared explained variation was 19%, and the residual variation 18%.

**Fig. 1**

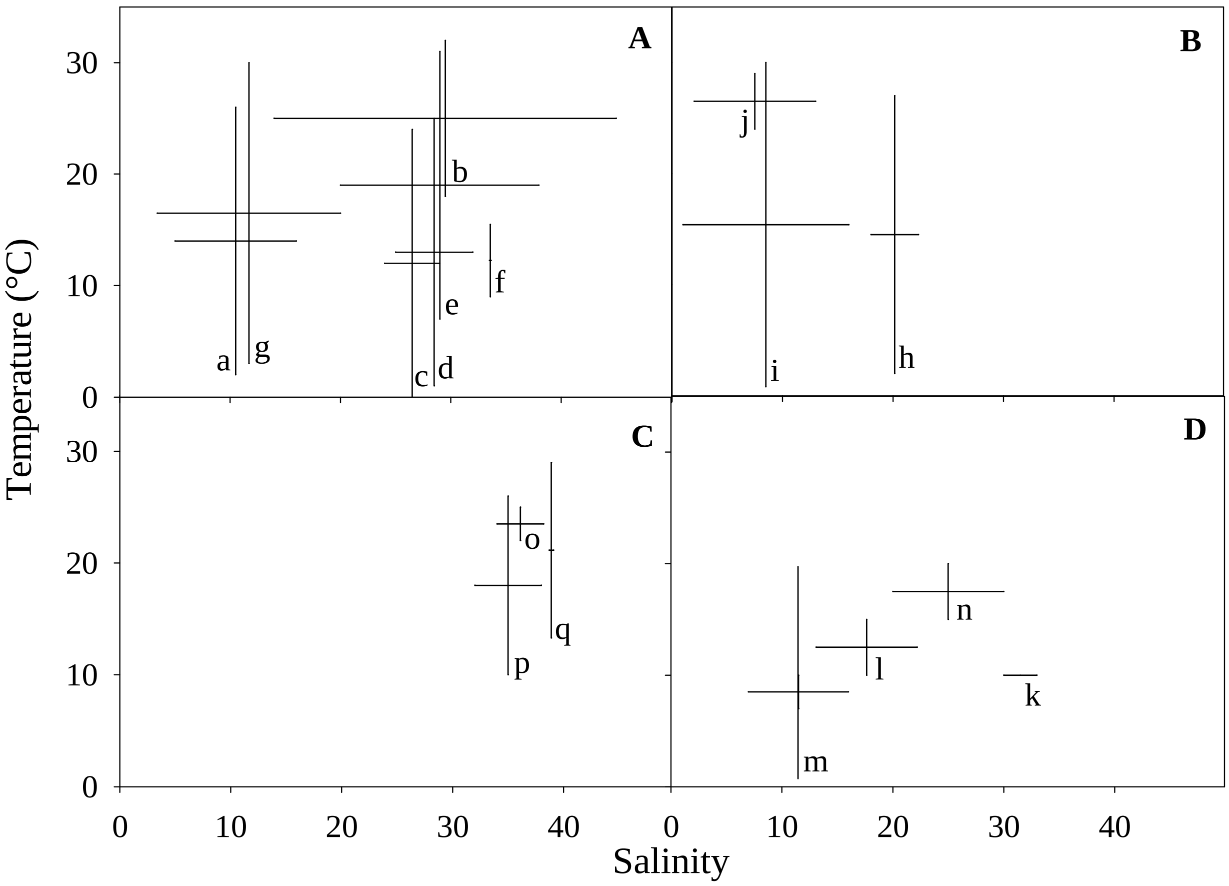

A
B
C
D
Temperature (°C)
Salinity
0
10
20
30
40
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q

**Fig. 2**

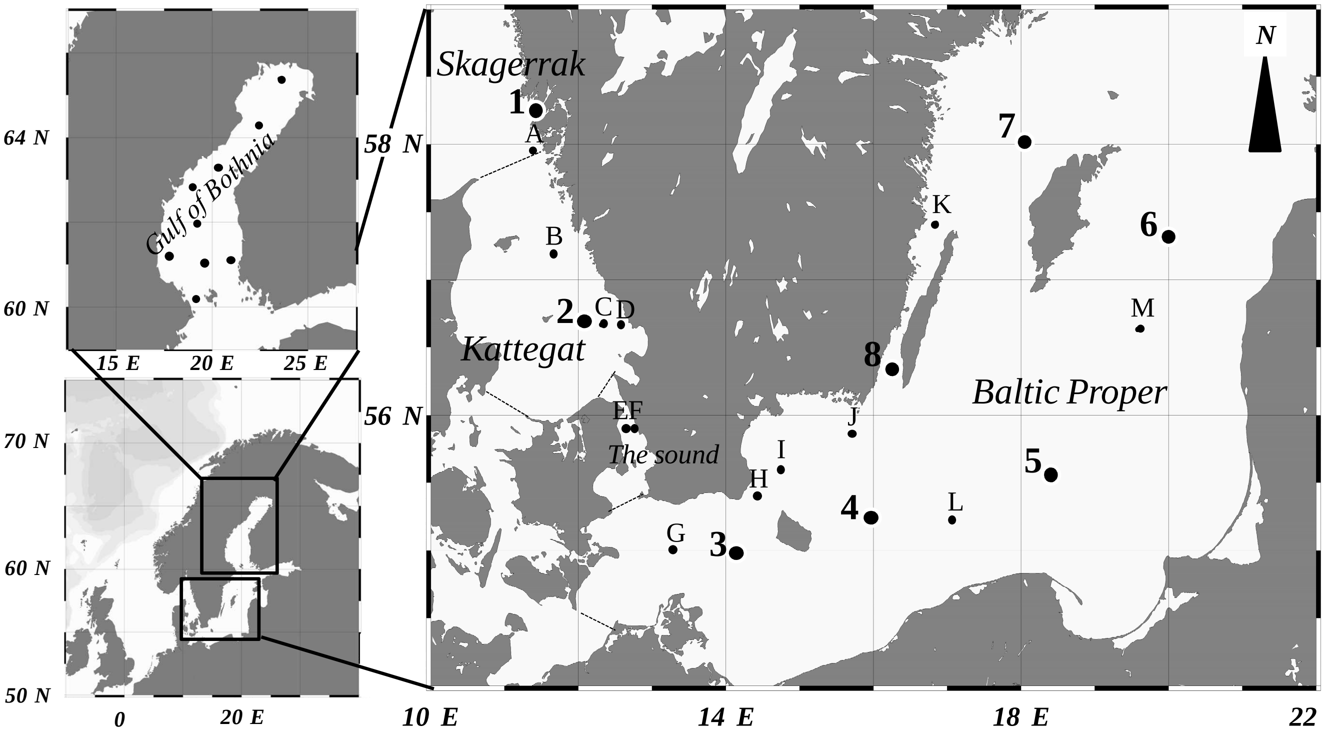

64 N
60 N
Gulf of Bothnia
15 E
20 E
25 E
70 N
60 N
50 N
0
20 E
Skagerrak
1
A
58 N
7
N
K
6
B
2
C
D
M
Kattegat
8
Baltic Proper
56 N
E
F
J
The sound
I
H
5
4
L
G
3
10 E
14 E
18 E
22

