

The Effect of Pore Water Chemistry on the Biodegradation of the *Exxon Valdez* Oil Spill

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Received: 9 August 2010 / Accepted: 8 November 2010 / Published online: 2 December 2010
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Abstract Knowledge of the oxygen and nutrient concentrations in an oil-contaminated Prince William Sound (PWS) beach is important for understanding of the oil persistence over two decades after the *Exxon Valdez* spill. It was traditionally believed that there was enough oxygen in the contaminated shorelines to sustain aerobic microbial metabolism of oil and that nutrients were the major factors limiting oil biodegradation. In the present study, we analyzed the oxygen and nutrients levels in both clean and oily areas on a PWS beach that was heavily contaminated by the *Exxon Valdez* oil spill. We found that the level of nitrogen and phosphorous were $0.454 \text{ mg-N L}^{-1}$ and $0.033 \text{ mg-P L}^{-1}$, respectively, which is not sufficient to fully support microbial growth, confirming that nutrient concentration was a major factor limiting oil transformation by biological ways. We also observed that the oxygen level varied from higher than 3 mg L^{-1} in the clean wells to about 1 mg L^{-1} (near anoxic level) in the oily wells. In addition, the lowest nitrate levels were observed at the oily wells. Altogether, these results suggest that the low level of efficient electron acceptors (oxygen and nitrate) detected at the oily spot is responsible for slow and potentially inefficient biodegradation of the oil.

Keywords Exxon Valdez · Oil spill · Biodegradation · Nutrients · Dissolved oxygen · Electron acceptors

Introduction

The *Exxon Valdez* oil spill in 1989 polluted over 1,100 miles of shorelines in Prince William Sound, the Gulf of Alaska,

and surrounding water bodies. Despite biodegradation of oil observed in the first years following the spill, 60 to 100 tons of subsurface oil (SSO) are still observed within the beaches of Prince William Sound (PWS) (Short et al. 2004, 2006). The oil is typically located under a layer of gravel, pebbles, and cobbles in a low porosity layer sitting 0.05 to 0.5 m below the surface (Owens et al. 2008; Taylor and Reimer 2008; Page et al. 2008; Boehm et al. 2008). The average thickness of the oil layer is about 0.1 m, but it could reach up to 0.22 m (Michel et al. 2006). Contaminated beaches have been shown to contain almost non-weathered oil residues and high concentrations of polycyclic aromatic hydrocarbons (PAHs) known to be highly toxic to wildlife (Carls et al. 2001; Peterson et al. 2003). The subsurface oil (SSO) in the beaches is distributed almost symmetrically around the mid-tide line (Short et al. 2006). However, Taylor and Reimer (2008), Boehm et al. (2008), and Page et al. (2008) suggest that the distribution is skewed toward the upper intertidal zone, and as such would result in smaller ecological impact. This is because the lower intertidal zone has a high ecological service due to high productivity and because it is where sea otters and marine birds forage.

The persistence of oil could be due to a variety of physical, chemical, and biological factors. Earlier studies provided evidence of efficient oil biodegradation following the first few years after the spill, which led to the eventual stoppage of cleaning activities (Neff et al. 1995).

More recently, Li and Boufadel (2010) reported that the beach EL056C on Eleanor Island consists of two layers, an upper layer with high permeability, overlaying a low permeability layer where the oil is trapped with limited exchange with the upper layer. They also noted that oxygen is lacking at oiled locations. Boufadel et al. (2010) further expanded the investigation by measuring the nutrient and oxygen concentration. They found that the oxygen concentration is

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around 1.0 mg L^{-1} at oiled locations and they also found that the nitrate concentration is low (around 0.02 mg L^{-1}) suggesting low of nitrification.

The objective of the present study was to evaluate the chemical factors affecting oil persistence at Beach SM006C. For this reason, measurement of dissolved oxygen (DO), nutrients (nitrogen and phosphorous), salinity, and pH were conducted. Previous studies on oil biodegradation in PWS beaches considered that the sediments were well oxygenated and that only nutrients were limiting biodegradation (Bragg et al. 1994; Pritchard and Costa 1991; Atlas and Bragg 2009b). The experimental results presented in the present study strongly suggest that, in addition to nutrients, the low levels of oxygen and other electron-acceptors might be a major factor limiting oil biodegradation in the beaches.

Experimental Section

Site Description

Our study was conducted on beach SM006C located on Smith Island. The coordinates of the beach are $147^{\circ} 24' 13.84'' \text{ W}$ and $60^{\circ} 31' 10.30'' \text{ N}$. This beach faces north, and is subjected to high wave energy (over 60 km fetch), as the waves travel mostly southwest. The across-shore length of the beach is about 120 m and the along-shore width is about 50 m. Based on the Shoreline Cleanup Assessment Technique manual (SCAT) (Short et al. 2004; Taylor and Reimer 2008), the transects defined for research purposes, and labeled “left” and “right”, are located on the left and right, respectively, of an observer standing on the beach and looking landward. A pond is located on the land side (about 30 m landward of the high tide line) close to the right side of the beach. The sediments of the beach are coarse, ranging from gravel (a few mm in diameter) to pebbles and cobbles (0.1 to 0.2 m diameter) interspersed between boulders (up to 1 m). A layer of armor (boulder, pebble, and clasts) was observed on the left side, and it disappeared moving seaward as the rock outcrop emerges. On the right, residues of degraded organic matter were detected between the upper and middle intertidal zone. The oil was observed on the left transect of the beach, at the middle intertidal zone, 0.1 to 0.4 m below the surface (Fig. 1A). This beach was heavily oiled immediately following the spill (Neff et al. 1995; Taylor and Reimer 2008). Recent studies on the same beach showed that the left side had heavy oil residue (HOR), while no free flowing oil was detected on the right side, according to ASTM classification (ASTM F1687-97 2003; Michel et al. 2006). On the left side of the beach, the oil was stranded under the layer of boulder and cobble and mixed with low porosity fine grain sediments. From a geomorphic point of view, this beach is very different from that studied by Boufadel et al. (2010), as this beach has exposure to high-energy waves.

