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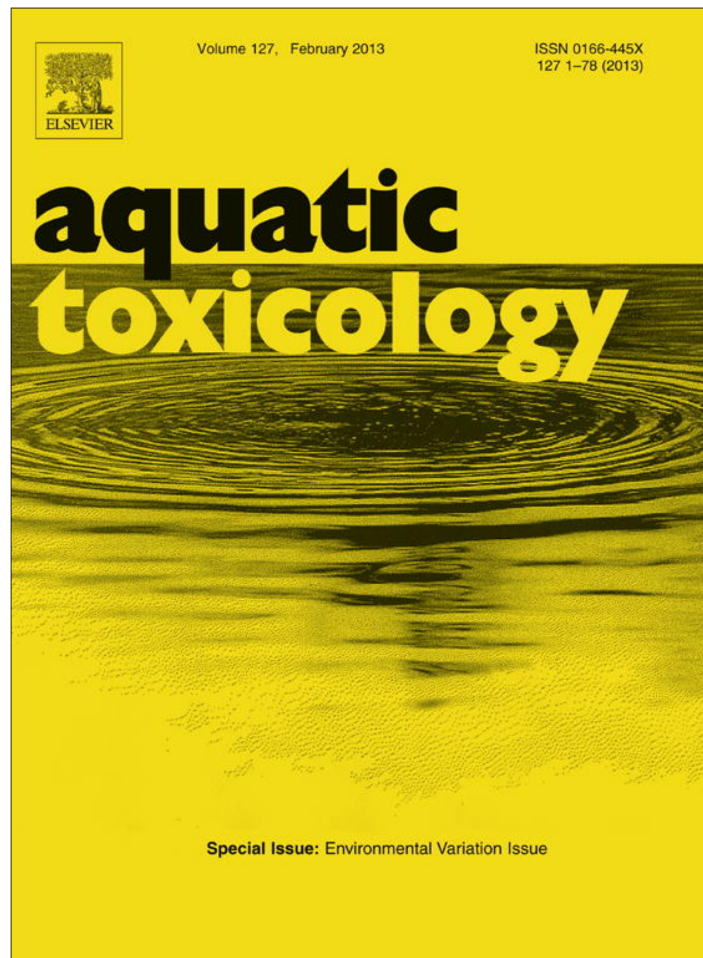

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Seasonal variation in biomarkers in blue mussel (*Mytilus edulis*), Icelandic scallop (*Chlamys islandica*) and Atlantic cod (*Gadus morhua*)—Implications for environmental monitoring in the Barents Sea

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ABSTRACT

In the Barents Sea, the limited data on biological relevant indicators and their responses to various anthropogenic stressors have hindered the development of a consistent scientific basis for selecting indicator species and developing practical procedures for environmental monitoring. Accordingly, the main aim of the present study was to develop a common set of baseline values for contaminants and biomarkers in three species, and to identify their strengths and limitations in monitoring of the Barents Sea. Blue mussel (*Mytilus edulis*), Icelandic scallop (*Chlamys islandica*) and Atlantic cod (*Gadus morhua*) were sampled from a north Norwegian fjord in March, June, September and December 2010. Digestive glands from the bivalve species and liver from Atlantic cod were analysed for biomarkers of oxidative stress (catalase [CAT], glutathione peroxidase [GPX], glutathione-S-transferase activities [GST], lipid peroxidation as thiobarbituric reactive substances [TBARS] and total oxyradical scavenging capacity [TOSC]), biotransformation (ethoxyresorufine-O-deethylase activity [EROD]) and general stress (lysosomal membrane stability [LMS]). Concentrations of polycyclic aromatic hydrocarbons (PAHs) and metals in the bivalves and PAH metabolites in fish bile were quantified. Finally, energy reserves (total lipids, proteins and carbohydrates) and electron transport system (ETS) activity in the digestive gland of the bivalves and liver of Atlantic cod provided background information for reproductive cycle and general physiological status of the organisms. Blue mussel and Icelandic scallop showed very similar trends in biological cycle, biomarker expression and seasonality. Biomarker baselines in Atlantic cod showed weaker seasonal variability. However, important biological events may have been undetected due to the large time intervals between sampling occasions. Physiological biomarkers such as energy reserves and ETS activity were recommended as complementary parameters to the commonly used stress biomarkers, as they provided valuable information on the physiological status of the studied organisms. Interpretation of the seasonality in oxidative stress biomarkers was in general difficult but TOSC and lipid peroxidation were preferred over the antioxidant enzyme activities. This study is the first reporting seasonal baseline in these three species in a sub-Arctic location. Overall, the Icelandic scallop was considered the most adequate organism for environmental monitoring in the Barents Sea due to the interpretability of the biomarker data as well as its abundance, ease to handle and wide distribution from the southern Barents Sea to Svalbard.

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1. Introduction

Biologically relevant indicators are required to monitor ecosystem status in relation to diffuse anthropogenic pollutants or acute accidental discharges. Responses and tolerances of individual taxa to various anthropogenic stressors have been extensively investigated in temperate systems (Collier and Varanasi, 1991; Aas and Klungsøyr, 1998; Baussant et al., 2009; Brooks et al., 2009), but few data exist for the Arctic and sub-Arctic regions (Chapman and

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Riddle, 2005; Nahrgang et al., 2010a,b). For the Barents Sea in particular, very limited long-term routine monitoring of contaminant levels and their biological effects have been carried out. This has hindered the development of a clear and consistent scientific basis for selecting indicator species and endpoints and developing practical procedures for their application in management (Anon., 2006). Nevertheless, increasing industrial activities in the high North, including shipping activities along the coast of Russia and Norway (Bambulyak and Frantzen, 2009), oil and gas fields in the Barents Sea such as the Goliat oil field close to the Norwegian coast (Øien et al., 2011), present an increasing risk to the environment. Therefore, the implementation of monitoring programmes is needed in the Barents Sea (Hasle et al., 2009).

Biomarkers complement contaminant analysis in aquatic organisms by providing first biological signals of exposure (Van der Oost et al., 2003). Several biomarkers are commonly used in international and national monitoring programmes to help identify adverse effects of xenobiotics in fish and blue mussels (OSPAR, 2007; Hylland et al., 2008; Brooks et al., 2011). The most widely accepted and used biomarkers in fish are the ethoxyresorufine-O-deethylase (EROD) and glutathione-S-transferase (GST) activities of phases I and II metabolism, respectively (Hylland et al., 2008; Nahrgang et al., 2010b). Furthermore, the quantification of PAH metabolites in fish bile has been shown to provide a relevant indication of the PAH bioavailable exposure in fish (Ariese et al., 1993; Aas et al., 1998, 2000; Nahrgang et al., 2010b). Biomarkers of oxidative stress, such as catalase (CAT), glutathione peroxidase (GPX) activities, and lipid peroxidation have been used for both bivalve and fish species, although their interpretation is usually difficult due to their implication in a wide range of biological functions (Van der Oost et al., 2003). The total oxyradical scavenging capacity (TOSC) assay is a helpful complement to regular oxidative stress biomarkers as it provides a direct assessment of an organism's ability to resist oxidative stress (Regoli and Winston, 1998). Finally, biomarkers of stress, such as the lysosomal membrane stability (LMS) assay, are considered general indicators of organism health (Moore et al., 2008) and are central parameters in integrated monitoring schemes developed for both fish and bivalves (Hylland et al., 2008).

Despite the considerable understanding of their links with contaminant exposure, the use of biomarkers is often limited by their strong variability due to natural biological and environmental cycles (Shaw et al., 2004; Depledge, 2009; Nahrgang et al., 2010a). Realistic interpretations of biomarker responses in environmental monitoring thus rely on extensive knowledge of the organisms' biological cycles and the biomarkers' seasonal baseline levels. The present study aimed to characterise baseline levels in contaminant burden and responses in biomarkers commonly used or recommended for biomonitoring (ICES, 2010) in three potential indicator species collected over four seasons, and to identify their strengths and limitations for application to environmental monitoring programmes in the Barents Sea.

Blue mussel (*Mytilus edulis*), Icelandic scallop (*Chlamys islandica*) and Atlantic cod (*Gadus morhua*) were selected as potential indicator species for the Barents Sea due to their abundance and wide distribution. Blue mussel and Atlantic cod have a temperate to boreal distribution and are already used in several monitoring programmes (Hylland et al., 2008; Brooks et al., 2011), and both species have been recorded as far north as Svalbard (Berge et al., 2005; Renaud et al., in press). Icelandic scallop, which is a sub-Arctic species distributed as far north as the Svalbard archipelago (Brand, 2006), has been less studied than blue mussel and Atlantic cod in ecotoxicology although some studies have reported biomarker responses following experimental contamination to petroleum-related compounds (Baussant et al., 2009; Hannam et al., 2009, 2010). There has been no study of seasonal variability in levels of biomarkers in any of these species, except for blue mussel (Viarengo et al., 1989; Harding et al., 2004; Hagger et al., 2010), nor have biomarkers used in temperate species been validated for sub-Arctic and Arctic environments.

Fig. 1. Map of sampling area.

Herein, we present baseline levels of the above-mentioned biomarkers and contaminant (PAHs and metals) body burden in blue mussel, Icelandic scallops and Atlantic cod collected in March, June, September and December 2010. Energy reserves (total lipids, total carbohydrates and total proteins) and the potential metabolic activity measured as the electron transport system (ETS) activity (Fanslow et al., 2001) served as general indicators of physiological status of the organisms. The results will contribute to the development of an environmental monitoring programme based on relevant bio-indicators and effect-levels as anthropogenic activities and risks for pollution increase in Arctic and sub-Arctic environments.

