Chemosphere 302 (2022) 134906

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Arsenic species in mesopelagic organisms and their fate during aquafeed processing

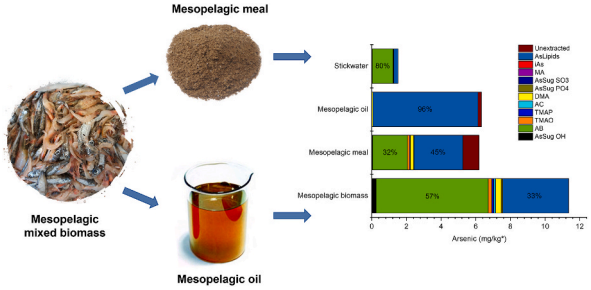
Jojo Tibon [a,b], Heidi Amlund [b], Ana I. Gomez-Delgado [a], Marc H.G. Berntssen [a], Marta S. Silva [a], Martin Wiech [a], Jens J. Sloth [a,b], Veronika Sele [a,*]

[a] Institute of Marine Research, P.O. Box 1870 Nordnes, NO-5817 Bergen, Norway
[b] National Food Institute, Technical University of Denmark, Kemitorvet, Building 201, DK-2800 Kgs. Lyngby, Denmark

HIGHLIGHTS

- Up to 12 arsenic species were detected in mesopelagic samples.
- Arsenobetaine comprised 70% and 50% of total As in crustaceans and fish, respectively.
- Mesopelagic mixed biomass comprised mainly of arsenobetaine and arsenolipids.
- Arsenolipids were transferred to meal and up-concentrated in oil when processed.
- Inorganic arsenic was <0.007 mg/kg ww in most samples.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Milena Horvat

Keywords:
Element speciation
Arsenobetaine
Arsenolipids
HPLC
ICP-MS
Novel feed ingredients

ABSTRACT

A responsible harvest of mesopelagic species as aquafeed ingredients has the potential to address the United Nations Sustainable Development Goal 14, which calls for sustainable use of marine resources. Prior to utilization, the levels of undesirable substances need to be examined, and earlier studies on mesopelagic species have reported on total arsenic (As) content. However, the total As content does not give a complete basis for risk assessment since As can occur in different chemical species with varying toxicity. In this work, As speciation was conducted in single-species samples of the five most abundant mesopelagic organisms in Norwegian fjords. In addition, As species were studied in mesopelagic mixed biomass and in the resulting oil and meal feed ingredients after lab-scale feed processing. Water-soluble As species were determined based on ion-exchange high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS). This was supplemented by extracting arsenolipids (AsLipids) and determining total As in this fraction. The non-toxic arsenobetaine (AB) was the dominant form in mesopelagic crustaceans and fish species, accounting for approximately 70% and 50% of total As, respectively. Other water-soluble species were present in minor fractions, including carcinogenic inorganic As, which, in most samples, was below limit of quantification. The fish species had a higher proportion of AsLipids, approximately 35% of total As, compared to crustaceans which contained 20% on average. The feed processing simulation revealed generally low levels of water-soluble As species besides AB, but considerable fractions of potentially toxic AsLipids were found in the biomass, and transferred to the mesopelagic meal and oil. This study is the first to report occurrence data of at least 12 As

* Corresponding author. P.O. Box 1870 Nordnes, NO-5817 Bergen, Norway.
E-mail address: Veronika.Sele@hi.no (V. Sele).

https://doi.org/10.1016/j.chemosphere.2022.134906
Received 17 March 2022; Received in revised form 26 April 2022; Accepted 6 May 2022
Available online 10 May 2022

species in mesopelagic organisms, thereby providing valuable information for future risk assessments on the feasibility of harnessing mesopelagic biomass as feed ingredients.

1. Introduction

The United Nations (UN) declared 2021–2030 as the Decade of Ocean Science, promoting sustainably harvested oceans as one of its goals. While the usage of traditional marine-based ingredients in aquafeed production has been reduced (Aas et al., 2019), plant-based raw materials can contain antinutritional factors and undesirable substances (e.g. pesticides and mycotoxins), introducing new risks to aquaculture (Glencross et al., 2020; Olsen et al., 2020). Alternative marine-based ingredients are now being explored. In lieu of pelagic fish as raw materials in the aquafeed industry, sustainable capture at low-trophic levels has been recommended (European Commission, 2017). The mesopelagic ecosystem is presumed to consist of species thriving between 200 and 1000 m below the sea surface – a biomass regarded as an unexploited resource for aquafeed production (Alvheim et al., 2020; Grimaldo et al., 2020; Olsen et al., 2020). However, prior to large-scale extraction of mesopelagic species, a holistic assessment is needed in terms of its impact on biodiversity and carbon sequestration (St. John et al., 2016).

Mesopelagic species were found to be high in proteins (Olsen et al., 2020), rich in omega-3 polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Nordhagen et al., 2020), a good source of Vitamin A and B_{12}, and also rich in calcium, selenium, iron, and iodine (Alvheim et al., 2020; Nordhagen et al., 2020). Among the potentially toxic elements, cadmium (Cd) and arsenic (As) have been reported at levels above the maximum limits (MLs) set in the European legislation for feed and feed materials, especially when the haul is mostly comprised of crustaceans (Olsen et al., 2020). Processing of mesopelagic biomass into aquafeed has the potential to modify the concentrations of undesirable substances, including As and Cd, in mesopelagic products, i.e. meal and oil (Wiech et al., 2020; Berntssen et al., 2021). Wiech et al. (2020) assumed in a theoretical worst-case approach that the total amount of As could end up in the protein fraction and consequently would exceed MLs defined in Directive 2002/32 EC and amendments (European Commission, 2002). However, in their succeeding study involving lab-scale feed processing of mesopelagic biomass, this was found to be not the case since As partitioned both in the oil and meal fractions and resulted in a dilution effect (Berntssen et al., 2021).

Arsenic is an element which is highly abundant in the marine environment, predominantly existing in marine animals as the non-toxic arsenobetaine (AB) (Francesconi and Edmonds, 1997). However, other forms are also present such as inorganic As (iAs) and the methylated species (methylarsonate (MA) and dimethylarsinate (DMA)), classified by the International Agency for Research on Cancer (IARC) as carcinogenic and possibly carcinogenic, respectively (IARC Working Group, 2012). Among available literature, iAs concentrations in mesopelagic species have, so far, only been reported by Wiech et al., 2020. While relatively low levels of iAs were reported (max 0.16 mg/kg ww), the species comprising the major remaining fraction of total As were neither identified nor quantified. However, this is of high relevance since other organic forms such as arsenosugars (AsSug) and arsenolipids (AsLipids), including their metabolites, were shown to exhibit neurotoxic and cytotoxic activity, and are frequently classified as potentially toxic As species (Feldmann and Krupp, 2011; Leffers et al., 2013; Witt et al., 2017). In addition, no study has been conducted yet regarding the fate of As species during feed processing. Such study is beneficial to verify if any up-concentration, dilution, or transformation to more toxic forms occur. This complex chemical nature of As highlights the importance of obtaining speciation data as basis for further risk assessment.

The present study aims to determine the organic As species, which were not included in earlier reports on total As in mesopelagic species (Wiech et al., 2020) and processed mesopelagic biomass (Berntssen et al., 2021). The specific objectives were to (1) provide occurrence data of As species in mesopelagic organisms and (2) give insight on the fate of As species during aquafeed processing, with an overarching goal of providing initial information for future risk assessments on the feasibility of harnessing mesopelagic biomass as feed ingredients.

2. Materials and methods

2.1. Sample collection and processing

2.1.1. Mesopelagic samples grouped by species

The mesopelagic single-species samples analyzed in this study were composed of fish species (1) glacier lanternfish (*Benthosema glaciale*) and (2) silvery lightfish (*Maurolicus muelleri*); also crustaceans including (3) the decapod genus *Pasiphaea* (*P. sivado*, *P. multidentata*, and *P. tarda*, (4) another decapod species *Eusergestes arcticus*, and (5) the euphausiid *Meganyctiphanes norvegica*, commonly known as the Northern krill. The samples were collected in December 2018 from three fjords on the western coast of Norway. At least 27 specimens of the same species were collected from each sampling location. These were pooled to form a composite sample (i.e. one pooled sample representing one sampling location). For each mesopelagic species, three pooled samples were analyzed (i.e. taken from three different sampling locations). The pooled samples were immediately homogenized after sorting the catch on board the research vessel 'Johan Hjort', distributed into different tubes, and stored at −20 °C. These were then freeze-dried upon arrival on shore and analyzed for water-soluble As, AsLipids, and iAs (Fig. 1a). Total As levels were presented and discussed in our earlier work (Wiech et al., 2020). Additional details regarding the samples were extensively described in Alvheim et al. (2020) and Wiech et al. (2020).

