

# Macrobenthic community response to the *Marenzelleria viridis* (Polychaeta) invasion of a Danish estuary

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**ABSTRACT:** We investigated the invasion of the non-native polychaete *Marenzelleria viridis* in a shallow Danish estuary, Odense Fjord. Three datasets with different spatial and temporal resolution were examined to describe the invasion of *M. viridis* and to investigate its effect on the native benthic community with focus on the 2 common polychaetes, *Nereis (Hediste) diversicolor* and *Arenicola marina*. *Marenzelleria viridis* colonized Odense Fjord rapidly, and within 3 yr it had spread to ~50 % of the estuary. The population development of *M. viridis* in Odense Fjord followed the 'boom-bust' pattern that is typical for many invaders. *M. viridis* is now firmly established and has reached an overall abundance of 100 to 200 individuals (ind.) m<sup>-2</sup> with local maxima of up to 1200 ind. m<sup>-2</sup>. Its distribution is apparently regulated by abiotic parameters that prevent its establishment in the oligohaline and more silty parts of Odense Fjord. There was a positive interaction between *M. viridis* and the native *A. marina*. Otherwise the introduction of *M. viridis* was synchronous with a decrease of several macroinvertebrates species, especially *N. diversicolor*. The latter is still the dominant species in Odense Fjord, but its density has decreased by >60 % in areas colonised by *M. viridis*. We do not expect that *N. diversicolor* disappears completely in this estuary, but it will probably be displaced to refuge areas where *M. viridis* cannot survive. Decrease in the population size of a key native species such as *N. diversicolor* might have significant ecological implications at the ecosystem level with respect to biodiversity and nutrient cycling.

**KEY WORDS:** Invasive species · *Marenzelleria viridis* · *Nereis (Hediste) diversicolor* · *Arenicola marina* · Benthic communities · Biological interactions · Polychaetes · Regime shift

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## INTRODUCTION

Central questions in studies of biological invasions are how and to which extent non-native species affect the recipient ecosystem (Reise et al. 2006). These questions have been increasingly relevant in the marine environment for 2 major reasons. (1) The number of non-native species is rising as a result of

increasing ship transportation (ballast water and hull fouling), aquaculture and man-made waterways (Leppäkoski & Olenin 2000, Gollasch 2006). (2) Non-native species constitute a possible cause for alteration of ecosystem processes and displacement of native species (Reise et al. 2006). Estuaries along the European coastal zone are 'hot spots' for introduction of non-native species (Zaiko et al. 2011). These areas

have often low species diversity and potentially empty niches resulting from their unpredictable environmental conditions (salinity, temperature and oxygen) concurrent with intense anthropogenic disturbances (Reise et al. 2006, Zaiko et al. 2011). It appears that benthic communities are particularly prone to colonization by non-native species since >50% of the 1000 species registered as non-native in European coastal waters are benthic macroinvertebrates (Gollasch 2006). These biological invasions may have implications for the ecological functioning of estuaries because benthic macroinvertebrates are components of the estuarine food web and important controllers of biological, chemical and physical processes in these environments (Kristensen et al. 2012).

Introduced polychaete species of the *Marenzelleria* genus are widespread in the Baltic Sea and Kattegat areas (Leppäkoski & Olenin 2000, Zaiko et al. 2011). To date 3 siblings have been identified here: *M. arctica* seems restricted to the most northern part, *M. neglecta* to the central and most southern part of the Baltic Sea, whereas only *M. viridis* is found at higher salinity in Danish waters at the mouth of the Baltic Sea (Blank et al. 2008, Christoffersen 2010). In some regions, the invasion of these species is associated with a negative effect because they rapidly became dominant and dramatically changed the benthic fauna communities (Zmudzinski 1996, Cederwall et al. 1999, Essink 1999, Zaiko et al. 2011). For example, *Marenzelleria* spp. invasion has been linked to low survival of the polychaete *Nereis (Hediste) diversicolor* (Essink & Kleef 1993, Kotta et al. 2001) and is therefore of special concern in areas where the latter is common. Conversely, *Marenzelleria* spp. invasion had only limited effects on the native benthic fauna in certain parts of the Dutch Wadden Sea and the Baltic Sea where it appears to constitute just an additional species (Essink & Dekker 2002). Thus, interactions of *Marenzelleria* spp. with other benthic species appear to vary greatly between regions and are likely related to the sensitivity of the native community and the environmental specificity of the habitat as well as the abundance and invasion stage (Reise et al. 2006, Zaiko et al. 2011).

Monitoring programmes in Denmark first confirmed the introduction of *Marenzelleria viridis* along the Danish west coast in the 1990's (Essink 1999, Thomsen et al. 2009). It subsequently became a successful invader in other Danish coastal areas and estuaries as its presence has now been confirmed throughout most Danish coasts in the Kattegat and Belt areas (Fig. 1). This is probably the result of a biological capacity of this species to establish success-

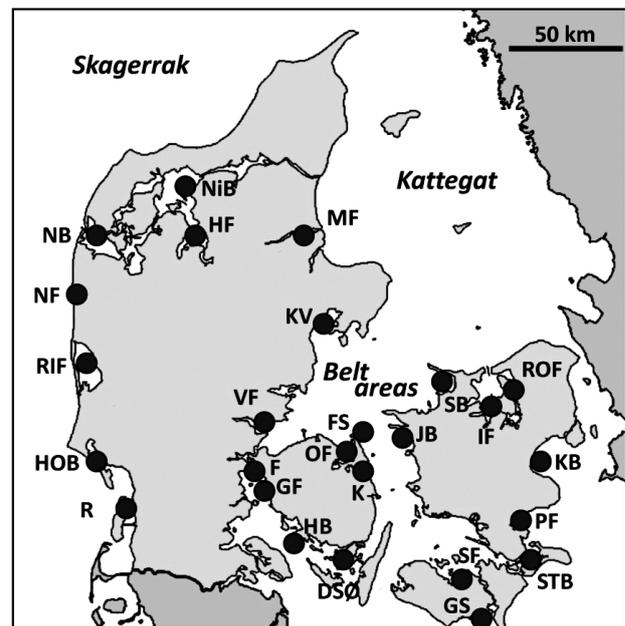


Fig. 1. *Marenzelleria viridis*. Occurrences of the non-native polychaete *M. viridis* recorded around Denmark between 2005 and 2010. Locations: Det Synfynske Øhav (DSØ), Fænøsund (F), Fynshoved (FS), Gølborg Fjord (GF), Guldborg Sund (GS), Helnæs Bugt (HB), Hjarbæk Fjord (HF), Ho Bugt (HOB), Isefjorden (IF), Jammerland Bugt (JB), Kalø Vig (KV), Kertinge Nor (K), Køge Bugt (KB), Mariager Fjord (MF), Nibe Bugt (NiB), Nissum Bredning (NB), Nissum Fjord (NF), Odense Fjord (OF), Præstø Fjord (PF), Ringkøbing Fjord (RIF), Roskilde Fjord (ROF), Rømø (R), Saksø Fjord (SF), Sejerøbugten (SB), Stege Bugt (STB), Vejle Fjord (VF). Data were compiled from the Ministry of Environment database, Christoffersen (2010), Zaiko et al. (2011) and authors' unpubl. data