2. Methods

2.1. Sample collection

All organisms were collected in a sub-Arctic location, near Tromsø, Northern Norway (70°N), in March, June, September and December 2010 (Fig. 1). Organisms were typically processed within 2 h (LMS assay), 4 h (all other biomarkers) and 6 h (body burden) following collection. Submerged blue mussels ($n = 60$ per sampling season) were collected by hand during low tide and transported to the laboratory facilities in buckets containing seawater soaked paper towels to provide a cool and moist environment. Icelandic scallop ($n = 60$ per sampling season, except $n = 35$ in March) were collected by triangular dredge from RV *Hyas* (University of Tromsø), maintained in tanks with running seawater until transportation to the laboratory and thereafter maintained in oxygenated seawater until dissection.Blue mussel haemolymph was collected from 20 individuals for the assessment of LMS by the method of neutral red retention (NRR, Section 2.2.1). Total wet weight including shell (±0.1 g wwt), length and width (±0.1 cm) were recorded from blue mussel and Icelandic scallop. Digestive glands were removed,

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weighed (±0.01 g wwt), snap frozen in liquid nitrogen and stored at −80 °C until analyses.

Atlantic cod was sampled onboard RV *Hyas* with bottom trawl (n = 21 and 20 in March and June, respectively) and fishing rod (n = 16 and 13 in September and December, respectively), and kept alive in tanks with running seawater until transportation to the laboratory and thereafter maintained in oxygenated seawater until dissection. Fish were sacrificed by a sharp blow to the head. Total length (±0.1 cm), total and somatic weights (±0.1 g wwt), liver and gonad weights (±0.01 g wwt) and gender were recorded. Liver slices and bile were placed into separate cryovials, snap frozen in liquid nitrogen, and stored at −80 °C prior to analysis.

The gonado-somatic index (GSI), the hepato-somatic index (HSI) and the Fulton's condition factor (K) were determined for Atlantic cod according to the equations:

$$GSI = 100 \times \frac{\text{total gonad wet weight}}{\text{sW}}$$

$$HSI = 100 \frac{\text{total liver wet weight}}{\text{sW}}$$

$$K = 100 \times \frac{\text{sW}}{L^3}$$

where sW is the somatic weight (g) and L the fork length (cm).

Due to limited amount of tissue for biomarker analyses, digestive glands from the bivalves were split into 4 batches of 15 samples per sampling point that were used for (1) energy reserves and ETS activity, (2) antioxidant defence enzymes and lipid peroxidation, (3) TOSC and (4) GST activity respectively. Each biomarker analysis is thus representative of 15 individuals per sampling month. For Atlantic cod, all analyses were performed on liver slices from each sampled fish.

2.2. Analyses

2.2.1. Determination of contaminant burden in total soft tissues of bivalves

Contaminant analyses were carried out by laboratories accredited for the methods used (Unilab Analyse, PAH, and ALS laboratory group, metals). For blue mussel, 2 pools of 10–15 individuals from each sampling point were analysed, while 3 pools, each consisting of 15 Icelandic scallops from each sampling point, were analysed. For PAH concentrations, each sample of pooled soft tissue (10–20 g wwt) was thoroughly ground and homogenised prior to analyses. Samples were weighed and a potassium hydroxide-methanol solution and an internal standard-mix of deuterated PAHs were added. The solution was boiled with reflux for 4 h (saponification), before filtration and extraction with pentane. Samples were purified using gel permeation chromatography (GPC), with dichloromethane as mobile phase. Samples were filtrated and further purified by solid phase extraction (SPE). Analyses were performed using a GC-MSD (Agilent 7890 GC with split/splitless injector, Aglient 7683 and Agilent 5975C, mass spectrometer with EI ion source). Blind samples were run parallel to all samples, and proficiency test samples (Quasimeme, Netherlands) were used as control samples. The limit of detection (LOD) was determined from analyses of a series of blank samples, processed along with real samples, and calculated as: LOD = (blank average) + 3 × (blank standard deviation). As concentrations of PAHs in bivalve tissues were in general close to or under the LOD, results were presented as min-max range of concentrations in the analysed pools instead of means and standard deviations (Table 1). Furthermore, for the calculation of the sum of NPDs (naphthalene, anthracene/phenanthrene, dibenzothiophene and their alkylated homologues) and 16 EPA-PAHs, half of the detection limit value was used for compounds that were below LOD.

Total lipid content in the bivalve soft tissue was quantified according to Folch et al. (1957) in order to relate eventual seasonal variations of PAHs to the lipid content. Homogenised samples were weighed; methanol was added, followed by chloroform. After filtration, an aqueous solution of potassium chloride was added for purification, before the extracts were centrifuged and the aqueous phase removed. The remaining organic phase was evaporated under a flow of nitrogen gas until dryness. The tube was then weighed, and the amount of extracted lipid was calculated as wet weight % of the initial sample.

For metal analyses, still frozen soft tissues were homogenised and freeze-dried. The dried samples were dissolved in concentrated nitric acid and hydrogen peroxide by microwave digestion (170 °C, 30 min) in sealed Teflon vessels. Cooled samples were transferred to test tubes and diluted to 10 ml. The samples were analysed by inductively coupled plasma sector field mass spectrometry. Analytical quality was confirmed through analyses of certified reference material bovine muscle powder (NIST 8414).

2.2.2. PAH metabolites in Atlantic cod bile

Preparation of hydrolysed bile samples was performed as described by Krahn et al. (1992). Briefly, bile (20 μl) was mixed with internal standard (triphenylamine) and diluted with de-mineralised water (50 μl) and hydrolysed with β-glucuronidase/arylsulphatase (20 μl, 1 h at 37 °C). Methanol (200 μl) was added and the sample was centrifuged and the supernatant was analysed.

The HPLC used was a Waters 2695 Separations Module with a 2475 fluorescence detector attached. The column was a Waters PAH C18 (4.6 mm × 250 mm) with 5 μm particles. The mobile phase consisted of a gradient from 40:60 acetonitrile:ammonium acetate (0.05 M, pH 4.1) to 100% acetonitrile at a flow of 1 ml/min, and the column was heated to 35 °C. Fluorescence was measured at the optimum for each analyte. 25 μl of extract was injected for each analysis.

2.2.3. Lysosomal membrane stability

Lysosomal membrane stability was measured in haemocytes of 20 blue mussels per sampling period, using the NRR procedure adapted from Lowe and Pipe (1994). Approximately 0.1 ml haemolymph was removed from the adductor muscle of the blue mussels with a syringe containing approximately 0.1 ml physiological saline (pH 7.3). The haemolymph/saline solution was placed in a microcentrifuge tube, from which a 40 μl sample was removed and pipetted onto the center of a microscope slide. The slide was left in a dark humid chamber for 15 min to allow adhesion of the cells to the slide. Excess liquid was removed from the slide after this time and 40 μl of neutral red solution was added. The neutral red solution was taken up inside the haemocytes and stored within the lysosomes. The ability of the lysosome to retain the neutral red solution was checked every 15 min by light microscopy (×40). The test was terminated and the time recorded when more than 50% of the haemocytes leaked the neutral red dye out of the lysosome into the cytosol.

2.2.4. Energy reserves

Total lipid, carbohydrate and protein contents were determined in digestive glands of blue mussel and Icelandic scallop and liver of Atlantic cod homogenised in a 0.1 M Trizma HCl/base buffer pH 7.5 containing, 0.4 M $MgSO_4$, 15% polyvinylpyrrolidone and 0.2% (w/v) Triton X-100 and centrifuged for 10 min (3000 × g, 4 °C). Lipids were extracted following Bligh and Dyer (1959). Briefly, the homogenates (200 μl) were mixed with 500 μl chloroform, 500 μl methanol and 250 μl dH_2O and centrifuged at 10,000 × g for 5 min. A 100 μl sample of the chloroform phase was then pipetted into glass reagent tubes, 500 μl of H_2SO_4 (95–97%)

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Table 1
Sum of NPDs and 16 EPA–PAHs (min–max, µg/kg wwt), extractable organic material (min–max, lipid content in % wwt), metals (mean ± standard deviation, mg/kg wwt) and dry weight content (mean ± standard deviation, %) in soft tissue pools from *Mytilus edulis* (Me, two pools of 10–15 individuals) and *Chlamys islandica* (Ci, three pools of 15 individuals) collected in March, June, September and December 2010. When PAHs concentrations in pools were below LOD, only the LOD value is presented. Letters (a and b) indicate significant difference ($p < 0.05$) within one species between sampling month. Statistical analyses were not run for PAHs.

	March	June	September	December
*Sum NPD				
Me	<24; 29	26; 35	31; 99	<24
Ci	28–39	27–35	<24–34	49–65
*Sum 16 EPA–PAHs				
Me	<5	<5	<5; 5.7	7.1; 22
Ci	11–17	8.6–9.1	5.7–6.3	8.0–9.2
Lipid content (% wet weight)				
Me	0.34; 0.42a	1.31; 1.62b	1.68; 2.01b	1.26; 1.5b
Ci	0.84–1.08a	1.37–1.41b	1.10–1.22a,b	1.07–1.22a,b
Metals (mg/kg wwt)				
Ag				
Me	0.004 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Ci	0.03 ± 0.01	0.02 ± 0.02	0.03 ± 0.002	0.04 ± 0.04
As				
Me	1.11 ± 0.25a	2.72 ± 0.63b	2.57 ± 0.11b	2.52 ± 0.02b
Ci	1.91 ± 0.36	1.77 ± 0.64	2.33 ± 0.38	2.29 ± 0.28
Cd				
Me	0.11 ± 0.03	0.25 ± 0.09	0.21 ± 0.03	0.23 ± 0.03
Ci	1.22 ± 0.37	1.14 ± 0.71	1.67 ± 0.65	1.27 ± 0.71
Cr				
Me	0.44 ± 0.01	2.59 ± 2.48	0.87 ± 0.05	0.45 ± 0.35
Ci	0.18 ± 0.06	0.14 ± 0.05	0.24 ± 0.1	0.52 ± 0.23
Cu				
Me	0.44 ± 0.17a	1.30 ± 0.07b	1.13 ± 0.08b	0.96 ± 0.09b
Ci	0.93 ± 0.43	0.97 ± 0.71	0.67 ± 0.20	0.56 ± 0.18
Hg				
Me	0.01 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
Ci	0.02 ± 0.004	0.02 ± 0.007	0.02 ± 0.002	0.02 ± 0.005
Ni				
Me	0.64 ± 0.01	2.95 ± 2.38	1.24 ± 0.13	0.61 ± 0.59
Ci	0.20 ± 0.03	0.15 ± 0.07	0.56 ± 0.63	1.71 ± 1.60
Pb				
Me	0.08 ± 0.01	0.13 ± 0.04	0.11 ± 0.02	0.10 ± 0.00
Ci	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.06 ± 0.03
Zn				
Me	5.92 ± 1.11a	10.52 ± 0.64b	11.03 ± 1.22b	10.55 ± 0.03b
Ci	23.16 ± 11.14	18.61 ± 10.85	17.28 ± 2.15	16.88 ± 1.91
Dry weight (%)				
Me	8.0 ± 1.4a	15.5 ± 3.7a,b	17.8 ± 0.8b	17.2 ± 1.2b
Ci	15.77 ± 2.06	15.07 ± 1.85	16.23 ± 0.64	17.30 ± 1.28