2.1.2. Mesopelagic mixed biomass of M. muelleri and M. norvegica and its processing

This study utilized a total of four mesopelagic biomass samples obtained from four different stations in the North Atlantic during a research cruise on board 'MS Birkeland' from September to November 2019. In an earlier work, biomass composed of *M. muelleri* and *M. norvegica* underwent lab-scale feed processing (Berntssen et al., 2021). Mixed biomass were either mechanically pressed or centrifuged, producing a liquid phase and a solid phase (mesopelagic meal) (Fig. 1b). The liquid phase was transferred to a separatory funnel and divided into mesopelagic oil and stickwater. The starting biomass and the resulting fractions from this previous study were subsequently analyzed for water-soluble As, AsLipids, iAs, and total As.

2.2. Analytical methods

2.2.1. Determination of iAs

The iAs concentration was determined by anion-exchange high-performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) based on EN 16802:2016 (CEN, 2016) and the work of Julshamn et al. (2012). Briefly, 0.2 g of freeze-dried sample or 1.0 g of wet material was weighed into a 13-mL polypropylene tube, followed by addition of 10 mL of 0.1 mM HNO_3 in 3% H_2O_2 (HNO_3, 65%; H_2O_2, 30%; Merck, Darmstadt, Germany). A sub-boiling distillation unit (Savillex, Eden Prairie, MN, USA) was used to further purify the HNO_3. The tubes were then subjected to vortex-mixing (MS 1, IKA, Staufen, Germany) and left to stand overnight. Thereafter, the tubes were placed in a shaking water bath

(OLS200, Grant, Cambridge, UK) set at 90 °C for 1 h (shaking speed at 100 rpm) and centrifuged for 10 min (1780×*g*; 5702, Eppendorf, Hamburg, Germany). Approximately 1 mL of the supernatant was collected using a 5-mL syringe (Henke-Sass Wolf, Tuttlingen, Germany) affixed with a needle, then filtered (0.45-μm PTFE; Sartorius, Göttingen, Germany) and transferred to an HPLC vial. Quantification was performed using a 1260 Infinity HPLC coupled to a 7900 ICP-MS (Agilent Technologies, Santa Clara, CA, USA) with an anion-exchange column (IonPac AS7, 2 × 250 mm; Dionex, Sunnyvale, CA, USA). Isocratic elution was carried out using 50 mM $(NH_4)_2CO_3$ in 3% CH_3OH adjusted to pH 10.3 with NH_3 ($(NH_4)_2CO_3$, reagent grade; CH_3OH, ≥ 99.97%; NH_3, 25%; Merck, Darmstadt, Germany). Quantification was based on chromatographic peak areas using an external calibration curve from an arsenate (As (V)) standard solution (1000 mg/L; Spectrascan Teknolab, Ski, Norway). Accuracy of results was verified using a rice certified reference material (ERM-BC211) and an in-house control sample of tuna fish tissue (BCR-627) (IRMM, Geel, Belgium). Data processing was performed using MassHunter 4.5 Workstation Software (v. C.01.05, Agilent Technologies, Santa Clara, CA, USA).

2.2.2. Determination of water-soluble As species

The quantification of water-soluble As species was carried out by cation- and anion-exchange HPLC-ICP-MS based on our earlier study (Tibon et al., 2021). Briefly, 0.2 g of sample was weighed into a 13-mL polypropylene tube, followed by addition of 5 mL of aqueous methanol (CH_3OH: H_2O, 50% v/v). The tubes were subjected to vortex-mixing and subsequently placed in a shaking water bath set at 90 °C for 30 min (shaking speed at 100 rpm). Afterwards, the tubes were placed in a centrifuge (1780×*g*) for 10 min. The supernatant was poured into a 5-mL syringe connected to a 0.45-μm PTFE filter and transferred to another 13-mL polypropylene tube. An aliquot was transferred into an HPLC vial and diluted accordingly with aqueous methanol (CH_3OH: H_2O, 50% v/v). Arsenic speciation was carried out using a 1260 Infinity HPLC coupled to a 7900 ICP-MS. A Metrosep C6 cation-exchange column (250 × 4.0 mm, 5 μm; Metrohm, Herisau, Switzerland) was used to separate cationic As species by employing gradient elution using pyridine-based mobile phases (0 and 50 mM at pH 2.7, 0.5% acetonitrile). For anionic As species, separation was performed using a PRP-X100 anion-exchange column (250 × 4.6 mm, 5 μm; Hamilton, Reno, NV, USA) and a corresponding gradient elution with carbonate-based mobile phases (0.5 and 60 mM at pH 9.3, 3% methanol). Quantification was achieved by preparing mixed standard solutions of As compounds (Tibon et al., 2021) and integrating chromatographic peak areas to generate external calibration curves. Certified reference materials of tuna fish tissue (BCR-627) and fish protein (DORM-4; National Research Council Canada, Ottawa, Ontario, Canada) were included in the analytical series for quality control. MassHunter 4.5 Workstation Software was used for data processing.

2.2.3. Extraction of AsLipids

AsLipids were estimated based on the approach of Freitas et al. (2020). Approximately 50 mg of oil/freeze-dried sample or 200 mg of wet material was weighed into a borosilicate glass tube (13 × 100 mm; DWK, Mainz, Germany), followed by addition of 1.5 mL of methanol and vortex-mixing for 5 s. Five mL of methyl *tert*-butyl ether (MTBE, HPLC grade; Merck, Darmstadt, Germany) was subsequently added. The glass tubes were capped and placed in a test-tube rotator (LD-79, LABINCO, Breda, the Netherlands) for 1 h to allow sufficient contact time between solvent and matrix. Thereafter, 1.25 mL of water (ultrapure quality with resistivity of 18.2 MΩ*cm) was added to the tubes and were left to stand for 10 min. The tubes were then centrifuged (1780×*g*) for 10 min. The upper layer (organic phase) was collected using glass Pasteur pipettes (150 mm; DWK, Mainz, Germany) with a rubber bulb and transferred to quartz digestion tubes (ultraWAVE, Milestone, Sorisole, Italy). The tubes were subsequently placed in a heated nitrogen evaporator (40 °C; Reacti-Therm, Thermo Fisher Scientific, Waltham, MA, USA) until a lipid pellet was obtained. These were then analyzed for total As as described in the succeeding section.

2.2.4. Total As analysis

Total As analysis was carried out by ICP-MS, as elaborated by Julshamn et al. (2007). Two mL of HNO_3 was added to quartz digestion tubes containing the lipid pellets or 0.2 g of freeze-dried sample in 500 μL of water. This was then followed by microwave digestion (UltraWAVE, Milestone, Sorisole, Italy). The heating program which lasted for 62 min involved gradual increase of temperature to 260 °C and 25 min of cooling. After allowing to cool, the digested solutions were quantitively transferred to a 25-mL volumetric flask, diluted to volume with water, and transferred to 50-mL centrifuge tubes. Analysis was performed using an iCAP Q ICP-MS (Thermo Fisher Scientific, Waltham,

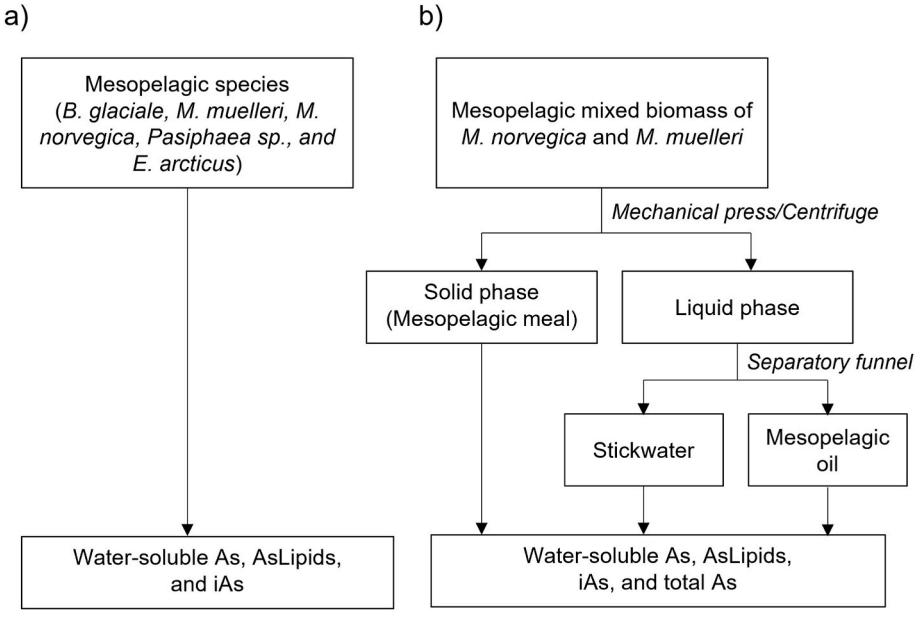


Fig. 1. An overview of the workflow, divided into (a) analysis of mesopelagic single-species samples and (b) processing and analysis of mesopelagic mixed biomass and resulting fractions (As – arsenic; AsLipids – arsenolipids; iAs – inorganic arsenic).

