Do polychaete digestive fluids affect the bioavailability of sediment-bound PAHs?

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Master Thesis in Ecotoxicology

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Preface

This thesis in ecotoxicology is part of the master's degree in biology at the University of Oslo. The work in this thesis was carried out at the Norwegian Institute for Water Research and at the department of Biology at the University of Oslo, from January 2011 to December 2012. The work was supervised by research scientist Anders Ruus (Norwegian Institute for Water Research). Ketil Hylland was the internal supervisor from the University of Oslo.

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Abstract

Marine organisms are exposed to a wide range of potentially hazardous substances, from natural and/or anthropogenic sources. Organisms in polluted sediments may accumulate high amounts of chemical contaminants and transfer these up the food chain. For a chemical to bioaccumulate, it needs to be bioavailable for accumulation. This study aimed to assess whether digestive fluids from the subsurface feeding polychaete *Arenicola marina* would affect the bioavailability of sediment-bound PAHs, and compare natural to artificial gut fluids.

Arenicola gut fluid, a BSA solution, an artificial gut fluid and seawater were incubated for 4 hours with two contaminated sediments and control sediment. The supernatants were then analyzed for 19 different PAHs using GC-MS. The solutions were also analyzed for protein content, lipase- and protease activity and surfactant properties. The results showed that the *Arenicola* gut fluid led to a small increase in the bioavailability of sediment-bound PAHs, and that the artificial gut fluids had a much greater effect on increasing the bioavailability of PAHs than *Arenicola* gut fluid. This may be partly due to a high *Arenicola* gut natural content of PAHs. The results also showed that the enzyme activity seemed to have little effect on the bioavailability of the PAHs, while the protein and bile salt content appeared to have a greater impact on the ability of the test solutions to increase the bioavailability of sediment-bound PAHs. The high bioavailability of some of the PAHs such as naphthalene and acenaphthylene may be attributed to their volatility in combination with the ability of the test solutions to increase their bioavailability. The results showed that one may be at risk of overestimating the bioavailability of gut fluids when using artificial gut fluids as a surrogate for real gut fluids when performing bioavailability tests.

Abbreviations

AP – Anthropogenic pollutants

BSA – Bovine serum albumin

DCM - Dichloromethane

FITC – Fluorescein isothiocyanate

GC-MS - Gas chromatography-mass spectrometry

GPC - Gel permeation chromatography

HCl – Hydrochloric acid (hydrogen chloride)

LC_{50} - Lethal concentration 50

N_2 – Nitrogen gas

Na – Sodium

Na_2SO_4 – Sodium sulfate

rpm – Rounds per minute

PAH – Polycyclic aromatic hydrocarbons

SEC - Size exclusion chromatography

Sim – Single ion monitoring

TOC – Total carbon content

1 Introduction

1.1 Background

Anthropogenic activities have caused inputs of huge quantities of organic pollutants to the aquatic environments over the last century (Lake et al. 1979; Voparil et al. 2004). Gas production, food manufacturing, incineration of industrial and domestic waste and power generation from fossil fuels are some the industrial activities resulting in the production of anthropogenic pollutants (AP) (Neff 1979). Due to their physical and geochemical properties, marine sediments around urban areas serve as basins for AP (Voparil and Mayer 2004), and an accumulation of the pollutants may take place in these areas and pose a threat to the organisms living in and above the sediments.

Polycyclic aromatic hydrocarbons (PAHs) are one of several classes of organic environmental toxicants which are present in marine environments due to both natural and anthropogenic activities (Hylland 2006; Onozato et al. 2010) (figure 1.1). They consist of two to several aromatic rings (Hazardous Substances Data Bank 2003; Jørgensen et al. 2008), with or without alkyl chains, and some are known to be carcinogenic and mutagenic (Neff 1979). Due to their low water solubility and hydrophobic properties, PAHs readily adsorb to particulate materials and solid surfaces in water (Neff 1979; Jørgensen et al. 2008). Adsorption occurs on substrates such as carbon, glass, silica, soil, clay and organic particles Therefore, PAHs may accumulate in marine sediments.

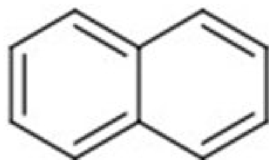
PAHs can be divided into two molecular weight classes based on chemical, physical, and biological properties; 2-3 ring aromatics and the higher molecular weight 4-7 ring aromatics. Low molecular weight PAHs will in general be acutely toxic to aquatic organisms, whereas the high molecular weight PAHs are acutely toxic to a lesser extent (Neff 1979).

Rossi and Neff (1978) performed acute toxicity tests of some PAHs to the polychaete *Neanthes arenaceodentata*. They observed that after 96 hours, the LC_{50} increased as the molecular weight of the PAH increased (naphthalene: 3.8 ppm; 2,6-Dimethylnaphthalene: 2.6 ppm; 2,3,6-Trimethylnaphtalene: 0.32 ppm; fluorene: 1 ppm; phenanthrene: 0.6 ppm), while the high molecular weight PAH (chrysene, benzo[a]pyrene and dibenz[a]anthracene) had no

measurable acute toxicity within the 96 hours. However, all of the 20-30 proven PAH carcinogens are in the high molecular weight group (Neff 1979).

The presence of PAH in the tissues of a wide range of aquatic organisms, although mainly invertebrates, indicates that these organisms are able to accumulate PAH present at low concentrations in the surrounding medium, food, or sediments (Neff 1985). In aquatic invertebrates, bioaccumulation is the net result of either passive or active uptake, and excretion of contaminants (Streit 1993). This means that bioaccumulation is a combination of bioconcentration and uptake from food. Accumulation may lead to an increased concentration of the substance in the organism than in the surrounding environment. For a chemical to bioaccumulate, it has to be bioavailable for accumulation (Ruus et al. 2005). Since fish and vertebrates have the ability to metabolize PAHs, biomagnification of these compounds is not an issue. However, invertebrates bioaccumulate PAHs and may therefore expose organisms that feed on them for high amounts of PAHs (Neff 1979).

a)



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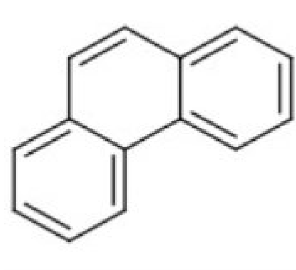

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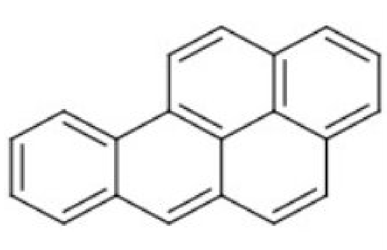

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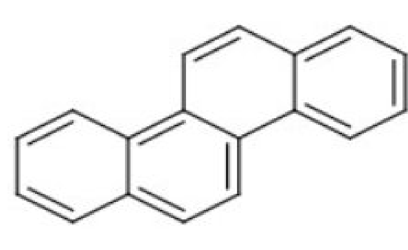


Figure 1.1 Ring structure of some selected PAH. Compounds are: a) naphthalene, (b) phenanthrene, (c) chrysene, and (d) benzo[a]pyrene.

Bioaccumulation is a complex phenomenon (figure 1.2), and knowledge cannot be obtained solely by chemical analysis of contaminants in sediments and biota. The different chemical and physical properties of the contaminants and the sediments will affect the bioavailability.

The interspecific differences in the uptake and excretion of the contaminants in the organisms may also be a factor that can complicate the quantification of bioaccumulation (Neff 1979; Mayer et al. 1996; Ruus et al. 2005).

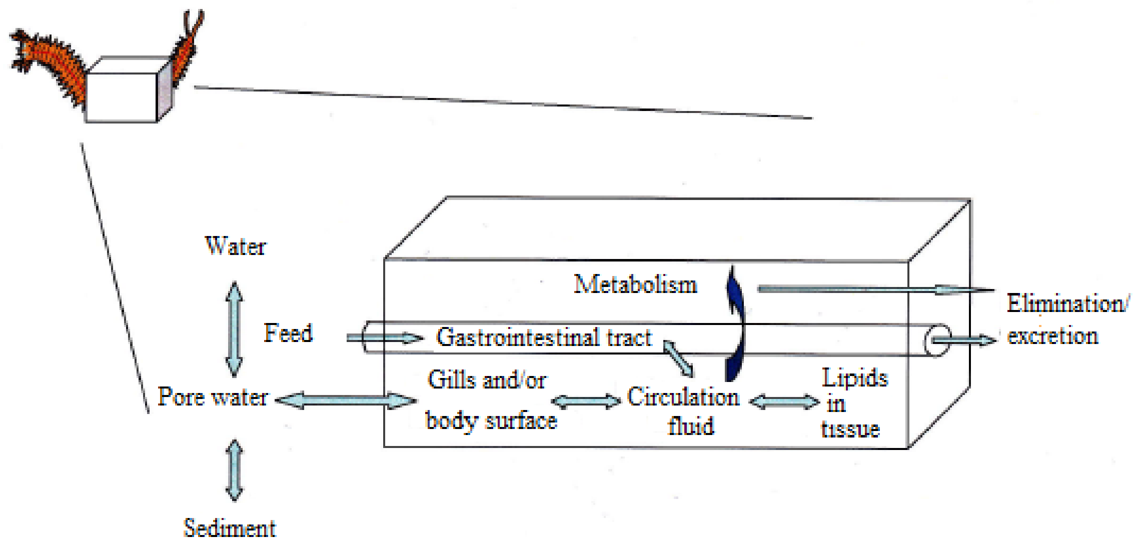


Figure 1.2 Schematic figure of the routes and mechanisms that theoretically are involved in bioaccumulation of PAH in benthic organisms. Figure modified from Ruus (2009).

Many benthic animals ingest sediment and remove organic materials from it as a source of nutrition. These animals are called deposit feeders. The lugworm, *Arenicola marina* (figure 1.3), is a deposit-feeding polychaete that lives in U-shaped burrows on the lower intertidal zones in sandy to muddy sediments, and are abundant along most Western European coasts (Kaag et al. 1998; Kristensen 2001).

Figure 1.3 *Arenicola marina*, a deposit-feeding polychaete.

During the last two decades, the lugworm has been commonly used in bioaccumulation and bioavailability assay (Mayer et al. 1996; Mayer et al. 1997; Lawrence et al. 1999; Voparil and Mayer 2000; Voparil et al. 2004; Voparil and Mayer 2004; Casado-Martinez et al. 2008). Lugworms are thought to be suitable organisms for the biomonitoring of sediments due to their limited capacity to biotransform organic contaminants. This means that they have a low mortality rate and high tolerance to environmental contaminants (Kaag et al. 1998).

Previous studies have shown that the digestive system of many benthic organisms is the main route of uptake for sedimentary contaminants (Landrum 1989; Leppanen and Kukkonen 1998). The bioavailability of sediment contaminants to any marine invertebrates will therefore rely upon the biochemical conditions of the digestive system. The intestinal fluids of deposit feeders have a high enzyme activity and have strong surfactant properties that may possibly increase the desorption of PAHs from sediments, thus making the PAHs more bioavailable (Mayer et al. 1997).

Voparil and Mayer (2000; 2004) observed that intestinal fluids from the lugworm *Arenicola marina*, had the capacity to make the PAHs in the sediment it ingested more bioavailable, and several studies have shown that polychaetes are able to bioaccumulate PAHs from the sediments they ingest (Leppanen 1995; Mayer et al. 1996; Morales-Caselles et al. 2008). Voparil and Mayer (2004) developed an artificial gut fluid, based on the digestive fluids of

the *Arenicola marina*, to serve as a surrogate for the real gut fluids and allow easier adoption of the in vitro incubation approach to bioavailability testing.

1.2 Aims of the study

Knowledge about the bioavailability of PAHs in sediment is essential for assessing the potential for bioaccumulation of PAH in marine food webs. Partly because there are uncertainties related to both uptake and excretion in evaluations of bioavailability and bioaccumulation of contaminants, EUs guidelines for risk assessment of polluted sediments contain additional safety factors for hydrophobic compounds.

The objective of this study was to clarify whether digestive fluids from the subsurface feeding polychaete *Arenicola marina* would affect the bioavailability of sediment-bound PAHs, and compare natural to artificial gut fluids.

2 Materials and Methods

2.1 Sediments

The three sediments used were collected in 2010 (Ruus et al. 2010).The two contaminated sediment samples were collected from Karmøy and Sunndalsfjord, and the uncontaminated control sediment was taken from Jeløya. The sediments from Karmøy and Sunndalsfjord were obtained using a 0.1 m^2 Van Veen grab, while the control sediment was obtained at the waterfront using a shovel. All sediments were stored at ~4°C after collection (Ruus et al. 2010). The sediments were homogenized by stirring with a paint stirrer, and transferred to small buckets. Before the experiments, they were re-homogenized, due to sedimentation, by stirring with a stainless steel spoon/spatula.

2.2 Animals

Lugworms (*Arenicola marina*) were imported from a commercial supplier in Scotland (Green Blue Fish Ltd, Scotland). The polychaetes were transported in a protective environment containing activated charcoal, and the gut fluids were extracted on the same day of arrival.

2.3 Methods

2.3.1 Extraction of gut fluids

The gut fluids were extracted using Pasteur pipettes. This was done by inserting the pipette gently into the posterior end of the lugworms (while alive), and letting capillary forces slowly draw the fluids from the gut (figure 2.1). The fluids were transferred to cryotubes and put on ice before they were eventually stored at -80°C until use.

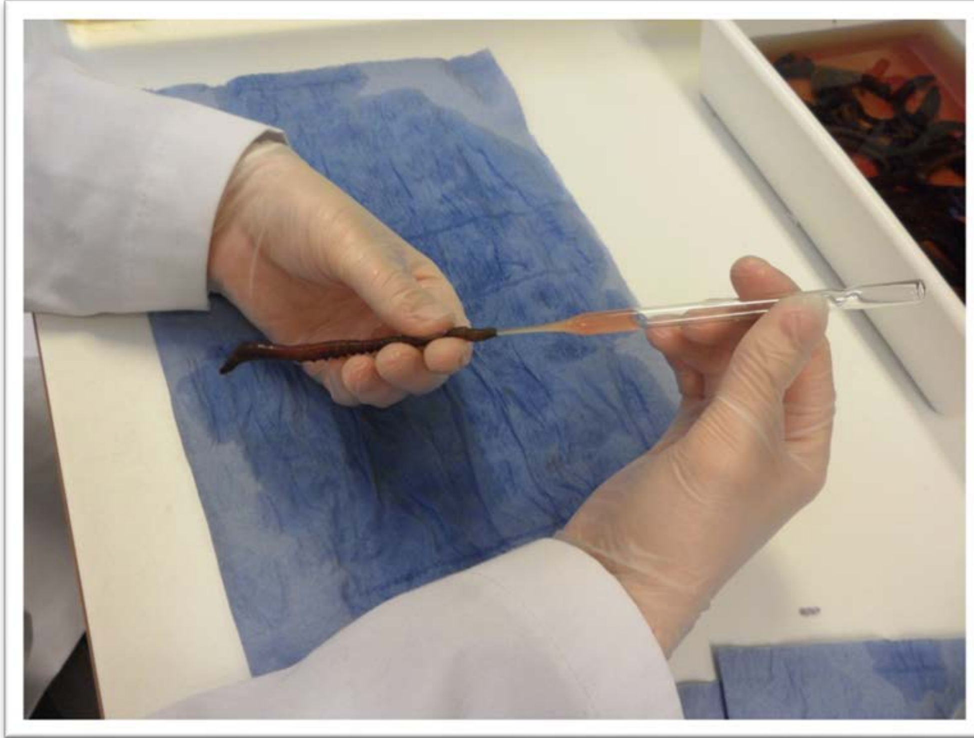


Figure 2.1 The extraction of gut fluids with a Pasteur pipette.

2.3.2 Preparation of test solutions

Artificial gut fluids were prepared as described by Voparil and Mayer (2004) with modifications. Two and a half grams of Bovine Serum Albumin (BSA) and 4.2 g of Na taurocholate were added to 500 mL of autoclaved and filtered seawater (see table 2.1.1 and 2.1.2). The same amount of BSA and seawater was used for the BSA solution (table 2.1.2). During the making of the solutions, the water was kept on ice. Both samples were mixed well, and the solutions were stored at 5°C until use. The real gut fluids were diluted 50:50 with the sterile seawater.

Four 75 mL dark glasses were filled with 24 mL of each solution, and stored at -20°C until gel permeation chromatography (GPC) and gas chromatography-mass spectrometry (GC-MSD) analysis.

Table 2.1.1 Properties for the seawater.

Seawater	
Origin	Drøbak
From depth	25-30
pH	7.85
PSU	22
Filtered through	GF/C filter with undefined pore size of 1.2 µm
Autoclaved	121°C for 15 min

Table 2.1.2 Content and conditions of the artificial gut fluids and BSA solution.

	artificial gut fluid	BSA solution
Seawater	500 mL	500 mL
Na taurocholate	4.2 g	-
BSA	2.5 g	2.5 g
pH	7.42	7.69

2.3.3 Orbital shaker experiment

The three sediments were mixed with four fluids; sterile seawater, sterile seawater with BSA, artificial gut fluid and real gut fluid, and there were four technical replicates of each treatment (table 2.2).

Table 2.2 The experimental setup for the orbital shaker experiment, with 4 technical replicates of each treatment.

	Seawater	BSA	Artificial gut fluid	Lugworm gut fluid
Control	4	4	4	4
Sunndalsfjorden	4	4	4	4
Karmøy	4	4	4	4

Nine grams x 4 of the control sediment, and 10 g x 4 of the Karmøy and Sunndalsfjord sediment were weighed out, and transferred to individual centrifugation tubes. To each treatment, there were added 24 mL of fluid. The target was to get approximately 6.5 g dry sediment to 24 mL of solution.

The centrifuge tubes were placed on an orbital shaker (KS501 digital orbital shaker; IKA Labortechnik®) for 4 hours at a speed of 160 rpm. Afterwards, the tubes were put in a centrifuge (Megafuge 1.0; Heraeus Sepatech®) at 3000 rpm for 15 min. The supernatants were extracted and added to labelled 75 mL sample vials. These were stored at -20°C.

2.3.4 GPC preparation

Gel permeation chromatography (GPC) is a type of size exclusion chromatography (SEC), which separates substances on the basis of molecular size.

The samples were transferred from the sample vials to centrifugation tubes. Dichloromethane (DMC) was added to the vials in order to detach any PAHs that might have been attached to the inner walls of the glass. This was also transferred to the centrifugation tubes. Hundred µL of PAH 162 internal standard (see table 2.3), with a concentration of 2 ng/mL, were added to each tube with a Gilson pipette. The centrifugation tubes were then put on an orbital shaker for 1 hour at a speed of 160 rpm.

The glasses were centrifuged at 2800 rpm for 10 min before the supernatant (DCM + PAH) were transferred to 60 mL vials with a Pasteur pipette. One teaspoon Na_2SO_4 was added to each vial, in order to get rid of possible water present in the samples. The liquid was gently transferred to new 60 mL vials, being careful not to pour any of the Na_2SO_4 into the new vials.

There were added droplets of iso-octane to the vials before they were placed in an evaporation machine (TurboVap LV; Zymark®). This was done to prevent the samples from drying out when concentrating the samples under N_2 at 35°C (water bath).

The samples were filtrated through 2 mL centrifuge filters. The filtered solutions were then added to 2 mL chromacol vials with caps, and placed in the GPC machine.

Table 2.3 The content of the internal standard PAH 162, dissolved in toluene.

Compound	Amount
NAP-D8	1 mL
ACNLED-D8	1 mL
DBTHi-D8	1 mL
PYR-D10	2 mL
BAA-D12	1 mL
PER-D12	0.5 mL
BiP-D10	507.6 µL

2.3.5 GC-MSD analysis

The presence of PAHs in the samples was investigated by GC-MSD analysis. GC-MSD is a technique that combines the separation power of GC (gas chromatography) and the detection power of MS (mass spectrometry).

After being through the GPC, the samples were evaporated under N_2 at 35°C (Turbo Vap LV; Zymark®) The remaining concentrated content was then added to 0.9 mL chromacol vials, and filled with DCM before being re-concentrated with the help of a reacti-vap evaporation machine (Reacti-Vap model 18780; Pierce®) and N_2.

<u>GC-MSD analysis:</u>
The analysis was done with assistance from Alfhild Kringstad and Andreas Sven Høgfeldt at the Norwegian Institute for Water Research. See table 2.4 and 2.5 for the GC-MS conditions. The PAHs were identified on the basis of SIM (single ion monitoring). The molecular ion of the compound was registered within a given time interval, and the compounds were quantified on the basis of an internal and an external standard.

Table 2.4 The GC-conditions.

GC-conditions	
Carrier gas	Helium
Column	J&W® DB5-MS (30 m x 0,25 mm i.d x 0,25 µm flim)
Constant flow	1.2 mL/min
Injection flow	1 µL
Injection temperature	280°C
Oven temperature program	60°C (2min) - 7°C/min - 250°C(0min) -15°C/min - 310°C(15min).
Solvent delay	9 min
Transfer line temp	280°C
Software	Chemstation

Table 2.5 MS-conditions.