**Fig. 3**

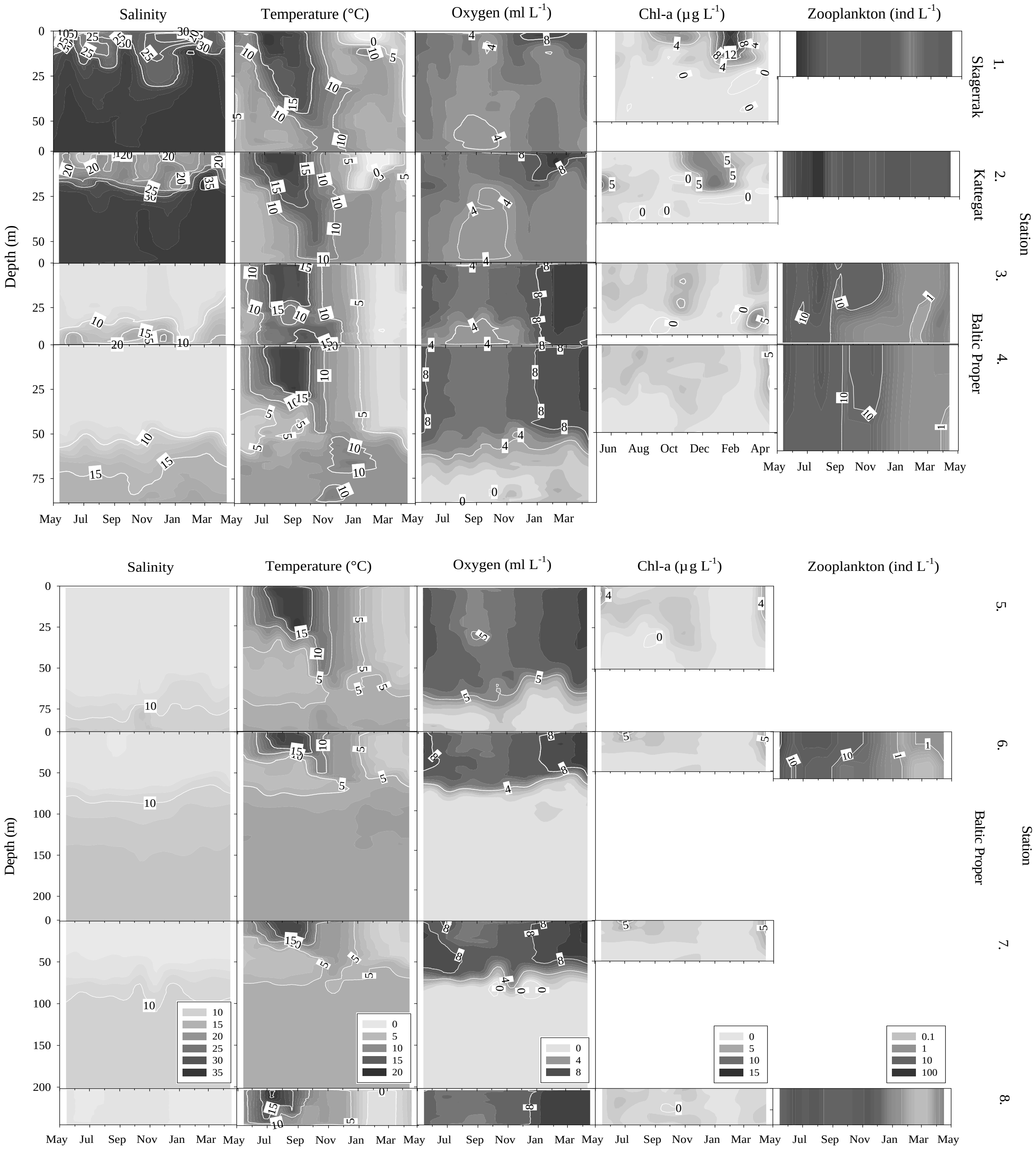

Salinity
Temperature (°C)
Oxygen (ml L-1)
Chl-a (µg L-1)
Zooplankton (ind L-1)
Depth (m)
Station
1. Skagerrak
2. Kattegat
3.
4.
Baltic Proper
5.
6.
7.
8.
May Jul Sep Nov Jan Mar May
Jun Aug Oct Dec Feb Apr
0 25 50 75 100 150 200
10
15
20
25
30
35
0
5
10
15
20
0
4
8
0
5
10
15
0.1
1
10
100

**Fig. 4**

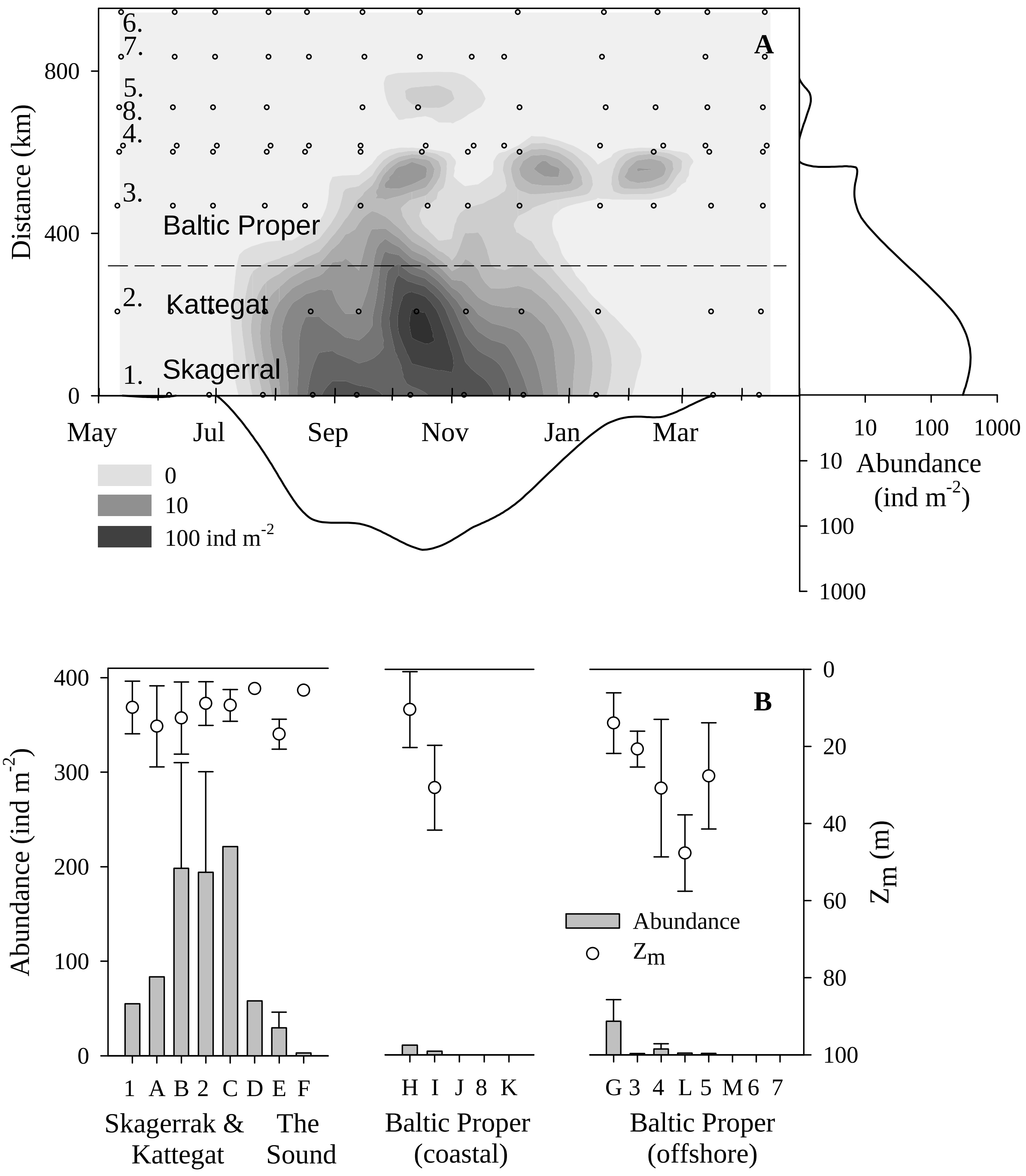

A
Distance (km)
0
400
800
6.
7.
5.
8.
4.
3.
Baltic Proper
2.
Kattegat
1.
Skagerral
May
Jul
Sep
Nov
Jan
Mar
0
10
100 ind m-2
10
100
1000
Abundance (ind m-2)
10
100
1000
B
Abundance (ind m-2)
0
100
200
300
400
Zm (m)
0
20
40
60
80
100
Abundance
Zm
1 A B 2 C D E F
Skagerrak & Kattegat
The Sound
H I J 8 K
Baltic Proper (coastal)
G 3 4 L 5 M 6 7
Baltic Proper (offshore)

**Fig. 5**

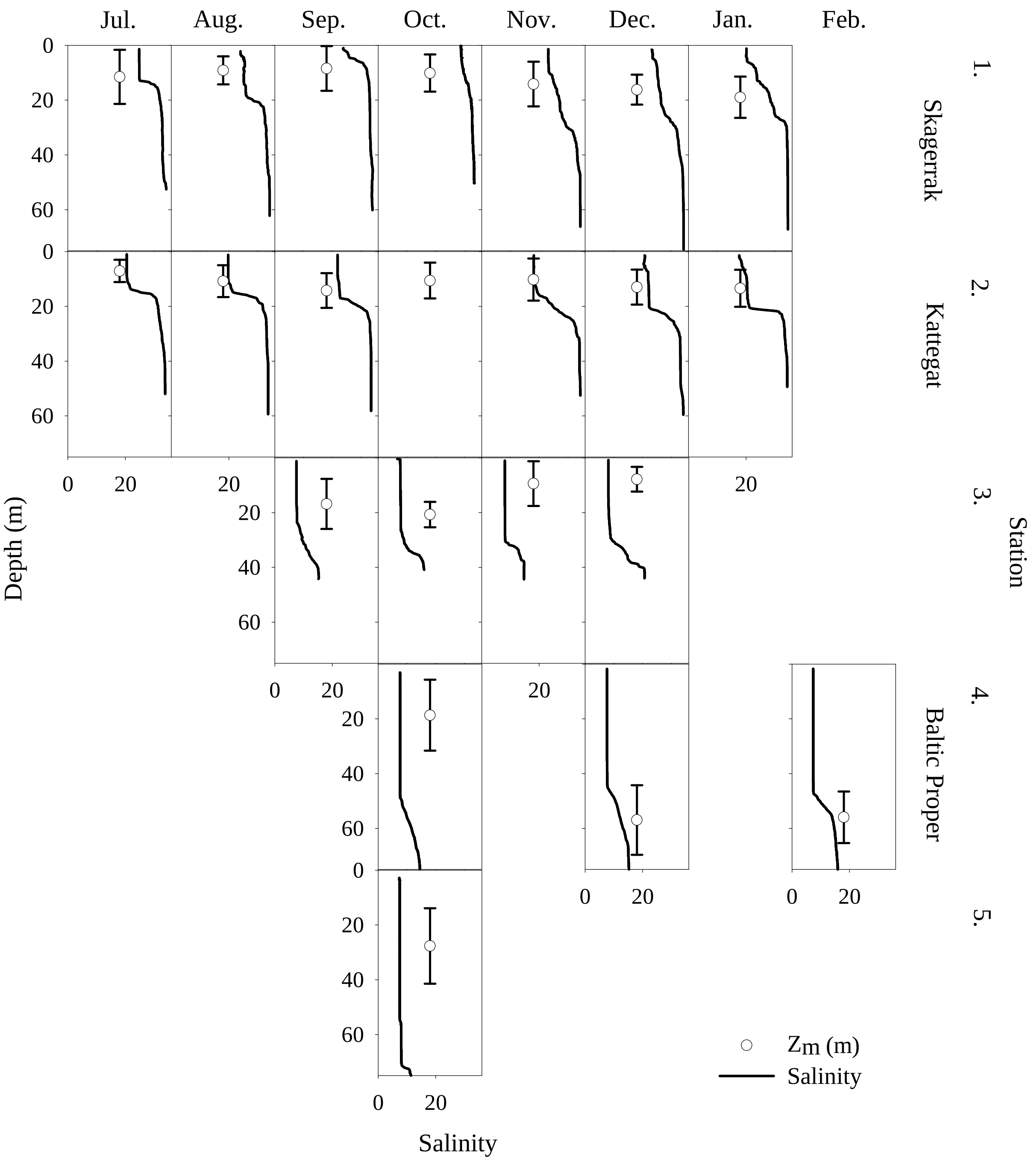

Jul.
Aug.
Sep.
Oct.
Nov.
Dec.
Jan.
Feb.
1.
Skagerrak
2.
Kattegat
3.
Station
4.
Baltic Proper
5.
Depth (m)
0
20
40
60
Salinity
Zm (m)
Salinity