Materials and Methods

Site Preparation

Due to the rocky nature of the beach and presence of boulders and cobbles within the beach sediments, it is extremely difficult to drive the sampling wells into the beach and driving the PVC pipes is practically impossible, hence one had to excavate to place the sensors and then refill the pits. However, if the concentration in the lower layer is sought, one needs to provide a sufficient time for the sediments to “heal” after excavation (i.e., to return to their original two-layer configuration). Otherwise, sensor measurements from the lower layer would be “contaminated” by water from the upper layer. We determined, based on measurements that we conducted in 2008, that a minimum period of 6 weeks is needed for the sediments to return to their initial configuration and the steady state was achieved (Li and Boufadel 2010). For this reason, we designed the field study in 2009 to have 9 weeks between the placement of the sensors and the measurements.

In total, six pits were dug for the purpose of monitoring the background DO, nutrients, salinity, and pH levels within the sediment pore water; three on the left transect which crossed the oily zone and three on the right transect which were clean (Fig. 1A and 1B). The surface elevation of the wells on each transect was selected to have one well in each intertidal zone (upper, middle and lower). The tidal range on this beach varied from 0 to 4.5 m and the high tide was approximately 1 m landward of the most landward wells, W1_1 and W2_1 (Fig. 1A and 1B).

The experimental approach was to dig the pits to about 5 feet (1.5 m) deep and place PVC pipe, a multiport sampling well (SP), and two sampling boxes (SBs) in the pits. The slotted PVC pipe was used as a protective sleeve for the pressure transducer. It allowed the water to move to the sensor while keeping the sediments out. The stainless steel multiport sampling well was used to collect water samples for nutrient analysis at different depths within the beach. The detail of the multiport sampling port is provided in the Appendix (Fig. 3). The sampling boxes were made out of PVC and could contain about 200 mL, which was adequate for DO sampling. The detail of the sampling boxes is provided in the Appendix (Fig. 4). The depth of SBs and SPs at each well is given in Table 1.

Sampling Methods

DO, nutrients, pH, and salinity of the pore-water samples were measured in this study. The DO levels were measured in the field, while the nutrient concentrations, pH, and salinity were determined in the lab.

The DO was measured in the field using a Thermo Scientific, RDO optical probe and ORION 4 Plus handheld meter

Fig. 1 (A) Topographic map of SM006C showing the location of the sampling wells in each transect. The oil was observed at the left transect in W1_2. (B) Location of the two transects on the beach during low tide

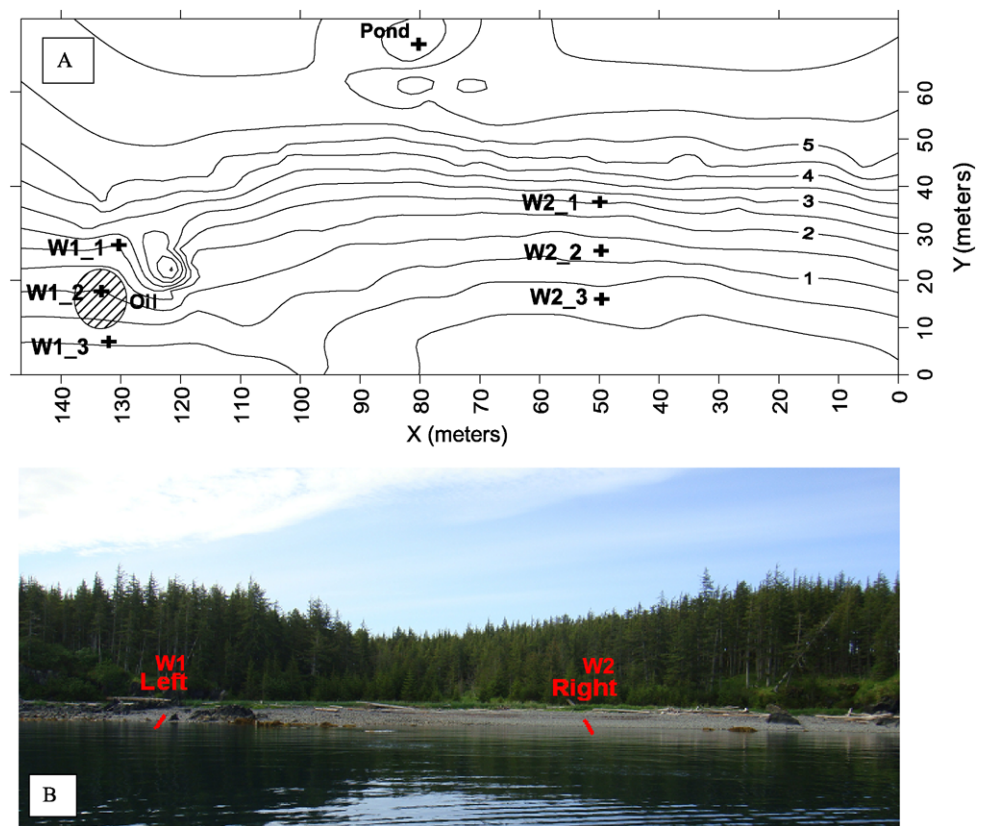


Table 1 Depth of the sampling ports and sampling boxes used along with the number of samples taken for different measurements

Location	W1_1	W1_2	W1_3	Pond	W2_1	W2_2	W2_3	Seawater
Port A depth (m)	0.42	0.62	0.61	–	0.62	0.49	0.53	–
Port B depth (m)	0.23	0.43	0.42	–	0.43	0.3	0.34	–
SB depth (m)	0.55	0.75	0.8	–	0.79	0.58	0.63	–
DO (numbers of samples)	2	2	2	1	4	4	3	–
Nutrients (numbers of samples)	22	36	32	6	23	23	20	7

