An evaluation of background levels and sources of polycyclic aromatic hydrocarbons in naturally spawned embryos of Pacific herring (Clupea pallasii) from Puget Sound, Washington, USA

James E. West a,⁎, Sandra M. O'Neill a, Gina M. Ylitalo b, John P. Incardona b, Daniel C. Doty a, Margaret E. Dutch c

a Washington Department of Fish and Wildlife, Fish Program/Marine Resources Division, 600 Capitol Way N, Olympia, WA 98501-1091, USA
b NOAA Fisheries, Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, WA 98112, USA
c Washington Department of Ecology, Marine Monitoring Unit, 300 Desmond Drive, PO Box 47600, Olympia, WA 98504-7600, USA

HIGHLIGHTS

• Our study documents exposure of herring embryos to polycyclic aromatic hydrocarbons.
• Embryos accumulated exogenous PAHs during their incubation in nearshore waters.
• Maternal transfer of PAHs accounted for some of the PAH body burden.
• Pyrogenic PAH patterns in embryos resembled sediments where they were spawned.
• Embryos from rural bays exhibited greatest PAH levels compared to other shore types.

ARTICLE INFO

Article history:
Received 14 July 2014
Received in revised form 12 August 2014
Accepted 13 August 2014
Available online 1 September 2014

Keywords:
Herring
Embryos
PAHs
Contaminants
Puget Sound
Maternal transfer

ABSTRACT

Pacific herring embryos spawned in nearshore habitats may be exposed to toxic contaminants as they develop, from exogenous sources in spawning habitats and from maternal transfer. Determining baseline concentrations of these toxic contaminants is important for evaluating the health of this species, especially during this sensitive life stage. In this study we compared concentrations of polycyclic aromatic hydrocarbons, or PAHs, in naturally spawned herring embryos from five spawning areas across Puget Sound. The summed values of 31 PAH analytes (Σ31PAH) in early- to late-stage development embryos ranged from 1.1 to 140 ng/g, wet weight. Σ31PAH concentrations increased with development time in embryos from one spawning area where the greatest concentrations were observed, and the relative abundance of PAH chemicals in late-stage embryos was similar to those in nearby sediments, suggesting accumulation from local environmental sources. PAHs in both sediments and late-stage embryos appeared to exhibit a pyrogenic pattern. Although maternal transfer of PAHs appeared to be a negligible source to embryos in spawning areas with the greatest embryo PAH concentrations, maternal transfer may have been the dominant source in embryos from spawning areas where the lowest levels of embryo-PAHs occurred. Chronic embryo mortality has been reported in spawning habitats where we observed the greatest concentration of PAHs in embryos, and necrotic tissue in herring embryos from one such location was similar in description to phototoxic PAH necrosis reported elsewhere for embryonic zebrafish.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Marine organisms that spawn in nearshore habitats may be exposed to a wide range of toxic contaminants entering the aquatic system from terrestrial sources. Of particular concern are compounds such as polycyclic aromatic hydrocarbons (PAHs) because of their potential to accumulate in intertidal and shallow subtidal habitats, where they can harm developing fish embryos (Rice et al., 2001). The major source of background PAHs in Puget Sound is woodstove and fireplace emissions, vehicle combustion emissions, and creosote-treated pilings (Ecology and King County, 2011). PAHs from acute pollution events such as oil spills can accumulate and persist in nearshore sediments as well, potentially causing a wide range of effects from immediate mortality to delayed, indirect effects in organisms that live or reproduce there (Peterson et al., 2003). There is a notable lack of field studies investigating PAHs in nearshore fishes, especially sensitive life stages such as...
embryos, from sources other than oil spills, including chronic inputs of pyrogenic PAHs from non-point pollution sources such as stormwater or other terrestrial inputs.