fully in new areas (Ricciardi & Rasmussen 1998). Species of the genus *Marenzelleria* have the required tolerance (Hahlbeck et al. 2000, Blank et al. 2004) to withstand salinity fluctuations and exposure to anoxia or even sulfidic events that are common in Danish estuaries (Conley et al. 2000). Furthermore, it has a high reproductive capacity (Essink & Kleef 1993) that enables it to spread rapidly and over long distances ( $>100 \text{ km yr}^{-1}$ ; Leppäkoski & Olenin 2000).

This study aims at establishing how the invasion of the polychaete *Marenzelleria viridis* affects the native benthic fauna in Danish estuaries. (1) We describe the past and current spatial population dynamics of *M. viridis* in the shallow Danish estuary, Odense Fjord. (2) We evaluate the implications of *M. viridis* for the native benthic fauna community. We focus on interactions between *M. viridis* and the 2 ecologically important native polychaete species in Danish estuaries, *Nereis diversicolor* and *Arenicola marina*.

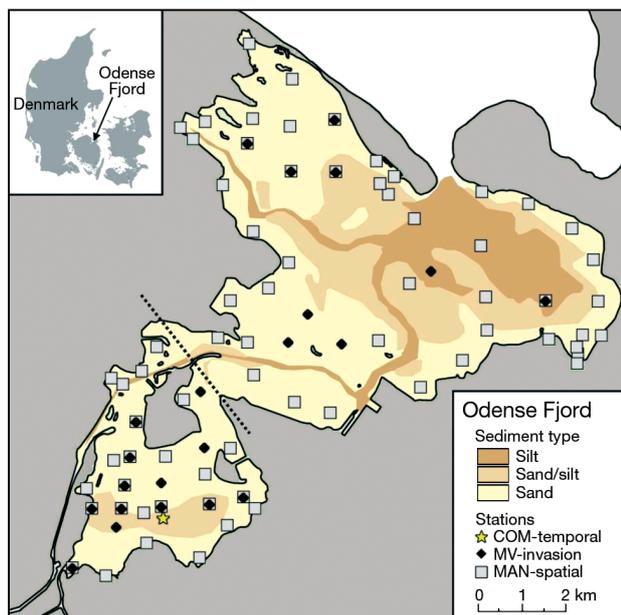


Fig. 2. Odense Fjord with sediment type and sampling locations of the 3 surveys: COM-temporal, MV-invasion and MAN-spatial (see 'Quantification of *M[.] viridis* invasion and its effect on the native fauna' for detailed explanations). Dashed line: separation between inner and outer part of the estuary

## MATERIALS AND METHODS

### Study site

Odense Fjord is a shallow microtidal estuary (sensu Conley et al. 2000) separated into 2 basins (Fig. 2). The inner basin covers  $\sim 11 \text{ km}^2$  with mean depth of  $\sim 1 \text{ m}$ , whereas the larger ( $\sim 50 \text{ km}^2$ ) outer basin has an average depth of  $\sim 3 \text{ m}$ . The main freshwater input to the estuary is the Odense River located at the head of the inner basin whereas seawater is exchanged through a narrow opening at the mouth of the estuary (Fig. 2; Riisgård et al. 2008). Consequently, the average salinity in the outer basin is higher ( $\sim 20$ ) than in the inner basin (10 to 15). The salinity in the inner basin is controlled by freshwater discharge from the Odense River and by a salt wedge through the deep ( $>6 \text{ m}$ ) shipping channel that runs throughout the estuary. Thus, the salinity in the inner part of the estuary occasionally drops  $<5$  during winter and spring when run-off from land is most intense. Sediments in the shallow areas mainly consist of low organic ( $<3\%$ ) fine sand, but the

deeper areas trap fine particles and form organic-rich ( $>10\%$ ) silty sediments (Fig. 2; Riisgård et al. 2008). As many other Danish estuaries, Odense Fjord is characterised by a widespread and high abundance of the polychaete *Nereis diversicolor* and local but dense population of *Arenicola marina* (Riisgård & Banta 1998, Conley et al. 2000, Riisgård et al. 2004). Recent surveys, based on  $>25$  stations, have shown that the benthic fauna in Odense Fjord is characterised by large abundances of the oligochaete *Tubificoides benedii* (on average 25%), crustacean *Corophium volutator* (10%), gastropods from the Hydrobiidae family (20%), and the polychaetes *N. diversicolor* (10%) and *Pygospio elegans* (20%). These species together constitute  $\sim 85\%$  of the total abundance (authors' unpubl.). The vegetation mainly consists of seagrasses (*Zostera marina* and *Ruppia maritima*) and macroalgae (*Fucus vesiculosus* and *Ulva lactuca*; Riisgård et al. 2008).

### Quantification of *Marenzelleria viridis* invasion and its effect on the native fauna

In order to describe the temporal and spatial dynamics of *Marenzelleria viridis* invasion and its effect on the benthic community structure in Odense Fjord, 3 different datasets were utilized (Table 1): (1) MV-invasion from spatial observations of *M. viridis* and *Nereis diversicolor* over a 10 yr period (1998 to 2007); (2) COM-temporal is a 25 yr long dataset (1983 to 2007) at 1 location of the benthic community associated with the introduction of *M. viridis*; (3) MAN-spatial is an extensive estuary-wide survey conducted in 2008 and 2010 to establish the current distribution and abundance of *M. viridis*, *Arenicola marina* and *N. diversicolor*. The MV-invasion and COM-temporal surveys were generated by the

Table 1. Summary of the 3 sampling designs used in this study. see 'Quantification of *M[.] viridis* invasion and its effect on the native fauna' for detailed explanations

Species	Station(s)	Period	Sampling	Replicate
<b>MV-invasion</b>				
<i>Marenzelleria viridis</i>	23	1998–2007	10	1
<i>Nereis diversicolor</i>				
<b>COM-temporal</b>				
Full community	1	1983–2007	37	10
<b>MAN-spatial</b>				
<i>Marenzelleria viridis</i>	70	2008 and 2010	1–2	4
<i>Arenicola marina</i>				
<i>Nereis diversicolor</i>				

County of Fyn as part of the National Monitoring Programme (Conley et al. 2002).

