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Oxylipin production during a mesocosm bloom of *Skeletonema marinoi*

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ABSTRACT

Numerous biological activities such as grazer defense and intraspecific signaling have been described for diatom oxylipins, fatty acid derived secondary metabolites produced by some diatom species. As the function and importance of these compounds are still controversial, the production of a subclass of these molecules, nonvolatile oxylipins, was studied during an induced bloom of *Skeletonema marinoi* (Sarno et Zingone) in a mesocosm setup. Reproductive parameters of one of the main grazers, *Calanus finmarchicus*, were also examined during the bloom. Oxylipins detected during the bloom were the same as those previously described for *S. marinoi* and were detected predominantly in the mesocosm inoculated with this diatom. Reproductive success of *C. finmarchicus* remained unaffected during the course of the bloom. This may have been due to a dilution effect by the availability of alternative suitable prey or to the limited exposure of the copepods to the oxylipins generated during the short bloom. Follow up laboratory studies showed that oxylipin composition changed both when the *S. marinoi* clone used for inoculation was grown in the laboratory and in comparison to the well-studied Adriatic clone of *S. marinoi*. These results highlight the necessity of quantitatively measuring oxylipin concentrations during diatom blooms at sea to be able to correctly evaluate their ecological significance.

1. Introduction

Diatom lipoxygenase products (collectively called oxylipins) have been suggested to serve numerous ecological functions. First proposed as an insidious grazing defense mechanism (Miralto et al., 1999), oxylipins have also been assigned a putative role in allelopathy (Ribalet et al., 2007a, 2008) and cell to cell signaling (Casotti et al., 2005; Vardi et al., 2006, 2008). The proposed defense mechanism is based on the detrimental impact of diatom diets on the reproductive success of calanoid copepods (reviewed by Ianora and Miralto, 2010). A reduction in hatching success of copepod eggs has been observed in laboratory experiments when females feed on diatom cultures (*e.g.* Ban et al., 1997; Ianora and Poulet, 1993) and in the field during diatom blooms (*e.g.* Ianora et al., 2004; Miralto et al., 2003). In contrast, other field studies have shown diatoms to be a suitable prey for supporting copepod growth and reproduction (Irigoien et al., 2002; Koski, 2007), pointing towards a dose-dependent response of copepods to oxylipins in the diet. In addition to reducing the hatching success of copepod eggs, maternal diets of certain diatom species lead to malformations in hatched nauplii such as missing or reduced limbs (Ianora et al., 2004; Poulet et al., 1995) as a consequence of an induced apoptotic process in naupliar tissue (Fontana et al., 2007; Poulet et al., 2003). These deleterious effects of maternal diatom diets have been linked to the production of polyunsaturated aldehydes (PUA) by diatoms showing anti-mitotic activity (Miralto et al., 1999). PUA are a subgroup of oxylipins which are rapidly formed via lipoxygenase pathways from C_{16}- and C_{20}-polyunsaturated fatty acids (PUFA) released from chloroplastic glycolipids and membrane phospholipids upon loss of cell integrity (Cutignano et al., 2006; d'Ippolito et al., 2003, d'Ippolito et al., 2004; Pohnert, 2000). PUA production in diatoms increases under nutrient stress in culture (Ribalet et al., 2007b, 2009) and induces apoptosis-like cell death in the diatom cells themselves (Casotti et al., 2005). Therefore oxylipins have also been suggested to function as signaling molecules under unfavorable conditions such as those encountered at the end of phytoplankton blooms (Casotti et al., 2005). Such a stress signal may be important in shaping phytoplankton population dynamics such as succession and bloom termination (Vardi et al., 2006).

Abbreviations: DHA, docosahexaenoic acid; EPR, egg production rate; FPR, fecal pellet production rate; NVO, nonvolatile oxylipins; PUA, polyunsaturated aldehydes; PUFA, polyunsaturated fatty acids.

