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Tissue accumulation and distribution of arsenic compounds in three marine fish species: relationship to trophic position

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In this study the accumulation and distribution of arsenic compounds in marine fish species in relation to their trophic position was investigated. Arsenic compounds were measured in eight tissues of mullet *Mugil cephalus* (detritivore), luderick *Girella tricuspidata* (herbivore) and tailor *Pomatomus saltatrix* (carnivore) by high performance liquid chromatography–inductively coupled plasma-mass spectrometry. The majority of arsenic in tailor tissues, the pelagic carnivore, was present as arsenobetaine (86–94%). Mullet and luderick also contained high amounts of arsenobetaine in all tissues (62–98% and 59–100% respectively) except the intestines (20% and 24% respectively). Appreciable amounts of dimethylarsinic acid (1–39%), arsenate (2–38%), arsenite (1–9%) and trimethylarsine oxide (2–8%) were identified in mullet and luderick tissues. Small amounts of arsenocholine (1–3%), methylarsonic acid (1–3%) and tetramethylarsonium ion (1–2%) were found in some tissues of all three species. A phosphate arsenoriboside was identified in mullet intestine (4%) and from all tissues of luderick (1–6%) except muscle. Pelagic carnivore fish species are exposed mainly to arsenobetaine through their diet and accumulate the majority of arsenic in tissues as this compound. Detritivore and herbivore fish species also accumulate arsenobetaine from their diet, with quantities of other inorganic and organic arsenic compounds. These compounds may result from ingestion of food and sediment, degradation products (e.g. arsenobetaine to trimethylarsine oxide; arsenoribosides to dimethylarsinic acid), conversion (e.g. arsenate to dimethylarsinic acid and trimethylarsine oxide by bacterial action in digestive tissues) and/or *in situ* enzymatic activity in liver tissue.

KEYWORDS: arsenic; marine fish; trophic position; speciation

Arsenobetaine has been identified as the major water-soluble arsenic compound present in the tissues of marine animals.[1] However, several other inorganic (arsenite and arsenate) and organic (arsenocholine, trimethylarsine oxide, tetramethylarsonium ion, dimethylarsinic acid, methylarsonic acid and arsenoribosides) arsenic compounds have also been identified in the tissues of marine animals.[1–4]

The assigning of marine animals into specific trophic positions based on their diet can be a useful tool for modelling transport processes.[5] Arsenobetaine has been identified in the tissues of marine animals from different trophic feeding positions, including planktivores, detritivores, herbivores and carnivores.[6–10] The only reported absence of arsenobetaine from a marine fish, possibly due to the unique fermentation of macroalgae in digestive tissues of this species, was by Edmonds *et al.*[4] from muscle and gut tissues of the silver drummer *Kyphosus sydneyanus*. At higher trophic positions (e.g. pelagic carnivores) the majority of arsenic is present as arsenobetaine,[11] whereas marine organisms at lower trophic positions (e.g. benthic detritivore and herbivore species) exposed through their diet to sediment, algae and seagrass may also contain appreciable quantities of other inorganic and organic arsenic compounds (e.g. arsenate, arsenite, trimethylarsine oxide, dimethylarsinic acid and arsenoribosides).[6,8,12,13] The diet of marine animals, and especially their association to sediments, is

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important in understanding the accumulation, distribution and cycling of arsenic compounds.[6]

A common practice when investigating arsenic compounds in marine animals is to examine only the muscle tissue, which is usually high in arsenobetaine.[1,6] This may result in an underestimation of the importance of other arsenic compounds in other tissues. In this study we have examined the tissues of three marine fish species from Lake Macquarie, New South Wales, Australia, to gain an understanding of the accumulation, distribution and cycling of arsenic compounds at different trophic feeding positions. Arsenic compounds were measured in eight tissues of mullet *Mugil cephalus* (detritivore), luderick *Girella tricuspidata* (herbivore) and tailor *Pomatomus saltatrix* (carnivore) by high performance liquid chromatography–inductively coupled plasma-mass spectrometry (HPLC–ICP-MS).

SPECIES DESCRIPTIONS

Mullet (*M. cephalus*)

Mullet are a benthic feeding detritivore fish species with a diet consisting of algae, seagrass detritus and organisms ingested with sediment. Large shoals are found in estuarine and coastal waters, distributed around the Australian mainland and northern Tasmania.

Luderick (*G. tricuspidata*)

Luderick are a herbivorous fish species that ingest large quantities of seagrass and algae. Large shoals can be found in the vicinity of seagrass beds and rocky outcrops, distributed mainly along the eastern coast of Australia.

Tailor (*P. saltatrix*)

Tailor are a pelagic carnivorous fish species that prey on shoals of small fish, such as mullet and herring. They are a migratory species that inhabit open-ocean waters and enter estuarine areas in search of food. They are distributed in waters and estuaries of the east, west and south coasts of Australia.

