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Recruitment of bloom-forming cyanobacteria from winter/spring populations in the Baltic Sea verified by a mesocosm approach

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Despite intense public and scientific interest in cyanobacterial blooms in the Baltic Sea, their origin and generation has not been well investigated. The aim of this work was to elucidate the overwintering strategies of *Aphanizomenon*, *Dolichospermum* and *Nodularia*. The presence of undetectably small start populations of these three genera in March 2009 and partly also in May 2006 was concluded from their growth in mesocosms. The results suggested that *Aphanizomenon* originates primarily from the surface-water population, whereas *Dolichospermum* from benthic akinetes. *Nodularia* was of particular interest as the potential of its extremely small winter/spring population to form large summer blooms was still under debate. The results verified that *Nodularia* does not develop in waters taken from below the halocline, but most likely overwinters in the upper water layers. This small planktonic start population alone, and not the benthic akinetes, may give rise to the summer blooms.

Introduction

Cyanobacterial blooms in the Baltic Sea are a cause for concern as they enhance eutrophication due to the ability of the bloom-forming filamentous species to fix nitrogen (Vahtera *et al*. 2007a). Moreover, filaments that sink to the bottom undergo remineralization (Heiskanen and Olli 1996), which leads to oxygen depletion and therefore phosphorus dissolution, thus stimulating cyanobacterial growth even more (Kuparinen and Tuominen 2001, Vahtera *et al*. 2010). The Baltic Sea, except its outer reaches, including the Bothnian Bay in the north and the Kattegat in the west, provides excellent conditions for the growth of nitrogen-fixing cyanobacteria because the inorganic nitrogen to phosphorus ratio is much smaller than the optimal Redfield ratio of 16 (Redfield *et al*. 1963) and indicates nitrogen limitation of phytoplankton growth. This condition favours the proliferation of nitrogen-fixing cyanobacteria in the Baltic Proper, typically in late spring, when excess phosphate is still available in its surface water. With increasing radiation and temperature, cyanobacterial growth accelerates such that wide-spreading blooms form by July or early August (Wasmund 1997, Kahru and Elmgren 2014). The nitrogen-fixing and bloom-forming species of the Baltic Sea are *Nodularia spumigena*, *Aphanizomenon* sp., and diverse *Dolichospermum* spp. As the genera *Nodularia* and *Aphanizomenon* contain only one bloom-forming species in the Baltic Sea (Laamanen *et al*. 2002, Lyra *et al*. 2005) and members of the

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genus *Dolichospermum* were not identified to the species level, in this study only the genus names are used in the following.

The cyanobacterial blooms in the Baltic Sea have been well investigated (Kononen and Nômmann 1992, Sellner 1997, Rolff 2000, Stal *et al*. 2003, Karlson *et al*. 2015, Svedén *et al*. 2016), but the pre-bloom conditions, especially cell recruitment, are still not well understood.

Aphanizomenon is present in the water all year round (furtive species, cf. Heiskanen 1998) and was found even under the ice in the Bothnian Bay (Laamanen 1996). Monthly samplings in the southern Baltic Sea did not reveal any akinete formation in this genus (Palińska and Surosz 2008). Thus, the vegetative filaments remaining in the water during winter may be sufficient for the perennation of this species (Jones 1979).

Dolichospermum overwinters as akinetes at the bottom of the Baltic Sea (Olli *et al*. 2005, Suikkanen *et al*. 2010), but it is not found in the ice (Laamanen 1996). In the study of Hellweger *et al*. (2008), ~90% of the *Dolichospermum* cells in the water column of a Siberian reservoir originated from the sediment, where they spent the preceding winter. The authors showed that the formation of akinetes is critical to the survival of the population on an annual basis.

