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Differences in microphytobenthos and macrofaunal abundances associated with groundwater discharge in the intertidal zone

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ABSTRACT: We investigated the impact of intertidal groundwater seepage on benthic microalgae and macrofauna in 4 study sites located in 2 large tidal flat ecosystems along the western coast of Korea by comparing the chemical, physical, and biological characteristics of 'glossy' seepage sites with those of nearby areas without visually distinct groundwater discharge (dry sediment surface). At 3 of the 4 sites, sediment properties as well as pore water chemistry were similar in groundwater seepage and dry areas. At the 4th study site, the groundwater seepage areas were more coarse-grained compared to the dry areas. Here, the groundwater seepage also had lower salinity and higher nutrient concentrations than the pore water of the dry area and the seawater in a nearby tide pool. Although diatoms were the dominant algal class in seepage and dry areas alike, the seepage areas in 3 of the sites had elevated contributions of other marker pigments such as chlorophyll (chl) *b* compared to the dry areas. Chl *a* concentrations were higher in all seepage areas compared to dry areas, and all dry areas had high pheophytin *a*:chl *a* ratios, indicating a substantial amount of degraded algal material. In the seepage areas of 3 of the sites, we found large numbers of the snail *Batillaria cumingi*, while crab burrows of *Scopimera* sp. were only present in the neighboring dry areas. Correlations of sediment chl *a* concentrations with physicochemical properties of the ambient pore water indicated that microphytobenthos responded specifically to groundwater seepage, which may provide shelter from desiccation and salt stress during emersion of the tidal flat. Our results suggest that globally common groundwater seepage significantly impacts the ecosystem structures and microphytobenthos production of the tidal flats.

KEY WORDS: Groundwater · Seepage · Microphytobenthos · Macrofauna · Intertidal · Pigments · Tidal flat

INTRODUCTION

The study of submarine groundwater discharge (SGD), e.g. in the form of groundwater seepage along a hydraulic gradient in the intertidal zone, has become increasingly widespread in recent years (Giblin & Gaines 1990, Miller & Ullman 2004, Hays & Ullman 2007). Groundwater is commonly enriched with nutrients (Johannes 1980, Johannes & Hearn 1985, Giblin & Gaines 1990, Slomp & van Capellen 2004) and other elements such as Ra (Moore 1996) and Rn, relative to river water, precipitation, or seawater. It can therefore have a significant impact on coastal marine primary production (Lapointe 1997, Gobler & Sañudo-Wilhelmy 2001, Carmichael & Valiela 2005). Recently, links between SGD and harmful algal blooms were found (Gobler & Sañudo-Wilhelmy 2001, Lee & Kim 2007, Lee et al. 2010). SGD can also influence benthic marine plant abundance and physiology (Maier & Pregnall 1990, Kamermans et al. 2002, Hwang et al. 2005a) and govern the distribution of macrobenthic species (Miller & Ullman 2004, Zipperle & Reise 2005, Dale & Miller 2008). However, data on the ecological effects of groundwater seepage on communities at the sediment–water interface, e.g. microphytobenthos, are sparse (Hagerthey & Kerfoot 1998, Carmichael & Valiela 2005, Blanco et al. 2008).

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Intertidal microphytobenthos, i.e. the assemblage of bottom-living microalgae such as diatoms, is an important contributor to the benthic food chain (MacIntyre et al. 1996, Kang et al. 2003). Through resuspension, microphytobenthos can be transferred to pelagic systems as well (De Jonge & van Beusekom 1992). Furthermore, microphytobenthos can regulate diffusive nutrient fluxes between sediment and the overlying water column (Sundbäck et al. 1991). The relationship between microphytobenthos and ambient nutrient concentrations is often unclear. Generally, benthic microalgae, particularly from muddy sediments, are not nutrient limited (Underwood & Kromkamp 1999), and nutrient enrichment via the water column does not seem to have any effect on microphytobenthos biomass (Hillebrand & Kahlert 2002). On the other hand, microphytobenthos cultures can be maintained well with natural porewater (Admiraal et al. 1982), and microphytobenthos mats in the sandy sediments of the intertidal zone increase in biomass if nutrients are introduced to them from the sediment (Flothmann & Werner 1992). Microphytobenthos biomass has previously been linked with groundwater discharge in freshwater (Hagerthey & Kerfoot 1998) and marine environments (Carmichael & Valiela 2005, Blanco et al. 2008), which was attributed to the supply of groundwater-derived nutrients.

The environmental consequences of intertidal groundwater discharge, which is enhanced by large tidal amplitudes, may be particularly strong for large tidal flats such as the extensive ones (~2850 km^2) bordering the Yellow Sea along the southwestern coast of Korea, with horizontal extensions of up to 10 km and tidal water level fluctuations of up to 10 m (Frey et al. 1987). Typically, they consist of muddy sediments that were deposited from the offshore Yellow Sea, and are bordered landward by coarse-grained beaches produced by coastal erosion (Ryu 2003). Because they are so highly productive, they act as a food source for migratory birds, and they also support a large sector of the Korean Fisheries industry (Deppe 2000). Similar to the German Wadden Sea, macrofaunal communities can be divided into 3 zones, but with higher biodiversity for being a low-energy environment (Frey et al. 1987). Due to the gently sloping tidal flat and the large semidiurnal tides, a vast intertidal zone is exposed to desiccation and salt stress, predation, and large temperature fluctuations (up to 25°C), for several hours per day (Deppe 2000). A series of recently conducted projects revealed that SGD is ubiquitous along the coastline of South Korea, and that this SGD has a significant impact on coastal marine biogeochemistry and water column productivity (Hwang et al. 2005a,b, Lee & Kim 2007, Lee et al. 2009, 2010). However, it is unknown how the groundwater seepage affects the benthic microalgal and macrofaunal community in the intertidal environment.

We therefore investigated the biogeochemical characteristics of intertidal groundwater discharge and associated patterns of the benthic community, in comparison with neighboring areas not exposed to groundwater, in 2 tidal flats along the western coast of Korea. These 2 tidal flats differ in the biogeochemical composition of their groundwater source, as well as in the sedimentary characteristics of the impacted seepage zones. Furthermore, the 2 environments were studied in different seasons, one during a spring microphytobenthos bloom when macrofauna was absent, and the other in mid-summer, with well-developed macrofaunal communities. This approach is an important advancement in studies on the effects of intertidal groundwater seepage, which in the past either focused on the algal or the macrofaunal communities. It is likely that the benthic intertidal community creates feedback loops that interact with the 'original' impact of the groundwater seepage: For example, increased microphytobenthos biomass may create larger grazing pressure, or seepage areas could attract burrowing organisms, which enhance the hydraulic conductivity even further (Dale & Miller 2008).