MS-conditions	
Temperature ion source	230°C
Temperature quadrupole	150°C

2.3.6 Sediment analysis

The analysis of the sediment samples was done with assistance from Erling Bratsberg, at the Norwegian Institute for Water Research.

The three sediments were dried out with sufficient amount of hydromatrix. The samples were put in 20 ml extraction cells, with cellulose filters in the top and bottom of the cell, and spiked with 400 µl PAH162 internal standard. The cells were put in an ASE 200 accelerated solvent extractor (Dionex) for extraction (see table 2.6 for the extraction conditions).

Table 2.6 Conditions for sediment extractions.

Extraction conditions:	
Temperature	100°C
Pressure	2000 psi
Solvents	50% cyclohexane and 50% DCM
Static extraction	5 min
Static cycles	3
Flush volume	60%
Purge time	60 seconds

After the extraction, the solutions were dried with Na_2SO_4 and concentrated down to 10 ml under N_2. Based on the colour of the extracts and the background information about the sediments (Ruus et al. 2005; Ruus et al. 2010), it was assumed that the extracts from the two contaminated sediments had high levels of PAHs. Therefore, it was added additional 400 ng internal standard to 0.5 ml of these extracts, and then analysed with the help of GC-MSD.

Sediment subsamples were freeze-dried, crushed and acidified (1N HCl), and analysed for total carbon content (TOC) by catalytic combustion at 1800°C in a Carlo Erba 1106 elemental analyser.

2.3.7 Protease activity

The protease activity in the five solutions (BSA-solution, artificial gut fluid-solution, seawater and gut fluids (both diluted 50:50 with seawater and undiluted)) were determined using Abcam's Protease Activity Assay Kit, with modifications. In this assay, there will be a reaction between proteases and fluorescein isothiocyanate (FITC)-labelled casein. When the proteases present in the samples cleave the FITC-casein substrate, it abolishes the quenching of the fluorescence label.

Fifty µl of test samples were added into a 96-well microtiter plates. Five µl Positive Control Solution and 45 µl Assay Buffer was used as positive control. Reagent background contained 50 µl Assay Buffer. To each well (not the standard), a reaction mix was added, containing 48 µl of Assay Buffer and 2 µl Protease Substrate Solution. There were four replicates for each sample, and three parallels for each standard series.

In addition, there were wells that included just 50µL of the test samples and 50µL of Assay buffer. This was done in order to see if there was any fluorescent substrate in the samples. There were four replicates of each sample.

The fluorescence were measured at Ex/Em = 485/530 nm at every minute for 30 minutes by a spectrophotometer (Synergy MX, Biotek).

The standards were: blind (0 nmol FITC standard), standard 1 (0.05 nmol FITC standard), standard 3 (0.1 nmol FITC standard), standard 4 (0.15 nmol FITC standard), standard 5 (0.2 nmol FITC standard) and standard 6 (0.25 nmol FITC standard). FITC standard was dissolved in Assay Buffer.

2.3.8 Lipase activity

The lipase activity in the five solutions was determined using Abcam's Lipase Detection Kit, with modifications. In this assay, the lipase activity will be observed as a change in the OxiRed probe absorbance, when the lipase hydrolyses a triglyceride substrate to glycerol.

The gut fluids (both diluted 50:50 with seawater and undiluted) were filtrated through 0.45 µm non-pyrogenic Sterile-R filters.

Fifty µL of test samples and control samples were added into a 96 microtiter plate. The control samples were added to the plate in order to correct for possible glycerol in the samples. Lipase positive control (5 µL lipase positive control and 45 µL Assay Buffer) was added to the plate. 100 µL of Sample Reaction Mix (table 2.7) was added to each well containing the Glycerol Standards, Lipase positive controls and test samples. Hundred µL Control Reaction Mix (table 2.7) was added to the wells containing sample control. There were four replicates for each sample, and three parallels for each standard series.

The samples were gently agitated by hand before the absorbance was read at 570 nm by a spectrophotometer (Synergy MX, Biotek).

The standards were: standard 1 (0 nmol glycerol), standard 2 (2 nmol glycerol), standard 3 (4 nmol glycerol), standard 4 (6 nmol glycerol), standard 5 (8 nmol glycerol) and standard 6 (10 nmol glycerol). The glycerol standard was diluted with Assay Buffer.

Table 2.7 Composition of the two reaction mixes (µL).

	Sample	Control
Assay Buffer	93	96
OxiRed Probe	2	2
Enzyme Mix	2	2
Lipase Substrate	3	-

2.3.9 Protein analysis

The amount of protein in the BSA-solution, artificial gut fluid-solution, seawater and gut fluids (both diluted 50:50 with seawater and undiluted) were determined as described by Lowry et al. (1951) with modifications. In this method there will be a colour reaction of proteins with an alkaline copper tartrate solution and Folin reagent.

Ten µL of standard/blank/samples were added into a 96-well microtiter plate. 25 µL of reagent A (alkaline copper tartrate solution) and 200 µL of reagent B (diluted Folin reagent) were added to each well. The plate was gently agitated by hand in order to mix the samples. Four replicates were made for each sample, and there were three parallels for each standard series. The samples were incubated for 15 minutes before the absorbance was read at 750 nm by a spectrophotometer (Synergy MX, Biotek).

The standards were: standard 1 (1.5 mg/mL BSA), standard 2 (1 mg/mL BSA), standard 3 (0.8 mg/mL BSA), standard 4 (0.4 mg/mL BSA) and standard 6 (0.2 mg/mL BSA). BSA was dissolved in 0.1 M Tris Buffer with pH 8.

2.3.10 Surfactant property

The surfactant property in BSA solution, artificial gut fluid, seawater and gut fluids (both diluted 50:50 with seawater and undiluted) were quantitatively determined by measuring the contact angle of droplets of the different solutions (see figure 2.2). Surfactants can form micelles, and can be detected by the ability of the surfactant-rich solution to maintain a low surface tension (i.e., contact angle). Three droplets of each of the different solutions were placed on parafilm and the sideview image of the droplets was photographed with a Nikon SMZ-U Zoom 1:10. The contact angle was measured with a protractor.

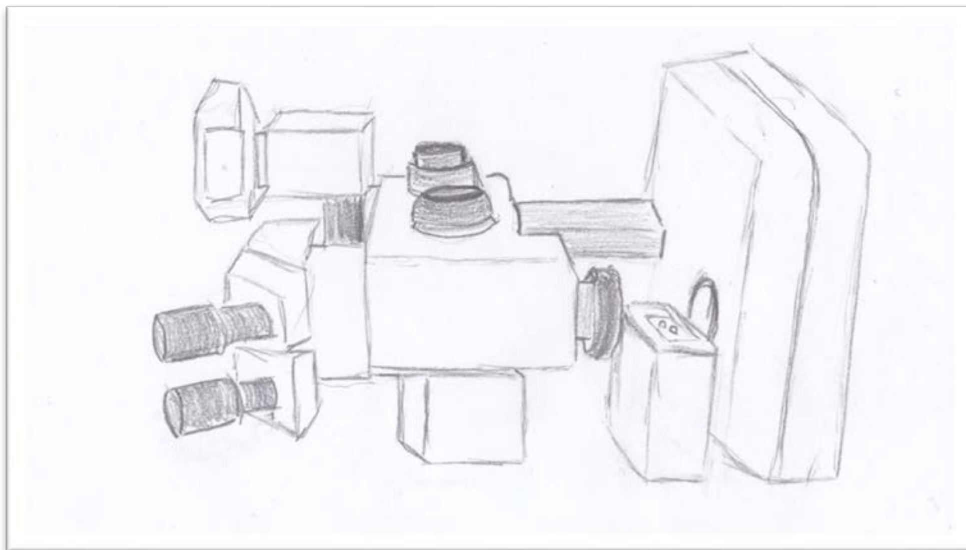


Figure 2.2 Microscope setup for the surfactant property measurement.

2.3.11 Statistical analysis

All figures were made using Graphpad Prism 5 (version 5.04). The data analyses were performed with JMP (version 10.0). Some of the data had values below the detection limit, and some of the data were not normally distributed, therefore the Kruskal-Wallis test was used to compare these groups. The Kruskal-Wallis test is a non-parametric method that compares several populations on the basis of independent samples from each population. The test ranks the data from all groups together and then applies one-way ANOVA to the ranks. A non-parametric multiple comparisons test (Wilcoxon comparison test) was done post-hoc to

determine which of the groups were different. The data that had homogenous variance were compared using one-way ANOVA and then each pair was compared with the Tukey's range test. The Tukey's range test is a multiple comparison method which is used in conjunction with ANOVA to find means that are significantly different from each other. P-values ≤ 0.05 was stated as significant.

3 Results

3.1 Protease activity

The artificial gut fluid had the highest protease activity, followed by the BSA solution. The activity was more than 10 times higher in the artificial gut fluid and BSA solution than in the seawater and the *Arenicola* gut fluid (both diluted with seawater and undiluted; table 3.1).

Table 3.1 The protease activity (mU/mL) in the different fluids. Technical replicates n = 4.

Fluid	**Activity (mU/mL)**
Seawater	38,5
BSA	449
artificial gut fluid	757,2
Arenicola gut fluid	12,9
Arenicola gut fluid diluted 50:50 with seawater	48,2

3.2 Lipase activity

The lipase activity was highest in the BSA solution, followed by the undiluted *Arenicola* gut fluid, seawater and diluted *Arenicola* gut fluid. The artificial gut fluid had the lowest lipase activity and was 8 times lower than the activity in the BSA solution (table 3.2).

Table 3.2 The activity of lipase (mU/mL) in the different fluids. Technical replicates n = 4.

Fluid	**Activity (mU/mL)**
Seawater	926,2
BSA	1924,2
artificial gut fluid	215,4
Arenicola gut fluid	1292,4
Arenicola gut fluid diluted 50:50 with seawater	753,9

3.3 Protein analysis

The highest content of protein was observed in the artificial gut fluid. The *Arenicola* gut fluid (undiluted) had the second highest amount of protein, followed by the BSA solution and the diluted *Arenicola* gut fluid. The seawater had no visible protein content (table 3.3).

Table3.3 The amount of protein (g/L) in the different solutions. Technical replicates n = 4.

Fluid	**Content (g/L)**
Seawater	0
BSA	4,93
artificial gut fluid	10,27
Arenicola gut fluid	8,53
Arenicola gut fluid diluted 50:50 with seawater	4,24

3.4 Surfactant activity

Seawater had the largest angle of 95° (figure 3.1 a, and table 3.4) followed by the BSA solution that had a angle of 84° (fig. 3.1 b and table 3.1) and the artificial gut fluid that had a angle of 72° (fig. 3.1 c and table 3.1). The *Arenicola* gut fluid had the smallest angle ranging from 61 to 71° (fig 3.1 d-g, and table 3.1).

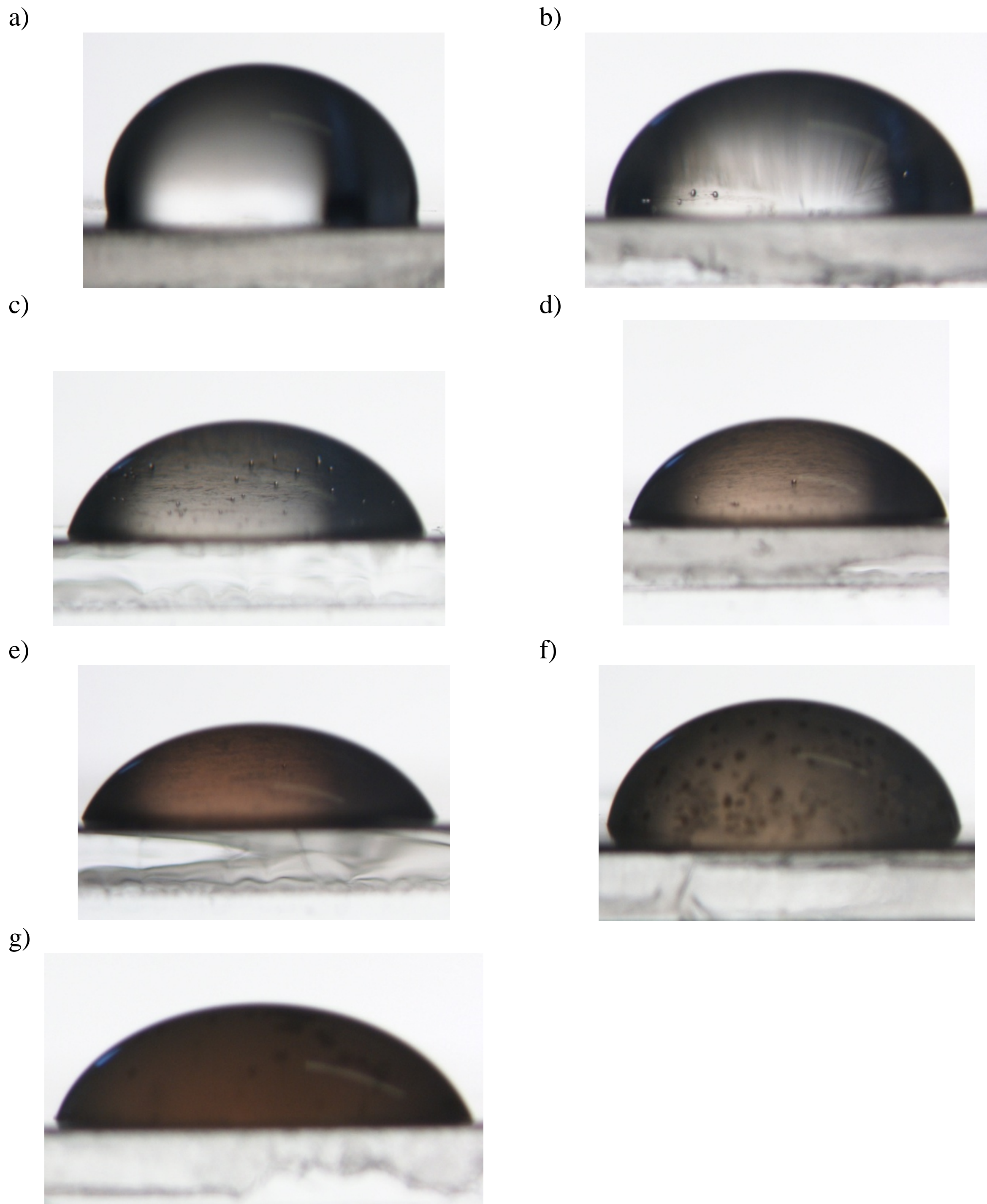


Figure 3.1 Microscopy pictures of droplets of a) seawater (n = 3), b) BSA solution (n = 3), c) artificial gut fluid (n = 3), d) filtered *Arenicola marina* gut fluid diluted 50:50 with seawater (n = 3) e) filtered *Arenicola marina* gut fluid undiluted (n = 3), f) unfiltered *Arenicola marina* gut fluid diluted 50:50 with seawater (n = 3) and g) unfiltered *Arenicola marina* gut fluid undiluted (n = 3); the replicates were technical replicates.

Table 3.4 The contact angle of the different solutions (n = 3).

Fluid	**Contact angle °**
Seawater	95
BSA	84
artificial gut fluid	72
Filtered *Arenicola* gut fluid diluted 50:50	63
Filtered *Arenicola* gut fluid	61
Unfiltered *Arenicola* gut fluid diluted 50:50	71
Unfiltered *Arenicola* gut fluid	62

3.5 Test sediments

3.5.1 General comparison of the test sediments

The control sediment had a concentration of PAH below 249.6 µg/kg dry weight. The sediment from the Sunndalsfjord and Karmøy had concentrations over 85 times higher than the control sediment (table 3.5).

Table 3.5 The amount of PAH (µg/kg dry weight) in each sediment.

Sediment	**Sum PAH µg/kg dry weight**
Control	<249.6
Sunndalsfjord	22230
Karmøy	22765

The Karmøy sediment had the highest content of total organic carbon (TOC), followed by Sunndalsfjord sediment. The control sediment had the lowest TOC.

Table 3.6 The total organic carbon (TOC) in sediment (%).

Sediment	**TOC (%)**
Control	0.64
Sunndalsfjord	1.33
Karmøy	1.55

3.5.2 The proportion of total amount of sediment-associated naphthalene that was desorbed from the sediment

There was a difference between the proportion of sediment-associated naphthalene desorbed from the sediments into the seawater ($p \leq 0.05$, $n = 4$; fig. 3.2a). Tukey's range test showed that the Karmøy sediment was not significantly different from the Sunndalsfjord sediment ($p > 0.05$). The control sediment was significantly different from the other sediments ($p \leq 0.05$).

There was a difference between the proportion of naphthalene desorbed from the sediment into the BSA solution ($p \leq 0.05$, $n = 4$; fig. 3.2b). Tukey's range test showed that there were significant differences between all the three sediments ($p \leq 0.05$). The same could be seen for the proportion of naphthalene in the artificial gut fluid (fig. 3.2c). There was a difference between the sediments ($p \leq 0.05$, $n = 4$), and Tukey's range test showed that there were significant differences between all three sediments ($p \leq 0.05$). There was no relative desorption of naphthalene from the sediment into the *Arenicola* gut fluid (fig 3.2d).

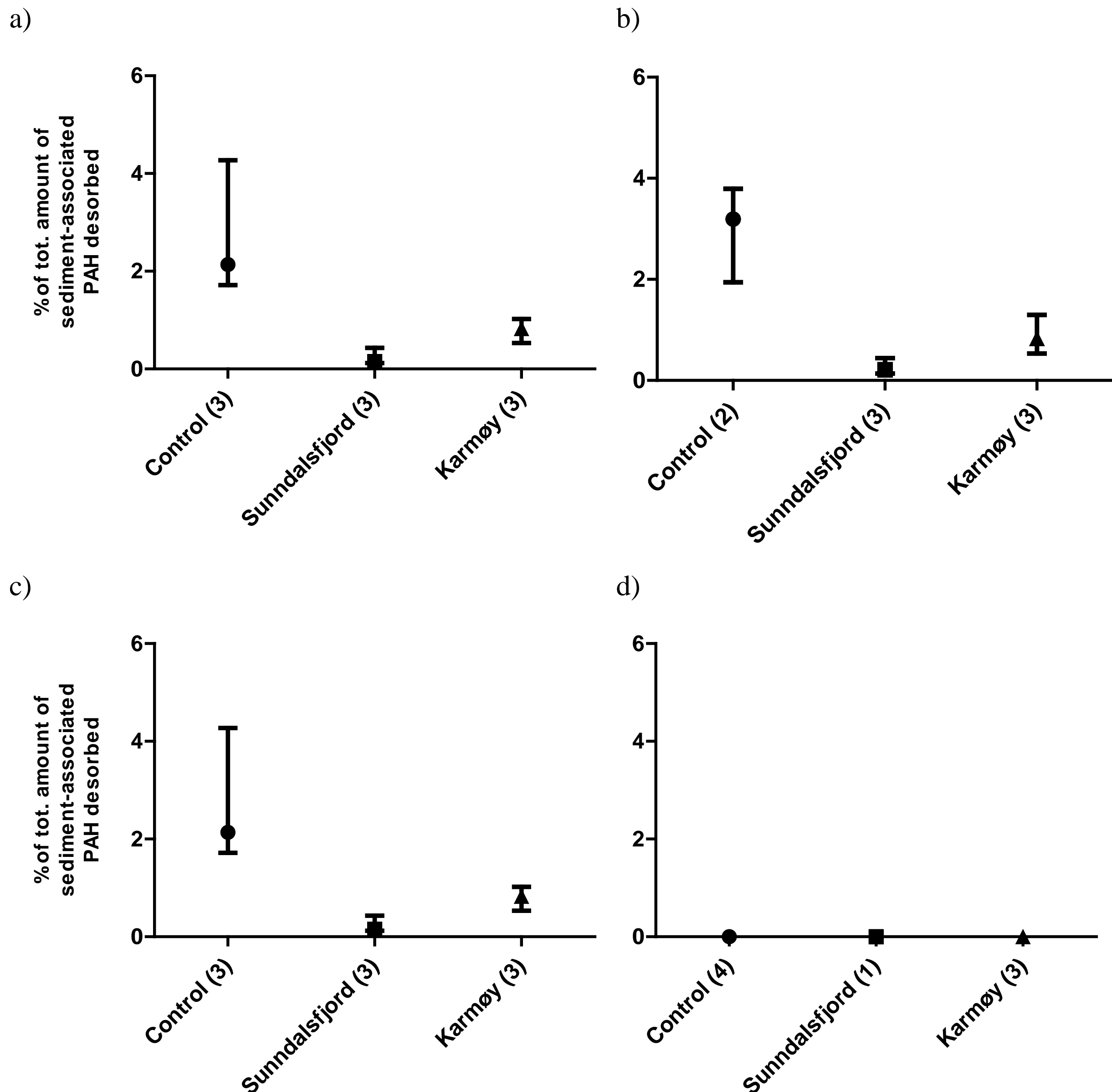


Figure 3.2 The proportion (%) of total amount of sediment-associated naphthalene that was desorbed from the control sediment (n = 4), Sunndalsfjord sediment (n = 4) and Karmøy sediment (n = 4) to; a) seawater, b) BSA, c) artificial gut fluid and d) *Arenicola* gut fluid; median and interquartiles. The numbers in the brackets denotes how many of the technical replicates that were below the detection value.