MATERIALS AND METHODS

Sample preparation

Five mullet (422–555 g), luderick (392–502 g) and tailor (640–725 g) were obtained from Lake Macquarie, New South Wales, Australia, on 15 May 2000. Fish were immediately placed into individual labelled acid-washed bags and frozen using dry ice.

Individual fish were dissected to remove eight tissues (muscle, liver, kidney, intestine, stomach, gonad, gill and heart) on separate plastic sheets; the implements were cleaned with 50% ethanol–water (v/v) between dissection and removal of individual tissues. Muscle tissue was removed from the left side of the fish between the pelvic and dorsal fins. Stomach and intestine tissues were opened and rinsed with deionized water to remove food and sediment particles. Individual tissues were rinsed with deionized water prior to being placed in acid-washed vials and frozen (−20 °C). Tissues were freeze dried for 72 h and ground to a fine homogeneous powder using a ZM 100 ultra centrifugal mill (Retsch, Germany). Individual tissues from five fish of the same species were combined for arsenic speciation, as there was insufficient dry mass (<0.01 g) of some tissues (i.e. heart, kidney and gonad) for analysis.

Extraction

Acetone extraction

Approximately 0.2–0.3 g of homogenized tissue was added to 50 ml polypropylene vials and 10 ml of acetone (HiPerSolv, BDH) added. The mixtures were shaken for 2 h and the supernatant removed after centrifuging at 3000 rpm for 15 min. The extraction procedure was repeated twice, with the supernatant removed after each centrifugation at 3000 rpm. After the final acetone extraction the residue pellet was dried under vacuum at room temperature (~25 °C).

Methanol–water extraction

Water-soluble arsenic compounds were separated from biological material by a microwave extraction procedure developed by Kirby *et al.*[14] Approximately 0.1–0.2 g of biological material was weighed into 50 ml polypropylene vials and 10 ml of 50% methanol (HiPerSolv, BDH)–deionized water (Milli-Q, Millipore) (v/v) added. Mixtures were loaded into the carousel of an MDS-2000 microwave oven (CEM, USA) (power 630 W) and heated to 70–75 °C for 5 min. The supernatant was removed after centrifuging at 3000 rpm for 15 min. The procedure was repeated twice more, with the combined supernatant from all three 50% methanol–water extractions used for arsenic speciation.

Total arsenic analysis

Fish tissues

Total arsenic concentrations in homogenized fish tissues were determined using a microwave digestion procedure published previously by Baldwin *et al.*[15] Approximately 0.07 g of freeze-dried tissue was weighed into 7 ml Teflon polytetrafluroacetate digestion vessels (A. I. Scientific, Australia) and 1 ml of concentrated HNO_3 (Aristar, BDH) added. Digestion occurred using an MDS-81D microwave oven (CEM, USA) and a time program consisting of three steps: 2 min at 600 W, 2 min at 0 W and 45 min at 450 W. After digestion, vessels were allowed to cool at room temperature (~25 °C) for approximately 60 min and then diluted with deionized water (Milli-Q, Millipore, Australia) to 10 ml in polyethylene vials. Digests were stored in a cool room (~0–5 °C) until total arsenic concentrations were determined with an Elan-6000 inductively coupled plasma mass spectrometer (Perkin–Elmer, Australia).

Acetone supernatant

Approximately 2 ml of combined acetone supernatant was weighed into a 7 ml Teflon polytetrafluroacetate digestion vessel (A. I. Scientific, Australia) and evaporated under vacuum at room temperature (~25 °C) to dryness. The residue was resuspended in 0.5 ml concentrated HNO_3 (Aristar, BDH) and digested by the procedure previously outlined for fish tissues. Extracts were diluted to 5 ml with deionized water (Milli-Q, Millipore, Australia) and total arsenic determined by ICP-MS (Perkin–Elmer, Australia).

Methanol–water supernatant

Approximately 2 ml of the combined methanol–water supernatant was evaporated to dryness at 50 °C using an RVC 2-18 rotational vacuum concentrator (CHRIST, Quantum, Australia). The residue was resuspended in 0.5 ml of 10% HNO_3 (Aristar, BDH) and further diluted with deionized water (Milli-Q, Millipore, Australia) to 5 ml prior to arsenic determination by ICP-MS (Perkin–Elmer, Australia).

Arsenic speciation

Arsenic standards

Arsenate and arsenite were prepared by dissolving sodium arsenate heptahydrate (Sigma–Aldrich, Australia) and sodium arsenite (Sigma–Aldrich, Australia) respectively in deionized ultrapure water (Milli-Q, Millipore, Australia). Dimethylarsinic acid (Sigma–Aldrich, Australia) and methylarsonic acid (Pfalz and Bauer, Germany) were prepared by dilution of sodium dimethylarsenic and disodium monomethyl arsenic in deionized water (Milli-Q, Millipore, Australia).