MATERIALS AND METHODS

Study sites. Due to changes in the hydraulic gradient at low tide, groundwater can form seepage areas in the intertidal zone that are often visually and geochemically distinct. The existence of these seepage areas has been described for several regions worldwide, such as the Perth region along the west coast of Australia, the German Wadden Sea, and beaches along the US East coast (Johannes 1980, Miller & Ullman 2004, Zipperle & Reise 2005, Santos et al. 2009). We investigated 2 intertidal zones on the Korean western coast: first, one station on Seokmo Island, northwest of Seoul (Fig. 1A), and second, 3 stations (Dolmori 1, Dolmori 2, and Songseokri) in Hampyeong Bay in the southern part of Korea (Fig. 1B). The average tidal ranges at Seokmo Island and Hampyeong Bay are 7.5 and 3.5 m, respectively. At both sampling locations, the study sites are comprised of sandy beaches bordering extensive mudflats on the seaward side. Groundwater seeps out along the border of sandy and muddy sediment, forming small pools that drain into larger tidal channels. Seepage areas such as the ones found in our study sites occur ubiquitously along the western and southern coast of Korea, and SGD is a major nutrient pathway in some of the embayments along the Korean southern coast previously investigated (Hwang et al. 2005a,b, Lee et al. 2009, 2010). For example, virtually every

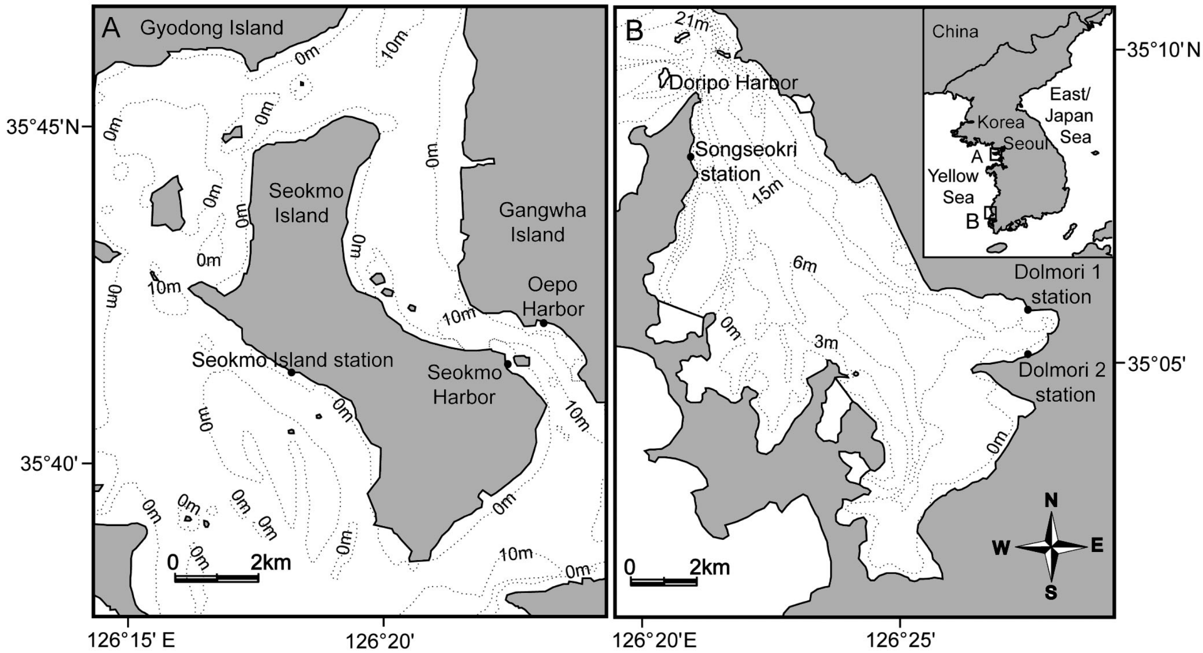

Fig. 1. Sampling stations at (A) Seokmo Island and (B) Hampyeong Bay

beach along the coast of Hampyeong Bay displayed visually distinct groundwater runoff.

At Seokmo Island, the beachface is only about 5 m wide and the border between sand- and mudflat is very distinct (Fig. 2 top), whereas the slope of the Hampyeong Bay coastline is gentler, the beachface is ~10 m wide and the borders of the seepage areas and adjacent tidal channels are less sharp (Fig. 2 bottom). Sampling at Seokmo Island took place on 21–22 March 2008 during spring tide (8 m) and in Hampyeong Bay on 30–31 July 2008, also at spring tide (4 m). The benthic algal community at the Seokmo Island station formed thin films on the surface of the muddy tidal flat. In Hampyeong Bay, the coarse sediment grains were covered with a greenish layer of attached algae, and at these stations, several stones of 2 to 3 cm diameter had attachments of *Enteromorpha* sp. Due to the low abundance of such stones, the macroalgae were not investigated in this study.

Significant macrofauna was absent in March during sampling in Seokmo Island, but in Hampyeong Bay in July, the snail *Batillaria cumingi* and the ghost crab *Scopimera* sp. dominated the area in high densities and were included in the descriptive survey.

Sampling protocols. Due to the different morphologies of the Seokmo Island and Hampyeong Bay tidal flats, we designed 2 types of sampling setups for a better characterization of the 2 environments (Fig. 2). For example, groundwater seepage at Seokmo Island occurred only on a narrow (10 to 30 cm) strip of permeable sediment along the shoreline, with the bulk of the groundwater being directly discharged onto the mudflat, whereas in Hampyeong Bay, the seepage area was more extensive, resulting in exposure of a larger permeable sediment interface. Consequently, at Seokmo Island, we investigated a mudflat community subject to (indirect) groundwater runoff from an adjacent seepage area, and in Hampyeong Bay, we sampled slightly muddy sand communities subject to direct groundwater seepage from below.

At the sampling station on Seokmo Island (Fig. 1A), we chose 3 distinct tidal channels into which groundwater seepage runoff was draining (hereafter referred to as 'groundwater seepage channels'), and divided them into channel centers and channel walls along transects perpendicular to the shoreline. At each transect, 3 stations were set: 1 adjacent to the seepage border, and the other 2 at 5 and 10 m distance towards the water line. At the seepage border, the channels were wide (30 to 50 cm) grooves with a depth of ~1 cm, and quickly deepened with increasing distance from shore: 5 m from the seepage border station, the seepage channels had a width of 10 to 20 cm and an approximate depth of 10 to 15 cm below the surrounding tidal flat, and at 10 m distance from shore, the depth of the channel increased to about 0.5 m. These small, meandering tributary tidal channels are stable over the course of several tides, but may vary seasonally (Wells et al. 1990). The mudflat areas next to the groundwater seepage channels at the station 5 m from the seepage border were defined as dry areas (Fig. 2 top). In Hampyeong Bay, we chose 3 sampling stations: Dolmori 1

