



Linking bacterial community structure to advection and environmental impact along a coast-fjord gradient of the Sognefjord, western Norway

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ABSTRACT

Here we present novel data on bacterial assemblages along a coast-fjord gradient in the Sognefjord, the deepest (1308 m) and longest (205 km) ice-free fjord in the world. Data were collected on two cruises, one in November 2012, and one in May 2013. Special focus was on the impact of advective processes and how these are reflected in the autochthonous and allochthonous fractions of the bacterial communities. Both in November and May bacterial community composition, determined by Automated Ribosomal Intergenic Spacer Analyses (ARISA), in the surface and intermediate water appeared to be highly related to bacterial communities originating from freshwater runoff and coastal water, whereas the sources in the basin water were mostly unknown. Additionally, the inner part of the Sognefjord was more influenced by side-fjords than the outer part, and changes in bacterial community structure along the coast-fjord gradient generally showed higher correlation with environmental variables than with geographic distances. High resolution model simulations indicated a surprisingly high degree of temporal and spatial variation in both current speed and direction. This led to a more episodic/discontinuous horizontal current pattern, with several vortices (10–20 km wide) being formed from time to time along the fjord. We conclude that during periods of strong wind forcing, advection led to allochthonous species being introduced to the surface and intermediate layers of the fjord, and also appeared to homogenize community composition in the basin water. We also expect vortices to be active mixing zones where inflowing bacterial populations on the southern side of the fjord are mixed with the outflowing populations on the northern side. On average, retention time of the fjord water was sufficient for bacterial communities to be established.

1. Introduction

Changes in bacterial community composition along environmental gradients, most notably salinity and nutrient availability, have been described in a large number of studies (i.e. Bordalo and Vieira, 2005; Crump et al., 2004; del Giorgio and Bouvier, 2002; Henriques et al., 2006; Herlemann et al., 2011; Hewson and Fuhrman, 2004; Kirchman et al., 2005). However, the advective transport that creates these gradients can also be a significant structuring force of bacterial community composition and succession (Wilkins et al., 2013). In a biogeographical perspective, variation patterns in microbial composition along a coast-fjord gradient is expected to be closely connected with physicochemical conditions, which again would select for a distance-decay relationship, making it difficult to differentiate between the effects of local environmental factors and dispersal by advective processes (Hanson et al., 2012). Coastal habitats such as estuaries and fjords are strongly

influenced by adjacent coastal/oceanic waters, terrestrial effluents and freshwater discharges, and therefore display steep physicochemical gradients, often enhanced by estuarine circulation processes. This makes them interesting sites for studying the effects of advection on the resident biological diversity.

Bacteria are major drivers of biogeochemical cycles in marine environments (Arrigo, 2004; Cho and Azam, 1988; Falkowski et al., 2008), and their diversity and community structure is thought to have major impacts on entire ecosystem functions (Gravel et al., 2012). In general, the Nordic coastal regions experience increased stress from human activity such as fisheries, nutrient runoff and pollution (Paasche et al., 2015). Thus increased knowledge of environmental conditions and factors forming the biology of estuaries, fjords and coastal areas is vital (Manzetti and Stenersen, 2010), not only for present, but also for predicting community responses to future environmental changes. Bacterial communities and abundances in estuaries have been

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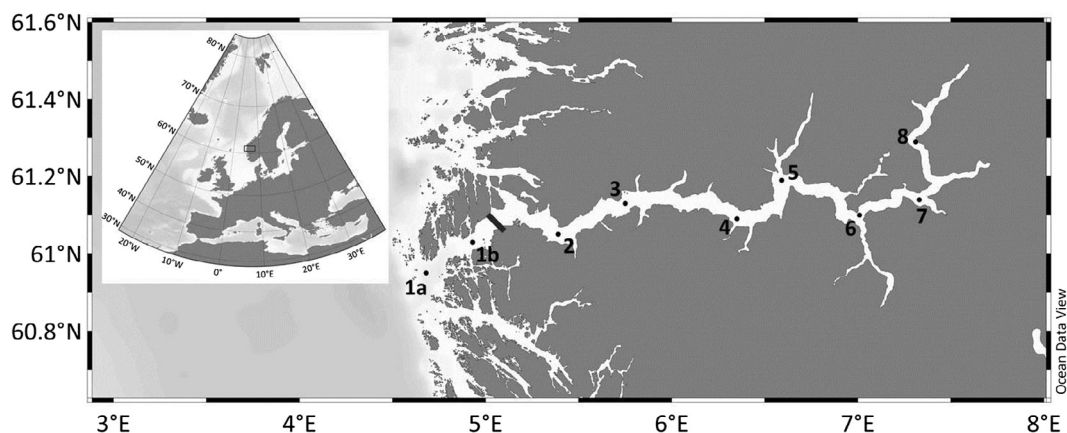


Fig. 1. Map of the Sognefjord with sampling locations marked. Station 1a was sampled only in May 2013, and station 1b in November 2012 only.

extensively studied in connection with spatial environmental gradients (Bordalo and Vieira, 2005; Crump et al., 2004; Henriques et al., 2006; Herlemann et al., 2011; Hewson and Fuhrman, 2004; Kirchman et al., 2005), yet knowledge of such couplings in classical fjords are limited (Gutiérrez et al., 2015).

Fjord-estuaries differ from conventional estuaries by displaying both horizontal physicochemical gradients caused by estuarine circulation, as well as strong vertical density gradients due to the presence of relatively shallow sills at the entrance, separating the deep basin waters from the adjacent coastal water (Matthews and Heimdal, 1980; Wassmann et al., 1996). It should be noted, however, that horizontal variability could occur in the deeper basin water due to incomplete renewal during restricted inflow periods. The brackish surface water of fjords contains autochthonous microbial communities that are also influenced by allochthonous bacteria and inorganic and organic matter derived from freshwater sources such as rivers, glacier melt-water and runoff from precipitation on land (Wilhelm et al., 2013; Gutiérrez et al., 2015). The freshwater often enters the main fjord at different sites depending on the distribution of side-arm fjords and rivers. The intermediate water directly below, usually displays biological and chemical compositions which reflects its coupling to the adjacent coastal water (Aure et al., 1996). The advective force from estuarine circulation promotes horizontal transport of nutrients, organic matter and microbes within the surface and intermediate water layers, and also leads to turbulence and mixing between them (i.e. entrainment) (Asplin et al., 1999; Aure and Stigebrandt, 1989; Aure et al., 1996; Erlandsson, 2008; Stigebrandt, 1976). In addition, fjords can also experience periods with stagnation in the bottom water (Gade and Edwards, 1980), and physical separation of water masses could follow due to the long, narrow nature of fjord basins and the presence of internal sills. This could be an important factor structuring resident bacterial communities, potentially leading to divergence between them.

The site for the present study, the Sognefjord, is the longest and deepest open (ice-free) fjord in the world, 205 km long, on average 4.5 km wide, and 1308 m deep, with a sill depth of 170 meters (Hermansen, 1974; Svendsen, 2006). The fjord exchanges water with the adjacent Norwegian Coastal Current (NCC) above sill depth (Asplin et al., 1999, 2014; Stigebrandt, 2012), and is highly stratified primarily due to freshwater runoff (Hermansen, 1974). Wind-driven exchange processes between offshore and inshore fjord waters are important for short-term variations of phytoplankton blooms in western Norwegian fjords (Braarud et al., 1974; Erga and Heimdal, 1984; Erga, 1989; Erga et al., 2012), and estuarine circulation and tidal motions significantly affects the horizontal transport of allochthonous and autochthonous zooplankton and animals (Aksnes et al., 1989; Basedow et al., 2004; Kaartvedt and Svendsen, 1990; Kaartvedt and Nordby, 1992; Tommasi et al., 2013).

Recently we assessed top-down versus bottom-up control of

bacterial diversity in the Sognefjord using ARISA for bacterial diversity measurements (Storesund et al., 2015). We found that the microbial diversity was strongly regulated by viruses (i.e. top-down controlled), while community composition was bottom-up controlled by competition for limiting growth substrates. However, in that study we focused on vertical distribution traits of bacterial diversity in the fjord, and did not take into account the influence of horizontal transport on the composition of bacterial communities in the different water layers. To improve our understanding of how fjord circulation affect the resident bacterial communities along a coast-fjord gradient, we here aim to (i) implement a high-resolution current model system for the Sognefjord area, (ii) identify horizontal gradients and patterns of bacterial communities, (iii) unravel the effects of individual environmental factors and advection on bacterial community composition, and (iv) resolve the relative contribution of allochthonous bacterial input to the total bacterial community composition in the fjord. To our knowledge such data have not been obtained earlier.

2. Materials and methods

2.1. Study site

The Sognefjord is situated on the west coast of Norway, bordering the northern part of the North Sea (Fig. 1), and displays a classic three-layer fjord structure, with surface, intermediate and basin water (Svendsen, 2006). At the surface, freshwater runoff mix with seawater to create the brackish top-layer (salinity ≤ 33) that moves out of the fjord (Stigebrandt, 1981). The intermediate layer consists of a mix of well oxygenated coastal water from the adjacent NCC and fjord basin water, and is characterized by salinities between 33 and 35 (Sætre et al., 2007). This layer will interact with the coastal water outside the fjord and horizontal internal pressure forces will create episodic currents appearing as long internal waves lasting several days (Asplin et al., 1999, 2014). The current direction can be both in-fjord or out-fjord, and typically in-fjord flow occurs after Southerly coastal winds (creating downwelling of relatively lighter water) and out-fjord flow occurs after Northerly winds (creating upwelling of relatively heavier water). Below sill depth we find basin water originating from Atlantic water, and salinity can be above 35 closely after deep-water renewal, but is gradually diluted over time by diffusion and mixing with water above due to ongoing tidal forcing, leading to less salty and dense water (Molvær and Gade, 1971; Stigebrandt and Aure, 1989). Generally, in the fjords the upper brackish layer (1–10 m deep) will be forced mainly by the freshwater runoff and winds, and in deep fjords the coupling with the water below is typically weak due to a strong halocline. The main portion of water mass transport in fjords is thus associated with circulation within the intermediate layer above sill-level, mainly forced by internal pressure differences between the fjord water and the coastal

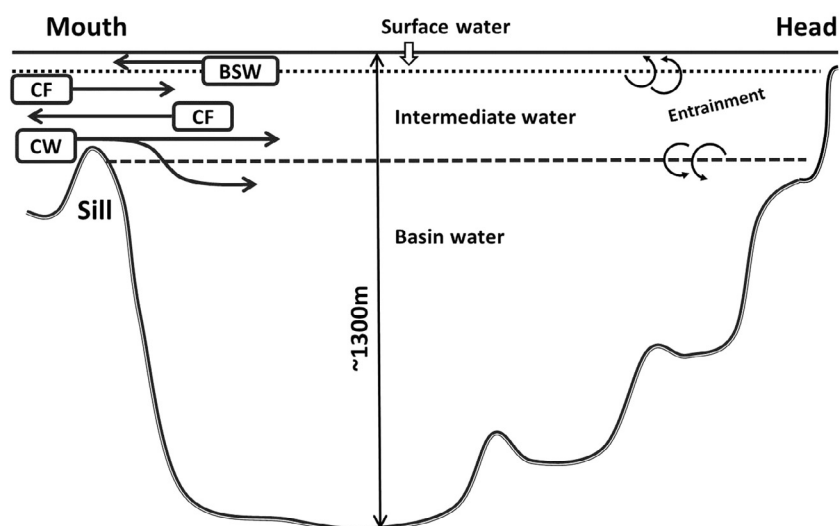


Fig. 2. Profile of the approximate bathymetry of the Sognefjord, with water masses and major currents displayed. BSW: Brackish Surface Water, CF: Compensational flow and CW: Coastal water.

water. The water in the deep basins below the sill depth will be more stagnant (Aure and Stigebrandt, 1989; Aure et al., 1996) (Fig. 2). In addition, water entering in the intermediate layer above the sill may be entrained into the basin water, creating a gradual transition between these water masses instead of a defined halocline. In this study, we defined the basin water as being deeper than 300 m.

2.2. Numerical current model

We have been using the current model system NorKyst800 developed by the Institute of Marine Research (Albretsen et al., 2011) to implement a higher resolution model for the Sognefjord area, abbreviated NorFjords. NorFjords is based on the ROMS hydrodynamical model (Regional Ocean Modeling System, www.myroms.org; Shchepetkin and McWilliams, 2005; Haidvogel et al., 2008). The numerical grid has a horizontal resolution of 160 m and we used 35 vertical s -coordinates where the densest resolution was chosen to be in the upper water masses (~ 50 m).