***Marenzelleria viridis* invasion (MV-invasion).** The temporal change in the spatial distribution of *M. viridis* was monitored over an estuary-wide grid of 23 stations separated by ~100 m (Fig. 2). Only 1 replicate sample was taken at each station either during spring or summer every year from 1998 to 2007 (Table 1). Sampling was conducted using a 30 cm long and 12.8 cm in diameter steel corer (0.0129 m<sup>2</sup>) to retrieve a representative portion of the sediment (0 to 25 cm depth). The sediment was sieved on the location through a 1 mm mesh, and retained material was immediately preserved in buffered 4% formaldehyde. In the laboratory, samples were washed clean of formaldehyde, and macrofauna was sorted after staining with Rose Bengal. Sorted macrofauna was then preserved in 70% ethanol before species identification and counting. Only results for *M. viridis* and *Nereis diversicolor* were considered in this study. Maps of the temporal and spatial dynamics of *M. viridis* invasion in Odense Fjord were generated with GIS software (MapInfo Professional 8.0).

**Benthic community temporal response (COM-temporal).** Temporal changes of the total benthic community were monitored at a ~0.9 m deep station in the inner part of the estuary (Fig. 2; 55.4512° N, 10.4617° E). A total of 10 replicates were taken within a radius of 20 m during each spring and autumn almost every year from 1983 to 2007 (Table 1). This survey was carried out with the sampling technique described for the MV-invasion survey.

Multivariate analyses of raw abundance data were done using the software package Primer 6 (Clarke & Gorley 2006). Prior to analyses, some of the taxonomically difficult groups were pooled to the lowest reliable taxonomic level (e.g. *Corophium* genus, Hydrobiidae family and *Polydora* genus). This operation has marginal consequences on the outcome of subsequent analysis (Sommerfield & Clarke 1995). To correct for the different levels of spatial aggregation of the species and downweigh the occurrence of erratic species, raw data were dispersion-weighted by dividing the abundance of each species by their respective index of dispersion, the variance to mean ratio, for each sampling date (Clarke et al. 2006a, Jørgensen et al. 2011). Subsequently, the data were square root transformed to retain the main community structure and lower the importance of abundant species (Clarke et al. 2006a). Changes in the community over time were determined by comparing the similarity of averages using the Bray-Curtis (BC) coefficient on which all subsequent multivariate tests

were based (Clarke et al. 2006b). Relationships among assemblages were visualised using non-metric multidimensional scaling (MDS) where the distance ranking between points on the diagram matches the BC similarity ranking between communities at different times (Clarke 1993). To test for changes in the community before (B) and after (A) the introduction of *Marenzelleria viridis*, the data were analysed using a 1-way analysis of similarity (ANOSIM). The procedure generates an R statistics that quantifies the degree of segregation between the groups and a p-value indicating the significance of the difference observed. R usually ranges between 0 and 1, and takes the value 1 when all communities within groups are more similar than any community between groups (Clarke 1993). The SIMPER technique was also used to identify the extent by which the 2 periods differed and how species contributed to this difference (Clarke & Gorley 2006). The most influential species were explored by the BVSTEP procedure by estimating the Spearman correlation of temporal variations for a chosen subset of species and the full fauna dataset. Furthermore, this procedure also identifies a subset of species having highly correlated temporal variations ( $\rho_{\text{Spearman}} > 0.95$ ) with the entire community (Clarke & Warwick 1998).

**Spatial distribution and interactions among polychaete species (MAN-spatial).** Two estuary-wide surveys were conducted to investigate the current spatial distribution of *Marenzelleria viridis* and 2 important native polychaete species in Odense Fjord, *Arenicola marina* and *Nereis diversicolor* (Riisgård & Banta 1998, Riisgård et al. 2004). These surveys consisted of 24 and 50 stations sampled in spring 2008 and autumn 2010, respectively (Table 1, Fig. 2). At all stations the density of polychaetes was determined from 4 replicate samples. Raw data from the 2 surveys were merged and in case of station-overlap, the data from the 2 surveys were averaged to provide a grid of 70 stations in total (Table 1). Samples were collected using a slightly larger corer (0.0177 m<sup>2</sup>) than in the other surveys. Samples were preserved as described above, but only *M. viridis*, *A. marina* and *N. diversicolor* were quantified. *Arenicola marina* density in 2008 was based on the corer technique whereas in 2010 it was supplemented by counting faecal casts inside a 0.5 × 0.5 m steel frame (Reise et al. 2001). For the large bodied lugworm, the latter method was assumed more representative than the corer technique. The frame technique may be inappropriate for detecting the smallest individuals <1 g (Valde-

marsen et al. 2011), but it provides better spatial resolution (max. resolution: 1 m<sup>2</sup>) compared to the core technique (max. resolution: 14 m<sup>2</sup>). A comparison between results obtained from both methods in 2010 showed a strong correlation between the methods for abundance >10 m<sup>-2</sup> ( $r = 0.9$ ,  $p < 0.001$ ) and that frame technique results represented ~40% of the results obtained with the corer. Data collected in 2008 were therefore corrected by this factor.

Maps of the spatial distribution of *Marenzelleria viridis*, *Nereis diversicolor* and *Arenicola marina* were constructed using GIS software (MapInfo Professional 8.0). The distribution was based on the inverse distance weighting interpolation method (Mitas & Mitasova 1999). Thus, the estuary was divided in 15 × 15 m cells in which the polychaete density was estimated from the distance weighted average of the polychaete abundance at the 70 stations. Since none of the examined polychaete species were observed in the fluid mud (authors' unpubl. data), hypothetical stations with zero abundance were added along the shipping channel.

## RESULTS

### *Marenzelleria viridis* invasion (MV-invasion)

*Marenzelleria viridis* was observed for the first time at one station in the inner part of Odense

Fjord in 2002 and it spread hereafter rapidly to most of the surveyed area (Fig. 3). In 2003 and 2004 it was observed on 25 and 75% of the monitoring stations, respectively. Subsequently, *M. viridis* was present at 50 to 75% of the monitoring stations until the end of the survey in 2007 (Fig. 3). *Marenzelleria viridis* was never observed at 5 stations located in the most inner parts of the estuary with lowest salinity and the silty areas (Figs. 2 & 3). Although the *M. viridis* abundance was site-specific, the temporal changes were similar at all stations. The rapid initial increase in abundance during the first 2 yr, reaching maximum values ranging from 150 to 3000 ind. m<sup>-2</sup>, was followed by a slow decrease from ~20 to 70% of this level at the end of the survey in 2007 (Fig. 4).