* For values below detection limit, ½ the detection limit was used to determine the sum of NPDs and 16 EPA–PAHs.

was added and the sample charred for 15 min at 200 °C. Samples were then diluted 1:6 in dH_2O distributed into microwells as 4 replicates and read spectrophotometrically at 340 nm. Lipid concentrations were determined against a glyceryl tripalmitate standard curve.

For protein and carbohydrate concentrations, trichloroacetic acid (15%) was mixed with 300 µl of the homogenates and centrifuged at 10,000 × *g* for 5 min. Pellets for total protein quantification were dissolved in 500 µl NaOH (1 N) and incubated 30 min at 60 °C for dissolution of the pellets. Subsequently, 300 µl of HCl (1.67 N) was added to neutralise the solution. Total protein concentration (mg/ml) was determined in 4 replicates, according to Bradford (1976) using bovine serum albumin (BSA) as a standard.

Total carbohydrate concentrations were quantified through the phenol sulphuric acid method according to Dubois et al. (1956). Supernatants (50 µl) were mixed with H_2SO_4 (95–97%; 200 µl) and phenol (50 µl), distributed into microwells as 4 replicates and read spectrophotometrically at 490 nm. Carbohydrate concentration was determined against a glycogen standard curve. Lipids, proteins and carbohydrates were converted into Joules per unit tissue wet weight (J/g wwt) according to their specific enthalpy of combustion (Gnaiger, 1983) i.e. 39.5, 24.0 and 17.5 kJ/g respectively.

2.2.5. Electron transport system (ETS) activity

The electron transport system activity (µmol O_2 consumed/min/g wwt) was measured on the same tissue homogenates as for the energy reserve, following King and Packard (1975) with major modifications as described in De Coen and Janssen (1997). Tissue homogenates (50 µl) were pipetted as 4 replicates into microwells in buffered substrate solution (50 µl, 100 mM Trizma HCl/base buffer pH 7.5, 0.3% Triton X-100) and NADH/NADPH (50 µl, 1.17 mM/250 µM). The reaction was started by adding 100 µl 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5 phenyl tetrazolium chloride (4 mg/ml). Absorbance was measured kinetically at 490 nm at 20 °C for 10 min.

2.2.6. Oxidative stress and antioxidant enzymes

For all oxidative stress biomarkers except GST activity, digestive glands and livers were homogenised with a Potter-Elvehjem type homogeniser in an ice-cold potassium phosphate buffer (100 mM, pH 7.5) containing 2.5% NaCl. Homogenates were centrifuged (10,000 × *g*, 4 °C) for 30 min and supernatants were subsequently stored at −80 °C. For GST activity, digestive glands and livers were homogenised with a Potter-Elvehjem type homogeniser in four volumes of ice-cold potassium phosphate buffer (100 mM, pH

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7.8) containing 0.15 M KCl. The homogenate was centrifuged at 10,000 × g for 30 min, split into subsamples and stored at −80 °C.

CAT activity was measured in duplicate and expressed in μmol/min/g digestive gland wwt for the bivalves and mmol/min/g liver wwt for Atlantic cod. The decrease in absorbance at 240 nm ($\varepsilon = 40\,M^{-1}\,cm^{-1}$) was recorded in quartz cuvette during 1 min at 20 °C after addition of 10 mM H_2O_2 to the diluted sample using a spectrophotometer LAMBDA 35 (Clairborne, 1985).

GPX activity was measured in duplicates and expressed as μmol/min/g tissue wwt (Livingstone et al., 1992). Briefly, 30 μl of homogenate was mixed with potassium phosphate buffer (100 mM, pH 7.5) containing 1 mM of EDTA and 1 mM of NaN_3 and GSH (1.5 mM) and 1 U of glutathione reductase and incubated 10 min at 20 °C. Then, the reaction was started by addition of 25 μl of NADPH (0.12 mM) and either cumene hydroperoxyde (4 mM, total GPX activity) or hydrogen peroxide (2 mM, selenium-dependent GPX activity). The decrease of NADPH was recorded during 1 min at 340 nm ($\varepsilon = 6.2\,mM^{-1}\,cm^{-1}$) and at 20 °C.

GST activity was measured in triplicate and expressed as μmol/min/mg cytosolic proteins (based on Habig et al., 1974). Homogenates were diluted 5-fold in ice cold potassium phosphate buffer (100 mM, pH 7.4), 50 μl of each sample was transferred to 96-microwell plates. Each plate contained a negative and a positive control purified porcine GST. The microplates were stored on ice prior to analysis. Reagents (2 mM CDNB, 1 mM GSH) were mixed and 200 μl added to the wells (containing cytosol samples, blanks, or positive controls). Absorbance was measured at 340 nm (ε CDNB–GSH conjugate = $9.6\,mM^{-1}\,cm^{-1}$) for 2 min at 22 °C. The cytosolic protein concentrations of the homogenates were determined by a procedure based on the Lowry method (Lowry et al., 1951), adapted for measurement with a plate reader and using bovine gamma globulin as the protein standard.

Lipid peroxidation was measured as thiobarbituric reactive substances (TBARS) according to Buege and Aust (1978). Briefly, a mixture of thiobarbituric acid (0.375%, w/v), trichloroacetic acid (15%, w/v) and HCl (0.25 N) was added to the samples. The samples were incubated at 100 °C for 15 min, centrifuged at 1000 × g for 10 min. TBARS were quantified at 535 nm ($\varepsilon = 0.156\,M^{-1}\,cm^{-1}$) and expressed as mmol TBARS/g tissue wwt.

2.2.7. Total oxyradical scavenging capacity (TOSC)

Bivalve digestive glands were homogenised with a Potter-Elvehjem type homogeniser in four volumes of ice-cold potassium phosphate buffer (100 mM, pH 7.4) with 2% NaCl (w/v). The homogenates were subsequently centrifuged at 50,000 × g for 120 min at 4 °C and the cytosolic fraction was stored at −80 °C until required. A separate reference was used for each sample, which was measured with each run on the gas chromatography equipment. TOSC was measured in 55 μg of cytosolic protein from each sample diluted in 800 μl of homogenizing buffer containing 2 mM α-keto-γ-methiolbutyric acid and 200 mM 2,2′ azobis (2-methylprpionamide) dihydrochloride. Glutathione was used as a positive control and homogenizing buffer was used as a blank. Ethylene gas production was measured at intervals of exactly 12 min from 36 to 84 min. The gas was quantified by measurement with a GC-FID (6890N, Agilent Technologies, California, USA) and a Supelco SPB-1 fused silica capillary column (30 m × 0.32 mm × 0.25 μm). Hydrogen, at a flow rate of 1.5 ml/min, was the carrier gas with a split ratio of 1:20 with an inlet temp of 160 °C, oven temp of 72 °C and a FID temp of 220 °C. The TOSC values were calculated using the equation: $TOSC = (100-(\int SA/\int Blk \times 100))/55$, where "∫SA" is the integral of the curve for the sample from the GC readout, "∫Blk" is the integral of the curve for the blank from the GC readout. The TOSC values were normalised with the cytosolic protein concentration and expressed as Unit TOSC per mg cytosolic protein. The cytosolic protein concentration of the samples where determined according to Lowry (1951) as described earlier.

2.2.8. EROD activity

Microsomes of Atlantic cod liver samples were prepared on ice with precooled equipment and solutions. Liver samples were homogenised in a potassium phosphate buffer (100 mM, pH 7.8) containing KCl (0.15 M), dithiothreitol (DTT, 1 mM), and glycerol (5%, v/v), using a Potter-Elvehjem type homogeniser. The homogenate was centrifuged (10,000 × g, 30 min) before the supernatant was recentrifuged (50,000 × g, 120 min). The microsomal fraction was obtained by suspending the resulting pellet in potassium phosphate buffer (100 mM, pH 7.8) containing KCl (0.15 M), DTT (1 mM), EDTA (1 mM), and glycerol (20%, v/v). EROD activity was assayed fluorometrically in a plate reader according to Stagg and Mcintosh (1998). Briefly, samples of microsomes were diluted to ∼2 mg/ml in buffer and pipetted (50 μl) in 6 technical replicates onto a 96-well microplate. Pre-prepared resorufin standards (duplicates) were then added to subsequent wells. The reaction mixture (200 μl) containing potassium phosphate buffer (100 mM, pH 8) and 3 μM 7-ethoxyresorufin was added to the sample wells, before NADPH solution (2.4 mM in final well volume of 275 μl) was added to initiate the reaction. Transformation of 7-ethoxyresorufin to resorufin was read at an excitation of 530 nm and fluorescence emission was measured at 590 nm. The EROD activity values were normalised to the microsomal protein content and expressed as pmol/min/mg microsomal protein. Protein concentrations were determined according to Lowry et al. (1951).