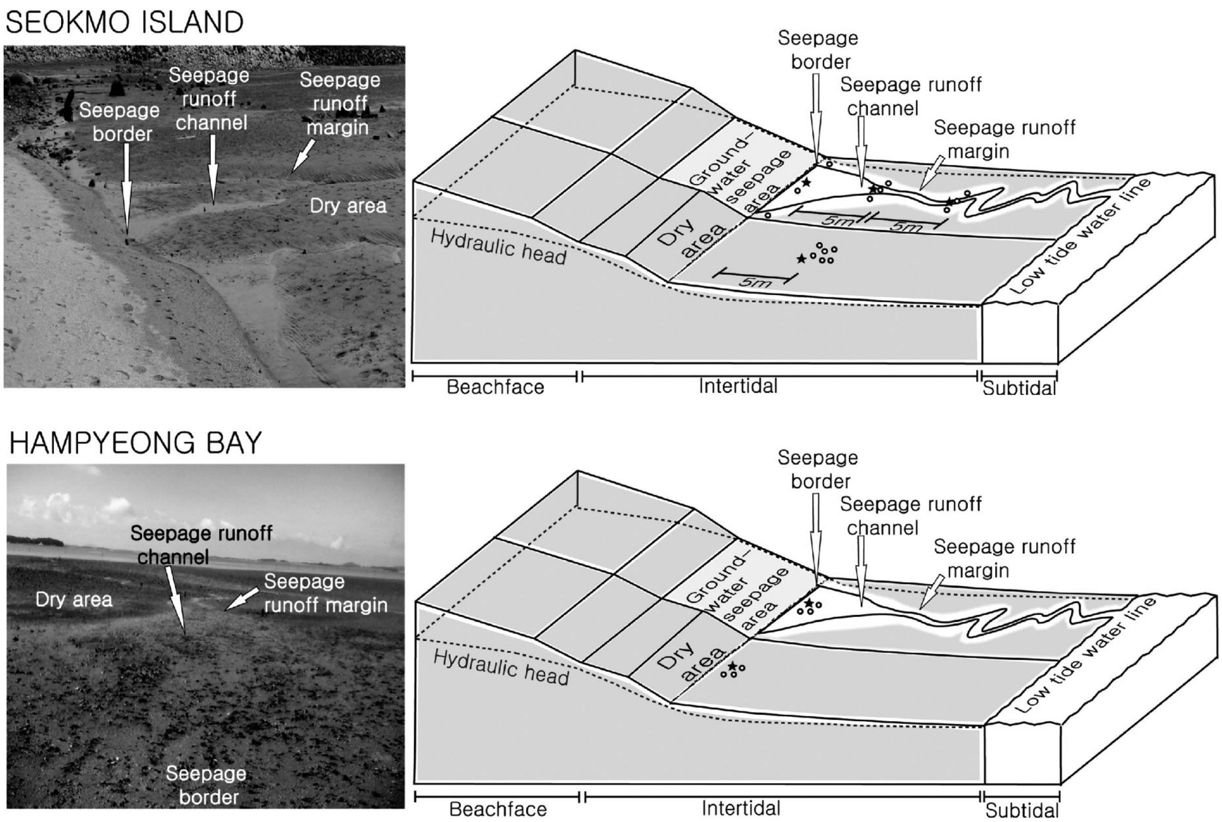

Fig. 2. Seepage and dry sites in Seokmo Island and Hampyeong Bay. Sediment (o) and groundwater (★) sampling locations

and Dolmori 2 at the bay head, and Songseokri at the bay mouth (Fig. 1B). Since all 3 stations showed similar groundwater seepage patterns, we applied the same sampling setup (Fig. 2 bottom). At these stations, no visible seepage margin could be found. Therefore, at Dolmori 1, Dolmori 2, and Songseokri, we only sampled visibly distinct 'seepage sites' and 'dry areas' with equal distances from land.

For pore water analysis in Seokmo Island, groundwater runoff was collected on both sampling days with syringes from each seepage channel at the 3 transect stations, and an additional pore water sample was collected thereafter by digging a shallow pit at the seepage border between mudflat and beachface. Samples were filtered through GF/F Whatman filters and stored frozen. In the dry areas, surface (upper 2 cm) sediments were collected and transported on ice back to the laboratory, where the pore water was extracted by centrifugation, filtered and stored frozen. Due to the low water content of the sediments at the dry site, all pore water samples were pooled into one sample. Salinity and temperature of the groundwater seepage were measured with an ISTEK conductivity sensor at each of the transect stations and at the seepage border. In Hampyeong Bay, multi-level pore water samplers were inserted into the permeable sediment, and samples at 2 cm depth intervals up to a depth of 20 cm were drawn using vacutainers and stored frozen until filtration and analysis in the laboratory. In addition, duplicate seawater samples were collected at high tide at both sampling locations, filtered and stored frozen. At the Seokmo Island station, one seawater sample was collected with a syringe from a tidal pool with ~10 cm depth and ~30 cm radius, ~5 m from the seepage border. Inorganic nutrients were measured with an Alliance Futura auto-analyzer (PO_4^{3-}, $Si(OH)_4$, NO_3^-, NO_2^-). Since we did not have a dedicated auto-analyzer set for NH_4^+, it was measured manually by spectrophotometry following phenolhypochlorite treatment (Solozarno 1969). All samples were calibrated against blanks (n = 2) and standard rows of each chemical species (n = 5), which were measured before and after the sample sets. NO_3^- and NO_2^- were measured together as NO_x and combined with NH_4^+, defined as dissolved inorganic nitrogen (DIN). PO_4^{3-} and $Si(OH)_4$ will hereafter be referred to as dissolved inorganic phosphorus (DIP) and silicon (DSi), respectively.

Sediments for pigment analysis were collected from the sampling points shown in Fig. 2, by gently inserting a 2 cm diameter cut-off syringe to a depth of 1 cm

(Seokmo Island) or 2 cm (Hampyeong Bay). The different sampling depths were chosen because of the differences in sediment grain size at the 2 different locations, which could potentially influence the light penetration and thus the depth distribution of actively photosynthesizing microphytobenthos. Samples were stored on dry ice in the field and at –80°C in the laboratory. Photosynthetic pigments were extracted based on a method modified from Grinham et al. (2007), where 100% chilled acetone (wt:vol ratio of 2:5) and a canthaxanthin internal standard were added to the samples, which were then stored at –20°C for 24 h. Thereafter, samples were sonicated for 8 min on ice and the resulting solution was centrifuged. The supernatant was then passed through 0.45 µm PTFE syringe filters (Waters Corporation). To 1 ml of sample, 300 µl of deionized water (DIW) were added, the sample was shaken, and immediately transferred to the HPLC (Waters 2487 absorbance detector, 436 nm). Analysis was conducted using a 3-solvent system (methanol/ammonium acetate; acetonitrile aqueous; ethyl acetate) with a binary linear gradient program. For calibration, standards for chlorophyll (chl) *a* and other pigments, respectively from Sigma (UK) and DHI (Institute for Water and Environment, Denmark) were used.

After pigment analysis, the sediment samples were dried and the dry wt was noted. Samples were then washed with 1 l of DIW through a 63 µm nylon mesh net to remove mud, then dried and weighed again to determine the mud content. Then, the washed sediment was dry-sieved for grain size analysis.

In Hampyeong Bay, an additional macrofaunal observation study was conducted to count abundances of the mud snail *Batillaria cumingi* and burrows of the ghost crab *Scopimera* sp. Three seepage areas ('glossy' sediment surface) and 3 dry areas (dry surface) of 2 to 3 m^2 size were selected, one 50×50 cm^2 quadrat was placed randomly within each of these patches, and snail and crab burrow numbers were determined.

RESULTS AND DISCUSSION

Sediment properties

In both Seokmo Island and Hampyeong Bay, sediment water contents were significantly higher in the seepage than in the dry sites (Fig. 3, Mann-Whitney *U*-test for each location: $p < 0.01$). Hampyeong Bay sediment was capable of sustaining higher water content than Seokmo Island sediment, probably due to the difference in sediment properties (grain size and sorting) and thus permeability (Fig. 3). Zipperle & Reise (2005) found elevated amounts of clay particles near fresh groundwater springs in the intertidal zone, probably transported vertically from a deeper clay layer. In our study, seepage sediments in Seokmo Island exhibited a lower mud content compared to dry sediments (Fig. 3), which is further elucidated by the dry-sieving data (Fig. 4). A clear difference (*t*-test: $p < 0.01$) between mud contents of seepage and dry sites such as seen in Seokmo Island could not be found in any of the Hampyeong Bay sampling stations, although at Dolmori 1, a shift was evident from coarse to medium sands as well as a slight increase in mud content from the seepage to the dry station. Songseokri had very coarse sediment overall, and the mud content at Dolmori 2 was slightly higher at the seepage sites than the dry areas (Fig. 4).