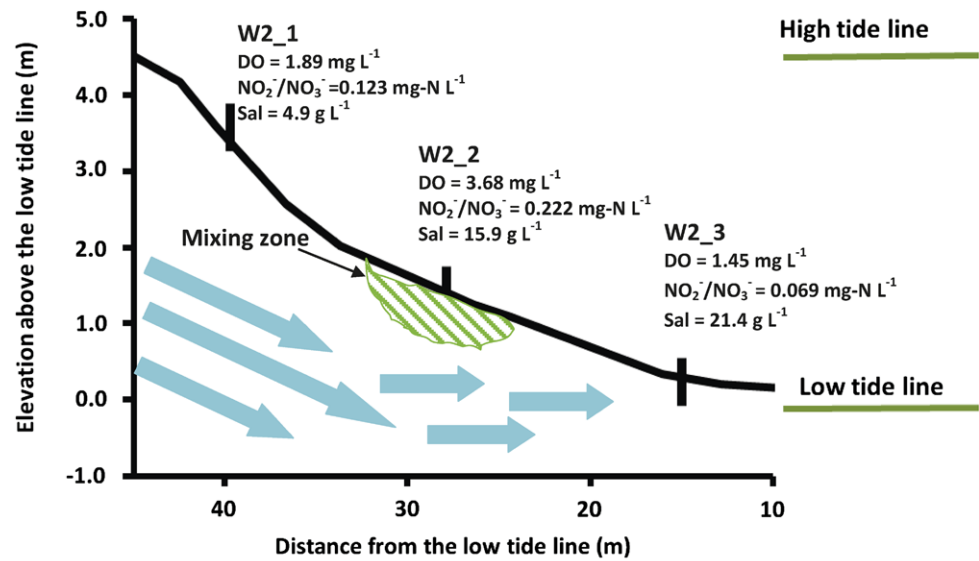
(Thermo Scientific, Beverly, MA). The water was pumped from the sampling boxes into the measuring chamber using a peristaltic pump (Masterflex, Cole-Parmer, Vernon Hills, IL) and the water was allowed to overflow from the measuring chamber. The details of the measuring chamber are provided in the [Appendix](#) (Fig. 5).

For sampling, one end of the peristaltic pump tubing (Masterflex, Cole-Parmer, Vernon Hills, IL) was lowered to the bottom of the sampling box and the other end was connected to the inflow of the measuring chamber prior to when the peristaltic pump started (Fig. 5). The initial flow rate was 0.33 L min^{-1} , and it was adjusted to have a steady flow with no air bubbles. Experiments showed that the steady state was achieved 10 min after running the pump; the first reading was recorded and considered as a representative value of the pore water.

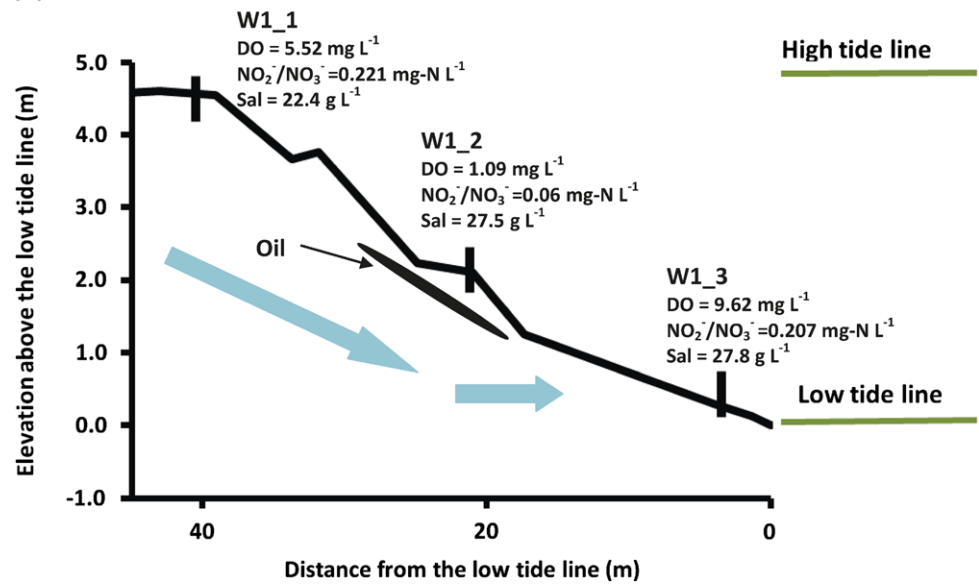
Nutrient samples were collected from SPs at different times, depending on accessibility (due to tide level) and resources. The samples were collected in 30 mL plastic bottles. The details of the sampling protocol are given in the [Appendix](#). On the beach, samples were kept on ice in a cooler and they were immediately frozen at -5°C once they arrived on board the research vessel (US Environmental Protection Agency 1983). The samples were air shipped to Temple University in dry ice to keep them frozen during shipping. Once in the lab, the samples were kept in a freezer at -20°C prior to analyses (Dore et al. 1996). Seven measurements were made in the lab: ammonia-N ($\text{NH}_3\text{-N}$), nitrite-N ($\text{NO}_2^- \text{-N}$), nitrite/nitrate-N ($\text{NO}_2^-/\text{NO}_3^- \text{-N}$), phosphate-P ($\text{PO}_4^{3-} \text{-P}$), silicate (SiO_2), pH, and salinity.

Fig. 2 Schematic of water flow within the beach on both transects. **(A)** On the right transect, the DO and salinity levels indicate that there is freshwater recharge into the beach and the mixing of the freshwater and seawater occurs close to W2_2. **(B)** On the left transect, there is less freshwater recharge and the salinity values are higher than on the right transect

(A) Right Transect



(B) Left Transect



Nutrient and Salinity Analysis of the Pore-Water Samples

Nutrients were analyzed using an AutoAnalyzer3 (Seal Analytical, Mequon, WI). The frozen samples were defrosted and kept in a fridge at 4°C in batches of 76 samples. At the time of analysis, the samples were taken out of the fridge, hand shaken for 15 seconds, and passed through 0.45 micron PTFE membrane filters (Puradisc™, Whatman, Florham, NJ) directly into the AutoAnalyzer3 cups. The segmented flow method was used (AutoAnalyzer3) and the concentrations were detected by colorimetric analysis. Ammonia

was measured using the Berthelot reaction (reaction of ammonia with phenol-hypochlorite) based on the formation of a blue-green colored complex measured photometrically at 660 nm (Grasshoff et al. 1999; Seal Analytical 2008). Nitrate in the solution was reduced to nitrite on a copper-cadmium reactor column (Grasshoff et al. 1999; Seal Analytical 2008). The nitrite reacted with sulfanilamide under acid conditions to form a purple azo dye and detected photometrically at 550 nm (Grasshoff et al. 1999; Seal Analytical 2008). Phosphate was measured following the Murphy and Riley (Grasshoff et al. 1999; Seal Analytical 2008) method

based on the development of a blue color by reaction of orthophosphate with molybdate and antimony, followed by reduction with ascorbic acid at $\text{pH} < 1$. Then the blue complex was read photometrically at 880 nm (Grasshoff et al. 1999; Seal Analytical 2008). Soluble silicate was determined by the reduction of siliconmolybdate to molybdenum blue by ascorbic acid at low pH. The complex formed was read photometrically at 820 nm (Grasshoff et al. 1999; Seal Analytical 2008).