3.5.3 The proportion of total amount of sediment-associated phenanthrene that was desorbed from the sediment

There was a significant difference between the proportion of phenanthrene desorbed from the sediments into the seawater, the BSA solution and the artificial gut fluid (seawater: $p \leq 0.05$, $n = 4$; BSA solution: $p \leq 0.05$, $n = 4$; artificial gut fluid: $H = 9.88$, $p \leq 0.05$, $n = 4$; fig. 3.3a-c). Pairwise comparison (Tukey's range test for the seawater and BSA solution; Wilcoxon comparison test for the artificial gut fluid) showed that there were significant differences

between all the three sediments for all the fluids ($p \leq 0.05$). There was no relative desorption of phentanthrene from the sediments into the *Arenicola* gut fluid.

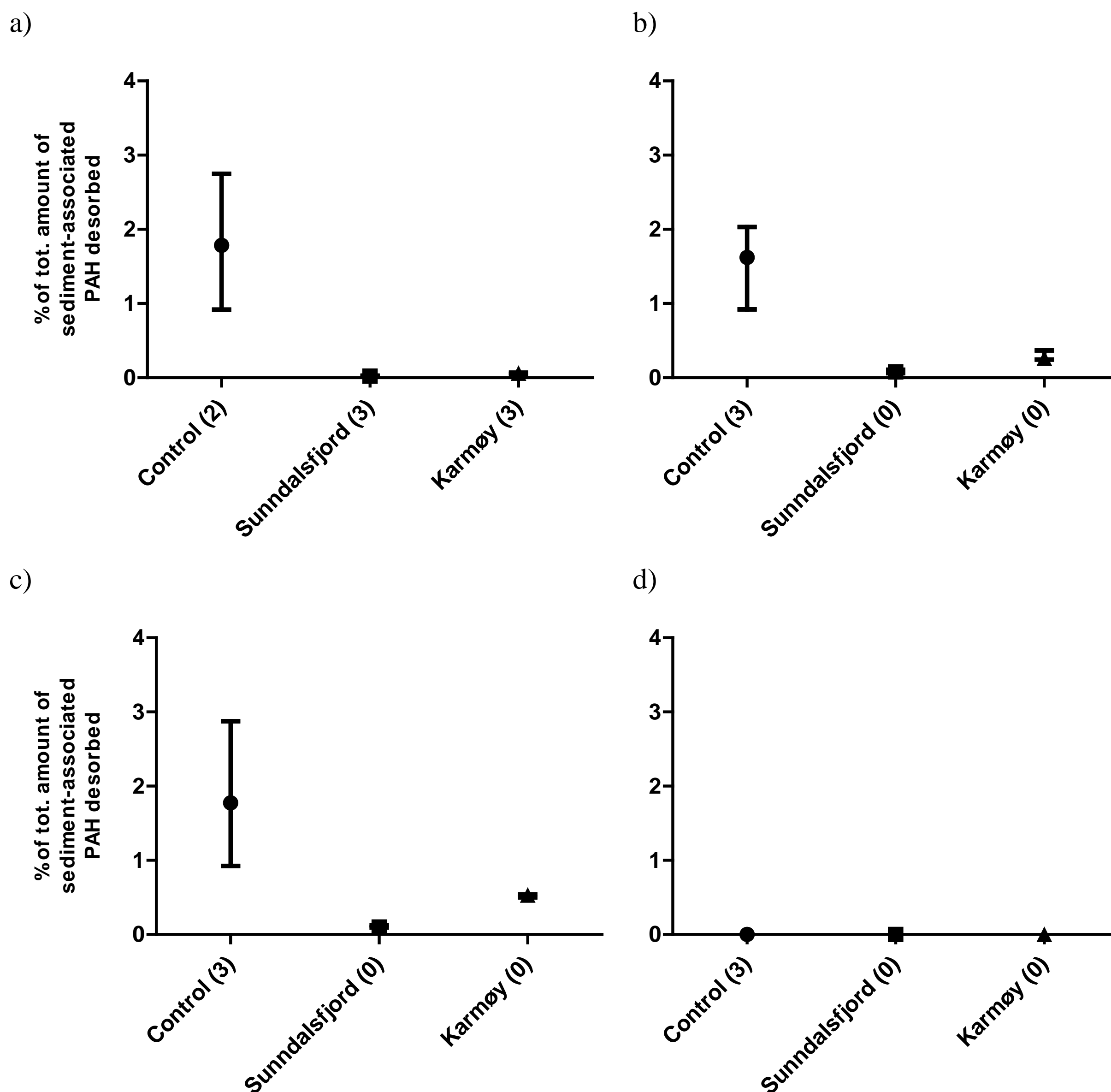


Figure 3.3 The proportion (%) of total amount of sediment-associated phenanthrene that was desorbed from the control sediment (n = 4), Sunndalsfjord sediment (n = 4) and Karmøy sediment (n = 4) to; a) seawater, b) BSA, c) artificial gut fluid and d) *Arenicola* gut fluid); median and interquartiles. The numbers in the brackets denotes how many of the technical replicates that were below the detection value.

3.5.4 The proportion of total amount of sediment-associated chrysene that was desorbed from the sediment

There was not a significant difference in the proportion of chrysene desorbed into the seawater between the sediments ($H = 4.19$, $p > 0.05$, $n = 4$; fig. 3.4a).

There was a significant difference in the proportion of chrysene desorbed from the sediments into the BSA solution ($p \leq 0.05$, $n = 4$; fig. 3.4b). Tukey's range test showed that there was a significant difference between the Karmøy sediment and Sunndalsfjord sediment ($p \leq 0.05$) and between the Karmøy sediment and the control sediment ($p \leq 0.05$). There was not a significant difference between the control sediment and the Sunndalsfjord sediment ($p > 0.05$).

There was a significant difference between the proportion of chrysene desorbed from the sediments into the artificial gut fluid ($p \leq 0.05$, $n = 4$; 3fig. 3.4c). Tukey's range test showed that there was a significant difference between the percentage in the Karmøy sediment and Sunndalsfjord sediment ($p \leq 0.05$) and between the Karmøy sediment and the control sediment ($p \leq 0.05$). There was not a significant difference between the control sediment and the Sunndalsfjord sediment ($p > 0.05$).

There was a significant difference between the proportion of chrysene desorbed from the sediments into the *Arenicola* gut fluid ($H = 9.41$, $p \leq 0.05$, $n = 4$; fig. 3.4d). Wilcoxon comparison test showed that there was a significant difference between the Karmøy sediment and Sunndalsfjord sediment ($p \leq 0.05$) and between the Karmøy sediment and the control sediment ($p \leq 0.05$). There was not a significant difference between the control sediment and the Sunndalsfjord sediment ($p > 0.05$).

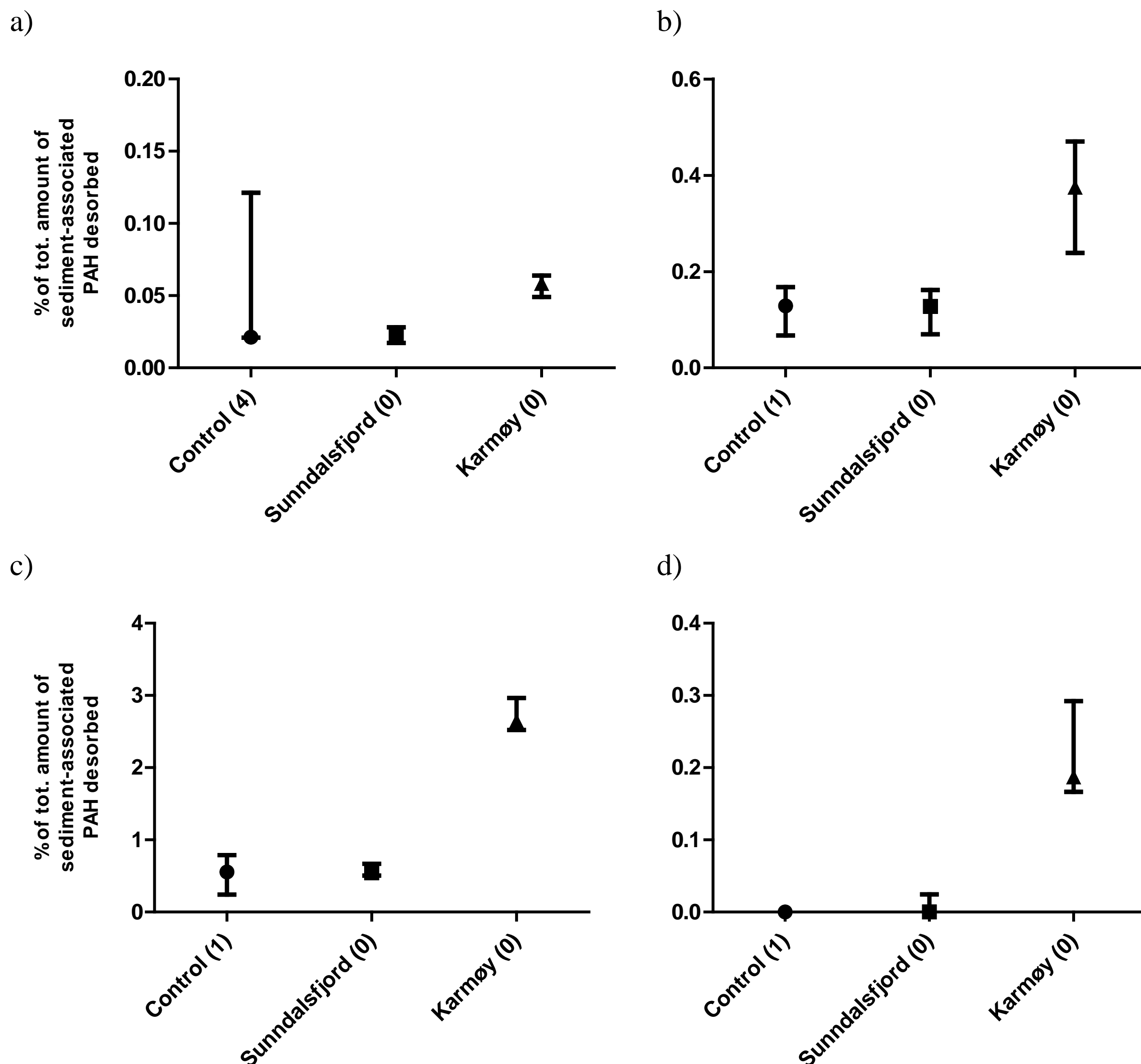


Figure 3.4 The proportion (%) of total amount of sediment-associated chrysene that was desorbed from the control sediment (n = 4), Sunndalsfjord sediment (n = 4) and Karmøy sediment (n = 4) to; a) seawater, b) BSA, c) artificial gut fluid and d) *Arenicola* gut fluid); median and interquartiles. The numbers in the brackets denotes how many of the technical replicates that were below the detection value.

3.5.5 The proportion of total amount of sediment-associated benzo[a]pyrene that was desorbed from the sediment

There was a significant difference between the proportion of benzo[a]pyrene desorbed from the sediments into the seawater ($H = 7.42$, $p > 0.05$, $n = 4$; fig. 3.5a). Wilcoxon comparison test showed that there was a significant difference between the Karmøy sediment and Sunndalsfjord sediment ($p \leq 0.05$) and between the Karmøy sediment and the control sediment ($p \leq 0.05$). There was not a significant difference was observed between the control sediment and the Sunndalsfjord sediment ($p > 0.05$).

There was a significant difference between the proportion of benzo[a]pyrene desorbed from the sediments into the BSA solution ($p \leq 0.05$, $n = 4$; fig. 3.5b). Tukey's range test showed that there was a significant difference between the Karmøy sediment and Sunndalsfjord sediment ($p \leq 0.05$) and between the Karmøy sediment and the control sediment ($p \leq 0.05$). There was not a significant difference between the control sediment and the Sunndalsfjord sediment ($p > 0.05$).

There was a significant difference between in the proportion of benzo[a]pyrene desorbed from the sediments into the artificial gut fluid ($p \leq 0.05$, $n = 4$; fig. 3.5c). Tukey's range test showed that there were significant differences between all three sediments ($p \leq 0.05$).

There was a significant difference between in the proportion of benzo[a]pyrene desorbed from the sediments into the *Arenicola* gut fluid ($H = 10.46$, $p \leq 0.05$, $n = 4$; fig. 3.5d). There was no relative desorption of benzo[a]pyrene from the control sediment or Sunndalsfjord sediment into the *Arenicola* gut fluid. There was a significant difference between the Karmøy sediment and the control sediment ($p \leq 0.05$) and between the Karmøy sediment and the Sunndalsfjord sediment ($p \leq 0.05$).

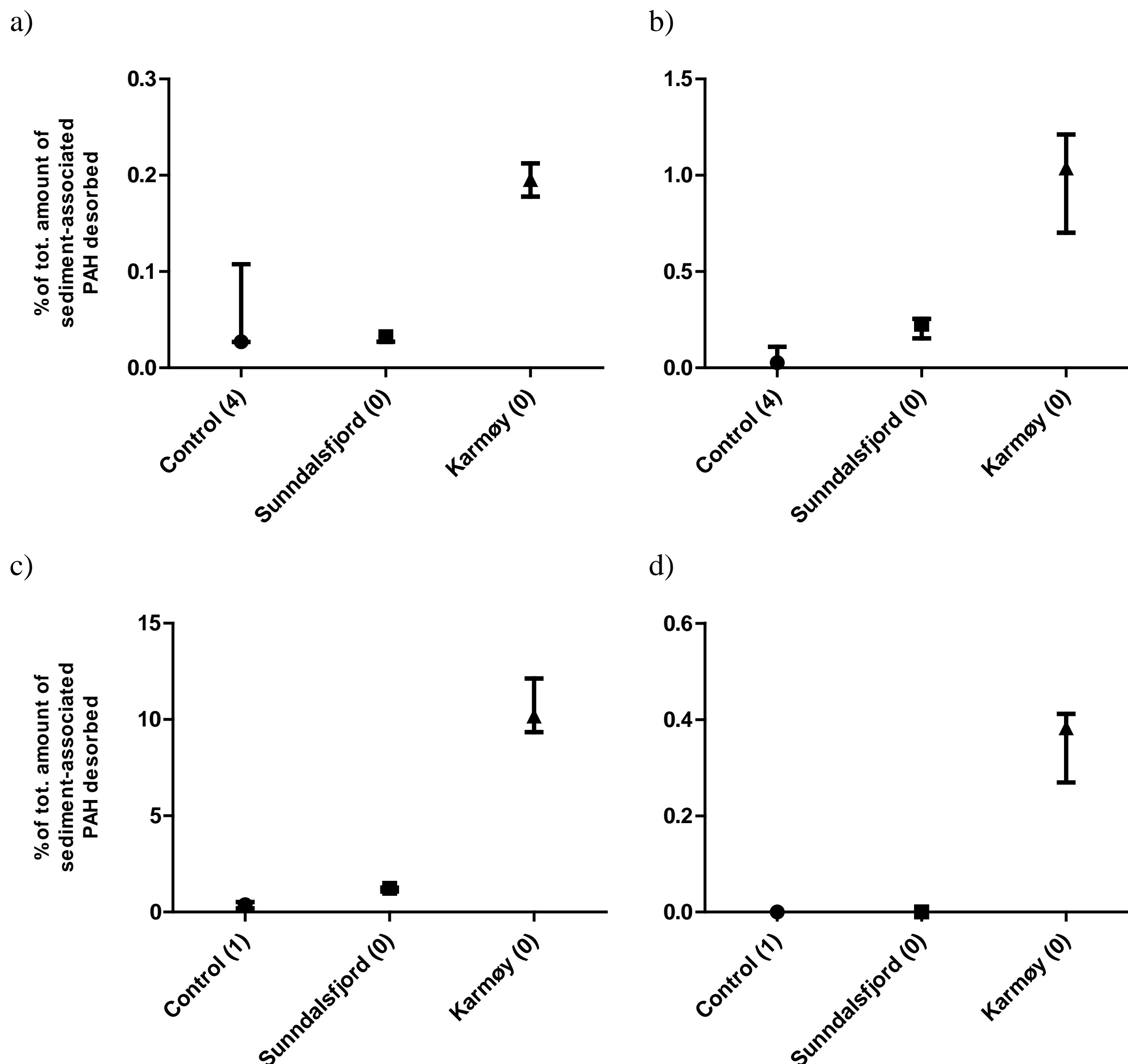


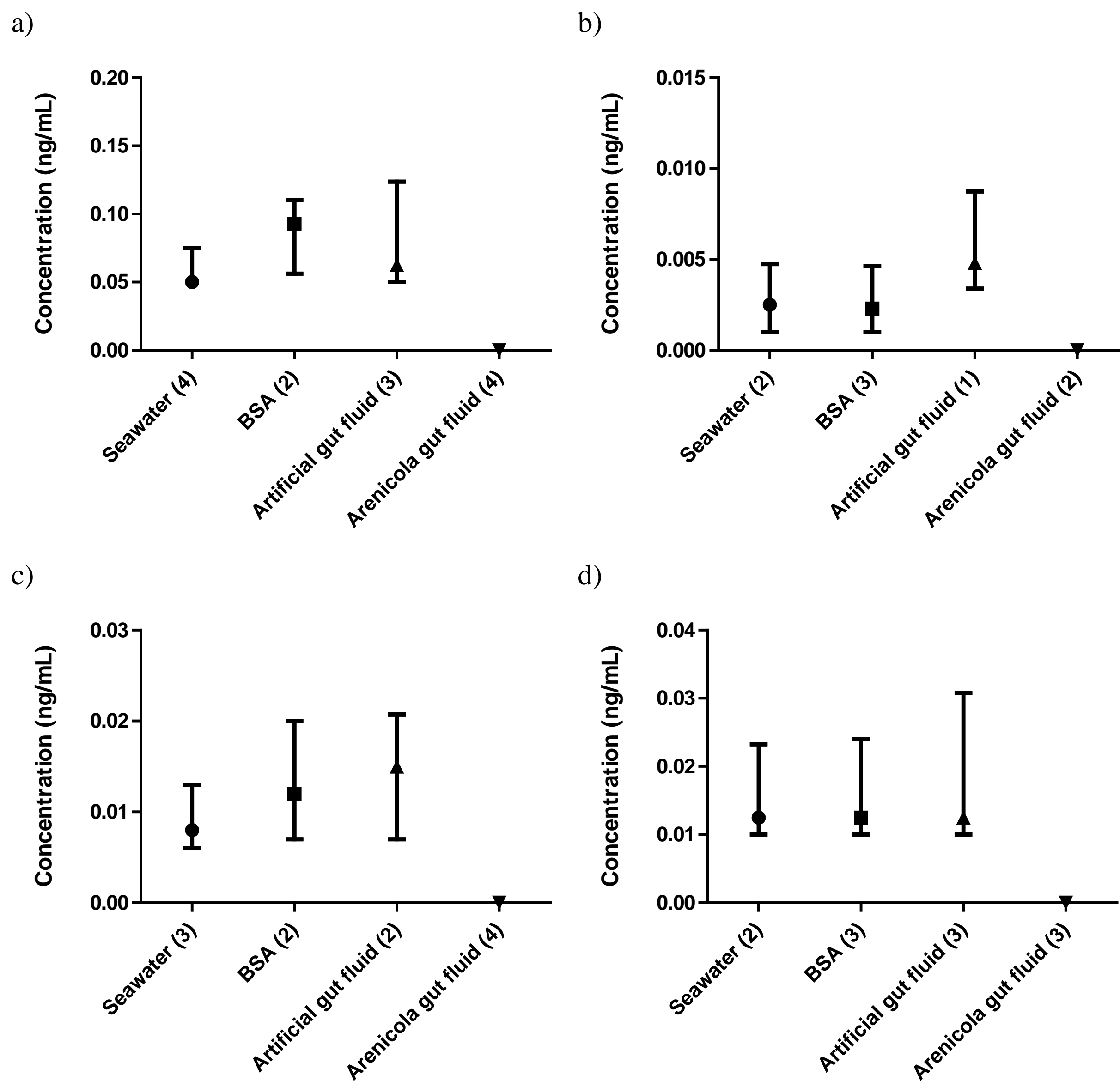
Figure 3.5 The proportion (%) of total amount of sediment-associated benzo[a]pyrene that was desorbed from the control sediment (n = 4), Sunndalsfjord sediment (n = 4) and Karmøy sediment (n = 4) to; a) seawater, b) BSA, c) artificial gut fluid and d) *Arenicola* gut fluid; median and interquartiles. The numbers in the brackets denotes how many of the technical replicates that were below the detection value.

