Helgol Mar Res (2012) 66:463–468
DOI 10.1007/s10152-011-0277-9

SHORT COMMUNICATION

The diatom *Mediopyxis helysia* Kühn, Hargreaves & Halliger 2006 at Helgoland Roads: a success story?

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Received: 10 January 2011 / Revised: 6 September 2011 / Accepted: 8 September 2011 / Published online: 28 September 2011

Abstract The Helgoland Roads phytoplankton long-term data set is one of the longest and the most detailed data sets in Europe, having provided continuous work-daily observations of phytoplankton abundance since 1962. These high frequency counts have undergone and are continuously subject to a high level of quality control. The Helgoland data set thus is useful in the evaluation of new records in phyto- and zooplankton. Here, we report the first appearance of the relatively recently described diatom *Mediopyxis helysia* in the Helgoland Roads counts. This species was first detected in Helgoland samples in March 2009. Importantly, it has rapidly become a prominent member of the Helgoland phytoplankton community. While in 2009 it only produced a moderate spring peak of 4,000 cells l^{-1}, it was one of the dominant diatoms in the 2010 spring bloom with *Mediopyxis* reaching cell densities above 300,000 cells l^{-1} and total chlorophyll concentrations exceeding 40 µg l^{-1}. In 2010, this species was repeatedly present throughout the year. There was no clear relationship between temperature and cell abundance with all *Mediopyxis* cells occurring at temperatures between 3 and 12°C. However, the extensive peak in 2010 was associated with a sudden drop in salinity, which could indicate that this bloom might have been the result of inflow of low salinity water into the area. This was supported by a laboratory growth experiment in which a clonal culture of *M. helysia* grew fastest at a salinity of 27 and slowest at a salinity of 31.5. Further long-term observations will be required to establish whether this species will become a regular feature at Helgoland and how this might affect the local food web.

Keywords Helgoland Roads · *Mediopyxis helysia* · Long-term trends · North Sea · Nutrients · Salinity

Communicated by H.-D. Franke.

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Introduction

The Helgoland Roads long-term phytoplankton data set is one of the longest and most detailed in Europe, as it is based on work-daily counts since 1962. It has also undergone a very rigorous taxonomic quality control and is therefore an invaluable tool for the detection of long-term trends in the composition of the phytoplankton community (Wiltshire and Dürselen 2004). These include not only the appearances of new species in the area, e.g. *Coscinodiscus wailesii* Gran and Angst, 1931 and *Thalassiosira punctigera* in the 1970s (Rick and Dürselen 1995), but also changes in the abundance and/or seasonality of existing species (Wiltshire et al. 2010). Although non-native species are often introduced either accidentally or on purpose (Reise et al. 2002), the immigration or transport of species into a new area is a normal process and often these new species remain inconspicuous components of the receiving community or establish without obvious adverse ecological or economic effects in the case of the diatom *Odontella sinensis*. The latter first appeared in the North Sea in 1903 possibly introduced by ship ballast water (Nehring 1998, Gomez 2008).

At Helgoland, one example of a relatively recent arrival, at least according to available published records, which has rapidly become a prominent member of the phytoplankton is the bipolar diatom *Mediopyxis helysia* Kühn, Hargreaves and Halliger 2006. This species was only formally described in 2006 by Kühn et al. (2006), and indeed, records of this perceived species before then are scant in the literature. It has been reported, e.g., from the Bay of Fundy (Martin and LeGresley 2008), and although no other published records are available, it is also present in several areas of the coastal North Sea (Filippart pers. com., van Beusekom pers. com.). Hoppenrath et al. (2009) cite a first record for Helgoland in June 2003. However, the species was not included in the checklist published in 2004 (Hoppenrath 2004) and was not recorded in the routine counts of the Helgoland Roads data series until it was categorically identified in March 2009. As *M. helysia* is a very large, conspicuous species, it is unlikely that it was previously overlooked and indeed rigorous evaluation of all the Helgoland Roads data categorically shows that there is no size class of unidentified diatoms of this size in the Helgoland Roads Long-term dataset.

Little is known about this species as a whole and in particular about its seasonality and physiological requirements. Therefore, the potential for *Mediopyxis* to further extend its range along European coasts is unknown. Here, we give a first account of the 'development' of *Mediopyxis helysia* since its first appearance in the Helgoland Roads time series, including an extensive bloom in spring 2010. We provide a first insight into its physiological tolerances with respect to salinity, as a possible explanation for the recent success of this species around Helgoland and in other parts of Europe.