2.3. Statistics

Statistical analyses were performed with Statistica 9.1. When requirements of normality and homogeneity of variances were met, a factorial ANOVA (season and gender) or one-way ANOVA (season) was used for fish and bivalve data, respectively. For Atlantic cod, no significant difference in biomarker baseline levels between genders was observed, thus all data were presented as mixed gender. A Tukey HSD post hoc test was used to test differences among the sampling months with $p \leq 0.05$ as the significance level applied for all analyses. When requirements for normality were not met, the nonparametric Kruskal–Wallis test was used with $p \leq 0.05$ as the significance level applied for all analyses. Correlations between variables were determined by the Pearson product–moment correlation. For PAH analyses, as levels were often below LOD, statistical analyses could not be run.

3. Results

3.1. Blue mussel

Blue mussels collected in March were significantly larger in size (length: 5.1 ± 0.1 cm) compared to the other months (length: 4.5 ± 0.1–4.7 ± 0.1 cm). Levels of most PAHs in blue mussel were below the LOD for all the different sampling seasons (Table 1). However, in September, concentration of NPD compounds in blue mussel increased concomitantly with an increase in the soft tissue lipid content. The sum of 16 EPA–PAHs did not show any remarkable variation. Levels of metals were in general low and only As, Cu and Zn showed significantly lower levels in mussels collected in March compared to the other seasons. These mussels also showed significantly lower soft tissue dry weights and lipid content.

Total lipids in blue mussel digestive gland were significantly higher in September and December compared to March and June (Fig. 2A). Individual variability in lipids in June was high. Carbohydrate peaked in June and decreased significantly from June to

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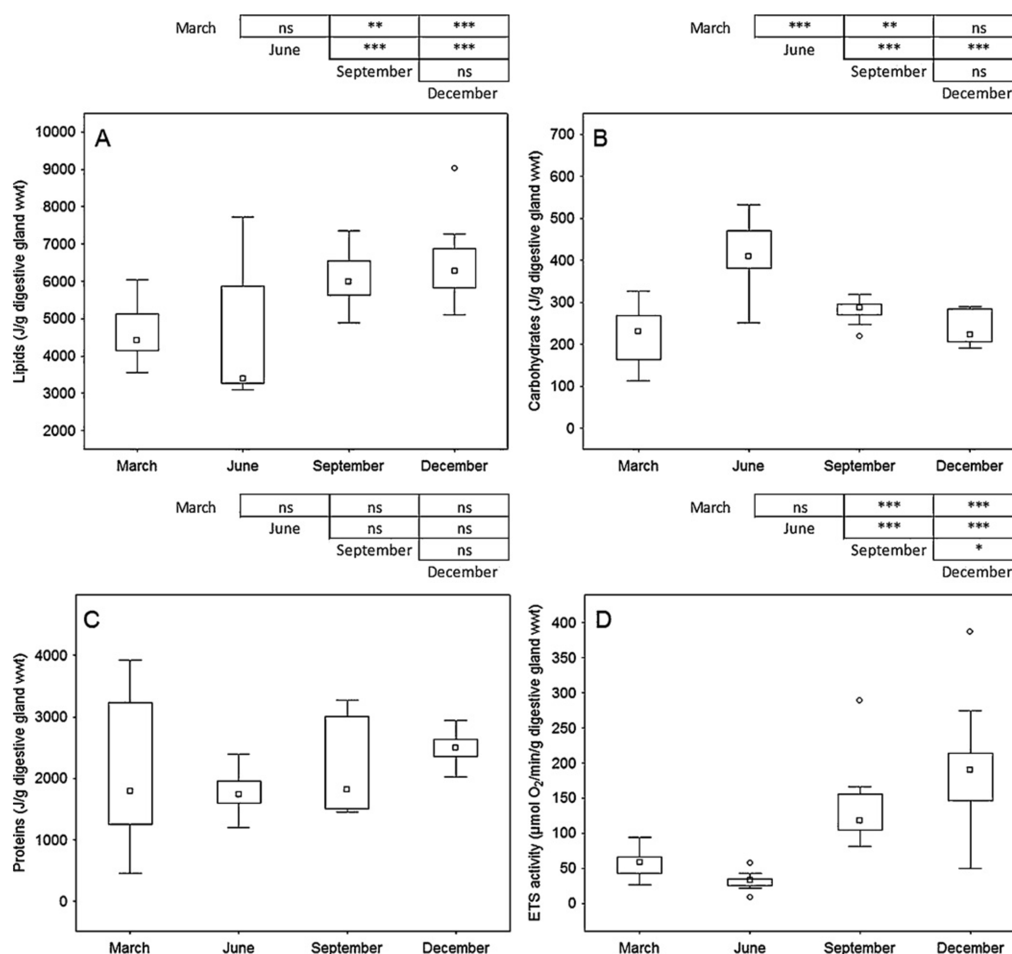

Fig. 2. A Lipid (J/g wwt), (B) carbohydrate (J/g wwt), (C) protein (J/g wwt) contents and (D) electron transport system activity (ETS, μmol O_2/min/g wwt) in the digestive gland of *Mytilus edulis*. Plots represent the median (square), 25–75% percentiles (box), non-outlier range (whisker), outliers (circle) and extreme values (asterisk). The inserted tables indicate significant differences (ns = non-significant, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) between sampling months.

September and remained low in December (Fig. 2B). There were no significant differences in total protein concentrations among seasons except higher levels in December compared to June (Fig. 2C). Furthermore, levels in March showed a high variability. Similarly to lipids, the ETS activity was lowest in March and June and increased significantly until December (Fig. 2D). Individual variability was also high in December. A significant correlation between ETS and lipids in the digestive gland ($r = 0.48$, $p < 0.001$) was found.

Neutral red retention time (NRRT) in blue mussel was highest in June (177 ± 3 min) and significantly lower in March (150 ± 10 min), September (135 ± 9 min) and December (132 ± 9 min). Antioxidant enzyme activities (CAT, GPX and GST) in blue mussel generally showed highest levels in March (Fig. 3). Both the total (Tot-) and selenium (Se-) dependent glutathione peroxidase (GPX) activities were high in March and decreased to reach the lowest levels in September (Fig. 3A). In December, the Se-GPX activity increased again to reach levels similar to March. Catalase activities in March showed a high individual variability compared to the other seasons (Fig. 3B). GST activities decreased significantly from March to September (Fig. 3C). Levels of TBARS in the digestive glands of blue mussel were maintained over the seasons, except for a significant decrease in September (Fig. 3D). Furthermore, a high individual variability in TBARS characterised the March, June and December samples. The TOSC ranged from 584 ± 85 to 726 ± 157 U/mg cytosolic proteins but levels did not vary significantly among months (Fig. 3E).

3.2. Icelandic scallop

Icelandic scallops collected in June and September were significantly larger in average size (length: 6.9 ± 0.1 and 7.0 ± 0.1 cm respectively) compared to those from March and December (length: 6.7 ± 0.1 and 6.5 ± 0.1 cm respectively). PAH concentrations in Icelandic scallops were slightly higher than those found in blue mussels (Table 1). The sum of NPD increased in December, due to high levels of C3-naphthalenes while levels of both NPDs and 16 EPA–PAHs were increased in March compared to June and September. In contrast to blue mussel, the levels in PAHs in Icelandic scallop soft tissue did not seem to follow the seasonal variation of the soft tissue lipid content which was lowest in March.

Levels of metals were in general low and showed no significant differences among months. The total dry weight did not vary significantly over the seasons.

Similarly to blue mussel, total lipids in Icelandic scallop digestive gland were significantly higher in September and December compared to March and June (Fig. 4A). Carbohydrates peaked in June and decreased to reach their lowest levels in September and December (Fig. 4B). No significant differences in total protein concentration were found among seasons (Fig. 4C). Furthermore, the ETS activity was increasing from June to December (Fig. 4D) with significant correlations to total lipids ($r = 0.67$, $p < 0.001$) and carbohydrates ($r = -0.62$, $p < 0.001$) in the digestive gland, similar to what was observed for blue mussel.