Pacific herring (Clupea pallasi) are one of several abundant small-bodied, schooling pelagic planktivorous fishes in Puget Sound that spawn in intertidal and shallow subtidal marine habitats. Herring deposit adhesive eggs in mass spawning events, typically on submerged aquatic vegetation such as eelgrass (Zostera spp.), kelp (e.g., Laminaria and Nereocystis) and fleshy red macroalgae. It is also not uncommon to see herring spawn on rocks, pilings or other abiotic or man-made structures. Timing of annual spawn periods and locations by herring is consistent and predictable for eighteen stocks that spawn in Puget Sound (Stick, 2005). Puget Sound herring typically begin spawning in January, and finish by late March. One spring spawning stock known as the Cherry Point stock spawns later, typically from April through early June in northern Puget Sound.

The abundance of Puget Sound herring populations, managed as 18 discrete stocks in Puget Sound (Stick and Lindquist, 2009) has varied widely over time, however the Cherry Point stock has exhibited a unique dramatic decline over the last 30 years (Gustafson et al., 2006; Stout et al., 2001). Changing environmental conditions, predation pressure, and other stressors such as pollution have been implicated, but the causes of the decline in the Cherry Point stock remain unknown (review by Gustafson et al., 2006; Landis et al., 2004). Two oil refineries and an aluminum smelter operate near spawning grounds for the Cherry Point herring stock, prompting concern that developing embryos may be exposed to contaminants, in particular PAHs that may further depress the stock or prevent recovery. Additionally, concern over chronic, unexplained mortality in herring embryos from three other spawning stocks (Quartermaster Harbor, Port Orchard/Madison, and Port Gamble) in Puget Sound has prompted investigations into the possibility of exposure of embryos to PAHs from creosote-pilings in the vicinity of herring spawning grounds.

Pacific herring are ecologically important in the inland marine waters of Washington and British Columbia (collectively known as the Salish Sea). They are important prey for virtually every large piscivorous fish in this ecosystem, including threatened or endangered species (Chinook salmon, Oncorhynchus tshawytscha and rockfishes, Sebastes spp.), as well as coho salmon (Oncorhynchus kisutch), lingcod (Ophiodon elongatus), and marine mammals such as harbor seals (Phoca vitulina) and harbor porpoise (Phocoena phocoena) and piscivorous seabirds such as western grebes (Aechmophorus occidentalis) and cormorants (Phalacrocorax spp.). Moreover, Pacific herring have been petitioned twice for protection under the U.S. Endangered Species Act because of concerns regarding their population health in the Salish Sea, resulting in detailed reviews of their status in Puget Sound (Gustafson et al., 2006; Stout et al., 2001).

Recent studies have highlighted the sensitive nature of developing fish embryos in marine nearshore environments, especially regarding exposure to PAHs (Incardona et al., 2009). These authors demonstrated how exposure to low levels of PAHs during incubation can kill herring embryos or impair their health. Similar toxicity and susceptibility have been reported for herring embryos exposed to creosote from pier pilings in San Francisco Bay (Vines et al., 2000). Related studies on Pacific herring (Barron et al., 2003) and zebrafish (Danio rerio; Hatlen et al., 2010) have documented enhanced toxicity of PAHs or other oil-components with exposure to ultraviolet light from sunlight.

Because herring can deposit eggs near sediments, their embryos may be exposed to PAHs contained therein. Sediments may act as a significant source of PAHs to overlying waters via diffusive flux (Sabin et al., 2010), and sediment-PAHs may be suspended by wave action and bioturbation. These nearshore habitats also experience PAH inputs from terrestrial sources, including stormwater runoff from the landscape and deposition from the atmosphere. Blue mussels (Mytilus spp.) from Puget Sound’s intertidal habitats have exhibited high PAH tissue residues compared to other sampling locations in the United States in a long-term study of pollution in nearshore marine habitats (Kimbro et al., 2008). Although PAH contamination of deeper subtidal sediments in Puget Sound has been broadly described (Long et al., 2003, 2005) an assessment of shallow nearshore sediments where herring and other species spawn is lacking.