Nodularia becomes undetectable in the surface water after the bloom (transitory species). It is extremely rare in water samples collected in winter (Wasmund 1997) but it may overwinter even within the ice (Laamanen 1996). Whether this rare occurrence in winter forms the basis for the huge blooms in summer is unknown. Moreover, although diazotrophic cyanobacteria grow slowly (e.g. Pechar 1992, Vahtera *et al*. 2007b), the outbreak of a *Nodularia* bloom is extremely fast and may involve the input of cells from deeper water layers that are mobilized by upwelling events, which are common features along certain coasts (Gidhagen 1987, Lehmann and Myrberg 2008, Wasmund *et al*. 2012). In an early investigation of the life cycle of *Nodularia spumigena* from a shallow Australian estuary, Huber (1984) and Huber (1985) found recruitment of that species from akinetes deposited in the sediment. Recruitment can be divided into three phases: germination, potential growth in the sediment, and migration to the pelagial (Karlsson-Elfgren and Brunberg 2004). The same processes are assumed to take place in the Baltic Sea (Kononen 1992, Sellner 1997, Heiskanen 1998, Hense and Beckmann 2006), although akinetes have been found only rarely (Albertano *et al*. 1996).

Thus, the overwintering strategy of *Nodularia* and the origin of the start populations for the development of a bloom development in the Baltic Sea are still not fully understood.

Suikkanen *et al*. (2010) used pelagial monitoring, sedimentation traps and germination experiments to identify the life cycle strategies of the three genera of interest. They demonstrated that *Dolichospermum* regularly germinates from sediment samples whereas *Aphanizomenon* is holoplanktonic. *Nodularia* germinates from akinetes only occasionally and seems to overwinter in the water in very low abundances. However, whether the small biomass of planktonic vegetative *Nodularia* trichomes present during the winter serves as an inoculant able to give rise to the large summer populations was left unanswered.

Therefore, the primary aim of my study was to determine the source of the *Nodularia* blooms in the open Baltic Sea. The following questions were addressed: (1) Do vegetative filaments remain in the water after the winter season? (2) If yes, is their biomass sufficient to serve as a seeding population for summer blooms?

Our working hypothesis was that *Nodularia* blooms recruit fully from a small overwintering population. The study, however, was not restricted to *Nodularia* but also included the other two bloom-forming cyanobacterial genera, *Dolichospermum* and *Aphanizomenon*.

The study area

The Baltic Sea is a rather shallow shelf-sea situated in northern Europe. It is not homogeneous but is divided horizontally into different basins and vertically by pycnoclines (Lass and Matthäus 2008). The large central part of the Baltic Sea, comprising the different basins but excluding the German and Danish Belt Sea and the gulfs, is called the "Baltic Proper." Due to its brackish nature, the bottom water, whose salinity

is high and oxygen concentration low, is isolated from the upper water layer by a halocline, which is situated at depths of 15–25 m in the western Baltic, 30–40 m in the Arkona Basin, 50–60 m in the Bornholm Basin, and 60–80 m in the eastern Gotland Basin. The water layer above the halocline is divided by a seasonal thermocline, which in summer forms at a depth of ~20 m. The water between the halocline and thermocline is called the "intermediate water". The salinity of the upper layers is largely affected by freshwater inputs from large rivers, leading to a salinity gradient ranging from 3 g kg^{-1} in the northern Bothnian Bay to ~18 g kg^{-1} in the Danish Straits, where the Baltic Sea is connected to the North Sea.

Methods

The mesocosm approach

In an approach completely different from that of Suikkanen *et al*. (2010), the presence of cyanobacteria in the water long before the summer bloom, at levels not detectable by routine monitoring, was examined in mesocosms. The eventual appearance of cyanobacteria would provide proof of the presence of seeding populations in the sampled water.

White, polyethylene, 100-l barrels (hereafter called "tanks") were used as mesocosms. They were filled with water taken at different Baltic Sea stations and from different depths as seeding populations may exist in different water layers. The water was taken in March 2009 and May 2006, and the phytoplankton development in the tanks was followed for up to 56 days and 49 days, respectively. Separate equipment was used to avoid cross-contamination from one tank to the next.

The samples were taken at the standard stations of the COMBINE monitoring programme of HELCOM, and the BMP (Baltic Monitoring Program) station number was used (HELCOM 2014: Annex C-1). As stations 285 and O11 had no HELCOM station numbers, the German nomenclature was adopted.

Two tanks were filled at each station with water from each depth. Each pair of tanks was marked with the same number. In case of the samples taken in March 2009 (numbered from 1 to 7), the distinction between replicates was made by adding "a" and "b" to the tank numbers. In case of the samples taken in May 2006 (numbered from 11 to 19), "+" was added to the numbers of tanks that received nutrient addition (*see* Tables 1 and 2). The water was collected using a HydroBios rosette sampler containing 12 5-l bottles and combined with a CTD Seabird 911+. Three hauls were necessary to fill two tanks. Each tank was filled with 90 litres of water and covered with a white, translucent lid.