3.6 Effect of the different fluids on PAH desorption from the test-sediments

3.6.1 PAH release from control sediment

The BSA solution and the artificial gut fluid had generally higher effect on the release of PAH from the control sediment, than the seawater and the *Arenicola* gut fluid (fig 3.6 a-s).
The *Arenicola* gut fluid did not appear to increase the bioavailability of PAHs from the control sediment (fig.3.6a-s), and there was a significant difference between the artificial gut

fluid and the *Arenicola* gut fluid for all the PAHs ($p \leq 0.05$), except for perylene ($p > 0.05$) (fig 3.6p).



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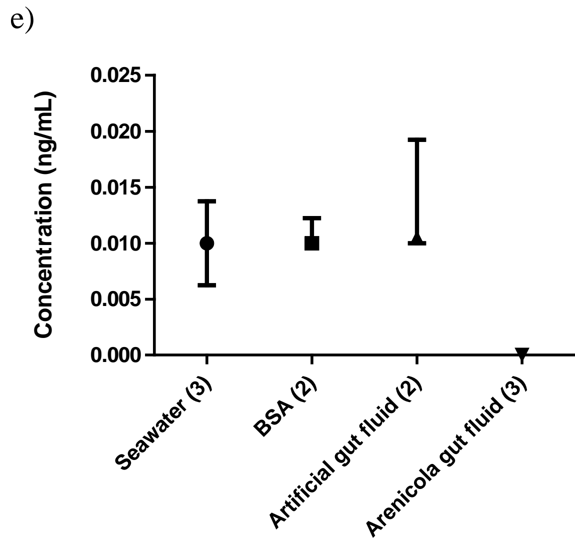

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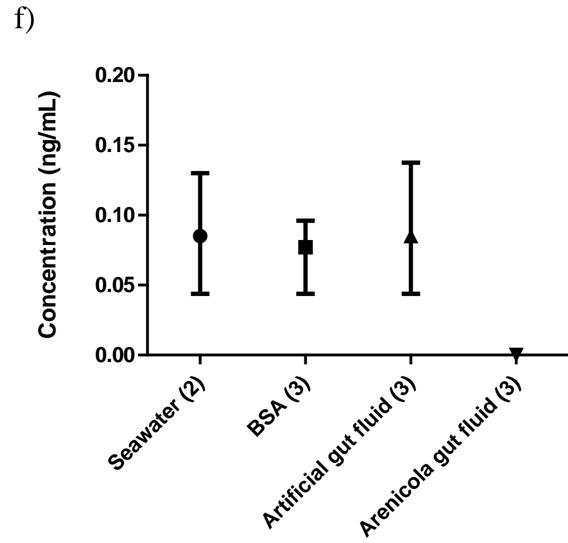

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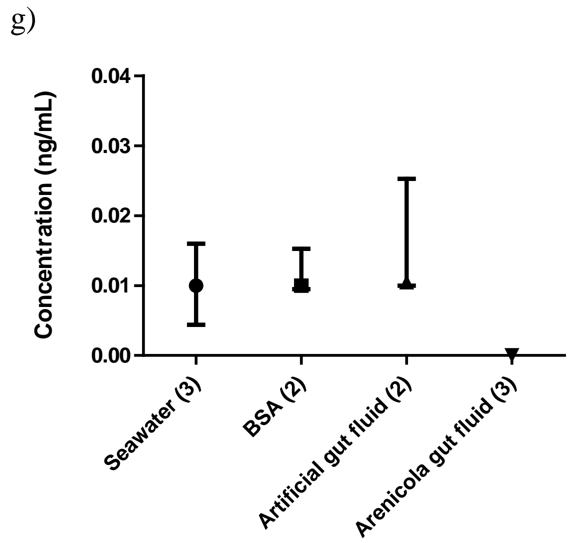

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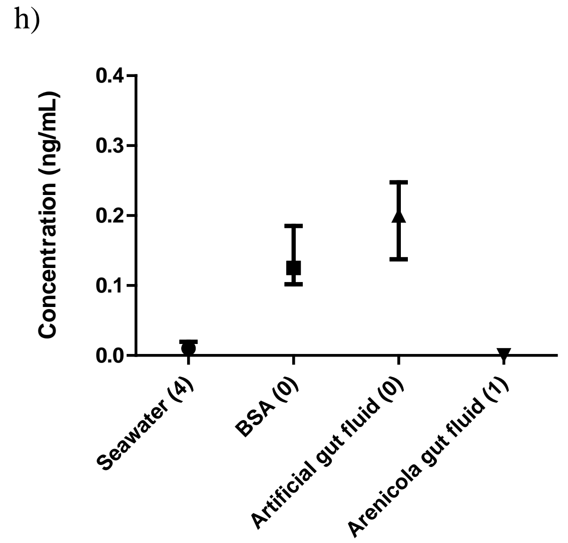

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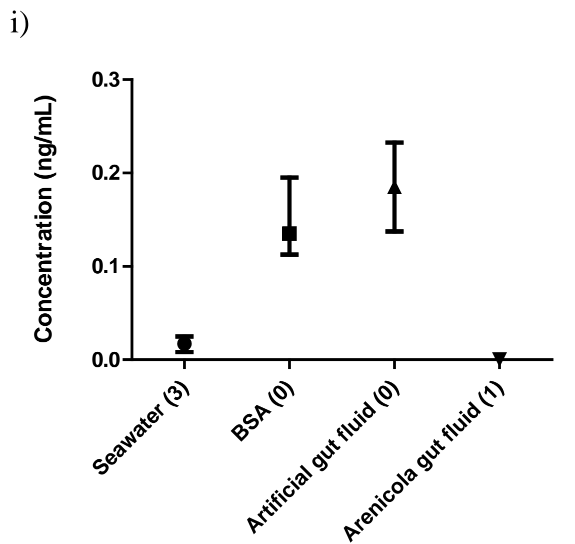

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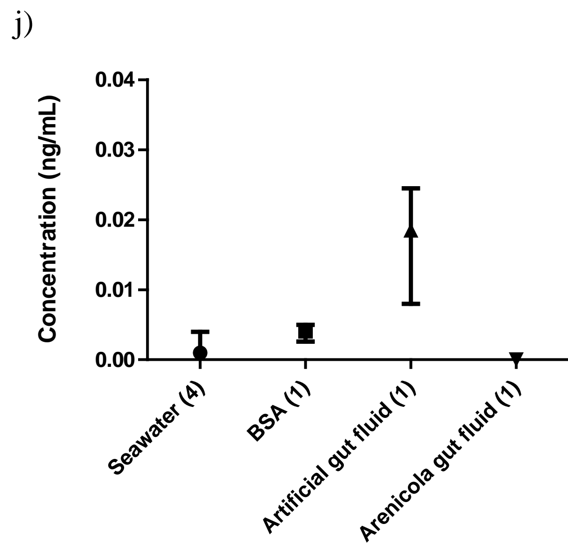

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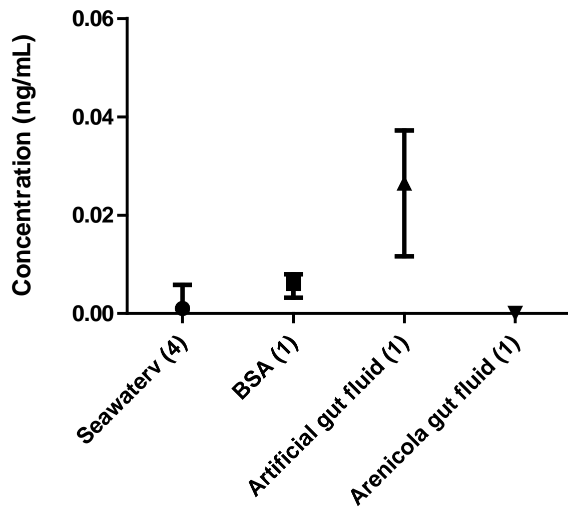

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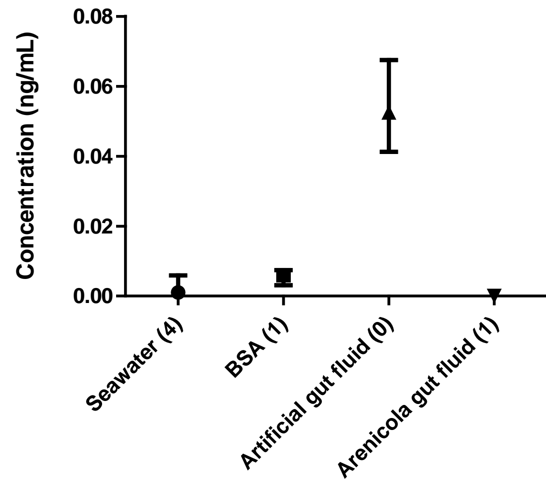

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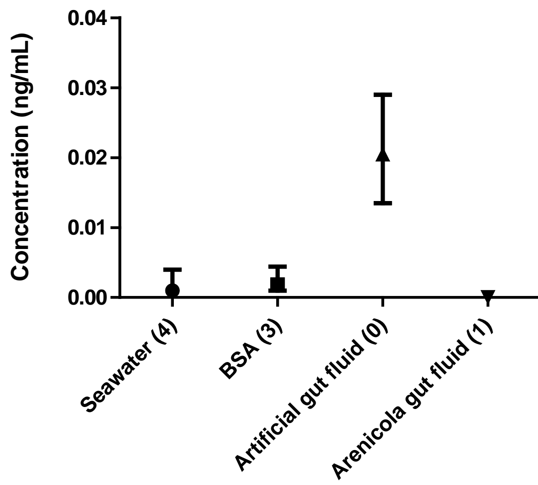

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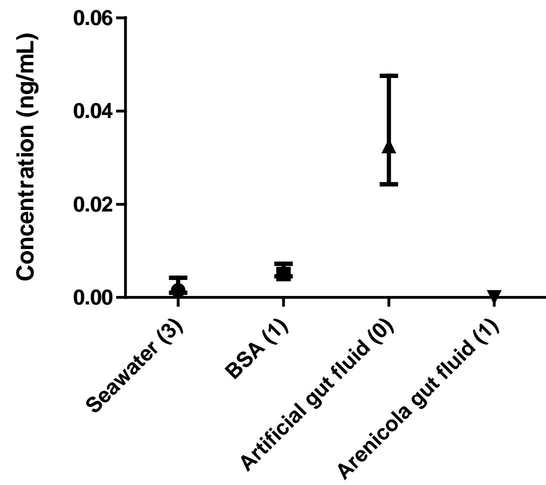

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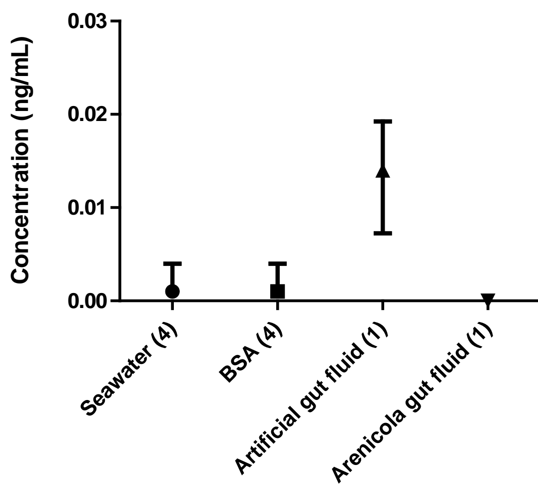

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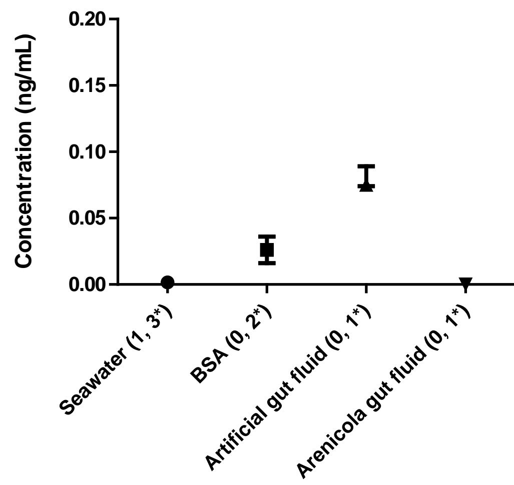

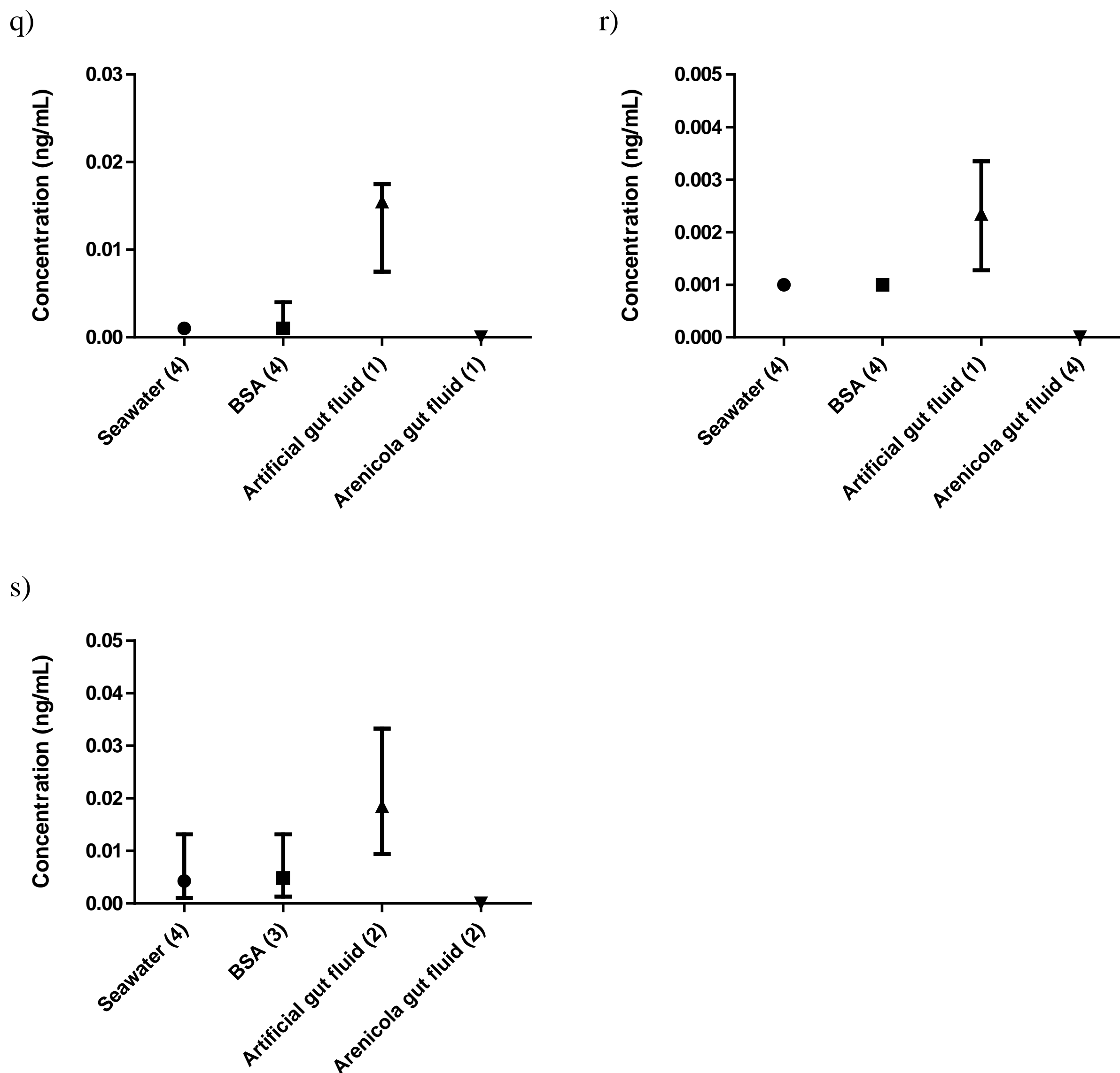

Figure 3.6 The concentration (ng/mL) of 19 different PAHs in the extracts of the four fluids (n = 4), after 4 hours of mixing with the control sediment; a) naphthalene, b) acenaphthylene, c) acenaphthene, d) fluorene, e) dibenzothiophene, f) phenanthrene, g) anthracene, h) fluoranthene, i) pyrene, j) benzo[a]anthracene, k) chrysene, l) benzo[b, j]fluoranthene, m) benzo[j,k]fluoranthene, n) benzo[e]pyrene, o) benzo[a]pyrene, p) perylene, q) ideno[1,2,3-cd]pyrene, r) dibenzo[a,c]anthracene and s) benzo[g,h,i]perylene; median and interquartiles. The numbers in the brackets denotes how many of the technical replicates that were below the detection value. (*) denotes the number of replicates that had interference.

There was a difference between the concentration of naphthalene in the different fluids following incubation with the control sediment ($H = 10,33$, $p \leq 0.05$, $n = 4$; fig 3.6a). Wilcoxon comparison test showed that there was a significant difference between the *Arenicola* gut fluid and seawater, as well as between the BSA solution and *Arenicola* gut fluid

($p \leq 0.05$). There was not a significant difference between the concentration in the seawater and in the BSA solution, in the seawater and in the artificial gut fluid, and in the artificial gut fluid and in BSA solution ($p > 0.05$).

There was a difference between the concentration of acenaphthylene in the different fluids following incubation with the control sediment ($H = 10.18$, $p \leq 0.05$, $n = 4$; fig 3.6b). Wilcoxon comparison test showed that there was a significant difference between the concentration in the seawater and *Arenicola* gut fluid ($p \leq 0.05$), the BSA solution and the *Arenicola* gut fluid ($p \leq 0.05$) and between the artificial gut fluid and the *Arenicola* gut fluid ($p \leq 0.05$). There was not a significant difference between in the seawater and the BSA solution, between seawater and the artificial gut fluid or between the BSA solution and the artificial gut fluid ($p > 0.05$).

There was a difference between the concentration of acenaphthene in the different groups ($H = 9.52$, $p \leq 0.05$, $n = 4$; fig. 3.6c). Wilcoxon comparison test showed that there was a significant difference between the concentration in the seawater and *Arenicola* gut fluid ($p \leq 0.05$), between the BSA solution and the *Arenicola* gut fluid ($p \leq 0.05$), as well as between the artificial gut fluid and the *Arenicola* gut fluid ($p \leq 0.05$). There was not a significant difference between the seawater and the BSA solution, between the seawater and the artificial gut fluid or between the BSA solution and the artificial gut fluid ($p > 0.05$).

There was a difference between the concentration of fluorene in the different groups ($H = 9.15$, $p \leq 0.05$, $n = 4$; fig 3.6d). Wilcoxon comparison test showed that there was not a significant difference between the concentration in the seawater and the BSA solution, between the seawater and the artificial gut fluid or between the BSA solution and artificial gut fluid ($p > 0.05$). There was a significant difference between the concentration in the *Arenicola* gut fluid and seawater, in the *Arenicola* gut fluid and BSA solution, as well as in the *Arenicola* gut fluid and the artificial gut fluid ($p \leq 0.05$).

There was a difference between the concentration of dibenzothiophene in the different groups ($H = 9.89$, $p \leq 0.05$, $n = 4$; fig 3.6e). Wilcoxon comparison test showed that there was not a significant difference between the seawater and BSA solution, between the seawater and artificial gut fluid or between the BSA solution and artificial gut fluid ($p > 0.05$). There was a

significant difference between the concentration in the *Arenicola* gut fluid and the other three fluids ($p \leq 0.05$).

There was a significant difference between the concentration of phenanthrene in the different groups ($H = 8.91$, $p \leq 0.05$, $n = 4$; fig. 3.6f). Wilcoxon comparison test showed that there was not a significant difference between the concentration in the seawater and BSA solution, between the concentration in seawater and in artificial gut fluid, as well as between concentration in the BSA solution and in the artificial gut fluid ($p > 0.05$). There was a significant difference between the *Arenicola* gut fluid and the artificial gut fluid, between the *Arenicola* gut fluid and seawater and between the *Arenicola* gut fluid and BSA solution ($p \leq 0.05$).

There was a difference between the concentration of anthracene in the different groups ($H = 9.71$, $p \leq 0.05$, $n = 4$; fig. 3.6g). Wilcoxon comparison test showed that there was a significant difference between the concentration in the *Arenicola* gut fluid and the other fluids ($p \leq 0.05$). There was not a significant difference between the seawater and BSA solution, between the seawater and the artificial gut fluid or between the BSA solution and artificial gut fluid ($p > 0.05$).

There was a difference between the concentration of fluoranthene between the different groups ($H = 13.54$, $p \leq 0.05$, $n = 4$; fig. 3.6h). Wilcoxon comparison test showed that there was not a significant difference between the concentration in the BSA solution and artificial gut fluid ($p > 0.05$). A significant difference was observed between the seawater and BSA solution, between the seawater and *Arenicola* gut fluid, between and seawater and artificial gut fluid ($p \leq 0.05$). There was a significant difference between the *Arenicola* gut fluid and artificial gut fluid, and between the BSA solution and *Arenicola* gut fluid ($p \leq 0.05$).