The pronounced decline in the overall abundance of *Nereis diversicolor* after 2001 corresponded with the introduction of *Marenzelleria viridis* in the estuary (Fig. 4). However, *N. diversicolor* remained throughout the survey period present at 75% of the examined 23 stations. The abundance of *N. diversicolor* at stations colonised by *M. viridis* on average dropped by 63% from an average of 1490 before 2002 to 550 ind. m<sup>-2</sup> after (Fig. 4). In contrast, the average abundance of this species at stations that were not colonised by *M. viridis* was >1000 ind. m<sup>-2</sup> throughout the survey period, except for 2002 and 2003 where the abundance reached 650 and 450 ind. m<sup>-2</sup>, respectively.

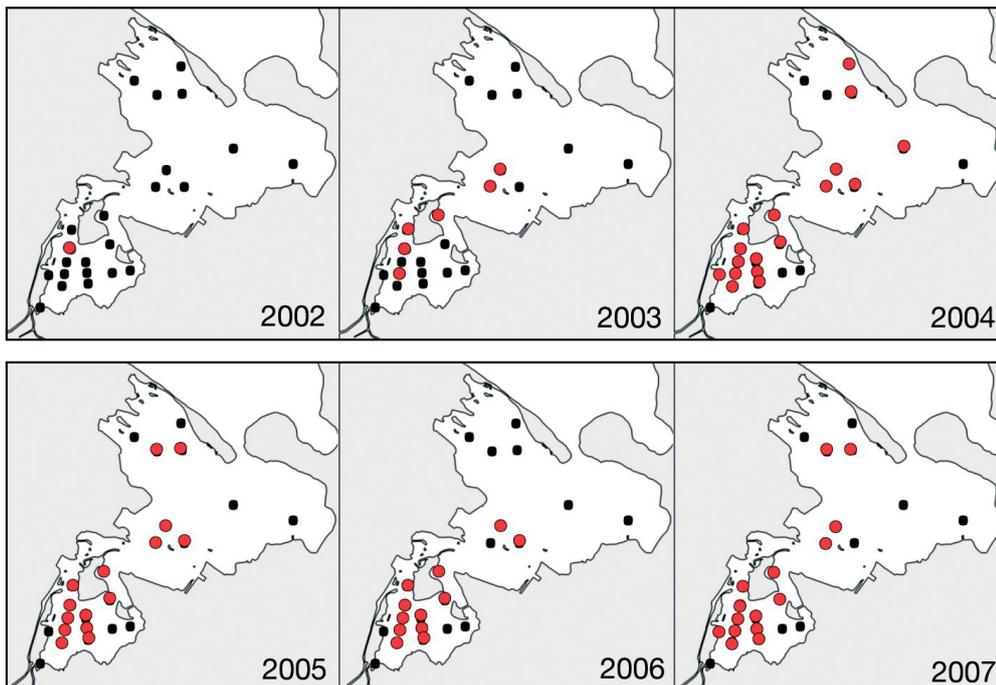


Fig. 3. *Marenzelleria viridis*. MV-invasion survey. Distribution and temporal change of non-native *M. viridis* in Odense Fjord from 2002 to 2007. Filled circles: presence (red) or absence (black) of *M. viridis*. *M. viridis* was not observed prior to 2002

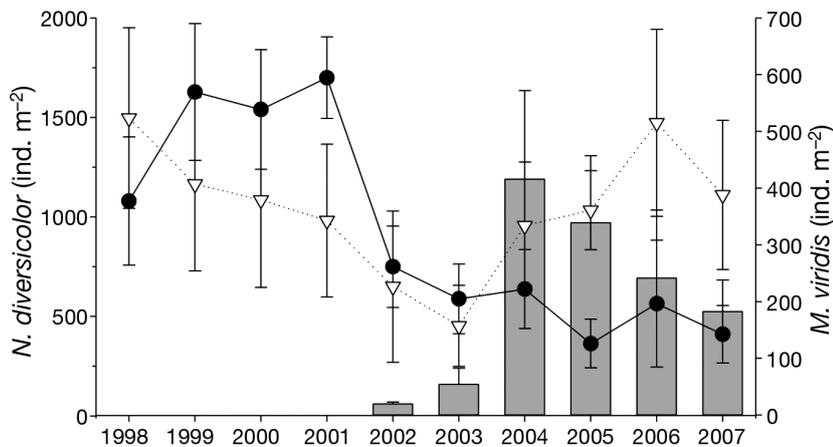


Fig. 4. *Marenzelleria viridis* and *Nereis diversicolor*. MV-invasion survey. Temporal change in *M. viridis* (grey columns) abundance in Odense Fjord represented by the average for the entire grid of 23 stations. Temporal change in density (means  $\pm$  SE) of *N. diversicolor* at stations colonised (●) and never colonised (▽) by *M. viridis*

#### Benthic community temporal response (COM-temporal)

More than 55 000 macroinvertebrate individuals belonging to 22 benthic taxa were identified over the 25 yr of the COM-temporal survey, and half of the identified species were polychaetes (Table 2). Overall, 95% of the total abundance consisted of species from the 3 taxonomic groups. The class Gastropoda was represented by the Hydrobiidae family that contributed 76% to the total species abundance (mainly *Hydrobia ulvae* and *Potamopyrgus antipodarum*), the class Polychaeta contributed 16% (mainly *Nereis diversicolor*), and the class oligochaeta contributed 3% (mainly *Tubificoides benedii*; Table 2).

*Marenzelleria viridis* was first recorded at the COM-temporal station in 2004 and its average abundance remained low (10 to 70 m<sup>-2</sup>). Nevertheless, there was a strong shift in the benthic community composition synchronous with its introduction. The MDS plot (Fig. 5a) shows that all dates before 2004 were clearly grouped together, indicating that temporal variations were minimal, although with some cyclic trends. A distinct change of the community was evident from 2003 to 2004 (ANOSIM,  $R = 0.93$ ,  $p < 0.001$ ). Subsequently, the community continued

to change as indicated by strong dissimilarities between consecutive dates until the end of the survey in 2007 (Fig. 5a). Overall, *M. viridis* introduction increased ( $p = 0.007$ ) the species richness (mean  $\pm$  SD) from  $9.5 \pm 2.0$  ( $n = 30$ ) to  $12 \pm 2.4$  ( $n = 7$ ). The SIMPER procedure identified that the community before and after 2004 was 60% dissimilar and the species contributing the most to this difference were *Nereis diversicolor* (27%) and *Arenicola marina* (10%). In fact, population changes of *N. diversicolor* alone were so influential that the BVSTEP procedure showed an almost perfect match between the temporal pattern of this species and that of the whole community ( $\rho_{\text{Spearman}} = 0.82$ ,

$p < 0.001$ ). While *N. diversicolor* was most abundant before 2004, its total density decreased >10-fold, from 1400 to 125 ind. m<sup>-2</sup> after introduction of *M. viridis* and *A. marina* (Fig. 5b-d, Table 2). The negative interaction between *N. diversicolor* and *A. marina* was nearly significant ( $r = -0.65$ ,  $p = 0.07$ ), whereas the relation between *N. diversicolor* and *M. viridis* was not significant ( $r = -0.34$ ,  $p = 0.41$ , Fig. 6). The abundances of *M. viridis* and *A. marina*, on the other hand, were strongly positively correlated ( $r = 0.83$ ,  $p = 0.01$ ; Fig. 6).