Experimental design in 2006

Sample water was taken during the monitoring cruise of r/v *Gauss* (4 May to 12 May 2006) at two stations in the northern Baltic Proper

Table 1. Experimental set-up in 2006. HC = halocline, TC = thermocline, FM = fluorescence maximum, +P +Fe = K_2HPO_4 and $FeCl_3$ added. Tank filling date is considered day 0.

Station	Station depth (m)	Tank filling date in 2006	Water origin depth (m)	Depth characteristics	Temp. (°C)	Salinity (‰)	Tank number	
							(blank)	(+P +Fe)
H3	459	9 May	1–9	above TC	5.4	6.4	11	11+
			48	HC–TC; FM	1.3	7.0	12	12+
285	128	9 May	1 + 10	above TC	5.0	6.8	13	13+
			40 + 55	HC–TC	1.8	7.2	14	14+
			80 + 120	below HC	5.4	10.5	15	15+
K2	89	10 May	12	above TC; FM	6.1	7.4	16	16+
			43	HC–TC; FM	1.7	8.5	17	17+
O11	22	11 May	6	above TC; FM	9.9	6.8	18	18+
			18	above bottom	3.7	8.1	19	19+

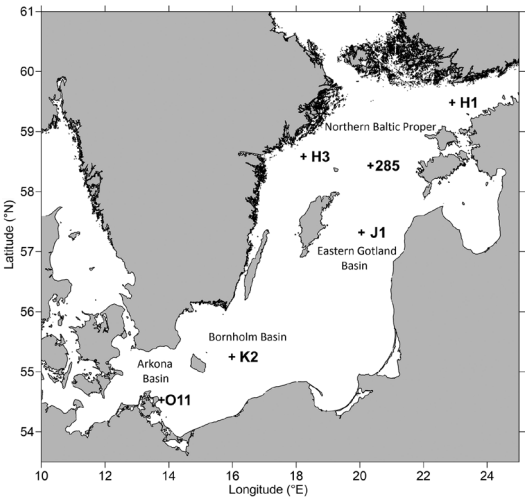

Fig. 1. Sampling stations for filling the tanks.

(station H3, Landsort Deep: 58°35´N, 18°14´E; station 285: 58°26.5´N, 20°20´E), one station in the Bornholm Basin (station K2: 55°15´N, 15°59´E), and one coastal station off the Island of Rügen (station O11: 54°32.1´N, 13°46.2´E; *see* Fig. 1 and Table 1). At each station, water was taken from different water layers: bottom (below the halocline), intermediate (between the halocline and thermocline), and surface (above thermocline). Within each water layer, the depth showing a fluorescence peak was chosen for sampling. At station 285, equal volumes of water taken from two depths were mixed to represent respective water layers (Table 1).

After the cruise, on 12 May 2006, the tanks were transferred to the institute and placed outdoors. From each sampling location, one tank was left without treatment whereas the replicate tank was spiked with 0.6 μmol K_2HPO_4, 1.8 μmol $FeCl_3$, and 1.7 μmol EDTA per litre to stimulate the growth of diazotrophic cyanobacteria, which have specific phosphorus and iron requirements (Moisander *et al*. 2003, Nausch *et al*. 2004, Sivonen *et al*. 2007). The aim was not to test a nutrient effect, but to avoid nutrient limitation and to attain the most possible yield for the detection of cyanobacteria growth. The nutrient additions are indicated by "+" next to tank numbers (Table 1). The tanks were stirred manually three times per week (Monday, Wednesday, Friday), each with its own stirrer. Water samples were taken from each tank after stirring 2, 4 and 7 weeks after the start of the experiment. Phytoplankton composition and biomass were analysed.

Experimental design in 2009

During the cruise of r/v *Alkor* (20 February to 17 March 2009), 14 tanks were filled according to the scheme shown in Table 2 and using the same procedure as in 2006. Water samples were taken at one station in the northern Baltic Proper (H1: 59°29´N, 22°54´E), one in the Gotland Deep (J1: 57°18.3´N, 20°4.5´E), and one in the Bornholm Deep (K2, same as in 2006). Unlike in 2006, nutrients were not added; therefore, the two tanks filled at each station with water taken from the same depth were true replicates ("a" and "b" after tank numbers). The tanks were transferred to the institute and placed outdoors. Sampling started on 18 March 2009, 9–14 days after filling. Prior to each of the seven samplings, carried out at weekly intervals, each tank was stirred with a separate stirring paddle.