The current study was designed as a first effort to estimate the magnitude and extent of PAH concentrations in spawned eggs of Pacific herring in Puget Sound, across a wide spectrum of shoreline types ranging from open, rural to industrial and residential embayments. We compared PAHs across location types by measuring tissue residues from ovaries and naturally spawned eggs. Our specific goals were to evaluate the magnitude and patterns of PAH concentration in herring embryos from five major Puget Sound spawning stocks, and determine the degree to which observed PAHs in embryos may be derived from maternal versus exogenous sources. Specifically, we (1) compared tissue residues of PAHs in embryos between five spawning areas in Puget Sound representing several shoreline types, and (2) compared accumulation of PAHs in spawned embryos with PAHs in eggs from the ovaries of pre-spawning herring. We focused further attention on one stock (Port Orchard/Madison) which has exhibited chronic embryo mortality at one of its spawning locations. At the Port Orchard/Madison spawning locations we (3) compared PAH residues in newly spawned embryos with embryos from the same location that had incubated in situ for an additional 7 days, and (4) compared the relative abundance of PAHs in embryos with PAHs in sediments over which eggs were spawned.

2. Methods

2.1. Sampling locations

We sampled spawned eggs from five herring stocks across a wide geographic area in Puget Sound and Hood Canal from 1999, and 2001–2003 (Fig. 1, Table 1). Specific spawned egg sampling sites within each stock spawning area were selected to cover as wide a range as possible of the spawning area used by herring during the sampled years. The closest distance between stock-specific spawning areas was 213 km (Cherry Point to Quartermaster Harbor) and the closest distance was 62 km (Port Orchard/Madison to Quartermaster Harbor — Fig. 1). Ovarian egg samples were also collected from four of these herring stocks. We sampled eggs from pre-spawning gravid females to compare with spawned eggs from nearby spawning habitats, for evaluation of whether PAHs may have been maternally transferred to embryos.

Shoreline type along spawning areas for each stock was qualitatively evaluated and classified with the aid of NOAA’s National Land Cover Database (Fry et al., 2011), with subtypes grouped into three broad categories: (1) rural, or largely undeveloped, (2) lightly developed, including residential, and (3) heavily developed industrial and urban. Spawning habitats were also distinguished as to whether they were situated in protected embayments (“bay”) or along open, unprotected shorelines (“open”). The Quilcene/Dabob stock represented the most rural (least developed) spawning area of all the locations we sampled, occurring primarily along an open shoreline (rural, open shoreline) in Hood Canal. Two of the stocks, Port Orchard/Madison and Quartermaster Harbor, spawn primarily along residential shorelines in the central basin of Puget Sound. The Port Orchard/Madison spawned egg samples were taken from a deeply incised, shallow embayment bounded by shoreline residences and a small marina (Hidden Cove — “residential embayment”) and along a rural, open shoreline (Point Bolin — see Fig. 1 inset). Quartermaster Harbor spawned egg samples were also taken from a deeply incised shallow embayment bounded primarily by shoreline residences, including a roadway that closely follows the shoreline. Two stocks spawn along shorelines where major industrial activities occur. The Cherry Point sample sites included spawning habitats along an open shoreline where three industrial shipping piers are located (“industrial, open shoreline”), and along an open shoreline absent the piers (“rural, open shoreline”). The Fidalgo Bay spawned-
Fig. 1. Sampling location for ovarian and spawned egg samples from five major spawning stocks in Puget Sound, Washington, USA. Inset shows the location of two separate spawning areas used by the Port Orchard/Madison spawning stock, Hidden Cove and Pt. Bolin, where additional sampling was conducted.

Table 1
Number of herring egg and embryo samples analyzed for PAHs, and spawning habitat shore-type for five major spawning stocks in Puget Sound, Washington, USA.