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The proposed role of PUA as an insidious defense mechanism against grazers by induction of a teratogenic effect is controversial (Flynn and Irigoien, 2009; Irigoien et al., 2002; Jónasdóttir et al., 1998). Its relevance in natural systems is still debated (Sommer, 2009) because of contradictory results obtained in field studies on the impact of diatoms on copepod reproduction (Irigoien et al., 2002; Pond et al., 1996; Sommer, 2009) and an absence of correlation between copepod reproductive success and PUA production by phytoplankton (Koski et al., 2008). More recently other oxylipins such as hydroxy-acids and epoxyalcohols have been characterized in diatoms (Barreiro et al., 2011; d'Ippolito et al., 2005; Fontana et al., 2007). These nonvolatile oxylipins (NVO) have been found to impair copepod reproduction in the laboratory by inducing apoptosis in nauplii similar to PUA (Fontana et al., 2007; Ianora et al., 2011) and have been linked to reduced hatching success during diatom blooms at sea (Ianora et al., 2008). They may therefore provide an explanation in some cases for the lack of correlation between PUA production and copepod reproductive success. Like PUA (Ribalet et al., 2007b; Vardi et al., 2006), NVO have been suggested to be involved in diatom bloom termination by functioning as a cell death signal under environmental stress (d'Ippolito et al., 2009).

In this study, mesocosms were used to study the production of NVO by a known producer of these metabolites, the marine diatom *Skeletonema marinoi* (formerly known as *S. costatum*, Sarno et al., 2005). In contrast to laboratory experiments with monocultures, the mesocosm setup allowed us to follow the development of the diatom bloom in a close to natural plankton community. The objective was to examine the production of oxylipins during the development and decline of a phytoplankton bloom, as well as to corroborate previous data on the negative effect of NVO on copepod reproductive success in the field (Ianora et al., 2008).

2. Materials and methods

2.1. Mesocosm setup

A mesocosm experiment was carried out from April 14th to 28th, 2008 at the mesocosm facility of the University of Bergen, Norway (www.bio.uib.no/lsf/inst2.html) as described in Barofsky et al. (2010). The mesocosm bags used in this study are mesocosms B, C, and F as described by Jónasdóttir et al. (2011), Koski et al. (2012), and Vidoudez et al. (2011a). These denominations are maintained to facilitate comparisons between the studies. Briefly, transparent polyethylene bags (11 m^3) were immersed in the bay outside Espegrend Marine Biological Field Station (Bergen, Norway) and filled with unfiltered seawater from 4 m depth from just outside the mesocosm bags. Seawater nutrient concentrations were low (<0.15 μmol L^{-1} nitrate, <0.04 μmol L^{-1} phosphate, <0.11 μmol L^{-1} silicate). Mesocosm bag B was enriched with nitrate and phosphate to initial concentrations of 4.21 μmol L^{-1} nitrate and 0.43 μmol L^{-1} phosphate. Mesocosm bag C was enriched to initial concentrations of 4.24 μmol L^{-1} nitrate, 0.36 μmol L^{-1} phosphate, and 3.53 μmol L^{-1} silicate. In mesocosm bag F, an exponentially growing culture of *S. marinoi* was inoculated at ~500 cells mL^{-1} initial concentration in addition to enrichment with nitrate to 4.06 μmol L^{-1}, phosphate to 0.37 μmol L^{-1}, and silicate to 3.35 μmol L^{-1}. The *S. marinoi* culture used for inoculation was the local strain isolated in 2006 and grown in bulk (10 L polyethylene bags) on f/2 medium (Guillard, 1975) on a 14 h:10 h light:dark cycle at 10 °C prior to inoculation.

2.2. S. marinoi cell counts

Concentrations of *S. marinoi* cells in the mesocosm bags were determined daily in triplicate with a CytoBuoy scanning flow cytometer (CytoBuoy, Woerden, Netherlands). Identification of *S. marinoi* was based on particle scan characteristics determined prior to the experiment. The number of cells was estimated following Takabayashi et al. (2006).

2.3. Oxylipin determination

For mesocosm B, phytoplankton samples were analyzed for days 6, 8, 10, and 12 of the mesocosm experiment. Samples from mesocosms C and F were analyzed for oxylipins on days 6–12, except for day 10 which was lost for mesocosm F. A sample from day 4 was analyzed additionally only for mesocosm F.