Materials and methods

Sampling location and methodology

The Helgoland Roads monitoring site (54°11.3′N, 7°54.0′E) is located in a channel between the main island of Helgoland and a small sandy island (the "Düne"). The water depth varies from 5 to 8 m depending on the tide. Further information about this site can be found in Franke et al. (2004). Sampling for phytoplankton began at this site in 1962 (Fig. 1).

Surface water samples are collected work-daily from the Helgoland Roads monitoring site using a bucket. The sample is mixed well before preserving a 100-ml subsample in 0.1% neutral Lugol's iodine solution. Samples are stored in an amber glass bottle before 25 ml is analysed using the Utermöhl method (Lund et al. 1958). These counts provide the baseline data for the present study. Additionally, live

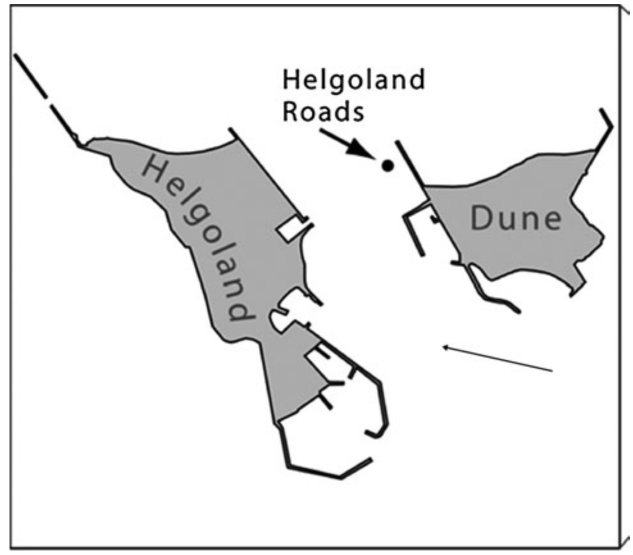

Fig. 1 Helgoland Roads sampling location (*arrow*) between the main island of Helgoland and the smaller dune

net samples are taken twice-weekly (20- and 80-µm mesh size). The images in Fig. 2 were taken from the 20-µm haul. They were produced using a Zeiss Axioskop 2 microscope and photographed with a Zeiss Axicam HCr digital camera at a magnification of ×400. Figure 2d, e show scanning electron micrographs of acid-cleaned samples.

Salinity measurements are taken work-daily from the same raw samples using an AUTOSAL salinometer (Gamma Analysen Technik GmbH).

Chlorophyll measurements

Chlorophyll concentrations are measured on a work-daily basis using a subsample of the raw water sample also used for the phytoplankton counts; 30 ml of the raw sample is routinely analysed in a BBE algal analyzer (BBE-Moldaenke GmBH, Kiel, Germany) that can detect four algal classes according to pigmentation.

The effects of salinity on the growth of *Mediopyxis* helysia

Clonal cultures of *Mediopyxis helysia* were established on 25th March 2010. They were isolated from net samples (20-µm mesh size) taken at Helgoland Roads monitoring station. Individual chains were picked from the samples and washed repeatedly with standard F/2 medium (Guillard and Ryther 1962). The newly established cultures were then grown continuously in F/2 medium in tissue culture flasks (75 cm^2, Ocean Scientific).

The growth of one clone isolated in March 2010 was tested at three different salinities each with three replicates per treatment. The salinities were chosen to reflect the salinities around the time of the phytoplankton peak in

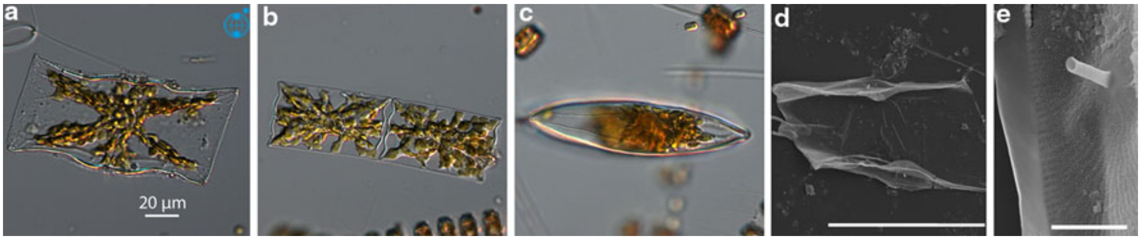

Fig. 2 Representative images of *Mediopyxis helysia* taken from net samples at the Helgoland Roads station: **a**, **b** Live cells in broad girdle view, note the difference in diameter, **c** live cell in valve view, **d**, **e** Scanning electron micrographs: **d** whole frustule, **e** Valve view of the central region showing the labiate process (*Scale bar* for **a**–**c** = 20 µm, **d** = 100 µm, in **e** = 5 µm)