MA, USA) equipped with an SC-4 DX autosampler (Elemental Scientific, Mainz, Germany). Quantification was achieved by generating an external calibration curve from mixed standard solutions containing As, and online internal standard addition of germanium (Spectrascan Teknolab, Ski, Norway). To evaluate accuracy of results, certified reference materials of lobster hepatopancreas (TORT-3; National Research Council Canada, Ottawa, Ontario, Canada) and oyster tissue (SRM 1566b; National Institute of Standards and Technology, Gaithersburg, MD, USA) were analyzed in duplicate for each analytical series. Data processing was facilitated through the Qtegra software (v. 2.10, 2018, Thermo Fisher Scientific, Waltham, MA, USA).

2.3. Quality assurance and control

The methods for the determination of iAs and total As are routine analyses at the Institute of Marine Research (Bergen, Norway) and are accredited by the Norwegian accreditation body according to NS-EN ISO/IEC 17025:2017. The method for water-soluble As species has been validated and method performance characteristics were presented in our previous work (Tibon et al., 2021). In the current study, analyses were done using either two or three replicates. An extraction blank was always included, and one of the calibration standards was injected periodically and at the end of the series to check for instrument drifts. The measured concentrations for the certified reference materials (CRM) were in good agreement with the certified values (Table S1). Results were within twice the standard deviation for the certified values, which is the acceptable limit in statistical control charts.

2.4. Processing factors

Processing factors (PF) were calculated based on the approach of Berntssen et al. (2021) and patterned after the European Food Safety Authority's (EFSA) definition (Scholz et al., 2018). EFSA uses PFs to give insight on the transfer of pesticide residues from raw agricultural commodities to processed products. Following the same approach, PF in this study will be expressed as the ratio of the concentration (in mg/kg) of the As species in produced mesopelagic meal (dw) or oil (ww) and the concentration (in mg/kg) of the As species in the raw mesopelagic biomass as starting material (dw). Mathematically, this is shown as:

$$PF = \frac{C_{meal\ (dw)\ or\ oil\ (ww)}}{C_{mesopelagic\ biomass\ (dw)}}$$

3. Results and discussion

3.1. Arsenic speciation in mesopelagic species

3.1.1. Total As

At least three pooled samples per species were analyzed and the average concentrations varied greatly, ranging from 2.2 to 28 mg/kg ww. The crustaceans (*M. norvegica*, *Pasiphaea* sp., and *E. arcticus*) generally had higher concentrations compared to the fish species (*B. glaciale* and *M. muelleri*), with one of the pooled samples of *M. norvegica* containing a total As of 52 mg/kg ww. However, since As species vary in toxicity, total As levels do not always give sufficient information, thus, calling for As speciation data.

3.1.2. Arsenobetaine

The mesopelagic samples contained at least 12 As species, of which AB was the most predominant form (Table 1). The fish species *B. glaciale* and *M. muelleri* had lower average AB concentrations at 2.2 ± 0.1 and 2.4 ± 1.0 mg/kg ww, respectively, while the crustaceans *M. norvegica* and *Pasiphaea* sp. had higher mean values at 14.2 ± 10.1 and 15.5 ± 10.7 mg/kg ww, respectively. This trend in AB concentration among the samples is very similar to total As concentration. Indeed, it was verified that a positive correlation ($R^2 = 0.996$, $p < 0.001$) exists between total As and AB (Fig. S1), similar to an earlier report for various types of seafood (Wolle et al., 2019b). When excluding the two highest points in Fig S1, the resulting R^2 is 0.965.

In several surveys of As species in seafood, it was observed that low-trophic marine animals such as shrimps contain higher AB concentrations than fish species which are positioned higher in the food chain (Ruttens et al., 2012; Wolle et al., 2019b; Luvonga et al., 2021). This has been generally attributed to their diet, habitat, and metabolic abilities (Kato et al., 2020). In the present work, while all samples were collected from the mesopelagic zone, the variation in As and AB concentrations could be explained by their differences in feeding behaviors. The fish species *B. glaciale* and *M. muelleri* are zooplanktivores, depending mostly on copepods, amphipods, and krill (García-Seoane et al., 2013). In contrast, crustaceans, such as *M. norvegica,* are omnivores which have been observed to scavenge the seabed for copepods and phytoplanktons (Schmidt, 2010), which can be significant sources of As. This is supported by another study which reported that elevated levels of total As and AB found in the shrimp *Metapenaeopsis palmensis* were due to their dependence on benthic food present in sediments (Zhang et al., 2018).

Table 1
Concentrations of total As and As species in mesopelagic samples (mg/kg ww, mean ± SD, $n = 3$).

As species	Fish species		Crustaceans		
	B. glaciale	*M. muelleri*	*M. norvegica*	*Pasiphaea* sp.	*E. arcticus*
Water-soluble species					
AsSug OH	0.077 ± 0.007	0.049 ± 0.005	0.15 ± 0.02	0.03 ± 0.01	0.077 ± 0.003
AB	2.2 ± 0.1 (2.1–2.2)[c]	2.4 ± 1.0 (1.6–3.5)[c]	14.2 ± 10.1 (8.0–25.8)[c]	15.5 ± 10.7 (7.8–27.7)[c]	5.3 ± 2.3 (4.4–7.9)[c]
TMAO	0.050 ± 0.004	0.08 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.007 ± 0.003
TMAP	0.007 ± 0.001	0.007 ± 0.001	0.14 ± 0.04	0.08 ± 0.04	0.042 ± 0.009
AC	0.048 ± 0.006	0.034 ± 0.007	0.05 ± 0.01	0.04 ± 0.04	0.049 ± 0.005
TETRA	<0.003	<0.003	0.005 ± 0.003	0.006 ± 0.003	<0.003
DMA	0.027 ± 0.008	0.29 ± 0.06	0.017 ± 0.003	0.013 ± 0.001	0.016 ± 0.006
AsSug PO4	<0.005	0.03 ± 0.01	0.09 ± 0.02	0.02 ± 0.01	0.04 ± 0.01
AsSug SO3	<0.003	<0.003	<0.003	<0.003	0.004 ± 0.001
MA	<0.003	0.007 ± 0.003	<0.003	0.002 ± 0.001	<0.003
iAs	<0.007	<0.007	0.06 ± 0.09	0.013 ± 0.007	<0.007
Unknowns	0.001 ± 0.001	0.007 ± 0.002	0.069 ± 0.003	0.04 ± 0.02	0.022 ± 0.002
AsLipids	1.4 ± 0.1	1.6 ± 0.3	2.8 ± 1.4	5.1 ± 4.7	2.1 ± 0.4
Sum[a]	3.8 ± 0.1	4.5 ± 0.8	17.5 ± 11.5	20.9 ± 15.5	7.6 ± 2.7
Total As	3.8 ± 0.1	5.1 ± 1.2	20.2 ± 13.6	21.2 ± 15.3	7.9 ± 3.0
Recovery (%)[b]	99 ± 3	88 ± 7	87 ± 2	99 ± 2	97 ± 4

[a] Sum = Sum of water-soluble As + AsLipids.
[b] Recovery = (Sum/Total As) *x* 100.
[c] Range of values.

It is well-known that AB is the most abundant form of As in marine organisms (Molin et al., 2015; Chen et al., 2020; Luvonga et al., 2020), usually accounting for at least 70% of total As (Francesconi and Raber, 2013). In the present study, the proportion of AB ranged from 45% to 75% of total As (Fig. 2). The crustaceans generally had higher percentage of AB (~70%) compared to the fish species (~50%). This agrees with an earlier report involving the North Pacific krill (*Euphausia pacifica*), a close relative of *M. norvegica*, wherein AB was the major As species (Shibata et al., 1996). In the same study, it was found that copepods (*Calanus* sp.) contained very little AB (0.21 mg/kg dw). Copepods are one of the principal food sources of *B. glaciale* (García-Seoane et al., 2013), which could explain the relatively lower fraction of AB in the fish species. As for the crustaceans, a study involving the common shrimp *Crangon crangon* showed that 42% of AB acquired through food was retained (Hunter et al., 1998). In contrast, exposure to water-borne AB only resulted in a small increase in concentration. AB has been detected in seawater, although at very low levels (0.5–10 ng/kg) (Glabonjat et al., 2018). This suggests that AB is mainly acquired from dietary sources rather than uptake from seawater.