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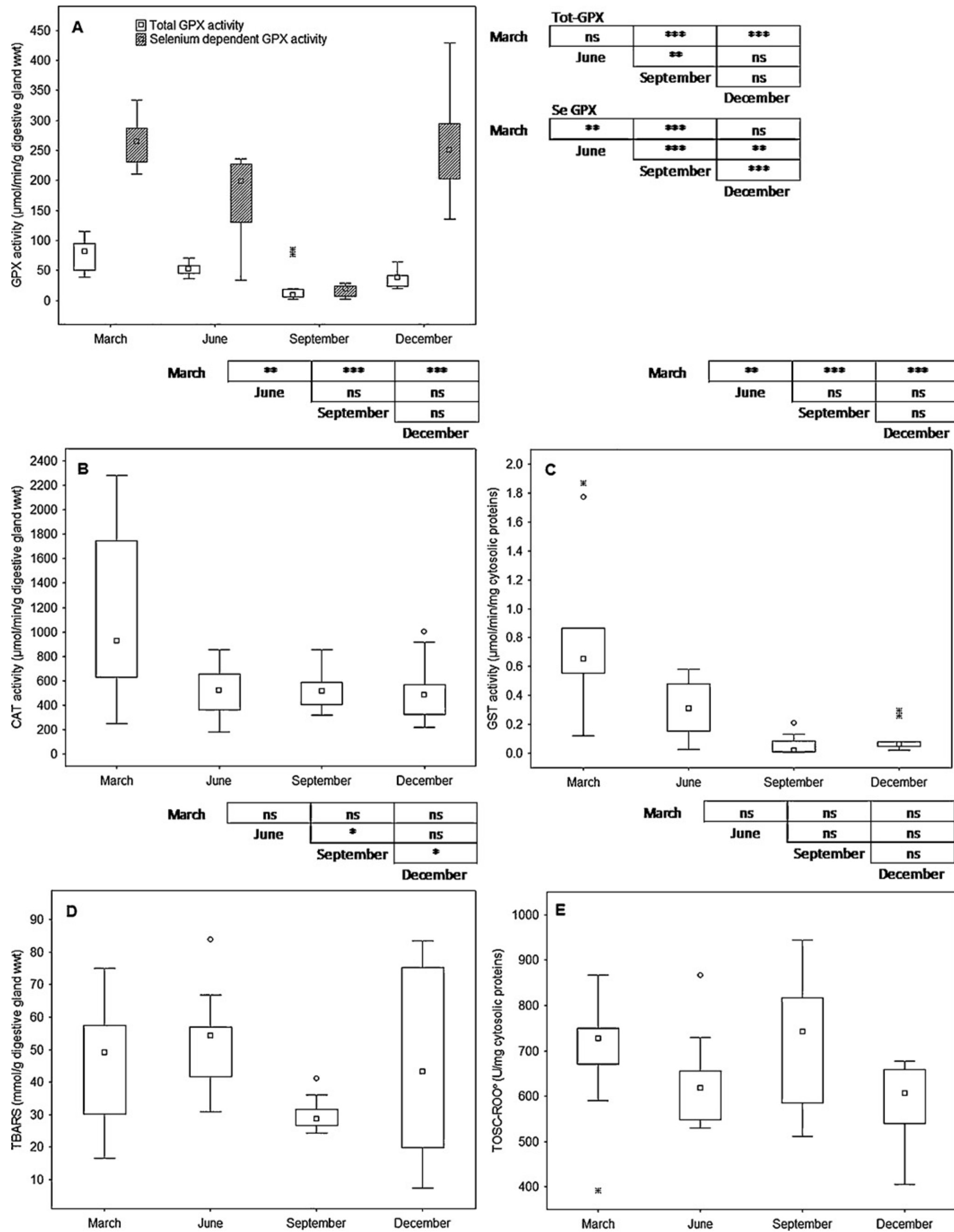

Fig. 3. Antioxidant defence enzyme activities and total oxyradical scavenging capacity (TOSC) in the digestive gland of *Mytilus edulis*. (A) Total and selenium dependent glutathione peroxidase (GPX, µmol/min/g wwt), (B) catalase (CAT, µmol/min/g wwt), (C) glutathione S-transferase (GST, µmol/min/mg proteins), (D) thiobarbituric-reactive substances (TBARS, mmol/g wwt) and (E) TOSC (U/mg proteins) towards peroxyl radicals. Plots and inserted tables as in Fig. 2.

Contrary to blue mussel where antioxidant enzymes were usually highest in March, levels of GPX and CAT activities were relatively constant in all seasons in Icelandic scallop (Fig. 5A and B). GST activity was significantly lower in December compared to September (Fig. 5C). TBARS showed a significant increase in levels from June to December (Fig. 5D). Finally, the TOSC of Icelandic scallop (780 ± 77–900 ± 83 U/mg cytosolic proteins) was in average more elevated than that of blue mussel and varied seasonally with maximum levels in September (Fig. 5E).

3.3. Atlantic cod

Atlantic cod caught in December were significantly smaller in average (length: 36 ± 2 cm, wet weight: 0.45 ± 0.09 kg) compared to the other sampling periods (length: 46 ± 2–51 ± 2 cm, wet weight: 0.96 ± 0.14–1.18 ± 0.11 kg). The sex ratio was almost equal in June and September (1.2 males per female), while more males were caught in March and December (3.0 and 2.6 males per females respectively). In March, 13 of the 21 sampled Atlantic cod were in a mature or maturing stage with significantly larger gonads (GSI = 8.8 ± 1.0%) compared to all other examined immature individuals (GSI < 0.2 ± 0.1%). The mature/maturing individuals also showed significantly higher HSI (2.6 ± 0.3%) compared to the immature individuals (1.2 ± 0.1%). The HSI was highest in June (3.4 ± 0.4%) and lowest in December (1.5 ± 0.2%). The Fulton's condition factor (K) was lower in December (0.74 ± 0.02%) than in the other months (0.78 ± 0.1–0.86 ± 0.03%). The large variability observed in June (0.78 ± 0.1%) was due to 3 individuals with

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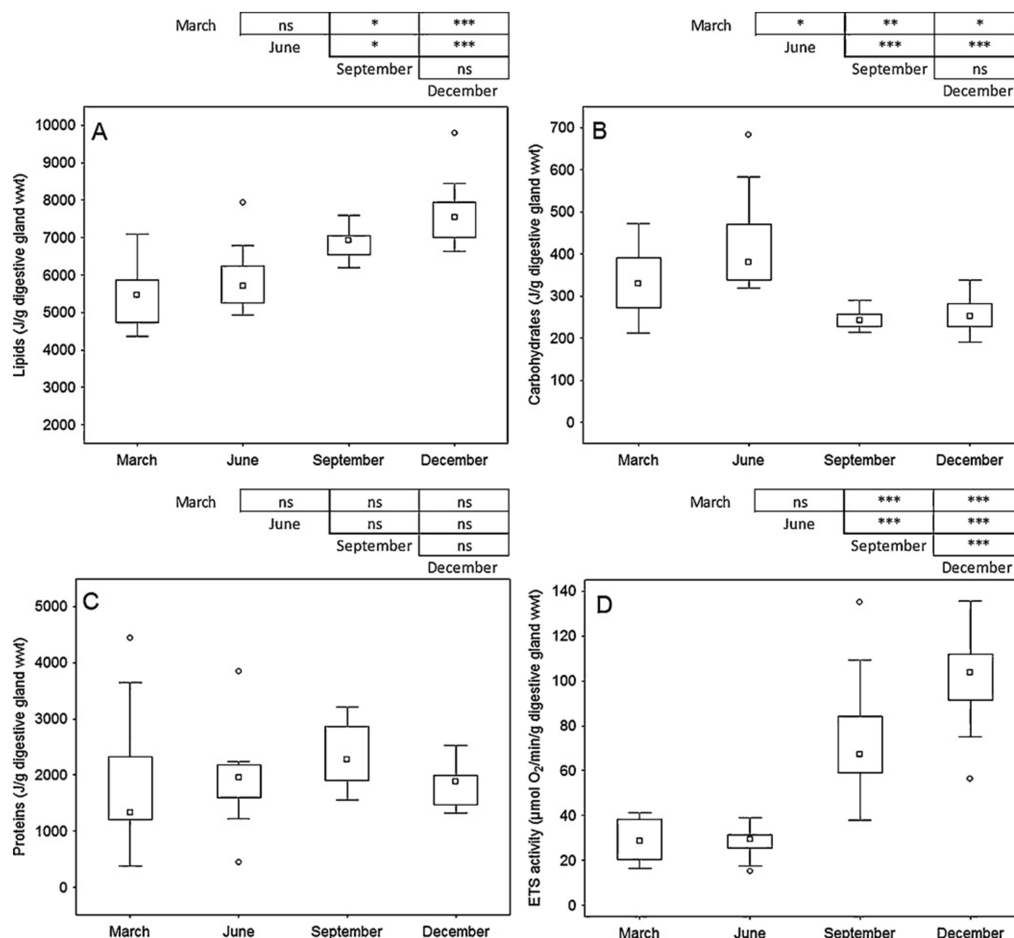

Fig. 4. A Lipid (J/g wwt), (B) carbohydrate (J/g wwt), (C) protein (J/g wwt) contents and (D) electron transport system activity (ETS, μmol O_2/min/g wwt) in the digestive gland of *Chlamys islandica*. Plots and inserted tables as in Fig. 2.

significantly lower condition factors (K = 0.43–0.56) than the rest of the June population (K = 0.83 ± 0.05).

The majority of the examined fish (60%) had empty stomachs in March compared to only 15% in June and none in September and December. Crustaceans such as the northern shrimp (*Pandalus borealis*), were the most represented prey, while some Atlantic cod also had ingested juvenile capelin (*Mallotus villosus*) and European plaice (*Pleuronectes platessa*).

Lipid content in Atlantic cod liver were highest in June compared to March, September and December (Fig. 6A). There were no differences in carbohydrates among seasons, but a large variability characterised the June samples (Fig. 6B). The total protein levels in Atlantic cod were lowest in June and September and highest in March and December (Fig. 6C). The ETS activity was significantly increased in September and December compared to March and June (Fig. 6D). Females in September (49 ± 8.6) and December (38 ± 3.3) showed a higher activity than males in September (62 ± 10.7) and December (52 ± 5.6) respectively, but these differences were not statistically significant. Furthermore, there was a significant relationship between the weight of the fish and the ETS activity in September (r^2 = 0.3192) and December (r^2 = 0.5479), but not in March and June.

The PAH metabolites 1-hydroxypyrene and 1-hydroxyphenanthrene were present in the bile of Atlantic cod while the metabolites 3-hydroxybenzo(a)pyrene and 2-hydroxynaphthalene were undetected (Fig. 7A). Levels of 1-hydroxypyrene were more elevated than 1-hydroxyphenanthrene and were highest in March and December. 1-Hydroxyphenanthrene metabolites were significantly elevated in March. Catalase activities showed significantly lower levels in September compared to the other seasons (Fig. 7B). Total and Se-dependent GPX activities were unchanged over the sampled months (Fig. 7C) as was GST activity (Fig. 7D). TBARS decreased significantly from March to June and remained low in December (Fig. 7E). Finally, EROD activity (not shown) was low (2.2 ± 3.7–7.0 ± 9.2 pmol/min/mg microsomal proteins) in Atlantic cod and did not show any significant seasonality.