Phytoplankton samples were collected by filtering 400–1000 mL of mesocosm water collected daily onto 1.0 μm polycarbonate filters (GE Water & Process Technologies, Trevose, PA, USA) by a vacuum pump. Filters were folded into 1.5 mL test tubes (Eppendorf, Hamburg, Germany), frozen in liquid nitrogen, and stored at −80 °C until analysis. Filters were suspended in 1 mL deionized H_2O (Millipore, Billerica, MA, USA) in the test tubes and sonicated for 1 min on ice. Filter material was removed and the cell lysate was extracted for oxylipin analysis according to Fontana et al. (2007). Briefly, 30 min after sonication, 1:1 v:v acetone (J. T. Baker, Deventer, Netherlands) was added to the cell lysate together with 20–30 μg 16-hydroxy-hexadecanoic acid (Sigma-Aldrich, St. Louis, MO, USA) used as an internal standard for quantification. The acetone/water phase was extracted three times with 1:1 v:v CH_2Cl_2 (Carlo Erba, Milan, Italy). The lower organic phase was recovered, dried over Na_2SO_4, filtered, and after removing the solvent under reduced pressure (Büchi Rotavapor R-114, Büchi Laboratory Equipment, Flawil, Switzerland) the organic material was derivatized with ethereal diazomethane. The resulting methyl esters were analyzed for nonvolatile oxylipins (NVO) by a Qtof-*micro* mass spectrometer (Waters SpA, Milan, Italy), equipped with an ESI source (positive mode) and coupled to a Waters Alliance HPLC system (d'Ippolito et al., 2009). Oxylipin concentrations were calculated based on the volume filtered and correspond to the potential production of all *S. marinoi* cells in 1 L of mesocosm water.

A similar protocol was used for the analysis of a laboratory culture (4 replicates) of the *S. marinoi* clone used for inoculation. This was collected by centrifugation (1000 *g*, 10 min, 4 °C) in a cooled centrifuge with a swing-out rotor (DR 15P, Braun Biotechnology International, Allentown, PA, USA) from a culture in stationary phase grown on f/2 medium on a 14 h:10 h light:dark cycle at 10 °C. The obtained pellet was suspended in 1 mL deionized H_2O (g sample)$^{-1}$ before sonication (Gerecht et al., 2011) and extracted as described above. Oxylipin production in this Norwegian clone was compared to oxylipin production in a clone of *S. marinoi* isolated in 1997 from the Northern Adriatic Sea (4 replicates) (Fontana et al., 2007; Gerecht et al., 2011).

2.4. Copepod reproduction experiment

C. finmarchicus females for copepod reproduction experiments were sorted from zooplankton samples collected approximately one nautical mile west of the mesocosm location in the nearby Raunefjord. A plankton net (diameter: 1 m, mesh size: 333 μm) with a 4 L non-filtering cod-end was towed obliquely from 50 to 0 m depth for collection and females sorted immediately under an inverted microscope in a temperature-controlled room (10 °C). For each mesocosm treatment 15 mature females were incubated individually in 50 mL Falcon culture flasks (BD Biosciences, San Jose, CA, USA) kept in a temperature-controlled room at *in situ* temperature (10 °C) and dim light. Females were transferred daily into new flasks with fresh mesocosm water from that day. Eggs as well as fecal pellets produced in 24 h were enumerated under an inverted microscope. Eggs were then left to hatch for 72 h at 10 °C after which they were fixed with 4% formalin before counting hatched nauplii, abnormal nauplii, and non-viable eggs. Abnormal nauplii were separated from normal nauplii based on incompleteness of limbs.

Statistical analyses (*t*-test) were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California, USA).

3. Results

3.1. Mesocosm experiment

A bloom of *S. marinoi* was successfully induced by inoculating mesocosm F with this alga. Cell concentrations of *S. marinoi* in this mesocosm increased from the inoculation value of ~500 cells mL^{-1} to ~60,000 cells mL^{-1} within seven days (Fig. 1C). At that point the bloom peaked and cell concentrations sank below inoculation values on day 11. In mesocosm B, enriched only with the nutrients nitrate and phosphate and in mesocosm C, enriched with nitrate, phosphate, and silicate, cell concentrations of *S. marinoi* remained below 1000 cells mL^{-1} (Fig. 1A,B). These values were similar to the concentrations found in the surrounding sea (Jónasdóttir et al., 2011).