The NorFjords model is forced by atmospheric fields, freshwater runoff and open boundary conditions towards the coastal ocean (including tidal forcing). Atmospheric fields were generated by the model WRF (Weather Research and Forecasting model; www.wrf-model.org Skamarock et al., 2008) using 3 km horizontal resolution and updated every 3 h. Daily values of freshwater discharges from the 250 main rivers in this area were provided by the Norwegian Water Resources and Energy Directorate (NVE). Open boundary conditions towards the coastal ocean, including the effects of tides and the highly stratified NCC, are taken from results of the NorKyst800 coastal ocean model archives as hourly values (Albretsen et al., 2011).

We have simulated two periods: November 1–20, 2012 and May 1–28, 2013. We applied one month spin up for both modelling periods initialized by results from the NorKyst800 model. Results are stored as hourly values of surface elevation and three dimensional fields of horizontal current components, salinity and temperature.

2.3. Sampling procedure and sample analyses

Parts of this dataset has been analysed previously, and sampling procedure, analyses of inorganic nutrients (nitrate (NO_3^-), nitrite (NO_2^-), orthophosphate (PO_4^{3-}) and silicate $\text{Si}(\text{OH})_4$), bacterial production (BP), DNA isolation, PCR and ARISA fingerprinting, are described extensively in Storesund et al. (2015), and a brief summary only is presented here. Water was sampled from six to twelve depths at eight stations along the longitudinal fjord-transect, using a General Oceanic Rosette with 20 L Niskin water bottles, coupled to a Sea Bird 911 CTD

probe. Samples for NO_3^- , NO_2^- , PO_4^{2-} and $\text{Si}(\text{OH})_4$ measurements were preserved using chloroform (200 μL per 50 mL sample) and analysed at the Institute of Marine Research, Bergen, using an autoanalyser (Alpkem-Lab) according to Parsons et al. (1984). Single-celled prokaryotes were counted using a flow cytometer (FACSCalibur). Samples were preserved with glutaraldehyde (25% (v/v), final concentration 0.5% (v/v)) for 30 min at 4 °C and flash-frozen in liquid N_2 until further processing (see Storesund et al., 2015 for further details). Bacterial production (BP) was measured as uptake of ^3H -Leucine using the microcentrifuge method (Smith and Azam, 1992) and leucine uptake was converted to carbon production assuming no isotope dilution (Simon and Azam, 1989). For DNA isolation 1 L of water per sample was filtered onto 0.22 μm polycarbonate filters. DNA was extracted using a modified version of the CTAB method with a bead-beating step prior to lysozyme treatment. ARISA fingerprinting was performed according to the protocol by Ramette (2009), using 20–25 ng DNA for each 50 μL reaction (triplicates of each sample) and primers ITSd; 5'-GTCGTAA-CAAGGTAGCCGTA-3', and ITSReub; 5'-GCCAAGGCATCCACC-3' (Ramette, 2009). Capillary electrophoresis was done on an ABI 3130 by the Center for Medical Genetics and Molecular Medicine (Haukeland University Hospital, Bergen, Norway). Peak size and area was determined using Peak Scanner (v1.0 Applied Biosystems), and peaks between 100 bp and 1000 bp were used for further analyses, with a bin size of 2 bp to separate between operational taxonomic units (OTUs). OTUs were considered valid if they were present in two or all of the triplicates.

2.4. Statistical analysis

Statistical analyses were done using the R-project software (R Core Team, 2013) and packages vegan (Oksanen et al., 2013) and ecodist (Goslee and Urban, 2007) unless otherwise stated. The relationship between bacterial community composition, geographical distance and environmental variables were tested using Mantel tests. Approximate distances and depths along the fjord transects were used to calculate distance matrices rather than longitude and latitude, as this gave a better resolution of the real distances between sampling locations. Species distance matrices based on presence-absence (representing richness) (Jaccard) were highly correlated with distance matrices based on relative abundance (representing evenness) (Bray-Curtis) data (Mantel, $r_M > 0.99$, $p = 0.0001$), and we chose to continue with the presence-absence distance matrices for further analyses as they are considered more conservative. Geographical distances were calculated in km, and distance matrices of environmental observations were calculated over pairwise dissimilarities between variables that were

standardised for each variable by subtracting the variable's mean value and dividing by mean absolute deviation (Kaufman and Rousseeuw, 2009).

To unravel the importance of individual environmental variables and separate this from geographical divergence of bacterial population similarities, we used multiple regressions on distance matrices (MRM) method (Legendre et al., 1994). Prior to the regression analyses, environmental datasets were tested for autocorrelation between variables using pairwise correlation analyses, and for high correlations ($r > 0.9$) we chose to use only one variable. NO_3^- , PO_4^{3-} and $\text{Si}(\text{OH})_4$ were strongly correlated, and we continued the analyses using NO_3^- only. Salinity and density were also highly correlated, and we used salinity for further analyses. Environmental variables that were not normally distributed were ln-transformed. NO_2^- showed undetectable levels in intermediate and basin samples from November, and was therefore excluded from these analyses. MRM analyses were run twice (10,000 permutations) using distance matrices of OTUs (presence-absence), geographical distances (km) and environmental variables. After the initial run, non-significant values were removed to reduce the effects of common response variables (Martiny et al., 2011), and the model was run again. Results from the second runs are reported.

2.5. Effects of advection on the bacterial community

2.5.1. Bacterial turnover and current speed

In order to determine if the bacterial community in the surface water had daily turnover rates on levels high enough to be affected by environmental conditions before being transported out of the fjord, we calculated the minimal bacterial growth which is required in the surface water for active growth to surpass advection out of the fjord. Average and maximum values of the surface current at 0–20 m depth in the Sognefjord has previously been estimated (Dupont and Aksnes, 2010; Lothe and Brørs, 2010), and these values were used to calculate transport time from the fjord head and from sampling stations situated at side-fjord outlets to the fjord sill (Eq. (1)).

$$\text{Transport time} = \frac{\text{distance (m)}}{\text{current speed} \left(\frac{\text{m}}{\text{d}}\right)} \quad (1)$$

where m is distance in meters, and d is days.

Further, average daily bacterial turnover rates in the brackish surface water were calculated from measurements of bacterial production and flow cytometry counts (Eqs. (2) and (3)).

$$\text{Number of bacteria produced (L}^{-1}\text{ h}^{-1}) = \frac{BP (\mu\text{g C L}^{-1}\text{ h}^{-1})}{2 \times 10^{-8} \mu\text{g C}} \quad (2)$$

$$\text{Average bacterial turnover (d)} = \frac{\text{number of bacteria produced (L}^{-1}\text{ h}^{-1})}{\text{bacterial abundance}} * 24 \quad (3)$$

where BP is average bacterial production measured in the surface water, C is carbon, d is days, and 2×10^{-8} is estimated μg carbon present per bacteria (Lee and Fuhrman, 1987). Bacterial abundance is average bacterial abundance measured using flow cytometry (FCM). All measurements used for these calculations are from the upper 20 m of the fjord, to correspond to the depths used by Dupont and Aksnes (2010) and Lothe and Brørs (2010).

2.5.2. Source-sink populations

To further elucidate the effects of advection on the bacterial community composition in the fjord, we used Sourcetracker (Knights et al., 2011) to find the percentage of bacteria in the fjord derived from freshwater sources and coastal water. Sourcetracker estimates the portion of a bacterial community (defined as a sink) that is derived from multiple pre-defined source populations, using a Bayesian approach. It was originally developed to identify the sources of

contaminants in metagenomics studies (Knights et al., 2011), but has also been successfully used to show the influence of advective processes on bacterial communities in Antarctic waters (Wilkins et al., 2013) and river systems (Staley et al., 2015). For the purpose of our study, the relative abundance of different OTUs in samples (ARISA fingerprinting) was used to compare the similarity between the stations along the transect. Two separate analyses were run as follows; (1) Only samples from November 2012 were used, and (2) Only samples from May 2013 were used. Samples from surface water (5–10 m depth) at the outlet of major sidearm-fjords (Stns 5, 6, 7 and 8, Fig. 1) and samples from coastal/Atlantic water outside the sill (Stn 1a/1b, Fig. 1) were defined as source populations, whereas all other samples were defined as sink populations. The approximate influence of source communities in the sinks were then predicted to give us a general idea of the effects of advective processes on community composition, as well as the amount of contact between communities in different layers of the fjord. The Sourcetracker data was visualized using ODV (Schlitzer, 2002, 2011).

3. Results

3.1. Fjord hydrography and distribution of inorganic nutrients and chl *a*

The longitudinal fjord transect can be separated into three main sections with respect to physiochemical properties. The outer part (outside the sill), mostly consisting of NCC water, is represented by Stn 1, the mid part by Stns 2, 3, 4 and the inner part by Stns 5, 6, 7, 8, which are heavily influenced by large freshwater outlets from the many fjord branches (Fig. 1). The most freshwater influenced part of the brackish layer extends down to only 5 m, and is possibly non-existent in winter due to reduced freshwater runoff and strong winds that lead to mixing with deeper layers. Below the brackish layer is an intermediate layer with a gradually increasing salinity (Fig. 3). In November, this intermediate layer is deeper (60–80 m) than in May (~40 m), and the temperature in November is warmer than the water above and below, while in May the temperature of the intermediate layer water is lower than the water above and below. The warm water in November is the reminiscence of the surface heating during summer, and the cold water in May is the reminiscence of the surface cooling during winter. Average values of salinity, temperature, NO_3^- , $\text{Si}(\text{OH})_4$, and PO_4^{3-} for the whole transect typically varied between 29 and 35, 7.5 and 9.6 °C, 1.2 and 13.6 $\mu\text{mol L}^{-1}$, 1.1 and 14.0 $\mu\text{mol L}^{-1}$, and 0.16 and 1.17 $\mu\text{mol L}^{-1}$, respectively, from surface to bottom (see Storesund et al., 2015). The vertical stratification becomes less pronounced towards the coast, which is typical for fjords, due to increased influence of various mixing mechanisms. Outside the sill, on the other hand, the water was more vertically homogeneous than further into the fjord (Fig. 3). The upper layers of the fjord displayed high variability in temperature and salinity between the two sampling times. This reflects the fact that a stronger stratification of the water column allows for internal waves and internal pressure forces to establish and develop. In our case this was confirmed by a more comprehensive water exchange in the intermediate layer in November than May. In general, NO_3^- , PO_4^{3-} and $\text{Si}(\text{OH})_4$ increased with depth and from the outer to the inner parts of the fjord at both sampling times (see Fig. 3, but only NO_3^- is shown as an example). However, we would normally expect higher levels of silicate relative to nitrate and phosphate in the surface layer inwards the fjord, and close to river outlets due to high content of silicate in rivers (see Erga et al., 2012). The reason why this was not the case in our study, could be that the inner stations were not situated near the freshwater discharges. Water below sill depth showed homogenous temperatures (~7.4 °C) and salinities (> 35.0) between the two sampling times. It should also be noted that oxygen-rich water associated with the intermediate layer in May appeared to reach down to approximately 600 m in the outer parts of the fjord close to the mouth, indicating some inflow and subduction of coastal water, while in the shallower inner part, which also receives material from the shallow

