INTRODUCTION

Sediments are a major repository for trace metals, especially in coastal regions, where metal concentrations can reach elevated levels in industrialized estuaries (Kennish 1997). Sediments are much more enriched in metals than the overlying water column. This enrichment provides a large source of metals to benthic organisms, potentially impacting their health or that of their predators. Many factors influence whether a particular metal is bioavailable, and the mechanisms involved are still being studied. Field studies have shown that benthic organisms can accumulate metals to high concentrations, including in industrialized coastal waters and salt marshes where killifish reside (Kennish 1997, França et al. 2005).

The bioavailability of sediment-bound metal to benthic organisms is dependent on the sediment geochemistry and phase-partitioning of the metal (Luoma 1989, Wang et al. 1999, Baumann & Fisher 2011) and the time of sediment exposure to the metal (Wang et al. 1999, Griscom et al. 2000, Baumann & Fisher 2011). Studies using radiotracers have shown that polychaetes, bivalves, and amphipods can assimilate sediment-bound metals (Wang et al. 1999, Schlekat et al. 2000, Griscom et al. 2002a, Baumann & Fisher 2011), and these organisms can act as a conduit for the trophic transfer of metals to higher trophic levels, including fish, birds, and potentially human consumers of seafood.

Within the planktonic food chain, the largest enrichment step occurs at the bottom of the food chain,
between the dissolved phase and phytoplankton (Fisher & Reinfelder 1995). Sinking planktonic debris enriched in metals can settle to sediments and serve as a source of metal for benthic animals in addition to the metals that sorb directly to the sediments themselves (Baumann & Fisher 2011).

The killifish, or mummichog, *Fundulus heteroclitus* inhabits estuaries, bays, and salt marshes along the eastern seaboard of the United States from the Gulf of St. Lawrence to northeastern Florida (Abraham 1985). Killifish gut content analyses have shown that they consume a varied diet, including algae, amphipods, copepods, polychaetes, nematodes, molluscs, crabs, eggs, plant material, and detritus (Kneib & Stiven 1978, Allen et al. 1994, McMahon et al. 2005). While gut content analyses have not shown that killifish actively consume sediment, some sediment may be accidently ingested while feeding on benthic prey, and the metals associated with this sediment may be bioavailable to killifish.

To investigate the bioavailability of sediment-bound and algal metals to killifish, we radiolabeled sediment from 3 contaminated field sites (Baltimore Harbor, Elizabeth River, and Mare Island) and the green alga *Dunaliella tertiolecta* with As, Cd, Cr, Hg(II), and methylmercury (MeHg). The transfer of these metals to killifish was assessed after the fish were intubated with sediment or algae. Following intubation, metal loss was monitored for 9 d, and kinetic parameters (assimilation efficiencies, or percentage of ingested material that crosses the gut lining, and loss rate constants) and tissue distributions of the ingested metal were determined. The kinetic parameters were used to calculate the trophic transfer factor (TTF), which describes the likelihood that a metal will be transferred from food to fish at this trophic step. The 3 sediment locations were chosen because they are all contaminated, have differing organic carbon content, grain size distribution, and geochemical properties (Baumann & Fisher 2011), and are part of a larger project comparing these sites. *D. tertiolecta* was the chosen alga because it has no cell wall, therefore minimizing the digestive complication of a walled cell.

The 3 metals (Cd, Cr, and Hg) and metalloid (As) were chosen based upon their chemical characteristics and environmental interest. Among the chosen trace elements, Hg is a Class B metal with a greater affinity for sulfur ligands than oxygen or nitrogen ligands, whereas As, Cd, and Cr are borderline metals (Nieboer & Richardson 1980). These metals are commonly found at elevated concentrations in estuarine sediments, particularly those near industrial areas (Kennish 1997), and their bioavailability is of interest for management of coastal ecosystems.

**MATERIALS AND METHODS**

**Study locations and sediment collection**

Sediment was collected by box coring from 2 contaminated sites in the Chesapeake Bay, the Elizabeth River (ER; Norfolk, VA; 36°12’32”N, 76°20’09”W) in May 2006 and Baltimore Harbor (BH; Baltimore, MD; 39°12’25”N, 76°31’41”W) in June 2007, and one contaminated site in San Francisco Bay, the Mare Island naval complex (MI; Vallejo, CA; 38°04’23”N, 122°14’91”W) in October 2007. The grain size distribution (described as the percentage of the coarse fraction >63 µm), organic carbon content, and background metal concentrations for each location are shown in Table 1. For grain size analysis, ~20 g of sediment was dried at 60°C to obtain a total dry weight, rehydrated, and separated into coarse (>63 µm) and fine (<63 µm) fractions by wet sieving. The coarse fraction was then dried at 60°C for 48 h to obtain dry weights (n = 3 per field site). Organic carbon content was calculated by combusting dry sediment at 450°C for 6 h; the percentage difference between the before and after dry weights was the percentage of organic carbon. Background metal concentrations were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) at the Trace Element Core Laboratory (Dartmouth College, Hanover, NH, USA) using an IAEA-433 reference standard.