Arsenobetaine, arsenocholine, trimethylarsine oxide and tetramethylarsonium ion were kindly supplied by Dr Erik Larsen (National Food Agency, Institute of Food Chemistry and Nutrition, Denmark). The phosphate, sulfonate, sulfate and glycerol arsenoribosides were isolated and quantified from the marine macroalgae *Fucus* (IAEA 140/TM) and *Ecklonia radiata*. Confirmation of the four arsenoribosides was achieved by using liquid chromatography–mass spectrometry–mass spectrometry (Perkin Elmer SCIEX API 300). The four arsenoribosides were separated using a Hamilton PRP-X100 anion-exchange column (250 mm × 4.1 mm, Phenomenex, USA) with an aqueous 20 mM ammonium carbonate buffer (pH 9.2). The *m/z* of 329, 483, 393, and 409 were selectively monitored to identify the glycerol, phosphate, sulfonate and sulfate arsenoribosides respectively.

Water-soluble arsenic speciation

Approximately 28 ml of combined methanol–water supernatant was evaporated to dryness at 50 °C using an RVC 2-18 rotational vacuum concentrator (CHRIST, Quantum, Australia). The residue was resuspended in deionized water (Milli-Q, Millipore, Australia) to an arsenic concentration of less than 500 ng ml^{-1} and filtered through a 0.45 μm Iso-Disc N-4-4 Nylon filter (Supelco, USA). Aliquots of 100 μl were injected onto an HPLC system consisting of a Perkin Elmer Series 200 mobile phase delivery and auto sampler system (Perkin Elmer, Australia).

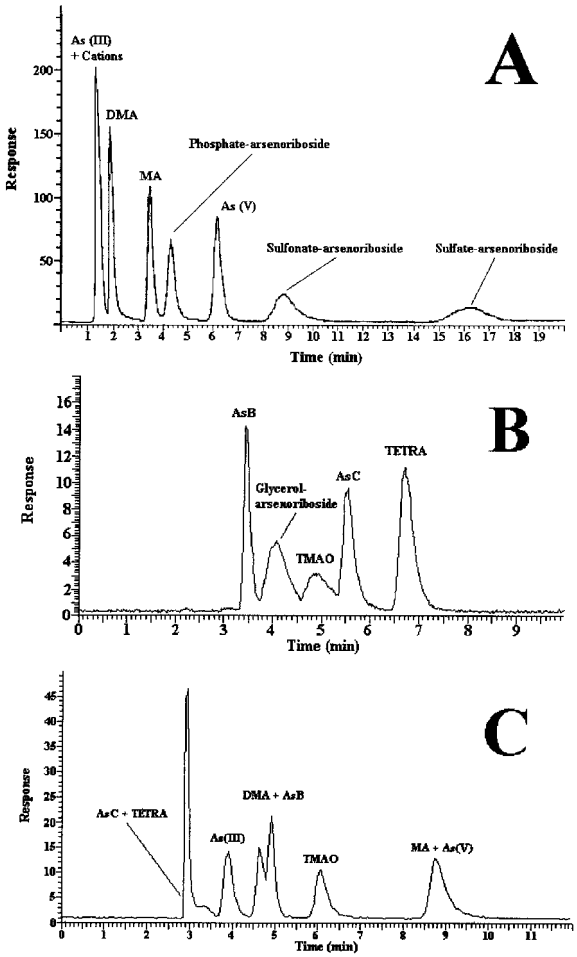

Figure 1. HPLC–ICP-MS chromatograms of standard arsenic compounds on (A) a Hamilton PRP-X100, (B) a Supelcosil LC-SCX and (C) a Supelcosil LC-SAX1.

A Hamilton PRP-X100 anion-exchange column (250 mm × 4.1 mm, Phenomenex, USA) and an aqueous 20 mM $NH_4H_2PO_4$ (Suprapur, Merck) mobile phase at pH 5.6 (flow rate: 1.5 ml min^{-1}; temperature: 40 °C) was used for the identification of arsenate [As(V)], dimethylarsinic acid (DMA), methylarsonic acid (MA), phosphate, sulfonate and sulfate arsenoribosides (Fig. 1A).