4. Discussion

4.1. Seasonality in bivalves

Contaminant levels (both PAHs and trace metals) in the bivalves were in general very low and did not correlate to any of the measured biomarkers (e.g. oxidative stress, EROD, LMS, etc.) in the present study. Blue mussels showed PAH levels in the same range or lower than those reported from locations considered as reference sites in environmental studies in temperate (e.g. Lowe and Fossato, 2000; Brooks et al., 2009; Hellou and Law, 2003) and sub-Arctic (Thomas et al., 1999) areas. PAH concentrations in Icelandic scallops were slightly higher than those found in blue mussels, probably reflecting local anthropogenic pollution of the Icelandic scallop sampling site located closer to Tromsø and along a shipping route. In blue mussel, higher levels in NPDs in September were concomitant with higher lipid contents in the soft tissues, suggesting the accumulation of these compounds in the lipid reserves of the organisms and no remarkable seasonality when normalised to the lipid content (Meador, 2003). In Icelandic scallops, sum of

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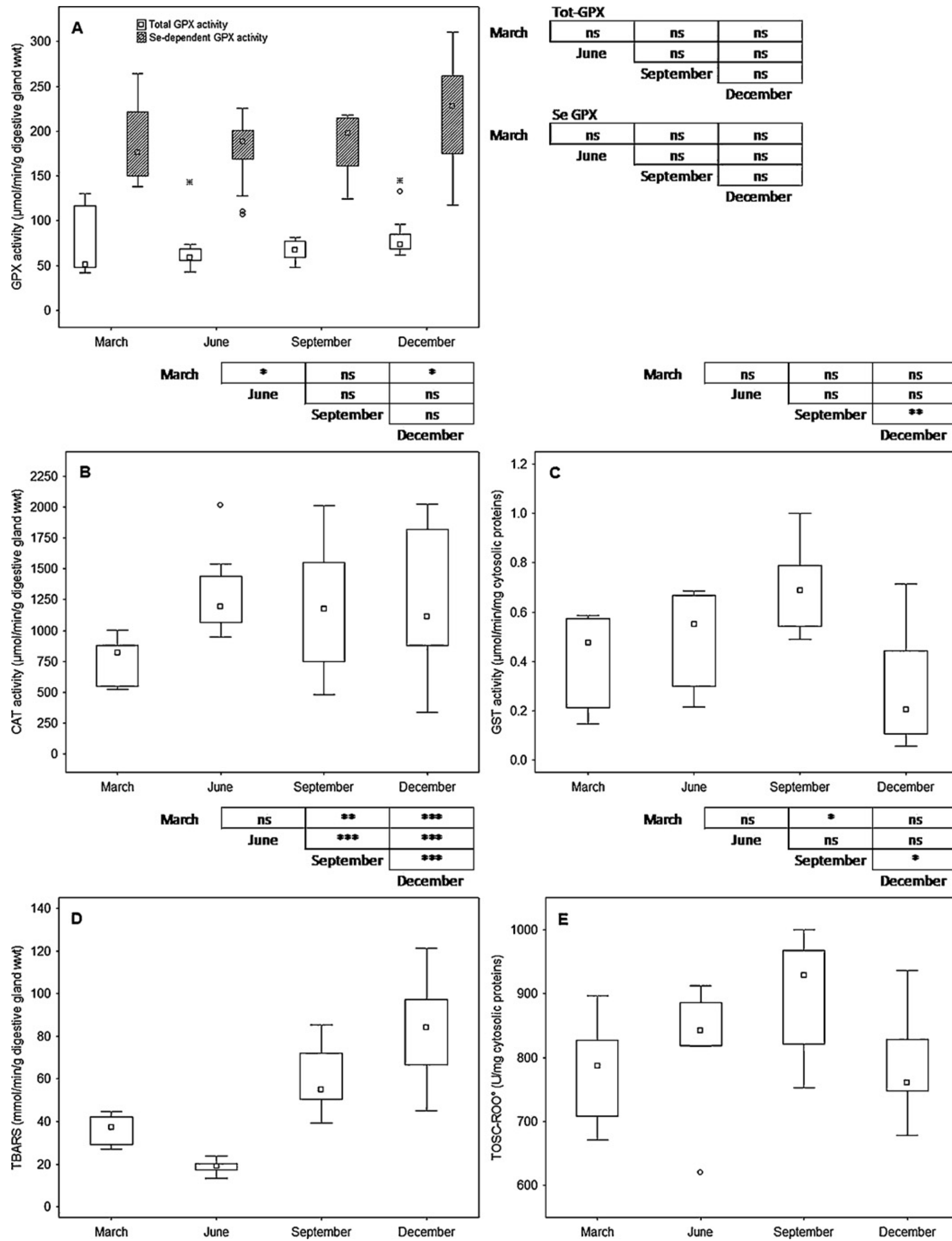

Fig. 5. Antioxidant defence enzyme activities and total oxyradical scavenging capacity (TOSC) in the digestive gland of *Chlamys islandica*. (A) Total and selenium dependent glutathione peroxidase (GPX, µmol/min/g wwt), (B) catalase (CAT, µmol/min/g wwt), (C) glutathione S-transferase (GST, µmol/min/mg proteins), (D) thiobarbituric-reactive substances (TBARS, mmol/g wwt) and (E) TOSC (U/mg proteins) towards peroxyl radicals. Plots and inserted tables as in Fig. 2.

NPDs and 16 EPA–PAHs did not seem to follow the seasonality in soft tissue lipid content, suggesting that other factors such as seasonal changes in direct exposure or feeding rates due to changes in food quality and quantity may be involved in the higher levels of NPD and 16 EPA–PAHs observed during the winter season i.e. December and March (Meador, 2003). Similarly, levels of metals in both blue mussel and Icelandic scallop were low and in the range of what has been reported in reference sites in temperate locations such as the Tyrrhenian Sea (Regoli, 1992), North Sea (Rank et al., 2007), and San Francisco Bay (Martin et al., 1984) as well as in Arctic locations such as the Kara Sea (Sericano et al., 2001). The lower levels of Cu, Zn and As in blue mussel in March could be explained by the decreased soft tissue dry weight at that time.

The similar seasonal pattern in energy reserves, with high levels of total carbohydrates in June in the digestive glands of both blue mussel and Icelandic scallop and an increase in lipids from June to December, indicated that in both bivalves, physiological events were influenced by common environmental cues such as food availability and seawater temperature (Fearman et al., 2009; Blicher et al., 2010). Following low food availability in winter, carbohydrate levels increased as a result of increased feeding in the interval between March and June in both bivalve species (Gabbott, 1976; Dare and Edwards, 1975; Pieters et al., 1980; Ren et al., 2003), corresponding also to the main growth period of shell, soma, gonads and gametes for the Icelandic scallop (Vahl, 1981). Icelandic scallop spawns at the end of June to beginning of July. Spawning coincides with increased food availability and high temperatures

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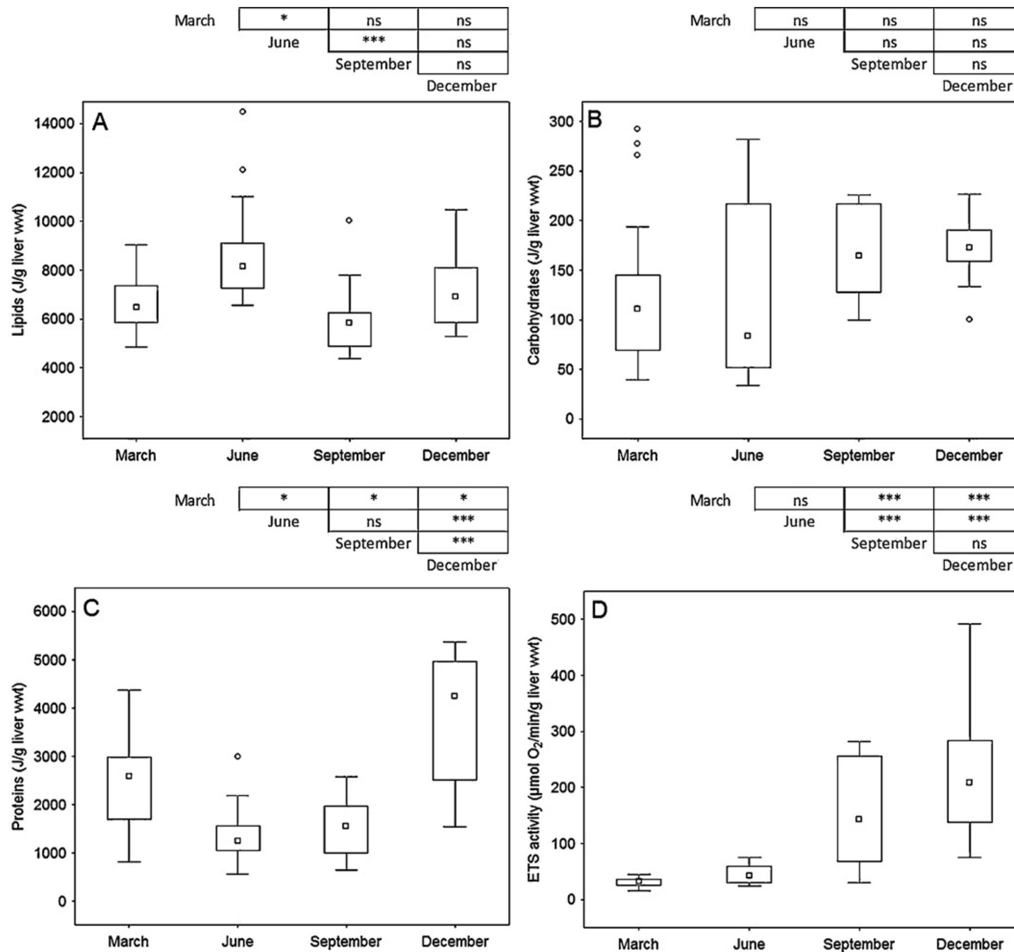

Fig. 6. A Lipid (J/g wwt), (B) carbohydrate (J/g wwt), (C) protein (J/g wwt) contents and (D) electron transport system activity (ETS, μmol O_2/min/g wwt) in the liver of *Gadus morhua*. Plots and inserted tables as in Fig. 2.