Water from each field location was collected using a trace metal clean pump, and the chemical properties (salinity, dissolved organic carbon [DOC] concentration, and background metal concentrations)
are shown in Table 2. The Hg background water concentration was also analyzed at Dartmouth College, and As, Cd, and Cr were analyzed at Rutgers Inorganic Analytical Core Laboratory (Rutgers University, New Brunswick, NJ, USA) using ICP-MS. All of the water was 0.2 µm sterile-filtered (Millipak 200, Millipore) before use. The sediment and water were held in the dark at 4°C until use.

### Fish maintenance

Field-collected (Taylor River, Hampton, NH, USA) killifish *Fundulus heteroclitus*, 59.5 ± 2.2 mm (mean ± SD) long and with a mean wet weight of 2.1 ± 0.3 g, were purchased from Aquatic Research Organisms and acclimated to experimental conditions for at least 4 wk prior to the start of the experiments. The fish were fed a diet of TetraCichlid™ cichlid flakes (Tetra Holding) and frozen bloodworms daily prior to the start of the experiments and were fed only bloodworms throughout the experiments. All fish were starved for 36 h prior to the start of the experiments to allow for total gut clearance. Fish were held at 18 ± 0.5°C on a 14:10 h light:dark cycle.

### Metal uptake from sediment

To prepare radiolabeled sediment, ~6 to 6.5 g of sediment from each of the 3 field locations were radiolabeled and left to age in the dark in sealed glass containers at room temperature (20 ± 1°C) for 7 d. Comparisons of wet and dry weights indicated that water comprised ~56% of the weight of sediment from BH, 63% from ER, and 52% from MI. Radioisotope additions per field location were 104 kilobecquerel (kBq) ^73^As, 40 kBq ^109^Cd, 245 kBq ^51^Cr, 33 kBq ^203^Hg(II), and 33 kBq MeHg; this corresponds to the following metal concentrations: 511 nM ^73^As, 103 nM ^109^Cd, 54 nM ^51^Cr, 195 nM ^203^Hg(II), and 195 nM MeHg. At the end of the 7 d sediment aging period, killifish were intubated with radiolabeled sediment (n = 5 per field site). To intubate killifish, radiolabeled sediment was added to a 3 cm³ syringe with a 16G1½ PrecisionGlide needle attached (Becton Dickinson), and the needle was carefully slid into intramedic non-radiopaque polyethylene tubing (internal diameter 1.57 mm, 5 cm long; Clay Adams). Fish were removed from the water, and the tubing was passed through the mouth and esophagus into the start of the intestine, where ~0.06 to 0.1 g of radiolabeled sediment was added. The fish were then returned to the water and not handled for 30 min to minimize stress and prevent sediment regurgitation. No feces were produced during this time, so the start of depuration was when the fish were intubated. No fish died or showed adverse effects (not feeding or abnormal swimming behavior) due to this intubation procedure.

After determining their initial radioactivity, the fish were returned to individual containers with non-radiolabeled water, collected from the same field location as the sediment, and fed non-radiolabeled bloodworms to purge their guts of radiolabeled sediment. Fish were radioassayed at regular intervals during the first 2 d and then once a day for the following 7 d to evaluate metal loss. At each sample time, feces and a 1 ml water sample were collected. The depuration water was changed after 1 d and then every other day to minimize any radioisotope leaching into the dissolved phase, either due to release from feces or excretion from the fish. At the end of the 9 d depuration period, the fish were euthanized using MS222 and dissected into head (including gills), viscera, and body (skeleton, fins, fillet, and skin). The radioactivity of each tissue compartment was determined, after which the samples were dried at 60°C for 48 h to determine dry weights.

Statistical analyses were conducted using IBM SPSS statistics software (v. 20). One-way ANOVA and Tukey post-hoc tests were conducted to identify significant differences (p < 0.05 or p < 0.01) between sediment location and kinetic parameters (assimilation efficiencies and loss rate constants) for each metal.

### Metal uptake from algae

The green alga *Dunaliella tertiolecta* (CCMP 1320) was uniformly radiolabeled with metals for 4 d in

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**Table 2. Water properties.** Salinity, dissolved organic carbon (DOC) concentration, and background metal concentrations (As, Cd, Cr, and total Hg) for water collected from Baltimore Harbor (BH), Elizabeth River (ER), and Mare Island (MI). For DOC concentration, values are means ± 1 SD; n = 3. For background metal concentrations, units are µg l⁻¹ for As, Cd, and Cr and ng l⁻¹ for Hg.