Table 2. COM-temporal survey. Macrofaunal composition (abundance m<sup>-2</sup>; means  $\pm$  SD) before and after the introduction of *M. viridis* in 2004. Only the 16 species that accounted for >5% of total counts are shown

Class	Species	1983–2003	2004–2007
Polychaeta	<i>Arenicola marina</i>	0.6 $\pm$ 2	70 $\pm$ 6
	<i>Eteone longa</i>	2 $\pm$ 6	28 $\pm$ 5
	<i>Nereis diversicolor</i>	1400 $\pm$ 500	125 $\pm$ 14
	<i>Heteromastus filiformis</i>	12 $\pm$ 10	30 $\pm$ 2
	<i>Marenzelleria viridis</i>	0	33 $\pm$ 4
	<i>Polydora</i> spp.	64 $\pm$ 131	32 $\pm$ 5
	<i>Pygospio elegans</i>	5 $\pm$ 14	18 $\pm$ 21
Bivalvia	<i>Cerastoderma glaucum</i>	29 $\pm$ 52	23 $\pm$ 6
	<i>Mya arenaria</i>	23 $\pm$ 31	1.2 $\pm$ 0.5
	<i>Mytilus edulis</i>	2 $\pm$ 5	0
Gastropoda	Hydrobiidae fam.	7200 $\pm$ 5300	6400 $\pm$ 600
	<i>Littorina</i> spp.	490 $\pm$ 640	220 $\pm$ 50
Insecta	Chironomidae indet.	8 $\pm$ 16	44 $\pm$ 10
Others	<i>Corophium</i> spp.	125 $\pm$ 200	15 $\pm$ 3
	<i>Nemertini</i> indet.	18 $\pm$ 33	35 $\pm$ 7
	<i>Tubificoides benedii</i>	180 $\pm$ 225	175 $\pm$ 20

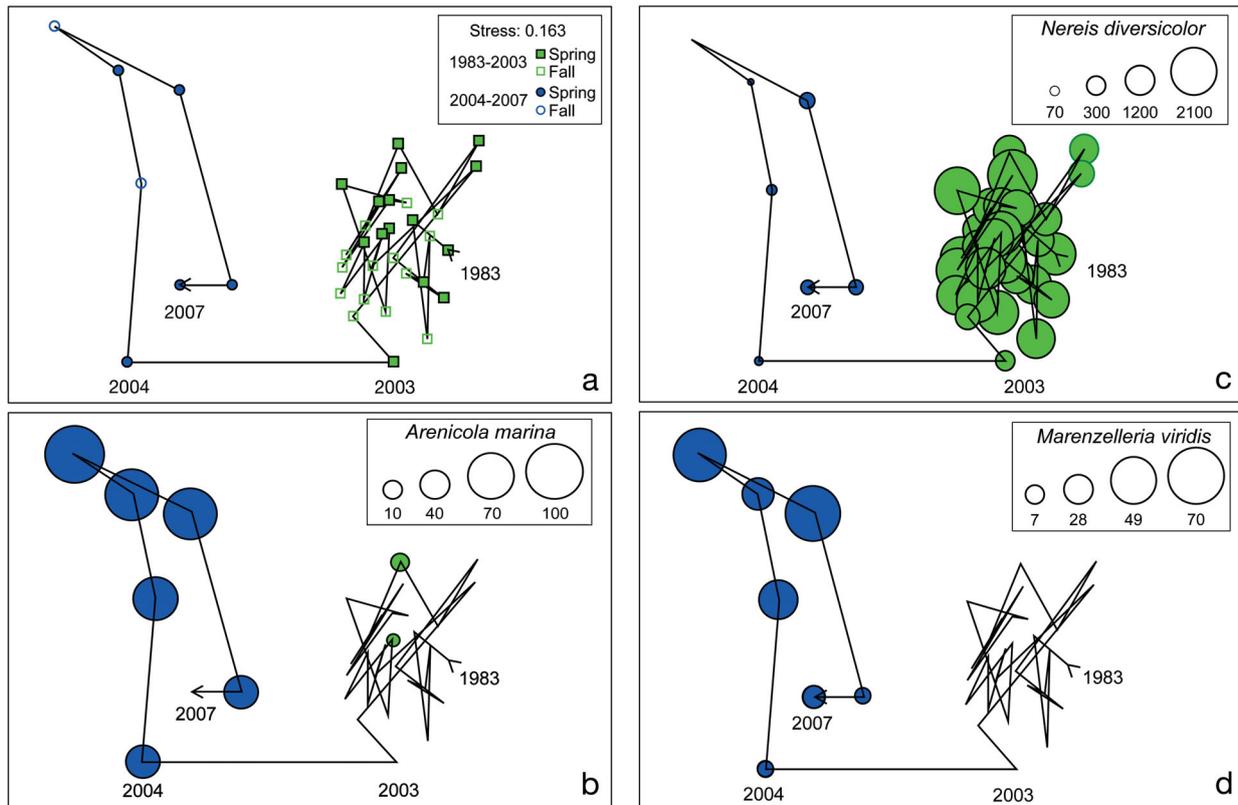


Fig. 5. *Marenzelleria viridis*, *Arenicola marina* and *Nereis diversicolor*. COM-temporal survey. (a) MDS ordination of temporal variations of the benthic community before (green squares) and after (blue circles) *M. viridis* introduction. The line connects communities that are consecutive in time. (b, c, d) Same MDS with superimposed circles representing the average abundance (ind. m<sup>-2</sup>, n = 10) of *A. marina*, *N. diversicolor* or *M. viridis*