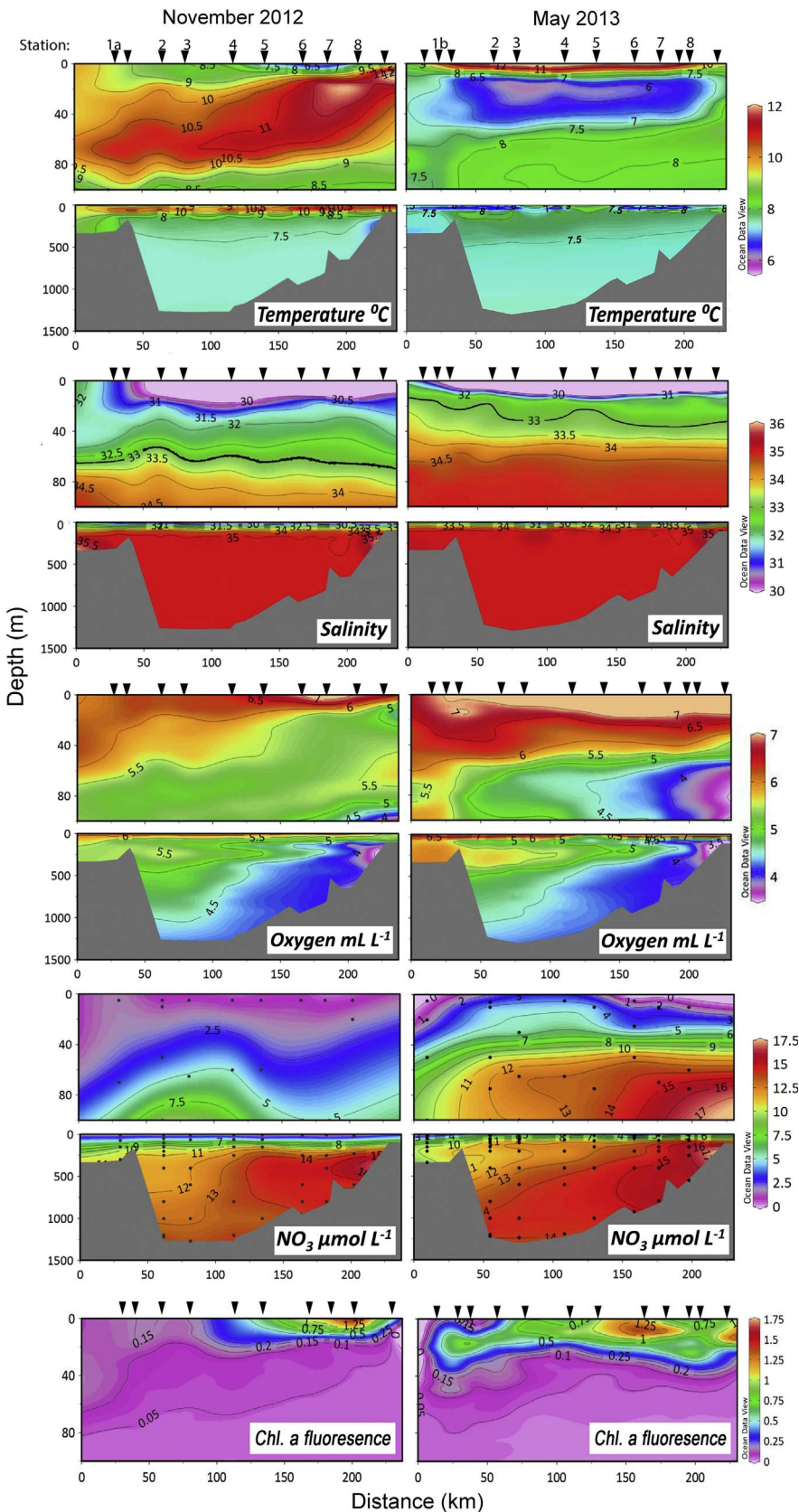


Fig. 3. Contour plots of Temperature, Salinity, Oxygen, NO_3 and chl *a* fluorescence measured in November 2012 and May 2013. Sampling stations are indicated by arrowheads and numbers or filled circles. On unmarked stations only CTD profiles were taken. The upper part of the plot indicates the upper 100 m of the fjord, whereas the lower part shows the entire water column. The 33 isohaline (marked in bold) indicates the transition between the brackish and intermediate layer.

fjord branches, oxygen levels decreased and nitrate increased within the deeper stagnating layers. During the melting season in May–June, these branches can give rise to the cold-water plumes observed in the upper 50 m along the transect.

Using chl *a* fluorescence along the transect (Fig. 3) as an indicator of phytoplankton biomass, the total biomass was lower in November than in May. In November, it was mainly restricted to the upper 10 m over the inner half of the fjord, whereas values higher than $0.5 \mu\text{g L}^{-1}$ were

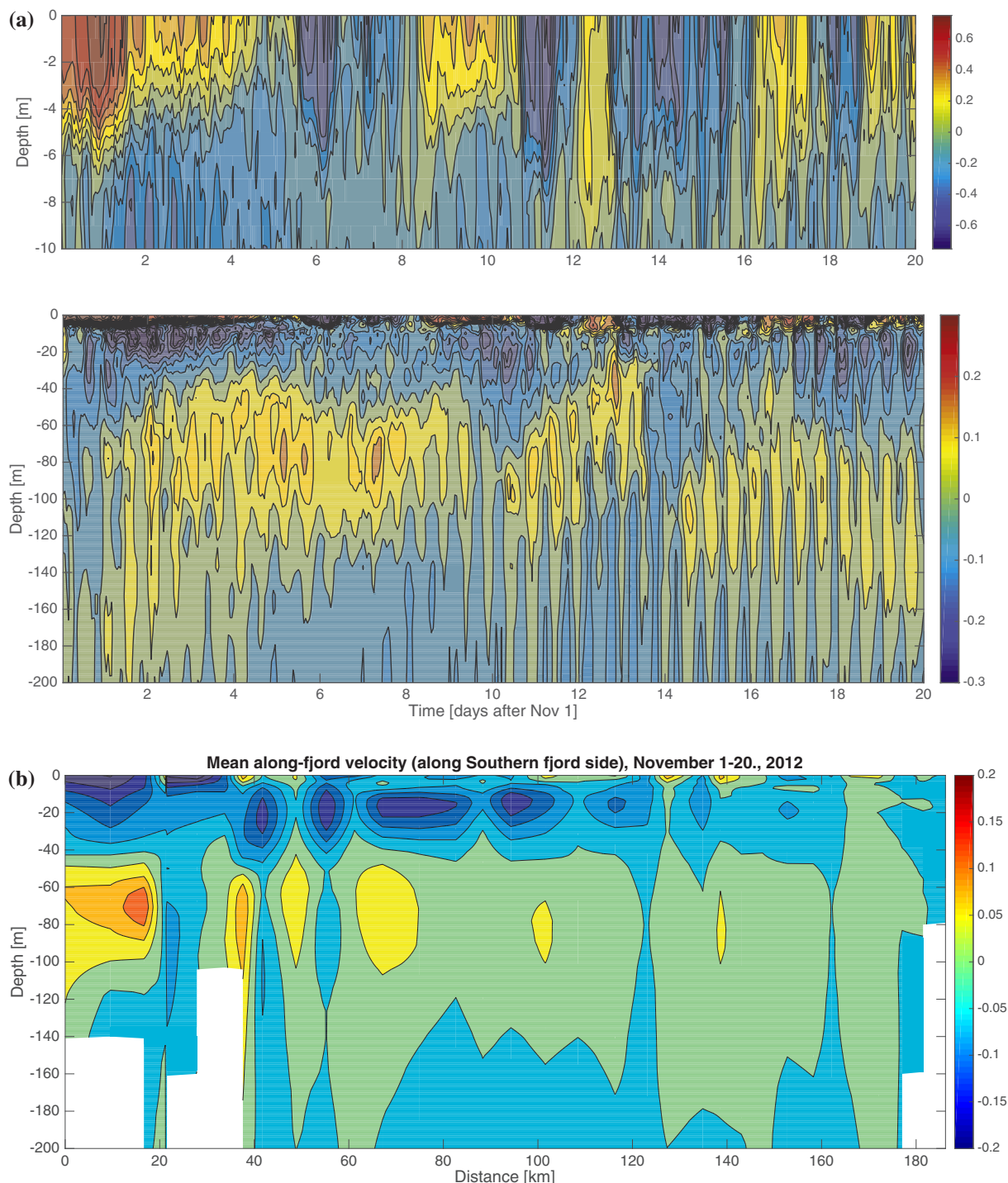


Fig. 4. (a) Time series of the along-fjord current component m s^{-1} in the upper 10 m and the upper 200 m respectively at the location St. 4 in the period November 1–20, 2012, from the numerical model results. Positive (red) and negative (blue) colors denote flow out of and into the fjord, respectively. (b) Vertical section along the southern side of the Sognefjord of the mean along-fjord current component [m/s] in the period November 1–20, 2012, from the numerical model results. The coast is to the left and the fjord head to the right. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

typical for the upper 20 m over the whole fjord length in May. In May, a subsurface chl *a* maxima had developed towards the mouth of the fjord.

3.2. Numerical modelling of currents

The water circulation of the Sognefjord is a result of several different forcing mechanisms. Most important is tide, wind, freshwater runoff, and internal pressure caused by differences in water column weight between the fjord and the coast. We have chosen to present the

along-fjord current at one location near Stn 4 (Fig. 1) to illustrate the mean circulation dynamics for the two periods (1–20 November 2012 and 1–28 May 2013). For the period 1–20 November 2012, we can clearly see the importance of the water flow in the intermediate layer on the fjord transportation, with a relatively persistent inflow between 10 and ~50 m (negative values, blue color) for the entire period, and with a slightly deeper and weaker outflow below, extending down to ~100 m depth (Fig. 4a). We also see the tidal motion as the high frequency oscillations (mainly consisting of the M2 diurnal component,

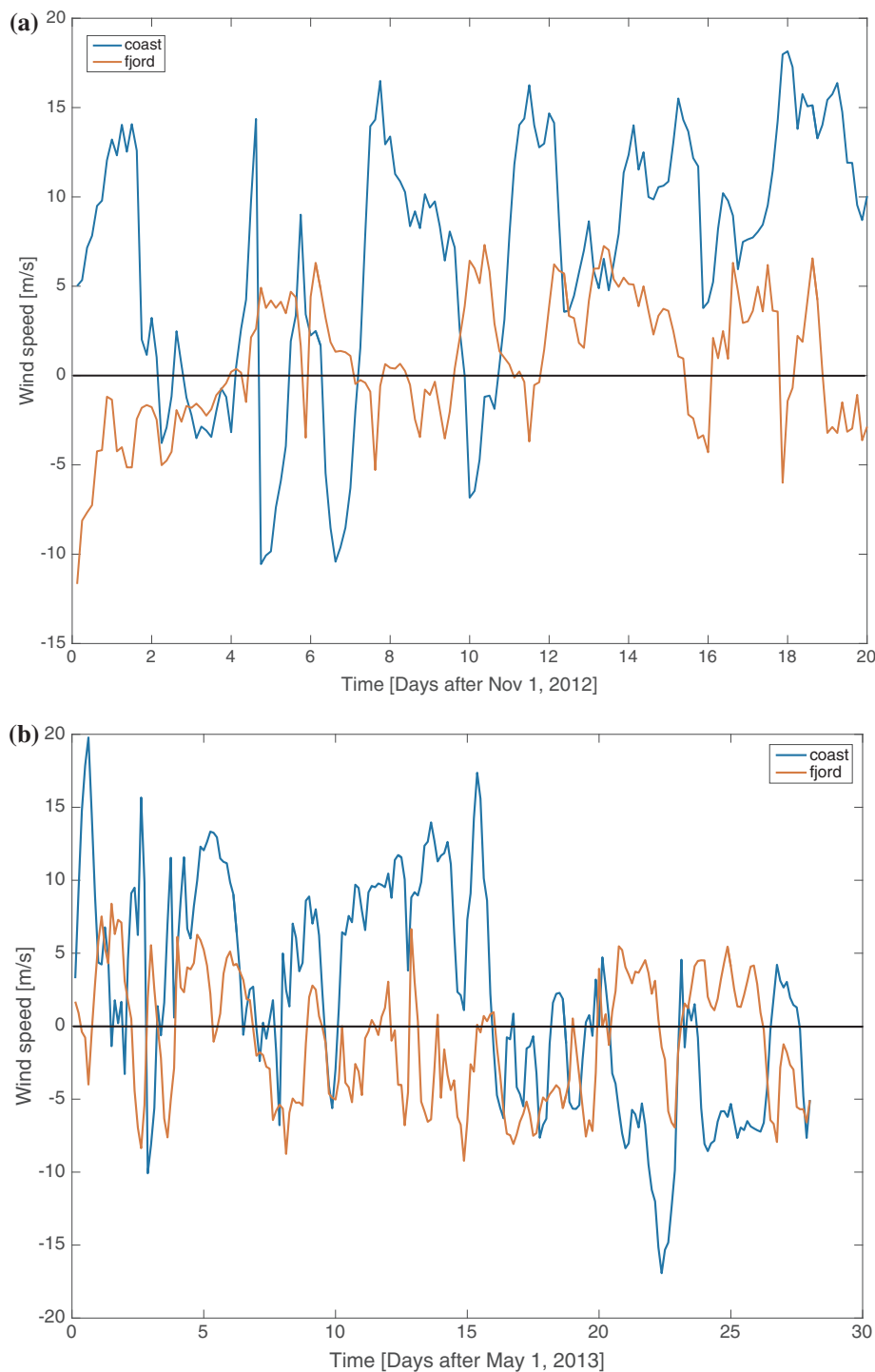


Fig. 5. Time series of the wind speed m s^{-1} for the Southerly component at the coast (blue line) and the along-fjord wind near St. 4 (red line) in the period November 1–20, 2012 (a) and for May 1–28, 2013 (b), from the numerical atmospheric model results. Positive values are towards the North or into the fjord. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

i.e. semidiurnal tidal component due to the moon), but with a relatively low amplitude ($0.05\text{--}0.1 \text{ m s}^{-1}$). The upper 5–10 m constitutes the brackish surface layer, which for the autumn period is mainly influenced by the wind. The water flow in the intermediate layer was regulated by numerous wind episodes at the fjord mouth, where along-coast Southerly winds typically lead to inflow in the upper part of the intermediate layer (Asplin et al., 1999). Time series of wind speed from the numerical wind model, show that several episodes with strong Southerly wind occurred in November 2012 (Fig. 5a). The duration of these episodes was limited to $\sim 1\text{--}2$ d (passage of low pressure atmospheric systems), while the duration of the inflow (Fig. 4a) was longer (3–5 days). The lateral extension of these currents in the intermediate

layer is about 150 km inwards the fjord, and current speed becomes weaker towards the fjord head (Fig. 4b).