<table>
<thead>
<tr>
<th>Salinity</th>
<th>DOC conc. (µM)</th>
<th>Background conc.</th>
<th>As</th>
<th>Cd</th>
<th>Cr</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>BH 7.6</td>
<td>219 ± 12</td>
<td>0.97</td>
<td>0.02</td>
<td>0.19</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>ER 19.5</td>
<td>384 ± 1.9</td>
<td>1.38</td>
<td>0.21</td>
<td>0.25</td>
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<tr>
<td>MI 22</td>
<td>169 ± 6.7</td>
<td>2.20</td>
<td>0.19</td>
<td>0.20</td>
<td>2.7</td>
<td></td>
</tr>
</tbody>
</table>
Radioisotopes and radioanalyses

High specific activity gamma-emitting radioisotopes were used in the present study (10.97 to 13.97 μCi μg⁻¹ ⁷³As, 13.88 to 14.43 μCi μg⁻¹ ¹⁰⁹Cd, 67.58 to 340.8 μCi μg⁻¹ ⁵¹Cr, and 0.55 to 3.22 μCi μg⁻¹ ²⁰³Hg(II)), and 0.55 to 3.22 μCi μg⁻¹ ⁴⁰MeHg. The ⁷³As (half-life \(t_{1/2} = 80.3\) d, as As(III)) and ¹⁰⁹Cd (\(t_{1/2} = 46.2\) d), both dissolved in 0.1 M HCl, were purchased from the Department of Energy (Los Alamos National Laboratory). ⁵¹Cr (\(t_{1/2} = 27.7\) d, as Cr(III)), dissolved in 0.5 M HCl, was purchased from PerkinElmer, and ²⁰³Hg(II) (\(t_{1/2} = 80.3\) d, as Hg(II)), dissolved in 1 M HCl) was purchased from Eckert & Ziegler Isotope Products. MeHg (CH₃⁻²⁰³Hg(II)) was synthesized in our laboratory using a method described by Rouleau & Block (1997) and held in deionized water. The radioisotopes were added in microliter quantities, and equimolar concentrations of sodium hydroxide were added to neutralize the acid. The pH remained unchanged after radioisotope additions. ¹⁰⁹Cd, ²⁰³Hg(II), and MeHg were single-labeled, while ⁷³As and ⁵¹Cr were double labeled.

Live fish were radioassayed using a Canberra deep-well NaI(Tl) γ-detector for no longer than 5 min to minimize stress on the fish. Initially, the propagated counting error was ≤ 5%, but after gut clearance, propagated counting errors could reach 25% due to much lower radioactivity caused by elimination of the radioisotope. Water, feces, and dissected fish tissue were radioassayed using an intercalibrated LKB Pharmacia-Wallac 1282 CompuGamma CS gamma-counter for 5 min ⁷³As, ¹⁰⁹Cd, and ⁵¹Cr) or 10 min ²⁰³Hg. The γ-emission of ¹⁰⁹Cd, ⁷³As, ²⁰³Hg, and ⁵¹Cr was detected at 22, 53, 279, and 320 keV, respectively. All sample counts were adjusted for background radioactivity and radioactive decay.

Modeling metal bioaccumulation in killifish

The steady-state concentration of metals in aquatic organisms can be determined using a well-described biokinetic model (Thomann 1981, Wang et al. 1996, Rein Felder et al. 1998). This model takes into account the uptake and loss of metals following aqueous and dietary exposure. We did not determine metal uptake from the aqueous phase in our study, but the dietary component of the model can be rearranged to calculate the TTF. The TTF estimates the likelihood for a metal to biomagnify at a particular trophic step, based on the ratio of metal in a predator compared to metal in its prey. A TTF > 1 indicates that biomagnification is likely at this trophic step, whereas a TTF < 1 indicates there is a low probability of biomagnification (Rein Felder et al. 1998). TTF is calculated as follows:

\[
TTF = \frac{(AE \cdot IR)}{k_{ef}}
\]  

where AE is the assimilation efficiency of the ingested metal (fraction), IR is the weight-specific ingestion rate (g g⁻¹ d⁻¹), and \(k_{ef}\) is the metal loss rate constant after dietary exposure (d⁻¹). Metals with a high AE and low \(k_{ef}\) have a greater probability of biomagnifying, compared to those with a low AE and high \(k_{ef}\).

The AE and \(k_{ef}\) for individual fish following sediment and algal intubations were calculated by exponentially regressing metal retention between 48 h
and 216 h of depuration. The AE was determined to be the y-intercept, and the $k_{ef}$ was the slope of the curve. The Cr AE after sediment intubation was calculated as the percentage remaining after 48 h of depuration, due to nearly complete elimination of the radioisotope. An average IR value was obtained from the literature (0.07 g g$^{-1}$ d$^{-1}$) (Prinslow et al. 1974).