The pH of the pore-water samples was measured using an ORION 4 Plus bench-top pH-meter (Thermo Scientific, Beverly, MA). The salinity of pore-water samples was measured using a digital refractometer (Salinity-300035, Sper Scientific, Scottsdale, AZ). The samples were filtered, about 1.5 mL of sample was poured into the measuring cup of the instrument, and the salinity was determined based on the refraction index.

Statistical analysis of the experimental results was performed. In order to compare the mean values of all wells, one way analysis of variance (ANOVA) was used. Once a significant difference between the wells in the beach was observed, the Tukey's honesty significant differences (HSD) test was applied to determine the well(s) that showed significantly different values from the rest of the wells. A paired Student *t*-test was used for the pairwise comparison between the values of two single wells and to compare the pond values with the ones of each well. The level of significance used was 95% ($\alpha = 0.05$).

Results

The background measurements of DO, nutrients, salinity, and pH are expected to help understanding the factors responsible for the lingering of the oil in the field. The DO levels were measured in the field and the nutrients, salinity, and pH values were measured in the lab. The number of samples for DO and nutrient analysis that were collected at each well is given in Table 1. The average values obtained from the two deepest ports for DO, nitrate/nitrite-N, ammonia-N, phosphate-P, silicate, pH, and salinity are presented in Table 2. The histograms of the obtained average values, with \pm standard deviations as error bars, are given in the Appendix (Fig. 6).

On the right (clean) transect, the DO concentration at the most landward well (W2_1) was $1.89 \pm 0.68 \text{ mg L}^{-1}$. Moving seaward to W2_2, the DO increased to $3.68 \pm 1.59 \text{ mg L}^{-1}$, then decreased to $1.45 \pm 0.42 \text{ mg L}^{-1}$ at W2_3. The DO in the pond behind the beach was 6.09 mg L^{-1} . On the left (oily) side, the DO at the most landward well (W1_1) was $5.52 \pm 2.35 \text{ mg L}^{-1}$, moving seaward to the oily well (W1_2), the DO dropped to $1.09 \pm 0.08 \text{ mg L}^{-1}$ (near anoxic condition). The DO at W1_3 was $9.62 \pm$

Table 2 The average of the values obtained from the two deepest ports for DO, nitrate-N, ammonia-N, phosphate-P, silicate, and salinity shown throughout the beach for each well. The samples used for seawater measurements were taken from the boat about 100 m offshore. The pond samples were collected 15 cm below the water surface

Location	W1_1	W1_2	W1_3	Pond	W2_1	W2_2	W2_3	Seawater
DO (mg L^{-1})	5.52 ± 2.35	$1.09 \pm 0.08^{(*)}$	$9.62 \pm 0.18^{(*)}$	6.09	$1.89 \pm 0.68^{(*)}$	3.68 ± 1.59	$1.45 \pm 0.42^{(*)}$	–
$\text{NO}_3^-/\text{NO}_2^-$ -N (mg-N L^{-1})	0.221 ± 0.167	$0.06 \pm 0.050^{(*)}$	0.207 ± 0.127	$0.1 \pm 0.060^{(*)}$	$0.123 \pm 0.092^{(*)}$	0.222 ± 0.178	$0.069 \pm 0.057^{(*)}$	0.037 ± 0.025
NH_4^+ -N (mg-N L^{-1})	0.263 ± 0.112	$0.345 \pm 0.171^{(*)}$	$0.245 \pm 0.102^{(*)}$	0.267 ± 0.244	$0.275 \pm 0.145^{(*)}$	$0.235 \pm 0.087^{(*)}$	$0.461 \pm 0.240^{(*)}$	0.253 ± 0.118
PO_4^{3-} -P (mg-P L^{-1})	0.02 ± 0.011	0.014 ± 0.012	0.021 ± 0.016	0.047 ± 0.057	$0.058 \pm 0.039^{(*)}$	0.024 ± 0.022	$0.061 \pm 0.04^{(*)}$	0.014 ± 0.009
SiO_2 (mg L^{-1})	$1.55 \pm 0.82^{(*)}$	$1.13 \pm 0.34^{(*)}$	$1.33 \pm 0.38^{(*)}$	$0.19 \pm 0.13^{(*)}$	$2.92 \pm 1.59^{(*)}$	$1.33 \pm 0.82^{(*)}$	3.25 ± 1.20	0.49 ± 0.17
pH	7.04 ± 0.54	7.08 ± 0.64	7.41 ± 0.71	$5.77 \pm 0.96^{(*)}$	$6.43 \pm 0.76^{(*)}$	6.91 ± 0.47	6.93 ± 0.44	7.12 ± 0.56
Salinity (g L^{-1})	22.4 ± 5.8	27.5 ± 3.6	27.8 ± 3.7	$2.5 \pm 1.05^{(*)}$	$4.9 \pm 3.7^{(*)}$	$15.9 \pm 7.4^{(*)}$	21.4 ± 6.0	27.6 ± 3.8

^(*)Denotes values that are statistically different from values at other wells based on Tukey's honesty significant differences (HSD) test

0.18 mg L⁻¹. Sampling at this well was only possible when the tide line was within 4 m of the well.

The ANOVA test showed that the mean DO values throughout the beach were significantly different from each other. Based on a paired *t*-test at 95% confidence level, it was found that the DO value at W1_1 is statistically different from the DO at W2_1 (*p*-value = 0.036), W2_3 (*p*-value = 0.025), and W1_2 (*p*-value = 0.0026); no significant difference was observed between W1_1 and W2_2.