spring 2010: 31.5, 29 and 27. Experiments were carried out in a constant temperature room at 15°C and a light period of 14:10 LD. Light was provided by a LUMILUX Plus Eco daylight lamp at approximately 40 µmol photosynthetically active radiation (PAR). The experiments were carried out in vent-capped tissue culture flasks. Each flask was inoculated from the stock culture so as to reach a starting concentration of approximately 100 cells ml^{-1} at t_0. The culture was then allowed to acclimate for 24 h before taking samples for t_0 cell densities. Once daily the flasks were rotated in the constant temperature room to avoid artifacts resulting from slightly differing light conditions near the edge and in the centre of the shelves.

Samples were then taken every 24 h for 5 days; 2 ml of sample was removed from each flask and fixed with neutral Lugols' iodine solution in brown Eppendorf tubes. On each sampling day, 1 ml per replicate was counted in a Sedgwick-Rafter counting chamber.

Statistical analysis

The results of the growth experiment were analysed using repeated-measures ANOVA in STATISTICA v9.1 (StatSoft). Prior to analysis, the data set was log-transformed and tested for homogeneity of variances using Cochran's *C* test. In addition, regression analyses were carried out on the log-transformed data to estimate growth rates (regression coefficient). Differences between individual treatments were analysed using the Student–Newman–Keuls post hoc test. Relationships between the biotic and abiotic factors were examined using linear regression (Multiple regression module in STATISTICA v9.1).

Results

Mediopyxis helysia morphology

Mediopyxis helysia was identified according to the original description in Kühn et al. (2006). These authors described one diagnostic character as the different morphology of the valve apices in this bipolar species: one pole usually has a round elevation, while the opposite valve pole terminates in an elongate, pointed horn (Fig. 2c). Healthy cells contained numerous plate-like chloroplasts. The protoplasm in some cells filled the whole frustule, but more commonly had retracted slightly to form a star-shaped structure. Cells in the Helgoland net samples occurred either as single cells, in pairs, or more rarely as chains of 4 or 6 cells. Similar chain lengths were observed in culture. Cell dimensions were extremely variable. In some cases, the apical axis greatly exceeded the pervalvar axis; in others, the opposite was observed (Fig. 2a, b).

Mediopyxis helysia abundance patterns

After its first detection in the Helgoland Roads long-term phytoplankton data series in March 2009, *Mediopyxis helysia* had two small peaks on April 20th and May 2nd (3,240 and 6,409 cells l^{-1}). It then occurred sporadically until November 2009. In 2010, it first appeared in January. A bloom then developed rapidly in April. Despite the rapid growth and high cell densities of *M. helysia*, the bloom in 2010 was by no means monospecific. On April 22 and May 6, the two days with the highest abundances of *M. helysia*, an additional 47 and 39 taxa, respectively, occurred in the samples (Data not shown). However, due to its large size, *Mediopyxis* still constituted 58% of the total carbon biomass on April 22nd and 91% on May 6th.

Total chlorophyll concentrations at that time peaked at over 40 µg chl l^{-1}. The bloom in April was associated with a drop in salinity. In the weeks preceding, the bloom salinity typically ranged from 31 to 33. During the week of maximum cell abundance, however, salinity had dropped to below 29. The relationship between *Mediopxis helysia* and salinity for the period 21st March 2009 (first appearance of *M. helysia*) to 31st August 2010 was significantly negative (regression analysis, $P < 0.001$, $y = -8{,}068.9x + 267009$). The peak abundance of *M. helysia* (306,289 cells l^{-1}) occurred on May 6th at a salinity of 28.6 (Fig. 3a). There was no clear trend of cell numbers with water temperature,