**Fig. 6**

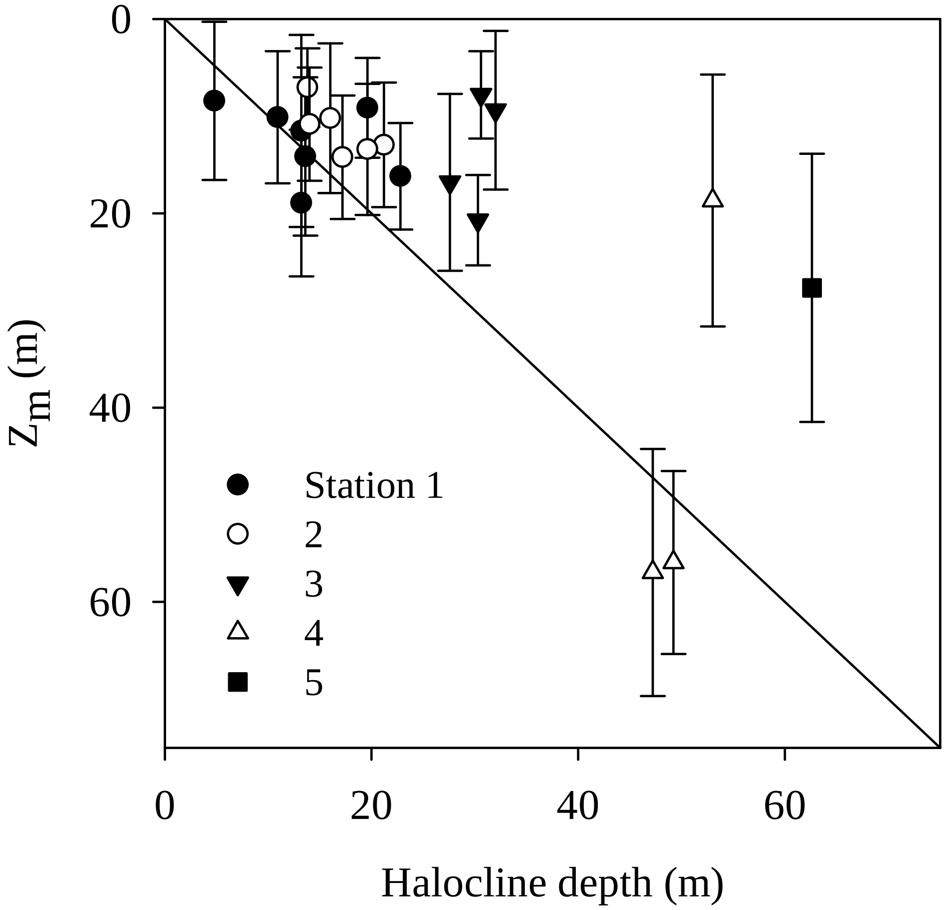


**Fig. 7**

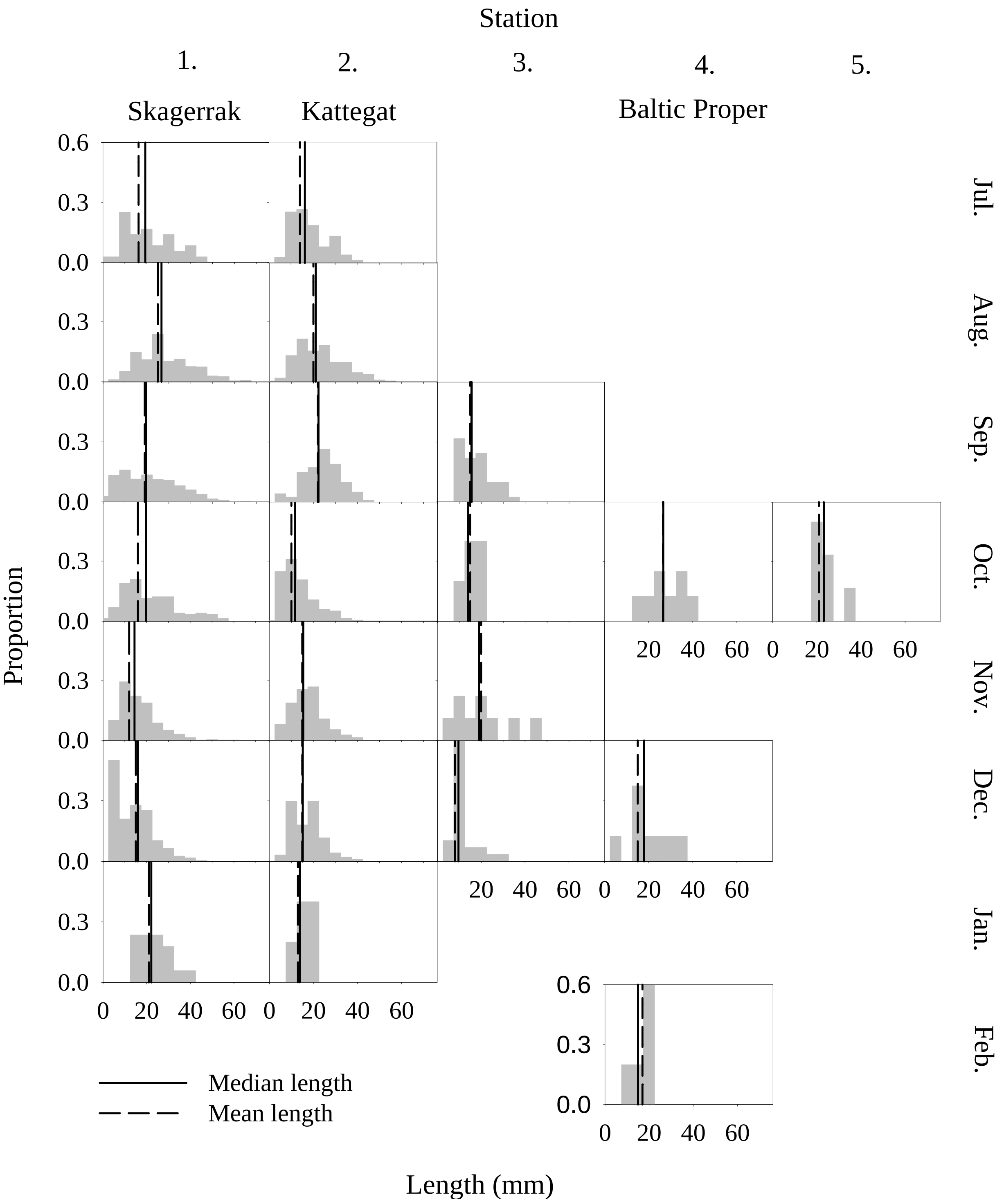

Station
1.
2.
3.
4.
5.
Skagerrak
Kattegat
Baltic Proper
Jul.
Aug.
Sep.
Oct.
Nov.
Dec.
Jan.
Feb.
Proportion
0.6
0.3
0.0
0
20
40
60
Median length
Mean length
Length (mm)