A Supelcosil LC-SCX cation-exchange column (250 mm × 4.6 mm, Supelco, USA) and an aqueous 20 mM pyridine (Extra Pure, Merck) mobile phase adjusted to pH 2.4 with formic acid (flow rate: 1.5 ml min^{-1}; temperature: 40 °C) was used for the identification of arsenobetaine (AsB), arsenocholine (AsC), trimethylarsine oxide (TMAO), tetra-

Table 1. Total, methanol–water-soluble and acetone-soluble arsenic

	As in sea mullet *M. cephalus*			As in luderick *G. tricuspidata*			As in tailor *P. saltatrix*		
Tissue	Total ($\mu g\ g^{-1}$)	Methanol–water-soluble (%)	Acetone-soluble (%)	Total ($\mu g\ g^{-1}$)	Methanol–water-soluble (%)	Acetone-soluble (%)	Total ($\mu g\ g^{-1}$)	Methanol–water-soluble (%)	Acetone-soluble (%)
Muscle	1.9	95	5	3.0	91	0	2.8	62	38
Liver	23.8	74	18	6.2	89	0	9.9	80	9
Stomach	3.3	58	9	2.9	86	0	2.6	87	0
Intestine	4.8	55	41	2.2	49	34	4.3	61	35
Kidney	6.6	61	0	2.2	61	0	4.3	65	5
Gonad	44.4	96	3	4.7	97	0	6.6	88	7
Gill	2.3	51	15	4.6	71	11	3.2	75	14
Heart	3.5	70	2	0.5	76	0	6.6	89	2

methylarsonium ion (TETRA) and the glycerol arsenoriboside (Fig. 1B).

A Supelcosil LC-SAX1 anion-exchange column (250 mm × 4.6 mm, Supelco, USA) and an aqueous 20 mM $NH_4H_2PO_4$ (Suprapur, Merck) mobile phase with 1% methanol (HiPerSolv, BDH)–deionized ultrapure water (Milli-Q, Millipore, Australia) (v/v) at pH 4.5 (flow rate: 1 ml min^{-1}; temperature: 40 °C) was used for the identification of arsenite [As(III); Fig. 1C). In the absence of glycerol arsenoriboside, arsenite can be more easily identified and quantified with this column [As(III) 3.83 min; glycerol arsenoriboside 3.94 min]. The remaining three main arsenoribosides elute after arsenobetaine on this column (sulfate 9.11 min; sulfonate 9.44 min; phosphate 9.72 min).

The eluant from HPLC columns was directed by polyether-ether-ketone (PEEK) capillary tubing into the cross-flow nebulizer of a Perkin Elmer Elan-6000 inductively coupled plasma mass spectrometer (Perkin Elmer SCIEX, Australia). Ion intensities were monitored at *m/z* 75, 77 and 82.

The chromatography package Turbochrom (Perkin Elmer, Australia) was used to quantify arsenic compounds by peak area. The spreadsheet package Excel (Microsoft, USA) was used for the determination of concentrations from calibration curves.

RESULTS

Total, methanol–water-soluble and acetone-soluble arsenic concentrations

Total arsenic concentrations in tissues ranged from 1.9 to 44.4 $\mu g\ g^{-1}$ (dry mass) for mullet, from 0.5 to 6.2 $\mu g\ g^{-1}$ (dry mass) for luderick and from 2.6 to 9.9 $\mu g\ g^{-1}$ (dry mass) for tailor (Table 1). The majority of arsenic in fish tissues was extracted in the methanol–water-soluble fraction (49–97%; Table 1). High amounts of acetone-soluble arsenic relative to total arsenic were found in some mullet (intestine 41%, liver 18% and gill 15%), luderick (intestine 34% and gill 11%) and tailor (muscle 38%, intestine 35% and gill 14%) tissues (Table 1).

Arsenic speciation

Arsenic speciation was performed on the methanol–water-soluble fraction; the percentage of each compound in relation to total arsenic in extracts is shown in Tables 2–4.

Table 2. Arsenic compounds in mullet *M. cephalus*

	Arsenic compound (%)								
Tissue	AsB	DMA	TMAO	As(V)	As(III)	TETRA	AsC	MA	Phosphate ribose
Muscle	89	9	2	0	0	0	0	0	0
Liver	76	19	2	0	0	1	0	1	0
Kidney	74	24	0	1	0	0	0	0	0
Stomach	80	13	0	3	2	0	1	1	0
Intestines	20	25	8	38	3	0	0	2	4
Gonad	99	1	0	0	0	0	0	1	0
Gill	85	4	4	3	1	0	2	1	0
Heart	62	32	4	1	0	0	0	1	0