<table>
<thead>
<tr>
<th>Herring spawning stock</th>
<th>Puget Sound Basin</th>
<th>Spawning ground shore-type and number of sites where spawned eggs(a) were sampled</th>
<th>Greatest distance between samples (km)(b)</th>
<th>Ovarian(c) egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cherry Point (1999, 2001)</td>
<td>Northern</td>
<td>Industrial, open (6 sites)</td>
<td>8.0</td>
<td>3</td>
</tr>
<tr>
<td>Fidalgo Bay (2001)</td>
<td>Northern</td>
<td>Industrial(d), bay (3 sites)</td>
<td>1.7</td>
<td>2</td>
</tr>
<tr>
<td>Port Orchard/Madison(e) (2001–2003)</td>
<td>Central</td>
<td>Residential, bay (5 sites)</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Quartermaster Harbor(e) (2001)</td>
<td>Central</td>
<td>Residential, bay (3 sites)</td>
<td>0.7</td>
<td>2</td>
</tr>
<tr>
<td>Quilcene Bay (2001)</td>
<td>Hood Canal</td>
<td>Rural, open (3 sites)</td>
<td>5.3</td>
<td>0</td>
</tr>
</tbody>
</table>

\(a\) Composite samples consisted of a homogenized mixture of 2 to 400 g (mean 100 g) of spawned eggs removed from spawning substrates.

\(b\) For all sites used by a spawning stock.

\(c\) Each ovarian egg sample comprised a homogenous mixture of a single ovary from each of ten fish.

\(d\) A petroleum refinery.

\(e\) Chronic embryo mortality has been reported from these stocks from small areas within the full extent of historically-used spawning shorelines.
egg samples were taken from adjacent to a petroleum trans-shipment pier to 1.7 km distant across a shallow bay ("industrial embayment"). Spawned-egg samples from three stocks (Cherry Point, Quilcene/Dabob) were widely dispersed within the spawning areas used by these stocks during the years sampled, ranging from 5.3 to 11 km between sampling sites. Spawned eggs from the other two stocks were concentrated in small areas during the years sampled, less than 2 km between sampling sites within their spawning areas (Fidalgo Bay and Quartermaster Harbor).

2.2. Spawned Egg (embryo) samples

Divers using SCUBA removed egg-laden vegetation from the substrate by hand, and placed samples into plastic bags underwater. All sampling sites were located in shallow subtidal habitats, within approximately one meter of the mean lower low water line. Bags were sealed underwater before surfacing to avoid potential contamination from PAHs from the overlying water column or surface-microalgal layer. On the boat a corner of the bag was cut to allow seawater to drain from the egg-laden vegetation, to reduce the potential for contact of eggs with the plastic from the bags. Bagged samples were then re-sealed and placed on ice, transported to the laboratory and held in a refrigerator until the following day, when eggs were manually separated from vegetation.

In the laboratory egg-laden vegetation was placed on clean aluminum foil, and eggs were removed one-by-one or in small clusters with solvent-rinsed stainless steel spatulas, and placed into pre-cleaned jars. Each sample-jar of spawned eggs was created by combining egg samples from substrates taken across a wide area, typically from 50 to 150 m. The number of eggs in each sample varied widely depending on spawn density and degree of difficulty in removing eggs from the substrate, however the mean mass of eggs was 100 g, representing many thousands of individuals in each sample. Samples were then stored in a −20 °C freezer until chemical analysis.

The length of time in days that embryos had incubated at each location was estimated by comparing samples with an illustrated developmental series. We observed a wide range of developmental stages in a few samples, indicating that several spawning events had occurred. In such cases we classified the sample according to the dominant stage. The incubation period for Puget Sound herring is thought to be 14 to 15 days, depending on environmental conditions.

To simplify analyses and avoid placing too much emphasis on the accuracy of estimating developmental stage, we grouped embryos based on three basic developmental stages: 1) early; body of the embryo shorter than the circumference of the egg (less than one complete coil), 2) mid; body of the embryo coiled inside the egg at least once, and 3) late; multiple body coils, embryo moving vigorously inside egg, hatching imminent. Additionally we noted whether embryos were potentially alive or dead. Living embryos were characterized by a translucent body suspended inside a transparent chorion (Fig. 2a). Dead embryos were easily identified both in the field and lab because the embryo (and subsequently the entire egg) quickly becomes opaque after death (Fig. 2b). We used these characteristics to qualitatively describe the condition of spawn (living or dead) in a sampling area during a dive.