In May 2013, the stratification of the intermediate layer was weaker than in November 2012 (Fig. 3), which is consistent with the relatively weaker water flow seen in this layer (Fig. 6a). Also in May 2013, Southerly coastal winds were dominating for the first 15 days (Fig. 5b), leading to an in-fjord flow between approximately 10 and 50 m depth. Towards the end of the month the coastal winds turned to Northerly, resulting in a shallow, but weak outflow just below the brackish layer (approximately from 10 to 30 m depth). However, the flow below these depths and down to ~ 100 m depth continued with an in-fjord direction. The lateral extension of these episodic currents could hardly be

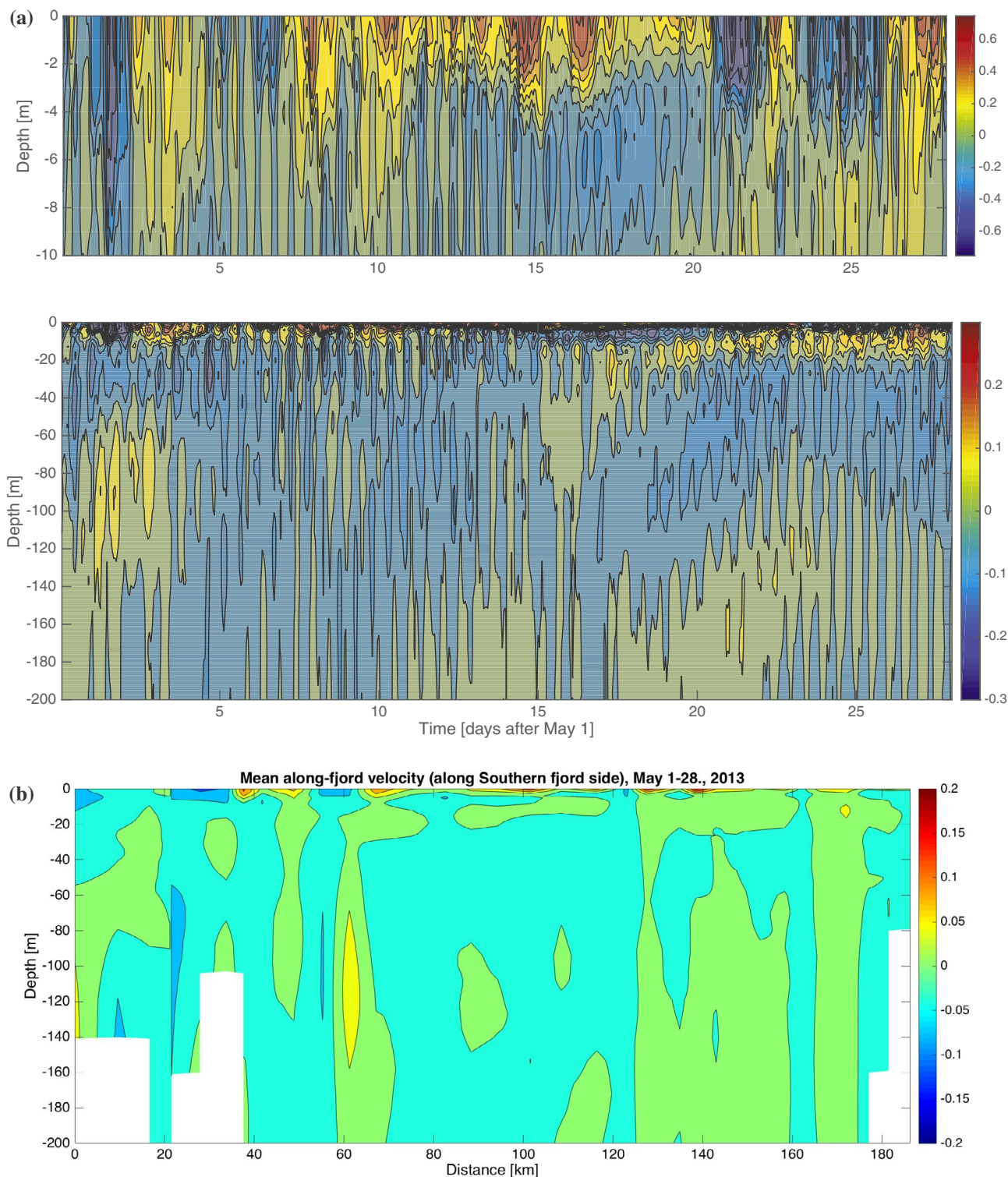


Fig. 6. a) Time series of the along-fjord current component m s^{-1} in the upper 10 m and 200 m respectively at the location St. 4 in the period May 1–28, 2013, from the numerical model results. Positive (red) and negative (blue) colors denote flow out of and into the fjord, respectively. (b) Vertical section along the southern side of the Sognefjord of the mean along-fjord current component m s^{-1} in the period May 1–28, 2013, from the numerical model results. The coast is to the left and the fjord head to the right. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

distinguished (Fig. 6b). With few exceptions (surface) current speed did not exceed 0.05 m s^{-1} .

Interestingly, we found that the current pattern along the fjord was not like a continuous water flow in one direction over a longer time period, but more like episodic formation of vortices (10–20 km wide), where circulation appears to be inwards at on one side of the fjord

(Southern) and outwards on the other side (Northern), and with low current speeds in between. In Fig. 7 the period 1–20 November 2012 is chosen as time case for this phenomenon, and it is representative for the water flow dynamics from the mouth of the fjord and inwards at 0 and 20 m depth (Fig. 7a and b, respectively). It should be noted that the surface layer of the main fjord receives significant quantities of water

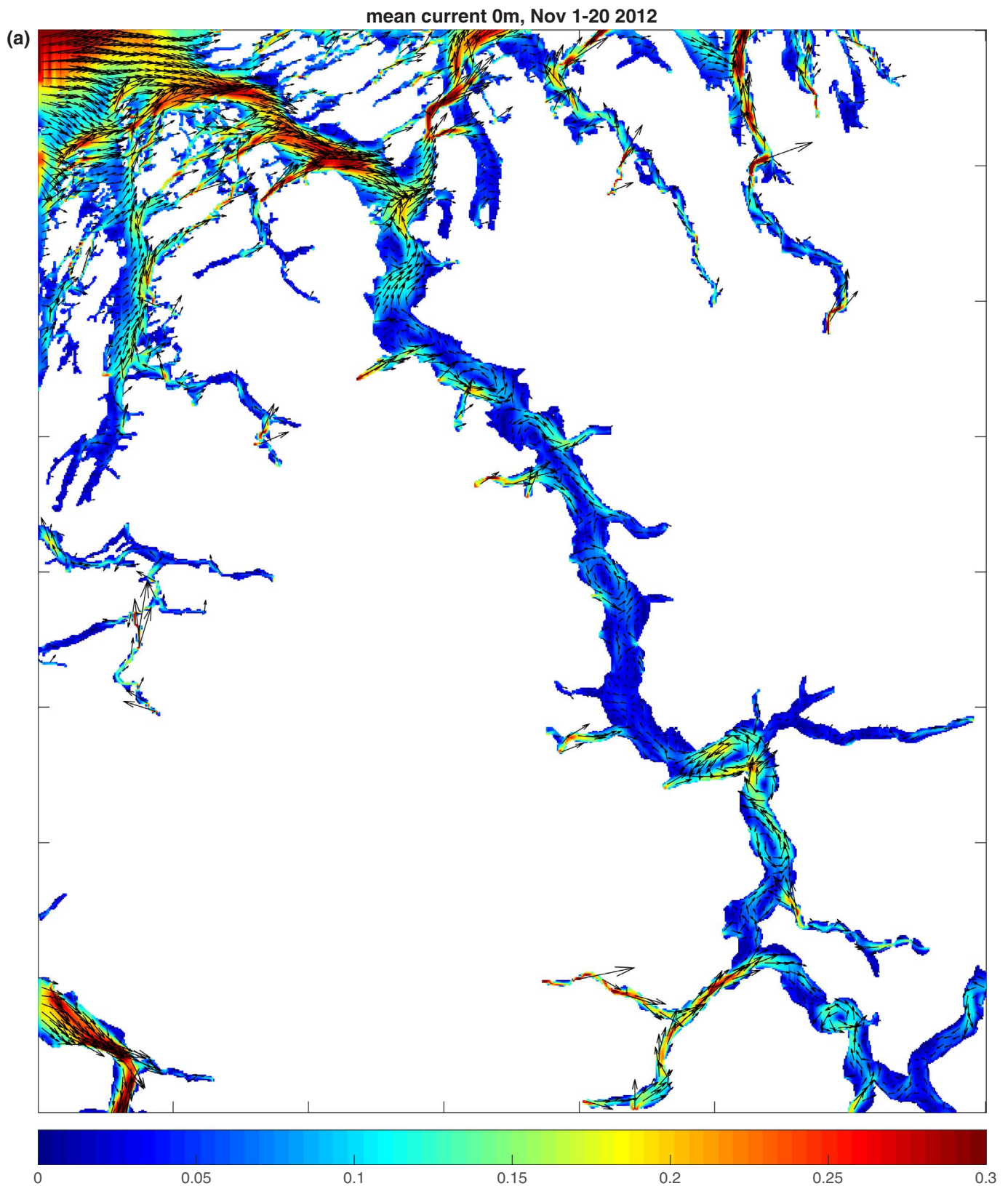


Fig. 7. Mean horizontal current m s^{-1} for the surface (a) and at 20 m depth (b) in the period November 1–20, 2012.

from the side fjords.

To illustrate the episodic water flow with periods of inflow, calm and outflow, respectively, the horizontal current at 20 m depth was followed during 300 h. The corresponding time development at St. 4 can be seen in Fig. 4a. From Fig. 8a we can see that around 80 h there is

a relatively long laterally inward current with a speed of 0.3 m s^{-1} , which is more than three times the mean. This inflow is generated at the coast and is propagating as an internal wave with a wave speed of approximately 0.5 m s^{-1} (Asplin et al., 1999). After 200 h the current in most of the fjord is calm (Fig. 8b), and around 300 h an episode with

mean current 20m, Nov 1-20 2012

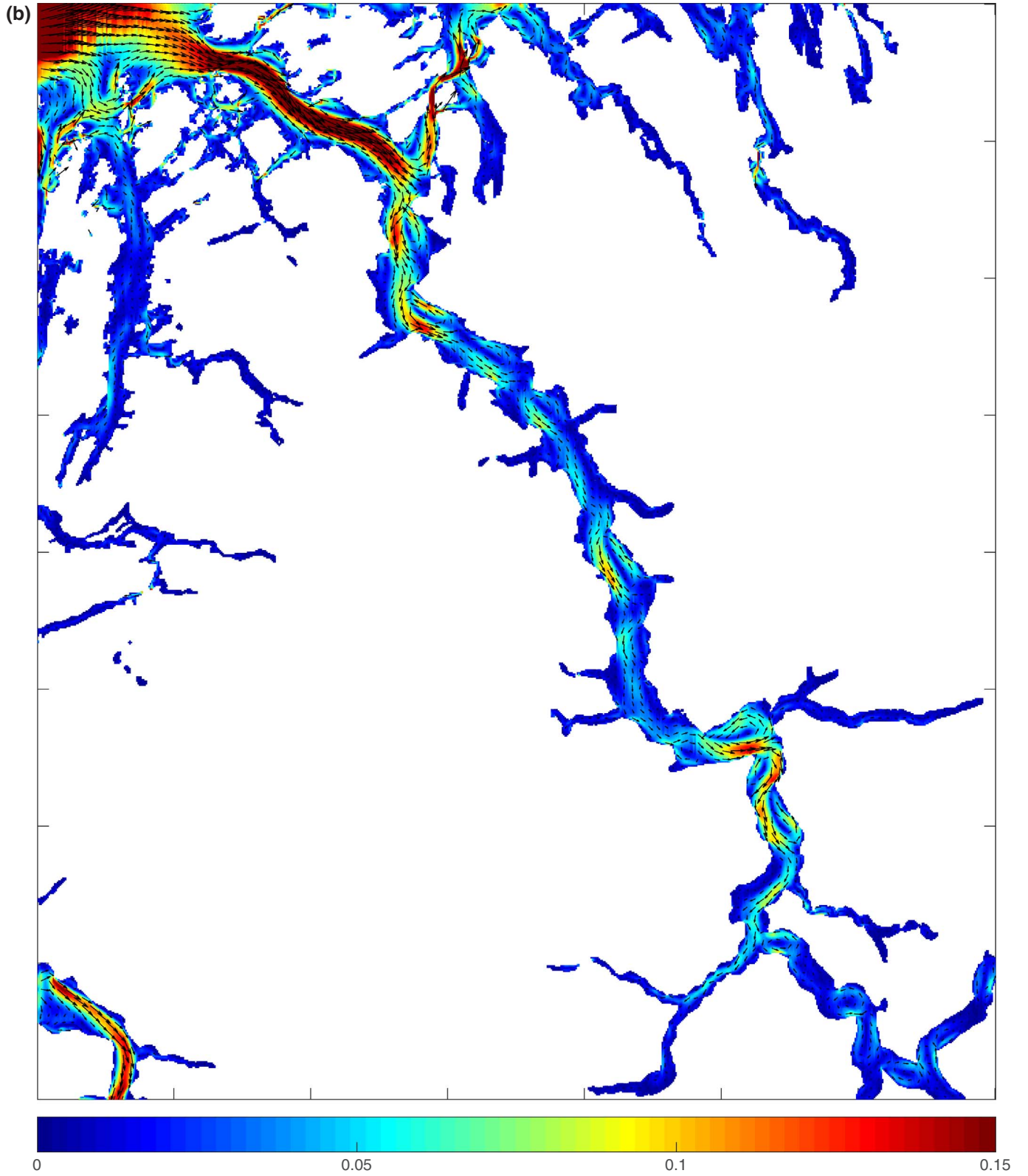


Fig. 7. (continued)