3.2. Oxylipins

The oxylipins detected in the mesocosm samples had been previously characterized from *S. marinoi* (Fontana et al., 2007). They included derivatives from C_{16}- and C_{20}-polyunsaturated fatty acids (PUFA). In the samples from the mesocosm enriched only in nitrate and phosphate (mesocosm B), no oxylipins were detected except for traces (0.02 nmol L^{-1}) of C_{20}-derivatives on day 6 (Fig. 1A). In the mesocosm enriched additionally in silicate (mesocosm C), oxylipins were detected on three occasions (days 8, 11, 12) (Fig. 1B) at concentrations below 1.5 nmol L^{-1}, and corresponded to slightly higher cell concentrations of *S. marinoi* in this mesocosm compared to mesocosm B. During the *S. marinoi* bloom in mesocosm F, two isomers of hydroxy-eicosapentaenoic acid (Rt = 17.8 and 18.7 min) and hydroxy-epoxy-eicosatetraenoic acid (Rt = 14.5 min) were detected (Fig. 2C). Also the C_{16}-oxylipin hydroxy-hexadecatetraenoic acid was present (Rt = 11.4) (Fig. 2C). Furthermore, some mesocosm samples contained an unusual fatty acid derivative with *m/z* at 381 (Rt = 20.8). This compound that showed UV absorption at 234 nm in agreement with the structure of a hydroxy-derivative of docosahexaenoic acid (DHA, $C_{22:6}$), was present in samples from mesocosm F, as well as in traces in samples from mesocosm C (Fig. 2).

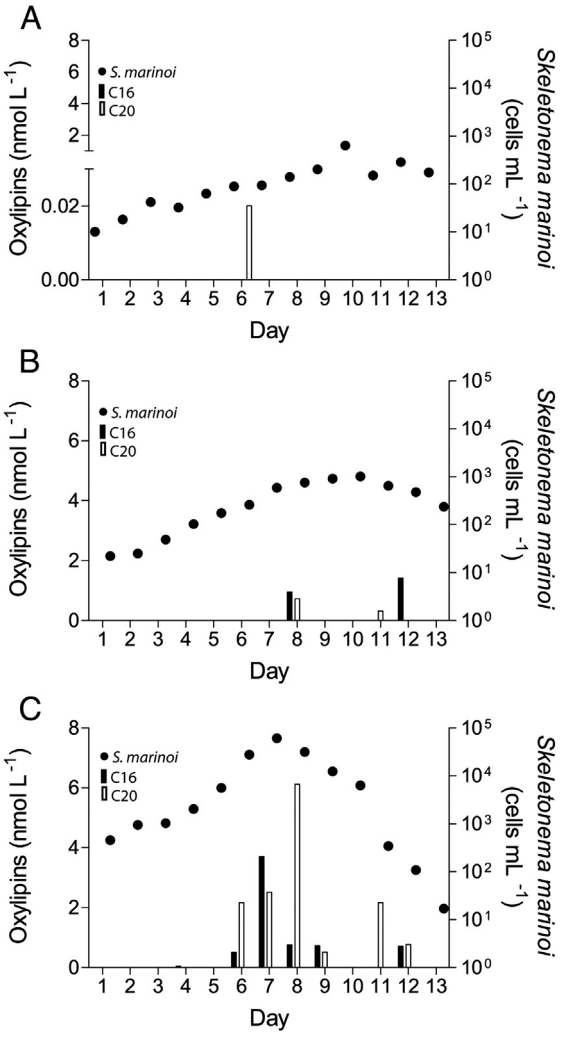

Fig. 1. Bloom development and oxylipin production of *Skeletonema marinoi* in A, mesocosm B, enriched in nitrate and phosphate; B, mesocosm C, enriched in nitrate, phosphate, and silicate; C, mesocosm F, enriched in nitrate, phosphate, silicate, and *S. marinoi*. Round symbols: cell concentrations of *S. marinoi*. Black bars: oxylipins deriving from C_{16}-PUFA and white bars: oxylipins deriving from C_{20}-PUFA.

In mesocosm F, the concentration of oxylipins measured in the collected samples depended on the presence of *S. marinoi* (Fig. 1C). Highest concentrations (6.8 nmol L^{-1}) were measured on day 8, one day after the peak of the bloom. The relative contributions of C_{16}- to C_{20}-derivatives varied throughout the bloom (Fig. 1C), but overall, C_{20}-derivatives dominated.

When the *S. marinoi* isolate used for inoculation was grown in the laboratory, this relationship of C_{16}- to C_{20}-derivates changed to ~2:1 (Fig. 3) because of a more diverse composition of C_{16}-oxylipins, including additional compounds such as hydroxy-hexadecatrienoic acid and hydroxy-epoxy-hexadecadienoic acid, which were not detected in mesocosm samples. The ratio of C_{16}- to C_{20}-derivatives observed in the Norwegian *S. marinoi* isolate (~2:1) differed from that measured in an Adriatic clone of *S. marinoi* used as a comparison (1997 clone in Gerecht et al., 2011). Although the same molecules were produced, the Adriatic clone showed an inverse relationship of ~1:2 in regard to C_{16}- and C_{20}-derivatives (Fig. 3). The Norwegian clone of *S. marinoi* produced nonvolatile oxylipins (NVO) at 0.86 ± 0.18 fmol $cell^{-1}$ when grown in the laboratory. This level is lower but not significantly different (*t*-test, $p = 0.18$) from the level of NVO produced by the Adriatic clone (1.4 ± 0.63 fmol $cell^{-1}$).