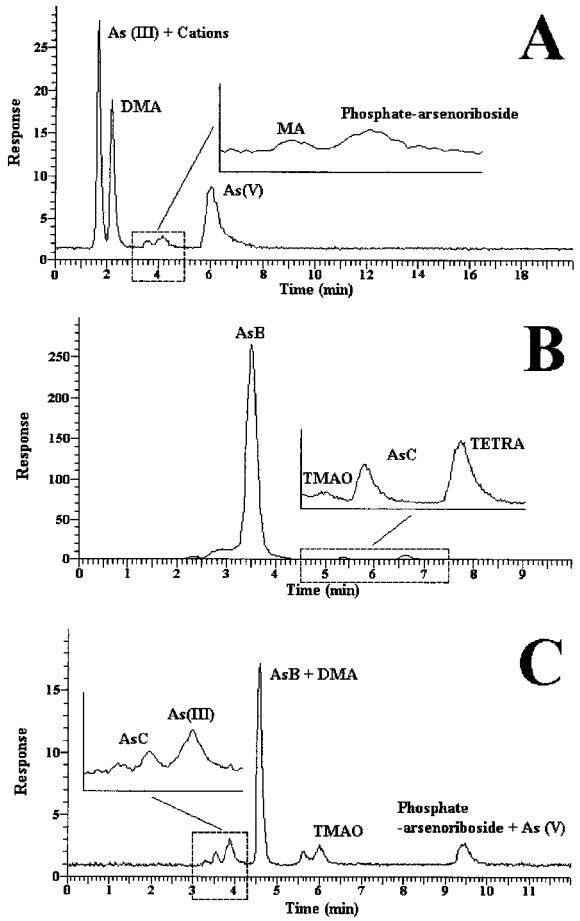

Figure 2. HPLC–ICP-MS chromatograms of (A) mullet intestine on a Hamilton PRP-X100 column, (B) tailor stomach on a Supelcosil LC-SCX column and (C) luderick stomach on a Supelcosil LC-SAX1 column.

Typical HPLC–ICP-MS fish tissue chromatograms are shown in Fig. 2.

*Mullet (*M. cephalus*)*

The majority of arsenic extracted from mullet tissues was arsenobetaine (62–98%), except in the intestines (20%). High amounts of arsenate were found in the intestines (38%), with trace amounts identified in stomach, gill, heart and kidney (1–3%) tissues. Dimethylarsinic acid was found in all tissues (1–32%), with the highest amounts identified in the heart (32%), intestine (25%), kidney (24%) and liver (19%) tissues. Small amounts of trimethylarsine oxide were identified in intestine, gill, heart, liver and muscle tissues (2–8%). Trace amounts of arsenocholine were found in gill (2%) and stomach (1%) tissues; arsenite was present in intestine, stomach and gill tissues (1–3%); tetramethylarsonium ion was found in liver tissue (1%); and methylarsonic acid was found in all tissues except kidney and muscle (1–2%). A small amount of phosphate arsenoriboside was found in the intestine tissue (4%).

*Luderick (*G. tricuspidata*)*

The majority of arsenic extracted from luderick tissues was found to be arsenobetaine (59–100%), except in the intestines (24%). Dimethylarsinic acid was found in all tissues except the muscle, with the highest amounts identified in intestine (39%), liver (15%) and gill (15%) tissues. High amounts of arsenate were found in intestine (20%) and stomach (16%), with small amounts identified in all tissues except muscle (2–6%). Small amounts of trimethylarsine oxide were found in gill (7%), liver (5%), kidney (3%), intestine (3%) and stomach (3%) tissues; and arsenite was found in all tissues except the muscle (1–9%). Trace amounts of arsenocholine were found in gill (2%), stomach (1%) and liver (1%) tissues; and methylarsonic acid was found in intestine (3%) and kidney (1%) tissues. Small amounts of phosphate arsenoriboside were found in all tissues (1–6%) except the muscle, with the highest amounts in intestine (6%) and stomach (4%) tissues. No tetramethylarsonium ion was identified in luderick tissues.

*Tailor (*P. saltatrix*)*

The majority of arsenic extracted from tailor tissues was

Table 3. Arsenic compounds in luderick *G. tricuspidata*

	Arsenic compound (%)								
Tissue	AsB	DMA	TMAO	As (V)	As (III)	TETRA	AsC	MA	Phosphate ribose
Muscle	100	0	0	0	0	0	0	0	0
Liver	67	15	5	4	5	0	1	0	3
Kidney	89	4	3	2	1	0	0	1	1
Stomach	59	7	3	16	9	0	1	0	4
Intestines	24	39	3	20	5	0	0	3	6
Gonad	83	4	0	6	3	0	0	0	3
Gill	68	15	7	5	1	0	2	0	1
Heart	83	7	0	6	3	0	0	0	1

Table 4. Arsenic compounds in tailor *P. saltatrix*

	Arsenic compound (%)								
Tissue	AsB	DMA	TMAO	As (V)	As (III)	TETRA	AsC	MA	Phosphate ribose
Muscle	94	6	0	0	0	0	0	0	0
Liver	93	5	0	0	0	1	0	0	0
Kidney	86	11	0	0	0	0	3	0	0
Stomach	86	8	1	1	0	2	1	1	0
Intestines	88	9	0	0	0	0	2	1	0
Gonad	95	4	1	0	0	0	0	0	0
Gill	90	7	0	0	0	2	1	0	0
Heart	96	3	0	0	0	1	1	0	0

found to be arsenobetaine (86–94%). Small amounts of dimethylarsinic acid were found in all tissues (3–11%). Trace amounts of arsenocholine were found in kidney (3%), intestine (2%), stomach (1%), gill (1%) and heart (1%) tissues; trimethylarsine oxide was found in gonad (1%) and stomach (1%) tissues; methylarsonic acid was found in intestine (1%) and stomach (1%) tissues; arsenate was found in stomach tissue; and tetramethylarsonium ion was found in stomach (2%), gill (2%), liver (1%) and heart (1%) tissues. No arsenite or arsenoribosides were found in the tissues of tailor.