**Fig. 8**

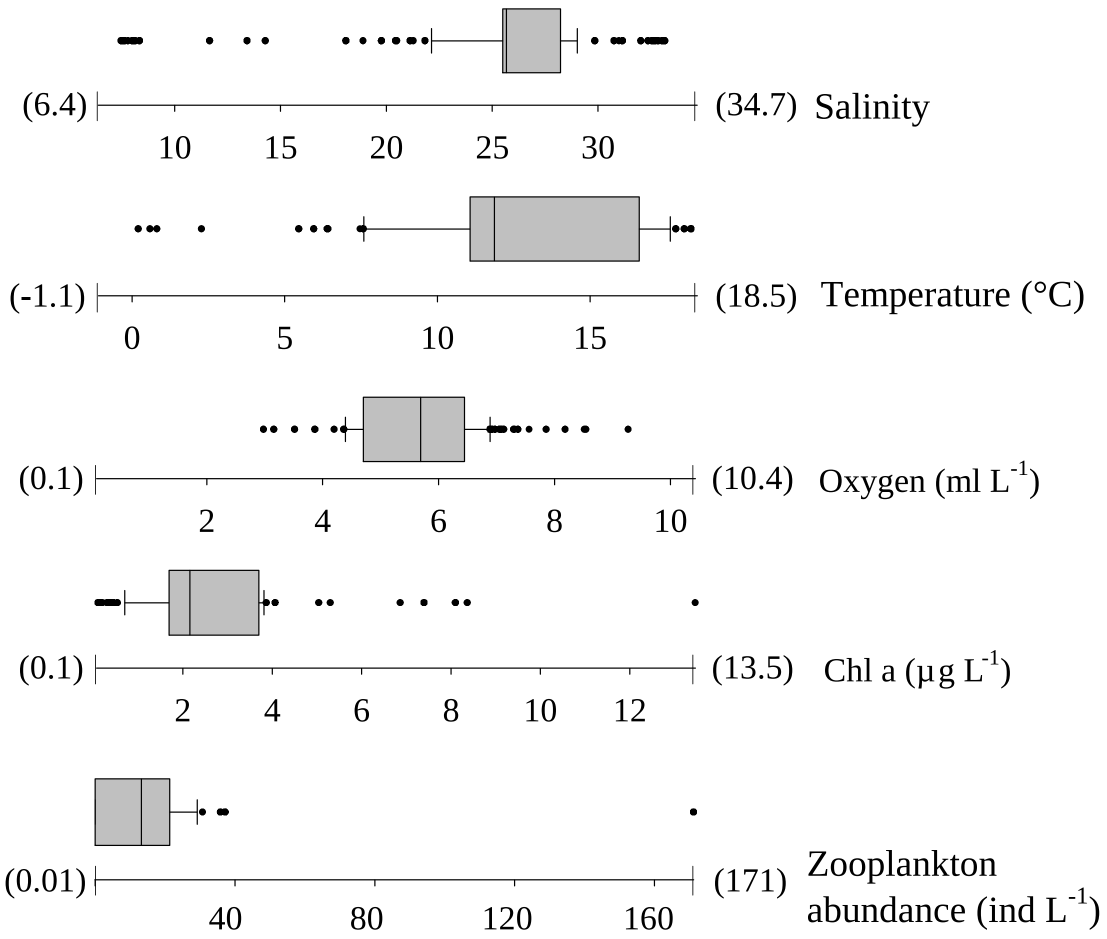
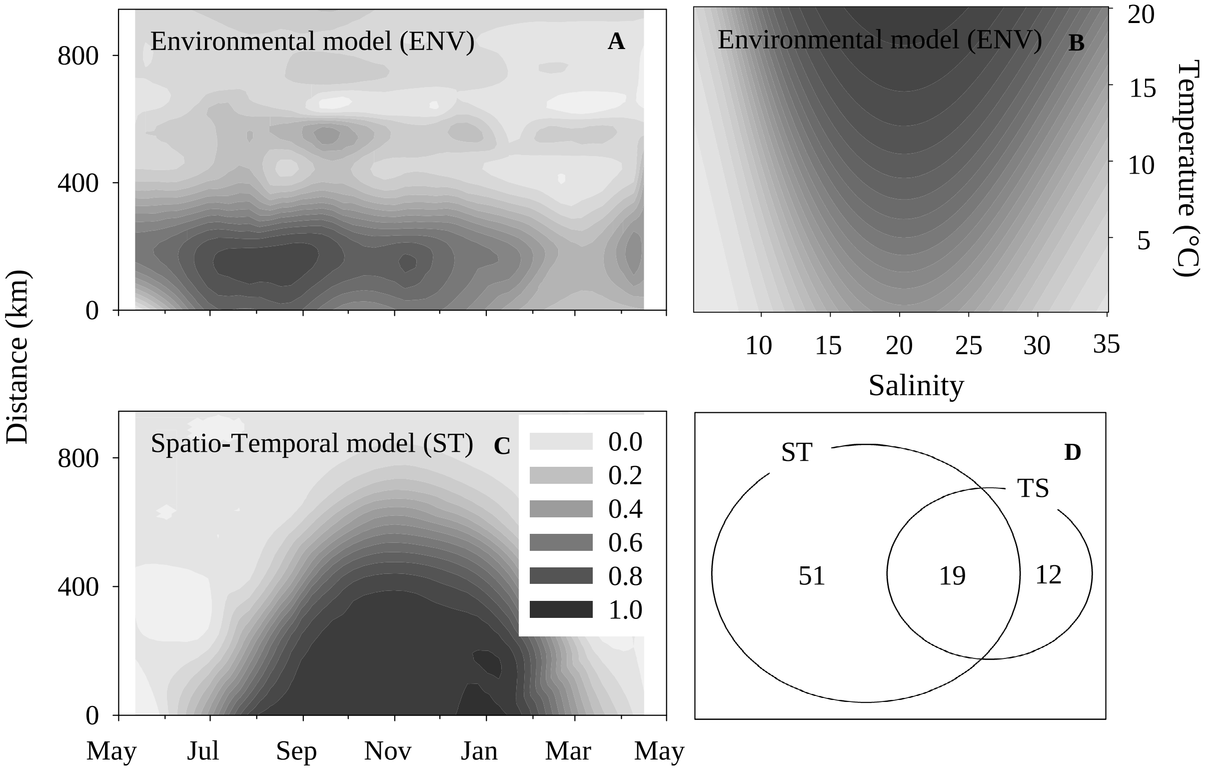
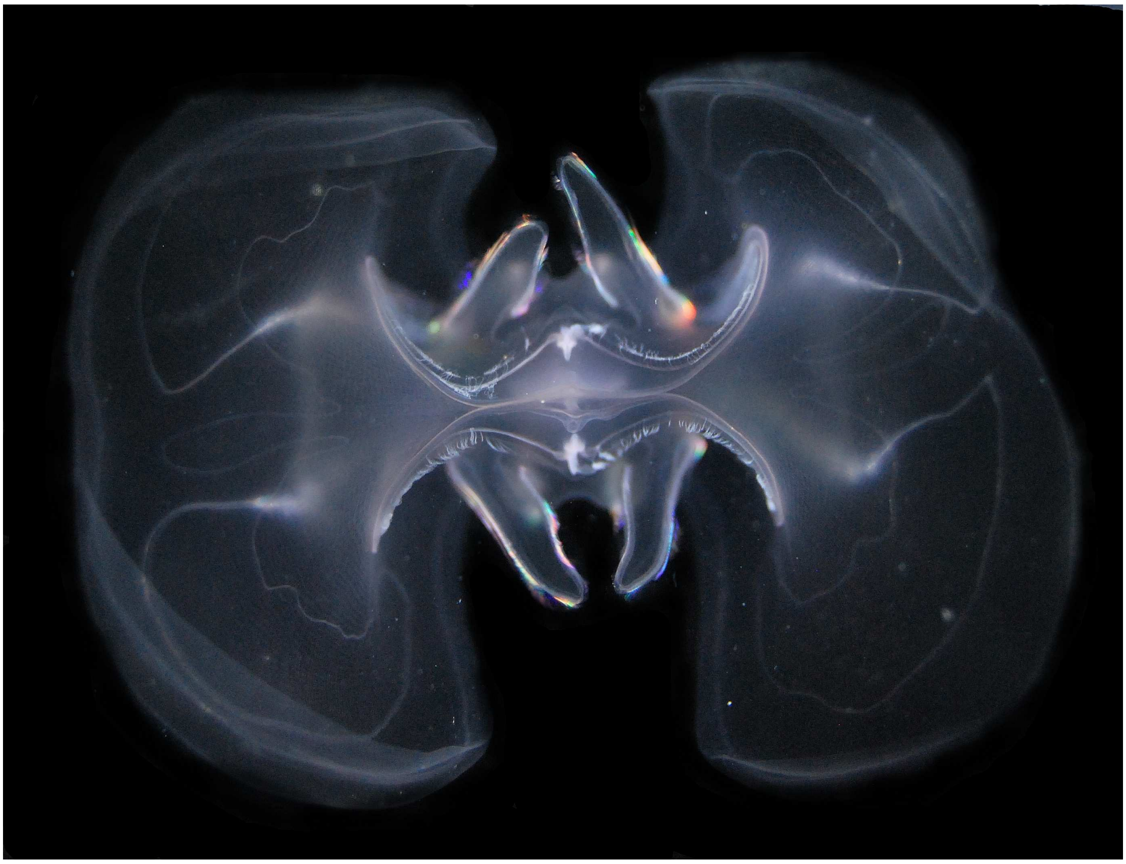

(6.4)
(34.7)
Salinity
10
15
20
25
30
(-1.1)
(18.5)
Temperature (°C)
0
5
10
15
(0.1)
(10.4)
Oxygen (ml L-1)
2
4
6
8
10
(0.1)
(13.5)
Chl a (µg L-1)
2
4
6
8
10
12
(0.01)
(171)
Zooplankton abundance (ind L-1)
40
80
120
160

**Fig. 9**


Environmental model (ENV)
A
Environmental model (ENV)
B
Spatio-Temporal model (ST)
C
D
Distance (km)
0
400
800
May
Jul
Sep
Nov
Jan
Mar
May
Temperature (°C)
5
10
15
20
Salinity
10
15
20
25
30
35
0.0
0.2
0.4
0.6
0.8
1.0
ST
TS
51
19
12

# Paper VI



**DRAFT MANUSCRIPT**

# Reproduction - a key to *Mnemiopsis leidyi's* invasion success

Jaspers, Cornelia[1*], Møller, Lene Friis[2] and Kiørboe, Thomas[1]

[1] Centre for Ocean Life, DTU Aqua, Technical University of Denmark, Kavalergården 6, 2920 Charlottenlund, Denmark