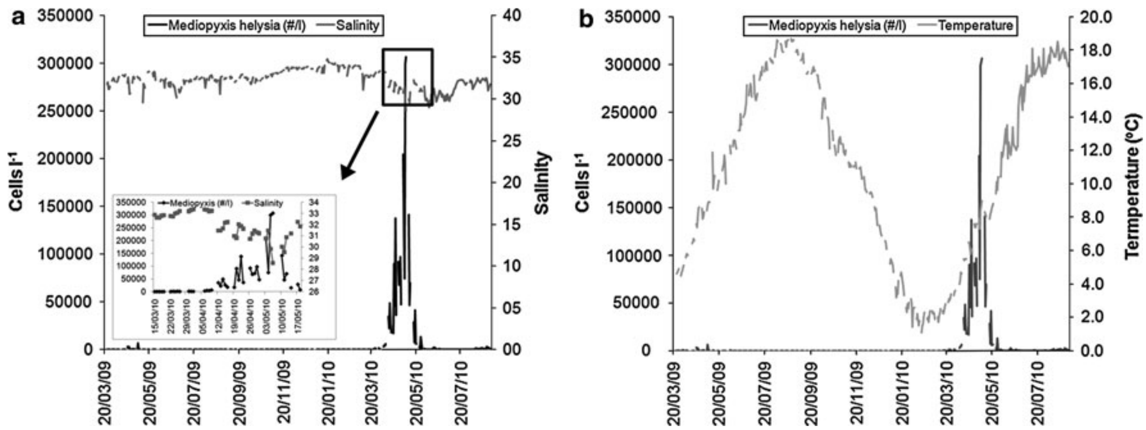

Fig. 3 Abiotic and biotic parameters as measured at Helgoland Roads between January 2009 and November 2010, plotted against *Mediopyxis* cell densities (*grey line*): **a** salinity (the *insert* shows a subset of the data from 15th March to 17th May 2010, covering the period of rapid decline in salinity and increase in Mediopyxis abundance), **b** surface water temperatures (°C)

but *M. helysia* in 2009 and 2010 occurred predominantly between temperatures of 3 and 12°C, apart from occasional records at temperatures below 3°C (Fig. 3b).

Long-term salinity patterns at Helgoland roads

A recent study by Wiltshire et al. (2010) has described a positive trend in the salinity around the island of Helgoland up to and including the year 2006. A regression analysis including data up to October 2010 has confirmed this trend ($P < 0.001$, $y = 31.15 + 0.118x$). Nevertheless, Helgoland is located in a very dynamic hydrographic environment that is influenced by different current regimes. Depending on which regime dominates, Helgoland is under the influence of offshore or coastal waters. This can cause very rapid increases or declines in salinity as shown in Fig. 3a.

The effect of salinity on growth rates of *Mediopyxis* helysia

The growth experiment showed clearly that *Mediopyxis helysia* grew significantly more quickly in the lowest salinities (similar to those prevailing around the time of its 2009 bloom) and slowest at salinity 31.5 (one-way repeated-measures ANOVA on log-transformed data: $F_{1,6} = 22.41$, $P = 0.002$, time intervals: $F_{5,30} = 109.37$, $P < 0.0001$). In all cultures, considerable growth was detectable even after 5 days, i.e. none of the cultures had reached stationary phase at the end of the experiment (Fig. 4). The calculated growth rates were almost identical at salinities 27 and 29 (0.983 and 0.983 d^{-1}, respectively) but only 0.88 d^{-1} at salinity 31.5. The SNK test revealed that for each salinity treatment, cell densities at t_0 were significantly lower than at all other time intervals. At t_5, however, salinity 27 differed significantly only from salinity 31.5 ($P = 0.0002$) but not salinity 29 ($P = 0.122$).

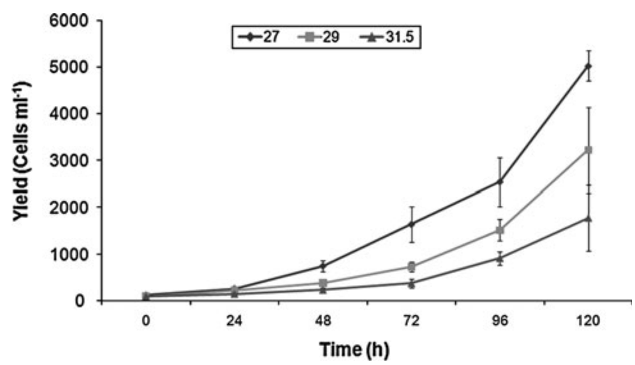

Fig. 4 Growth experiment with a clonal *Mediopyxis helysia* culture at three salinities: 27, 29 and 31.5, $n = 3$, *error bars* = ±1 standard deviation