The biological half-life ($t_{b_{1/2}}$, defined as the time it takes for 50% of a metal to be excreted from the body) of a metal can be calculated to estimate a metal’s residence time in an organism as follows:

$$t_{b_{1/2}} = \ln 2 / k_{ef}$$ (2)

RESULTS

Assimilation and retention of metals after sediment intubation

After intubation with radiolabeled sediment, the AE values were highest for MeHg (10 to 14%), followed by Hg(II) (1.9 to 4.1%), As (0.8 to 1.7%), and Cd (0.04 to 0.3%), and lowest for Cr (0.01 to 0.03%) (Table 3). The AE values for each field location did not differ significantly from each other for Cr, Hg(II), and MeHg (p > 0.05), but the values did differ for As (p < 0.05; ER vs. MI) and Cd (p < 0.01; BH vs. ER).

Metal elimination from killifish followed a biphasic loss pattern: during the first 24 h of depuration, the rapid loss corresponded to gut clearance of unassimilated metal, while the slower loss for the remaining 8 d corresponded to the physiological turnover of assimilated metal (Fig. 1). Nearly all of the metal bound to sediment from the 3 field locations was eliminated during the first 24 h of depuration. At the end of the 9 d depuration, the percentage of original metal retained was 0.5 to 0.9% As, 0.03 to 2% Cd, 0.05 to 0.06% Cr, 1.1 to 1.9% Hg(II), and 9.1 to 14% MeHg. Loss rate constants ($k_{ef}$) for Hg(II) (0.074 to 0.113 d$^{-1}$), As (0.057 to 0.097 d$^{-1}$), and Cd (0.074 to 0.089 d$^{-1}$) were similar to one another and significantly higher than for MeHg (0.004 to 0.020 d$^{-1}$) (Table 3). The $k_{ef}$ values for Cr could not be calculated due to nearly complete elimination of the radioisotope. The $k_{ef}$ values for each field location did not differ significantly from one another for As, Cd, and Hg(II) (p > 0.05) but did differ for MeHg (p < 0.01; ER vs. MI).

Assimilation and retention of metals after algal intubation

Following intubation with radiolabeled algae, the AE values were highest for MeHg (82%), followed by Hg(II) (18%), As (15%), and Cd (10%), and lowest for Cr (0.7%). For all metals, AEs were higher after intubation with algae than with sediment. If the sediment AE values for the 3 field locations are averaged, the AEs are 12-, 56-, 35-, 5.6-, and 6.8-fold higher for As, Cd, Cr, Hg(II), and MeHg, respectively, after intubation with algae (Table 3).

Metals were eliminated from killifish following the same biphasic loss pattern noted after intubation with sediment (Fig. 2). Within the first 24 h, 80% of As, 89% of Cd, 90% of Cr, 74% of Hg(II), and 18% of MeHg had been eliminated. At the end of the 9 d depuration, the percentage of original metal retained was 0.1 to 0.9% As, 0.03 to 2% Cd, 0.07 to 0.1% Cr, 1.1 to 1.9% Hg(II), and 9.1 to 14% MeHg. Loss rate constants ($k_{ef}$) for Hg(II) (0.074 to 0.113 d$^{-1}$), As (0.057 to 0.097 d$^{-1}$), and Cd (0.074 to 0.089 d$^{-1}$) were similar to one another and significantly higher than for MeHg (0.004 to 0.020 d$^{-1}$) (Table 3). The $k_{ef}$ values for Cr could not be calculated due to nearly complete elimination of the radioisotope. The $k_{ef}$ values for each field location did not differ significantly from one another for As, Cd, and Hg(II) (p > 0.05) but did differ for MeHg (p < 0.01; ER vs. MI).

### Table 3. Fundulus heteroclitus. Assimilation efficiencies (AE) and loss rate constants ($k_{ef}$) calculated for killifish after intubation with radiolabeled sediment from 3 sites and algae. n = 5 for sediment and 9–10 for algae. BH: Baltimore Harbor, ER: Elizabeth River, MI: Mare Island, nd: not determined

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
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<td>As</td>
<td>BH</td>
<td>1.3</td>
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<td>0.018</td>
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<td>ER</td>
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<td>0.010</td>
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<td></td>
<td>MI</td>
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<td>1.0–2.3</td>
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<td>0.004</td>
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<td>Cd</td>
<td>BH</td>
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<tr>
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<td>BH</td>
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<td>Hg(II)</td>
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<tr>
<td>MeHg</td>
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<td>73–90</td>
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</table>
depuration period, the percentage of original metal retained was 2.3% As, 7.3% Cd, 0.3% Cr, 8.8% Hg(II), and 76% MeHg. The $k_{ef}$ values were highest for As (0.223 d$^{-1}$), followed by Cr (0.119 d$^{-1}$), Hg(II) (0.085 d$^{-1}$), and Cd (0.041 d$^{-1}$), and lowest for MeHg (0.009 d$^{-1}$) (Table 3). These $k_{ef}$ values were lower than that calculated for Cd and higher than that calculated for As following sediment intubation, whereas the values were comparable for Hg(II) and MeHg.