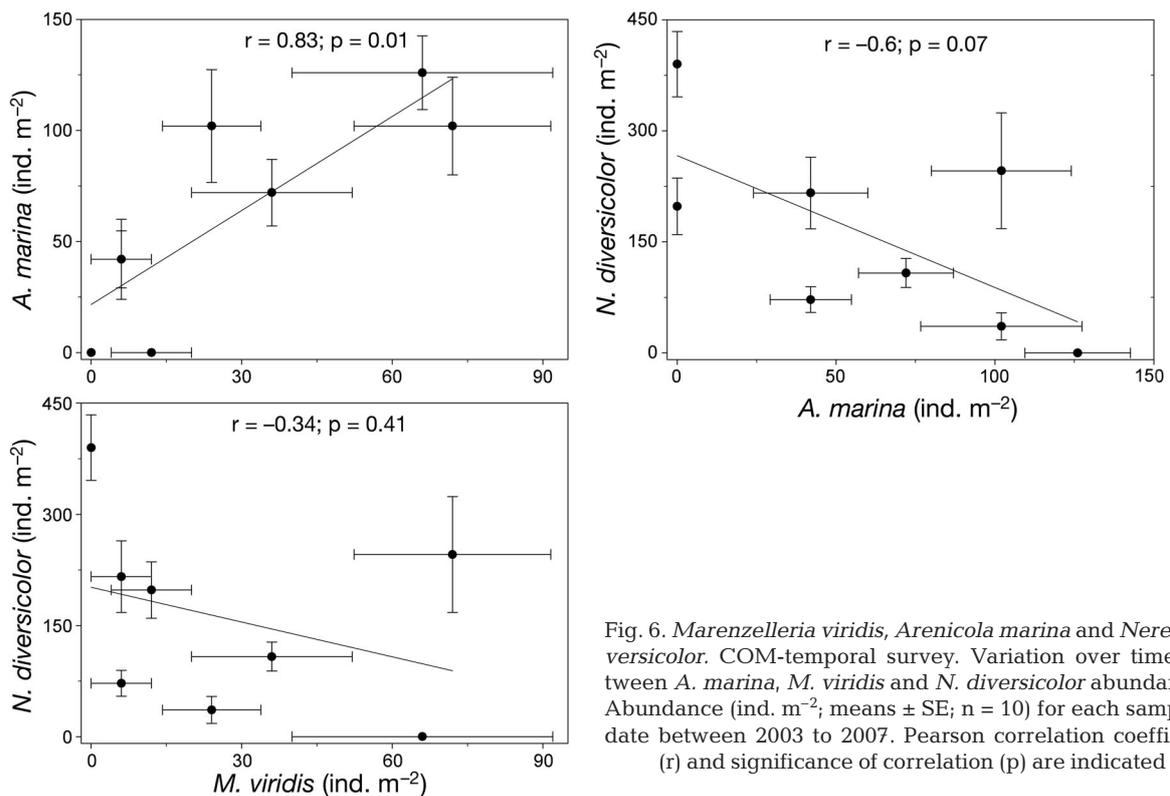


Fig. 6. *Marenzelleria viridis*, *Arenicola marina* and *Nereis diversicolor*. COM-temporal survey. Variation over time between *A. marina*, *M. viridis* and *N. diversicolor* abundances. Abundance (ind. m<sup>-2</sup>; means  $\pm$  SE; n = 10) for each sampling date between 2003 to 2007. Pearson correlation coefficient (r) and significance of correlation (p) are indicated

Table 3. *Marenzelleria viridis*, *Arenicola marina* and *Nereis diversicolor*. MAN-spatial survey. Populations of the 3 species in Odense Fjord. Proportion (%) of stations occupied by each species is reported as well as their average (maximum) population abundance (ind. m<sup>-2</sup>) in the inner (28 stations) and outer (42) parts of the estuary

Odense Fjord	<i>Marenzelleria viridis</i>		<i>Arenicola marina</i>		<i>Nereis diversicolor</i>	
	Proportion	Abundance	Proportion	Abundance	Proportion	Abundance
Inner	37	80 (1174)	21	4 (29)	100	1148 (3013)
Outer	58	123 (523)	50	10 (85)	60	267 (3169)
Whole	50	107	38	7.6	76	607

Besides the role of *Nereis diversicolor*, the BVSTEP procedure suggested that the crustacean *Corophium* spp., the bivalve *Cerastoderma glaucum* and the gastropods *Littorina* spp. and Hydrobiidae contributed to the overall community change by a decrease in abundance ( $\rho_{\text{Spearman}} = 0.95$ ,  $p < 0.001$ ; Table 2). Hydrobiidae and *Littorina* spp. were negatively correlated with *A. marina* ( $r = -0.87$ ,  $p = 0.04$ , and  $r = -0.60$ ,  $p = 0.01$ , respectively) and *M. viridis* ( $r = -0.76$ ,  $p = 0.003$ , and  $r = -0.70$ ,  $p = 0.05$ , respectively).

#### Polychaete spatial distribution (MAN-spatial)

*Marenzelleria viridis* was present at 50% of all 70 surveyed stations (Table 3). It was most common in the outer part of the estuary where it was found at 58% of the 42 stations mainly in sandy and silty/sandy sediment (Figs. 2 & 7). The presence of *M. viridis* was more restricted in the inner part of the estuary where it was found at 37% of the 28 stations, mainly in areas near the shipping channel. The average *M. viridis* density in Odense Fjord was ~107 with local maxima of up to ~1200 ind. m<sup>-2</sup> (Table 3, Fig. 7).

*Arenicola marina* was observed at 27 out of the 70 stations. Similarly to *Marenzelleria viridis*, it was

more commonly found in the outer part of the estuary (50%) compared to stations located in the inner part of the estuary (21%). The highest abundance of the large bodied lugworm was 85 ind. m<sup>-2</sup> in the outer part of the estuary. The average abundance calculated from the 70 stations was ~8 ind. m<sup>-2</sup> (Table 3, Fig. 7).

*Nereis diversicolor* was found at 76% of all stations and was therefore the most widespread and abundant of the 3 polychaete species (Table 3). Only *N. diversicolor* was present at all 28 stations located in silt areas and at all stations in the inner part of Odense Fjord (Figs. 2 & 7). Its average density for the entire estuary was ~600 ind. m<sup>-2</sup>, but reached >3000 ind. m<sup>-2</sup> at certain locations in particularly the inner part (Table 3, Fig. 7).