3.1.3. Other water-soluble As species

Other water-soluble species were present in minor concentrations in the mesopelagic samples, accounting for less than 10% of total As (Table 1, Fig. 2). Inorganic As levels were generally less than 0.007 mg/kg ww (LOQ), with the highest concentration found in *M. norvegica* at 0.06 ± 0.09 mg/kg ww. Among the water-soluble As species, DMA was the second most abundant in *M. muelleri* at 0.29 ± 0.06 mg/kg ww, accounting for approximately 6% of total As. Methylarsonate was only found in *M. muelleri* and *Pasiphaea* sp., albeit at very low levels. Trimethylarsoniopropionate (TMAP) was found in higher concentrations in crustaceans compared to the fish species, ranging from 0.042 ± 0.009 to 0.14 ± 0.04 mg/kg ww. These levels are comparable to those obtained by Wolle et al. (2019b) for different shrimp species, where TMAP concentrations ranged from 0.003 to 0.037 mg/kg. Also, in their study, elevated levels of TMAP (as high as 0.8 mg/kg) were found in several species of crab. It is, however, difficult to conclude whether TMAP is characteristic of crustaceans since only a handful of As speciation studies have measured TMAP (Sloth et al., 2003; Leufroy et al., 2011; Wolle et al., 2019b). Most studies focus on the most common As species such as As (III), As (V), DMA, MA, and AB (Ruttens et al., 2012; Schmidt et al., 2018), hence, the current work hopes to bridge this gap.

Trimethylarsine oxide (TMAO) was slightly higher in *B. glaciale* and *M. muelleri*, while arsenocholine (AC) and tetramethyl arsonium ion (TETRA) were present in trace levels or below LOQ (Table 1). A few unknown peaks were also detected in the chromatograms (Fig. S2) and their concentrations were estimated using the calibration curve of the nearest eluting standard. As for the AsSug, glycerol-arsinoylriboside (AsSug OH) was detected in all samples, ranging from 0.03 ± 0.01 to 0.15 ± 0.02 mg/kg ww. Phosphate-arsinoylriboside (AsSug PO4) was also found in all samples except for the fish species *B. glaciale*. Sulfonate-

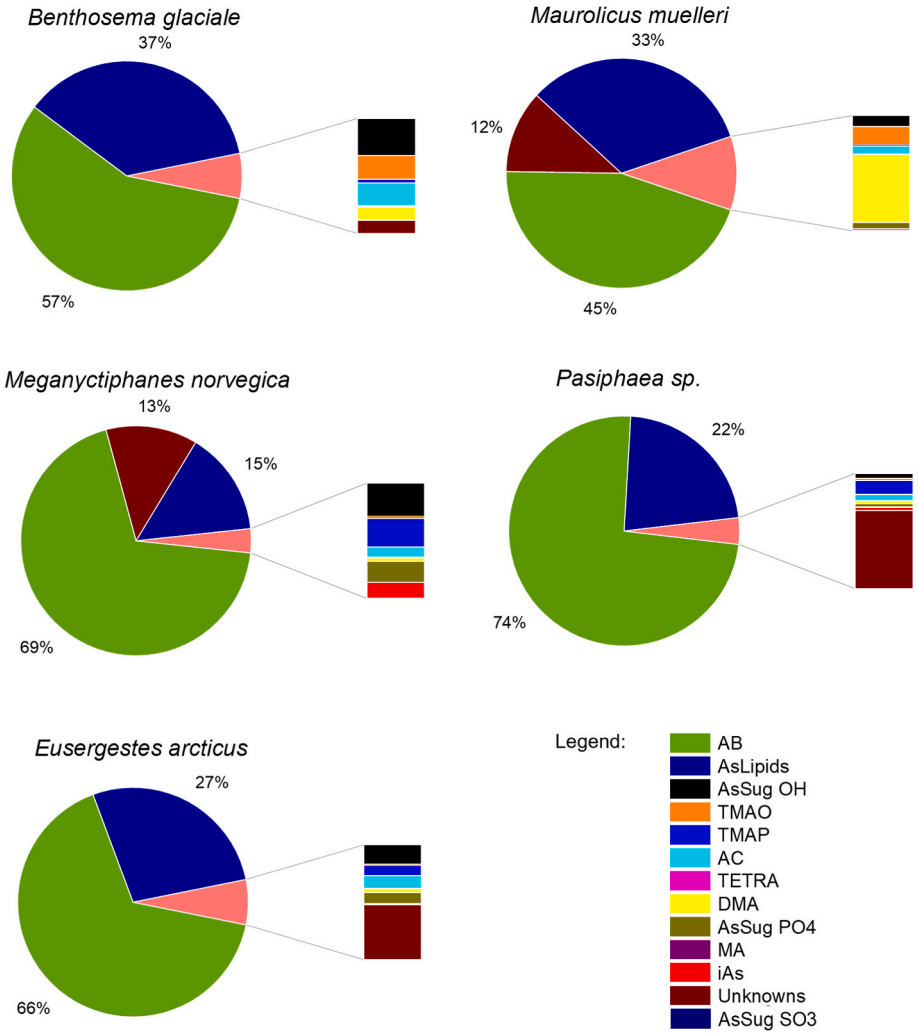


Fig. 2. Arsenic species profile in the mesopelagic samples. Arsenic species fractions are given in % = (concentration of As species/total As) *x* 100. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

arinoylriboside (AsSug SO3) was only detected in the crustacean *E. arcticus*. Sulfate-arsinoylriboside (AsSug SO4) was not found in any of the samples, which was unexpected. Glacier lanternfish (*B. glaciale*) are known to prey on copepods which are rich in AsSug SO4 (Shibata et al., 1996). The absence of AsSug SO4 in the mesopelagic fish samples could suggest that this AsSug is biotransformed to other As forms in the mesopelagic food web. The varying presence of AsSug in crustaceans could be attributed to their preference for different phytoplanktons as food. In contrast, Wolle et al. (2019b) did not find any AsSug in shrimp samples, while they were found in crabs and clams.

3.1.4. AsLipids

Notable concentrations of As in the lipid fraction, corresponding to AsLipids, were found in all samples. Lowest mean concentration was found in the fish species *B. glaciale* at 1.4 ± 0.1 mg/kg ww (Table 1), while the highest level was observed in the crustacean *Pasiphaea* sp. at 5.1 ± 4.7 mg/kg ww. In general, higher concentrations of AsLipids were found in crustaceans (*M. norvegica*, *Pasiphaea* sp., *E. arcticus*) compared to mesopelagic fish species (*B. glaciale* and *M. muelleri*). However, looking at the proportion of AsLipids relative to total As (Fig. 2), *B. glaciale* and *M. muelleri* contained 33%–37% AsLipids, while the crustaceans only had around 20% AsLipids. AsLipids are usually associated with 'fatty' fish such as herring (Lischka et al., 2013) and blue whiting (Taleshi et al., 2014), among others. In the work by Wiech et al. (2020), the mean fat contents for *B. glaciale* and *M. muelleri* were 14% and 18%, respectively, which were two to three times higher than the fat content in the crustaceans. The higher fat content in the fish species could hence explain the larger proportion of AsLipids compared to the crustaceans.

AsLipids can occur as arsenic-containing hydrocarbons (AsHC), arsenic-containing fatty acids (AsFA), and AsSug phospholipids (AsPLs), among others (Sele et al., 2012; Luvonga et al., 2020). AsHCs were the major AsLipids in salmon (*Salmo salar*), specifically oxo-analogs of AsHC 332, AsHC 360, and AsHC 404 (Xiong et al., 2022). In contrast, AsFA 362, AsFA 448, and AsFA 528 are more common in tuna fillet, and AsFA 360 and AsFA 422 in kelp (Liu et al., 2021). Planktons collected from the North Atlantic revealed prevalence of AsPLs, mainly AsPL 958, AsPL 978, and AsPL 1006 (Glabonjat et al., 2021). In a study involving the Mediterranean mussel (*M. galloprovincialis*), traces of AsPLs and arsenic-phytol derivatives (AsPT) were found (Freitas et al., 2020). These AsLipids were initially reported in algae, which suggest that transfer of AsLipids occur through the diet (Freitas et al., 2020). It would be interesting to conduct further studies on mesopelagic samples involving identification of AsLipids since they can provide an insight regarding the distribution of AsLipids at the bottom of the food chain.

3.1.5. Arsenic mass balance

Arsenic recovery was calculated by comparing the sum of water-soluble As and AsLipids with the total As. Overall, good recoveries were obtained for the mesopelagic samples, ranging from 87 ± 2% to 99 ± 3% (Table 1). This suggests that the method for water-soluble As species was nicely complemented by the extraction technique used for the estimation of AsLipids.