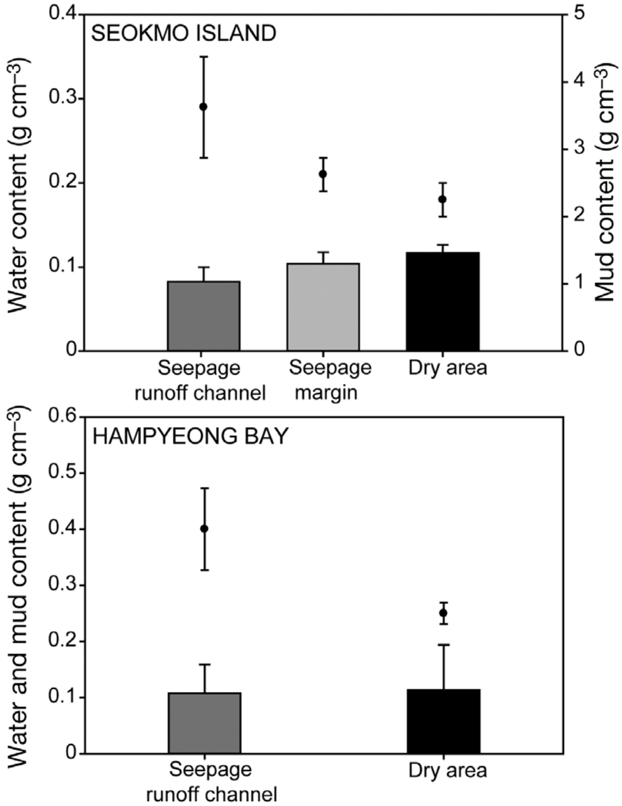

Fig. 3. Water (●) and mud (bars) content of sediments in Seokmo Island (top) and Hampyeong Bay (bottom). Error bars = SD of means

Nutrient patterns in sea- and groundwater

The seawater samples from Seokmo Island displayed unusually low salinities and very high nutrient concentrations, except for 1 seawater sample from a tidal pool in the mudflat, which had elevated levels of salinity and decreased nutrient concentrations (Fig. 5). There was no large freshwater source besides the groundwater seepage in this area, but the (surface) seawater

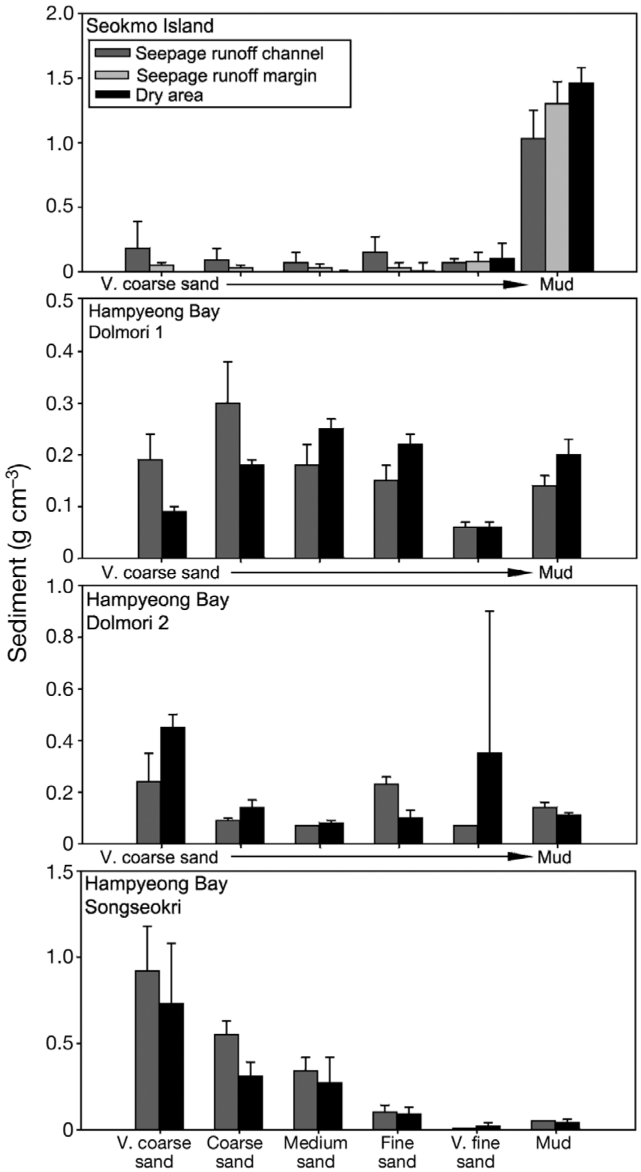

Fig. 4. Sediment grain sizes of seepage and dry sites at all 4 stations. Error bars = SD of means

samples were taken from a pier in the vicinity of the sampling site and probably influenced by the fresh groundwater plume. In general, scatter plots of pore water nutrients showed that groundwater was a net source of nutrients such as DSi, DIN, and DIP to the intertidal as well as to the bordering seawater area in both sampling locations.

The majority of nitrogen species was comprised of NO_3^- and NO_2^-, with a very small NH_4^+ component, which is common for groundwater seepage (Johannes 1980). Whilst Seokmo Island groundwater seepage was influenced by a fresh component and had an average salinity of 19.3 ± 7.1, the groundwater in the Hampyeong Bay Stns was more saline (26.0 ± 5.8). In addition, the difference in salinity and nutrient concentrations between the seepage and the dry sites in Seokmo Island was extreme, but no such clear distinction could be found at any of the Hampyeong Bay Stns (Fig. 5). Although the samples in Hampyeong Bay were extracted with multilevel pore water samplers, we chose to display the data points as scatter plots because pore water profiles showed inconsistent patterns of variation for salinity and nutrients with depth. It is likely that the pore water in the top 20 cm of the coarse sediment found in Hampyeong Bay (Fig. 4) is easily disturbed by tidal oscillations and wave-induced circulation, thus causing the heterogeneous depth profiles.

Seokmo Island seepage runoff was characterized by high DSi, moderate DIN, and low DIP concentrations (Si:N:P = 515:111:1). Predominantly fresh groundwater discharge such as the one found in Seokmo Island is, compared to groundwater, which consists mainly of recirculated seawater, commonly enriched with DSi and DIN, but DIP-depleted and can turn DIP into the locally limited nutrient (Slomp & van Capellen 2004). On the other hand, Hampyeong Bay seepage was moderate in DSi and DIP, and high in NO_x, in a ratio close to the Redfield/Brzezinski ratio (Si:N:P = 17:23:1). Since Hampyeong Bay is dominated by extended tidal flats that are subject to turbulent mixing during immersion, it is feasible that the observed nutrient concentrations were produced by remineralisation of marine organic matter that had been buried in the sediments (Ehrenhauss et al. 2004, Santos et al. 2009). In addition, the low-salinity groundwater samples in Hampyeong Bay also exhibited low DSi concentrations, indicating that DSi might have originated from a marine source (Fig. 5).