[2]University of Gothenburg, Department of Biological and Environmental Sciences – Kristineberg, 45178 Fiskebäckskil, Sweden

*Corresponding author: coja@aqua.dtu.dk

**Keywords**: Invasive species, ctenophore, reproduction, egg production rate, hatching success, food dependent egg production, starvation

## ABSTRACT

Although the comb jelly *Mnemiopsis leidyi* is characterized by high growth rates and a large reproduction capacity, the contribution of reproduction to its invasion success in European waters has not yet been studied in detail. Here we present a combination of laboratory and *in situ* measurements of *M. leidyi's* egg production and growth capacity. Cohort experiments show that energy allocation changes during maturation. During the first two weeks after hatch growth rates are high with 0.4 $d^{-1}$ but are reduced to 1/4$^{th}$ thereafter, coinciding with metamorphosis and onset of egg production. Similarly, *in situ* egg production rates show that egg production and size scale with two different slopes. Hence, while small animals allocate more energy to growth compared to egg production, adults allocate more energy to egg production than to growth, but this shift occurs at a larger for field than laboratory reared animals. Half-saturation concentration for egg production was reached at low food concentrations > 10-20 µgC $L^{-1}$ for both small and large sized *M. leidyi*. Starved animals maintained high reproduction rates for the first 24 hours and kept reproducing for consecutive days. Maximum egg production rates were > 11,000 *M. leidyi* $d^{-1}$ in high saline areas of the Baltic Sea. Hatching success is high and 60-90 % of eggs hatch within 48 hours. The combination of these results might explain why population blooms are observed and sustained in localized areas where water exchange is limited and zooplankton food resources are quickly depleted due to high grazing pressure by *M. leidyi*.

## INTRODUCTION

Life-history traits such as fast growth, high reproduction rates and phenotypic plasticity have been shown to make invasive species competitive especially under circumstances where natural predators are lacking or the ecological niche of the invader is not occupied leading to high resource availability (Sax and Brown 2000; Facon et al. 2006; Sorte et al. 2010). The comb jelly *Mnemiopsis leidyi,* native to the east coast of America (Purcell et al. 2001), is an example of a successful invasive species which has recently reached a globally wide expansion range (as reviewed in Costello et al. 2012). It shares many characteristics of a successful invasive r-strategy species especially due to its high growth rates (Stanlaw et al. 1981) and large reproduction potential (Kremer 1976). Furthermore, it is a self-fertilizing hermaphrodite thereby offsetting the Allee effect which may be encountered by and limit sexually reproducing invasive species during colonization (Berec et al. 2007; Tobin et al. 2011).

Although *M. leidyi* is characterized by high feeding rates and large reproduction capacity, the contribution of reproduction to its invasion success in northern Europe has not yet been studied in detail. Laboratory investigations have shown that egg production is highly food dependent at high temperatures (Reeve et al. 1989) but in contradiction to this, large ctenophore blooms with active recruitment are commonly observed in semi-enclosed systems where food resources get quickly depleted (e.g. Riisgård et al. 2007; Javidpour et al. 2009a,b; Riisgård et al. 2010). The mechanisms behind this have not yet been addressed. Here we address the effect of food concentration on reproduction rates in *M. leidyi* in the laboratory and investigate the effect of starvation on reproduction and size development. Further, cohort experiments in the laboratory were conducted to investigate reproductive parameters, including the onset and energy allocation to growth and egg production.

## MATERIALS AND METHODS

**Laboratory cohort experiments** - To investigate growth and energy allocation in *Mnemiopsis leidyi*, a cohort of laboratory reared animals was followed for a total of 40 days following hatch at a salinity of 33 and 19.5±0.5˚C. Adult *M. leidyi* were allowed to spawn (n=5) in GFF filtered water over night and removed from the spawning container the next morning. One day after, 150 larvae were collected in the morning and 10 larvae were incubated in 0.32 L squared tissue culture bottles (Falcon$^{©}$) each with GFF filtered sea water and fed with newly hatched *Acartia tonsa* nauplii at a target start concentration of 100µgC L$^{-1}$ every second day or daily along with quantification of eggs. Carbon content of *A. tonsa* was estimated from lengths (Berggreen et al. 1988) accounting for shrinkage due to Lugol preservation (Jaspers and Carstensen 2009). As animals grew, bottle volumes were increased to 0.7L and later 7.5L buckets along with increased size range of *A. tonsa*. Target food concentration remained at 100µgC L$^{-1}$ as start concentration, however, no food controls were performed and animals probably had lower food concentration as clearance was likely to have exceeded 30% of the bottle volume. Incubations were conducted at a 12:12 light dark cycle. Up to day 20, bottles were incubated on a plankton wheel, while thereafter 5 bottles remained on the plankton wheel and animals were starved, while the other ten incubation bottles were transferred to 7.5L buckets each. Mortality was low and accounted for while calculating average egg production *M. leidyi*$^{-1}$ d$^{-1}$. Animals were sized and checked for presence of eggs every second day until day 34 after hatch, and on a daily basis thereafter. *M. leidyi* were sized either with a stereomicroscope and ocular ruler or by calibrated pictures and image analysis using the freeware ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2011) with triplicate measurements for each animal. For calculations, *M. leidyi* length measurements were converted to carbon biomass by C (mg) = 0.0017xoral-aboral length (mm) $^{2.01}$ (Sullivan and Gifford 2004) and carbon content of *M. leidyi* eggs was assumed to be 0.1µgC egg$^{-1}$ (Reeve et al. 1989). For estimating specific growth rates, regression analyses on logarithmic transformed data were performed where the slope equals the instantaneous, specific growth rate (g) with the daily specific growth rate calculated as $e^{(g-1)}$ which yield similar values if $g \leq 0.5$ d$^{-1}$. Specific egg production rates (µgC µgC$^{1}$ d$^{-1}$) are presented, scaled to the average carbon content of the cohort on the respective day.

***Field investigation of egg production rates*** **-** *M. leidyi* were individually collected by snorkelling in Gullmar Fjord, Skagerrak (58°15′N 11°24′E) during 7 sampling events (28.9.2010 - 10.9.2010), always in the evening (c. 6 pm). Animals (n=97) were individually transferred to 4 or 7.5L GFF filtered seawater (salinity of 22.5) at 16.5˚C and incubated at a cycle with 9 hours dark (10pm to 7am) and 15 hours light (7am to 10pm) in a temperature controlled room, similar to *in situ* conditions. Experiments were started at 7 pm and individual egg production was followed over 24 hours. To prevent egg cannibalism, all animals were transferred to new GFF filtered sea water after 12 hours. Eggs were concentrated by reverse filtration (45µm), preserved in 2% acidified Lugol solution and later enumerated over the full 24 hour incubation period.

To follow the approximate time of egg production during 24 hours, eggs were enumerated for the night incubation (7 pm to 7 am) and compared with daytime production (7am to 7 pm) (for n=27 *M. leidyi*). To assess egg hatching success, a subset of eggs for each of the 7 experimental days (n=5-8 *M. leidyi* $d^{-1}$) was incubated for additionally 24 and 48 hours. Water from the bottom of the egg production buckets (c. 30 mL) was transferred to 50 mL Kautex bottles by use of a wide mouthed pipette and Lugol solution was added after 24 and 48 hours, always at 7 am. Eggs and larvae were later enumerated and hatching success assessed by the ratio of larvae to total number of eggs and larvae. At the termination of all experiments, animals were measured with a calliper (oral-aboral and total length in mm) and their displacement volume was measured in a graduated cylinder, ranging from 250 to 25 mL depending on the animals size. All animals were photographed.

Handling controls (n=4) were performed with a total of 180 eggs. The egg disappearance after incubation for 12 to 20 hours in 2 to 4L containers following reverse filtration and enumeration after 2% acidified Lugol preservation was negligible with a loss rate of 0.56% and has therefore not been corrected for.

***Laboratory controlled food dependent egg production and starvation* -** Experiments were conducted from a new *M. leidyi* cohort spawned in August 2010 from laboratory cultures at Kristineberg, Sweden. Laboratory cultures were fed with *Acartia tonsa* cultures and kept at natural light conditions, 19±0.5˚C and a salinity of 33. Carbon content of *A. tonsa* was estimated as described above.

Animals were starved overnight (12-16 hours) before experiments were started (always between 10-12 am). Egg production over 24 hours was measured over two consecutive days with 4 to 5 *M. leidyi* kept individually in 20 L containers. The first 24 hour period corresponds to food acclimatization (data not presented) and eggs as well as remaining *A. tonsa* were enumerated after the first and second 24 hour incubation periods. Clearance was assured not to exceed 30% of the container volume to ensure constant prey concentrations. Simultaneous food controls were performed and the mean prey concentration was estimated assuming exponential decay over time. Two size classes of *M. leidyi* were analyzed, small (16.4±2.7mm) and large (21.4±2.7mm), respectively, and egg production rates are presented as volume specific rates. Volume regression (see methods above) is given by *M. leidyi* volume $(\text{mL})=0.0009\text{x}\text{length}(\text{mm})^{2.84}$ ($R^2=0.89$, $F_{1,27}=237.4$, $p<0.0001$, n=28, size range 7.9-25.5mm) based on laboratory animals. Food concentration affected egg production following Holling Type II functional response curve which was fitted to the data and the half saturation concentrations were calculated as 1/b.