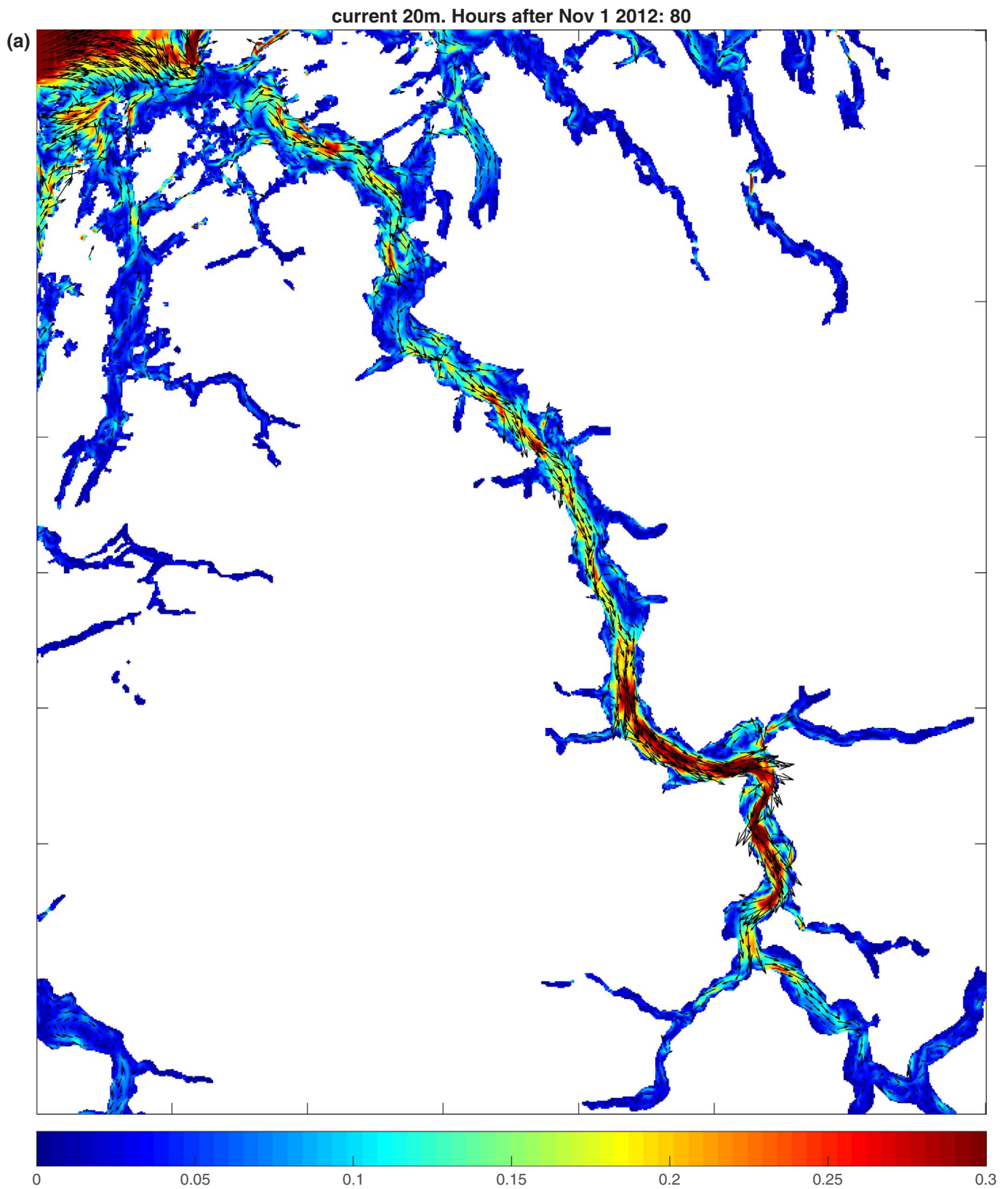


Fig. 8. Horizontal current m s^{-1} at 20 m depth for respectively 80 h (a), 200 h (b) and 300 h (c) after November 1, 2012, illustrating episodes of inflow, calm and outflow of water in the Sognefjord according to the numerical current model.

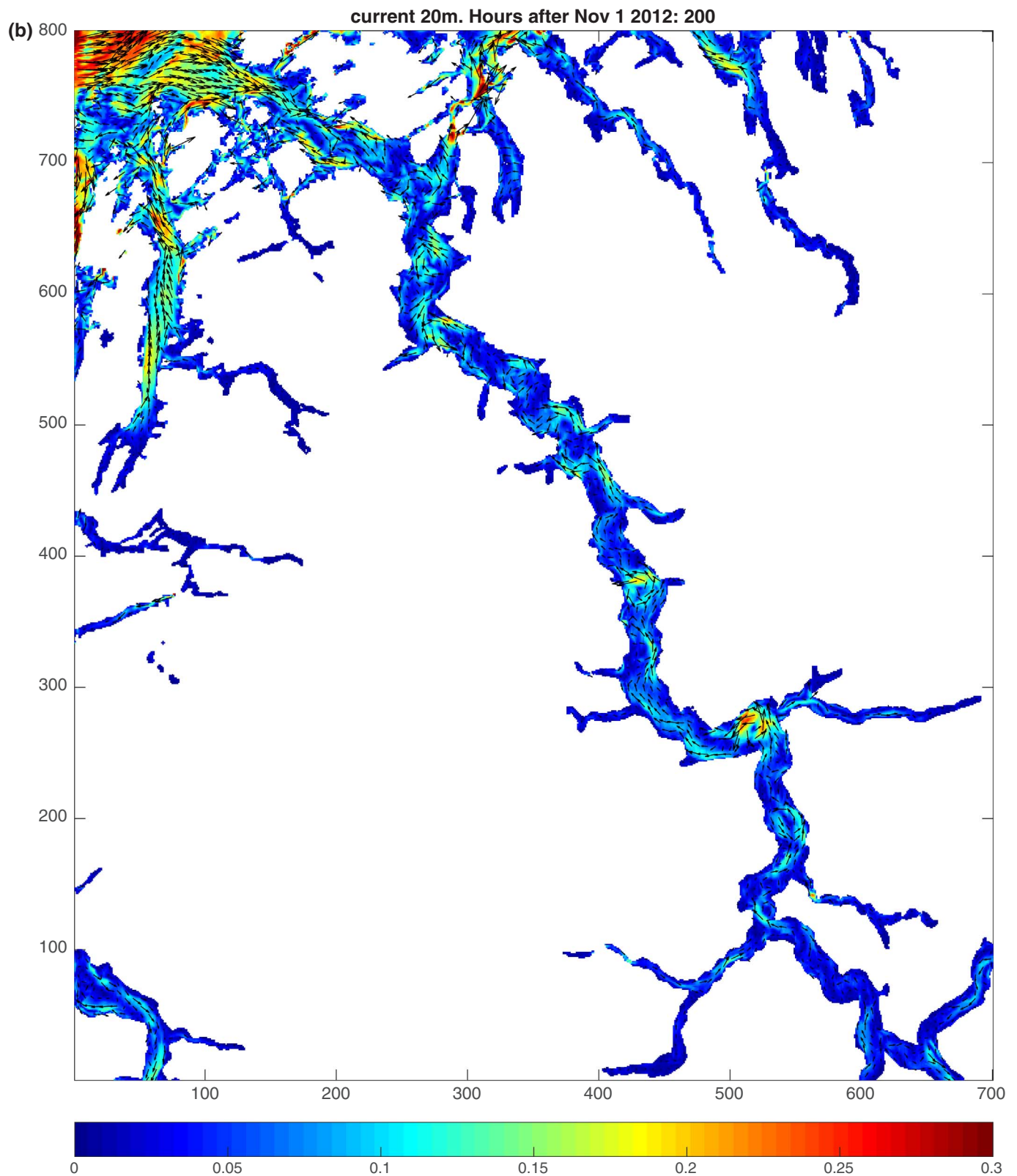


Fig. 8. (continued)

outfjord current is established covering almost the whole fjord length (Fig. 8c).

3.3. Bacterial abundance and production

Bacterial production did not differ between samples taken from the

different water masses in November, whereas they in May were significantly higher in the surface water compared with basin and intermediate water samples. Bacterial abundances measured by FCM indicated lower abundances in the basin water than in the surface and intermediate water. For details on these results, see Storesund et al. (2015).

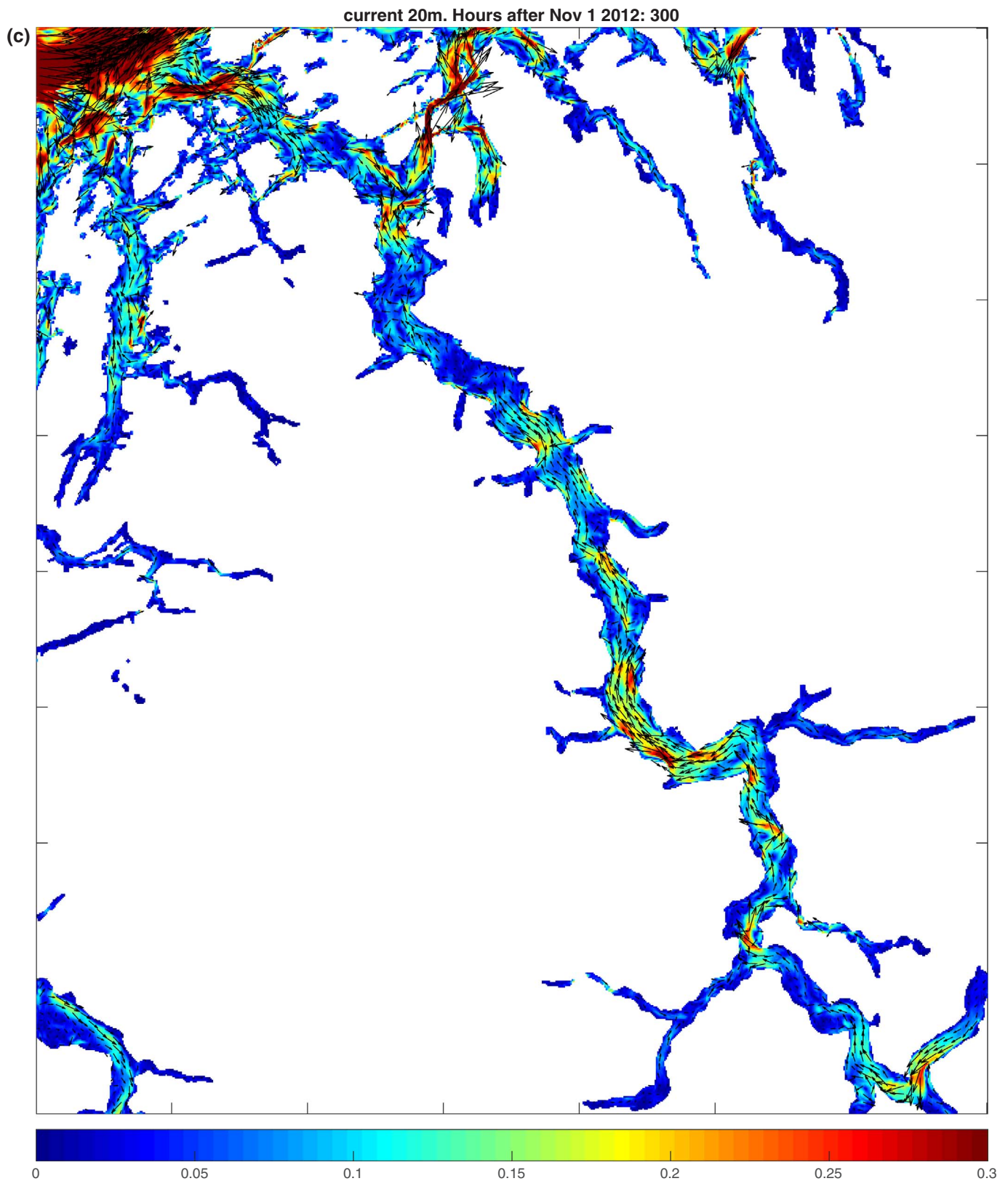


Fig. 8. (continued)

3.4. Relationships between bacterial community similarity, distance and environmental variables

In the surface water, the Mantel correlations showed contrasting results, with bacterial community differences in November showing

high significant correlations with environmental variations, and no significant correlation with geographic distances, whereas bacterial community differences in May showed significant correlations with geographic distances, but not with environmental variables (Table 1). Unravelling the relative importance of factors influencing bacterial

Table 1

Results from Mantel correlations and multiple regressions on distance matrices (MRM). Mantel correlations between the bacterial distance matrix and physical distances in km (r_M dist) and a distance matrix of environmental variables (r_M env) are listed. MRM model results indicates differences in bacterial community composition explained by the whole model (R^2), salinity (Sal), temperature in C° (Temp), nitrate (NO_3^-), chlorophyll *a* fluorescence (chl *a* fl.) and oxygen concentration (O_2). *n* = number of samples included in the respective analyses. All environmental variables except salinity were log-transformed. Stars indicate significance of the results.