Additional effort was focused on Port Orchard/Madison spawning sites because of chronic mortality reported from Hidden Cove (Washington Department of Fish and Wildlife, unpublished studies), sites because of chronic mortality reported from Hidden Cove (Spawning sites, Hidden Cove (bay) and Pt. Bolin (open). Hidden Cove sites showing high ΣPAH values and high mortality in 2002 were also re-sampled in 2003 for morphological analysis. Embryos from five replicate sites in Hidden Cove were sampled and fixed in Stockard’s solution over an eight-day period of their development, and inspected for gross pathology using a Nikon SMZ800 stereomicroscope (maximum 63× magnification) in the lab.

2.3. Ovarian egg samples

Ovarian eggs were taken from herring collected from predictable pre-spawning aggregations near to where spawning occurred. Fish were collected at night using a midwater trawl. To reduce potential variability in ΣPAH concentration in maternal sources related to fish age, females were selected by size in the field to retain putative 3-year-olds. This age class is typically the most abundant for the stocks we sampled. Age of each fish was later estimated in the lab using scale-anuli counts, and the mean age of fish contributing eggs to ovarian egg composite samples was computed. Female herring were wrapped in foil, bagged in plastic and iced within 2 h of capture, and transported on-ice to the laboratory within 12 h of capture. Herring were dissected in the lab and ovarian eggs placed in pre-cleaned jars. Eggs from ten females were combined to create composite samples; two to three composite samples, termed “ovarian egg” for each of four stocks were analyzed for PAHs (Table 1).

2.4. Chemical analysis of ovarian egg and spawned egg (embryo) samples

Prior to chemical analysis, spawned-egg and ovarian-egg samples were individually homogenized using a tissue grinder. The homogenized egg samples (1.0–2.0 g) were then weighed and extracted by one of two different methods: (1) homogenization with sodium sulfate and dichloromethane using a tissue grinder (Sloan et al., 1993) or (2) extraction of tissue using dichloromethane in an accelerated solvent extraction procedure (Sloan et al., 2005) after the addition of surrogate standards (naphthalene-d8,acenaphthene-d10, and benzo[α]pyrene-d12 at 1.7 ng/μl for each compound). Each sample extract was cleaned up on a single stacked, gravity-flow silica gel/alumina column to remove any highly polar compounds present in the sample. Using high-performance size-exclusion liquid chromatography, the PAHs were separated from the bulk lipid and other biogenic material present in each sample, and the cleaned extract was analyzed for parent and alkylated PAHs using a low-resolution quadrupole GC/MS system equipped with a 60-meter DB-5 GC capillary column and an electron impact mass spectrometer in selected-ion monitoring mode. The instrument was calibrated using sets of up to ten multi-level calibration standards of known concentrations.

Thirty-one PAH compounds were selected for quantitation based on their designation by the US Environmental Protection Agency as priority pollutants (fourteen parent, or C0 compounds), and their utility in evaluating diagnostic patterns (one parent compound, dibenzo[b, fluorine, C1-throug6 H-fluorine, phenanthrene, C1- through C4-phenanthrenes/anthracenes, dibenzothiophene, C1- through C3-dibenzoanthiophenes, and retene. Naphthalene and C1- through C4-naphthalenes were co- detected in herring egg samples, but also occurred in solvent
blanks, presumably related to uncontrollable ambient sources during processing. Naphthalene concentrations in embryo and ovary samples ranged from 1.2 to 5.5 ng/g wet weight, and from 0.87 to 4.8 ng/g in solvent blanks. Concentrations of C1- through C4-naphthalenes in embryos and ovaries ranged from 0.14 to 6.8 wet weight, and from 0.32 to 3.0 ng/g in solvent blanks. Because of the uncertainty introduced by naphthalene compounds in blanks, all naphthalene and naphthalene homologues were excluded from analyses.