There was a significant difference between the concentration of pyrene between the different groups ($H = 13.37$, $p \leq 0.05$, $n = 4$; fig. 3.6i). Wilcoxon comparison test showed that there was a significant difference between the concentration in the *Arenicola* gut fluid and in the artificial gut fluid, as well as in the *Arenicola* gut fluid and in BSA solution ($p \leq 0.05$). The seawater was significantly different from the other fluids ($p \leq 0.05$). There was not a

significant difference between the concentration in the BSA solution and in the artificial gut fluid ($p > 0.05$).

For benzo[a]anthracene (fig. 3.6j), there was a significant difference between the concentration in the different groups following incubation with sediment ($H = 13.02$, $p \leq 0.05$, $n = 4$). Wilcoxon comparison test showed that there was a significant difference between the *Arenicola* gut fluid and the seawater, between the BSA solution and artificial gut fluid ($p \leq 0.05$), as well as between seawater and artificial gut fluid ($p \leq 0.05$). There was not a significant difference between the concentration in the seawater and in the BSA solution ($p > 0.05$), nor between the concentration in the BSA solution and in artificial gut fluid ($p > 0.05$).

After mixing with the control sediment, there was a difference between the concentration of chrysene in the different groups ($H = 13.25$, $p \leq 0.05$, $n = 4$; fig. 3.6k). Wilcoxon comparison test showed that there was not a significant difference between the seawater and the BSA solution, or between the BSA solution and the artificial gut fluid ($p > 0.05$). A significant difference was observed between the *Arenicola* gut fluid and sweater, BSA solution and artificial gut fluid ($p \leq 0.05$) and between the seawater and artificial gut fluid ($p \leq 0.05$).

There was a difference between the concentration of benzo[b,j]fluoranthene in the different groups ($H = 13.45$, $p \leq 0.05$, $n = 4$; fig. 3.6l). Wilcoxon comparison test showed that there was not a significant difference between the concentration in the seawater and BSA solution ($p > 0.05$). The *Arenicola* gut fluid had a significantly different concentration than the other fluids ($p \leq 0.05$). There was a significant difference between the concentration in the artificial gut fluid and BSA solution, and in the artificial gut fluid and seawater ($p \leq 0.05$).

After mixing with the control sediment, there was a difference between the concentration of benzo[j,k]fluoranthene in the different groups ($H = 13.36$, $p \leq 0.05$, $n = 4$; fig. 3.6m). Wilcoxon comparison test showed that there was not a significant difference between the concentration in the seawater and the BSA solution ($p > 0.05$). There was a significant difference between the concentration in the *Arenicola* gut fluid and the other three fluids ($p \leq 0.05$). There was a significant difference between the concentration in the artificial gut fluid and BSA solution as well as between the artificial gut fluid and seawater ($p \leq 0.05$).

There was a difference between the concentration of benzo[e]pyrene in the different groups ($H = 13.88$, $p \leq 0.05$, $n = 4$; fig. 3.6n). Wilcoxon comparison test showed that there was not a significant difference between concentration in the seawater and BSA solution ($p > 0.05$). There was a significant difference between the *Arenicola* gut fluid and the other fluids ($p \leq 0.05$). A significant difference was observed between the concentration in the artificial gut fluid and BSA solution, and between the artificial gut fluid and seawater ($p \leq 0.05$).

After mixing with the control sediment, there was a difference between the concentration of benzo[a]pyrene in the different groups ($H = 13.16$, $p \leq 0.05$, $n = 4$; fig. 3.6o). Wilcoxon comparison test showed that there was not a significant difference between the concentration in the seawater and BSA solution ($p > 0.05$). There was a significant difference between the *Arenicola* gut fluid and the other fluids ($p \leq 0.05$), between the artificial gut fluid and BSA solution, as well as between the artificial gut fluid and seawater ($p \leq 0.05$).

After mixing with the control sediment, there was not a significant difference in the concentration of perylene between the different groups ($H = 7.6$, $p > 0.05$, $n = 1-3$; fig 3.6p).

There was a significant difference between the concentration of ideno[1,2,3-cd]pyrene in the different groups ($H = 13.93$, $p \leq 0.05$, $n = 4$; fig. 3.6q). Wilcoxon comparison test showed that there was not a significant difference between the concentration in the seawater and BSA solution ($p > 0.05$). There was a significant difference between the *Arenicola* gut fluid and the other solutions ($p \leq 0.05$), between the artificial gut fluid and BSA solution, as well as between artificial gut fluid and seawater ($p \leq 0.05$).

After mixing with the control sediment, there was a significant difference between the concentration of dibenzo[a,c]anthracene between the different groups ($H = 13.42$, $p \leq 0.05$, $n = 4$; fig. 3.6r). Wilcoxon comparison test showed that there was not a significant difference between the BSA solution, the artificial gut fluid and seawater ($p > 0.05$). The *Arenicola* gut fluid had a concentration significantly different from the other fluids ($p \leq 0.05$).

After mixing with the control sediment, there was a difference between the concentration of benzo[g,h,i]perylene in the different groups ($H = 11.24$, $p \leq 0.05$, $n = 4$; fig. 3.6s). Wilcoxon comparison test showed that the *Arenicola* gut fluid had a concentration significantly different

from the other fluids ($p \leq 0.05$). There was not a significant difference between the concentration in seawater and the BSA solution, between the BSA solution and artificial gut fluid or between the seawater and the artificial gut fluid ($p > 0.05$).

3.6.2 PAH release from Sunndalsfjord sediment

The artificial gut fluid generally had the greatest effect on the release of PAHs from Sunndalsfjord sediment, followed by the BSA solution (fig 3.7a-s). The seawater and *Arenicola* gut fluid had low ability to desorb the PAHs from the sediment compared to the BSA solution and artificial gut fluid (fig. 3.7a-s). For several of the PAHs (fig 3.7a, b, c, d, e, f, g, h, i, j, o and p) the *Arenicola* gut fluid had no ability to increase the bioavailability of PAHs from Sunndalsfjord sediment. There was a significant difference between the concentration in the artificial gut fluid and the *Arenicola* gut fluid for all the PAHs ($p \leq 0.05$; fig 3.7 a-s).

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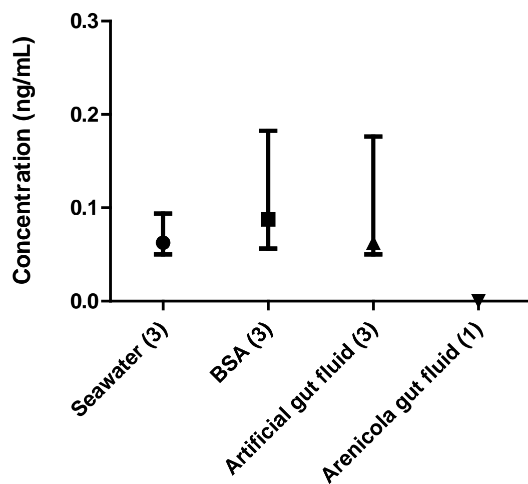

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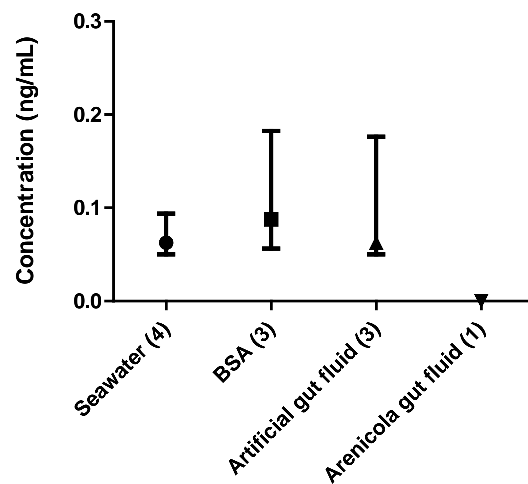

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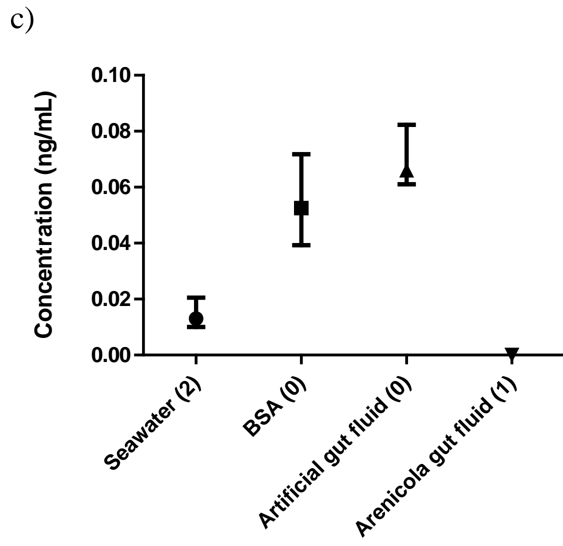

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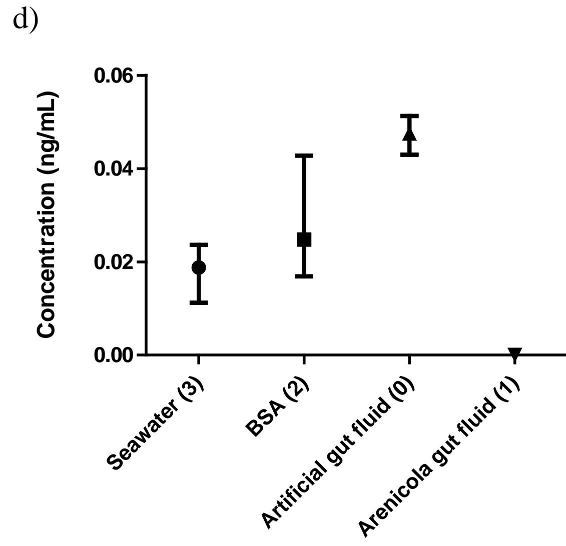

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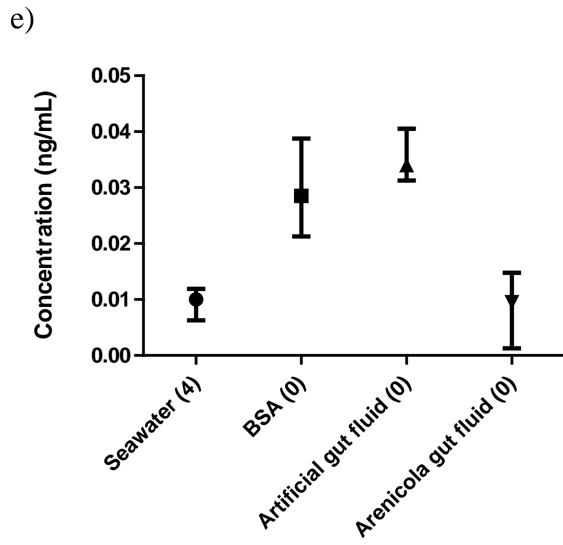

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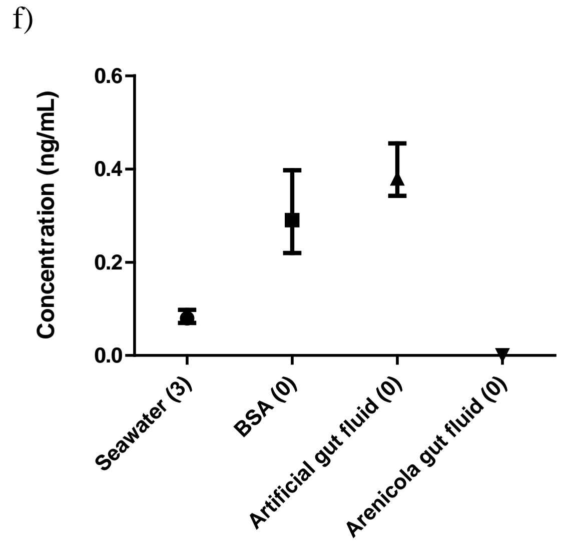

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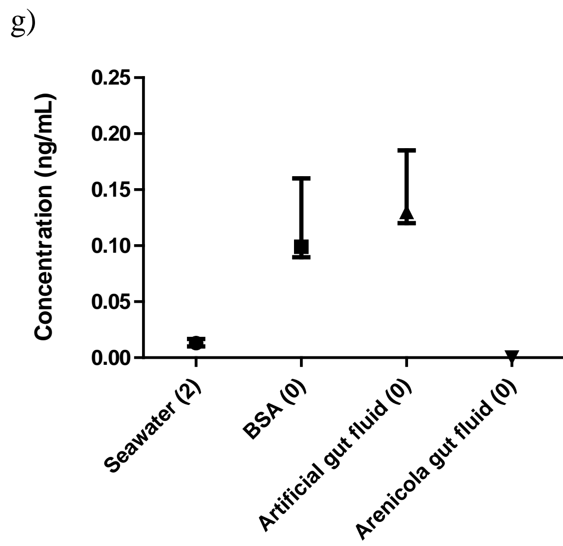

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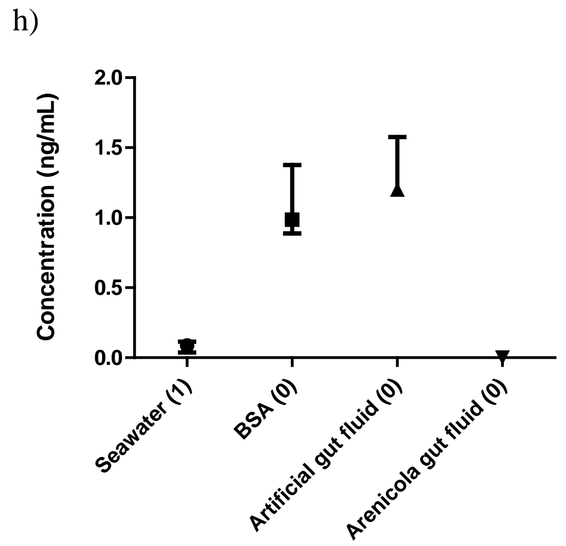

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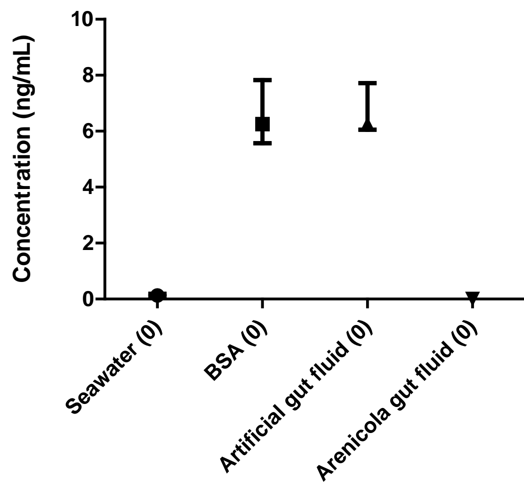

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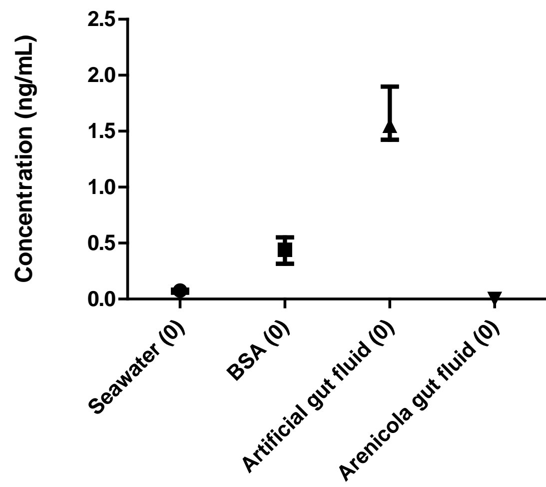

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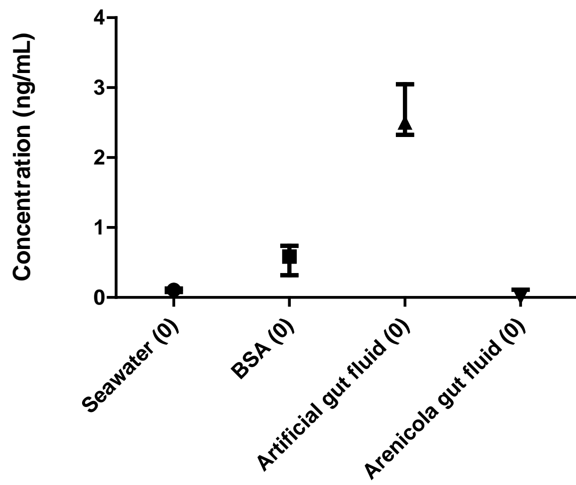

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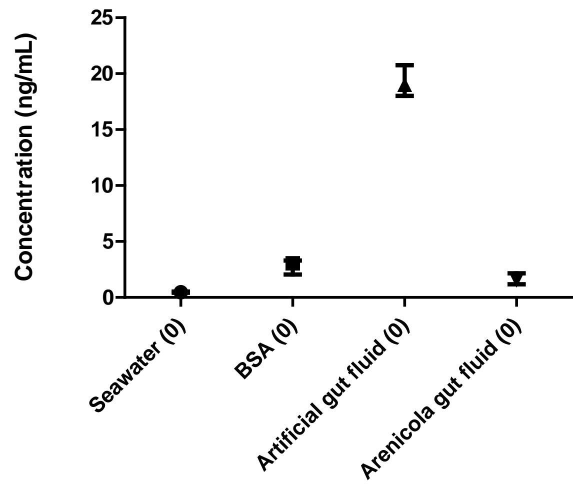

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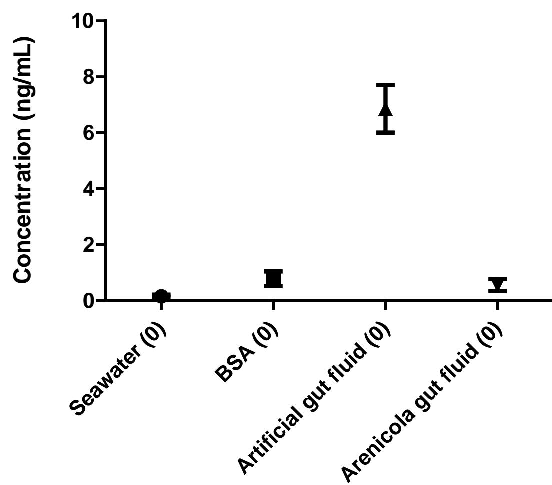

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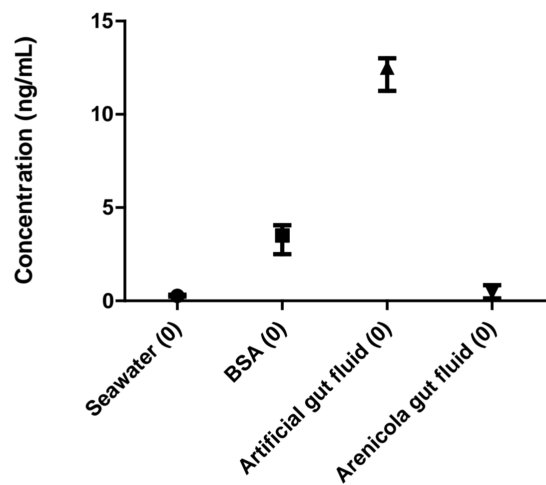

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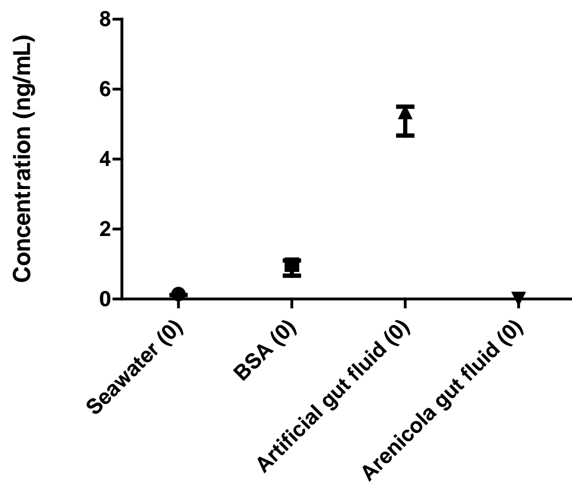

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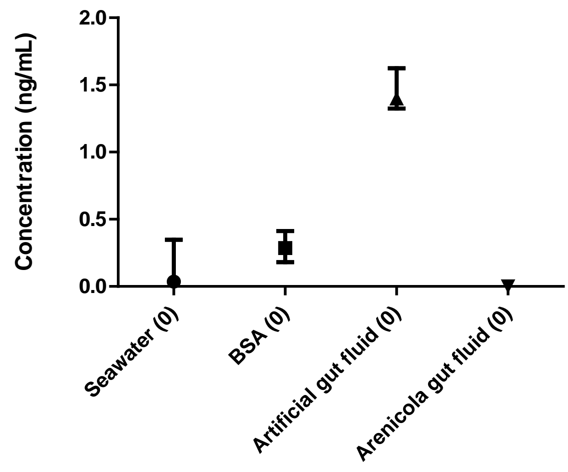