Month/water mass	<i>n</i>	Mantel correlations		MRM: whole model/standard partial regression coefficients						
		r_M dist	r_M env	R^2	Distance (km)	Sal	Temp	NO_3^-	chl <i>a</i> fl.	O_2
<i>November</i>										
Surface	10	0.29	0.88 ^{***}	0.86 ^{***}	0.17 [†]	-0.45 [†]	0.65 ^{**}	0.27 [†]	0.52 ^{***}	
Intermediate	8	0.58 [†]	0.74 ^{***}	0.77 ^{***}				0.58 ^{***}		
Basin	14	0.22 [†]	0.63 ^{**}	0.37 ^{**}						0.61 ^{**}
<i>May</i>										
Surface	8	0.59 ^{**}	0.22	0.47 ^{**}	0.69 ^{**}					
Intermediate	14	0.60 ^{***}	0.66 ^{***}	0.64 ^{***}			0.36 ^{**}	0.55 ^{***}	0.30 ^{**}	
Basin	14	0.21	0.68 ^{**}	0.39 ^{**}						0.49 ^{***}

P-values:

- * $P < 0.05$.
- ** $P < 0.005$.
- *** $P < 0.0005$.

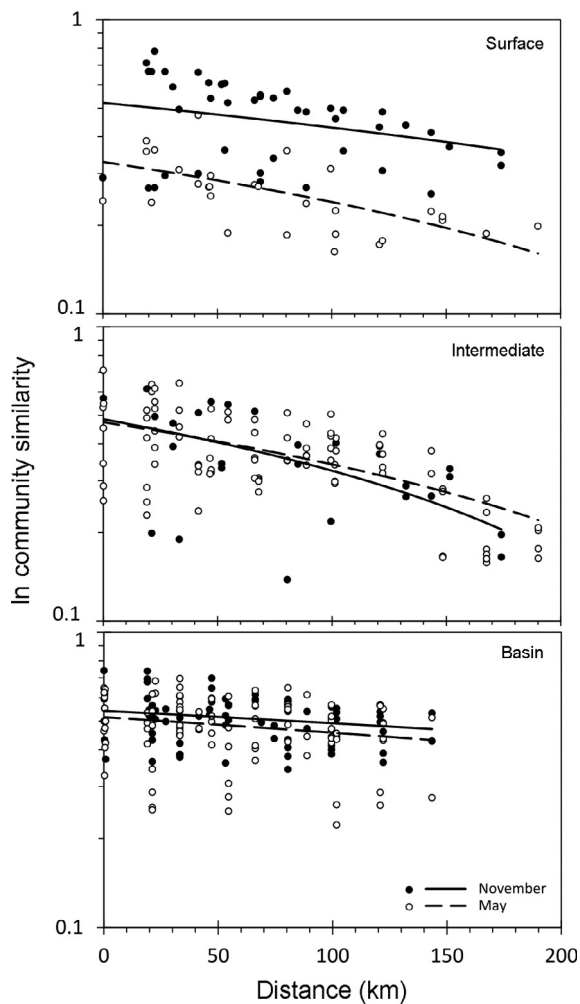


Fig. 9. Distance-decay curves for the bacterial community in November (solid line) and May (dashed line) in the three different water layers, indicating bacterial community composition changes at different depths with increasing distance in the fjord. Calculations are based on the logarithm (ln) of community similarity calculated from 1 – Jaccard distance matrices.

community composition using MRM models indicated higher influence from environmental variables in November than in May. In November, the partial regression coefficients of salinity, temperature, NO_3^-

concentration and chl *a* fluorescence showed high correlations with bacterial community composition, and geographic distance also showed a lower, but significant, correlation (Table 1). In strong contrast, only geographic distance was a significant factor influencing the surface water community composition in May. Overall community similarity was also higher throughout the surface water in November than in May, while for the intermediate and basin water the community was rather similar at the two time points, but there was a tendency of a stronger decrease in similarity for the intermediate water inwards the fjord in November than in May (Fig. 9).

In the intermediate water, Mantel correlations indicated that both geographic distance and environmental variations were highly correlated with bacterial community composition at both sampling times (Table 1). However, the MRM models showed that the partial regression coefficients for temperature, NO_3^- concentration, and chl *a* fluorescence were significant in May, and NO_3^- in November, whereas geographic distance was not significant at either sampling time (Table 1).

For the basin samples, Mantel correlations showed that differences in bacterial community composition were highly correlated with environmental differences both in November and in May. Community composition showed a low significant correlation with geographic distances in November, and no significant correlation in May (Table 1). The MRM models supported this, as oxygen content, decreasing inwards the fjord, was the only significantly correlated partial regression coefficients at both sampling times.

3.5. Relative contribution of allochthonous bacteria to fjord bacteria

The Sourcetracker analyses indicated varying influences of allochthonous bacterial communities from freshwater runoff and offshore coastal and Atlantic water on the overall bacterial community composition in the fjord (Fig. 10, Table 2). Bacterial communities from freshwater runoff and coastal surface water appeared to have a higher impact on the surface bacterial community in November (Fig. 10a and b) than in May (Fig. 10d and e). Coastal and Atlantic water bacterial communities influenced both the surface and intermediate layers (Fig. 10c and f, Table 2). The sources for OTUs in the basin water were for the most part not associated with bacterial communities defined as allochthonous.

4. Discussion

We have identified clear changes in bacterial community composition by depth along the coast-fjord gradient in the Sognefjord, and

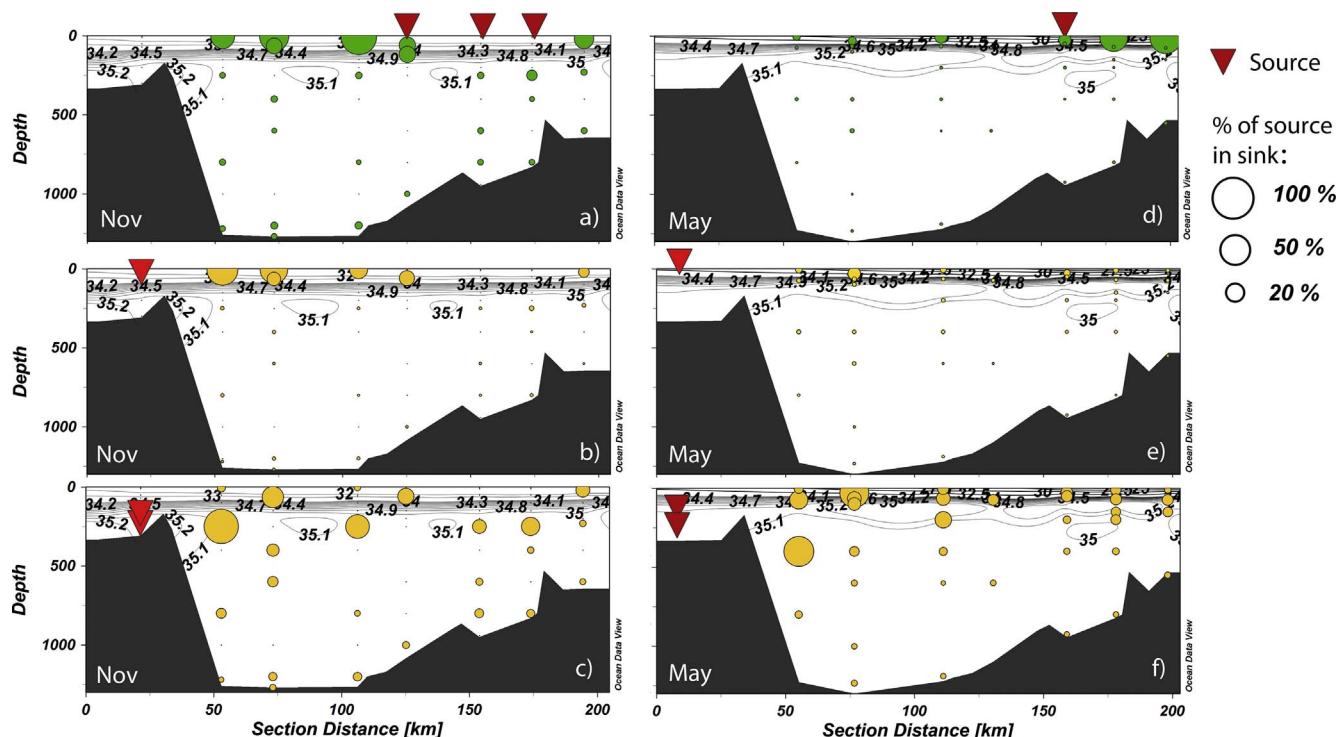


Fig. 10. Illustration of how allochthonous bacterial communities enter the fjord in November 2012 (a–c) and May 2013 (d–f), and mixes with the resident bacterial community as estimated using Sourcetracker. Arrowheads indicate source samples. Each circle represents one sample, the size of the circle indicates how much (%) of the community that is influenced by the source communities. Samples marked green indicate freshwater influence from water located at the entrance to side-fjords. Yellow circles indicate Coastal water/Atlantic water influence from samples located outside the sill. Contours indicate salinity gradients in the fjord. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

linked these to variations in the environment and to geographic distance in different water layers. Results from numerical modelling of current speed and direction for the upper 200 m of the fjord have been used to illustrate how the water circulation influences bacterial distribution along the fjord.

4.1. Advection as a structuring force in the surface and intermediate layers

Current simulations revealed high temporal and spatial variations in speed and direction along the coast-fjord gradient across the upper 200 m (Figs. 4b and 6b). With respect to in and out-fjord transport of bacteria, the modelling results show that on average the advective forces contribute stronger in November than May, which is consistent with a stronger influence of coastal and Atlantic bacterial communities in the intermediate layer in November than May (see Section 3).

Variations in environmental factors along the fjord transect appear to have an overall higher impact on bacterial community composition in the fjord than geographic distances. The exception was in the surface

water in May, when only geographic distance was significantly correlated with bacterial community differences. This could be due to the natural variations in freshwater input to the fjord, which fluctuates throughout the year, but normally peaks in May–June due to snow melting (Sætre et al., 2007), and give rise to a strong estuarine circulation (Fig. 10). Increased freshwater input increases current speed in the surface layer, strengthens stratification and advection, and leads to restricted vertical exchange of organic matter (OM), and increased transport of particles out of the fjord (Aure and Stigebrandt, 1989; Wassmann, 1984; Wassmann et al., 1996).

Even if our results from numerical modelling show that on average, horizontal transport is more discontinuous than continuous over distance, the water flow within a certain layer could contribute significantly to the displacement of bacterial populations. Thus, during the period 1–20 November, the mean current speed was around 0.1 m s⁻¹ over the depth interval 10–20 m, and current direction was inward (Fig. 4b). During the whole period of 20 d this represents a total distance of 173 km from the coast. For the depth interval 40–120 m, on the

Table 2
Contribution (%) of freshwater-runoff and Atlantic/Coastal-water OTUs to fjord bacterial communities in November and May. Mean, standard deviation (std), minimum (Min) and maximum (Max) values are shown.