On the right transect, the concentration of nitrate/nitrite-N at W2_1 was 0.123 ± 0.092 mg-NL⁻¹. Moving seaward to W2_2, the concentration increased to 0.222 ± 0.178 mg-NL⁻¹, and then it decreased to 0.069 ± 0.057 mg-NL⁻¹ at the most seaward well (W2_3). On the left transect, the nitrate/nitrite-N level at W1_1 was 0.221 ± 0.167 mg-NL⁻¹. It decreased significantly at W1_2 (oily well) to 0.06 ± 0.050 mg-NL⁻¹, and then increased again to 0.207 ± 0.127 mg-NL⁻¹ at W1_3. The nitrate/nitrite-N level of the seawater samples was 0.037 ± 0.025 mg-NL⁻¹. The nitrate/nitrite-N level in the pond behind the beach was 0.1 ± 0.060 mg-NL⁻¹. Statistical analysis showed that there was a significant difference between the average nitrate/nitrite-N values in the beach. The paired *t*-test results showed that the pond was significantly different from W2_2 (*p*-value = 0.009), W1_1 (*p*-value = 0.008) and W1_3 (*p*-value = 0.004).

The ammonia-N levels on the right transect were 0.275 ± 0.145 mg-NL⁻¹ at W2_1, 0.235 ± 0.087 mg-NL⁻¹ at W2_2, and 0.461 ± 0.240 mg-NL⁻¹ at W2_3. On the oily side, the ammonia-N level was 0.263 ± 0.112 mg-NL⁻¹ at W1_1; it increased to 0.345 ± 0.171 mg-NL⁻¹ at W1_2, and then it decreased to 0.245 ± 0.102 mg-NL⁻¹ at W1_3. The ammonia levels for the seawater and the pond water were 0.253 ± 0.118 mg-NL⁻¹ and 0.267 ± 0.244 mg-NL⁻¹, respectively. The statistical analysis showed that the mean ammonia-N values were significantly different from each other. However, no significant difference was observed between the ammonia values of the pond and the wells in the beach.

The phosphate levels at W2_1 was 0.058 ± 0.039 mg-PL⁻¹, which decreased when moving seaward to W2_2 (0.024 ± 0.022 mg-PL⁻¹) and increased again moving seaward to W2_3 (0.061 ± 0.04 mg-PL⁻¹). The phosphate concentration was 0.020 ± 0.011 mg-PL⁻¹ at W1_1; it decreased slightly to 0.014 ± 0.012 mg-PL⁻¹ at W1_2, and then it increased to 0.021 ± 0.016 mg-PL⁻¹ moving seaward to W1_3. The phosphate concentration of seawater was detected as 0.014 ± 0.009 mg-PL⁻¹. The phosphate concentration of the pond (0.047 ± 0.057 mg-PL⁻¹) was consistent with the values observed at W2_1 and W2_3. The statistical analysis showed that the mean values of phosphate were significantly different within the beach. As with ammonia, no significant difference was observed between the phosphate values of the pond and the wells within the beach.

Silicate is a constituent of sediment and seawater, which can be used to trace the source of the nutrients in the beach (Ullman et al. 2003). On the right transect, the silicate concentration was 2.92 ± 1.59 mg L⁻¹ at W2_1; it decreased to 1.33 ± 0.82 mg L⁻¹ at W2_2, and then it increased again to 3.25 ± 1.20 mg L⁻¹ at W2_3. The same trend was also observed on the left transect; the silicate concentration was 1.55 ± 0.82 mg L⁻¹ at W1_1, it decreased to 1.13 ± 0.34 mg L⁻¹ at W1_2, and then it increased again to 1.33 ± 0.38 mg L⁻¹ at W1_3. The statistical analysis showed that the values of the wells were significantly different from each other. The paired Student's *t*-test showed that the silicate values of the pond water were significantly different from the ones of all of wells in the beach.

The pH values on the right transect were generally lower than those on the left transect. The pH value at W2_1 was 6.43 ± 0.76, which increased to 6.91 ± 0.44 at W2_2 and 6.93 ± 0.47 at W2_3. On the left transect, the pH value at W1_1 was 7.04 ± 0.54, which was close to the value at W1_2 (7.08 ± 0.64). Then the pH increased to 7.41 ± 0.71 when moving seaward to W1_3. The pH of the pond and the seawater were found to be 5.77 ± 0.96 and 7.12 ± 0.56, respectively. Our measurements are in agreement with Feely et al. (2008), who observed lower than average ocean pH in Northern Pacific. The statistical analysis showed significant differences between the pH of the wells of the beach.

On the right transect, the observed salinity at W2_1 was 4.9 ± 3.7 g L⁻¹; moving seaward, the salinity increased to 15.9 ± 7.4 g L⁻¹ at W2_2 and increased to 21.4 ± 6.0 g L⁻¹ at W2_3, where the salinity was expected to decrease. On the left transect, the salinity of W1_1 was 22.4 ± 5.8 g L⁻¹; moving seaward to W1_2, it increased to 27.5 ± 3.6 g L⁻¹ and remained almost constant when moving to W1_3 (27.8 ± 3.7 g L⁻¹). The salinity in the pond was found to be 2.5 g L⁻¹ and that of the seawater was 27.6 ± 3.8 g L⁻¹. Our measurements are in agreement with the seawater salinity at PWS found in literature. The reported PWS salinity values vary between 33.00 g L⁻¹ in the middle of the sound to 20.00 g L⁻¹ close to the shorelines, which receives freshwater run off (Gay and Vaughan 2001; Vaughan et al. 2001; Bang and Mooers 2003; Li and Bo-ufadel 2010). The ANOVA analysis showed that the salinities within the beach were significantly different from each other. The paired student's *t*-test showed significant differences between the salinities of the pond and within the beach.

The water temperature during the study was between 12.2 and 13.7°C. Although the optimum temperature for oil microbial biodegradation is considered to be approx. 15°C (Margesin and Schinner 1998), the microbial community in the arctic environment is adapted to low temperature (0–5°C) and significant biodegradation of oil has been recorded in this range of temperature (Zhu et al. 2001). Therefore, it

can be fairly assumed that the summer temperature was not too low to slow oil biodegradation.

The distance between the two transects (about 80 m), as well as the measurement of water levels, concentrations of nutrients, and salinity in the beach, confirms that groundwater flow interaction between the two transects was inexistent.