3.3. Copepod reproduction experiment

Fecal pellet production rate (FPR) of *C. finmarchicus* females incubated with mesocosm water from mesocosm F was highest during the *S. marinoi* bloom (days 5–10), whereas in females incubated with mesocosm water of mesocosms B and C, FPR increased towards the end of the experiment (Fig. 4A). Egg production rate (EPR) was similar among females incubated with water from the three mesocosms with highest egg production during days 8–10 (Fig. 4B).

Hatching success of eggs produced by *C. finmarchicus* females was high throughout the experiment in all treatments (Fig. 5A). Also, the percentage of abnormal nauplii was continuously low (Fig. 5B). Cannibalized eggs, as identified by the presence of empty egg envelopes in fecal pellets, were practically absent (data not shown).

4. Discussion

The current mesocosm setup provided an excellent opportunity to study diatom oxylipin production within a complex, close to natural food web. Oxylipin production by the phytoplankton community depended on the presence of *S. marinoi* in the phytoplankton assemblage with highest potential concentrations measured during the bloom of *S. marinoi* in the mesocosm inoculated with this species. Although the nonvolatile oxylipins (NVO) produced by the *S. marinoi* clone used for inoculation were the same as those that have been previously characterized in the *S. marinoi* clone isolated from the

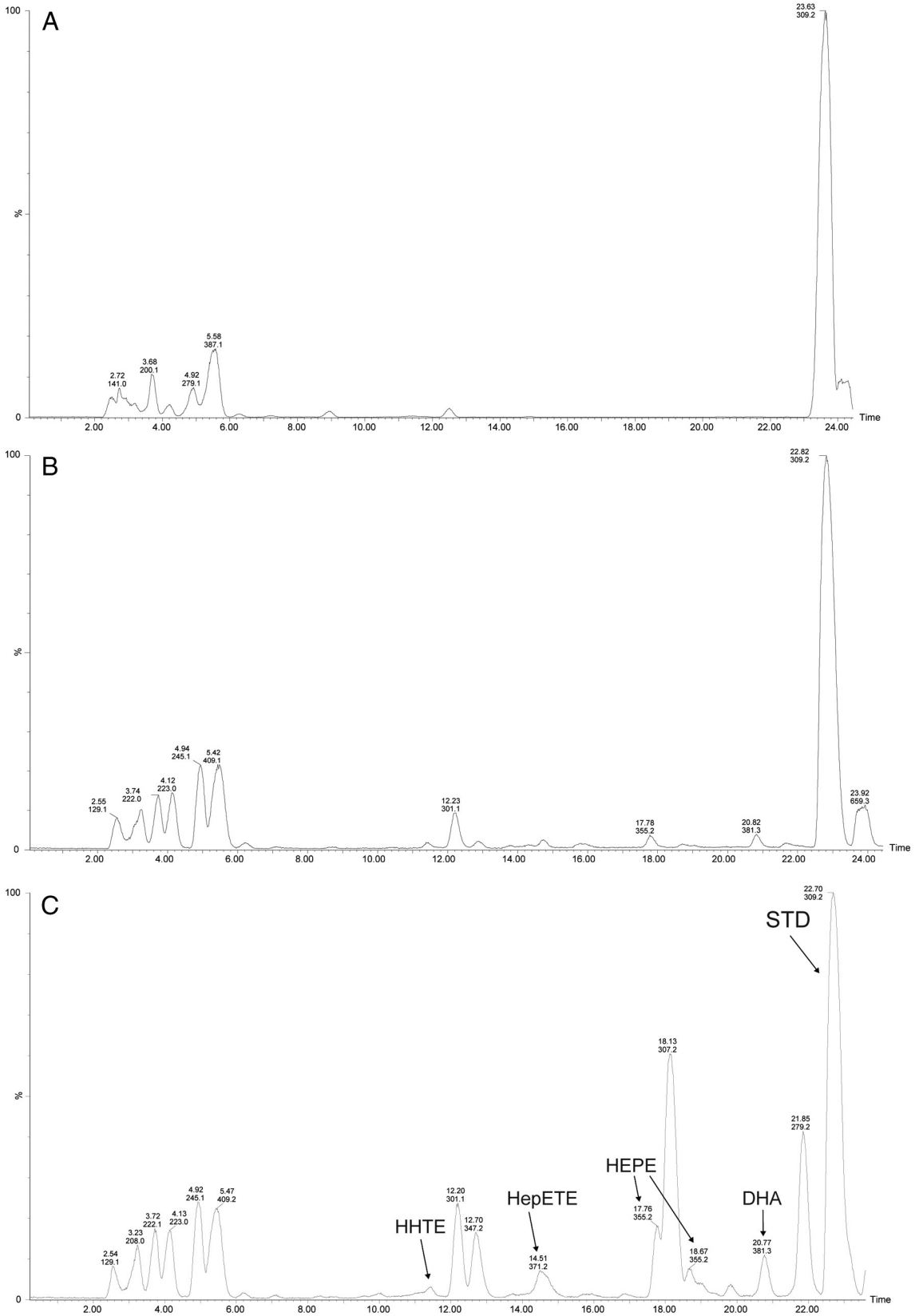

Fig. 2. Representative LC-MS chromatograms of samples collected from A, mesocosm B; B, mesocosm C; and C, mesocosm F during the height of the bloom in mesocosm F (day 8). The identified oxylipin peaks hydroxy-hexadecatetraenoic acid (HHTE), hydroxy-epoxy-eicosatetraenoic acid (HepETE), hydroxy-eicosapentaenoic acid (HEPE), as well as the docosahexaenoic acid (DHA) derivative, and the internal standard 16-hydroxy-hexadecanoic acid (STD) are indicated by the arrows.