	Freshwater runoff				Atlantic/Coastal (5 m)				Atlantic/Coastal (100–300 m)				Unknown source			
	Mean	Std	Min	Max	Mean	Std	Min	Max	Mean	Std	Min	Max	Mean	Std	Min	Max
November																
Surface (n = 5)	39.6	24.0	15.9	75.5	28.4	21.6	6.7	56.9	75.3	29.7	40.3	98.8	24.7	29.7	1.2	59.7
Intermediate (n = 6)	5.1	4.4	2.1	14.4	2.6	3.8	0.5	11.1	35.0	22.5	6.2	72.9	65.0	22.5	27.1	93.8
Basin (n = 14)	2.1	0.5	1.4	3.2	0.5	0.2	0.2	0.8	6.6	2.3	3.8	12.2	93.4	2.3	87.8	96.2
May																
Surface (n = 15)	21.6	22.0	3.2	58.6	3.3	2.8	0.9	9.4	13.1	16.4	2.7	49.5	62.1	22.1	37.0	89.5
Intermediate (n = 13)	0.6	0.2	0.3	0.8	0.8	0.2	0.3	1.2	9.1	4.4	3.6	19.8	89.5	4.6	78.4	95.3
Basin (n = 14)	0.5	0.2	0.2	1.1	0.5	0.2	0.2	1.1	5.9	12.6	1.3	53.0	93.1	12.8	45.4	98.2

other hand, the water flow was outwards at an average speed of 0.02 m s^{-1} , meaning that during 20 d, bacterial cells within this layer will be transported 34 km towards the coast.

Previous studies of current strength in the upper 20 m of the Sognefjord have estimated average speed to be $0.03\text{--}0.08 \text{ m s}^{-1}$ (Dupont and Aksnes, 2010; Lothe and Brørs, 2010), and maximum speed at 0.77 m s^{-1} (Lothe and Brørs, 2010), which are also consistent with current speeds measured in another western Norwegian fjord, the Lysefjord (Aure et al., 2007). However, our results from numerical modelling show that it is not realistic to assume a maximum speed measured at one site to be valid for the whole fjord system. With an average current speed of 0.1 m s^{-1} , which is in accordance with the numerical model results, bacterial turnover in the fjord needs to be less than 27.3 d at the head (Supplementary Table A), and 13.7 d at Stn 5 (approximately half way into the fjord), to attain a net growth in the fjord before being flushed out, and thus have the chance to be influenced by local environmental variations. Even if current speed could approach 0.7 m s^{-1} (Fig. 4a), this will be restricted to vortices or episodes of limited size and/or duration. On the other hand, these vortices could be considered as active mixing zones, where inflowing bacterial populations on the southern side of the fjord are mixed with the outflowing populations on the northern side.

We measured average bacterial community turnover in the surface layer to be 3.9 d in November, and 4.0 d in May (see Section 2.5.1, Eq. (3)), meaning that given realistic current speeds over the length of the fjord, surface bacterial community growth rates would be more than sufficient to achieve a net biomass increase within the fjord. Differences in community composition along the coast-fjord transect in the upper water layer would then mainly be affected by input of allochthonous bacteria, advection, entrainment and vertical mixing, and end-member mixing with underlying water masses. In this context, the current vortices probably contribute strongly to mixing of allochthonous populations of coastal and fresh water origin. End-member mixing is the conservative mixing of dissimilar water layers (and their respective bacterial communities) over large spatial scales (Hewson et al., 2006). In fjords, end-member mixing is common, as water from the deeper compensation inflow entrains into the outflowing surface water, making this brackish water gradually more saline from head to mouth (Stigebrandt, 1981, 2012), contributing to the observed linear decrease in community similarity with distance in May (Fig. 9). Aure et al. (1996) concluded that stratified fjords respond to density changes in the adjacent coastal waters, and that the water exchange is most easily observed in the intermediary water layers, where the circulation is often an order of magnitude greater than the estuarine circulation above (Aure and Stigebrandt, 1989). Our findings highlight the fundamental importance of physical forcing for growth dynamics and structuring of microbial communities in fjord ecosystems.

4.2. Impact from environmental factors on bacterial community composition

We observed a high significant correlation between bacterial community differences and inorganic nutrient (NO_3^-) concentrations in the surface and intermediate water (surface water in May excepted) (Table 1). Inorganic nutrients are often considered to be limiting factors in the upper, photic zone of marine environments (Hecky and Kilham, 1988), and variations in concentration can affect bacterial community composition (Pinhassi et al., 2006). In particular, competition with autotrophs for limiting inorganic nutrients such as NO_3^- could affect bacterial community structure (Cherrier et al., 1996; Joint et al., 2002), and indeed, the MRM models indicated that strength of chl *a* fluorescence was linked to differences in bacterial community composition along the coast-fjord gradient in the Sognefjord. Alternatively, algal derived DOC may have promoted growth of certain bacterial taxa, leading to the observed correlation of bacterial community composition with chl *a* fluorescence.

The MRM models explain a large portion of the bacterial community variability in the fjord, but it should be noted that much of the variability remains unexplained, especially in the basin water. This could be due to variables that were not included in the models, such as DOC, which was measured at some stations, but was excluded from the MRM model analyses due to missing values. However, we have previously shown that DOC did not differ significantly between sampling stations or water layers in the fjord (Storesund et al., 2015). In addition, DOC represents a large variety of complex organic compounds (Benner, 2002), and equal concentrations in different samples, does not necessarily mean equal availability of the carbon present in them. Also, fjord basin waters can due to the isolated position be considered more conservative with respect to environmental impact (with the exception of vertical transport) than the upper water masses, and it is therefore not given that expanding the MRM model would give us further answers.

ARISA fingerprinting is subjected to the same biases as other PCR based methods, such as favouring certain taxa over others (Wintzingerode et al., 1997). Archaea and bacterial taxa that do not possess the intergenic spacer region, such as the Planctomycetes, are also excluded from the analyses (Chow et al., 2013). It should thus be noted that the measured OTU richness is not a true representation of the total bacterial richness in the fjord, which is most likely underestimated (Bent et al., 2007; Dunbar et al., 2000). However, ARISA has been successfully used to estimate relative bacterial richness and diversity in different environments, and is considered to be a robust method for investigating beta-diversity (Danovaro et al., 2006; Hewson and Fuhrman, 2004; Hewson et al., 2006).

4.3. Bacterial community homogeneity in the basin water

We expected to find some distance-decay in bacterial community similarity in the basin water of the Sognefjord, both due to its length, several internal basins separated by sills (Fig. 1), and because the infrequent advective exchanges would facilitate stagnant water masses below sill level and lead to increased residence time for the bacterial populations. Therefore, the lack of a significant correlation between geographical distance and bacterial community composition in the basin water was surprising. Even low levels of dispersion between communities can counteract the effects of local selection and lead to reduced beta-diversity (Declerck et al., 2013), and potential distance-decay relationships may thus be counteracted through seeding between communities by advection and introduction of allochthonous species (Martiny et al., 2006). In the Sognefjord, the similarity in community composition throughout the basin, with no distinct local communities, is therefore concurrent with the presence of some dispersal, possibly in the form of internal waves or advective currents. Results from our numerical modelling revealed a weak mean inflow (0.02 m s^{-1}) of water below sill depth (170–700 m) at Stn 4 between 1 and 20 November 2012 (data not shown). Alternatively, the time-span since the last major extensive exchange of basin water, which was in the winter of 2009/2010 (approximately two years prior to our sampling) as a result of an extreme negative North Atlantic Oscillation (NAO) index (Osborn, 2011), was too short for discernible geographical differences in bacterial community composition to evolve.

Oxygen concentration, which decreased along the coast-fjord transect both in November and May (Fig. 3), was the only significant variable connected to bacterial community composition in the basin water. Decreasing oxygen concentrations are often linked to ageing water masses as POM is mineralized (Aure and Stigebrandt, 1989), and higher bacterial activity in the inner part of the fjord than in the outer part could explain the lower oxygen concentrations seen there. The inner part of the Sognefjord is shallower and more heavily influenced by side-fjords than the outer part, and water from side-fjords meeting the main fjord water can lead to turbulent mixing and thus increased vertical transport and increased nutrient availability and mineralization in the basin at the inner stations.

4.4. Conclusions

Coastal regions experience elevated stress from human activity, and increased knowledge of environmental conditions and factors shaping the biology of fjords and coastal areas is crucial. Distinct circulation patterns together with the strong vertical zonation typical for the deep Norwegian fjords make them ideal sites for studying the effect of advective processes on bacterial community growth and distribution. In the Sognefjord, we found that although bacterial community composition was indeed highly linked to geographic distance and advective forces in the surface water in May, individual environmental factors appeared to have a higher impact on bacterial community composition in November, and also in the deeper layers of the fjord. Our study emphasises the strong coupling between coastal and fjord bacterial communities through advection that introduced allochthonous species to the upper layers of the fjord, but not to the basin water. We have also shown that the water flow along the fjord is more discontinuous than continuous, appearing as distinct episodes lasting for only hours to a few days each. Therefore advection of bacteria also should be considered to follow a more episodic pattern, meaning that strong horizontal transport during one limited time period is followed by a calm period, and that also the flow direction changes within a relatively short time frame.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.pocean.2017.09.002>.

References

Aksnes, D.L., Aure, J., Kaartvedt, S., Magnesen, T., Richard, J., 1989. Significance of advection for the carrying capacities of fjord populations. *Mar. Ecol. Prog. Ser.* 50, 263–274.

Albretsen, J., Sperrevik, A.K., Staalstrøm, A., Sandvik, A.D., Vikebø, F., Asplin, L., 2011. *NorKyst-800 Report No. 1—User Manual and Technical Descriptions*. Tech. Rep. *Fisken og Havet* 2/2011.

Arrigo, K.R., 2004. Marine microorganisms and global nutrient cycles. *Nature* 437, 349–355.

Asplin, L., Salvanes, A.G.V., Kristoffersen, J.B., 1999. Nonlocal wind-driven fjord-coast advection and its potential effect on plankton and fish recruitment. *Fish. Oceanogr.* 8, 255–263.

Asplin, L., Johnsen, I.A., Sandvik, A.D., Albretsen, J., Sundfjord, V., Aure, J., Boxaspen, K.K., 2014. Dispersion of salmon lice in the Hardangerfjord. *Mar. Biol.* 161, 216–225. <http://dx.doi.org/10.1007/s00227-013-1075-5>.

Aure, J., Stigebrandt, A., 1989. On the influence of topographic factors upon the oxygen consumption rate in sill basins of fjords. *Estuar. Coast. Shelf Sci.* 28, 59–69.

Aure, J., Molvær, J., Stigebrandt, A., 1996. Observations of inshore water exchange forced by a fluctuating offshore density field. *Mar. Pollut. Bull.* 33, 112–119.

Aure, J., Strand, Ø., Erga, S.R., Strohmeier, T., 2007. Primary production enhancement by artificial upwelling in a western Norwegian fjord. *Mar. Ecol. Prog. Ser.* 352, 39–52.

Basedow, S.L., Eiane, K., Tverberg, V., Spindler, M., 2004. Advection of zooplankton in an Arctic fjord (Kongsfjorden, Svalbard). *Estuar. Coast. Shelf Sci.* 60, 113–124.

Benner, R., 2002. Chemical composition and reactivity. In: Hansell, D.A., Carlson, C.A. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, pp. 59–90.

Bent, S.J., Pierson, J.D., Forney, L.J., 2007. Measuring species richness based on microbial community fingerprints: the emperor has no clothes. *Appl. Environ. Microbiol.* 73, 2399–2401.

Bordalo, A.A., Vieira, M.E.C., 2005. Spatial variability of phytoplankton, bacteria and viruses in the mesotidal salt wedge Douro Estuary (Portugal). *Estuar. Coast. Shelf Sci.* 63, 143–154.

Braarud, T., Føyn Hofsvang, B., Hjelmsfoss, P., Överland, A.-K., 1974. The natural history of the hardangerfjord: 10. The phytoplankton in 1955–56. The quantitative phytoplankton cycle in the fjord waters and in the offshore coastal waters. *Sarsia* 55, 63–98.

Cherrier, J., Bauer, J., Druffel, E., 1996. Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern North Pacific surface waters. *Mar. Ecol. Prog. Ser.* 139, 267–279.

Cho, B.C., Azam, F., 1988. Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature* 332, 441–443.

Chow, C.-E.T., Sachdeva, R., Cram, J.A., Steele, J.A., Needham, D.M., Patel, A., Parada, A.E., Fuhrman, J.A., 2013. Temporal variability and coherence of euphotic zone bacterial communities over a decade in the Southern California Bight. *ISME J.* 7, 2259–2273.

Crump, B.C., Hopkinson, C.S., Sogin, M.L., Hobbie, J.E., 2004. Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time. *Appl. Environ. Microbiol.* 70, 1494–1505.