3.2. Processing of mesopelagic biomass into aquafeed

3.2.1. As species

The distribution of As species in mesopelagic biomass and resulting fractions is presented in Fig. 3 (see Table S2 for tabulated values). The discussion on total As and its compliance to existing MLs in animal feed and feed materials (Directive, 2002/32 EC and amendments) was already presented in an earlier work (Berntssen et al., 2021). The focus of this section will therefore be on the organic As species. The main As compound found in the initial mesopelagic biomass was AB, accounting for 57% of total As (Fig. 3). The same can be observed for the stickwater wherein AB attributes for 80% of total As. In contrast, the mesopelagic

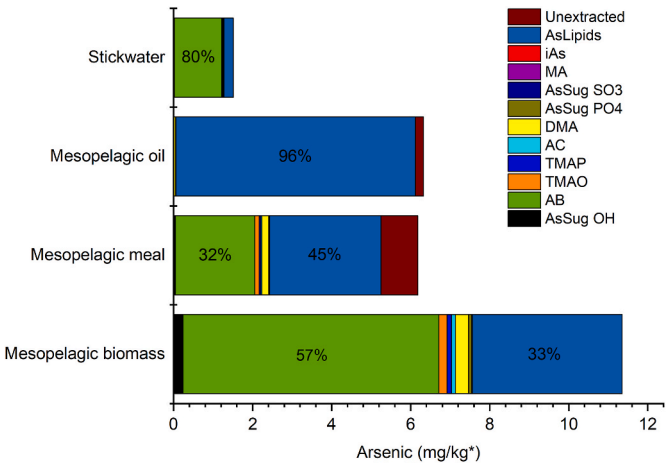


Fig. 3. Bar graph showing the fractions of As species (% = (concentration of As species/total As) *x* 100) in the mesopelagic biomass and produced mesopelagic meal, oil, and stickwater after feed processing (*Unit is in mg/kg dw for mesopelagic biomass and meal; mg/kg ww for mesopelagic oil and stickwater). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

meal only had approximately 32% of total As in the form of AB. This is a notable difference compared to fish meals produced from herring and blue whiting where the water-soluble As accounted for 71%–93% of total As (Pétursdóttir et al., 2018). In the present study, the majority of As in the mesopelagic meal was found as AsLipids (45% of total As). It can be assumed that AsLipids would partition mostly with the mesopelagic oil. However, the results show that AsLipids tend to bind also with the solid phase after the extrusion process. This agrees with an earlier study wherein residual lipids were found in fish meal processed from another species of lanternfish (*Benthosema pterotum*) (Haque et al., 1981). Fish meal typically end up having varying lipid content after production, which also dictates which type of fish protein concentrate it will be classified under (Einarsson et al., 2019; Hilmarsdottir et al., 2020). It can be presumed that AsLipids contribute to the total lipid content in fish meal. The final lipid content is highly dependent on the quality of the raw material and process parameters (Hilmarsdottir et al., 2020), which could also dictate the distribution of AsLipids in the produced meal and oil. In fish meals produced from capelin, Amayo et al. (2011) found AsHC 332, AsHC 360, AsHC 404 as the major AsLipids. Another study on herring and blue whiting fish meals reported the same set of AsLipids as the dominant species (Pétursdóttir et al., 2018).

As expected, mesopelagic oil was mostly comprised of AsLipids (~96%). Minor concentrations of DMA and MA were found, which can just be degradation products of AsLipids, as seen in previous studies (Amayo et al., 2014; Pétursdóttir et al., 2018). The presence of AsLipids in different types of fish oil has been described extensively in literature. Fish oil from Peruvian anchoveta (*Engraulis ringens*) contained AsHC 332 and AsHC 360 as major AsLipids, and AsFA 250, AsFA 278, AsFA 292 as minor species (Pereira et al., 2016). Similarly, Sele et al. (2014) found AsHC 332, AsHC 360, and AsHC 404 as the prevalent As compounds in commercial fish oil samples of blue whiting and anchovy. In contrast, krill oil mostly contained AsFA 362, AsFA 390, and AsFA 436 among others (Liu et al., 2021). Comparing the AsLipids found in fish meal (Pétursdóttir et al., 2018) and fish oil (Sele et al., 2014), both from blue whiting, it can be observed that the same set of AsLipids were present as dominant species. It appears that the partitioning of AsLipids does not follow a specific pattern (e.g. AsHC in fish meals and AsFA in fish oils).

As for the stickwater, AsLipids were the second most abundant species, accounting for approximately 16% of total As. The presence of AsLipids in stickwater is expected since stickwater was still found to contain lipids (approximately 2%) after centrifugation in a commercial fish meal production (Hilmarsdottir et al., 2020). In aquafeed ingredient

processing, stickwater is usually further concentrated by evaporating the water and then centrifuged, producing an oil and another concentrate. The oil is normally added to the first oil extracted, while the dried concentrate is mixed with the press cake, and eventually further dried until the final fish meal is produced (Hilmarsdottir et al., 2020).

Among the hydrophilic As species, DMA was the second most abundant in mesopelagic biomass and meal, representing 3% of total As. AsSug OH was also present in the biomass, meal, and stickwater, ranging from 0.8% to 2.5% of total As. AsSug PO4 was only found in the biomass and meal, while AsSug SO3 was only detected in the starting biomass (Table S2). The absence of AsSug in the mesopelagic oil suggests that AsSug only have affinity with the solid and aqueous phases. Other organic As forms were found in trace levels in the mesopelagic biomass and resulting fractions. Inorganic As concentrations were all less than 0.007 mg/kg ww (LOQ). Most of the As species present in mesopelagic biomass were also observed in mesopelagic meal. Arsenic species detected as trace compounds in mesopelagic biomass, specifically MA and AsSug SO3, seem to have degraded and/or transformed to other As forms as they were not detected in quantifiable levels in the resulting fractions. As for the As mass balance, good recoveries were obtained overall, ranging from 85 ± 3% to 119 ± 8% (Table S2). The higher recoveries obtained could be due to overestimation of the AsLipids fraction, e.g. that some water-soluble As species were co-extracted with the AsLipids.

It should be clarified that a process mass balance for As, i.e. As in the starting mesopelagic biomass is equal to the sum of the As in the meal, oil, and stickwater fractions, was not possible to calculate due to lack of data on the weights of the resulting fractions.

3.2.2. *Processing factors*

PFs were calculated following the approach of Berntssen et al. (2021) to verify possible up-concentration (PF > 1) or dilution/removal/degradation (PF < 1) of As species during aquafeed ingredients processing (Table 2). For the mesopelagic meal, results suggest that all compounds were diluted/removed/degraded after processing. In contrast, an up-concentration was observed for AsLipids in mesopelagic oil wherein a PF of 1.4 was calculated. The opposite was seen in stickwater where AsLipids were diluted. This is expected due to their different polarities, with stickwater being mostly water with some particles. In the mesopelagic meal, the dilution effect was more pronounced for the AsSug, having low PFs (0.2). In particular, AsSug SO3 was detected in low levels in the starting mesopelagic biomass but was absent in the resulting fractions. While not completely conclusive, a dilution in AsSug could suggest transformation to other forms. In a study involving macroalgae, AsSug were found to degrade to DMA, MA, and As (V) (Duncan et al., 2015). Similarly, Wolle et al. (2019a) reported a matrix-induced transformation of spiked AsSug to their thiolated counterparts in finfish and crustaceans. Degradation of AsSug is possible with the aid of marine microbes, through acid or base hydrolysis, or exposure to gastric-type conditions (Chen et al., 2020; Luvonga et al., 2020). If indeed the AsSug underwent transformation in this study, this could explain the slightly higher PFs in mesopelagic meal for the simple methylated arsenicals (i.e. DMA, MA).

Normally, PFs are applied to processes which remove or reduce compounds due to exclusion of certain parts of the commodity, e.g. removal of rice hull and non-edible parts of the fruit (Scholz et al., 2018). The use of PFs in this study is not conventional but was applied to provide an indicative value of how levels of As species are affected during aquafeed processing.

Table 2
Processing factors of As species indicating up-concentration (PF > 1) or dilution/removal/degradation (PF < 1) during processing; expressed as median (range), $n = 4$.