Egg production during starvation was followed for up to 11 days for small (13±0.7 mm) and large (23±0.6 mm) *M. leidyi*. Water was changed every day, though eggs were only enumerated on day 1-4 and day 11 for large and day 1 and 2 for small *M. leidyi* during the starvation period. Size development of the adults was measured based on picture analysis (see above).

## RESULTS

***Laboratory cohort experiments* -** Energy allocation to growth and egg production of laboratory reared *M. leidyi* under constant food and temperature conditions showed two distinct growth phases during the first 6 weeks after hatch. First, a fast initial growth phase until they were c. two weeks old and a subsequent reduced growth phase during which metamorphosis (e.g. lobe development) and the start of continuous egg production was observed (Fig. 1A). Egg production was first noted at an age of 2.5 weeks; animals were observed to regularly produce eggs when they reached c. 6.5 mm in oral aboral length.

The specific growth rate during the initial growth phase was high with 0.4 $d^{-1}$, while only 0.08 $d^{-1}$ during the subsequent period for *M. leidyi* larvae older than 2 weeks (Fig. 1B). The specific egg production rate was low between week 2.5 and 5 but substantially increased thereafter, reaching the highest rate of 0.1 $d^{-1}$ on the last sampling day when larvae were 39 days old.

***Field investigation of egg production rates* -** Investigation of *in situ* reproduction capacity of *M. leidyi* during late August 2010 confirmed that the population in the Skagerrak was actively reproducing. Egg production scaled with animal size, although characterized by two different slopes. Small animals showed a steeper slope, hence higher egg production dependency on size than large animals. Thus, animals between 10 – 20 mm oral-aboral lengths allocated more energy to growth than to egg production (Fig. 2). Maximum measured egg production was 11,250 eggs *M. leidyi* $d^{-1}$ corresponding to 250 eggs mL *M. leidyi* $d^{-1}$, based on direct volume measurements.

Significantly more eggs were produced during the dark period; hence > 98% of the eggs were found to be produced during the night (Table 1). Although we do not know when spawning occurred precisely, egg cleavage primarily consisting of first or second cleavage stages, suggesting that eggs were newly spawned and presumably not older than c. 6 hours when investigated at 7 am. For the first 24 hours (plus presumably 6 hours), between 19 and 29 % of the eggs were hatched, while after an additional 24 hours, 60-88% of all eggs were hatched (Table 2).

***Laboratory controlled food dependent egg production* -** Food concentration had a significant effect on reproduction rates for both small and large sized *M. leidyi* and followed a Holling Type II functional response curve (Fig. 3). Half saturation concentration for egg production was reached at low food concentrations of 10 and 23 µgC $L^{-1}$ for small and large sized *M. leidyi*, respectively. Reproduction rates for the same food concentrations showed a significant difference between the two size groups (Fig. 3).

***Starvation* -** After 24 hours without food, egg production rates remained high and were not significantly different from reproduction rates under fed conditions (Fig. 4). However, during the subsequent days without food, egg production decreased. After 2 days of starvation, egg production rates were still 40% of the reproduction rates observed under fed conditions. No eggs were found after 11 days without food and the oral-aboral length was reduced by 33±6% during this period (Fig. 4). This corresponds to a volume reduction of 67±9% at 19.5±0.5˚C.

## DISCUSSION

***Energy allocation to growth and eggs*** - Energy allocation to somatic growth and egg production for newly hatched *M. leidyi* larvae including the period of metamorphosis shows two different growth patterns. Until development of lobes, animals grow at a high instantaneous rate of 0.41 $d^{-1}$, equivalent to a daily specific growth rate of 0.49 % $d^{-1}$. This is similar to previously published growth rates for newly hatched *M. leidyi* larvae of 0.37 to 0.54 $d^{-1}$ (Stanlaw et al. 1981) when corrected for temperature differences (Hansen et al. 1997). We observed a change in growth rate at > day 12; Stanlaw et al. (1981) also registered such a change but at day 4 after hatch; however, at higher temperatures and without noting morphological changes or onset of egg production.

During metamorphosis, growth rates were drastically reduced and remained low during start of high egg production, leading to a somatic growth rate of only 1/4th compared to the initial growth phase. Reeve et al. (1989) found that for large sized *M. leidyi* energy allocation to growth always exceeded energy allocation to egg production. In their study at 26˚C, egg production was <2% $d^{-1}$ of the *M. leidyi* carbon biomass (Reeve et al. 1989).

On our last sampling day, specific egg production and specific growth rates were similar but the sum of both was still only half the specific growth rate as observed during the initial growth phase. One reason for the difference may be that we have underestimated the specific egg production rate due to underestimating the carbon content of eggs. So far, no direct carbon measurements of *M. leidyi* eggs are available in literature, and Reeve et al. (1989) estimated a carbon content of 0.1 µgC for *M. leidyi* eggs. Direct carbon measurements of newly hatched *M. leidyi* larvae show carbon weights of 0.2-0.3 µgC (Stanlaw et al. 1981; Sullivan and Gifford 2004). This is much lower than carbon weights measured for *Bolinopsis mikado* eggs (700µm) of 0.57 µgC $egg^{-1}$ (Kasuya et al. 2008). *M. leidyi* eggs are slightly smaller with 500-600 µm (Jaspers et al. submitted). This indicates that the carbon content of eggs is likely to be factor 2 to 3 higher. If we assume a carbon content of 0.3 µgC $egg^{-1}$, this would lead to a specific egg production of 0.3$d^{-1}$ on the last sampling day and adding the daily specific growth rate, this would match up indicating that energy allocation to egg production accounts for the drastically reduced growth rate at the end of the metamorphosis period. Hence, although we need further investigations to confirm carbon concentrations of *M. leidyi* eggs, it calls for a higher energy allocation to egg production than to growth as *M. leidyi* matures.

Furthermore, it is suggested that the shift in energy allocation between growth and reproduction, which occurs at different sizes in laboratory and field caught animals, might explain some of the large scatter in previous investigations of egg production (for review see Purcell et al. 2001).

***Interactions between food concentration, egg production and starvation*** - Previous work suggests that egg production in *M. leidyi* is highly sensitive to food conditions (Reeve et al. 1989). From model predictions, food concentrations of 24µgC $L^{-1}$ were estimated to be sufficient to sustain an actively reproducing ctenophore population (Kremer and Reeve 1989). This is in the same range where we found half-saturation food concentrations for egg production in *M. leidyi*. Similar to our findings, Graham et al. ( 2009) suggested that high egg production rates were maintained in field collected animals even though mesozooplankton biomass was low. They concluded that egg production is not as sensitive to food availability as previously thought (Graham et al. 2009).

In the Kattegat, average zooplankton concentrations during summer are c. 40µgC $L^{-1}$ and between April to November food concentrations are generally >10-20µgC $L^{-1}$ (Zervoudaki et al. 2009). This shows that zooplankton biomasses are generally above the half-saturation food concentrations for *M. leidyi* egg production and suggests that egg production in *M. leidyi* is not food limited in high saline Baltic Sea areas during summer and autumn. Reeve et al. (1989) observed that no eggs were produced after

2-4 days without food at 26˚C. In the present study, but at 7˚C lower temperatures, we found that egg production was still 40% of un-starved conditions after 2 days of starvation. Futhermore, we found that animals were shrinking while reproducing under short term starvation, which suggests that they channelled energy to egg production and a new generation.

Another mechanism to explain high active recruitment under bloom with depleted food conditions is that ctenophore larvae have a different food size spectrum than adults composed of microzooplankton and microphytoplankton prey (Sullivan and Gifford 2004) and thereby avoid competition with adults.

***Life-history and egg production*** - We show that *M. leidyi* has a synchronized spawning behaviour with > 98 % of the eggs produced during night. This might be interpreted as a adaptation to avoid and swamp visual predators like fish, but this hypothesis has to be investigated in more detail. Hatching success in single parent incubations was high with 60 - 80 % of the eggs being hatched within 48 hours. Such a high hatching success is explained by *M. leidyi* being a self-fertilizing simultaneous hermaphrodite.

Another advantage of hermaphroditism, which may be an additional explanation why *M. leidyi* is such a successful invader, is that self-fertilization offsets a possible Allee effect during early stages of colonization which may be a bottleneck for sexually reproducing invasive species due to problems finding a mate (Berec et al. 2007; Tobin et al. 2011). However, longer-term consequences of self-fertilization may be inbreeding but it is unknown how *M. leidyi* out-breeds and to what extend cross-fertilisation is important and secured on population level. A recent investigation suggested that the North Sea population stems from the Baltic Sea due to reduced allelic richness (genetic diversity) in North Sea *M. leidyi* (Reusch et al. 2010). However, the offshore population in the North Sea around Helgoland might face more dilute *M. leidyi* abundances, hence self-fertilization in those areas might be more pronounced compared to coastal SW Baltic Sea or Kattegat where blooms are regularly observed (Javidpour et al. 2009 a,b; Riisgård et al. 2010). Therefore active out-breeding might be a source of higher allelic richness in the Baltic Sea due to higher population densities compared to offshore North Sea waters. Further investigations are necessary to illuminate this.

***Conclusions*** - Our results show that a key to the success of *M. leidyi* is its high reproduction capacity. Combined with short hatching time and high hatching success *M. leidyi* can rapidly respond to favourable conditions by spawning. Egg production can be maintained for some days even though food resources become limited, hence during situations with food shortage, adults shrink and channel energy into egg production, by which they maximize their fitness. Although egg production is not highly sensitive to food concentrations, food has a significant impact on reproduction rates. The results help reconcile observed large population blooms which are maintained in localized areas where water exchange is limited and food resources get quickly depleted by the high grazing impact exhibited by *M. leidyi*.