**Tissue distribution and corresponding metal concentrations**

Table 4 shows the tissue distribution of As, Cd, Hg(II), and MeHg as the percentage of total body burden and radioactivity concentration (Bq g$^{-1}$ dry weight) associated with each tissue compartment at the end of depuration after intubation with radiolabeled sediment and algae. The tissue distribution for Cd at BH and all Cr experiments could not be determined due to low detection in the fish. After intubation with sediment, As and MeHg were predominantly associated with the body (70–73% and 42–43%, respectively), whereas Cd and Hg(II) were associated with the viscera (81–97% and 68–91%, respectively). The percentage of the body burden associated with each tissue compartment did not vary among the field locations, except for Hg(II) in the head (4–22%) and viscera (68–91%). Radioactivity concentrations were highest in the viscera for Cd, Hg(II), and MeHg and nearly evenly concentrated between the viscera and body for As.

Following algal intubation, As and MeHg were predominantly associated with the body (48% and 49%, respectively), while Cd and Hg(II) were associated with the viscera (81% and 77%, respectively). This is the same distribution pattern as observed after sediment intubation. Radioactivity concentrations were highest in the viscera for all metals. Radioactivity concentrations could not be compared among metals and between sediment and algae due to exposure to different metal concentrations.

**Modeling metal bioaccumulation in killifish**

The $t_{b_{0.5}}$ of metals in killifish was highest for MeHg (35–173 d), followed by similar $t_{b_{0.5}}$ values for As, Cd, and Hg(II) (7.1–12, 7.8–9.4, and 6.1–9.4 d, respectively) after intubation with radiolabeled sediment (Table 5). Assuming it takes 7 half-lives for all of the assimilated metal to be excreted, MeHg would be retained for 243 to 1213 d, As for 50 to 85 d, Cd for 55 to 66 d, and Hg(II) for 43 to 66 d. After intubation with radiolabeled algae, the $t_{b_{0.5}}$ was highest for MeHg (77 d), followed by Cd (17 d), Hg(II) (8.2 d), and Cr (5.8 d), and lowest for As (3.1 d) (Table 5). This corresponds to a retention time of 539 d for MeHg, 118 d for Cd, 57 d for Hg(II), 41 d for Cr, and 22 d for As.

The TTF values were <1 for As, Cd, Hg(II), and MeHg after sediment intubation (except ER MeHg; TTF = 2.0) regardless of field location, indicating that these metals would not be expected to biomagnify from sediments in killifish. TTF values could not be calculated for Cr due to nearly complete elimination of the radioisotope. After intubation with algae, MeHg was the only metal with a TTF > 1 (TTF = 6.4), indicating that MeHg would be expected to biomagnify, whereas As, Cd, Cr, and Hg(II) had TTF < 1, indicating that these metals would not be expected to biomagnify (Table 5).
DISCUSSION

Assimilation of metals after sediment intubation

To our knowledge, the present study is the first to investigate the bioavailability of several metals associated with contaminated sediment for fish. Because no significant differences were noted in the TTF of metals among the 3 sediments (except ER MeHg), it is apparent that sediment geochemistry differences among the 3 sediment sites (Baumann & Fisher 2011) did not influence the AE and $k_{ed}$ of each metal in killifish. The AE values calculated for killifish in the present study are much lower than those calculated for deposit-feeding polychaetes and marine bivalves that ingested radiolabeled sediment. AE values in polychaetes ranged from 43 to 83% for MeHg, 7 to 30% for Hg(II), 1.5 to 59% for Cd, 1.2 to 12% for As, and 0.7 to 4.6% for Cr (Wang et al. 1998, 1999, Baumann & Fisher 2011). AE values in bivalves ranged from 5 to 87% (generally >30%) for MeHg, 6 to 35% for Cd, <1 to 20% for Cr, and 1 to 9% for Hg(II) (Gagnon & Fisher 1997, Wang et al. 1997, Griscom et al. 2000, 2002a). No literature values could be found to compare As values in bivalves to those calculated for killifish in the present study. For all of the metals investigated in the present study, the sediment AE values are lower than the algal AE values; this observation was also noted in another study using the clam *Macoma baltica* (Griscom et al. 2002a). The difference in AE between sediment and algae could be due in part to what fraction the metal is bound to in the sediment. In algae, the metal is bound to the more labile organic matter, whereas in the sediment, little metal is bound to labile organic matter, and this metal is much less bioavailable. It should also be noted that because the fish were intubated and did not feed naturally, the AE values may be