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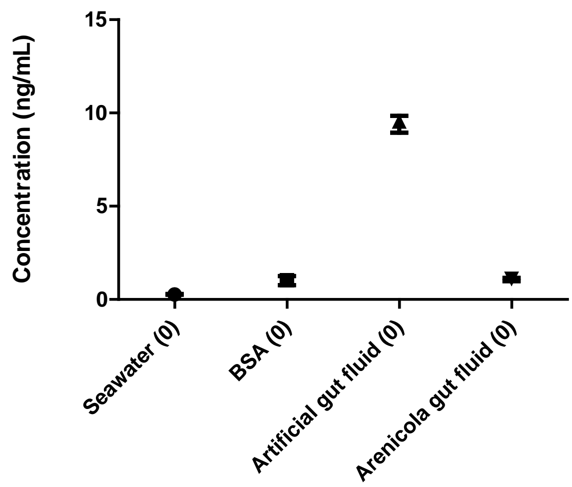

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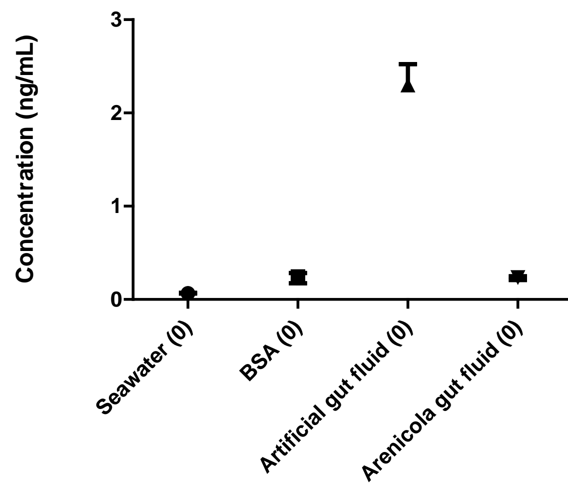

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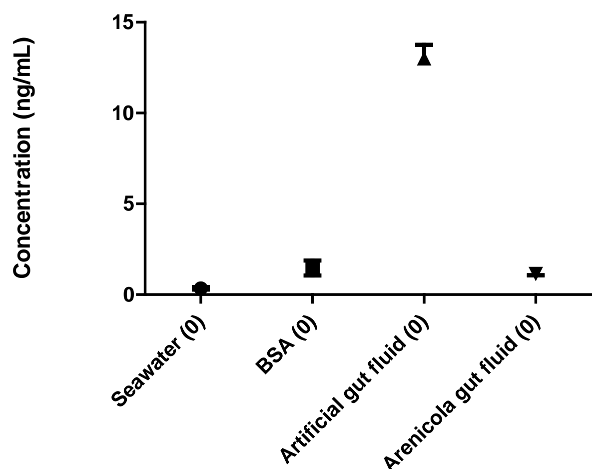

Figure 3.7 The concentration (ng/mL) of 19 different PAHs in the extracts of the four fluids (n = 4), after 4 hours of mixing with the Sunndalsfjord sediment; a) naphthalene , b) acenaphthylene, c) acenaphthene, d) fluorene, e) dibenzothiophene, f) phenanthrene, g) anthracene, h) fluoranthene, i) pyrene, j) benzo[a]anthracene, k) chrysene, l) benzo[b, j]fluoranthene, m) benzo[j,k]fluoranthene, n) benzo[e]pyrene, o) benzo[a]pyrene, p) perylene, q) ideno[1,2,3-cd]pyrene, r) dibenzo[a,c]anthracene and s) benzo[g,h,i]perylene; median and interquartiles. The numbers in the brackets denotes how many of the technical replicates that were below the detection value.

For naphthalene (figure 3.7a), there was a difference between the concentration in the different fluids ($H = 9.40$, $p \leq 0.05$, $n = 4$). Wilcoxon comparison test showed that the *Arenicola* gut fluid had a concentration significantly different from the other fluids ($p \leq 0.05$), while there was not a significant difference between the BSA solution, artificial gut fluid and the seawater ($p > 0.05$).

There was a difference between the concentration of acenaphthylene (fig 3.7b) in the different groups ($H = 9.40$, $p \leq 0.05$, $n = 4$). Wilcoxon comparison test showed that there was a significant difference between the *Arenicola* gut fluid and the other fluids ($p \leq 0.05$), while there was not a significant difference between the BSA solution, seawater and artificial gut fluid ($p > 0.05$).

There was a difference between the concentration of acenaphthene in the different groups ($H = 13.48$, $p \leq 0.05$, $n = 4$; fig.3.7c). Wilcoxon comparison test showed that there was not a significant difference between the BSA solution and the artificial gut fluid ($p > 0.05$). There were significant differences between the seawater and the other solutions ($p \leq 0.05$), between

the *Arenicola* gut fluid and artificial gut fluid ($p \leq 0.05$), and between the *Arenicola* gut fluid and BSA solution ($p \leq 0.05$).

For fluorene, there was a difference between the concentration in the different fluids following incubation with Sunndalsfjord sediment ($H = 12.49$, $p \leq 0.05$, $n = 4$; fig. 3.7d). Wilcoxon comparison test showed that there was not a significant difference between the seawater and the BSA solution ($p > 0.05$), or between the BSA solution and the artificial gut fluid ($p > 0.05$). There was a significant difference between the concentration in the *Arenicola* gut fluid and the other solutions ($p \leq 0.05$), and between the seawater and the artificial gut fluid ($p \leq 0.05$).

For dibenzothiophene, there was a difference between the concentration in the different fluids ($H = 11.53$, $p \leq 0.05$, $n = 4$; fig. 3.7e). Wilcoxon comparison test showed that there was not a significant difference between the seawater and the *Arenicola* gut fluid ($p > 0.05$), or between the BSA solution and the artificial gut fluid ($p > 0.05$). There was a significant difference between the concentration in the artificial gut fluid and *Arenicola* gut fluid, in the *Arenicola* gut fluid and the BSA solution, in the seawater and artificial gut fluid and in the seawater and the BSA solution ($p \leq 0.05$).

After mixing with the Sunndalsfjord sediment, there was a difference between the concentration of phenanthrene in the different fluids ($H = 13.41$, $p \leq 0.05$, $n = 4$; fig. 3.7f). Wilcoxon comparison test showed that there was not a significant difference between the BSA solution and the artificial gut fluid ($p > 0.05$). There was a significant difference between the *Arenicola* gut fluid and the artificial gut fluid, seawater and BSA solution ($p \leq 0.05$), between the seawater and BSA solution, as well as between the seawater and the artificial gut fluid ($p \leq 0.05$).

There was a significant difference between the concentration of anthracene in the different fluids ($H = 13.50$, $p \leq 0.05$, $n = 4$; fig. 3.7g). Wilcoxon comparison test showed that there was not a significant difference between the concentration in the BSA solution and in the artificial gut fluid ($p > 0.05$). There was a significant difference between the *Arenicola* gut fluid and the other fluids ($p \leq 0.05$), as well as between the seawater and BSA solution and artificial gut fluid ($p \leq 0.05$).

There was a difference between the concentration of fluoranthene in the different groups ($H = 13.54$, $p \leq 0.05$, $n = 4$; fig. 3.7h). Wilcoxon comparison test showed that there was not a significant difference between the BSA solution and the artificial gut fluid ($p > 0.05$). There was a significant difference between the concentration in the *Arenicola* gut fluid the other fluids ($p \leq 0.05$), and between the seawater and BSA solution, as well as between the artificial gut fluid and seawater ($p \leq 0.05$).

After mixing with the Sunndalsfjord sediment, there was a difference between the concentration of pyrene in the different groups ($H = 12.90$, $p \leq 0.05$, $n = 4$; fig.3.7i). Wilcoxon comparison test showed that there was not a significant difference between the BSA solution and the artificial gut fluid ($p > 0.05$). There was a significant difference between the concentration in the *Arenicola* gut fluid and in the other fluids ($p \leq 0.05$). There was a significant difference between the seawater and BSA solution, as well as between the seawater and artificial gut fluid ($p \leq 0.05$).

For benzo[a]anthracene (fig 3.7 j), there was a difference between the concentration in the different fluids following incubation with sediment ($H = 14.33$, $p \leq 0.05$, $n = 4$). Wilcoxon comparison test showed that there were significant differences between all the groups ($p \leq 0.05$).

After mixing with the Sunndalsfjord sediment, there was a difference between the concentration of chrysene in the different groups ($H = 13.25$, $p \leq 0.05$, $n = 4$; fig. 3.7k). Wilcoxon comparison test showed that there was a significant difference between the artificial gut fluid and the *Arenicola* gut fluid, between the BSA solution and seawater ($p \leq 0.05$), between the BSA solution and the artificial gut fluid ($p \leq 0.05$), and between the seawater and the BSA solution ($p \leq 0.05$). There was not a significant difference between the seawater solution and the *Arenicola* gut fluid ($p > 0.05$).

After mixing with the Sunndalsfjord sediment, there was a significant difference between the concentration of benzo[b, j]fluoranthene in the groups ($p \leq 0.05$, $n = 4$; fig. 3.7l). Tukey's range test showed that there were significant differences between the concentrations in all the solutions ($p \leq 0.05$).

There was a significant difference between the concentration of benzo[j,k]fluoranthene in the different groups ($p \leq 0.05$, $n = 4$; fig. 3.7m). Tukey's range test showed that there was not a significant difference between the BSA solution and the *Arenicola* gut fluid ($p > 0.05$). There was a significant difference between the concentration in the artificial gut fluid and in the other solutions ($p \leq 0.05$), and in the seawater and in the other solutions ($p \leq 0.05$).

There was a difference between the concentration of benzo[e]pyrene in the different fluids ($H = 12.72$, $p \leq 0.05$, $n = 4$; fig. 3.7n). Wilcoxon comparison test showed that there was not a significant difference between the seawater and the BSA solution ($p > 0.05$). A significant difference was observed between the *Arenicola* gut fluid and other fluids ($p \leq 0.05$), and between the artificial gut fluid and BSA solution, as well as between the artificial gut fluid and seawater ($p \leq 0.05$).

After mixing with the Sunndalsfjord sediment, there was a difference between the concentration of benzo[a]pyrene in the different groups ($H = 14.46$, $p \leq 0.05$, $n = 4$; fig. 3.7o). Wilcoxon comparison test showed that there was a significant difference between all the groups ($p \leq 0.05$).

There was a significant difference between the concentration of perylene (fig. 3.7 p) in the different groups ($p \leq 0.05$, $n = 4$). There was a significant difference between the artificial gut fluid and the other fluids ($p \leq 0.05$), and not a significant difference between the BSA solution, *Arenicola* gut fluid and seawater ($p > 0.05$).

There was a difference between the concentration of ideno[1,2,3-cd]pyrene (fig. 3.7q) in the different fluids ($p \leq 0.05$, $n = 4$). Tukey's range test showed that there was not a significant difference between the BSA solution and the *Arenicola* gut fluid ($p > 0.05$). There was a significant difference between the concentration in the artificial gut fluid and in the seawater, in the BSA solution and in the artificial gut fluid ($p \leq 0.05$), in the seawater and in BSA solution ($p \leq 0.05$), as well as in the seawater and in *Arenicola* gut fluid ($p \leq 0.05$)

There was a difference between the concentration of dibenzo[a,c]anthracene in the different fluids ($H = 12.80$, $p \leq 0.05$, $n = 4$; fig. 3.7r). Wilcoxon comparison test showed that there was not a significant difference between the BSA solution and the *Arenicola* gut fluid ($p > 0.05$).

There was a significant different between the concentration in the artificial gut fluid and in the seawater, in the BSA solution and in artificial gut fluid, in the seawater and in the BSA solution, as well as in the seawater and in the *Arenicola* gut fluid (all $p \leq 0.05$).

There was a difference between the concentration of benzo[g,h,i]perylene (fig. 3.7s) in the different fluids ($H = 13.21$, $p \leq 0.05$, $n = 4$). Wilcoxon comparison test showed that there was not a significant difference between the BSA solution and the *Arenicola* gut fluid ($p > 0.05$). There was a significant difference between the *Arenicola* gut fluid and the artificial gut fluid, between the *Arenicola* gut fluid and seawater, between the seawater and the BSA solution and artificial gut fluid, as well as between the BSA solution and artificial gut fluid (all $p \leq 0.05$)

3.6.3 Release from Karmøy sediment

The artificial gut fluid had the greatest effect on release of most of the PAHs from the sediment, followed by the BSA solution (fig 3.8a-s). The seawater and *Arenicola* gut fluid had a lower ability to increase the bioavailability of the PAHs than the BSA solution and artificial gut fluid (fig. 3.8a-s). For several of the PAHs (fig 3.8 a, b, c, d, f and g) the *Arenicola* gut fluid had no ability to increase the bioavailability of the PAHs. There was a significant difference between the concentrations in the artificial gut fluid and the *Arenicola* gut fluid for all the PAHs ($p \leq 0.05$), and there was a significant difference ($p \leq 0.05$) between the *Arenicola* gut fluid and the BSA solution for all the PAHs, expect for chrysene and benzo[e]pyrene ($p > 0.05$; fig. 3.8k and 3.8n)

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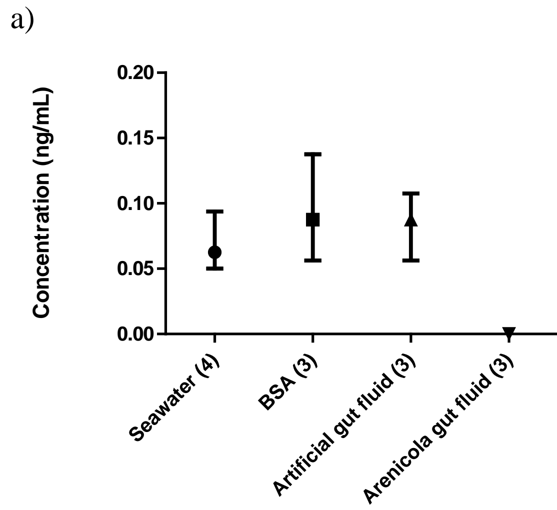

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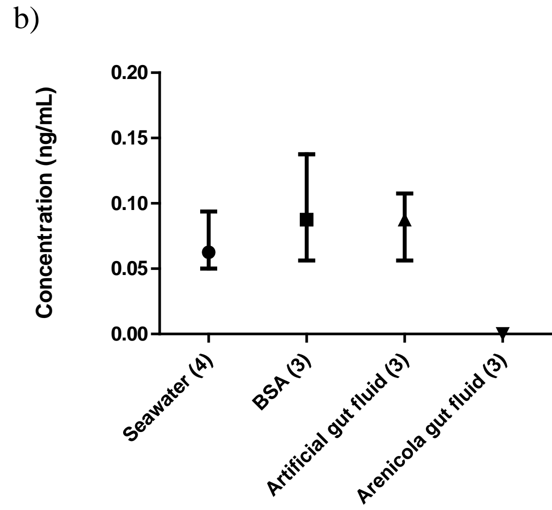

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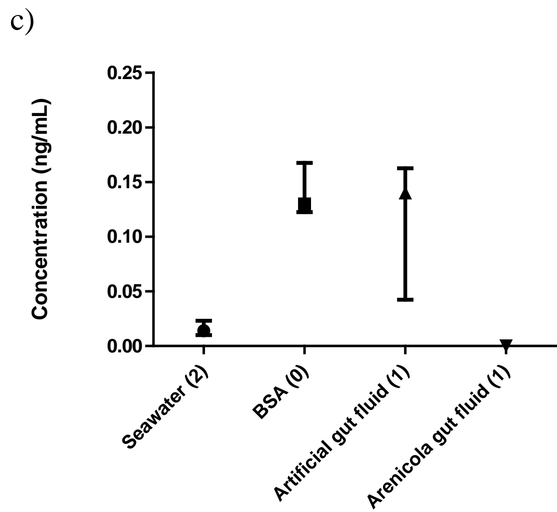

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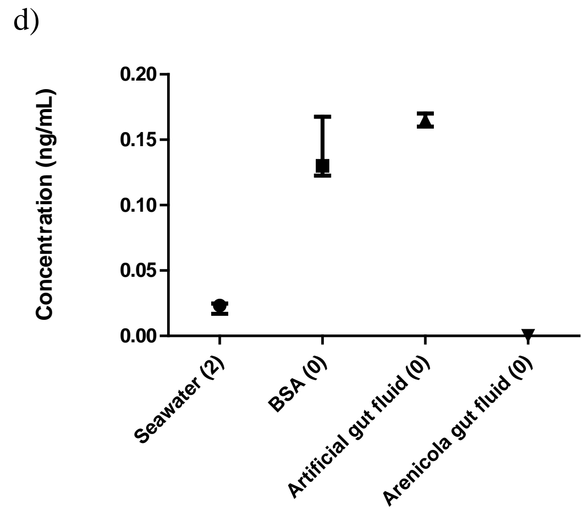

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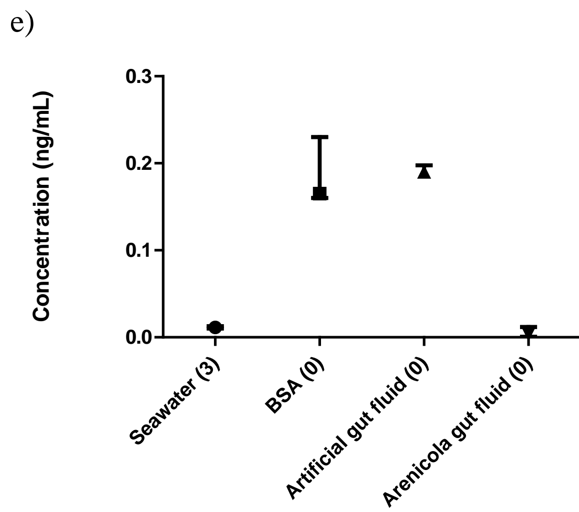

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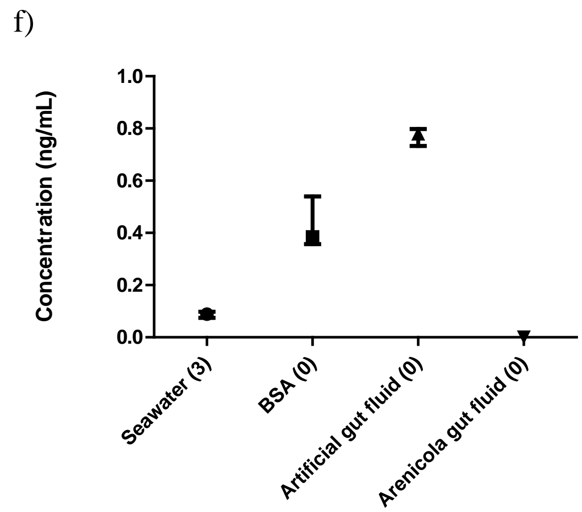

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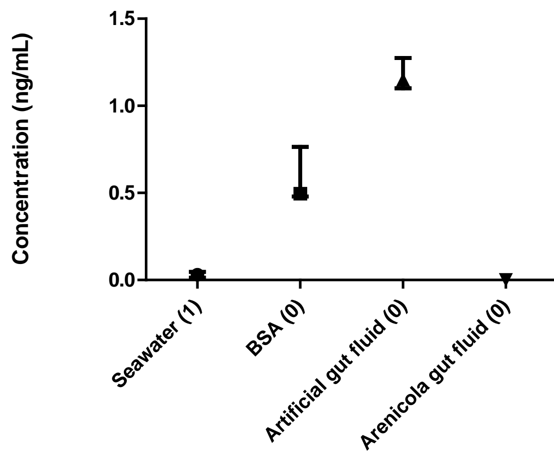

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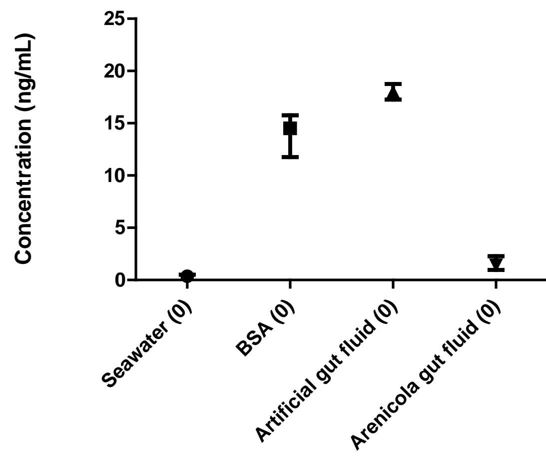

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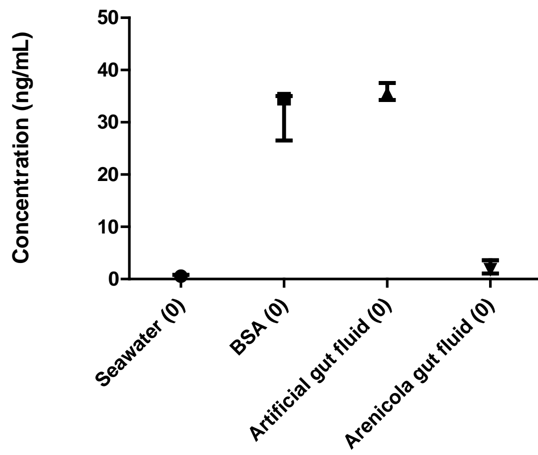