	Mesopelagic meal	Mesopelagic oil	Stickwater
AB	0.3 (0.3–0.4)		0.19 (0.16–0.22)
TMAO	0.6 (0.5–0.6)		0.10 (0.05–0.14)
TMAP	0.4 (0.3–0.4)		0.18 (0.17–0.19)
DMA	0.6 (0.5–0.8)	0.2 (0.1–0.4)	0.02 (0–0.03)
MA	0.4 (0.2–0.8)	0.2 (0.1–0.4)	
AC	0.2 (0.2–0.2)		0.12 (0.08–0.14)
AsSug OH	0.2 (0.2–0.2)		0.10 (0.08–0.12)
AsSug PO4	0.2 (0–0.4)		
AsSug SO3			
AsLipids	0.8 (0.6–0.9)	1.4 (1.2–2.6)	0.06 (0.04–0.09)

4. Feed and food safety implications

Due to their abundance and nutritional composition, the sustainable harvest of mesopelagic species has the potential to address micronutrient deficiency and contribute to food and feed security (Alvheim et al., 2020; Nordhagen et al., 2020; Olsen et al., 2020). However, a thorough risk assessment is needed due to inherent undesirable substances, including As. The negative reputation associated with As in terms of toxicity is mainly due to its inorganic forms, arsenite (As (III)) and arsenate (As (V)), classified by IARC as carcinogenic (IARC Working Group, 2012). The current EU food legislation (Commission Regulation (EC) No 1881/2006) only has set maximum limits for iAs, specifically in rice and products derived therefrom (European Commission, 2006). As for products intended for animal feed, Directive 2002/32/EC only imposes limits for total As, but the legislation also specifies that it should be possible to demonstrate that the iAs content is below 2 ppm (European Commission, 2002). The current study revealed low levels of iAs in all samples, showing compliance to applicable regulations.

Arsenobetaine, on the other hand, is generally considered non-toxic (Kaise et al., 1985; Sabbioni et al., 1991). AB was the most abundant As compound in the mesopelagic single-species samples and was present in high concentrations especially among crustaceans. So despite it was reported in our previous study that total As concentrations for some crustaceans exceeded the limits in feed legislation (Wiech et al., 2020), the current work shows that AB made up majority of the As species and does not pose a toxicological concern based on latest assessment (IARC Working Group, 2012). On the other hand, AsLipids were present in significant proportions in *B. glaciale* and *M. muelleri*. The lab-scale feed processing study demonstrated that a considerable fraction of AsLipids bound to the mesopelagic meal. While levels were generally low, potential neurotoxicity and cytotoxicity were reported in *in-vitro* and *in-vivo* studies where AsHCs were observed to exhibit similar or stronger toxicity than iAs, while AsFAs were generally less toxic than AsHCs (Meyer et al., 2014a, 2014b; Witt et al., 2017; Muller et al., 2018). A limitation of our study was that only estimates of bulk AsLipids concentration were provided. Since the toxicity of AsLipids also vary per species (Witt et al., 2017; Muller et al., 2018), future work should focus on complete analytical characterization of individual AsLipid compounds present in mesopelagic samples by HPLC-ICP-MS or coupling HPLC to high-resolution mass spectrometry (HPLC-HRMS).

An up-concentration of AsLipids was seen in the mesopelagic oil. The mesopelagic biomass used to produce the oil contains Northern krill, among others (Berntssen et al., 2021). Krill oil from Antarctic krill is currently considered as an alternative source of EPA and DHA and has gained approval from EFSA as a novel food ingredient (EFSA, 2009). In the EFSA Scientific Opinion, while it was reported that iAs is < 0.1 mg/kg, the Working Group recognized the need for organic arsenic data (EFSA, 2009). The data from the present study show that mesopelagic oil from Northern krill (and silvery lightfish) comprises mainly of potentially toxic AsLipids and does not contain AB, contrary to what was suggested in previous reports (EFSA, 2009). This corroborates an earlier study which detected the presence of AsFAs in krill oil (Liu et al., 2021). These findings, again, highlight the importance of As speciation, especially in novel ingredients intended for human and animal consumption. In the aquafeed industry, the produced fish oil usually undergoes decontamination procedures to remove organic contaminants such as

dioxins and dioxin-like PCBs (Knutsen et al., 2017). These additional processing steps can further reduce the level of certain AsLipids in the final fish oil product, as Sele et al. (2013) reported.

The AsSug were present in trace levels and their distribution varies among mesopelagic organisms. The feed processing experiment also suggests possible transformation of AsSug to other forms. Arsenosugars in their native forms are considerably less toxic than iAs (Leffers et al., 2013). However, these are bio-accessible to human and could yield metabolites which were demonstrated to induce cytotoxic effects (Feldmann and Krupp, 2011; Taylor et al., 2017; Chen et al., 2020; Luvonga et al., 2020).

5. Conclusion

Arsenic species were found in varying concentrations in the mesopelagic single-species samples, processed mesopelagic meal and oil. The non-toxic AB was the major As compound in mesopelagic fish and crustaceans, while AsLipids were also found in significant concentrations. Other As species, including the carcinogenic iAs, were present in low levels. The feed processing study demonstrated transfer of potentially toxic AsLipids from the mesopelagic biomass to both mesopelagic meal and oil, providing a novel insight regarding the partitioning of As during aquafeed processing. Due to the prevalence of AB, it can be presumed that the use of mesopelagic resources as feed ingredients will not pose any arsenic-related hazards. However, the possible adverse effects of AsLipids cannot be neglected and needs to be further studied. An overall assessment regarding the suitability of mesopelagic species whether as food or feed ingredient in light of As compounds is challenging due to (1) lack of occurrence data for As species, (2) lack of toxicological data for the less common As species, partly due to unavailability of compound standards which hampers toxicity studies, and (3) the surrounding issues on exploitation of mesopelagic resources, specifically impacts on biodiversity and carbon sequestration. A holistic evaluation is needed, and the current study aims to contribute by providing valuable As speciation data which can be used for future risk assessments on the feasibility of harnessing mesopelagic biomass as feed ingredients.

Author contributions statement

Jojo Tibon: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Heidi Amlund:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Ana I. Gomez-Delgado:** Investigation, Writing – review & editing. **Marc H. G. Berntssen:** Resources, Writing – review & editing. **Marta S. Silva:** Resources, Writing – review & editing. **Martin Wiech:** Resources, Writing – review & editing. **Jens J. Sloth:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Veronika Sele:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was part of the project “Metals, arsenic and arsenic species in novel marine feed resources” (Project no. 15333), which was funded by the Ministry of Trade, Industry and Fisheries (NFD), Norway.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.134906.

References

Aas, T.S., Ytrestøyl, T., Åsgård, T., 2019. Utilization of feed resources in the production of Atlantic salmon (Salmo salar) in Norway: an update for 2016. Aquacult. Rep. 15 https://doi.org/10.1016/j.aqrep.2019.100216.

Alvheim, A.R., Kjellevold, M., Strand, E., Sanden, M., Wiech, M., 2020. Mesopelagic species and their potential contribution to food and feed security-A case study from Norway. Foods 9. https://doi.org/10.3390/foods9030344.

Amayo, K.O., Petursdottir, A., Newcombe, C., Gunnlaugsdottir, H., Raab, A., Krupp, E. M., Feldmann, J., 2011. Identification and quantification of arsenolipids using reversed-phase HPLC coupled simultaneously to high-resolution ICPMS and high-resolution electrospray MS without species-specific standards. Anal. Chem. 83, 3589–3595. https://doi.org/10.1021/ac2005873.

Amayo, K.O., Raab, A., Krupp, E.M., Feldmann, J., 2014. Identification of arsenolipids and their degradation products in cod-liver oil. Talanta 118, 217–223. https://doi.org/10.1016/j.talanta.2013.09.056.

Berntssen, M.H.G., Thoresen, L., Albrektsen, S., Grimaldo, E., Grimsmo, L., Whitaker, R. D., Sele, V., Wiech, M., 2021. Processing mixed mesopelagic biomass from the North-east atlantic into aquafeed resources; implication for food safety. Foods 10, 1265. https://doi.org/10.3390/foods10061265.

CEN, 2016. Foodstuffs – Determination of Elements and Their Chemical Species – Determination of Inorganic Arsenic in Foodstuffs of Marine and Plant Origin by Anion-Exchange HPLC-ICP-MS: European Committee for Standardization. EN 16802: 2016.

Chen, J., Garbinski, L.D., Rosen, B., Zhang, J., Xiang, P., Ma, L.Q., 2020. Organoarsenical compounds: occurrence, toxicology and biotransformation. Crit. Rev. Environ. Sci. Technol. 50, 217–243. https://doi.org/10.1080/10643389.2019.1619375.

Duncan, E.G., Maher, W.A., Foster, S.D., 2015. The formation and fate of organoarsenic species in marine ecosystems: do existing experimental approaches appropriately simulate ecosystem complexity? Environ. Chem. 12, 149–162. https://doi.org/10.1071/EN14124.