Photosynthetic pigment abundance and distribution

The benthic algae at Seokmo Island formed visible, golden brown mats typical for epipelic diatoms along the seepage border. The colouration decreased with increasing distance from the seepage area, a trend also supported by the distribution patterns of the analyzed pigments. Chl *a* concentrations decreased from a range of 5–132 mg m^{-2} in seepage runoff channels to 5–33 mg m^{-2} at the channel margins and to 2–12 mg m^{-2} in the dry areas (Fig. 6A). The sediments in the tide pool in Seokmo Island were not sampled, but a visually distinct diatom film, like the one found at the seepage border, was absent.

Out of the total (n = 33) collected in Seokmo Island, 4 samples, one each from the seepage runoff and the dry site, and 2 from the seepage runoff margin, displayed

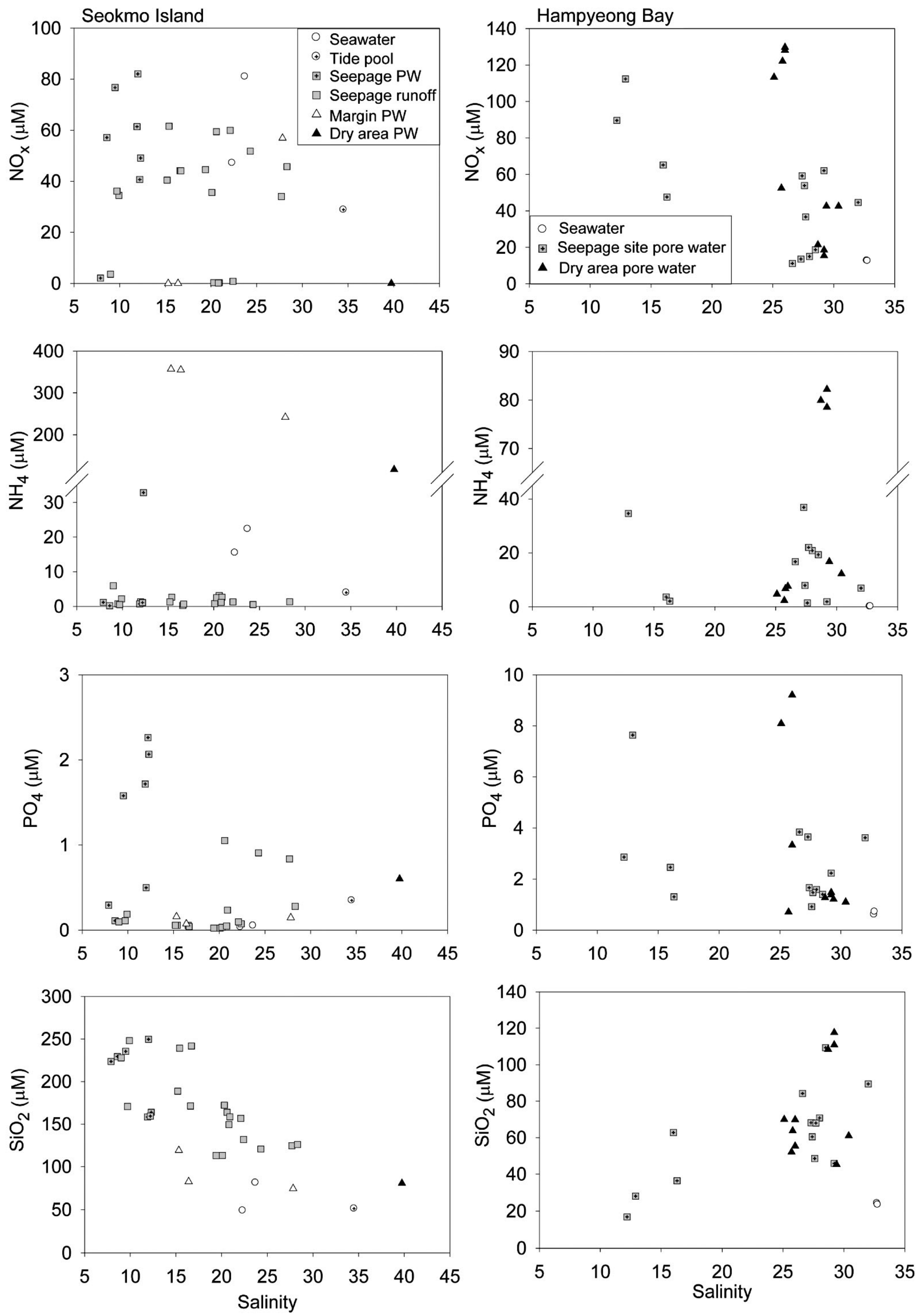

Fig. 5. Nutrient concentrations of seawater, groundwater and pore water in Seokmo Island and Hampyeong Bay

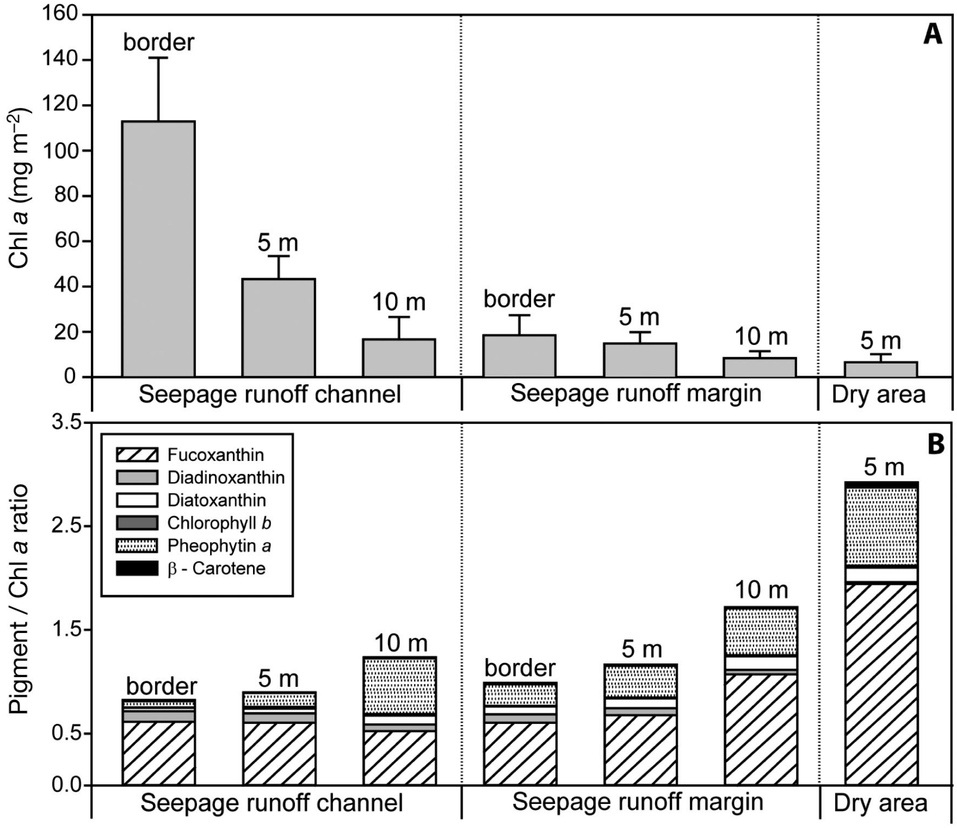

Fig. 6. Photosynthetic pigment distribution patterns in sediments of Seokmo Island: (A) mean chl *a* concentrations, (B) mean ratios of accessory pigments over chl *a*. Error bars = SD of means. Labels above the bars = distance from the seepage border

very low chl *a* (0.1 to 2 mg m^{-2}) and high pheophytin *a* (15 to 83 mg m^{-2}) concentrations, indicating chl *a* degradation to pheophytin *a*. We treated these 4 samples as outliers and did not include them in our statistical analyses or overall interpretation of groundwater seepage-related abundance patterns.