Northern Adriatic Sea (Fontana et al., 2007), there was a larger contribution of derivatives from C_{16}-polyunsaturated fatty acids (PUFA) to NVO production in this clone compared to the Adriatic clone when both were grown under the same conditions in the laboratory. These findings highlight metabolic differences between clones of the same species. These differences may be due to changing

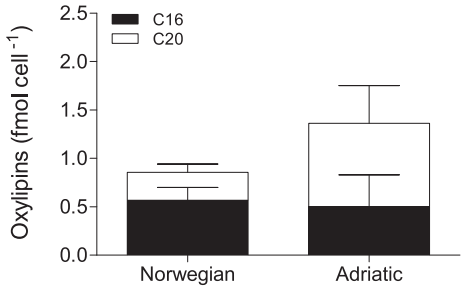

Fig. 3. Contributions of C_{16}- and C_{20}-PUFA derivatives to oxylipins in laboratory cultures of the *Skeletonema marinoi* clone used for inoculation of the mesocosm (Norwegian), and a clone of *S. marinoi* isolated from the Adriatic Sea (Adriatic). Black bars: C_{16}-derivates; white bars: C_{20}-derivatives. Bars are means ± sd.

availability of fatty acid precursors, to varying enzymatic activity and affinity or a combination of both factors. Interestingly, there were also differences in oxylipin composition between samples collected from the mesocosms and from the same clone grown in culture. Although the relationship of C_{16}- to C_{20}-derivatives in the Norwegian clone was ~2:1 in culture, in the samples collected from the mesocosms, C_{20}-derivatives had an overall higher contribution. Vidoudez et al. (2011a) showed a similar pattern for polyunsaturated aldehydes (PUA) from the same mesocosm setup. These authors detected higher levels of the C_{20}-derived heptadienal compared to the C_{16}-derivatives octatri- and octadienal. This indicates that metabolic profiles can change depending on whether species are grown in pure culture or in natural communities. The predominance of C_{20}-derivatives in mesocosm samples may be due to a spurious metabolism of fatty acids made available from other sources in a mixed natural community. Dinoflagellates, haptophytes, and ciliates were all abundant in mesocosm F along with *S. marinoi* (Jónasdóttir et al., 2011) and could have contributed to the fatty acid pool available to lipoxygenase enzymes for oxylipin production.