<table>
<thead>
<tr>
<th>Table 4. <em>Fundulus heteroclitus</em>. Tissue distribution of metals in killifish at the end of 9 d depuration after intubation with radiolabeled sediment and algae. Values are the percentage of total body burden and radioactivity concentrations (Bq g⁻¹ dry weight) associated with each tissue compartment (head, viscera, or body). Tissue partitioning of Cr and BH Cd could not be determined due to nearly complete elimination of the radiisotope. Values are means ± 1 SE; n = 5 for sediment and 9–10 for algae. BH: Baltimore Harbor, ER: Elizabeth River, MI: Mare Island, nd: not determined</th>
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**Fig. 2. Fundulus heteroclitus.** Mean (±1 SE) loss of As, Cd, Cr, Hg(II), and MeHg from killifish over 9 d following intubation with radiolabeled algae. n = 9–10
underestimated because the sediment was forced into the intestine during the intubation procedure.

The lower AE values noted for killifish compared to deposit-feeding polychaetes and bivalves could be a result of the difference in gut physiology among these organisms. The pH of gut fluid in worms (pH 6.88 in *Nereis succinea*; Ahrens et al. 2001) and bivalves (pH 5.0 in the clam *Macoma balthica* and 5.6 in the mussel *Mytilus edulis*; Griscom et al. 2002b) is neutral or mildly acidic, whereas the pH of killifish gut fluid is mildly acidic to alkaline. A study by Babkin & Bowie (1928) determined that the intestinal fluid in fasting killifish has a pH between 8.0 and 9.2, and fish have a pH between 8.4 and 9.0 after feeding on clams. More recently, Wood et al. (2010) found a comparable fasting pH (7.7) but determined that the pH of the intestinal fluid was 5.7 in seawater killifish and 6.8 in freshwater killifish 1 to 3 h after feeding on fish pellets. The pH can influence the solubility of metal from the sediment fraction to which it is bound; metals bound to the acid-volatile sulfide (AVS) and iron-oxide fractions are extracted in the low pH of the clam and mussel gut fluid, and the proportion of metal extracted is greater in the clam, which has a more acidic gut fluid (Griscom et al. 2002b). Baumann & Fisher (2011) calculated that 13 to 42% of As, 33 to 50% of Cd, and 75 to 91% of Cr was bound to the AVS and iron- and manganese-oxide fractions 2 d after the sediment from the 3 field locations used in the present study were radiolabeled directly. The pH of the killifish intestinal fluid would not be expected to affect the bioavailability of metal bound to the AVS and iron-oxide fractions. Furthermore, the killifish does not have a stomach (Babkin & Bowie 1928); because the stomach secretes gastric acid, the absence of the stomach can provide some explanation for the higher pH of the intestinal fluid. Worms also have a high concentration of amino acids in their digestive fluid, which can solubilize metals from sediment, and surfactants that can solubilize polycyclic aromatic hydrocarbons (Mayer et al. 1996, Ahrens et al. 2001). Mayer et al. (1996) investigated the solubility of metals in the gut fluid of the lugworm *Arenicola marina* and the sea cucumber *Parastichopus californicus*; the lugworm solubilized more metal as a result of a much higher dissolved amino acid concentration. This has also been observed in fish: when sturgeon and catfish gut fluid were exposed to sediment labeled with MeHg, the sturgeon solubilized more MeHg in the gut fluid due to a higher concentration of amino acids (Leaner & Mason 2002a).

### Assimilation of metals after algal intubation

The wide range of AE values observed after killifish were intubated with radiolabeled algae (0.7% for Cr to 82% for MeHg) indicates there is large variability in AE among the metals. The general ranking of AE (MeHg > Hg(II) > As > Cd > Cr) is identical to the ranking observed after sediment intubation. To our knowledge, the present study is the first to calculate AE values after fish have consumed radiolabeled algae for As, Cd, Cr, and Hg(II). Leaner & Mason (2004) calculated an AE of 90% for the sheepshead minnow *Cyprinodon variegatus* fed MeHg radiolabeled pellets of the green alga *Tetraselmis*. Our AE value of 82% could be slightly lower because the sheepshead minnows were fed naturally, whereas the killifish used in the present study were intubated. The Cd, Hg(II), and MeHg AE values calculated from the present study fall within the range of literature values for freshwater and marine fish fed zooplankton and worm prey (2.7–39%, 8–51%, and 56–95%, respectively) (Reinfelder & Fisher 1994, Ni et al. 2000, Xu & Wang 2002, Wang & Wong 2003, Pickhardt et al. 2006, Mathews & Fisher 2008, Dutton & Fisher 2010, 2011). Our calculated As AE (15%) is higher than that calculated for killifish fed amphipod
prey (9.4%; Dutton & Fisher 2011), and our calculated Cr AE (0.7 %) is at the lower end of the range observed for fish fed amphipod and worm prey (0.2 to 19%; Ni et al. 2000, Dutton & Fisher 2011).