Table 2. Experimental set-up in 2009. HC = halocline. Tank filling date is considered day 0.

Station	Station depth (m)	Tank filling date in 2009	Water origin depth (m)	Depth characteristics	Temp. (°C)	Salinity (‰)	Tank number
H1	81	4 March	1	surface	1.1	6.1	1a, 1b
			16	above HC	1.6	6.4	2a, 2b
			80	above bottom	5.7	10.2	3a, 3b
J1	249	6 March	1	surface	3.2	7.5	4a, 4b
			50	above HC	3.2	7.5	5a, 5b
K2	89	9 March	1	surface	3.2	7.8	6a, 6b
			82	above bottom	9.1	15.8	7a, 7b

Incubation conditions of the tanks

In 2006 and 2009, the tanks were incubated outside, along the northern wall of the institute's building, which sheltered them from direct solar radiation. The light transmittance of the tank material (white polyethylene) was determined by measuring the light intensity inside and outside the tanks with the spherical sensor of the LI-COR data logger LI-1000. Photosynthetic active radiation (PAR) was reduced by both the wall and the lid of the tank by about 50%, which corresponded to the light intensity at approximately 2 m water depth in the sea.

The temperature in the tanks in 2006 ranged from 6.2 °C (12 May) to 16.5 °C (30 June) and in 2009 from 1.9 °C (26 March) to 11.2 °C (15 April). The ranges for both years were within the natural temperature range for the corresponding season, although the diurnal temperature range in the tanks may have been wider than that of the sea. However, the incubation conditions were not expected to impede the growth of the cyanobacterial seeding populations.

Determination of phytoplankton biomass

The water samples (250 ml) used to analyse phytoplankton biomass and composition were preserved with 1 ml of acetic Lugol solution. Subsamples (25 ml) were allowed to stand to settle the particles according to the traditional method of Utermöhl (1958). Phytoplankton was counted and assigned to taxa and size classes using an inverted microscope (Zeiss Axiovert 100). The biomass was calculated according to stereometric formulas as recommended in HELCOM (1988, 2014).

In 2009, a second subsample comprising 25 or 50 ml of each sample was additionally counted by another person to improve the representativeness of the results. The mean of the two replicate countings was calculated. Empty or aberrant parts of the filaments were excluded from the analyses. The detection limit for the use of a 25-ml counting chamber and one filament of 5 μm width and 100 μm length is 0.08 μg l^{-1}.

Results

Recruitment of cyanobacteria in spring

The growth of cyanobacteria during the tank experiments in 2006 and 2009 suggested the presence of seeding populations already in the water although their concentrations were often below the detection limit of the routinely used monitoring method.

Early in the year (March 2009), the succession started with *Aphanizomenon*, followed by *Nodularia* (Fig. 2). At the start of the experiment, *Aphanizomenon* was found only in the water from station K2 used to fill tanks 6a and b (Fig. 2c), and its concentration was very low (1.4 μg l^{-1}; 2700 μm filament length counted in a 25 ml counting chamber). The growth of *Aphanizomenon* was strongest in the surface water, including the water taken from above a shallow halocline (16 m) at station H1. *Nodularia* also grew in the surface water and in water from above but not below the halocline. By contrast, *Dolichospermum* was primarily found in water from above the bottom and above the halocline (Fig. 2a), but with a much weaker presence in the surface water.

In late spring (May 2006), *Aphanizomenon* was present already at the beginning of the experiment in tanks 11, 11+, 13, 13+, 16, 16+, and 18+ (Fig. 3). Again, *Aphanizomenon* occurred originally only in tanks which contained the surface water. Surprisingly, it became undetectable by day 28 or 49, irrespective of phosphate addition, and was replaced by *Nodularia*. In tank 18+, *Aphanizomenon*, whose biomass was extremely low, disappeared immediately and was not replaced by any other species. Later in the experiment after May 2006, *Aphanizomenon* developed also in waters taken from greater depths (tanks 12, 14, 14+, 15 and 19), which suggested that seeding populations from those depths were much smaller leading to a retarded growth to detectable biomass.