DISCUSSION

The majority of arsenic in fish tissues was extracted in the methanol–water-soluble fraction (Table 1). This is consistent with other reported studies on marine animals, including fish.[16–18]

As previously reported for other studies of marine fish,[19–21] relatively low inorganic arsenic concentrations were found in fish tissues, except for the digestive tissues of mullet and luderick (Tables 2 and 3). The higher accumulation of inorganic arsenic in digestive tissues of benthic feeding fish has previously been reported by Maher *et al.*[6] and is thought to be associated with uptake from sediment.

In all tissues, except the intestine of mullet and luderick, most of the methanol–water-soluble arsenic was present as arsenobetaine (Tables 2–4). This compound was first identified in the tail muscle tissue of the western rock lobster *Panulirus cygnus* in 1977,[22] and has since been shown to be the major arsenical present in almost all marine animals from different trophic positions.[1,3,9]

The pelagic carnivore, tailor, accumulated the majority of arsenic in its tissues as arsenobetaine (≤86%; Table 4). Pelagic carnivore species exposed mainly to arsenobetaine through their diet would be expected to accumulate this compound in their tissues. Francesconi *et al.*[23] found that marine fish readily accumulate arsenobetaine when exposed through their diet. Mullet (detritivore) and luderick (herbivore) would also accumulate ingested arsenobetaine, but may also be exposed to other inorganic (e.g. arsenite and arsenate associated with sediments) and organic (e.g. dimethylarsinic acid, trimethylarsine oxide and arsenoribosides) arsenic compounds through their diet.[6]

It is unlikely that fish from this study accumulated arsenobetaine directly from seawater or marine sediments, as this compound has been reported to be readily degraded to dimethylarsinic acid, trimethylarsine oxide and inorganic arsenic in these environments.[24–27]

The pathway(s) for the synthesis of arsenobetaine is still to be determined.[1] Edmonds and Francesconi[28] have proposed a pathway for the synthesis of arsenobetaine, from the microbial degradation of arsenoribosides in marine sediments. The intermediates in this pathway (i.e. dimethylarsinylethanol, dimethylarsinylacetic acid and arsenocholine) may then be available to detritivore and herbivore species for conversion to arsenobetaine.[1,29,30] Goessler *et al.*[8] have given support for this pathway, finding that the herbivore gastropod *Austrocochlea constricta* (which feeds mainly on macroalgae *Hormosira banksii* containing arsenoribosides) has most of its arsenic as arsenobetaine. At present there is no conclusive evidence to support this as the major pathway for the formation of arsenobetaine in the marine environment.

Edmonds[31] has proposed an alternative pathway for the synthesis of arsenobetaine within marine organisms. He suggests that dimethylarsinic acid might replace ammonium ions in the biosynthesis of amino acids. Thereby, 'arsenylation' of pyruvate, paralleling the biosynthesis of alanine, or glyoxylate could lead to the synthesis of arsenobetaine. Appreciable quantities of dimethylarsinic acid were found in fish tissues from all three trophic positions in this study; however, further experimentation is required to determine if this pathway can explain the high abundance of arsenobetaine in marine animals.

Arsenocholine, a possible intermediate in the pathway proposed by Edmonds and Francesconi[28] for the synthesis of arsenobetaine, was identified at low concentrations (<2%) in some fish tissues from all three trophic positions (Tables 2–4). The low accumulation of arsenocholine is consistent with

that reported for other marine animals.[9,32] Arsenocholine may be present in fish tissues as a result of ingestion and/or the degradation of arsenoribosides in digestive tract tissues. Francesconi *et al.*[23] have reported that the marine fish yellow eye mullet *Aldrichetta forsteri* retained approximately 39% of the arsenocholine obtained through their diet. However, the rapid conversion of arsenocholine to arsenobetaine prevents the accumulation of this compound in tissues.