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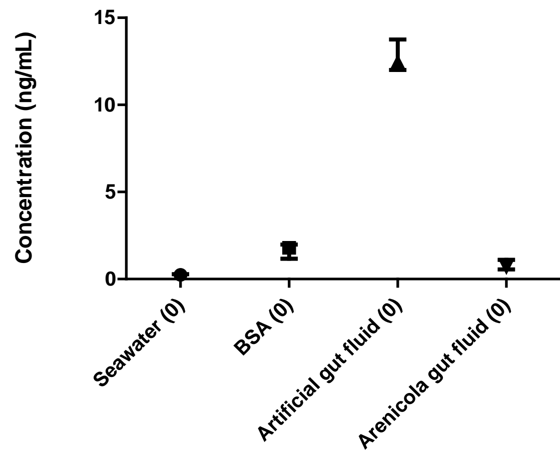

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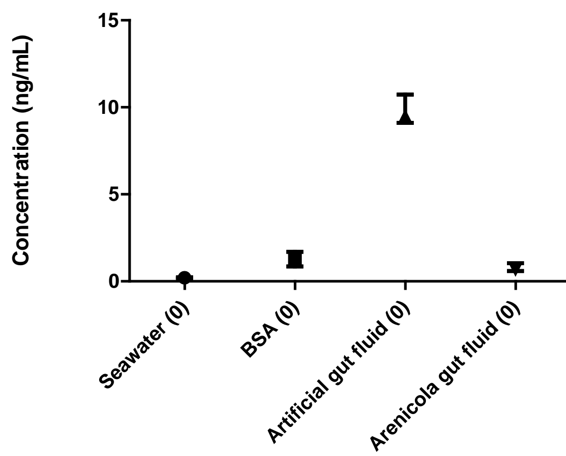

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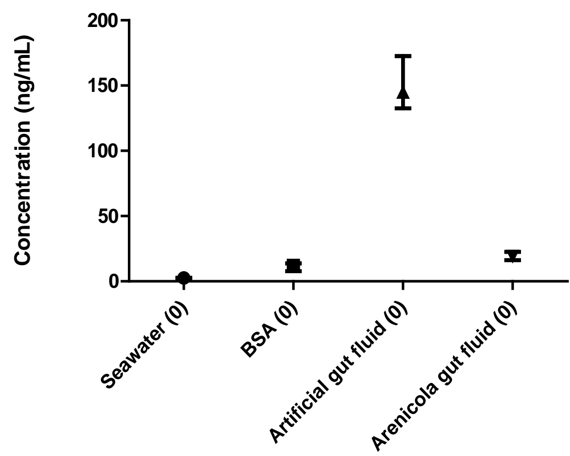

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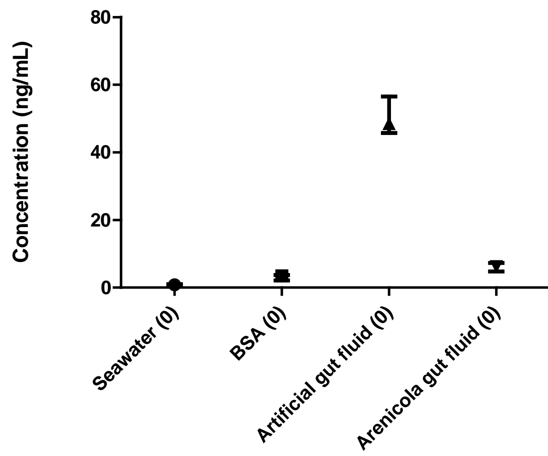

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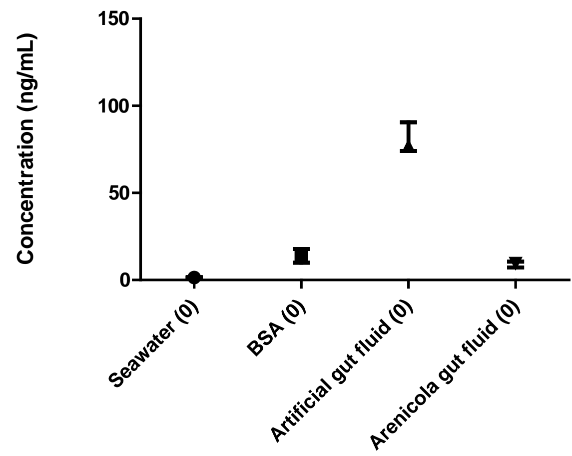

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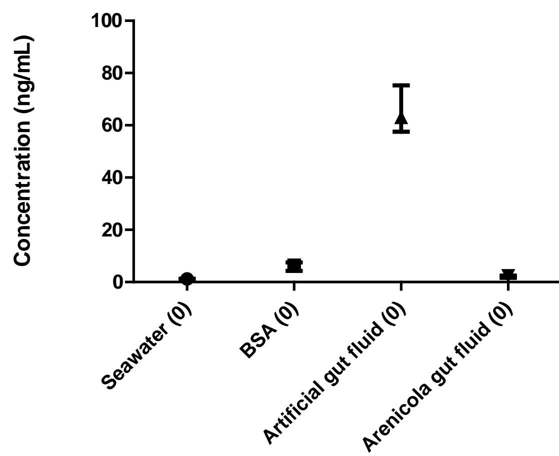

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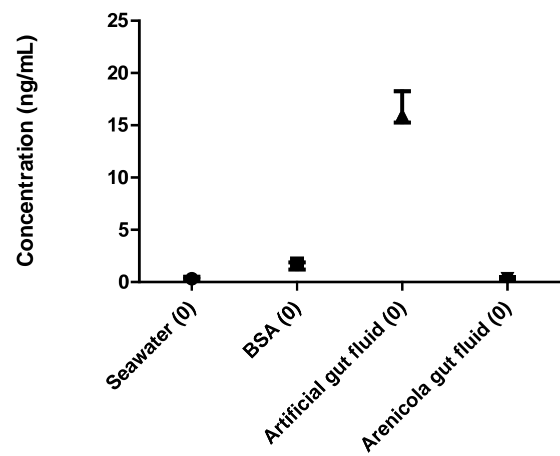

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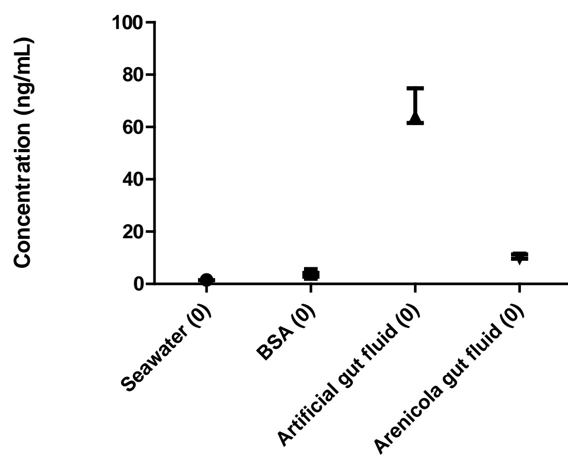

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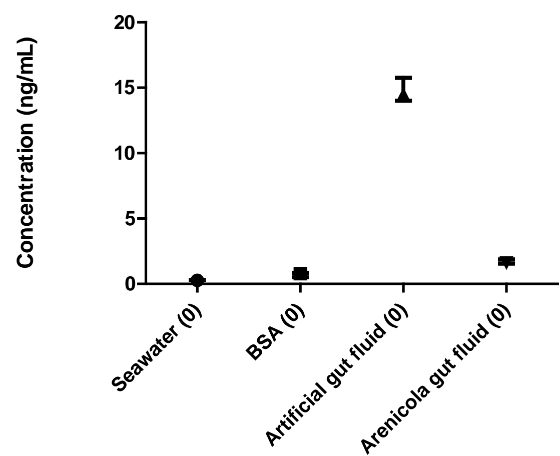

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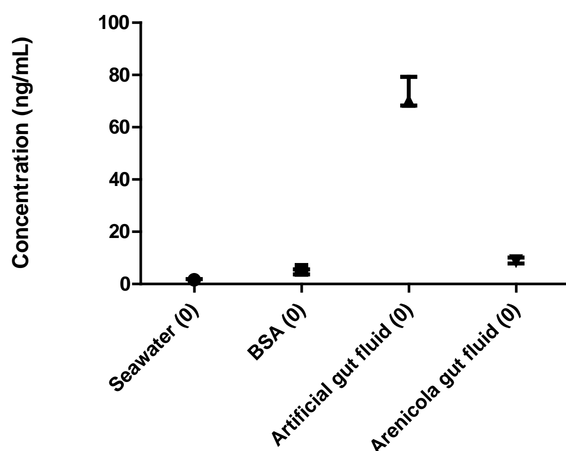

Figure 3.8 The concentration of 19 different PAHs in the extracts of the four fluids(n=4), after 4 hours of mixing with the Karmøy sediment. ; a) naphthalene, b) acenaphthylene, c) acenaphthene, d) fluorene, e) dibenzothiophene, f) phenanthrene, g) anthracene, h) fluoranthene, i) pyrene, j) benzo[a]anthracene, k) chrysene, l) benzo[b, j]fluoranthene, m) benzo[j,k]fluoranthene, n) benzo[e]pyrene, o) benzo[a]pyrene, p) perylene, q) ideno[1,2,3-cd]pyrene, r) dibenzo[a,c]anthracene and s) benzo[g,h,i]perylene; median and interquartiles. The numbers in the brackets denotes how many of the technical replicates that were below the detection value.

There was a significant difference between the concentration of naphthalene (fig. 3.8a) in the different fluids ($H = 10.33$, $p \leq 0.05$, $n = 4$). Wilcoxon comparison test showed that there was a significant difference between the concentration in the *Arenicola* gut fluid and the other fluids ($p \leq 0.05$). There was not a significant difference between the BSA solution, artificial gut fluid and seawater ($p > 0.05$).

There was a difference between the concentration of acenaphthylene (fig. 3.8b) in the different fluids ($H = 9.46$, $p \leq 0.05$, $n = 4$). There was a significant difference between the *Arenicola* gut fluid and the other groups ($p \leq 0.05$). There was not a significant difference between the BSA solution, artificial gut fluid and seawater ($p > 0.05$).

There was a difference between the concentration of acenaphthene (fig. 3.8c) in the different fluids ($H = 11.54$, $p \leq 0.05$, $n = 4$). Wilcoxon comparison test showed that there was not a significant difference between the BSA solution and the artificial gut fluid ($p > 0.05$), nor between the seawater and the artificial gut fluid ($p > 0.05$). The *Arenicola* gut fluid had a

concentration significantly different from the other fluids ($p \leq 0.05$), and there was a significant difference between the BSA solution and the seawater ($p \leq 0.05$).
For fluorene (fig. 3.8d), there was a difference between the concentration in the different fluids ($H = 13.31$, $p \leq 0.05$, $n = 4$). Wilcoxon comparison test showed that there was not a significant difference between the BSA solution and the artificial gut fluid ($p > 0.05$). There was a significant difference between the *Arenicola* gut fluid other fluids ($p \leq 0.05$), and between the seawater and BSA solution, as well as between the seawater and the artificial gut fluid ($p \leq 0.05$).

There was a significant difference between the concentration in the different fluids following incubation with sediment ($H = 12.11$, $p \leq 0.05$, $n = 4$; fig. 3.8e). Wilcoxon comparison test showed that there was not a significant difference between the *Arenicola* gut fluid and seawater ($p > 0.05$), or between the BSA solution and artificial gut fluid ($p > 0.05$). There was a significant difference between the concentration in the *Arenicola* gut fluid and in the BSA solution ($p \leq 0.05$), in the *Arenicola* gut fluid and in the artificial gut fluid ($p \leq 0.05$), in the seawater and in the artificial gut fluid, as well as in the seawater and in the BSA solution ($p \leq 0.05$).

After mixing with the Karmøy sediment, there was a difference between the concentration of phenanthrene (fig. 3.8f) in the different groups ($H = 14.33$, $p \leq 0.05$, $n = 4$). Wilcoxon comparison test showed that there was a significant difference between the concentration of phenanthrene in all of the fluids ($p \leq 0.05$).

There was a difference between the concentration of anthracene (fig. 3.8g) in the different groups ($H = 14.37$, $p \leq 0.05$, $n = 4$). Wilcoxon comparison test showed that there was a significant difference between all the fluids ($p \leq 0.05$).

There was a difference between the concentration of fluoranthene (fig. 3.8 h) in the different fluids ($p \leq 0.05$, $n = 4$). There was not a significant difference between the seawater and the *Arenicola* gut fluid ($p > 0.05$). Tukey's range test showed a significant difference between the artificial gut fluid and the other solutions ($p \leq 0.05$). The BSA solution was also significantly different from the other fluids ($p \leq 0.05$).

There was a significant difference between the concentration of pyrene (fig. 3.8i) in the different fluids ($H = 13.27$, $p \leq 0.05$, $n = 4$). There was not a significant difference between the BSA solution and the artificial gut fluid ($p > 0.05$). Wilcoxon comparison test showed that the seawater and the *Arenicola* gut fluid were significantly different from each other in addition to being significantly different from the BSA solution and artificial gut fluid ($p \leq 0.05$).

After mixing with the Karmøy sediment, there was a significant difference between the concentration of benzo[a]anthracene (fig. 3.8j) in the different fluids ($p \leq 0.05$, $n = 4$). Tukey's range test showed that there were significant differences between the concentrations in all the groups ($p \leq 0.05$).

There was a significant difference between the concentration of chrysene (fig. 3.8k) in the different fluids after mixing with the Karmøy sediment ($p \leq 0.05$, $n = 4$). Tukey's range test showed that there was not a significant difference between the BSA solution and the *Arenicola* gut fluid ($p > 0.05$). There was a significant difference between the artificial gut and the other fluids ($p \leq 0.05$), and between the seawater and BSA solution, as well as between the seawater and the *Arenicola* gut fluid ($p \leq 0.05$).

There was a significant difference between the concentration of benzo[b,j]fluoranthene (fig. 3.8l) in all the groups ($p \leq 0.05$, $n = 4$). Tukey's range test showed that there were significant differences between all the groups ($p \leq 0.05$).

There was a significant difference between the concentration of benzo[j,k]fluoranthene (fig. 3.8m) in the different fluids ($p \leq 0.05$, $n = 4$). Tukey's range test showed that there were significant differences between all the groups ($p \leq 0.05$).

There was a difference between the concentration of benzo[e]pyrene (fig 3.8n) in the different groups ($p \leq 0.05$, $n = 4$). Tukey's range test showed that there was not a significant difference between the BSA solution and *Arenicola* gut fluid ($p > 0.05$), while the test showed that there was a difference between the other groups ($p \leq 0.05$).

After mixing with the Karmøy sediment, there was a difference between the concentration of benzo[a]pyrene (fig. 3.8o) in the different fluids ($p \leq 0.05$, $n = 4$). There was a significant difference between all the groups ($p \leq 0.05$). This was shown with Tukey's range test. There was a significant difference between the concentration of perylene (fig. 3.8p) in the different groups ($p \leq 0.05$, $n = 4$). Tukey's range test showed that there was not a significant difference between the seawater and the *Arenicola* gut fluid ($p > 0.05$). The artificial gut fluid and the BSA solution were significantly different from each other ($p \leq 0.05$), as well as from the seawater and the *Arenicola* gut fluid ($p \leq 0.05$).

There was a difference between the concentration of ideno[1,2,3-cd]pyrene (fig. 3.8q) in the different fluids ($p \leq 0.05$, $n = 4$). Tukey's range test showed that there were significant differences between all the fluids ($p \leq 0.05$).

There was a difference between the concentration of dibenzo[a,c]anthracene (fig. 3.8r) in the different groups ($p \leq 0.05$, $n = 4$). Tukey's range test showed that there was a significant difference between all of the fluids ($p \leq 0.05$).

There was a difference between the concentration of benzo[g,h,i]perylene (fig. 3.8s) in the different groups ($p \leq 0.05$, $n = 4$). Tukey's range test showed that there were significant differences between all the groups ($p \leq 0.05$).

4 Discussion

4.1 Characterization of *Arenicola* gut fluid

4.1.1 Protease and lipase activity

The *Arenicola* gut fluid had a low protease activity (where the undiluted gut fluid had a lower activity than the diluted gut fluid,) and a high lipase activity. This does not correspond with the protease and lipase activity in the gut of detritivorous polychaetes, but correspond with the lipase and protease activity in the gut of carnivorous polychaetes reported earlier (Michel et al. 1984; Mayer et al. 1997). Mayer et al. (1997) measured fluorescence for both lipase and protease. We chose a different approach. We used fluorescence to estimate the protease activity, while absorbance was used to measure lipase activity. There was also a difference in the choice of substrates used for the tests, which may have given different results. Precipitation in the wells could affect the fluorescence measurements. The diluted gut fluid had some lower lipase activity than the undiluted fluid. The small reduction in activity may be due to the dilution with seawater, which had the lowest lipase activity of the fluids. The opposite was seen for the protease activity; the diluted gut fluid had a higher protease activity than the undiluted gut fluid. This could be because the diluted fluids had less particulate matter than the undiluted gut fluid, and therefore not affect the fluorescent measurement as much as the undiluted gut fluid.

The artificial gut fluid and the BSA solution had protease activities more than 10 times higher than the *Arenicola* gut fluid. The artificial gut fluid had a lipase activity 8 times lower than the *Arenicola* gut fluid, while the BSA solution had an activity not that different from the undiluted gut fluid. The BSA may contain enzymes, since it is a protein extracted from bovine serum plasma. This could be the reason for the high lipase activity in the BSA solution and artificial gut fluid, and the high protease activity in the BSA solution. However, the artificial gut fluid had a lipase activity much lower than the BSA solution, and should at the very least have had the same activity.

4.1.2 Protein content

There was a difference between the protein content in the diluted gut fluid and in the undiluted gut fluid. The ratio between the two fluids corresponded well with what we would expect, since the concentration of protein in the seawater was below the detection limit for the method (0.5 µg protein/cm^3). The protein content in the diluted *Arenicola* gut fluid was similar to the protein content in the BSA solution, while the protein content in the undiluted gut fluid was slightly less to the protein content in the artificial gut fluid.

The BSA solution and artificial gut fluid had different content of protein. Each solution was added the same amount of BSA, and should therefore have shown the same content of protein after analysis. A possible reason for this could be the sodium taurocholate hydrate. This bile salt has an amine group and forms taurine if hydrolysed (Rosch et al. 2008). This amine group may have caused the elevated protein content in the artificial gut fluid.

Voparil and Mayer (2004) conclude that the artificial gut fluid may be administered a less concentrated BSA solution, when mimicking the protein content in the gut fluids of *Arenicola marina*, which corresponds well with the results.

4.1.3 Surfactant properties

The *Arenicola* gut fluid had contact angles that were 20-30° higher than for commercial surfactants which are 30-40° (Mayer et al. 1996), but they were 30-40° lower than for pure seawater. Lower contact angles indicate greater spreading of a droplet and therefore have a higher surfactant property (Mayer et al. 1996). This indicates a relatively high surfactant property in the gut fluid. The gut fluids also exhibited surfactant properties by frothiness during pipetting.

The BSA solution and artificial gut fluids had angles greater than the undiluted *Arenicola* gut fluid, but had angles smaller than the seawater. Voparil and Mayer (2004) used sodium taurocholate hydrate in order to create solutions with the same critical micelle dilution as *Arenicola marina* gut fluids. Bile salts main functions are to form micelles and emulsify dietary fat (Sand et al. 2006). This bile salt is most likely the reason for the difference

between the contact angle for the BSA solution and for the artificial gut fluid. However, both solutions showed surfactant properties by frothiness during pipetting.

W could see that the artificial gut fluid and the unfiltered and diluted *Arenicola marina* gut fluid had similar contact angles. This does not correspond with Voparil and Mayer (2004). Voparil and Mayer used undiluted *Arenicola marina* gut fluids, where the undiluted *Arenicola marina* exhibited a contact angle similar to the contact angle for the artificial gut fluid. In this study, the undiluted *Arenicola marina* gut fluid had a contact angle smaller than the angle for the artificial gut fluid, and hence should have a higher surfactant property than the artificial gut fluid.

4.2 Test sediments

We would expect to see approximately the same proportion of PAHs desorbed from both the contaminated sediments, since they did not differ much in PAH content. However, what saw was that the Sunndalsfjord sediment was significantly different from the Karmøy sediment for the high molecular weight PAHs (chrysene and benzo[a]pyrene). The Sunndalsfjord sediment was not significantly different from the control sediment, which had a PAH content far less than the contaminated sediments. For the low molecular weight PAHs (naphthalene and phenanthrene), the control sediment showed a higher release than the contaminated sediments. The Karmøy sediment had the second highest release of naphthalene and phenanthrene.