Growth potential of the cyanobacteria

The data from March 2009 (Fig. 2) showed strong cyanobacterial growth, with an increase in some

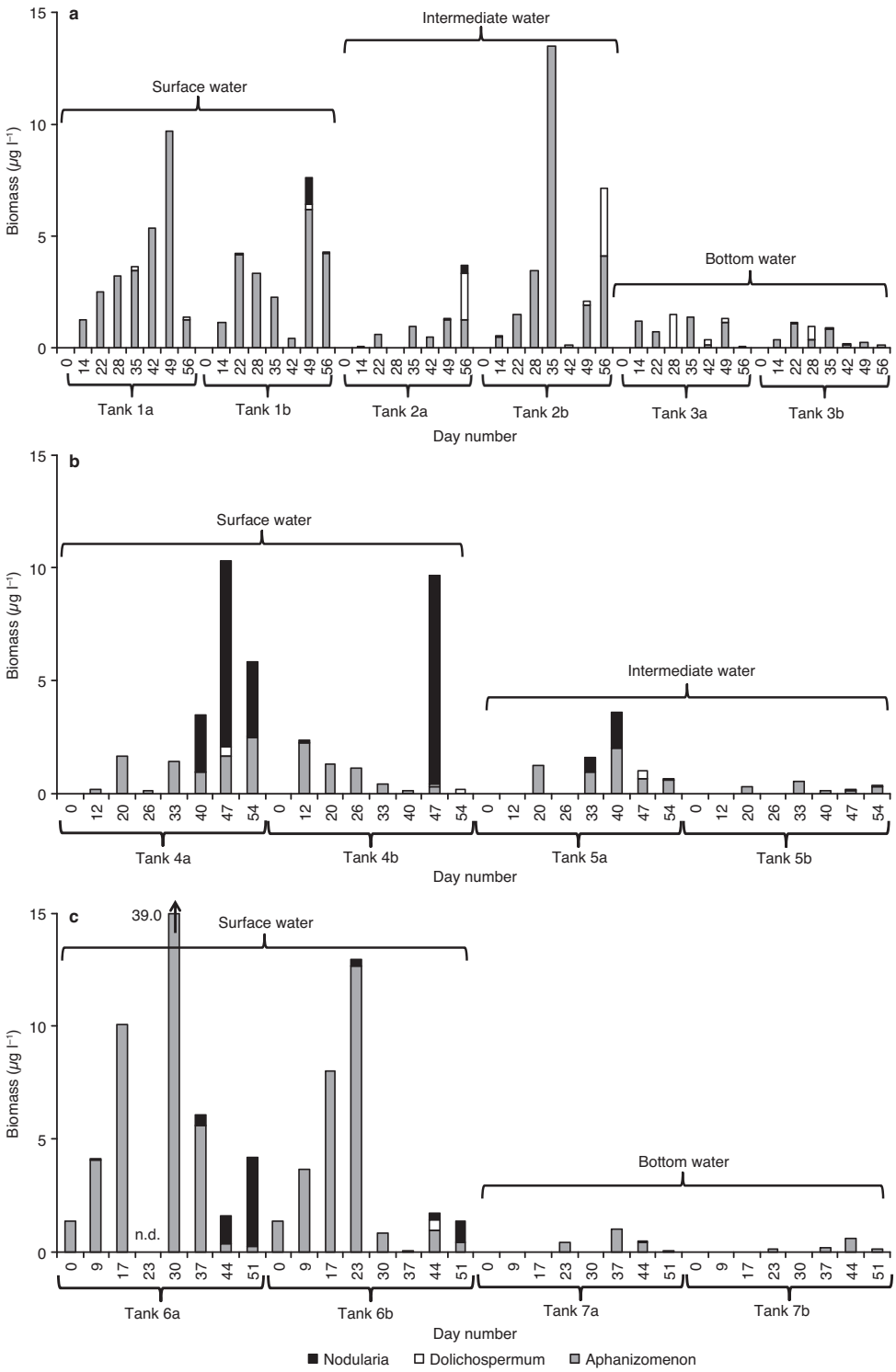

Fig. 2. Biomass development of *Nodularia*, *Dolichospermum* and *Aphanizomenon* (**a**) from 4 March to 29 April 2009 in tanks with water from station H1 (northern Baltic Proper), (**b**) from 6 March to 29 April 2009 in tanks with water from station J1 (eastern Goltland Basin), and (**c**) from 9 March to 29 April 2009 in tanks with water from station K2 (Bornholm Basin) (cf. Table2).