## ACKNOWLEDGEMENTS

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**Table 1.** Egg production during night (dark) and day (light) differs significantly (t=5.38, p<0.0001, df=52). In experiment 4, one animal (14.7 mm oral aboral length) produced 3 eggs during day time (21%).

| Experiment | Animals | Eggs | Night production % | |
|---|---|---|---|---|
| | *n* | *n* | *average* | *SD* |
| 1 | 5 | 11351 | 99.7 | 0.5 |
| 2 | 5 | 3780 | 99.9 | 0.2 |
| 3 | 5 | 2303 | 99.8 | 0.2 |
| 4 | 12 | 16508 | 97.8 | 6.1 |

**Table 2.** Hatching success of *Mnemiopsis leidyi* eggs from field caught animals in Skagerrak, Gullmar Fjord, late Aug. - Sept. 2010 at 16.5˚C.

| Experiments | Total eggs | hatched 24h | hatched 48h |
|---|---|---|---|
| *n* | *n* | *%* | *%* |
| 5 | 2049 | 26 ± 11.3 | 88 ± 5.0 |
| 5 | 2333 | 21 ± 9.1 | 60 ± 22.9 |
| 4 | 540 | 19 ± 11.3 | 68 ± 11.6 |
| 5 | 2051 | 29 ± 15.2 | 82 ± 6.0 |

**Fig. 1.** Egg production rates from cohort experiments of *Mnemiopsis leidyi* kept at a salinity of 33, 19.5±0.5˚C and c. 100µgC $L^{-1}$ for 40 days after hatch. A) Size development of oral-aboral and total length as semi log plot along with average individual egg production $d^{-1}$ (±sd). B) Specific growth and egg production with regressions based on <12 days ($F_{1,5}$=2862, p<0.0001) and ≥ 12 days ($F_{1,16}$=352, p<0.0001). *No egg counts were conducted on day 40.

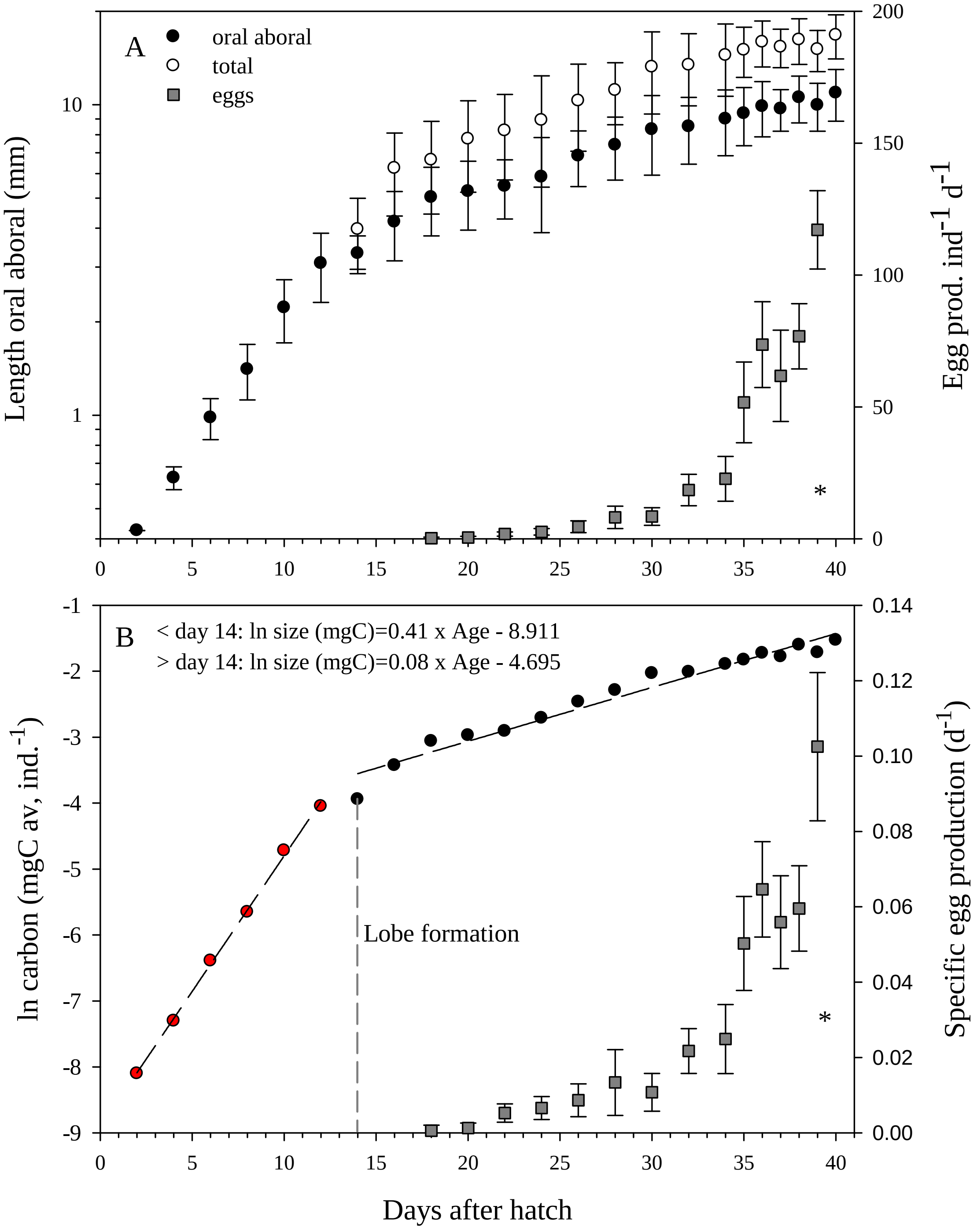


**Fig. 2.** Egg production rates of wild caught *Mnemiopsis leidyi* in Skagerrak, Gullmar Fjord, Sweden, between 29 Aug. and 10 Sep. 2010 (n=97 including six zero-observations and a total of 148,816 eggs counted). Salinity was constant at 22.5 and *in situ* temperatures were c. 16.5˚C. Regression is based on >22mm animals (n=62) ($R^2$=0.66, $F_{1,60}$=116.67, p<0.0001, $Y_0$=-2.6973, a=3.7986). Small *M. leidyi* showed a different energy allocation to egg production than large ones and < 22mm (n=29, range: 10-22mm) were not included in the regression. Further, six zero observations in the size range from 8-18 mm (n=6) are not shown in the figure. Grey squares correspond to average size dependent egg production rates from the cohort experiment > 5.5mm oral-aboral lengths, regression ($R^2$=0.93, $F_{1,11}$=128.3, p<0.0001, $Y_0$=-4.58, a=6.37).