The large range in AE values from algae observed among the metals could be due to varying cellular distributions of the metals in Dunaliella tertiolecta cells. Numerous studies have found that the AE of ingested elements in herbivores is related to the cytoplasmic content of these elements in algae, first noted for diatoms by Reinfelder & Fisher (1991), although variations in cellular distributions of elements among different algal taxa have been noted (Ng et al. 2005), and the relationship to herbivore AE may vary. This relationship between cellular distribution of metals and AE in herbivores may explain our findings, where the MeHg AE is 4.6-fold higher than that for Hg(II). Pickhardt & Fisher (2007) found that 59 to 64% of MeHg is associated with the cytoplasm in freshwater phytoplankton, whereas only 9 to 16% of Hg(II) is associated with the cytoplasm and is therefore less assimilable when ingested. Furthermore, it has been found that >98% of Cr is bound to algal cell surfaces, and when fed to the mussel Mytilus edulis, the AE ranged between 0.2 and 1.3% (Wang & Fisher 1996), comparable to our low Cr AE value of 0.7%. This relationship has also been observed when fish were fed zooplankton; AE values were lower in fish when a large proportion of the metal was associated with the zooplankton exoskeleton. For example, 97% of Cd in copepods was bound to the exoskeleton, and fish that fed on these copepods assimilated 2.7%, due to the fish being unable to digest the exoskeleton (Reinfelder & Fisher 1994). This was also noted when killifish were fed radiolabeled amphipods and worms, with higher AE values for Cr and Hg(II) after the fish were fed soft-bodied worms (Dutton & Fisher 2011).

**Loss of metals after sediment and algal intubations**

The $k_{ef}$ values after sediment and algal intubations were similar for Hg(II) and MeHg, higher for As after algal intubation, and higher for Cd after sediment intubation. The physiological turnover rate of metals probably reflects the turnover rates of the tissues in which the metals reside. The tissue distributions of Hg(II) and MeHg were similar for sediment and algal diets (Table 4), and therefore, their $k_{ef}$ did not vary between the diets. In contrast, more As and less Cd was in the viscera following the algal diet than the sediment diet (Table 4), matching their $k_{ef}$ patterns. This suggests that the $k_{ef}$ of each metal was principally related to the loss of metal from the viscera.

The algal As $k_{ef}$ value calculated in the present study (0.223 d$^{-1}$) is similar to that calculated after killifish were fed radiolabeled amphipods (0.287 d$^{-1}$), whereas the sediment $k_{ef}$ values were ~3-fold lower (Dutton & Fisher 2011). The calculated algal $k_{ef}$ for Cd (0.041 d$^{-1}$) falls within the range calculated in other studies in which fish were fed worm and zooplankton prey (0.03–0.073 d$^{-1}$; Xu & Wang 2002, Mathews & Fisher 2008, Dutton & Fisher 2010, 2011). The Cr algal $k_{ef}$ value calculated in the present study (0.119 d$^{-1}$) is 1.9-fold higher than after killifish were fed radiolabeled worms (0.064 d$^{-1}$; Dutton & Fisher 2011). The sediment and algal $k_{ef}$ values calculated in the present study for Hg(II) and MeHg (0.074 to 0.113 and 0.004 to 0.020 d$^{-1}$, respectively) fall within the range of those calculated in other studies using freshwater and marine fish fed zooplankton and worm prey (0.003 to 0.194 d$^{-1}$ for Hg(II) and 0.007 to 0.018 d$^{-1}$ for MeHg; Pickhardt et al. 2006, Mathews & Fisher 2008, Dutton & Fisher 2010, 2011).

**Tissue distribution of metals**

The tissue distribution of the metals investigated in the present study fall within the range of values calculated in other studies when fish were fed radiolabeled prey. Of all of the metals examined, Cd shows the greatest variability in literature values. Our study concluded that 81 to 97% of Cd remains associated with the viscera, which is similar to values calculated in another killifish study (85% associated with the intestine; Dutton & Fisher 2011) and mangrove snapper Lutjanus argentimaculatus (81%; Xu & Wang 2002) but much higher than the percentage calculated for the Atlantic silverside Menidia menidia (13 to 16%; Dutton & Fisher 2010), striped bass Morone saxatilis (20%; Baines et al. 2002), and killifish (50%; Mathews & Fisher 2008). This range of values indicates that some fish are better protected against the gastrointestinal uptake of Cd than others. Cd shares the same gastrointestinal uptake pathway as Ca, and elevated levels of Ca reduce the uptake of Cd (Franklin et al. 2005).