EFSA, 2009. Safety of ‘Lipid extract from Euphausia superba’ as a novel food ingredient Safety of ‘Lipid extract from Euphausia superba’ as a novel food ingredient. EFSA J. 7 https://doi.org/10.2903/j.efsa.2009.938.

Einarsson, M.I., Jokumsen, A., Bæk, A.M., Jacobsen, C., Pedersen, S.A., Samuelsen, T.A., Palsson, J., Eliasen, O., Flesland, O., 2019. Nordic Centre of Excellence Network in Fishmeal and Fish Oil. https://doi.org/10.5281/zenodo.3243334.

European Commission, 2002. Commission Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on Undesirable Substances in Animal Feed. Official Journal of the European Union, Brussel, Belgium.

European Commission, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. Official Journal of the European Union, Brussels, Belgium.

European Commission, 2017. Food from the Oceans - How Can More Food and Biomass Be Obtained from the Oceans in a Way that Does Not Deprive Future Generations of Their Benefits? (Luxembourg).

Feldmann, J., Krupp, E.M., 2011. Critical review or scientific opinion paper: arsenosugars-a class of benign arsenic species or justification for developing partly speciated arsenic fractionation in foodstuffs? Anal. Bioanal. Chem. 399, 1735–1741. https://doi.org/10.1007/s00216-010-4303-6.

Francesconi, K.A., Edmonds, J.S., 1997. In: Sykes, A.G. (Ed.), Arsenic and Marine Organisms. Academic Press, Inc.

Francesconi, K.A., Raber, G., 2013. Arsenic in Foods: Current Issues Related to Analysis, Toxicity and Metabolism. In: Persistent Organic Pollutants and Toxic Metals in Foods, pp. 414–429.

Freitas, F.P., Raber, G., Jensen, K.B., Nogueira, A.J.A., Francesconi, K.A., 2020. Lipids that contain arsenic in the Mediterranean mussel, Mytilus galloprovincialis. Environ. Chem. 17, 289–301. https://doi.org/10.1071/EN19213.

García-Seoane, E., Dalpadado, P., Vázquez, A., 2013. Feeding ecology of the glacier lanternfish benthosema glaciale (actinopterygii, myctophidae) in the flemish cap (North Atlantic ocean). Hydrobiologia 717, 133–146. https://doi.org/10.1007/s10750-013-1579-5.

Glabonjat, R.A., Raber, G., Holm, H.C., Van Mooy, B.A.S., Francesconi, K.A., 2021. Arsenolipids in plankton from high- and low-nutrient oceanic waters along a transect in the North atlantic. Environ. Sci. Technol. 55, 5515–5524. https://doi.org/10.1021/acs.est.0c06901.

Glabonjat, R.A., Raber, G., Van Mooy, B.A.S., Francesconi, K.A., 2018. Arsenobetaine in seawater: depth profiles from selected sites in the North atlantic. Environ. Sci. Technol. 52, 522–530. https://doi.org/10.1021/acs.est.7b03939.

Glencross, B.D., Baily, J., Berntssen, M.H.G., Hardy, R., Mackenzie, S., Tocher, D.R., 2020. Risk assessment of the use of alternative animal and plant raw material resources in aquaculture feeds. Rev. Aquacult. 12, 703–758. https://doi.org/10.1111/raq.12347.

Grimaldo, E., Grimsmo, L., Alvarez, P., Herrmann, B., Møen Tveit, G., Tiller, R., Slizyte, R., Aldanondo, N., Guldberg, T., Toldnes, B., Carvajal, A., Schei, M., Selnes, M., 2020. Investigating the potential for a commercial fishery in the Northeast Atlantic utilizing mesopelagic species. ICES (Int. Counc. Explor. Sea) J. Mar. Sci. 77, 2541–2556. https://doi.org/10.1093/icesjms/fsaa114.

Haque, A., Pettersen, J., Larsen, T., Opstvedt, J., 1981. Fishmeal and oil from lantern fish (myctophidae) with special emphasis on protein quality. J. Sci. Food Agric. 32, 61–70. https://doi.org/10.1002/jsfa.2740320110.

Hilmarsdottir, G.S., Ogmundarson, Ó., Arason, S., Gudjónsdóttir, M., 2020. The effects of varying heat treatments on lipid composition during pelagic fishmeal production. Processes 8, 1142. https://doi.org/10.3390/pr8091142.

Hunter, D.A., Goessler, W., Francesconi, K.A., 1998. Uptake of arsenate, trimethylarsine oxide, and arsenobetaine by the shrimp Crangon crangon. Mar. Biol. 131, 543–552. https://doi.org/10.1007/s002270050346.

IARC Working Group, 2012. Arsenic, metals, fibres, and dusts. IARC Monogr. Eval. Carcinog. Risks Hum. 100, 11–465.

Julshamn, K., Maage, A., Norli, H.S., Grobecker, K.H., Jorhem, L., Fecher, P., 2007. Determination of arsenic, cadmium, mercury, and lead by inductively coupled plasma/mass spectrometry in foods after pressure digestion: NMKL interlaboratory study. J. AOAC Int. 90, 844–856. https://doi.org/10.1093/jaoac/90.3.844.

Julshamn, K., Nilsen, B.M., Frantzen, S., Valdersnes, S., Maage, A., Nedreaas, K., Sloth, J. J., 2012. Total and inorganic arsenic in fish samples from Norwegian waters. Food Addit. Contam. Part B Surveill 5, 229–235. https://doi.org/10.1080/19393210.2012.698312.

Kaise, T., Watanabe, S., Itoh, K., 1985. The acute toxicity OF arsenobetaine. Chemosphere 14, 1327–1332. https://doi.org/10.1016/0045-6535(85)90153-5.

Kato, L.S., Ferrari, R.G., Leite, J.V.M., Conte-Junior, C.A., 2020. Arsenic in shellfish: a systematic review of its dynamics and potential health risks. Mar. Pollut. Bull. 161, 111693 https://doi.org/10.1016/j.marpolbul.2020.111693.

Knutsen, H.K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hoogenboom, L., Nebbia, C.S., Oswald, I.P., Petersen, A., Rose, M., Roudot, A.C., Schwerdtle, T., Vleminckx, C., Vollmer, G., Wallace, H., Lundebye, A.K., Metzler, M., Colombo, P., Hogstrand, C., 2017. Assessment of decontamination processes for dioxins and dioxin-like PCBs in fish oil by physical filtration with activated carbon. EFSA J. 15 https://doi.org/10.2903/j.efsa.2017.5081.

Leffers, L., Ebert, F., Taleshi, M.S., Francesconi, K.A., Schwerdtle, T., 2013. In vitro toxicological characterization of two arsenosugars and their metabolites. Mol. Nutr. Food Res. 57, 1270–1282. https://doi.org/10.1002/mnfr.201200821.

Leufroy, A., Noel, L., Dufailly, V., Beauchemin, D., Guerin, T., 2011. Determination of seven arsenic species in seafood by ion exchange chromatography coupled to inductively coupled plasma-mass spectrometry following microwave assisted extraction: method validation and occurrence data. Talanta 83, 770–779. https://doi.org/10.1016/j.talanta.2010.10.050.

Lischka, S., Arroyo-Abad, U., Mattusch, J., Kuhn, A., Piechotta, C., 2013. The high diversity of arsenolipids in herring fillet (Clupea harengus). Talanta 110, 144–152. https://doi.org/10.1016/j.talanta.2013.02.051.

Liu, Q., Huang, C., Li, W., Fang, Z., Le, X.C., 2021. Discovery and identification of arsenolipids using a precursor-finder strategy and data-independent mass spectrometry. Environ. Sci. Technol. 55, 3836–3844. https://doi.org/10.1021/acs.est.0c07175.

Luvonga, C., Rimmer, C.A., Yu, L.L., Lee, S.B., 2020. Organoarsenicals in seafood: occurrence, dietary exposure, toxicity, and risk assessment considerations - a review. J. Agric. Food Chem. 68, 943–960. https://doi.org/10.1021/acs.jafc.9b07532.

Luvonga, C., Rimmer, C.A., Yu, L.L., Lee, S.B., 2021. Determination of total arsenic and hydrophilic arsenic species in seafood. J. Food Compos. Anal. 96, 103729 https://doi.org/10.1016/j.jfca.2020.103729.

Meyer, S., Matissek, M., Müller, S.M., Taleshi, M.S., Ebert, F., Francesconi, K.A., Schwerdtle, T., 2014a. In vitrotoxicological characterisation of three arsenic-containing hydrocarbons. Metallomics 6, 1023–1033. https://doi.org/10.1039/c4mt00061g.