The same sediments were used in Ruus et al (2010) bioaccumulation test, and they discovered that the Sunndalsfjord sediment had a higher organic carbon/water partition coefficient (K_{oc}) than the sediment from Karmøy. This indicated that the PAHs had a stronger adsorption to the Sunndalsfjord sediment than to the Karmøy sediment. In addition, Ruus et al (2010) determined the ratio between the predicted biota-to-sediment accumulation factors (BSAFs) and observed BSAFs in sediment exposed organisms. The predicted BSAFs were based on simple organic carbon sorption models. The ratio indicated that the PAHs had a stronger particle association in the Sunndalsfjord sediment than the Karmøy sediment.

The strong particle association in the Sunndalsfjord sediment is most likely attributed to the strong absorption behaviour of black carbon (soot and charcoal) and organic carbon. This is in accordance with previous work, which shows that sediments from areas with high anthropogenic activities have similar behaviour due to relatively high soot and charcoal content (Cornelissen et al. 2006; Oen et al. 2006). It may be that there was more black carbon in the Sunndalsfjord sediment and control sediment, than in the Karmøy sediment. This could explain why there was a higher desorption from the Karmøy sediment than from the other two, even though the Karmøy sediment had the highest TOC.

The relatively high desorption of naphthalene and phenanthrene may to some extent be explained by the high release from the sediment, but it is of great importance that they are so volatile (Neff 1979).

4.3 PAHs extracted by fluids

The *Arenicola* gut fluid did not appear to increase the bioavailability of the PAHs from the control sediment. The concentration of PAHs in the *Arenicola* gut fluid after incubating with the Sunndalsfjord- and Karmøy sediment was low, which indicate that the gut fluid was inefficient in regards to increasing the bioavailability of the PAHs. However, the gut fluid did appear to increase the bioavailability for most of the sediment-bound PAHs more than the seawater. This corresponds with previous studies (Mayer et al. 1996; Voparil and Mayer 2000; Timmermann and Andersen 2003; Voparil et al. 2004; Voparil and Mayer 2004; Mayer et al. 2007) and is in accordance with the hydrophobic nature of PAHs (Neff 1979; Neff 1985; Knutzen 1995; Ferguson and Chandler 1998)

The *Arenicola* gut fluid had a lesser effect on the bioavailability of the PAHs in comparison with the artificial gut fluid. This does not correspond with Voparil and Mayer (2004). The artificial gut fluid showed a great ability in increasing the bioavailability of the PAHs in the three sediments, and had a greater effect on the bioavailability than the other three fluids. Voparil and Mayer (2004) concluded that the artificial gut fluid could serve as a surrogate for the real gut fluids in bioavailability tests. According to our findings, the result from a bioavailability test using the artificial gut fluid would overestimate the gut fluids ability to increase the bioavailability. Interestingly, the *Arenicola* gut fluid was more similar to the BSA

solution in its ability to increase the bioavailability of the sediment-bound PAHs, rather than the artificial gut fluids. This may be explained by the dilution of the *Arenicola* gut fluid with seawater. However, we would expect the *Arenicola* gut fluid and the artificial gut fluid to have relatively the same effect on the bioavailability in conjunction with the surfactant analysis. The difference may be due to differences in the effect of the sodium taurocholate hydrate. This bile salt may be more effective in desorbing the PAHs from the sediment than the surfactant agents in the real gut fluids.

There were also some differences in the ability to desorb from the sediments between the PAH. The low molecular weight PAHs are more volatile than the high molecular weight PAHs, and are therefore more readily released into the fluids (Neff 1979). Example, naphthalene is a volatile PAH, and is known to be present in the air, both indoors and outdoors due to anthropogenic sources (Neff 1979; World Health Organization International Agency for Research on Cancer 2002). The high concentration of naphthalene in the seawater may be due to air contamination rather than as a result of the shaker experiment with the sediments. It is also known that the low molecular weight PAHs are less hydrophobic than the high molecular PAHs (Neff 1979). This could be the explanation for the relatively high concentrations of PAHs seen in the seawater for the 2-3 ringed PAHs.

4.4 The methods

The *Arenicola marina* gut fluid sample blank had a higher concentration of PAHs than the test solutions for many of the samples. This resulted in negative values for every PAH in the *Arenicola marina* gut fluid after being incubated with the control sediment, 11 out of 19 PAHs after incubation with the Sunndalsfjord sediment and 6 out of 19 PAHs after incubating with the Karmøy sediment. These values were therefore set as nil. The high concentration of PAHs in the *Arenicola* sample blank was most likely due to remnants of particulate matter in the sample. During the sampling procedure for gut fluids, the fluid collected may have contained fluids from the vascular and coelomic system, as well as particles from the gut lining. This corresponds with the results. The gut fluid that incubated with the control sediment had lower concentrations than the sample blank. This is probably because the samples were centrifuged, and the particulate remnants were spun out of the solution with the sediment particles.

In addition to this, many of the replicates were under the detection value. These values were set as half the detection value, and are therefore not real numbers, but estimated values. It should be noted that this may have affected the outcome of the results.

It may be suggested that the orbital shaker experiment should have had a longer duration than 4 hours in order to reach equilibrium between the sediments and the fluids. However, studies (Weston and Mayer 1998; Lawrence et al. 1999) show that the gut retention time of the lugworm is between 1-2hours, and Kermack (1955) observed that the time for the food to pass through the gut of an *Arenicola marina* was 14 min. Weston and Mayer (1998) showed that the proportion of PAHs extracted with *Arenicola* gut fluids was constant from 20 min-4 h. The duration of 4 hours was therefore set as a compromise between the desire to reach equilibrium and the actual gut retention time of the lug worm. Our decision to let the fluids incubate with the sediments for 4 hours should therefore not give an underestimate.

4.5 Conclusions

This study showed that the *Arenicola* gut fluid led to minor increases in the bioavailability of sediment-bound PAHs, and that the artificial gut fluid had a much greater effect on increasing the bioavailability of the PAHs than the *Arenicola* gut fluid. This difference may partly be due to the high *Arenicola* sample blank, and that the *Arenicola* gut fluid was diluted with seawater.

The enzyme activity seemed to have little effect on the bioavailability of the PAHs, though they were present in the gut fluids. The protein and bile salt amount seemed to have a greater impact on the fluids ability to increase the bioavailability of the sediment-bound PAHs, than the enzymes. The amount of carbon in the sediments may affect the bioavailability of the sediment-bound PAHs.

Low molecular weight PAHs desorb more readily from the sediment into the fluids, than the high molecular weight PAHs. It is therefore important to screen for both high- and low molecular weight PAHs when doing a bioavailability test in order to avoid false positive/negative results.

The results in this thesis showed that the gut fluids of *Arenicola marina* led to an increase in the desorption of PAHs from sediments. This should be taken into account when risk assessing polluted sediments. One should also be aware that when using artificial gut fluids, you may be at risk of overestimating the bioavailability.

4.6 Future work

One should centrifuge the samples prior to the experiments to avoid particulate remnants interfering with the analysis, and one could extract the gut fluids with a syringe through the tissue, rather than with a Pasteur pipette in order to reduce the possibility of collecting tissue from the gut lining and fluids from the coelomic and vascular system. Instead of using lipase and protease kits from a commercial supplier, it would be interesting to see if the analysis done as Mayer et al. (1996) would give a different result. One should avoid diluting the gut fluids by collecting fluids from more individuals and have more replicates in order to secure more accurate estimates.

It would be interesting to do bioavailability tests on other invertebrates with different feeding methods, in combination with a bioaccumulation study, in order to see the differences between the organisms and to see how much of the desorbed PAHs get accumulated. The gut fluids could also be analysed for other properties, such as lipids and esterases, to see if they have an impact on the fluids ability to increase the bioavailability. It would also be interesting to check if the time of the lugworms last feeding would have an impact on the properties of the gut fluid.

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Appendix

Equipment

Heraeus Sepatech Megafuse 1.0
IKA Labotechnik KS501 digital orbital shaker
IKA Labortechnik MS2 Minishaker
Meterlab CDM92 conductivity meter
Meterlab PHM220 LAB pH meter
Millipore SAS Millipore water purification system

GC-MSD:
Agilent J&W DB5-MS Column (30m x 0.25 mm i.d x 0.25 µm flim)
Agilent Technologies HP Autosampler model 7686
Agilent Technologies HP Gaschromatograph model 6890N
Agilent Technologies HP Mass selective detector model 5973 MSD
Restec corp. 4mm split injector with glasswool

GPC:
Environgel GPC Clean-up Column; 19x300mm and 19x150mm
Shimadzu Chromatopac C-R8A Integrator
Waters 2695 Separations Module
Waters 486 Tunable Absorbance Detector
Waters Fraction Collector

Protein, Protease and Lipase analysis:
Labnet Accublock Mini
Biotek Synergy MX

Sediment analysis:
Dionex ASE 200 Accelerated Solvent Extractor

Dionex Solvent controller

Micelle analysis:
Nikon SMZ-U Zoom 1:10
Nikon DS-5M

TOC analysis:
Carlo Erba 1106 elemental analyser

Chemicals

Listed according to manufacturer:

Abcam (Cambridge, England)
Lipase detection kit, Protease activity Assay kit

Agilent Technologies (Santa Clara, USA)
Hydromatrix

Bio-Rad (Oslo, Norway)
Protein Assay reagent A, Protein Assay reagent B

Dr. Ehrenstorfer GmbH (Augsburg, Germany)
Biphenyl D10, Naphthalene D8

Merck (Darmstadt, Germany)
Iso-octane P.A, Sodium sulfate (Na_2SO_4), Toluene P.A

Rathburn Chemicals Ltd (Walkerburn, Scotland)
Dichlorormethane HPLC-grade (DCM),

Sigma Aldrich (Oslo, Norway)

Albumin bovine serum (BSA), Sodium taurocholate hydrate, Trizma® Base, Trizma® Hydrochloride reagent grade

Ultra scientific (North Kingstown, RI, USA)

Acenaphthylene D8, Benzo(a)anthraceneD12, Dibenzothiophene D8, Perylene D12, Pyrene D10

Raw data:

Sediment analysis:

	TTS/%	NAP-Sm	ACNLE-Sm	ACNE-Sm	FLE-Sm	DBTHI-Sm	PA-Sm
	%	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.
	B 3	H 2-3	H 2-3	H 2-3	H 2-3	H 2-3	H 2-3
Control	75	11	<2	3.3	5.3	2.7	18
Sunndal	63	180	<10	380	200	120	1600
Karmøy	69	41	<10	110	55	39	570
	ANT-Sm	**FLU-Sm**	**PYR-Sm**	**BAA-Sm**	**CHR-Sm**	**BBJF-Sm**	**BKF-Sm**
	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.
	H 2-3	H 2-3	H 2-3	H 2-3	H 2-3	H 2-3	H 2-3
Control	5.5	34	25	12	18	23	10
Sunndal	270	2500	2100	1300	2000	3000	1200
Karmøy	140	1500	1400	990	1400	4300	1600
	BEP-Sm	**BAP-Sm**	**PER-Sm**	**ICDP-Sm**	**DBA3A-Sm**	**BGHIP-Sm**	**Sum PAH**
	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.	µg/kg t.v.
	H 2-3	H 2-3	H 2-3	H 2-3	H 2-3	H 2-3	Beregnet*
Control	13	14	23	11	2.8	16	<249.6
Sunndal	1800	1900	540	1300	340	1500	22230
Karmøy	2400	2400	690	2100	530	2500	22765

PAH concentration:

		NAP-V	ACNLE-V	ACNE-V	FLE-V	DBTHI-V
		ng/l	**ng/l**	**ng/l**	**ng/l**	**ng/l**
		H 2-2	**H 2-2**	**H 2-2**	**H 2-2**	**H 2-2**
Blank dest.water (seawater batch		<200	<3	<20	<45	<25
Control repl. 1 seawater		<100	<2	<12	<20	<10
Control repl. 2 seawater		<100	4	14	26	15
Control repl. 3 seawater		<100	<2	<12	<20	<20
Control repl. 4 seawater		<150	<10	<20	<30	<20
Sunndalsfj repl. 1 seawater		<200	3,2	<20	<45	<25
Sunndalsfj repl. 2 seawater		<100	<3	22	24	<10
Sunndalsfj repl. 3 seawater		<100	4,3	16	<20	<20
Sunndalsfj repl. 4 seawater		<150	<10	<20	<30	<20
Karmøy repl. 1 seawater		<200	<3	<20	<45	<25
Karmøy repl. 2 seawater		<100	<3	25	25	13
Karmøy repl. 3 seawater		<100	<2	18	24	<20
Karmøy repl. 4 seawater		<150	<10	<20	<30	<20
Blank dest.water (BSA batch)		<200	<3	<20	<45	<25
Kontroll repl. 1 BSA		110	<2	14	<20	10
Kontroll repl. 2 BSA		110	3,6	22	27	13
Kontroll repl. 3 BSA		<100	<2	<12	<20	<20
Kontroll repl. 4 BSA		<150	<10	<20	<30	<20
Sunndalsfj repl. 1 BSA		<200	15	76	<45	40
Sunndalsfj repl. 2 BSA		210	15	59	48	35
Sunndalsfj repl. 3 BSA		<100	11	46	27	22
Sunndalsfj repl. 4 BSA		<150	<10	37	<30	21
Karmøy repl. 1 BSA		<200	<3	180	180	250
Karmøy repl. 2 BSA		<100	7,1	130	120	160
Karmøy repl. 3 BSA		150	<2	120	130	160
Karmøy repl. 4 BSA		<150	<10	130	130	170
		NAP-V	**ACNLE-V**	**ACNE-V**	**FLE-V**	**DBTHI-V**
		ng/l	**ng/l**	**ng/l**	**ng/l**	**ng/l**
		H 2-2	**H 2-2**	**H 2-2**	**H 2-2**	**H 2-2**
Blank dest.vann (art. gut-f)		<200	<3	<20	<45	<25
Control repl. 1 artificial gut-f		140	10	20	<20	11
Control repl. 2 artificial gut-f		<100	4,6	21	36	22
Control repl. 3 artificial gut-f		<100	3	<12	<20	<20
Control repl. 4 artificial gut-f		<150	<10	<20	<30	<20
Sunndalsfj repl. 1 artificial gut		210	36	87	52	42
Sunndalsfj repl. 2 artificialgut		<100	23	68	49	36

Sunndalsfj repl. 3 artificial gut		<100	24	60	46	32
Sunndalsfj repl 4 artificial gut		<150	31	64	42	31
Karmøy repl. 1 artificial gut-flu		<200	36	<20	170	190
Karmøy repl. 2 artificial gut-flu		110	35	140	160	190
Karmøy repl. 3 artificial gut-flu		<100	39	140	170	190
Karmøy repl. 4 artificial gut-flu		<150	44	170	160	200
Blank dest.water (Arenicola G-F		<200	<3	<20	<45	<25
Control repl. 1 Arenicola G-F		<100	6,7	<12	<20	11
Control repl. 2 Arenicola G-F		<100	5,9	<12	<20	<10
Control repl. 3 Arenicola G-F		<100	<2	<12	<20	<20
Control repl. 4 Arenicola G-F		<150	<10	<20	<30	<20
Sunndalsfj repl. 1 Arenicola G		280	35	30	63	43
Sunndalsfj repl. 2 Arenicola G		190	26	39	51	33
Sunndalsfj repl. 3 Arenicola G		190	25	30	41	42
Sunndalsfj repl. 4 Arenicola G		<150	41	<20	<30	20
Karmøy repl. 1 Arenicola G-F		250	31	43	63	42
Karmøy repl. 2 Arenicola G-F		<100	13	<12	30	21
Karmøy repl. 3 Arenicola G-F		<100	<2	100	45	30
Karmøy repl. 4 Arenicola G-F		<150	28	33	43	33
Seawater straight to analysis		<200	<3	<20	<45	<25
BSA straight to analysis		<200	<3	<20	<45	<25
Artificial gut-fluid straight to analysis		<200	<3	<20	<45	<25
Arenicola G-F straight to analysis		360	440	120	140	28

		ANT-V	**FLU-V**	**PYR-V**	**BAA-V**	**CHR-V**	**BBJF-V**
		ng/l	**ng/l**	**ng/l**	**ng/l**	**ng/l**	**ng/l**
		H 2-2	**H 2-2**	**H 2-2**	**H 2-2**	**H 2-2**	**H 2-2**
Blank dest.water (seawater batch		<9	<30	<25	<2	3,5	2,9
Control repl. 1 seawater		<5	<20	<15	<2	<2	<2
Control repl. 2 seawater		18	<20	24	<2	<2	<2
Control repl. 3 seawater		<20	<20	<20	<2	<2	<2
Control repl. 4 seawater		<20	<50	<50	<10	<15	<15
Sunndalsfj repl. 1 seawater		17	120	200	85	130	550
Sunndalsfj repl. 2 seawater		16	77	120	71	98	450
Sunndalsfj repl. 3 seawater		<20	98	140	80	120	490
Sunndalsfj repl. 4 seawater		<20	<50	91	54	72	430
Karmøy repl. 1 seawater		50	550	870	300	230	2700
Karmøy repl. 2 seawater		37	380	550	240	170	2200

Karmøy repl. 3 seawater		23	360	550	240	230	2300
Karmøy repl. 4 seawater		<20	360	590	240	190	2800
Blank dest.water (BSA batch)		<9	<30	<25	<2	<2	<2
Kontroll repl. 1 BSA		9,3	200	210	5	8,1	7,2
Kontroll repl. 2 BSA		17	110	120	2,4	2,7	2,6
Kontroll repl. 3 BSA		<20	140	150	3,1	4,7	4,7
Kontroll repl. 4 BSA		<20	99	110	<10	<15	<15
Sunndalsfj repl. 1 BSA		180	1500	8300	570	760	3300
Sunndalsfj repl. 2 BSA		100	860	5400	290	260	1800
Sunndalsfj repl. 3 BSA		98	1000	6400	490	670	3300
Sunndalsfj repl. 4 BSA		87	970	6100	390	500	2800
Karmøy repl. 1 BSA		850	16000	35000	1900	1400	11000
Karmøy repl. 2 BSA		480	11000	24000	1000	710	6600
Karmøy repl. 3 BSA		510	15000	35000	2000	1800	14000
Karmøy repl. 4 BSA		480	14000	34000	1700	1300	13000
		ANT-V	**FLU-V**	**PYR-V**	**BAA-V**	**CHR-V**	**BBJF-V**
		ng/l	**ng/l**	**ng/l**	**ng/l**	**ng/l**	**ng/l**
		H 2-2	**H 2-2**	**H 2-2**	**H 2-2**	**H 2-2**	**H 2-2**
Blank dest.vann (art. gut-f)		<9	<30	<25	<2	<2	<2
Control repl. 1 artificial gut-f		11	250	240	26	40	71
Control repl. 2 artificial gut-f		30	240	210	20	29	57
Control repl. 3 artificial gut-f		<20	160	160	17	24	48
Control repl. 4 artificial gut-f		<20	130	130	<10	<15	39
Sunndalsfj repl. 1 artificial gut		200	1700	8200	2000	3200	18000
Sunndalsfj repl. 2 artificialgut		140	1200	6000	1500	2300	18000
Sunndalsfj repl. 3 artificial gut		120	1200	6200	1600	2400	20000
Sunndalsfj repl 4 artificial gut		120	1200	6300	1400	2600	21000
Karmøy repl. 1 artificial gut-flu		1100	18000	35000	13000	9100	s140000
Karmøy repl. 2 artificial gut-flu		1200	17000	34000	12000	9100	s130000
Karmøy repl. 3 artificial gut-flu		1100	18000	36000	12000	9900	s150000
Karmøy repl. 4 artificial gut-flu		1300	19000	38000	14000	11000	s180000
Blank dest.water (Arenicola G-F		<9	<30	<25	<2	<2	<2
Control repl. 1 Arenicola G-F		<5	43	52	4,4	10	25
Control repl. 2 Arenicola G-F		6,6	35	58	4,9	9,3	38
Control repl. 3 Arenicola G-F		<20	32	51	4,5	11	31
Control repl. 4 Arenicola G-F		<20	<50	<50	<10	<15	<15
Sunndalsfj repl. 1 Arenicola G		43	580	1700	350	580	3200
Sunndalsfj repl. 2 Arenicola G		33	470	1200	260	410	2600
Sunndalsfj repl. 3 Arenicola		50	440	1200	310	420	2900

G							
Sunndalsfj repl. 4 Arenicola G		20	490	1600	320	430	3800
Karmøy repl. 1 Arenicola G-F		89	3700	6500	1600	1600	21000
Karmøy repl. 2 Arenicola G-F		59	2100	3300	870	1000	17000
Karmøy repl. 3 Arenicola G-F		47	2400	3900	1000	1100	20000
Karmøy repl. 4 Arenicola G-F		54	2800	4500	1000	1100	25000
Seawater straight to analysis		<9	<30	<25	<2	<2	<2
BSA straight to analysis		<9	<30	<25	<2	<2	<2
Artificial gut-fluid straight to analysis		<9	<30	<25	<2	2,3	<2
Arenicola G-F straight to analysis		99	1200	2400	350	430	1500