## DISCUSSION

### Past and present distribution of *Marenzelleria viridis*

**Invasion.** The population dynamics of *Marenzelleria viridis* in Odense Fjord as observed by the MV-invasion survey followed the 4 phase 'boom-bust' population development typical of many invaders

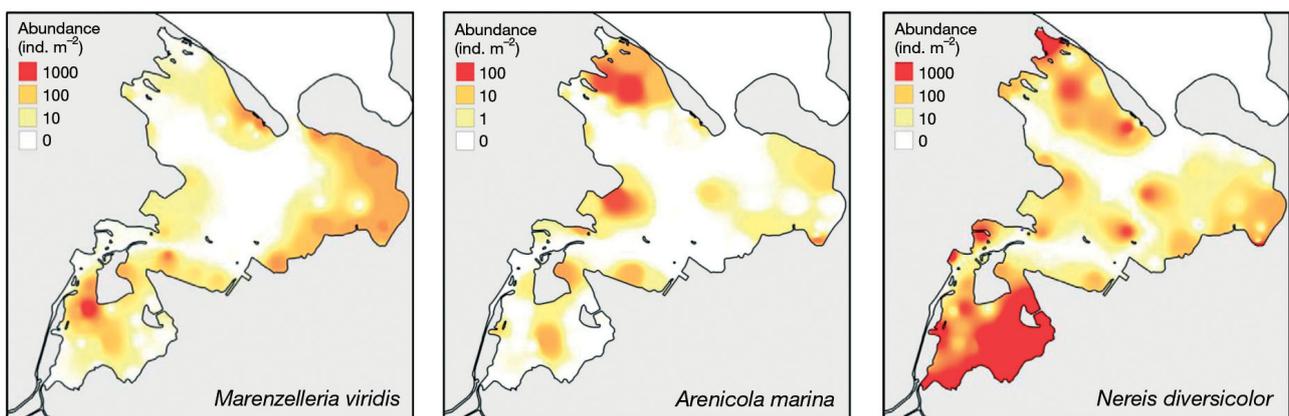


Fig. 7. *Marenzelleria viridis*, *Arenicola marina* and *Nereis diversicolor*. MAN-spatial survey. Populations of the 3 species in Odense Fjord for Years 2008 to 2010. Note: scales differ for each panel

(Fig 4; Essink & Dekker 2002, Reise et al. 2006). The first phase corresponds to the initial inoculation of the new species into the ecosystem. This occurred for *M. viridis* near the shipping channel in the inner part of Odense Fjord in 2002. Its pelagic larvae may have been introduced through the release of ballast water from ships or brought by the subsurface inflow of high saline seawater from the connecting sea (Blank et al. 2004, Josefson & Hansen 2004). The second phase is a lag period where the newly established species after a successful introduction maintains a low but stable population for some time (Essink & Dekker 2002, Reise et al. 2006). The duration of this phase is variable, but ecosystems with low species richness and high anthropogenic pressure tend to have shorter lag-phases because of their higher invasibility (Reise et al. 2006, Zaiko et al. 2011). The lag-phase observed for *M. viridis* in the species-poor and eutrophic Odense Fjord was 1 to 2 yr and is among the shortest reported in the literature for *Marenzelleria* spp. (Essink & Dekker 2002). The third phase is typically expressed by rapid population growth (Essink & Dekker 2002, Reise et al. 2006). This phase was obvious in Odense Fjord as a 10-fold increase of *M. viridis* abundance from 2003 to 2004. Such development has been reported elsewhere for both *M. viridis* (Essink & Dekker 2002) and *M. neglecta* (Zettler et al. 2002) and has been attributed to the availability of an empty niche or unutilised food resources (Essink & Dekker 2002, Reise et al. 2006). In the 4th phase of introduction, the population levels off to lower intermediate values (Essink & Dekker 2002, Reise et al. 2006). The reasons of such decline remain hypothetical (Reise et al. 2006), but it could be attributed to decline of genetic population quality (consanguinity) or increased predation from birds and fish (Essink & Kleef 1993, Essink & Dekker 2002). Thus, at the end of the MV-invasion survey in 2007, the estimated average abundance of *M. viridis* in Odense Fjord was reduced to ~50% of that in 2004. The population dynamics of *M. viridis* suggests that the invasion in Odense Fjord lasted 5 yr and was at its final stage in 2007. According to the 'boom-bust' population dynamic (Essink & Dekker 2002), it is likely that densities measured in this estuary will remain in this range and will not reach levels observed in the Baltic (>9000 ind. m<sup>-2</sup>; Norkko et al. 2012). This is supported by the current average abundance of *M. viridis* measured in the estuary wide survey (MAN-spatial), but only future surveys of the area can provide more certainty on this matter.

**Spatial distribution.** The MV-invasion survey also indicates that *Marenzelleria viridis* spread from a

single population near the shipping channel in the inner part to the entire 60 km<sup>2</sup> estuary within 2 yr (Fig. 3). A single introduction was probably sufficient for *M. viridis* to expand its geographical range. Compared to native polychaetes, it has the ability to spread rapidly due to a high reproductive capacity (up to 40 000 eggs female<sup>-1</sup>) and larvae with a 1 to 3 mo pelagic stage (Essink & Dekker 2002). Once introduced at 1 location, *M. viridis* generally maintained its presence throughout the duration of the MV-invasion survey. The small sample size used in this survey (1/78 m<sup>2</sup>) may have caused the minor temporal fluctuations in the grid occupation (50 to 75%) by occasionally overlooking its presence at stations with low abundance.

The current distribution of *Marenzelleria viridis* obtained from the estuary-wide survey (MAN-spatial) confirms the generally successful establishment of this polychaete over the entire estuary. Both the historical (MV-invasion) and current spatial (MAN-spatial) distribution of *M. viridis* in Odense Fjord could arise from the limits of its environmental tolerance. It was not found at 2/3 of the stations in the inner part of the estuary where the low winter salinity (<5 psu) probably was beyond its physiological range (George 1966, Bochert 1997, Blank et al. 2004). The distribution of *M. viridis* in the outer estuary could be regulated by sediment conditions as salinity in this area remains >15 psu at all seasons. Its absence in impermeable silty sediments (Figs. 2, 3 & 7) was probably a consequence of its specific burrowing lifestyle. It can probably only sustain adequate ventilation of the deep J- or I-shaped burrows and thus provide sufficient oxygen supply in permeable sandy sediments (Quintana et al. 2011).