Discussion

The beach fills with seawater during the rising tide. As the tide recedes, the water moves seaward within the beach and discharges to the sea through the beach surface (Urish and McKenna 2004; Li et al. 2008; Li and Boufadel 2010). The landward recharge from the freshwater aquifer is also affected by the tide, with the highest recharge occurring during low tide (Li and Boufadel 2010). The DO is introduced to the beach during seawater recharge and depleted with the seaward water movement. Therefore, even in beaches with no oil, the DO level would be highest at the upper intertidal zone and decreases moving seaward. This trend was more or less observed in this study (Fig. 2). It is likely that the presence of oil caused a further decrease in the DO in the middle intertidal zone (W1_2). The significantly high DO at W1_3 was highly depending on the tide level, which is due to the fact that area filled and drained completely with the tide (based on the inability to get water samples at low tide). This suggests that this well was located in a high permeability zone.

On the right transect, the most landward well, W2_1, has a low DO, which is in contrast with the most landward well on the left (W1_1). The near anoxic level at W2_1 suggests that the pond is recharging to the right side of the beach and DO is depleted moving from the pond to this well. This was also supported with the observed nutrient levels as well as lower salinity and pH levels.

The salinity measurements suggest two sources of water recharge into the beach (Fig. 2). On the right transect, the DO was low at the most landward well, increased at the middle well, and decreased again at the most seaward well. This suggests that the middle portion of the beach fills up from the sea with oxygen-laden water and that the water entering the beach near the high tide line propagates seaward below the mixing zone around W2_2 and exits seaward of it. This is the “freshwater tube” that Boufadel (2000) noted based on laboratory beach studies. It appears that the tube exits in the beach landward of W2_3 as the salinity at that well is equal to that of seawater. The low oxygen at W2_3 indicates that little seawater enters the beach at that location.

On the left transect, the small decrease of salinity at W1_1 indicates a small freshwater recharge. The freshwater seems inexistent within the rest of the beach. The lowest DO was observed at the oily well (W1_2), which suggests

that little exchange with oxygen-rich seawater is taken place at that location.

The seawater nitrate/nitrite-N was lower than the levels observed in the beach. The high average concentration of nitrate detected on the beach ($0.150 \text{ mg-N L}^{-1}$) suggests that either the beach received high levels of nitrate from inland or nitrification of reduced nitrogen occurred in the beach where aerobic conditions prevailed. Input of reduced nitrogen may happen in the beach due to the decomposition of the organic matter and/or microbial fixation or through groundwater recharge from inland, vegetated with alder trees (Cooper 1942; Postgate 1998; Atlas and Bragg 2009b). The lower nitrate concentrations observed in the oily well could be explained by anaerobic degradation of oil components using nitrate as electron acceptor, i.e., denitrification. On the right transect, the low nitrate/nitrite-N level suggests that the low nitrate groundwater is recharging the beach.

The seawater ammonia level was close to the pond and the beach values, except on two wells that showed significantly higher levels (W2_3 and W1_2); these two wells are also the ones showing the lower DO levels in the entire beach. The high concentration of ammonia in W1_2 (oily) and W2_3 is in agreement with the near anoxic conditions recorded there as low oxygen concentration frequently prevents the oxidation of ammonia by nitrification, resulting in its accumulation (Ullman et al. 2003; Slomp and Van Cappellen 2004).

The seawater phosphate level measured was comparable to the values obtained in previous Prince William Sound and Delaware Bay studies (Eslinger et al. 2001; Ullman et al. 2003) studies. The significantly higher concentration of phosphate at wells, W2_1 and W2_3, along with lower concentration of DO and nitrate at these wells is consistent with the occurrence of anoxic conditions in the beach (Ullman et al. 2003; Slomp and Van Cappellen 2004). The dissolved inorganic phosphate in groundwater tends to precipitate under oxic condition by reaction with ferric iron forming iron phosphate (Slomp and Van Cappellen 2004).

Mixing of seawater and groundwater in a beach typically results in higher concentration of silicate at upper and lower intertidal zones (Ullman et al. 2003). At W1_2 (the oily well), the silicate level was significantly lower than the other two wells, which suggests that the exchange between seawater and sediments pore water at this well was restricted. On the right transect, the silicate concentrations at W2_1 and W2_3 were significantly different from the rest of the wells in the beach, which could be explained by mixing of seawater and aquifer discharge at these wells.

Atlas and Bragg (2009b) conducted measurements on this beach by driving piezotubes into the beach. They reported an average nitrate-N concentration of 0.24 mg-N L^{-1} and DO concentration larger than 9.0 mg L^{-1} . The average nitrate-N value that they reported is comparable to our average nitrogen concentration of $0.454 \text{ mg-N L}^{-1}$ (based on

the nitrate and ammonia). The main difference between the Atlas and Bragg's study (2009b) and our study resides in DO measurements and it can be explained by two factors. First, in our study, we placed the sensors in the beach nine weeks prior to the measurements, providing enough time for the beach to retrieve its natural state. We are not sure that such was done by Atlas and Bragg (2009b). In addition, piezotubes driven into the beach would inevitably hit cobbles on the way and disturb the path; hence, the deep sampling would be contaminated by the water from shallower depths. Second, the nutrient and oxygen samples were taken above the oil layer by Atlas and Bragg (2009b), although our samples were taken below the oil layer.

Our results showed that the nitrogen concentrations observed in the beach were an order of magnitude lower than the optimum concentration (2 to 10 mg L⁻¹) for biodegradation of oil hydrocarbons (Venosa et al. 1996; Boufadel et al. 1999; Du et al. 1999; Zhu et al. 2001). Also our study showed that although the N:P ratio in the beach was greater than the optimum N:P ratio for oil biodegradation (around N:P = 10) (Liebeg and Cutright 1999), the concentrations of nitrogen and phosphate were an order magnitude lower than the recommended levels for biodegradation, suggesting that both nitrogen and phosphate are limiting biodegradation.

The present study showed that DO and nutrients are both limiting factors for biodegradation. Stone (1992) reported that the addition of fertilizers only resulted in highly variable results for the treatment of contaminated beaches in PWS. It was also suggested that the low bioavailability and mass transfer limitations were additional factors hindering biodegradation (Page et al. 2008; Taylor and Reimer 2008; Atlas and Bragg 2009a, 2009b). In such a case, the addition of oxygen and nutrients might not enhance the biodegradation of oil. We believe this to be a valid concern that can be addressed by field studies to evaluate the feasibility of bioremediation.

Acknowledgements This work was supported by Exxon Valdez Oil Spill Trustee Council under Project Number 070836. However, it does not necessarily reflect the views of the Council, and no official endorsement should be inferred.