Discussion

Should *Mediopyxis helysia* have bloomed at Helgoland before 2006, it would have been recorded as an unidentified species or as part of an unidentified size class. Since this species is very large and distinctive (the apical axis can exceed 100 µm) and is therefore easily recognizable, it is unlikely that it was previously overlooked completely. The old data set was carefully evaluated to check that an algal species in this size class was not overlooked. However, we, like with any new sightings of species, cannot preclude that it did not occur elsewhere and in another water body of the German Bight. We have no knowledge of the distribution of this diatom prior to 2006. The only conceivable species it could have been mistaken for is the diatom *Helicotheca tamesis*, however, as less than 50 records of this species appear in the entire Helgoland Roads data set and all of them at cell numbers of below 5,000 cells l^{-1}, it is extremely unlikely that *Mediopyxis* blooms of the magnitude seen in 2010 were "missed" in the past (data not shown).

The high biomass blooms appearing since 2009 therefore remain a novel feature of the Helgoland phytoplankton, and although there is virtually no information in the literature about the distribution of this species from before or indeed after its formal description (the only published record is from the Bay of Fundy (Martin and LeGresley 2008), there are now anecdotal accounts of *Mediopyxis* blooms in many coastal areas including in the North Sea.

At Helgoland, it not only has recently been recorded for the first time, but also appears to be increasing in numbers rapidly. After very moderate cell peaks in 2009, it formed extensive and prolonged blooms in 2010. The analysis of the Helgoland Roads data has shown that *Mediopyxis helysia* produced its peak during a sudden decrease in salinity and its abundance displayed a generally negative relationship with salinity throughout. That it might be adapted to low salinities was also supported by the results of the growth experiment in which *Mediopyxis* grew best at a salinity of 27 and 29 and by its occurrence in areas of frequently reduced salinities, e.g. in the Wadden Sea around the island of Sylt, where an annual average salinity of 28 has been reported (van Beusekom et al. 2008). Moreover, it has to be emphasized that even at the highest salinity growth rates were still high and could facilitate bloom formation. Whether a bloom actually develops will then also depend on the growth characteristics of other species co-occurring and therefore potentially competing with *Mediopyxis*.

It is too early to judge whether this diatom will establish a permanent presence in the Helgoland plankton community or whether it remains associated with particular environmental settings such as salinity anomalies. But its population development at Helgoland resembles that of *Coscinodiscus wailesii* in 1977 (Boalch and Harbour 1977, Boalch 1994), which first occurred only in small numbers but then started forming massive blooms that were not consumed by any predator. In the meantime, *C. wailesii* is a permanently re-occurring member of the phytoplankton community and has also become part of the zooplankton food spectrum at Helgoland Roads, although it is not consumed by microzooplankton.

The waters around Helgoland represent a very dynamic environment influenced by Atlantic inflow as well as coastal waters at different times (Hickel 1998, Stockmann et al. 2010) and salinity frequently exceeds 30 with an overall long-term trend towards higher salinities (Wiltshire et al. 2010), which has been re-confirmed by the present study using data up to October 2010. If, as the results of the growth experiment suggest, it is low salinity waters that *Mediopyxis* is adapted to, a permanent establishment of this diatom would appear unlikely.

However, as this diatom is very large, it is probably not edible for the majority of microzooplankton species (ciliates and dinoflagellates), which are often considerably smaller than the diameter of *M. helysia* (Hansen 1992, Hansen et al. 1994). Such large prey is particularly problematic for those microzooplankton species using direct engulfment or pallium feeding to capture prey. A release from grazing could compensate for suboptimal environmental growth conditions allowing this species to flourish. If *Mediopyxis* blooms are going to establish as a permanent and conspicuous component of the Helgoland phytoplankton community, the potential impact on the local food web could therefore be considerable. But negative consequences of this 'invasion' are not inevitable. While *Coscinodiscus wailesii*, for instance, turned out to be harmful as it could also clog up fishing nets, another large introduced species *Odontella sinensis* (Greville) Grunow, 1884 seemed to have no adverse effects (Boalch 1994). Further long-term observations in combination with more detailed physiological studies will be required to gain a firmer grasp of the dynamics of this species at Helgoland Roads and its potential impact on other coastal areas. These experiments will have to include greater salinity ranges, tests of the combination of different environmental factors on growth and the potential effects of grazers. This short communication also highlights the necessity of basing the interpretation of possible long-term trends not on species counts alone but combining them with physiological laboratory studies.

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