The sum of high molecular weight (HMW) PAHs included detected values of fluoreanthene, pyrene, C1-fluoranthene/pyrene, benz[a]anthracene, chrysene/triphenylene, C1- through C4-chrysenes/benz[a]anthracenes, benzo[b]fluoranthene, benzo[j]fluoranthenes/benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indenopyrene, dibenz[a,h]anthracene, and benzo[g,h,i]perylene. The sum of all 31 PAH compounds (Σ31PAH) was calculated as a simple sum of all detected analytes. Detection limits ranged from 0.17 to 0.53 ng/g, wet weight for these samples with a mean of 0.28 ng/g wet wt. Gravimetric analysis of total extractible lipids (%) and dry weight measurements of the herring egg samples were determined as described in Sloan et al. (2005). Total extractible lipid concentrations ranged narrowly in embryos across locations (mean of 1.3% to 2.0%; Table 3). Lipid-adjusted PAH results were comparable to wet weight results, hence we presented only wet weight results for simplicity.

A method blank and two National Institute of Standards and Technology (NIST) Standard Reference Material Organics in Mussel (SRM 1974a or SRM 1974b) were analyzed with each batch of 10–14 egg samples to evaluate performance based on quality assurance criteria (Sloan et al. 2005).

![Fig. 2. a–d. (a) healthy herring embryos approximately four days after fertilization, attached to a stem of algae, (b) necrotic or dead embryos, (c) an apparently healthy four-day-old embryo removed from its chorion, and (d) four-day-old dechorionated embryo with arrows indicating necrotic (opaque, or high-contrast) tissues.](image)

**Table 2**

Frequency of occurrence of low molecular weight (LMW) and high molecular weight (HMW) polycyclic aromatic hydrocarbon compounds in ovarian egg and spawned egg samples from Pacific herring.

<table>
<thead>
<tr>
<th>LMW compounds</th>
<th>Mean % of total</th>
<th>HMW compounds</th>
<th>Mean % of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthylene (ACY)</td>
<td>63.4%</td>
<td>Fluoranthene (FLA)</td>
<td>90.2%</td>
</tr>
<tr>
<td>Acenaphthene (ACE)</td>
<td>92.7%</td>
<td>Pyrene (PYR)</td>
<td>80.5%</td>
</tr>
<tr>
<td>Fluorene (FLU)</td>
<td>87.6%</td>
<td>C1-FLA/PYR a</td>
<td>58.5%</td>
</tr>
<tr>
<td>C1-FLU</td>
<td>82.9%</td>
<td>Benz[a]anthracene (BAA)</td>
<td>41.5%</td>
</tr>
<tr>
<td>C2-FLU</td>
<td>51.2%</td>
<td>Chrysene (CHR) b</td>
<td>46.3%</td>
</tr>
<tr>
<td>C3-FLU</td>
<td>34.1%</td>
<td>C1-CHR/BAA c</td>
<td>36.6%</td>
</tr>
<tr>
<td>C2-CHR/BAA</td>
<td>17.1%</td>
<td>C3-CHR/BAA</td>
<td>9.8%</td>
</tr>
<tr>
<td>C1-DBT</td>
<td>41.5%</td>
<td>C4-CHR/BAA</td>
<td>0%</td>
</tr>
<tr>
<td>C2-DBT</td>
<td>58.5%</td>
<td>Benzo[b]fluoranthene (BBF)</td>
<td>53.7%</td>
</tr>
<tr>
<td>C3-DBT</td>
<td>53.7%</td>
<td>Benzo[k]fluoranthene (BKF) d</td>
<td>48.8%</td>
</tr>
<tr>
<td>Phenanthrene (PHN)</td>
<td>100%</td>
<td>Benzo[a]pyrene (BAP)</td>
<td>46.3%</td>
</tr>
<tr>
<td>C1-PHN/ANT e</td>
<td>97.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2-PHN/ANT</td>
<td>78.0%</td>
<td>Indeno-pyrene (IDP)</td>
<td>43.9%</td>
</tr>
<tr>
<td>C3-PHN/ANT</td>
<td>65.9%</td>
<td>Dibenz[a,h]anthracene (DBA)</td>
<td>19.5%</td>
</tr>
<tr>
<td>C4-PHN/ANT</td>
<td>31.7%</td>
<td>Benzo[g,h,i]perylene (BGHIP)</td>
<td>53.7%</td>
</tr>
</tbody>
</table>

a. Alkyl-fluoranthenes and alkyl-pyrenes were inseparable and so are reported together.