#### **Effects of *Marenzelleria viridis* on the infauna community**

**Perspectives for *Nereis diversicolor*.** The MV-invasion survey revealed that the introduction of *Marenzelleria viridis* was synchronous with a 60% decrease in abundance of *N. diversicolor* in Odense Fjord. Nevertheless, *N. diversicolor* remains the prevailing polychaete species in this estuary (Fig. 4, Table 3). It is possible that *M. viridis* limited the abundance of *N. diversicolor* through competition for food and space (Kristensen 1988, Essink 1999) or reducing the recruitment and survival of juveniles by ventilation-driven upward percolation of sulphide rich water from burrows (Woodin et al. 1998, Kotta et al. 2001, Quintana et al. 2011). Alternatively, the

overall abundance of *N. diversicolor* in Odense Fjord may have declined following recurrent hypoxic events in the early 2000s as in other Danish estuaries (Greve et al. 2005, Conley et al. 2007), creating an empty niche for the opportunistic (Essink & Dekker 2002) and anoxia tolerant (Hahlbeck et al. 2000) *M. viridis*. Once established, *M. viridis* may have prevented the return of *N. diversicolor* to previous population levels observed at locations that were never colonised by *M. viridis*. Nevertheless, *N. diversicolor* is not likely to disappear completely from Odense Fjord as a consequence of the *M. viridis* invasion. Instead, it may coexist in low abundances with *M. viridis* or retreat to refuges where *M. viridis* cannot survive, e.g. low saline areas with silty sediment. It will be interesting to follow whether the currently established *Nereis* and *Marenzelleria* zones in Odense fjord are stable over time.

**Relations with other infauna.** The profound decline in the abundance of *Nereis diversicolor* after 2001 was not only correlated with the occurrence of *Marenzelleria viridis*, but also related to a concurrent expansion of the lugworm *Arenicola marina*. Antagonism between *A. marina* and *N. diversicolor* has often been reported and is primarily attributed to the continuous sediment mixing and thus physical disturbance induced by *A. marina* (Flach 1992, Zipperle & Reise 2005, Volkenborn & Reise 2006). However, the low population of *N. diversicolor* maintained after the subsequent disappearance of *A. marina* suggests that *M. viridis* may prevent the return of *N. diversicolor* (Fig. 5) as discussed above. The lack of correlation between *M. viridis* and *N. diversicolor* may be masked by the strong effect of *A. marina* that acted in concert with the negative influence of *M. viridis*.

Introduction of *Marenzelleria viridis* was according to the COM-temporal survey negatively correlated with the gastropods, *Littorina* spp. and Hydrobiidae. The interactions involved in these relations are unknown, but the mechanisms mentioned for its interaction with *N. diversicolor* may apply for the other species too. However, reworking activities of *A. marina* are also suggested to affect species like *Cerastoderma glaucum*, *Corophium* spp., *Littorina* spp. (Flach 1992) and have certainly contributed to the decline observed for these species in the COM-temporal survey.

The strong positive correlation between *Arenicola marina* and *Marenzelleria viridis* observed in the COM-temporal survey indicates that they may facilitate each other. This was unexpected and is to our knowledge the first observation of such a relation between these 2 species. However, *M. viridis* may

mobilize nutrients from the sediment and stimulate microphytobenthic primary production and thereby increase food availability for *A. marina* (Riisgård & Banta 1998, Kotta et al. 2001, Kristensen et al. 2011). The relation also suggests that *M. viridis* is able to survive and thrive well under the disturbance regime of *A. marina*, while several other infaunal species are severely hampered (Flach 1992, Riisgård & Banta 1998). It is possible that *M. viridis* is capable of forming burrows deeper than those of *A. marina* thereby avoiding the strong surface disturbance. Conversely, *A. marina* may facilitate *M. viridis* by excluding potentially competitive species, including *N. diversicolor*, and thereby maintaining an 'empty' niche for *M. viridis* introduction (Flach 1992, Essink & Dekker 2002).

It is important to note that recent massive disappearance of the seagrass *Zostera marina* in the outer part of the estuary may have created more suitable sites for *Arenicola marina* (Valdemarsen et al. 2011). There, the positive relationship between the *Marenzelleria viridis* and *A. marina* may therefore have facilitated the introduction of *M. viridis* and strengthened the competitive pressure on *N. diversicolor*.

### Ecological implications

The polychaete *Marenzelleria viridis* is undoubtedly a new and persistent species in Odense Fjord. Irrespective of the consequences for the native benthic community, the ecological implications of such invasion must be assessed thoroughly. However, information from other studies on the consequences of *Marenzelleria* invasions must be evaluated carefully due to confusion in discriminating between *Marenzelleria* sibling species (Blank et al. 2008). Recent evidence indicates that the ecological function of *M. neglecta* from low saline eastern Baltic Sea areas is markedly different from that of *M. viridis* from high saline western Baltic Sea and North Sea areas (Norkko et al. 2012), probably owing to their different life traits, e.g. burrowing depth, sediment preference and bio-irrigation (Renz et al. unpubl. data). Unfortunately, only a few recent studies are available for understanding the ecological effects of *M. viridis* in Danish coastal waters (Quintana et al. 2007, 2011, Kristensen et al. 2011).

The present study indicates that the invasive *Marenzelleria viridis* in Odense Fjord is competitively superior to the native *Nereis diversicolor*. This effect is more apparent when abiotic (e.g. anoxia) or biotic (e.g. *Arenicola marina*) disturbance events

favour *M. viridis* compared to *N. diversicolor*. There are possible positive aspects related to the introduction of *M. viridis* in Odense Fjord, such as increased species richness and an additional food source for fish (Essink & Kleef 1993, Rosa et al. 2008), with implications on resilience and stability of the system (Schlaepfer et al. 2011). However, population changes in important key species such as *N. diversicolor* and *A. marina* as a consequence of the *M. viridis* invasion may have significant ecological consequences given that bioturbation (ventilation and particle reworking) by these 3 polychaete species is very different, both in scale and intensity (Kristensen et al. 2012). This may significantly affect the biological (Valdemarsen et al. 2011, Delefosse & Kristensen 2012), chemical (Banta et al. 1999, Kristensen et al. 2011) and physical (Volkenborn et al. 2007) environment in Odense Fjord.

**Acknowledgements.** We thank E. Glob from the Nature Management and Water Environmental Division of the Fyn County and C. B. Pedersen from the Ministry of Environment of Odense for providing access to their historical database. We are also grateful for assistance in the field by M. D. Kristiansen, T. Lange and T. Reuss Schmidt, and in the laboratory by D. Crunelle, P. Gandolfo Castiñeira, B. Sweeten Elemans, D. Sørensen and K. Wendelboe. We thank K. Povidisa, M. Holmer, K. Reise, J. L. S. Hansen and 3 anonymous reviewers for improving an earlier version of the manuscript and M. Anderson for her suggestions on some of the unpublished statistical analysis. This study was supported by the Danish Strategic Science Foundation (contract no. 09-063190) and the Danish Council for Independent Research (contract no. 272-08-0577 and no. 09-071369).

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Editorial responsibility: Richard Osman,  
Edgewater, Maryland, USA

Submitted: March 9, 2012; Accepted: May 14, 2012  
Proofs received from author(s): July 30, 2012