Ratios of accessory pigment over chl *a* concentrations revealed relative increases in fucoxanthin, pheophytin *a* and diatoxanthin, and a relative decrease of diadinoxanthin from seepage runoff channels to seepage runoff margins and the dry area. The same trend was found within the seepage runoff channels, as well as the seepage runoff margins from the seepage border towards the offshore sites (Fig. 6B). Fucoxanthin is a major pigment in diatoms, and in unialgal diatom cultures, the fucoxanthin:chl *a* ratio ranges from 0.6 to 0.8 (Brotas & Plante-Cuny 2003), which is similar to the values we found in our seepage runoff and seepage margin areas (Fig. 6B). At the dry sites, on the other hand, the average fucoxanthin:chl *a* ratio was 2. Such a high ratio is generally found in diatoms from subtidal, rather than intertidal, sediments, where it is associated with low irradiance (Cibic et al. 2007, and references therein). Phytoplankton diatom communities also often display ratios of fucoxanthin:chl *a* > 1, depending on irradiation, nutrient regime and life stage (Schlüter et al. 2000, Henriksen et al. 2002), but to our knowledge, this has not been found for intertidal benthic diatoms (Rijstenbil 2003). In addition to the elevated fucoxanthin:chl *a* ratios, pheophytin *a* made up to 70 % of the pigment composition in the dry area at Seokmo Island. Contrastingly, sediments of the groundwater seepage were characterized by high concentrations of chl *a* and low concentrations of fucoxanthin and pheophytin *a* (Fig. 6). A scenario that could explain the patterns observed in the dry areas would be an 'import' of detrital diatom material, in which chl *a* is largely degraded to pheophytin *a*. If this algal material were from a pelagic or subtidal source, it could have higher fucoxanthin concentrations as well. A deposition of algal detritus during high tide would occur in seepage areas and dry areas alike, but it is not likely to accumulate in the seepage areas due to the constant water flow.

The microphytobenthic environment at the Hampyeong Bay stations was different from Seokmo Island. Upon visual inspection, epipelic diatom mats (i.e. thin, golden-brown films of motile diatoms) were absent; instead, the coarse sediment grains were covered with a greenish algal layer. Overall, chl *a* concentrations increased from a range of 39–89 mg m^{-2} in dry areas to 83–216 mg m^{-2} in seepage runoff channels (Fig. 7A). Fucoxanthin:chl *a* ratios were lower, ranging from 0.2

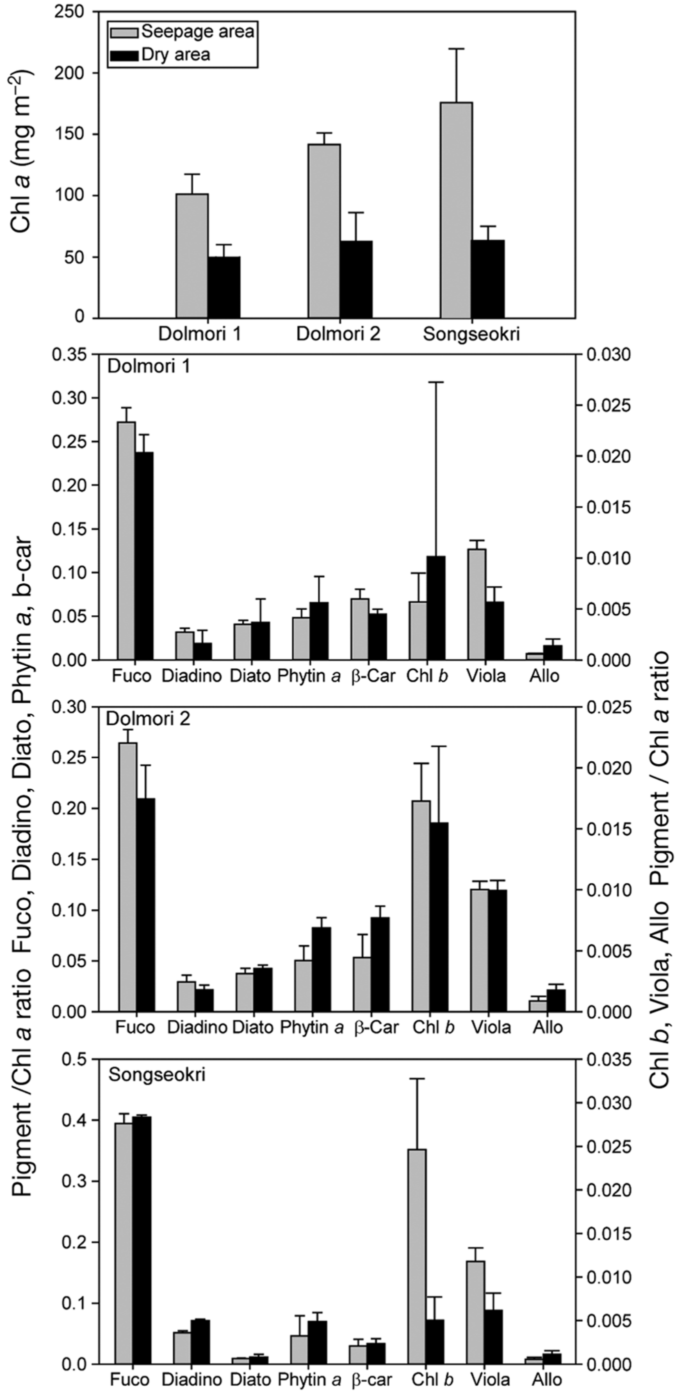

Fig. 7. Photosynthetic pigment distribution patterns in Hampyeong Bay sediments: (A) mean chl *a* concentrations for all stations, and mean ratios of accessory pigments over chl *a* in (B) Dolmori 1, (C) Dolmori 2 and (D) Songseokri Stns. Error bars = SD of means. Note the different *y* axes for pigment ratios chl *b*, violaxanthin (Viola) and alloxanthin (Allo)

to 0.4 in both seepage areas and dry areas for all stations. Diatoms were still the dominant algal group, as shown by the high fucoxanthin:chl *a* and diadinoxanthin:chl *a* ratios compared to those of other pigments. However, the ratios of some accessory pigments, e.g. alloxanthin (cryptophytes), violaxanthin (chlorophytes, euglenophytes), and chl *b* (chlorophytes, prasinophytes), over chl *a* were higher in Hampyeong Bay compared to Seokmo Island, indicating a taxonomically more diverse microphytobenthos (Fig. 7). Furthermore, chl *b*:chl *a* ratios at the Dolmori 2 and Songseokri seepage sites were higher compared to the respective dry areas. The differences in the ratios between groundwater seepage sites and dry areas reflect a change in the relative contributions of different algal taxonomic classes to the microphytobenthic communities.

Despite abovementioned differences, the dry areas of all Hampyeong Bay Stns did display elevated pheophytin *a*:chl *a* ratios compared to seepage runoff sites (Fig. 7B–D), which was consistent with the pattern found in Seokmo Island (Fig. 6A). Alloxanthin:chl *a* ratios were also higher in the dry areas, and together with the elevated pheophytin *a*:chl *a* ratios, they were probably the result of detritus deposition from the water column, e.g. from cryptophytes (Brotas & Plante-Cuny 2003).