A phosphate arsenoriboside was identified in mullet intestine and from all luderick tissues except muscle (Tables 2 and 3). Other studies have also reported the presence of arsenoribosides in marine animals, including fish.[6,13,33] Since arsenoribosides are not directly synthesized by marine animals, their accumulation in tissues is believed to be associated with ingestion of phytoplankton and/or the presence of symbiotic algae.[1,13,33] Arsenoribosides have been reported to degrade with the aid of microorganisms to arsenocholine and dimethylarsinic acid.[1] The presence of a phosphate arsenoriboside in luderick tissues suggests that this compound is accumulated to some degree from digestive tissues without degradation to dimethylarsinic acid and/or arsenocholine. Luderick is a herbivore that may be exposed to arsenoribosides through the ingestion of seagrass and/or algae. The isolation of a phosphate arsenoriboside from the intestine tissue of the detritivore mullet suggests accumulation may be occurring from their diet of seagrass and algae detritus; however, it is rapidly degraded, possibly in the liver, prior to circulation.

The presence of an arsenoriboside and arsenobetaine together in tissues of luderick and mullet suggests that conversion to the latter compound may be occurring within marine animals. Other studies have also identified arsenobetaine and arsenoribosides together in the tissues of detritivore and herbivore marine animal species, including fish.[6,8,13] However, Cooney and Benson,[34] in a study of the lobster *Homarus americanus*, failed to report any direct conversion to ^{74}As-arsenobetaine when fed a macroalgae diet consisting of ^{74}As-arsenoribosides.

Dimethylarsinic acid was identified in all fish tissues, except the muscle of luderick (Tables 2–4). The presence of dimethylarsinic acid in these tissues may occur as a result of microbial degradation of arsenobetaine ingested with food.[35] Dimethylarsinic acid in mullet and luderick tissues may also occur as a result of the degradation of arsenoribosides and/or methylation of inorganic arsenic ingested with their food. High amounts of inorganic arsenic (Tables 2 and 3), especially in mullet digestive tissues, probably from ingested sediments while feeding, may be further methylated to dimethylarsinic acid by microorganisms following a pathway initially proposed by Challenger.[36] The methylation of inorganic arsenic to methylarsonic acid and dimethylarsinic acid has been suggested to be a detoxification mechanism in marine animals that protects against potentially toxic interactions from arsenite and arsenate with important cell components.[3,37] Dimethylarsinic acid taken up from the digestive tract can then be distributed by blood to accumulate in other tissues.[6]

Only trace amounts of tetramethylarsonium ion ($\leq$2%) were identified in fish tissues from all three trophic positions (Tables 2–4). High concentrations of this compound have been reported in some gastropod and mollusc species.[38–40] The synthesis of the tetramethylarsonium ion has been proposed to occur from the degradation of arsenobetaine and/or methylation of trimethylarsine oxide or trimethylarsine.[6,39,40] The small quantity of tetramethylarsonium ion found in this study suggests that this pathway is not important for arsenic in marine fish.

Relatively high concentrations of trimethylarsine oxide were identified in mullet and luderick tissues (Tables 2 and 3). The presence of trimethylarsine oxide, especially in digestive tissues, may be due to microbial methylation of ingested inorganic arsenic and/or degradation of arsenobetaine. Edmonds and Francesconi[12] found that trimethylarsine oxide accumulated in the tissues of estuary catfish *Cnidoglanis macrocephalus* and school whiting *Sillago bassensis* after oral administration of arsenate. The authors attributed this accumulation of trimethylarsine oxide to the conversion of arsenate by microorganisms in digestive tissues. The detritivore mullet and herbivore luderick may be exposed to inorganic arsenic as a consequence of their feeding habits. Mullet feed by sucking up detritus from the sediment benthic layer, whereas luderick consume seagrass that can contain a fine sediment layer adhering to its surface. Some macroalgae contain large quantities of arsenate, and it is likely that algae consumed by detritivore and herbivore species contain inorganic arsenic as well.

Trimethylarsine oxide may also be produced by the degradation of arsenobetaine by microorganisms.[41–43] The absence of trimethylarsine oxide from the tissues of tailor, the pelagic carnivore, which consumes the majority of its arsenic as arsenobetaine, suggests that degradation of arsenobetaine to trimethylarsine oxide is not a major pathway in marine fish. However, degradation pathways of arsenic in fish may be species specific.

Arsenic compounds present in fish tissues may be different depending on trophic position (diet) and/or their association with marine sediments. Pelagic carnivore fish species consuming mainly arsenobetaine accumulate arsenic in their tissues as this compound. Detritivore and herbivore fish species also accumulate arsenobetaine, with other arsenic compounds (e.g. arsenate, dimethylarsinic acid, trimethylarsine oxide and arsenoribosides) following ingestion, degradation and/or conversion of arsenic from their diet or sediments.

REFERENCES

1. Francesconi K and Edmonds J. *Oceanogr. Mar. Biol. Annu. Rev.* 1993; **31**: 111.
2. Cullen W and Reimer K. *Chem. Rev.* 1989; **89**: 713.