Meyer, S., Schulz, J., Jeibmann, A., Taleshi, M.S., Ebert, F., Francesconi, K.A., Schwerdtle, T., 2014b. Arsenic-containing hydrocarbons are toxic in the in vivo model Drosophila melanogaster. Metallomics 6, 2010–2014. https://doi.org/10.1039/c4mt00249k.

Molin, M., Ulven, S.M., Meltzer, H.M., Alexander, J., 2015. Arsenic in the human food chain, biotransformation and toxicology–Review focusing on seafood arsenic. J. Trace Elem. Med. Biol. 31, 249–259. https://doi.org/10.1016/j.jtemb.2015.01.010.

Muller, S.M., Ebert, F., Raber, G., Meyer, S., Bornhorst, J., Huwel, S., Galla, H.J., Francesconi, K.A., Schwerdtle, T., 2018. Effects of arsenolipids on in vitro blood-brain barrier model. Arch. Toxicol. 92, 823–832. https://doi.org/10.1007/s00204-017-2085-8.

Nordhagen, A., Rizwan, A.A.M., Aakre, I., Moxness Reksten, A., Pincus, L.M., Bokevoll, A., Mamun, A., Haraksingh Thilsted, S., Htut, T., Somasundaram, T., Kjellevold, M., 2020. Nutrient composition of demersal, pelagic, and mesopelagic fish species sampled off the coast of Bangladesh and their potential contribution to food and nutrition security-the EAF-nansen programme. Foods 9. https://doi.org/10.3390/foods9060730.

Olsen, R.E., Strand, E., Melle, W., Nørstebø, J.T., Lall, S.P., Ringø, E., Tocher, D.R., Sprague, M., 2020. Can mesopelagic mixed layers be used as feed sources for salmon aquaculture? Deep Sea Res. Part II Top. Stud. Oceanogr. 180, 104722 https://doi.org/10.1016/j.dsr2.2019.104722.

Pereira, É.R., Kopp, J.F., Raab, A., Krupp, E.M., Menoyo, J.D.C., Carasek, E., Welz, B., Feldmann, J., 2016. Arsenic containing medium and long chain fatty acids in marine fish oil identified as degradation products using reversed-phase HPLC-ICP-MS/ESI-MS. J. Anal. At. Spectrom. 31, 1836–1845. https://doi.org/10.1039/c6ja00162a.

Pétursdóttir, Á.H., Rodrigues de Jesus, J., Gunnlaugsdóttir, H., Feldmann, J., 2018. Quantification of labile and stable non-polar arsenolipids in commercial fish meals and edible seaweed samples. J. Anal. At. Spectrom. 33, 102–110. https://doi.org/10.1039/c7ja00333a.

Ruttens, A., Blanpain, A.C., De Temmerman, L., Waegeneers, N., 2012. Arsenic speciation in food in Belgium. J. Geochem. Explor. 121, 55–61. https://doi.org/10.1016/j.gexplo.2012.07.003.

Sabbioni, E., Fischbach, M., Pozzi, G., Pietra, R., Gallorini, M., Piette, J.L., 1991. Cellular retention, toxicity and carcinogenic potential of seafood arsenic. I. Lack of cytotoxicity and transforming activity of arsenobetaine in the BALB/3T3 cell line. Carcinogenesis 12, 1287–1291. https://doi.org/10.1093/carcin/12.7.1287.

Schmidt, K., 2010. Food and Feeding in Northern Krill (Meganyctiphanes Norvegica Sars). Elsevier, pp. 127–171.

Schmidt, L., Landero, J.A., Novo, D.L.R., Duarte, F.A., Mesko, M.F., Caruso, J.A., Flores, E.M.M., 2018. A feasible method for as speciation in several types of seafood by LC-ICP-MS/MS. Food Chem. 255, 340–347. https://doi.org/10.1016/j.foodchem.2018.02.079.

Scholz, R., Van Donkersgoed, G., Herrmann, M., Kittelmann, A., Von Schledorn, M., Graven, C., Mahieu, K., Van Der Velde-Koerts, T., Anagnostopoulos, C., Bempelou, E., Michalski, B., 2018. Database of processing techniques and processing factors compatible with the EFSA food classification and description system FoodEx 2 Objective 3: European database of processing factors for pesticides in food. EFSA Supporting Publications 15. https://doi.org/10.2903/sp.efsa.2018.en-1510.

Sele, V., Amlund, H., Berntssen, M.H., Berntsen, J.A., Skov, K., Sloth, J.J., 2013. Detection of arsenic-containing hydrocarbons in a range of commercial fish oils by GC-ICPMS analysis. Anal. Bioanal. Chem. 405, 5179–5190. https://doi.org/10.1007/s00216-013-6925-y.

Sele, V., Sloth, J.J., Holmelid, B., Valdersnes, S., Skov, K., Amlund, H., 2014. Arsenic-containing fatty acids and hydrocarbons in marine oils - determination using reversed-phase HPLC-ICP-MS and HPLC-qTOE-MS. Talanta 121, 89–96. https://doi.org/10.1016/j.talanta.2013.12.049.

Sele, V., Sloth, J.J., Lundebye, A.-K., Larsen, E.H., Berntssen, M.H.G., Amlund, H., 2012. Arsenolipids in marine oils and fats: a review of occurrence, chemistry and future research needs. Food Chem. 133, 618–630. https://doi.org/10.1016/j.foodchem.2012.02.004.

Shibata, Y., Sekiguchi, M., Otsuki, A., Morita, M., 1996. Arsenic compounds in Zoo- and phyto-plankton of marine origin. Appl. Organomet. Chem. 10, 713–719.

Sloth, J.J., Larsen, E.H., Julshamn, K., 2003. Determination of organoarsenic species in marine samples using gradient elution cation exchange HPLC-ICP-MS. J. Anal. At. Spectrom. 18, 452–459. https://doi.org/10.1039/b300508a.

St. John, M.A., Borja, A., Chust, G., Heath, M., Grigorov, I., Mariani, P., Martin, A.P., Santos, R.S., 2016. A dark hole in our understanding of marine ecosystems and their services: perspectives from the mesopelagic community. Front. Mar. Sci. 3 https://doi.org/10.3389/fmars.2016.00031.

Taleshi, M.S., Raber, G., Edmonds, J.S., Jensen, K.B., Francesconi, K.A., 2014. Arsenolipids in oil from blue whiting Micromesistius poutassou–evidence for arsenic-containing esters. Sci. Rep. 4, 7492. https://doi.org/10.1038/srep07492.

Taylor, V., Goodale, B., Raab, A., Schwerdtle, T., Reimer, K., Conklin, S., Karagas, M.R., Francesconi, K.A., 2017. Human exposure to organic arsenic species from seafood. Sci. Total Environ. 580, 266–282. https://doi.org/10.1016/j.scitotenv.2016.12.113.

Tibon, J., Silva, M., Sloth, J.J., Amlund, H., Sele, V., 2021. Speciation analysis of organoarsenic species in marine samples: method optimization using fractional factorial design and method validation. Anal. Bioanal. Chem. 413, 3909–3923. https://doi.org/10.1007/s00216-021-03341-4.

Wiech, M., Silva, M., Meier, S., Tibon, J., Berntssen, M.H.G., Duinker, A., Sanden, M., 2020. Undesirables in mesopelagic species and implications for food and feed safety-insights from Norwegian fjords. Foods 9. https://doi.org/10.3390/foods9091162.

Witt, B., Meyer, S., Ebert, F., Francesconi, K.A., Schwerdtle, T., 2017. Toxicity of two classes of arsenolipids and their water-soluble metabolites in human differentiated neurons. Arch. Toxicol. 91, 3121–3134. https://doi.org/10.1007/s00204-017-1933-x.

Wolle, M.M., Conklin, S.D., Wittenberg, J., 2019a. Matrix-induced transformation of arsenic species in seafoods. Anal. Chim. Acta 1060, 53–63. https://doi.org/10.1016/j.aca.2019.02.027.

Wolle, M.M., Stadig, S., Conklin, S.D., 2019b. Market basket survey of arsenic species in the top ten most consumed seafoods in the United States. J. Agric. Food Chem. 67, 8253–8267. https://doi.org/10.1021/acs.jafc.9b02314.

Xiong, C., Glabonjat, R.A., Al Amin, M.H., Stiboller, M., Yoshinaga, J., Francesconi, K.A., 2022. Arsenolipids in salmon are partly converted to thioxo analogs during cooking. J. Trace Elem. Med. Biol. 69, 126892 https://doi.org/10.1016/j.jtemb.2021.126892.

Zhang, W., Guo, Z., Song, D., Du, S., Zhang, L., 2018. Arsenic speciation in wild marine organisms and a health risk assessment in a subtropical bay of China. Sci. Total Environ. 626, 621–629. https://doi.org/10.1016/j.scitotenv.2018.01.108.