Since we sampled both seepage and dry areas at similar distances from the shoreline, we assumed that exposure times during low tide and thus light availabilities were similar. However, changes in the relative abundances of the xanthophyll cycle pigments diadinoxanthin and diatoxanthin between the seepage sites and dry areas were evident for the Seokmo Island Stn and for 2 of the Hampyeong Bay Stns. At all of these stations, diadinoxanthin was negatively correlated with diatoxanthin (Pearson correlation, $r^2 = 0.494$, $p < 0.01$; Hampyeong Bay: $r^2 = 0.375$, $p < 0.01$). Diadinoxanthin can be transformed into the heat-dissipating diatoxanthin in benthic algal communities for photoprotection (Demers et al. 1991, Rijstenbil 2003), which could indicate a higher radiation stress of the microphytobenthos at the dry areas during emersion.

Generally, microphytobenthos is associated with muddy sediments, which are common in sheltered environments and provide nutrient-rich substrates (Underwood & Kromkamp 1999, Facca et al. 2002). Seokmo Island and Dolmori 1 had low mud contents and high chl *a* concentrations in the seepage areas, and at Songseokri Stn, with the lowest mud content, the chl *a* concentrations in the seepage areas exceeded those of all other studied sites (Fig. 7A). Coarser sediment has higher irradiance levels, which may be favored by microphytobenthos, but chl *a* concentration at Songseokri's dry area, which had a similar sediment grain size as the seepage site, was >60% lower (Fig. 7A). Our

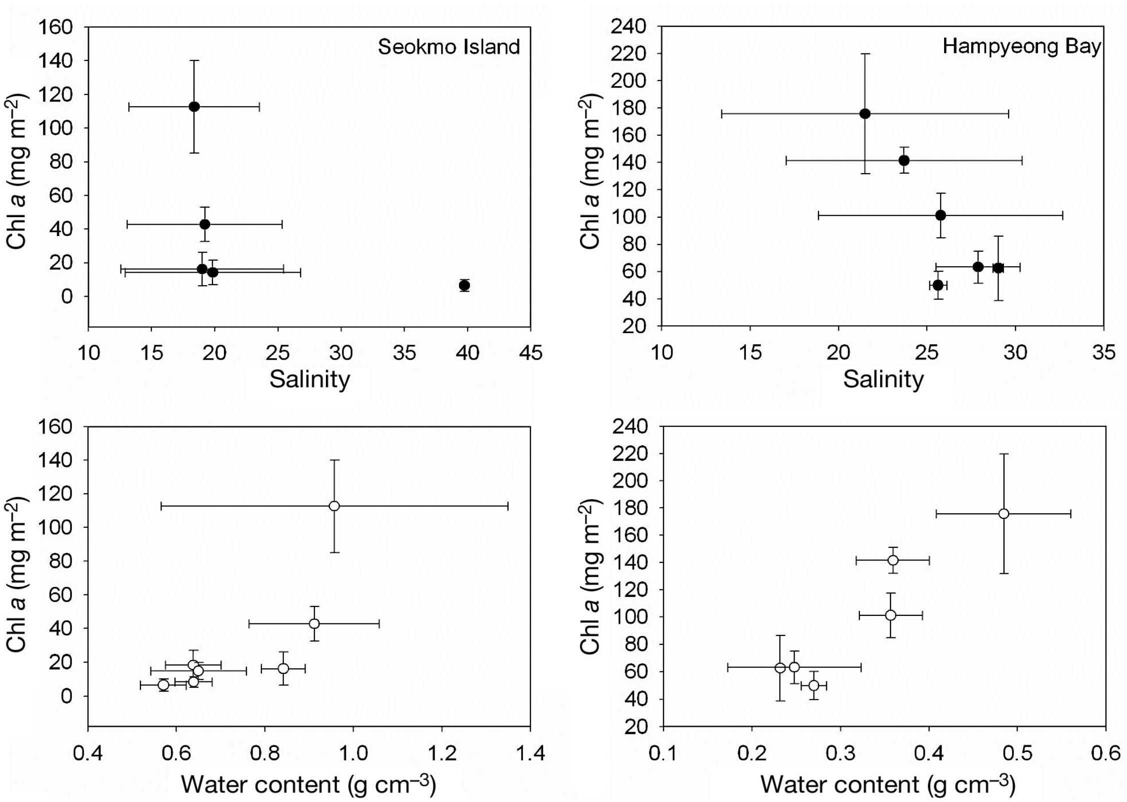

Fig. 8. Averaged regression for salinity (top, ●) and water content (bottom, ○) vs. chl *a* concentrations in Seokmo Island (left panels) and Hampyeong Bay (right panels). Error bars = SD of means

results therefore indicate that sediment grain size is of little influence in governing the microphytobenthos abundance and distribution at the study sites.

It is more likely that the physical properties of groundwater seepage are advantageous for microphytobenhos in that they provide protection from desiccation, heat and salt stress: in Fig. 8, averaged regressions indicate a negative relationship between chl *a* concentrations and salinity, and a positive relationship between chl *a* concentrations and sediment water content. Groundwater seepage areas become shelters similar to tide pools, but with added benefits, for example relatively constant salinity and temperature, and elevated nutrient concentrations (Dale & Miller 2007). However, nutrient concentrations and microphytobenthos abundance did not correlate significantly (Pearson correlation) in either Seokmo Island or the Hampyeong Bay locations. In the seepage margins and dry areas of Seokmo Island, NH_4^+ replaced NO_x as the dominant form of nitrogen, and silicate and phosphate concentrations were high in all 3 environments (Fig. 5). On the other hand, nutrient compositions as well as overall concentrations in dry and seepage areas were similar in all of the Hampyeong Bay Stns (Fig. 5). It should be noted, however, that in the dry areas of Hampyeong Bay, the pore water saturation level was ~10 cm below the sediment surface. Therefore, the water content in the dry areas was stagnant or decreasing due to evaporation, while groundwater seepage provided a constant water and nutrient flux. In this respect, groundwater seepage is advantageous over ‘regular’ water-saturated microenvironments such as tide pools, which are stagnant and often experience large increases in temperature, salinity and pH that can inhibit algal growth (Rasmussen et al. 1983). Although we did not measure chl *a* concentrations in the sediments of the tide pool at Seokmo Island Stn, visible diatom mats were absent, and the water in the pool had a higher salinity and lower nutrient concentrations than the nearby groundwater seepage (Fig. 5). Microalgal biofilms under stagnant water conditions can experience nutrient depletion (Underwood & Kromkamp 1999), but advective fluxes, for example in the form of groundwater seepage, could overcome this limitation. Advective flushing rates between 400 to 600 l m^{-2} d^{-1} increase photosynthesis rates due to a constant carbon supply (Cook & Røy 2006). These rates are in the range of intertidal groundwater seepage along sandy beaches (Uchiyama et al. 2000, Ullman et al. 2003). Groundwater discharge runoff in Seokmo Island and Hampyeong Bay was high enough to form small streams (~6000 to 8000 l m^{-2} d^{-1}). With such high flow speeds, intertidal groundwater seepage could affect the benthic boundary layer (Cook & Røy 2006).