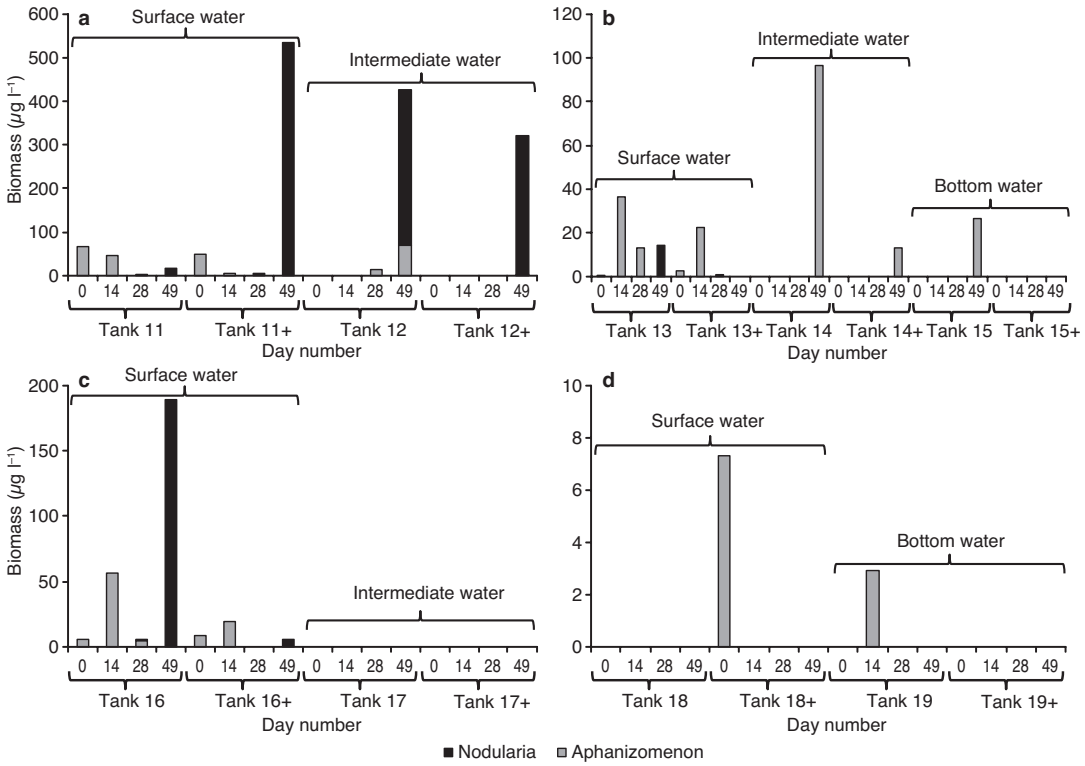

Fig. 3. Biomass development of *Nodularia* and *Aphanizomenon* from 12 May (day 0) to 30 June 2006 (day 49) in water from (**a**) station H3 (Landsort Deep), (**b**) station 285 (northern Baltic Proper), (**c**) station K2 (Bornholm Basin), and (**d**) station O11 (Arkona Basin, coastal) (cf. Table 1). *Dolichospermum* was not detected in the samples. Note the different scales of the *y*-axes.

of the tanks from mostly undetectable seeding populations to a biomass of ~10 μg l^{-1} within 3–7 weeks. The maximum biomass measured in tank 6a on day 30 was 39 μg l^{-1}. If this growth intensity is also achieved in the field, then this biomass may be reached by the beginning of April or at the latest by the beginning of May. In fact, my own monitoring data from May reveal *Aphanizomenon* biomass at this concentration (e.g. Wasmund *et al*. 2016). A biomass of ~40 μg l^{-1} can also be calculated from May values given by Rolff *et al*. (2007). In the experiment started in May 2006, *Aphanizomenon* occurred at the start of the experiment in all tanks filled with surface water, except in tank 18 (Fig. 3). *Aphanizomenon* biomass amounted to 66 and 49 μg l^{-1} in tanks 11 and 11+, respectively, filled at station H3 on 9 May 2006. This biomass can certainly grow to bloom concentrations by July.