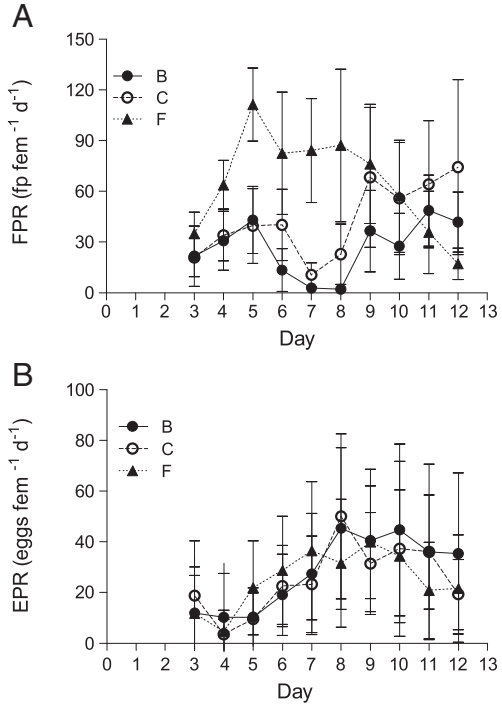

Fig. 4. A, fecal pellet production rate (FPR) and B, egg production rate (EPR) of *Calanus finmarchicus* during the experiment. Full circles: FPR and EPR of copepods incubated with water from mesocosm B; empty circles: with water from mesocosm C; full triangles: with water from mesocosm F. Symbols are means ± sd.

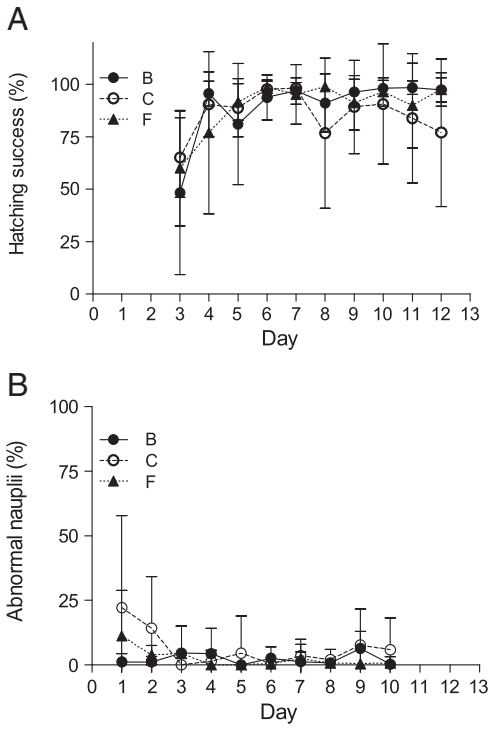

Fig. 5. A, hatching success and B, percentage of abnormal nauplii produced by *Calanus finmarchicus* during the experiment. Full circles: Hatching success and percentage of abnormal nauplii of copepods incubated with water from mesocosm B; empty circles: with water from mesocosm C; full triangles: with water from mesocosm F. Symbols are means ± sd.

In agreement with this hypothesis, the putative docosahexaenoic acid (DHA)-derived peak detected in the mesocosm samples has so far not been identified from pure *S. marinoi* cultures in our laboratory (Barreiro et al., 2011; Fontana et al., 2007; Gerecht et al., 2011). DHA was, indeed, one of the most abundant fatty acids in the mesocosms (Jónasdóttir et al., 2011). However, the production of oxylipins is not governed only by the presence of precursor fatty acids. Jónasdóttir et al. (2011) showed similar concentrations of the precursor for C_{20}-oxylipins, EPA, in mesocosms B and C on day 8 even though C_{20}-oxylipins where detected only in mesocosm C and not in B. Similarly, EPA concentrations had decreased by less than half until the end of the experiment in mesocosm F whereas C_{20}-oxylipins were produced mainly during the peak of the bloom. This lack of correlation between oxylipin production and fatty acid precursors implies an enzymatic control over oxylipin production which is corroborated by the correlation between the presence of *S. marinoi* and potential oxylipin concentrations. However, the different metabolic profiles measured from the same clone grown in culture and collected from the mesocosm suggest that lipoxygenase enzymes can use fatty acid precursors from other sources in a mixed community leading to a different array of oxylipins than in a pure culture. This then also implies caution when extrapolating data on secondary metabolites obtained in culture to the field as a more complex fatty acid pool from other microbial sources is available when field samples are collected. This underlines the necessity of measuring actual metabolite composition and concentrations at sea.