(Skreslet and Brun, 1969; Thorarinsdóttir, 1993). The exact timing of gametogenesis and spawning of the blue mussel population from the present study is unknown, but occurs also from late June to July in populations from eastern Norway (Duinker et al., 2008). The depletion of carbohydrate in the digestive gland in September and December may indicate a net loss of energy associated with a reduction of food abundance or quality (Gabbott, 1976; Vahl, 1981). Furthermore, blue mussel and Icelandic scallop showed comparable patterns in ETS activities that were increasing during the autumn and winter. The ETS activity reflects the potential cellular respiration or oxidative metabolic rate of tissues or organisms (Smith and Chong, 1982; Cammen et al., 1990), and is usually found increased during months of high food availability and temperature (Fanslow et al., 2001) contradicting the present data. However, the significant correlations found between ETS and the energy reserves in both bivalves suggested a close link between metabolic activity and energy allocation to, for instance, gametogenesis (Hawkins et al., 1985) or lipid biosynthesis (Zandee et al., 1980). Increased metabolic rates in blue mussel in the autumn and winter were reported previously (e.g. Widdows and Bayne, 1971; Bayne, 1973) and attributed to gametogenesis and high maintenance costs in periods of low food abundance and quality.

Seasonal variation in the NRRT in blue mussel with lower retention times observed during the autumn and winter supported the hypothesis of an increased physiological stress of the organisms with a negative energy balance in this period of low food abundance and quality. Baseline levels in NRRT have previously been shown to vary seasonally due to changes in food availability, water temperature, salinity and reproduction (Hauton et al., 1998; Harding et al., 2004). For instance, blue mussel from the Exe Estuary (UK), showed maximum retention times (30 and 60 min) during gamete development that were well below those found in the present study (>130 min) (Hagger et al., 2010). In general, most levels reported in the literature show relatively lower retention times in blue mussel than in the present study (Bocchetti and Regoli, 2006; Moschino et al., 2011). This may be mainly explained by higher contaminant levels and higher seawater temperatures in more temperate locations. At the present location, contamination was very low and seasonal variation in temperature (Wexels Riser et al., 2010) did not seem to influence the NRRT during the summer. However, the slight but significant increase in NRRT in June could be linked to fresh food input (Tremblay et al., 1998; Zhang et al., 2006; Koukouzika et al., 2009). Nevertheless, the NRRT in blue mussel remained over 120 min at all sampled seasons, indicating that the mussels were in a healthy state at all sampling seasons (OSPAR, 2007; Moore et al., 2006).

The range of biomarker levels (CAT, GPX and GST activities) from the present study was generally in the same range as values reported in blue mussels from other reference sites or control groups (experimental studies) along the North Tyrrhenian Sea (Regoli, 2000), Atlantic coast of France (Manduzio et al., 2004) and Baltic Sea (Kankaanpää et al., 2007) and in Icelandic scallops collected around Svalbard (Baussant et al., 2009). TOSC levels in bivalves of the present study were similar to levels in Icelandic scallop from the White Sea (Regoli et al., 2000) and higher that in mussels and clams from the Adriatic Sea (Bocchetti and Regoli,

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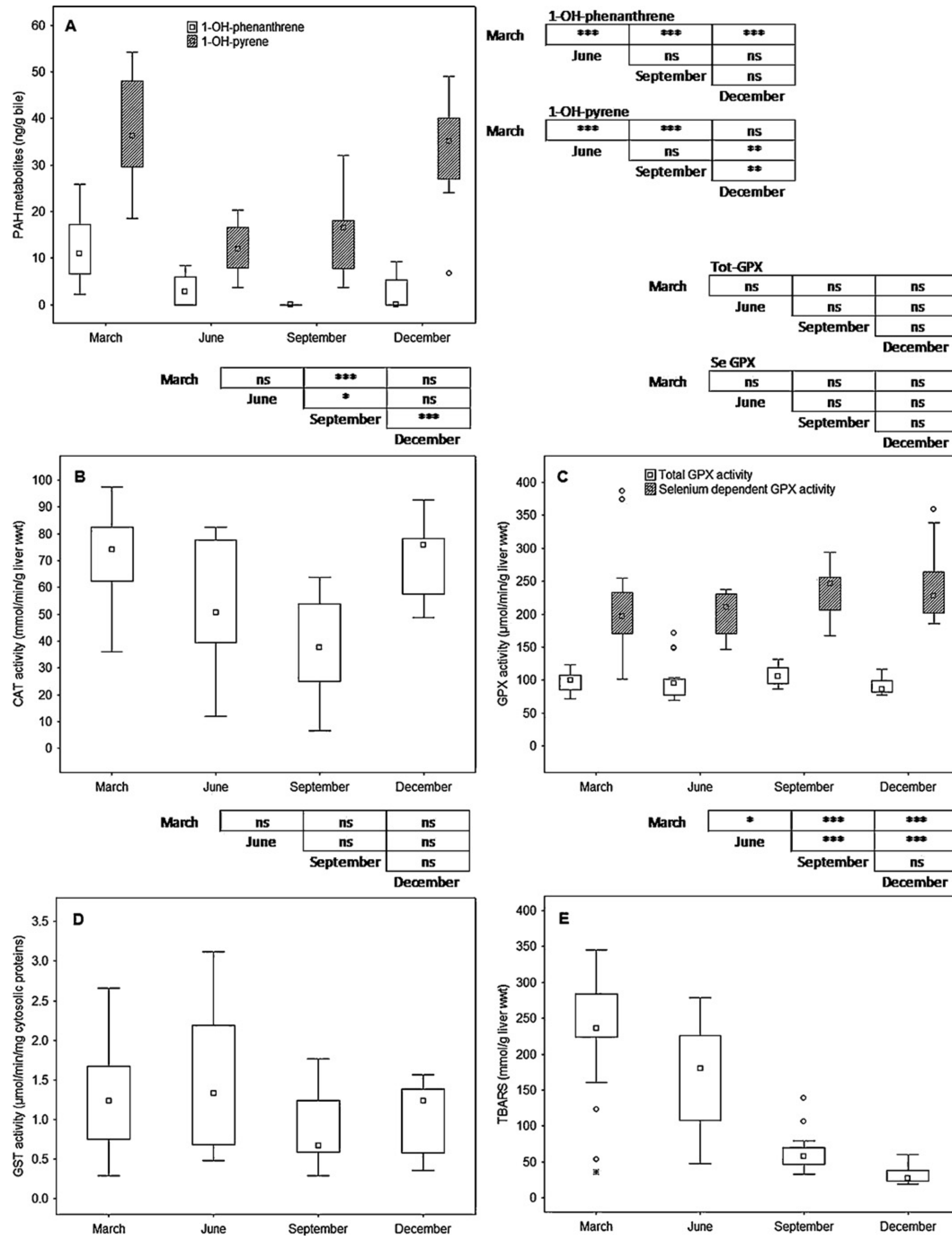

Fig. 7. (A) Polycyclic aromatic hydrocarbons metabolites 1-hydroxypyrene and 1-hydroxyphenanthrene (ng/g bile) in the bile of *Gadus morhua* and antioxidant defence enzyme activities in the liver of *Gadus morhua*, (B) catalase (CAT, mmol/min/g wwt), (C) total and selenium dependent glutathione peroxidase (GPX, µmol/min/g wwt), (D) glutathione S-transferase (GST, µmol/min/mg proteins) and (E) thiobarbituric-reactive substances (TBARS, mmol/g wwt). Plots and inserted tables as in Fig. 2.

2006; Bocchetti et al., 2008). Regoli et al. (2000) suggested that increased TOSC levels in polar compared to temperate organisms were due to exposure to higher oxidative pressure in environments characterised by low seawater temperature and high levels of dissolved oxygen.

The seasonal pattern in antioxidant defence activities differed slightly between the two bivalve species and showed also different seasonal trends compared to species from other locations. For instance, the lack of seasonality in GPX activities and only very weak seasonal variations in other biomarkers (CAT, GST) in Icelandic scallops, agreed with trends reported in blue mussel by Manduzio et al. (2004). By contrast, blue mussels in the present study showed decreasing levels for GPX and GST activities towards the summer, comparable to the seasonal patterns of antioxidant biomarkers (CAT, GST, GPX and TOSC) reported for the bivalves *Mytilus galloprovincialis* and *Tapes philippinarum* from the Adriatic Sea (Bocchetti et al., 2008). Seasonal changes in antioxidant defences may be linked to age, reproductive cycle, food availability and water temperature (Viarengo et al., 1991; Canesi and Viarengo, 1997; Sheehan and Power, 1999; Hagger et al., 2010). The observed increase in the antioxidant enzymes in blue mussel in March, but not in Icelandic scallop, may be a direct effect of the age difference between blue mussels collected in March and the other seasons, as individuals were significantly larger. An increase in the antioxidant defence system during the summer months, when temperature is increased and food abundant, was supported by the increase in TOSC and low TBARS, but not by the antioxidant enzyme activities, in Icelandic scallop. Due to the strong individual variability in

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TBARS and TOSC in blue mussel, no link between oxidative damage and free radical scavenging capacity could be detected. The significant increase in TBARS in Icelandic scallop in September and December could have been partially caused by the production of an excessive amount of reactive oxygen species in the digestive glands, through increased metabolic activity (ETS) (Nelson and Cox, 2008). This was also supported by a reduced antioxidant defence system (TOSC), during maximum levels of TBARS in December (Fig. 5). The joint measure of TBARS and TOSC seemed to provide a better picture of the oxidative stress balance of the organisms throughout the year than the measure of CAT, GPX and GST activities. The cellular mechanisms behind the antioxidant defence system are complex and include both specific enzymes and small molecules that have different cell location and roles, and that may act in cooperation (Di Giulio and Meyer, 2008). Thus, the change in levels in certain enzymes does not necessarily indicate a change in the capacity to cope with pro-oxidant pressures. Low seasonal changes observed for CAT and GPX activities may be considered an advantage for their use in environmental monitoring. However, the general complexity of their responses even in controlled exposure studies (reviewed by Van der Oost et al., 2003) makes these biomarkers less relevant or reduces the interpretability of their variability in environmental monitoring contexts.