The abundance and distribution patterns of the total chl *a* concentrations found in Seokmo Island and Hampyeong Bay both indicate that seepage runoff can create a microenvironment beneficial for intertidal microphytobenthos (Fig. 6A & 7A). Our results are in congruence with other research in different aquatic environments, and our observed chl *a* concentrations in the groundwater seepage areas were in the high range of those found in other studies (Hagerthey & Kerfoot 1998, Carmichael & Valiela 2005, Blanco et al. 2008), which emphasizes the ubiquity of this phenomenon in a large variety of ecosystems. So far, no attempt has been made to quantify groundwater-fuelled benthic primary productivity, or the impact of increased microphytobenthos biomass on the tidal food web. In the case of our 2 study sites, the potential impact is high since microphytobenthos is a major contributor to the food web of the southern (Kang et al. 2003) and western coast of Korea due to (1) the vast tidal flat areas, which are emersed during low tide, and (2) the high turbidity of the coastal seawater, which limits phytoplankton productivity. However, in Seokmo Island, the groundwater seepage occurred only at one beach of ~80 m length, and the microphytobenthos was composed of epipelic diatoms, which are easily resuspended during high tide. Contrastingly, in Hampyeong Bay, the microalgae were attached to the coarse sand grains, and export through resuspension was limited. The whole bay is lined with beaches submerged in groundwater seepage. For example, in Dolmori 1, ~60 % of the beach was subject to groundwater seepage that on average supported a microphytobenthos biomass twice as high as in the nearby dry areas. In addition, we only investigated a small area of the tidal flat which has horizontal extensions of up to 2 km, and which has large areas also subject to the groundwater runoff. Such an increase in benthic microalgal biomass is not negligible. A concurrent study on the impacts of groundwater seepage on the overall nutrient balance of Hampyeong Bay revealed that the microphytobenthos community is capable of substantial nutrient removal when the phytoplankton abundance in the water column is low (Waska & Kim unpubl.). Finally, Hampyeong Bay is a relatively pristine environment where large cities are absent, and impacts may be much larger in urbanized watersheds where groundwater is influenced by septic tanks (Carmichael & Valiela 2005).

Macrofaunal distribution patterns

We observed large differences in the abundance of the benthic snail *Batillaria cumingi* and the ghost crab *Scopimera* sp. between the seepage sites and dry sites in Hampyeong Bay. Both are organisms that inhabit ecological niches typical for tidal flat ecosystems: *B. cumingi* is commonly found in tidal mud flats along the coasts of China, Korea and Japan, and was introduced to the US West coast in the 1930s, where it was found to be the 'ecological equivalent' of the native snail *Cerithidea californica* (Whitlatch & Obrebski 1980). It is known to be a deposit feeder that preferably ingests benthic diatoms, and occasionally, detrital material of larger plants (Whitlatch & Obrebski 1980). Crabs of the genus *Scopimera* sp. create burrows in sandy areas on the high shore where they hide during high tide, and feed in droves in water-saturated areas near the water line during low tide, which is a behavior also commonly found in other ocypodid crabs inhabiting tidal flats (Koga 1995).

Snail abundances were high (>1000 ind. m^{-2}) in seepage sites, but snails were absent in dry areas of Dolmori 1 and Songseokri, and much lower in the dry areas than in the seepage areas of Dolmori 2. Contrastingly, crab burrows were absent in all seepage areas, but present in all dry areas (Table 1). The number of samples per station was small (n = 3 for seepage and dry areas, respectively), but statistics run for all stations pooled showed that snail and crab burrow abundances significantly differed between seepage and dry sites (Mann-Whitney *U*-test, $p < 0.01$). *Batillaria cumingi* had its highest abundances at the seepage runoff channel sites of stations Dolmori 1 and 2, and its lowest abundances at the Seongseokri seepage runoff channel site (Table 1). This trend is concurrent with a trend of decreasing sediment mud content from Dolmori 1 to Dolmori 2 and Songseokri (Fig. 4). The diet of this deposit feeding gastropod is predominantly based on benthic diatoms, which it ingests together with surficial soft sediment (Whitlatch & Obrebski 1980). Similarly, the number of crab burrows was highest in the dry areas of Dolmori 1, intermediate in Dolmori 2, and lowest in Songseokri (Table 1).

Both *Batillaria cumingi* and *Scopimera* sp. have low tolerances to low salinities (Jiang & Li 1995, Koga 1995), but the salinities found in our pore water samples were relatively high and constant (26.0 ± 5.8) and thus tolerable for both species. In addition, low salinities should decrease not only the number of crab burrows, but also the number of *B. cumingi* individuals in the seepage areas, which was not the case in this study. In more rocky or gravelly environments, *B. cumingi* is often found in tidal pools (Adachi & Wada 1999), analogous to the accumulation of high numbers of individuals that we found in the groundwater seepage areas. On the other hand, *Scopimera* sp. burrows are generally common in the upper tidal flats but not in water-saturated areas of the tidal flat (Koga 1995).

Table 1. Influence of intertidal groundwater seepage on benthic macrofaunal abundances. (C): crustaceans, (G): gastropods, (P): polychaetes. Errors = SD of mean

Location	Species	Seepage	Non-seepage	Reference
Möwenbergwatt, German Wadden Sea	*Nereis* 2 spp. (P)	1711 ± 917	144 ± 133	Zipperle & Reise (2005)
	Arenicola marina (P)	–	21 ± 26	
	Scoloplos armiger (P)	–	300 ± 212	
Hampyeong Bay, Yellow Sea				Present study
Dolmori 1	*Batillaria cumingi* (G)	1782 ± 1108	–	
	Scopimera sp. (C) [a]	–	215 ± 105	
Dolmori 2	*Batillaria cumingi* (G)	1006 ± 581	9 ± 8	
	Scopimera sp. (C) [a]	–	16 ± 5	
Songseokri	*Batillaria cumingi* (G)	368 ± 109	–	
	Scopimera sp. (C) [a]	–	1 ± 2	

[a]Only burrow numbers were counted

Thus, our results indicate that sediment water saturation causes the observed distribution patterns. However, it should be noted that pore water in some of the other tidal flats of Hampyeong Bay displayed large fluctuations in salinity over the course of 3 seasons (Waska & Kim unpubl.), for example from 5.9 in summer to 32.7 in fall 2008 in a beach in the northern part of the bay. Longer-lived benthic structures such as crab burrows could be the result of the organisms avoiding previous lower salinities, whilst the motile *B. cumingi* might quickly accumulate in seepage sites when salinities reach the tolerable range found in this study. The apparent de-coupling of salinity and macrofaunal distribution patterns through temporal variation in groundwater biogeochemistry has been hypothesized in another recent study on polychaete abundances in groundwater seeps by Dale & Miller (2008), and long-term monitoring studies will be necessary to determine temporary lags in organism responses.

Microphytobenthos vs. macrofauna in groundwater seepage areas

In Hampyeong Bay, chl *a* concentrations were very high in seepage areas despite the high numbers of *Batillaria cumingi* (Fig. 7A, Table 1). Amongst these seepage areas, the station with the highest snail numbers, Dolmori 1 (Table 1), displayed the lowest chl *a* concentration (Fig. 7A) in contrast to the station with the lowest snail numbers, Songseokri, which also had the highest chl *a* concentrations. Similarly, the dry area with the highest number of crab burrows in Dolmori 1 had the lowest chl *a* concentrations, whereas at Songseokri, only 1 to 2 burrows were counted and the chl *a* concentrations were higher as well (Fig. 7A, Table 1). The presence of grazers was therefore somewhat important in determining chl *a* concentrations in the different stations in Hampyeong Bay but not significant compared to the influence of groundwater seepage.

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