There was no negative effect of the *S. marinoi* bloom on copepod reproductive success. Egg production rates (EPR) followed the development of the *S. marinoi* bloom in the inoculated bag and decreased in the declining phase even though there was an increasing biomass of alternative protozoan prey (Jónasdóttir et al., 2011). These authors reported high EPR during the same mesocosm study until the end of the experiment. However, differences in EPR may be due to the different incubation protocols between the studies. Hatching rates of the produced eggs remained high and abnormal nauplii were rarely

detected. The copious production of fecal pellets in *C. finmarchicus* females during the *S. marinoi* bloom, suggests that females were indeed feeding on *S. marinoi* as FPR tends to be high for copepods feeding on diatoms (Ceballos and Ianora, 2003; Ianora and Poulet, 1993). Barofsky et al. (2010) have also shown active feeding of *C. finmarchicus* on *S. marinoi* in the same mesocosm setup applying a qPCR method. However, a number of previous studies on feeding of *C. finmarchicus* in similar mesocosms at this location have shown persistent selectivity for larger prey such as ciliates and rotifers over diatoms (*e.g.* Nejstgaard et al., 1997, 2001b), making it likely that copepods were also feeding on protozoan prey, possibly leading to a dilution effect of potentially toxic prey (Halsband-Lenk et al., 2005; Turner et al., 2001). Another possible explanation for the continued high hatching success in the presence of oxylipins may be the short duration of the bloom (5 days). Although *S. marinoi* in the laboratory usually induces a rapid reduction in hatching success (Barreiro et al., 2011; Ceballos and Ianora, 2003), a short bloom coupled with alternative prey may weaken possible negative effects of oxylipin producing diatoms. Furthermore, different species of copepods will have different sensitivities to different species of diatoms. In fact, in the review by Ban et al. (1997), the authors reported that *S. marinoi* did not negatively affect either fecundity or hatching success of *C. finmarchicus* in the St. Lawrence estuary. Differences in sensitivity to oxylipins have recently been shown also among geographically distinct populations of *C. helgolandicus* (Lauritano et al., 2012). These authors observed differential expression of stress-related genes among three populations of *C. helgolandicus* when exposed to oxylipin producing diatoms, suggesting a population-dependent response of copepods to these metabolites. Furthermore, negative effects of oxylipins will depend on the concentrations of oxylipins that copepods are exposed to. Unfortunately, very little information is available in the literature about the actual concentrations of PUA and/or NVO during blooms at sea. The only other study carried out so far which measured the concentration of these metabolites is a study by Vidoudez et al. (2011b), which measured PUA concentrations during a bloom of *S. marinoi* in the Adriatic Sea. Although the potential concentrations of PUA (assuming lysis of all cells) were high and similar to the present study (up to 28 nmol L^{-1}; Vidoudez et al., 2011b), concentrations of *S. marinoi* was two orders of magnitude lower during the Adriatic bloom compared to the present study (maximum concentrations of 0.7×10^6 *vs.* 60×10^6 cells L^{-1}). This implies that PUA production per cell in the Adriatic Sea was up to 40 fmol $cell^{-1}$ whereas in this study the maximum production of PUA per cell was only 1.3 fmol $cell^{-1}$ (Vidoudez et al., 2011a). Even if we include NVO in this calculation, the potential oxylipin levels of single *S. marinoi* cells are still an order of magnitude lower than during the bloom in the Adriatic Sea. In contrast to Jónasdóttir et al. (2011), who classified the present mesocosm bloom as strong in regard to potential PUA production, this analysis would argue that *S. marinoi* was a weak producer of oxylipins during this bloom, relative to the Adriatic bloom, based on the low potential levels of oxylipins produced per cell. It remains a field of further study whether the overall potential of a phytoplankton population to produce oxylipins or the potential of single diatom cells to produce these metabolites is more important for copepod reproduction. However, as *C. finmarchicus* was likely not feeding exclusively on *S. marinoi* (Nejstgaard et al., 2001a), we would argue that potential oxylipin levels of single cells are important as these determine whether copepods eat more or less "toxic" cells.

To conclude, this study presents one of the first studies of diatom oxylipin production that addresses the more recently discovered PUFA-derived hydroxy-acids and epoxyalcohols, NVO, in an almost natural setting. Further studies would benefit greatly from quantifying levels of oxylipins during diatom blooms in the field to be able to correctly evaluate their potential ecological role. This study provides an important first attempt at quantifying oxylipin production and can be used as a comparison for subsequent studies. However, the possible contamination by oxylipins, *e.g.* the DHA product, deriving from substrates made available by other sources from natural assemblages needs to be taken into consideration. Low potential levels of oxylipins may be a reason why the role for these metabolites in grazer defense could not be confirmed by the present study and again underlines the necessity to start evaluating oxylipin production by phytoplankton in a quantitative manner.

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