In conclusion, both bivalves showed very similar patterns in their energy levels and metabolic activities that could be linked to their natural biological cycle. The interpretability of these biomarker responses can be considered an advantage for their use in environmental monitoring. In opposite, biomarkers of oxidative stress, such as antioxidant enzyme activities, lipid peroxidation and TOSC, have received much less attention and the interpretation of their seasonal variability remains more difficult. Thus, further studies should focus on the mechanisms behind the seasonal patterns observed.

4.2. *Seasonality in Atlantic cod*

A majority of the Atlantic cod caught in March were mature or maturing, with a high GSI.

In June, Atlantic cod were in a better condition as indicated by a high Fulton's condition factor and HSI together with maximum lipid levels in the liver. Lipid and protein content in the liver and the HSI decreased in September and remained low in December, suggesting that energy expenditure was higher than energy intake. This contradicts Eliassen and Vahl (1982) who showed that Atlantic cod from Balsfjord (close to sampling location in the present study) had an increase in liver weight and energy content from October to December in both mature and immature fishes, followed by a decline from January as energy was allocated to the gonads in mature fishes and metabolic processes in both mature and immature fishes.

The increase in ETS activity from June to December suggested increased metabolic activity in the liver and thus energy expenditure that may have been partly due to the transfer of energy from the liver to the gonads and gametogenesis (Dahle et al., 2003). The higher ETS activity in females in September and December, although not statistically significant, may reflect the higher energetic investment of females through for instance vitellogenesis (Lambert and Dutil, 2000; Dahle et al., 2003), which is supported by the decrease in liver lipid content. Interestingly, ETS activity in Atlantic cod liver was negatively correlated to fish size in September and December in accordance with other studies (King and Packard, 1975; Smith and Chong, 1982; Miller, 2009), but not in March and June. Major physiological events that were not identified in the present study, occurring in March and June may have limited ETS activity in the liver of all fishes independently of size.

Antioxidant enzymes (CAT, GPX and GST) activities showed little seasonal variability. Catalase activity was significantly elevated during the gonadal development and spawning season (December and March) but remained low during the resting phase in September. High levels of CAT and TBARS may reflect enhanced oxidative stress during reproductive events. In general, biomarkers are significantly affected by temperature and salinity (Menezes et al., 2006; Cailleaud et al., 2007). As mentioned earlier, the lack of strong seasonality in biomarker responses in Atlantic cod, and also to some extend in the bivalves species, may be due to weak seasonal changes in temperature and salinity at the sampling location (Wexels Riser et al., 2010), in comparison to more temperate location such as the Mediterranean Sea.

Some PAH metabolites were present in the bile, indicating some biotransformation processes. However, levels were very low (Grung et al., 2009; Holth et al., 2009) and did not correlate with EROD activity. The seasonal variation observed for PAH metabolites could be attributed to the momentary feeding status of the organisms at collection, as bile is accumulated in the gallbladder during reduced feeding, thereby increasing the concentration of PAH metabolites (Richardson et al., 2004). In March, most of the fishes had empty stomachs and highest levels in PAH metabolites. Nevertheless, the seasonal variation of PAH metabolites in the bile could not be correlated to any other biomarker response, and was unlikely to be responsible of any adverse effects considering the low values.

EROD activities ($<7.0 \pm 9.2$ pmol/min/mg microsomal proteins) in Atlantic cod were very low in the present study, despite of the presence of some PAH metabolites in the bile. EROD activity was, however, in the same range of values previously reported in this species (Aas et al., 2000; Lyons et al., 2011), although some studies have reported higher levels of baseline EROD activity in Atlantic cod (Goksøyr et al., 1994; Sturve et al., 2006).

Surprisingly, EROD activity did not show any significant changes and remained very low throughout the year. These findings contrasted the high seasonality in EROD activity reported in polar cod (*Boreogadus saida*) by Nahrgang et al. (2010a) with close-to-zero levels in winter and spring to high levels during summer when food availability was important. Indeed, EROD activity is known to be affected by many environmental and biological factors (Goksøyr, 1995), including the reproductive cycle (Stegeman et al., 1982; Larsen et al., 1992), diet (Jimenez and Burtis, 1989; George and Henderson, 1992), temperature (Andersson and Förlin, 1992; Sleiderink et al., 1995) and age of the organisms (Sleiderink et al., 1995). Low levels in EROD activity and a lack in seasonality may be explained by the accumulation of certain trace metals in the liver of Atlantic cod, known to inhibit the biotransformation metabolism (e.g. Benedetti et al., 2007). However, this hypothesis cannot be verified with the available data.

4.3. *Recommendations for environmental monitoring*

The seasonal patterns in biomarkers between bivalves were very similar, while Atlantic cod showed marked differences both in levels and patterns. To the best of our knowledge, no data have been published reporting on the seasonal baseline levels of commonly used biomarkers for Atlantic cod, although this fish is widely used in environmental monitoring along the coast of Norway (Hylland et al., 2008; Balk et al., 2011; Brooks et al., 2011). The relatively low seasonal variability in baseline levels of biomarkers in Atlantic cod can be regarded as a strong advantage for environmental monitoring because any deviation can indicate anthropogenic impact. However, the present study only shows a snapshot of some physiological and biochemical parameters during four seasons and important biological events were most likely undetected due to the large sampling window. Thus

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an increased sampling frequency is necessary in order to determine if the observed lack of variability in biomarkers is consistent throughout the year.

Seasonal variations in biomarker baseline levels are acceptable in environmental monitoring as long as the causes (reproduction, temperature, etc.) and timing are understood. Although the data were based only on four sampling periods, the measure of the energy reserves and the potential metabolic activity provided useful information to determine the seasonal physiological status of these species and for the interpretation of the pollutant biomarker. Thus, these parameters should be considered complementary to the more routine pollutant biomarkers (oxidative stress, biotransformation, etc.) in future environmental monitoring procedures. Nevertheless, important gaps in knowledge remain concerning the biological cycle of these bivalve species in the Barents Sea. It is therefore important to further investigate the effect of organism size, age and maturity status on the biomarker responses, as they are known to influence biomarker levels. Long-term monitoring data of the physico-chemical environment (temperature, salinity, etc.) of the sampling locations would also help in the interpretation of the biological data. A thorough sampling design should include the measurement of physico-chemical variables of the environment as well as a thorough characterisation of the selected organisms (gender determination, maturity status and age if possible). Furthermore, all endpoints should be analysed in the same tissues in order to correlate contaminant burden with biomarker responses.

The preferred biomarkers (TBARS, TOSC, LMS) in the present study were those that were easiest to interpret in relation to their seasonality and which are known to increase response with contaminant burden, opposite to the antioxidant enzyme activities characterised by a bell-shaped response (Viarengo et al., 2007). In order to validate these biomarkers for their use in monitoring programmes in the Barents Sea, future studies should investigate how well these biomarkers respond to contaminants and define threshold levels for effects. Furthermore, it is also important to determine how pollutant induced responses may behave in conjunction with natural variability (e.g. synergic, antagonistic effects, etc.). Finally, other biomarkers known for their sensitivity to contaminants and recommended for biological monitoring in invertebrates and fish, such as biomarkers of genotoxicity (DNA adducts, comet assay) or endocrine disruption (vitellogenin) should be considered in future studies (ICES, 2010).

From the three species selected in the present study as sentinel species for the Barents Sea, the two selected bivalves seemed to be the most relevant indicator species. Bivalves are often better as environmental indicators than fishes due to their sessile lifestyle, high filtration capacity and direct contact with the sediment where pollutants accumulate. Furthermore, the seasonal variation in biomarkers of the bivalves was easier to interpret than that of Atlantic cod. Both bivalve species were convenient species to sample and handle in the laboratory. However, blue mussel often provided too little tissue per individual for analysis of a battery of biomarkers, and Icelandic scallop was in that sense more appropriate. Although blue mussel have recently been reported on the western coast of Spitsbergen (Svalbard) (Berge et al., 2005), the wider geographical distribution of Icelandic scallop and its abundance makes it an ideal indicator species for environmental monitoring in the entire Barents Sea as it is found from the north Norwegian fjord systems to north of Svalbard. The main drawback of using Icelandic scallop in environmental monitoring is the lack of long-term monitoring data and studies reporting biomarker responses to pollutant in this species. In contrast, there are increasing numbers of biomarkers with established assessment criteria for blue mussel, which are based on many years of monitoring data (ICES, 2008, 2010, 2011).

Considering the increasing oil and gas production and transport in Barents Sea, both on the Norwegian and Russian side, this paper provides the first biomarker baseline data set in these sub-Arctic species. At the light of the recent dramatic oil spills that have taken place in Europe (Erika, Prestige) and in the US (Deep Water Horizon), we are stressing the need for strengthening such a study, with an international approach such as ICES, which has been established in other areas (i.e. North Sea, Baltic), and most importantly, by establishing an interactive collaboration with the Russian Institutes for paving the future of a common Norwegian-Russian biomonitoring programme for the Barents Sea.

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