Danovaro, R., Luna, G., Dell'Anno, A., Pietrangeli, B., 2006. Comparison of two fingerprinting techniques, terminal restriction fragment length polymorphism and automated ribosomal intergenic spacer analysis, for determination of bacterial diversity in aquatic environments. *Appl. Environ. Microbiol.* 72, 5982–5989.

Declerck, S.A.J., Winter, C., Shurin, J.B., Suttle, C.A., Matthews, B., 2013. Effects of patch connectivity and heterogeneity on metacommunity structure of planktonic bacteria and viruses. *ISME J.* 7, 533–542.

del Giorgio, P.A., Bouvier, T.C., 2002. Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. *Limnol. Oceanogr.* 47, 471–486.

Dunbar, J., Ticknor, L.O., Kuske, C.R., 2000. Assessment of microbial diversity in four southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. *Appl. Environ. Microbiol.* 66, 2943–2950.

Dupont, N., Aksnes, D.L., 2010. Simulation of optically conditioned retention and mass occurrences of *Periphylla periphylla*. *J. Plankton Res.* 32, 773–783.

Erga, S.R., Heimdal, B.R., 1984. Ecological studies on the phytoplankton of Korsfjorden, western Norway. The dynamics of a spring bloom seen in relation to hydrographical conditions and light regime. *J. Plankton Res.* 6, 67–90.

Erga, S.R., 1989. Ecological studies on the phytoplankton of Boknafjorden, western Norway. II. Environmental control of photosynthesis. *J. Plankton Res.* 11, 785–812.

Erga, S.R., Szebiyonga, N., Frette, Ø., Hamre, B., Aure, J., Strand, Ø., Strohmeier, T., 2012. Dynamics of phytoplankton distribution and photosynthetic capacity in a western Norwegian fjord during coastal upwelling: effects on optical properties. *Estuar. Coast. Shelf Sci.* 97, 91–103.

Erlandsson, C.P., 2008. Vertical transport of particulate organic matter regulated by fjord topography. *J. Geophys. Res.: Biogeosci.* 113.

Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The microbial engines that drive Earth's biogeochemical cycles. *Science* 320, 1034–1039.

Gade, H.G., Edwards, A., 1980. Deep water renewal in fjords. In: Freeland, H., Farmer, D., Levings, C. (Eds.), *Fjord Oceanography*. Springer, US, pp. 453–489.

Goslee, S.C., Urban, D.L., 2007. The ecodist package for dissimilarity-based analysis of ecological data. *J. Stat. Softw.* 22, 1–19.

Gravel, D., Bell, T., Barbera, C., Combe, M., Pommier, T., Mouquet, N., 2012. Phylogenetic constraints on ecosystem functioning. *Nat. Commun.* 3, 1117.

Gutiérrez, M.H., Galand, P.E., Moffat, C., Pantoja, S., 2015. Melting glacier impacts community structure of Bacteria, Archaea and Fungi in a Chilean Patagonia fjord. *Environ. Microbiol.* 17, 3882–3897.

Haidvogel, D.B., Arango, H., Budgell, W.P., Cornuelle, B.D., Curchitser, E., Di Lorenzo, E., Fennel, K., Geyer, W.R., Hermann, A.J., Lanerolle, L., Levin, J., McWilliams, J.C., Miller, A.J., Moore, A.M., Powell, T.M., Shchepetkin, A.F., Sherwood, C.R., Signell, R.P., Warner, J.C., Wilkin, J., 2008. Ocean forecasting in terrain-following coordinates: formulation and skill assessment of the Regional Ocean Modeling System. *J. Comput. Phys.* 227, 3595–3624.

Hanson, C.A., Fuhrman, J.A., Horner-Devine, M.C., Martiny, J.B., 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat. Rev. Microbiol.* 10, 497–506.

Hecky, R., Kilham, P., 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.* 33, 796–822.

Henriques, I.S., Alves, A., Tacão, M., Almeida, A., Cunha, Â., Correia, A., 2006. Seasonal and spatial variability of free-living bacterial community composition along an estuarine gradient (Ria de Aveiro, Portugal). *Estuar. Coast. Shelf Sci.* 68, 139–148.

Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J.* 5, 1571–1579.

Hermansen, H.O., 1974. *Sognefjordens hydrografi og vannutveksling* (In Norwegian). Cand. real. thesis. University of Bergen, Bergen.

Hewson, I., Fuhrman, J.A., 2004. Richness and diversity of bacterioplankton species along an estuarine gradient in Moreton Bay, Australia. *Appl. Environ. Microbiol.* 70,

- 3425–3433.
- Hewson, I., Steele, J.A., Capone, D.G., Fuhrman, J.A., 2006. Temporal and spatial scales of variation in bacterioplankton assemblages of oligotrophic surface waters. *Mar. Ecol. Prog. Ser.* 311, 67–77.
- Joint, I., Henriksen, P., Fonnes, G.A., Bourne, D., Thingstad, T.F., Riemann, B., 2002. Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. *Aquat. Microb. Ecol.* 29, 145–159.
- Kaartvedt, S., Svendsen, H., 1990. Impact of freshwater runoff on physical oceanography and plankton distribution in a Western Norwegian fjord: an experiment with a controlled discharge from a hydroelectric power plant. *Estuar. Coast. Shelf Sci.* 31, 381–395.
- Kaartvedt, S., Nordby, E., 1992. Impact of a controlled freshwater discharge on zooplankton distribution in a Norwegian fjord. *J. Exp. Mar. Biol. Ecol.* 162, 279–293.
- Kaufman, L., Rousseeuw, P.J., 2009. *Finding Groups in Data: An Introduction to Cluster Analysis*. vol. 344 John Wiley & Sons.
- Kirchman, D.L., Dittel, A.I., Malmstrom, R.R., Cottrell, M.T., 2005. Biogeography of major bacterial groups in the Delaware Estuary. *Limnol. Oceanogr.* 50, 1697–1706.
- Knights, D., Kuczyński, J., Charlson, E.S., Zaneveld, J., Mozer, M.C., Collman, R.G., Bushman, F.D., Knight, R., Kelley, S.T., 2011. Bayesian community-wide culture-independent microbial source tracking. *Nat. Meth.* 8, 761–763.
- Lee, S., Fuhrman, J.A., 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Appl. Environ. Microbiol.* 53, 1298–1303.
- Legendre, P., Lapointe, F.J., Casgrain, P., 1994. Modeling brain evolution from behavior: a permutational regression approach. *Evolution* 1487–1499.
- Lothe, A.E., Brørs, B., 2010. Mulighetstudie for kryssing av Sognefjorden Opedal-Lavik. Estimert på Bølger og strøm. SINTEF rapport nr. SBF IN F10208 (in Norwegian). SINTEF, p. 19.
- Manzetti, S., Stenersen, J.H.V., 2010. A critical view of the environmental condition of the Sognefjord. *Mar. Pollut. Bull.* 60, 2167–2174.
- Martiny, J.B., Eisen, J.A., Penn, K., Allison, S.D., Horner-Devine, M.C., 2011. Drivers of bacterial β -diversity depend on spatial scale. *Proc. Natl. Acad. Sci.* 108, 7850–7854.
- Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., 2006. Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.* 4, 102–112.
- Matthews, J.B.L., Heimdal, B.R., 1980. Pelagic productivity and food chains in fjord systems. In: Freeland, H., Farmer, D., Levings, C. (Eds.), *Fjord Oceanography*. Springer, US, pp. 377–398.
- Molvær, J., Gade, H.G., 1971. Vannutveksling og vannfornyning i Sognefjorden: en vurdering av fortykning og transport av utlutningsprodukter fra ovnsbunner: rapport for A/S Årdal & Sunddal verk, mars 1971 (In Norwegian). University of Bergen, Geophysical Institute, Bergen.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. *Vegan: Community Ecology Package*. R package Version 2.0-9. Available from < <http://CRAN.R-project.org/package=vegan> > .
- Osborn, T.J., 2011. Winter 2009/2010 temperatures and a record-breaking North Atlantic Oscillation index. *Weather* 66, 19–21.
- Paasche, O., Osterblom, H., Neuenfeldt, S., Bonsdorff, E., Brander, K., Conley, D.J., Durant, J.M., Eikeset, A.M., Goksoyr, A., Jonsson, S., Kjesbu, O.S., Kuparinen, A., Stenseth, N.C., 2015. Connecting the seas of Norden. *Nat. Clim. Change* 5, 89–92.
- Parsons, T.R., Maita, Y., Lalli, C.M., 1984. Ch. 1: Nutrients. In: Lalli, T.R.P.M.M. (Ed.), *A Manual of Chemical & Biological Methods for Seawater Analysis*. Pergamon: Amsterdam, pp. 3–28.
- Pinhassi, J., Gómez-Consarnau, L., Alonso-Sáez, L., Sala, M.M., Vidal, M., Pedrós-Alió, C., Gasol, J.M., 2006. Seasonal changes in bacterioplankton nutrient limitation and their effects on bacterial community composition in the NW Mediterranean Sea. *Aquat. Microb. Ecol.* 44, 241–252.
- Ramette, A., 2009. Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. *Appl. Environ. Microbiol.* 75, 2495–2505.
- Schlitzer, R., 2002. Interactive analysis and visualization of geoscience data with Ocean Data View. *Comput. Geosci.* 28, 1211–1218.
- Schlitzer, R., 2011. *Ocean Data View*. Available from. <<http://odv.awi.de>> .
- Shchepetkin, A., McWilliams, J.C., 2005. The Regional Ocean Modeling System: a split-explicit, free-surface, topography following coordinates ocean model. *Ocean Model.* 9, 347–404.
- Simon, M., Azam, F., 1989. Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol. Prog. Ser.* 51, 201–213.
- Skamarock, W.C., Klemp, J.B., Dudhia, J., Gill, D.O., Barker, D.M., Duda, M.G., Huang, X.-Y., Wang, W., Powers, J.G., 2008. A description of the Advanced Research WRF version 3. NCAR Technical Note 475. < http://www.mmm.ucar.edu/wrf/users/docs/arw_v3.pdf > .
- Smith, D.C., Azam, F., 1992. A simple, economical method for measuring bacterial protein synthesis rates in seawater using [³H]-leucine. *Multilingue* 6, 107–114.
- Staley, C., Gould, T.J., Wang, P., Phillips, J., Cotner, J.B., Sadowsky, M.J., 2015. Species sorting and seasonal dynamics primarily shape bacterial communities in the Upper Mississippi River. *Sci. Total Environ.* 505, 435–445.
- Stigebrandt, A., 1976. Vertical diffusion driven by internal waves in a sill fjord. *J. Phys. Oceanogr.* 6, 486–495.
- Stigebrandt, A., 1981. A mechanism governing the estuarine circulation in deep, strongly stratified fjords. *Estuar. Coast. Shelf Sci.* 13, 197–211.
- Stigebrandt, A., Aure, J., 1989. Vertical mixing in basin waters of fjords. *J. Phys. Oceanogr.* 19, 917–926.
- Stigebrandt, A., 2012. Hydrodynamics and circulation of fjords. *Encyclopedia of Lakes and Reservoirs*. Springer, pp. 327–344.
- Storesund, J.E., Erga, S.R., Ray, J.L., Thingstad, T.F., Sandaa, R.A., 2015. Top-down and bottom-up control on bacterial diversity in a western Norwegian deep-silled fjord. *FEMS Microbiol. Ecol.* 91 (7).
- Svendsen, S.W., 2006. *Stratification and Circulation in Sognefjorden*. M.Sc thesis. University of Bergen, Bergen.
- Sætre, R., Gjertsen, K., Brøker, K., 2007. *The Norwegian Coastal Current: Oceanography and Climate*. Tapir Academic Press, Trondheim.
- Team, R.C., 2013. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria 3-900051-07-0. Available from < <http://www.R-project.org/> > .
- Tommasi, D., Hunt, B.P.V., Pakhomov, E.A., Mackas, D.L., 2013. Mesozooplankton community seasonal succession and its drivers: Insights from a British Columbia, Canada, fjord. *J. Mar. Syst.* 115–116, 10–32.
- Wassmann, P., 1984. Sedimentation and benthic mineralization of organic detritus in a Norwegian fjord. *Mar. Biol.* 83, 83–94.
- Wassmann, P., Svendsen, H., Keck, A., Reigstad, M., 1996. Selected aspects of the physical oceanography and particle fluxes in fjords of northern Norway. *J. Mar. Syst.* 8, 53–71.
- Wilhelm, L., Singer, G.A., Fasching, C., Battin, T.J., Besemer, K., 2013. Microbial biodiversity in glacier-fed streams. *ISME J.* 7, 1651–1660.
- Wilkins, D., van Sebille, E., Rintoul, S.R., Lauro, F.M., Cavicchioli, R., 2013. Advection shapes Southern Ocean microbial assemblages independent of distance and environment effects. *Nat. Commun.* 4.
- Wintzingerode, F.V., Göbel, U.B., Stackebrandt, E., 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol. Rev.* 21, 213–229.