Dolichospermum became apparent in the experiment from March 2009 on day 28 in tanks 3a and 3b and after 44 days in some other tanks, but biomass was still low. *Dolichospermum* was not noticed in May 2006.

Whether the small pelagic seeding population of *Nodularia* also had the potential to grow to bloom concentrations was a question to be solved. *Nodularia* became visible in the samples from March 2009 after day 30 in tanks containing surface water and intermediate water (Fig. 2). The strongest growth of this genus, to a biomass of almost 10 μg l^{-1} by day 47, occurred in the surface water of station J1. However, no noticeable concentrations of *Nodularia* were found at the very beginning of the experiment commenced in May 2006. In tanks 12 and 12+, even as late as day 28, *Nodularia* filaments had not been found, but 3 weeks later biomasses of 357 and 320 μg l^{-1} developed (Fig. 3a). The biomass in tanks 11+ and 16 increased from < 5 μg l^{-1} on day 28 to 534 and 189 μg l^{-1} on day 49, respectively. Considering the bloom threshold of 200 μg l^{-1}, as defined

by Wasmund (1997), *Nodularia* originating only from pelagic seeding populations could reach bloom concentrations within 3 weeks. Although the conditions in the tanks may be more favourable to bloom formation than are field conditions, this result reveals the potential of *Nodularia* to form fully developed blooms from overwintering pelagic seeding populations.

Discussion

Aphanizomenon

The holoplanktonic life cycle of *Aphanizomenon* includes overwintering in the form of vegetative filaments without akinetes (Palińska and Surosz 2008). Suikkanen *et al*. (2010) found this genus in samples taken between March and November and in an additional large-volume sample from December. Their data showed that *Aphanizomenon* cells did not germinate from akinetes. My data established the presence of *Aphanizomenon* in the water already in March and therefore presumably also in winter. The highest growth potential was in the upper water layers and the lowest in the deep-water layers. In a previous tank experiment conducted by Wasmund *et al*. (2012) in July 2009, *Aphanizomenon* was strongly present in the surface water of the eastern Gotland Basin, but not in the intermediate water. This genus declined by the end of our experiments in most of the tanks. Walve and Larsson (2010) attributed the collapse of *Aphanizomenon* blooms to phosphorus shortages.

Dolichospermum

Among the three genera examined in this study, the biomass of *Dolichospermum* was the smallest. The sporadic appearance did not allow conclusions on the recruitment mode. However, there is no doubt that akinetes play a decisive role in the recruitment of this genus. Seed beds of *Dolichospermum* may be stronger in the northern Baltic Proper (tanks 2 and 3) than in the southern Baltic Proper (Fig. 2).

The various conditions leading to akinete formation were discussed extensively by Suikkanen *et al*. (2010). Wasmund *et al*. (2012) demonstrated the intense growth of *Dolichospermum* in upwelled water (tanks U8 and U8+ in that study) and in intermediate water originating from a depth of 30 m (tanks I4a and I4b in that study), suggesting the importance of upwelling for *Dolichospermum* bloom development.

Nodularia

Identifying the source of summer *Nodularia* blooms was the focus of this study. Kononen (1992) and Sellner (1997) suggested akinete formation as the most likely overwintering strategy for this genus and that germination from akinetes is triggered by temperatures > 16 °C (cf. Huber 1984). This mechanism is unlikely in the deep water of the Baltic Proper, where the temperature is typically < 7 °C, and especially because *Nodularia* seems to develop primarily in the open sea, not in coastal areas (Niemistö *et al*. 1989). In microcosm experiments with inoculated sediment, Karlson *et al*. (2012) discovered resuspension of *Nodularia* by amphipod bioturbation, which may be one factor affecting recruitment. According to Suikkanen *et al*. (2010), *Nodularia* germinates from akinetes only occasionally and blooms develop also from trichomes that overwinter in the water column.