3. Maher W and Butler E. *Appl. Organomet. Chem.* 1988; **2**: 191.
4. Edmonds J, Shibata Y, Francesconi K, Rippingale R and Morita M. *Appl. Organomet. Chem.* 1997; **11**: 281.
5. Bernhard M and Andreae M. Transport of trace metals in marine food chains. In *Changing Metal Cycles and Human Health*. Springer: Berlin, Heidelberg, New York, 1984; 143–467.
6. Maher W, Goessler W, Kirby J and Raber G. *Mar. Chem.* 1999; **68**: 169.
7. Francesconi K and Edmonds J. *Croat. Chem. Acta* 1998; **71**: 343.
8. Goessler W, Maher W, Irgolic K, Kuehnelt D, Schlagenhaufen C and Kaise T. *Fresenius J. Anal. Chem.* 1997; **359**: 434.
9. Larsen E, Pritzl G and Hansen S. *J. Anal. At. Spectrom.* 1993; **8**: 1075.
10. Hanaoka K, Fujita T, Matsuura M, Tagawa S and Kaise T. *Comp. Biochem. Physiol.* 1987; **86**: 681.
11. Francesconi K and Edmonds J. In *Arsenic in the Environment: Cycling and Characterisation*, Advances in Environmental Science and Technology, vol. 26. 1994; 221–261.
12. Edmonds J and Francesconi K. *Sci. Total Environ.* 1987; **64**: 317.
13. Morita M and Shibata Y. *Anal. Sci.* 1987; **3**: 575.
14. Kirby J, Maher W, Elwood M and Krikowa F. *J. Anal. At. Spec.* In preparation.
15. Baldwin S, Deaker M and Maher W. *Analyst* 1994; **119**: 1701.
16. Maher W. *Comp. Biochem. Physiol. C* 1985; **80**: 199.
17. Maher W. *Comp. Biochem. Physiol. C* 1985; **82**: 433.
18. Maher W and Batley G. *Appl. Organomet. Chem.* 1990; **4**: 419.
19. Shinagawa A, Shiomi K, Yamanaka H and Kikuchi T. *Bull. Jpn. Soc. Sci. Fish.* 1983; **49**: 75.
20. Maher W. *Mar. Poll. Bull.* 1983; **14**: 308.
21. Branch S, Ebdon L and O'Neil P. *J. Anal. At. Spectrom.* 1994; **9**: 33.
22. Edmonds J, Francesconi K, Cannon J, Raston C, Skelton B and White A. *Tetrahedron Lett.* 1977; **18**: 1543.
23. Francesconi K, Edmonds J and Stick R. *Sci. Total Environ.* 1989; **79**: 59.
24. Hanaoka K, Goessler W and Irgolic K. *Chemosphere* 1997; **35**: 2463.
25. Hanaoka K, Koga H and Tagawa S. *Comp. Biochem. Physiol. B* 1992; **101**: 595.
26. Hanaoka K, Motoya T, Tagawa S and Kaise T. *Appl. Organomet. Chem.* 1992; **6**: 139.
27. Hanaoka K, Yamamoto H, Tagawa S and Kaise T. *Appl. Organomet. Chem.* 1988; **2**: 371.
28. Edmonds J and Francesconi K. *Experientia* 1987; **43**: 553.
29. Edmonds J and Francesconi K. *Appl. Organomet. Chem.* 1988; **2**: 297.
30. Edmonds J and Francesconi K. *Nature* 1981; **289**: 602.
31. Edmonds J. *Bioorg. Med. Chem. Lett.* 2000; **10**: 1105.
32. Lau B, Michalik P, Porter C and Krolik S. *Biomed. Environ. Mass Spectrom.* 1987; **14**: 723.
33. Edmonds J, Francesconi K, Healy P and White A. *J. Chem. Soc. Perkin Trans. 1* 1982; 2989.
34. Cooney R and Benson A. *Chemosphere* 1980; **9**: 335.
35. Hanaoka K, Motoya T, Tagawa S and Kaise T. *Appl. Organomet. Chem.* 1991; **5**: 427.
36. Challenger F. *Chem. Rev.* 1945; **36**: 315.
37. Tamaki S and Frankenberger W. *Rev. Environ. Contam. Toxicol.* 1992; **124**: 79.
38. Shiomi K, Kakehahi Y, Yamanaka H and Kikuchi T. *Appl. Organomet. Chem.* 1987; **1**: 177.
39. Francesconi K, Edmonds J and Hatcher B. *Comp. Biochem. Physiol. c* 1988; **90**: 313.
40. Cullen W and Dodd M. *Appl. Organomet. Chem.* 1989; **3**: 79.
41. Norin H, Christakopoulos A, Sandstrom M and Ryhage R. *Chemosphere* 1985; **14**: 313.
42. Kaise T, Hanaoka K and Tagawa S. *Chemosphere* 1987; **16**: 2551.
43. Hanaoka K, Motoya T, Tagawa S and Kaise T. *Appl. Organomet. Chem.* 1991; **5**: 427.