Appendix

Material and Methods Details

Sampling Equipment Details

The PVC pipe had an inner diameter of one inch (0.025 m), and was slotted across over its whole length to allow water passage. A pressure transducer (Mini-Diver DataLogger, Schlumberger, Sugar Land, TX) was placed at the bottom of each PVC pipe to record the water pressure at 10 min

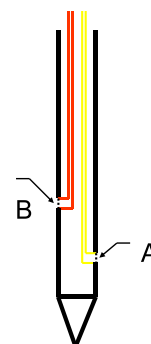
interval. The barometric pressure, monitored by an air-pressure sensor (DL-500 BaroLogger, Schlumberger, Sugar Land, TX), was subtracted from the readings of the pressure transducers to obtain the water level. No rainfall occurred during the field measurement period in August 2009.

The multiport sampling wells were made of stainless steel and contained ports at various levels. The ports were spaced at intervals of 0.19 m and were labeled A, B, C, and D from the bottom up. Each port was connected via a steel tube that extended to the top of the pipe. A 1/8 inch (3.2 mm) inner diameter Tygon tube (Cole-Parmer, Vernon Hills, IL) was placed on each of the steel tubings, and it was connected to a Luer-Lok three-way valve (Cole-Parmer, Vernon Hills, IL). To prevent blockage by fine sediments and guarantee good hydraulic connection between the beach pore water and the water inside the well, the ports were wrapped with a fine stainless steel screen.

The sampling box (SB) consisted of two perforated concentric cylinders made of PVC, schedule 40 (Online Resources, Fig. 3). The chamber between them was filled with Sand #16 (Alaska Sand and Gravel, Anchorage, AK). The diameter of the sand grains ranged from 0.21 to 1.41 mm with an average size of 0.88 mm. The uniformity coefficient was 1.68, a low value that indicates a uniform particle-size sand. Both cylinders were covered with mesh size 100 (opening diameter 150 µm) steel screen. The diameter of the inner cylinder was 0.05 m (2 inches) and the length was 0.15 m (6 inches), giving a total volume of 200 mL. The inner diameter of the outer cylinder was 0.1 m (4 inches), and considering the thickness of the inner cylinder wall, the spacing between the cylinders was around 15 mm. Twelve SBs (six serving as backup) were designed, built, and placed in each pit for the dual purpose of measuring the DO of pore-water and as a backup for taking water samples in case the sampling ports (SPs) were clogged.

The measuring chamber was a 60-mm long and 50-mm (2-inch) wide (ID) PVC pipe, schedule 40. The volume of the chamber was around 120 mL. Half-inch fiberglass plates (70 × 70 mm²) were glued to the top and bottom of the pipe with silicon glue. One hole (5/8 inch (15.9 mm) ID) was drilled and threaded in each of the top and bottom plates for the insertion of brass pipe and fitting allowing

Fig. 3 The schematic detail of the multiport well. The deepest port was labeled A, and the next port is 19 cm above the lower port



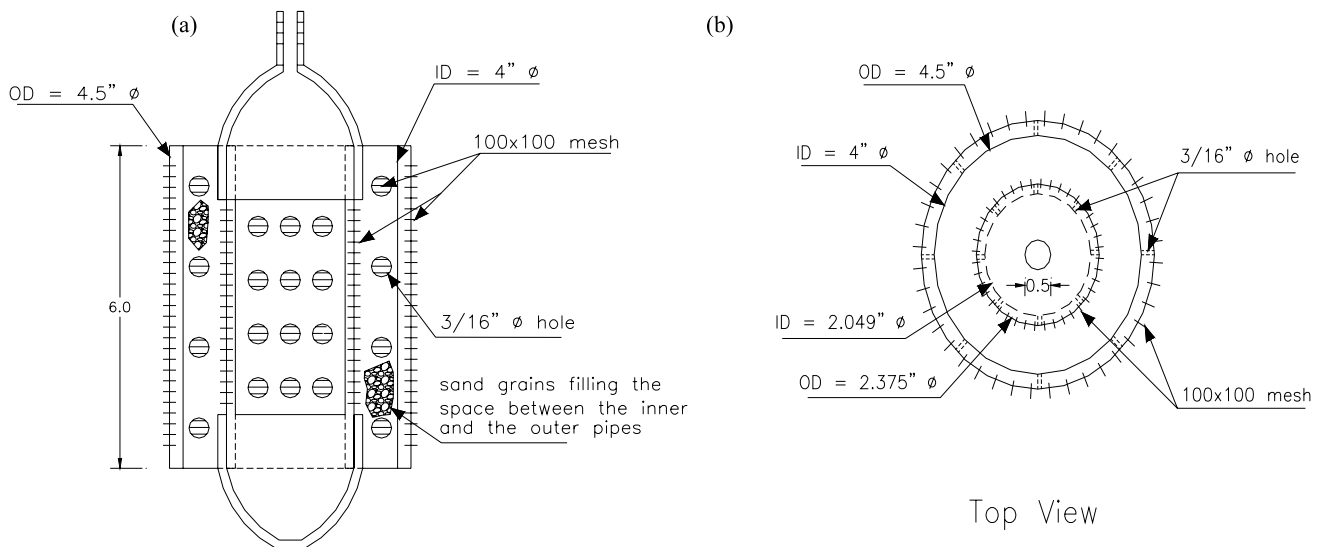


Fig. 4 The sampling box (SB) used for water sampling. **(a)** A vertical cross section of the SB, **(b)** top view

the water to flow through the chamber. A third hole (3/4 inch (19.05 mm) diameter) was drilled in the top plate and threaded for the insertion of the DO probe. Brass pipes and connections were used and all connections were sealed with Teflon tape.

Sampling Protocol

The water samples for nutrient study were collected using a new pre-sterilized 60 mL syringe each time from SPs. The sampling started from the port closest to the surface and moved to the deeper ports. The samples were put in 30 mL high density polypropylene bottles (Nalgene, Fisher Scientific, Pittsburgh, PA). All the bottles were acid-washed (in 10%-HCl acid bath for 18 hours) and rinsed 3 times with DI water prior to the field sampling. The first volume (60 mL in the syringe) taken from each port was wasted. An additional volume of 25 mL was used to rinse the bottles. Once the rinsing was finished, the remaining water in the syringe was disposed, and a new water sample was taken from the port using the same syringe and transferred into the rinsed bottle. The bottles were filled up to the neck to leave enough room for expansion during freezing. All bottles were labeled indicating the beach, pit, depth, time, and date of sampling.