The fact that *Nodularia* grew only in tanks filled with water from above the halocline and remained below the detection limit in tanks filled with water from below the halocline leads me to the conclusion that the planktonic start population of *Nodularia* present during winter is sufficient for bloom development, without additional input from akinetes. It is unlikely that the winter population originated from akinetes that had germinated already in winter. The winter population may have accumulated at a certain depth, e.g., in the pycnocline, or it may have somehow overwintered attached to other organisms or debris, as reported by Huber (1984). Suspension in the water or its discontinuous layers, such as the halocline, will be supported by gas vesicles as long as a minimum number of them remain intact (Walsby *et al*. 1995). Therefore, it seems likely that *Nodularia* arises in the central open sea from pelagic seed banks (Smayda 2002) and

not from seed beds of the sediment. Probably, an obligatory akinete stage as suggested in the life-cycle model for cyanobacteria by Hense and Beckmann (2006) has no relevance for bloom formation.

The question is whether the small *Nodularia* biomass present during winter — ~0.5 ng l^{-1} in the surface water (Suikkanen *et al*. 2010) — is sufficient to give rise to the summer bloom. *Nodularia* developed already in the tanks of the March 2009 experiment, and it grew from a very low biomass of vegetative trichomes to reach a bloom concentration of 200 μg l^{-1} or more within three weeks in May 2006. This allows the conclusion that *Nodularia* blooms may originate from hibernating vegetative filaments alone.

Succession

My experiments also demonstrated succession of the three genera. Due to the advantage conferred by its survival in the water during winter, *Aphanizomenon* was the dominating cyanobacteria in the early phase of the bloom. This early appearance of *Aphanizomenon* was observed both in nature and in the tanks. *Aphanizomenon* grows slowly starting from its relatively high spring biomass until it reaches its summer bloom density, whereas *Nodularia* grows quickly from extremely low spring concentrations after a lag-phase and finally outcompetes *Aphanizomenon*. A lag-phase in the *Nodularia* growth was also reported by Lehtimäki *et al*. (1997). The growth of *Nodularia* and the disappearance of *Aphanizomenon* observed in this study were also described by Wallström *et al*. (1992) in their mesocosm experiments.

Aphanizomenon benefits from higher phosphorus concentrations whereas *Nodularia* is a better competitor at low phosphorus concentrations (Degerholm *et al*. 2006, Vahtera *et al*. 2007b). This may be one reason for the early growth of *Aphanizomenon* and the dominance of *Nodularia* in the late successional state. Moreover, *Nodularia* prefers higher irradiances, temperatures, and salinities than *Aphanizomenon* (Lehtimäki *et al*. 1997). The akinete-based *Dolichospermum* populations get most of their nutrients from the sediment (Hellweger *et al*. 2008); they appear rather late in the succession.

Experimental conditions

Quantification of cyanobacterial growth in field studies is problematic because of the high patchiness and the advection in waterbodies. Lab studies with cultures do hardly represent natural conditions. Mesocosms are an easily manageable approach to follow the development in a separated natural water body under field conditions. Processes such as grazing, competition and nutrient limitation are included on purpose. Admittedly, artefacts may appear after a few weeks. A problematic issue is the low precision of the counting method, owing to the small sample size that can be analysed microscopically. However, the primary aim of my experiments was not to quantify cyanobacterial growth but to observe qualitatively whether cyanobacteria can recruit from winter water.

Stal *et al*. (2003) showed that solar irradiance is the main factor controlling growth of *Nodularia* in the Baltic Sea. As deep circulation could not be reproduced in the tanks, the enclosed phytoplankton received more light than under natural conditions. Kononen *et al*. (1996) reported that a shallow mixed layer of 5 m depth stimulated *Nodularia* but the high irradiance may have damaged other phytoplankton organisms. This experimental factor may have supported a shift in the tank experiments from *Aphanizomenon* to *Nodularia*. Generally the mesocosm approach may stimulate cyanobacterial growth and therefore supports our intention to receive detectable amounts of biomass from undetectable seeding populations.

Conclusions

Using a mesocosm approach, I could assert that overwintering seeding populations of *Nodularia* were present in March in the upper water layers, but not below the halocline. The low *Nodularia* biomass, undetectable at the beginning of the experiment commenced in May 2006, reached bloom concentrations in some tanks by the end of the experiment. It proves that pelagic seeding populations alone may give rise to the *Nodularia* blooms. It seems that akinetes are not an obligatory precondition for these blooms.

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