b. Coeluted with triphenylene.

c. Alkyl-chrysenes and alkyl-benz[a]anthracenes were inseparable and so are reported together.

d. Coeluted with benzo[j]fluoranthene.

e. Alkyl-phenanthrenes and alkyl-anthracenes were inseparable and so are reported together.

f. 1-methyl-7-isopropyl phenanthrene.

118

J.E. West et al. / Science of the Total Environment 499 (2014) 114–124
Concentrations of individual PAH analytes measured in SRM 1974a and 1974b were in excellent agreement with the reference values published by NIST. Other quality control samples met established laboratory criteria.

2.5. Sediment sampling

Sediments were sampled by Washington State Department of Ecology (WADOE) from two locations in Hidden Cove within approximately 30 m of the sites where embryos were taken for the early- to late-stage PAH comparison (see Fig. 1 inset). The top 2 cm of sediments were collected from a van-Veen grab and analyzed for PAHs as described in WADOE’s standard operating procedure EAP039 for sampling marine sediments.\(^3\) PAHs in sediments were analyzed using capillary column by gas chromatography/mass spectrometry following guidance from the US Environmental Protection Agency (USEPA) described in their method 8270, SW-846.\(^4\) Alkylated PAH homologues were added to the EPA list to match the compounds measured in herring embryos. Sediment data generated for this study are unpublished (personal communication; Margaret Dutch, Washington Department of Ecology).

3. Results

At least one PAH compound was detected in all ovarian and embryo samples; the concentration of \(\Sigma_{31}\text{PAH}\) varied in ovarian eggs from 6.1 ng/g wet wt. (Cherry Point) to 13 ng/g wet wt. (Quartermaster Harbor) and in embryos from 1.1 ng/g wet wt. (early/mid stage, Quilcene Bay) to 140 ng/g wet wt. (late stage, Port Orchard/Madison — Table 3). Although PAHs were detected in every sample, the \(\Sigma_{31}\text{PAH}\) concentration in some samples resulted from summing only a few individual PAH analytes near the limit of quantitation.

3.1. Maternal transfer of PAHs: comparison of ovarian eggs and spawned eggs

Maternal transfer of PAHs to eggs, estimated as the ratio of the mean \(\Sigma_{31}\text{PAH}\) in early/mid stage embryos to mean \(\Sigma_{31}\text{PAH}\) in ovarian eggs for a given stock, appeared to represent the dominant source of PAHs for Cherry Point, the location exhibiting the lowest overall \(\Sigma_{31}\text{PAH}\) (Table 3). The \(\Sigma_{31}\text{PAH}\) concentration in early- to mid-stage embryos was roughly equivalent between ovarian and embryos for that stock, at approximately 8 ng/g wet weight (ratio of 0.95). Early- to mid-stage embryos from the other three stocks had \(\Sigma_{31}\text{PAH}\) concentrations that were 1.4 to 2.0 times higher in embryos than ovarian eggs, and the late-stage embryos from Port Orchard/Madison exhibited 6.7 times greater \(\Sigma_{31}\text{PAH}\) concentrations than ovarian eggs.

---

Table 3

Comparison of total PAH concentration (ng/g wet weight) in spawned and ovarian eggs from five herring stocks in Puget Sound, Washington, USA.