Analytical Methods Quality Assurance

For each nutrient compound a four point calibration curve was obtained. In order to check the precision of the procedure, each 10th sample was re-analyzed. Drift and carry-over errors were also accounted for by checking the standard solutions every 24 samples. In addition, every 36 samples, quality control cups (DI water cup and one highest standard



Fig. 5 Dissolved oxygen measuring setup during operation. The DO probe (black) is immersed in the measuring chamber (transparent cylinder with red top and bottom)

Table 3 Detection limit of the different analytical methods used

Measuring method	Detection limit
RDO probe (DO)	0.01 mg L ⁻¹
Ammonia (NH ₃)-N	0.56 µg L ⁻¹
Nitrate (NO ₂ /NO ₃)-N	0.21 µg L ⁻¹
Phosphate (PO ₄)-P	0.29 µg L ⁻¹
Silicate (SiO ₂)	1.8 µg L ⁻¹
pH	0.02
Refractometer (salinity)	1 g L ⁻¹

solution cup) were placed with samples to control the analytical measurements.

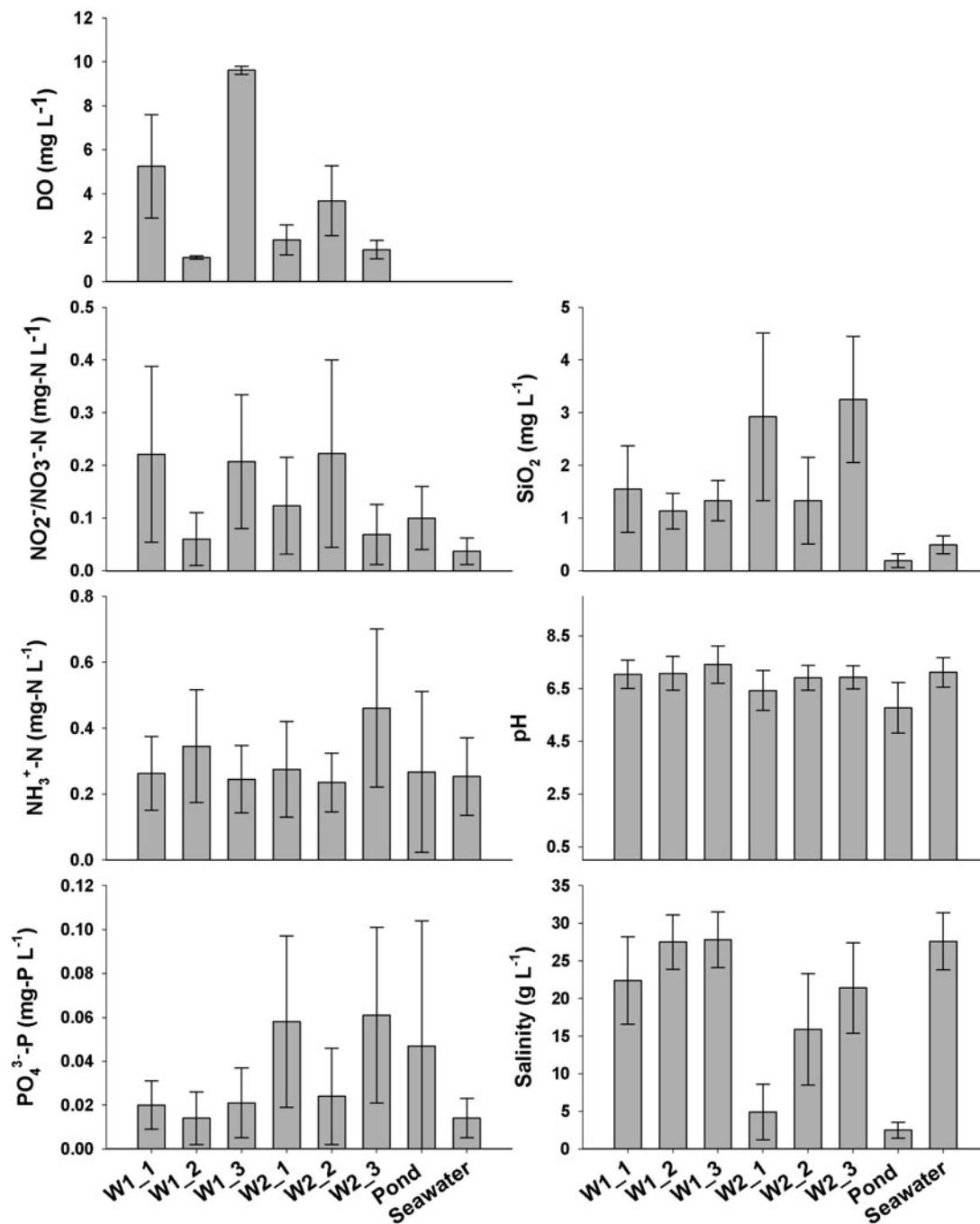


Fig. 6 Dissolved oxygen (DO), nutrients concentrations, pH, and salinity values recorded in different plots within the beach are presented. The *error bars* show \pm standard deviations. The data obtained

at two groups of monitoring points (i.e., clean- vs. oily-region points) were compared using statistical tests

The DO probe was calibrated using the water-saturated air calibration method. The zero calibration was conducted in zero oxygen solution. A 60 g L⁻¹ solution of sodium sulfite was prepared, the already calibrated DO probe was then placed in the solution and zero calibration was achieved. The water-saturated air calibration was performed daily in the field prior to sampling.

The pH electrode and meter were self-calibrating, though the accuracy was checked every 20 samples by using commercial pH 4, 7 and 10 buffer solutions. Every 10th samples was re-measured to control the precision.

The refractometer was calibrated using DI water. The refraction index of DI water was between 1.3329 and 1.3331. Different salinity control solutions (10, 20, 35 and 50 g L⁻¹)

were made to check the instrument accuracy. DI water and one of the salinity control solution were measured after every 10 sample measurements.

The minimum detection limits of different analytical methods and instruments used for measuring the DO, salinity, pH and nutrients are reported in Table 3.

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