The different tissue distributions of Hg(II) and MeHg indicate that MeHg can more readily pass across the intestinal wall, after which it is redistributed around the body via the blood and accumulates in the fillet, which is sulfur-rich. A study by Leaner & Mason (2002b) found that MeHg binds to cysteine and crosses the intestine via an amino acid uptake pathway in the channel catfish Ictalurus punctatus.
The higher percentage distribution and concentration of MeHg in the body poses a risk to killifish predators, including the blue crab and striped bass (Kneib 1986, Hartman & Brandt 1995), and therefore potentially human consumers. The tissue distribution of Hg(II) and MeHg presented in our study are comparable to other literature values. For Hg(II), Pickhardt et al. (2006) found that 92 to 96% of Hg(II) was associated with the viscera in mosquitofish Gambusia affinis and 67.5 to 73% in redear sunfish Lepomis microlophus, 28 to 72% was associated with the viscera in the Atlantic silverside (Dutton & Fisher 2010), and 81 to 84% was associated with the body in the redear sunfish and 68% in mosquitofish (Pickhardt et al. 2006), 51 to 57% in the Atlantic silverside (Dutton & Fisher 2010), and 51 to 58% in 2 other killifish studies (Mathews & Fisher 2008, Dutton & Fisher 2011), which are comparable but slightly higher than our values of 42 to 49%.

Like MeHg, As was also redistributed around the body, where 48% was associated with the body after algal intubation and 70 to 73% after sediment intubation. The reason for this difference is not apparent to us, although it is noteworthy that very little As was acquired from either diet. Literature values for the tissue distribution of As in fish are limited. One laboratory study found that 62% of As was associated with the body following a 9 d depuration after acquiring As from amphipods (Dutton & Fisher 2011). Another study analyzed field-collected herring, cod, and flounder and found that As accumulates in the fillet (Larsen & Francesconi 2003). Arsenate is known to behave as a phosphate analog in phytoplankton, sharing the same uptake pathway (Sanders & Windom 1980), and after arsenate is taken up, it is reduced to a variety of organoarsenic species, including arsenobetaine (Neff 1997). A recent study found that the phosphate transporter, NaPi-IIb1, is most likely responsible for arsenate accumulation in zebrafish tissues (Beene et al. 2011). Speciation of As in field-collected fish found that 89 to 100% of As in the muscle tissue was present as arsenobetaine, whereas arsenate accounted for 0%; however, 0 to 38% of the As in the intestine was present as arsenate (Kirby & Maher 2002). We did not speciate As in the fish tissue in the present study, so we cannot conclude if this is the case for killifish. The tissue distribution data from the present study and others indicate that once the metal has been solubilized from the ingested prey or substrate, it is remobilized around the body in the same way, regardless of the source.

**Biomagnification of metals in killifish**

The TTF values were <1 after sediment intubation for As, Cd, and Hg(II) at all 3 field locations and for MeHg at BH and MI, indicating that these metals are not expected to biomagnify from sediment to killifish. Killifish intubated with sediment from ER had a TTF > 1, indicating that MeHg will biomagnify in this field location. TTF values could not be calculated for Cr due to elimination of the radioisotope. After intubation with algae, MeHg was the only metal expected to biomagnify (TTF = 6.4) due to a high assimilation and low elimination rate. In comparison, As, Cd, Cr, and Hg(II) had a TTF < 1 due to low assimilation and high elimination rates. The IR of sediment and algae is most likely lower than the 0.07 g⁻¹ g⁻¹ d⁻¹ value used to calculate the TTF in the present study. Therefore, the TTF of metals bound to sediment and algae are likely to be even lower than the values calculated here. MeHg associated with ER sediment and algae will not biomagnify at this trophic step if the IR of ER sediment is <0.04 g⁻¹ g⁻¹ d⁻¹ and the IR of algae is <0.011 g⁻¹ g⁻¹ d⁻¹.

To our knowledge, the present study is the first to calculate TTF values after exposure to radiolabeled sediment and algae, so no comparisons can be made to other studies. Following exposure to radiolabeled algae, Hg(II) and MeHg TTF values are similar to those calculated for killifish that consumed radiolabeled amphipods and worms, while the TFF values are higher for As and Cd and lower for Cr (Dutton & Fisher 2011). The TTF values following exposure to radiolabeled sediment compared to amphipods and worms were lower for Cd at all 3 locations and for MeHg at BH and MI but were similar for As at all 3 locations and for MeHg at ER. The Hg(II) TTF values were similar to values calculated after killifish consumed amphipods but higher than that calculated after killifish consumed worms (Dutton & Fisher 2011).

**Sediment and algae as a source of metals to killifish**

Metals can accumulate to high concentrations in industrialized coastal regions. The present study concludes that metals bound to sediment are not a significant direct source of metals to fish due to their low bioavailability. This reduces the likelihood of health implications to the killifish themselves and their predators. Killifish do not actively consume sediment, and the risk of metal accumulation from incidental uptake while consuming benthic prey is minimal. Algae, however, can be an important food
source for killifish, especially in salt marshes (Kneib & Stiven 1978, Kneib 1986), and can be a significant source of metal, especially MeHg.

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