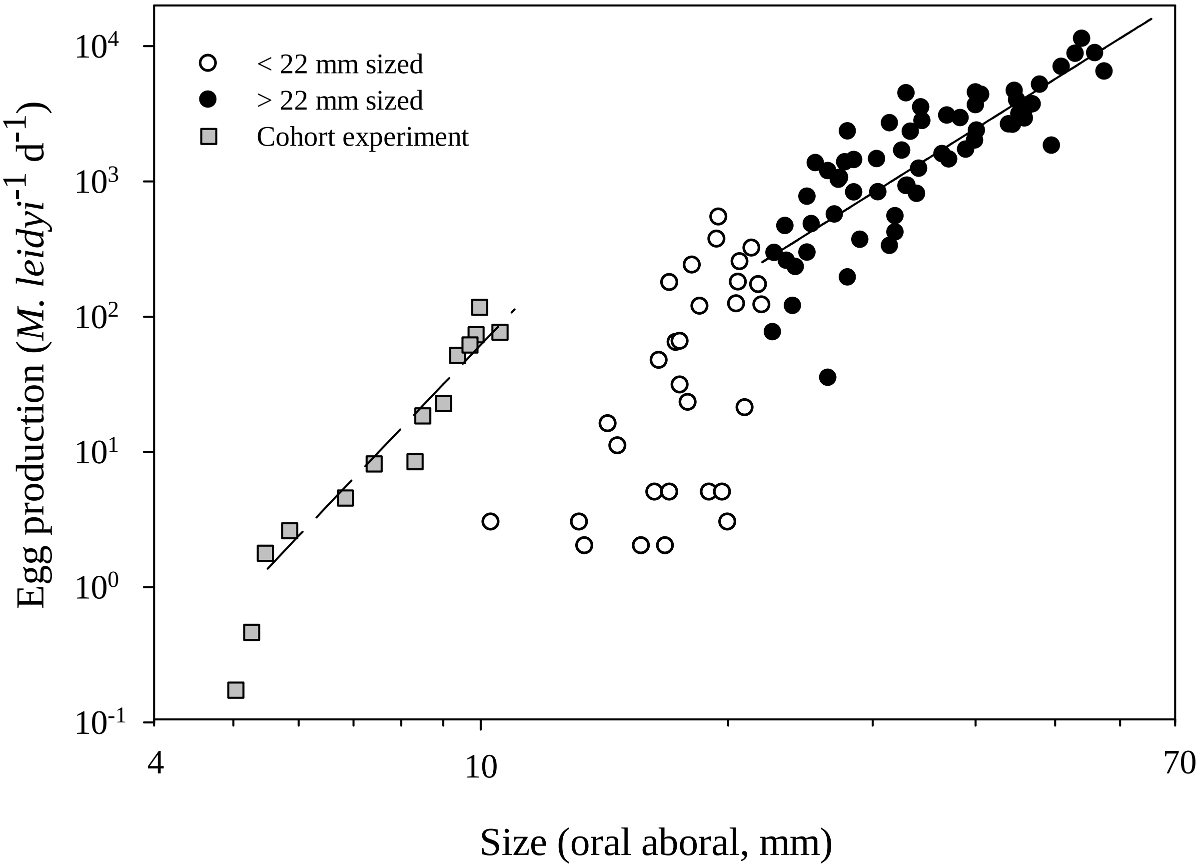


**Fig. 3.** Food dependent, volume specific egg production of laboratory reared *Mnemiopsis leidyi* at salinity 33, 19±0.5˚C. *M. leidyi* oral aboral lengths were converted to volume by (volume (mL *M.leidyi*) = 0.0009 x length (mm)$^{2.84}$ ($R^2$=0.89, $F_{1,27}$=237.4, p<0.0001, n=28, size range 7.9-25.5mm, 23PSU). Results are based on the second 24 hour incubation period. Volume specific egg production (±SE) are shown with 95% confidence bands of Holling type II response curve for A) small (16.4±2.7mm, $F_{1,22}$=22.189, p=0.0001) and B) large (21.4±2.7mm, $F_{1,13}$=10.78, p=0.0059) sized *M. leidyi*. Half-saturation concentration for egg production was reached at low food concentrations of 10 and 23 µgC L$^{-1}$ for small and large sized animals, respectively.

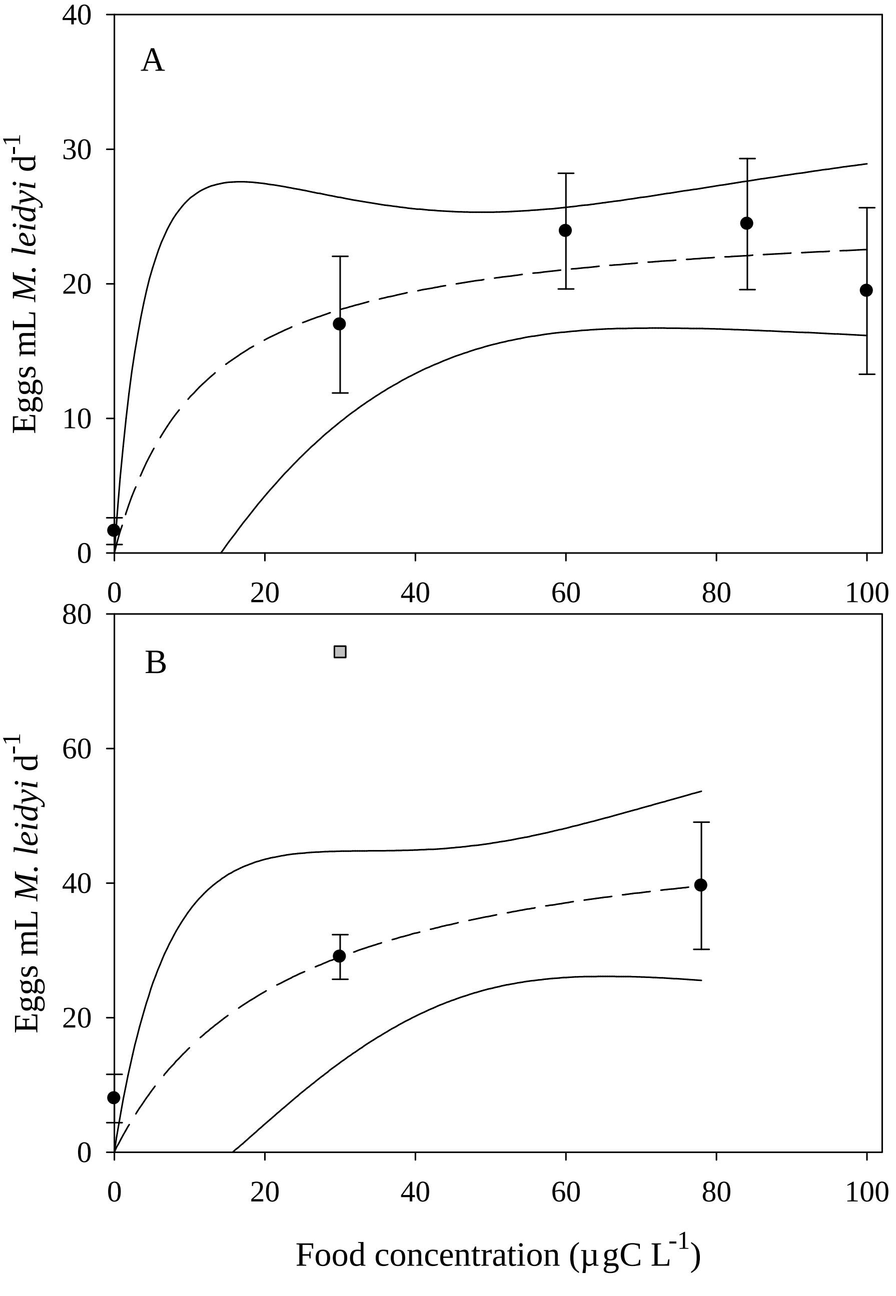
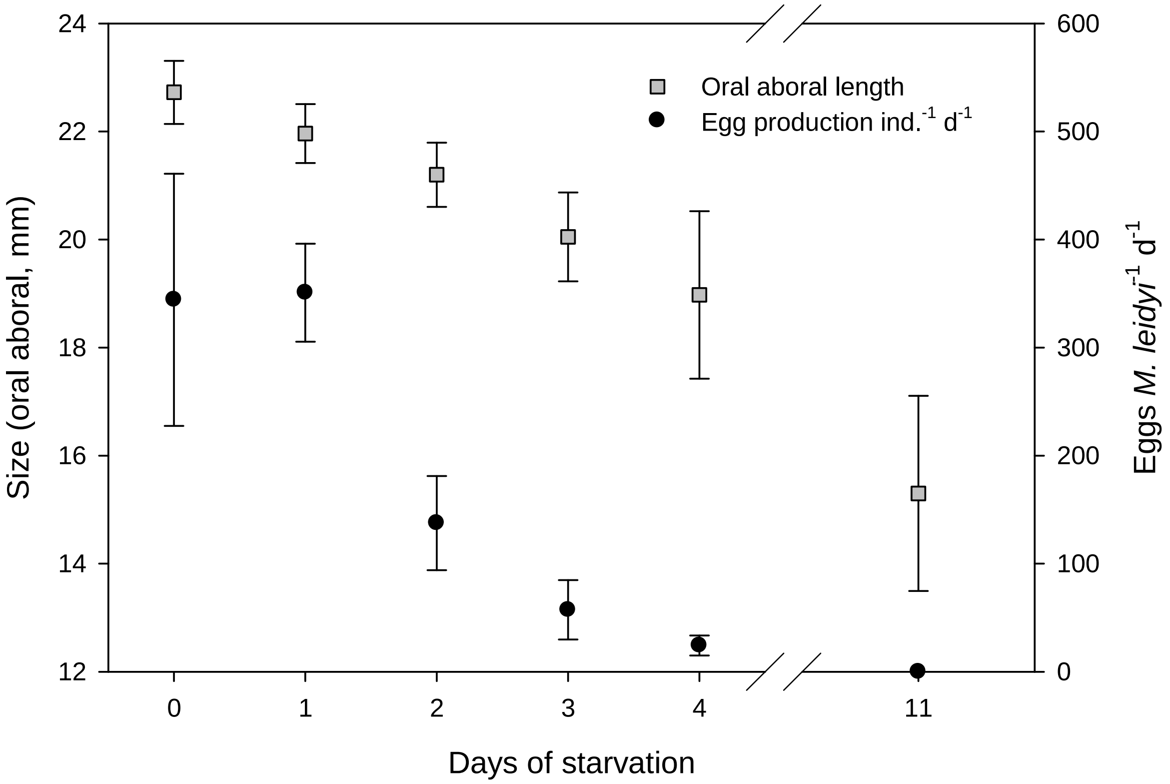


**Fig. 4.** *Mnemiopsis leidyi* size development (oral-aboral, mm) and egg production presented as average ±SD. Day zero indicates pre-conditioned egg production at 80µgC $L^{-1}$ (n=4). Incubations started in the morning between 10 to 12 am. Size and egg production differ significantly with time of starvation (1-way ANOVA: eggs $F_{5,18}=32.65$, $p<0.0001$; sizes $F_{5,18}=23.47$, $p<0.0001$). Egg production between non-starved and day 1 of starvation does not show a significant difference neither when compared per animal (t-test t=0.104, df=6, p=0.92), nor on a volume specific basis (t=0.51, df=6, p=0.6283). Egg production on day 2 is 40% of the production under un-starved conditions (Day 0). After 4 days without food, average reproduction rate is c. 10% of rates observed under fed conditions. No egg production was observed after 11 days of starvation. Average oral-aboral size at the start of the experiment (Day 0) was 22.7±0.6 mm and 15.3±1.8 mm after 11 days without food, corresponding to a reduction in oral aboral length of 33±6.4% or a volume reduction of 67±8.8%.



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**DTU Aqua**
**National Institute of Aquatic Resources**
**Technical University of Denmark**

**Jægersborg Allé 1**
**2920 Charlottenlund**
**Tlf. +45 35 88 33 00**
**www.aqua.dtu.dk**