<table>
<thead>
<tr>
<th>Herring spawning stock</th>
<th>Adult age/embryo developmental stage(^a)</th>
<th>Total extr. lipids (%)(^b)</th>
<th>n</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>sd</th>
<th>Ratio of mean (\Sigma_{31}\text{PAH}), spawned:ovarian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cherry Point</td>
<td>Ovarian 3.2 years</td>
<td>3.3</td>
<td>3</td>
<td>6.1</td>
<td>9.9</td>
<td>8.3</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spawned Early/mid</td>
<td>1.4</td>
<td>10</td>
<td>3.4</td>
<td>20</td>
<td>7.9</td>
<td>4.9</td>
<td>0.95</td>
</tr>
<tr>
<td>Fidalgo Bay</td>
<td>Ovarian 3.0 years</td>
<td>3.0</td>
<td>2</td>
<td>7.5</td>
<td>11</td>
<td>9.3</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spawned Early/mid</td>
<td>1.3</td>
<td>3</td>
<td>14</td>
<td>18</td>
<td>16</td>
<td>2.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Port Orchard/Madison</td>
<td>Ovarian 2.8 years</td>
<td>2.5</td>
<td>2</td>
<td>9.0</td>
<td>9.5</td>
<td>9.5</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spawned Early/mid</td>
<td>1.4</td>
<td>6</td>
<td>9.0</td>
<td>17</td>
<td>13</td>
<td>3.0</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Spawned Late</td>
<td>1.6</td>
<td>4</td>
<td>5.2</td>
<td>140</td>
<td>64</td>
<td>48</td>
<td>6.7</td>
</tr>
<tr>
<td>Quartermaster Harbor</td>
<td>Ovarian 2.9 years</td>
<td>3.0</td>
<td>2</td>
<td>9.2</td>
<td>13</td>
<td>11</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spawned Early/mid</td>
<td>2.0</td>
<td>3</td>
<td>15</td>
<td>26</td>
<td>22</td>
<td>6.3</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Spawned Mid</td>
<td>1.0</td>
<td>3</td>
<td>1.1</td>
<td>6.0</td>
<td>3.7</td>
<td>2.5</td>
<td>na</td>
</tr>
</tbody>
</table>

\(^a\) For ovarian eggs, mean age in years of females from which eggs were sampled. For spawned eggs, the number of estimated days post fertilization (dpf) according to the dominant stage in each sample grouped into three classes: early (1–4 dpf), mid (5–8 dpf) or late (9–12 dpf).

\(^b\) Total extractible lipids as % of wet weight, measured gravimetrically after Sloan et al. (2005).

---

Fig. 3. a–b. Comparison of mean \(\Sigma_{31}\text{PAHs}\) (± 95% confidence intervals) in early/mid stage embryos across (a) herring stock and across (b) shore type. Lowercase a,b, and c indicate statistically significant differences between groups. Numbers in parentheses indicate sample size. “nt” indicates not tested with ANOVA because of low sample size (n = 1).
eggs, suggesting accumulation of exogenous PAHs subsequent to spawning.

3.2. PAH accumulation in embryos and observed mortality

Overall, \( \Sigma_{31} \) PAH for the early/mid stage spawned eggs was greatest from Quartermaster Harbor (22 ng/g), Fidalgo Bay (16 ng/g), and Port Orchard/Madison (13 ng/g), intermediate from Cherry Point (7.9 ng/g) and lowest from Quilcene (3.7 ng/g) wet wt, (ANOVA of natural log-transformed \( \Sigma_{31} \) PAH by stock, \( F_{(4,20)} = 8.9, p < 0.001 \), with Tukey’s Honestly Significant-Difference pairwise analysis; Fig. 3a). Although low sample sizes precluded a statistical comparison of \( \Sigma_{31} \) PAH across all shore-types, embryos from residential bay and industrial bay shore-types exhibited a significantly greater concentration than the rural open shore-type, and the industrial open shore type was intermediate between these groups (Fig. 3b).

Exogenous, local PAH sources appeared to dominate \( \Sigma_{31} \) PAH in embryos from Hidden Cove, the residential bay site used by the Port Orchard/Madison stock, and the only stock for which we sampled both early and late embryos. \( \Sigma_{31} \) PAH increased roughly 1.4-fold from ovarian to early-mid embryos (comparing the offshore white bar with light gray residential bay for that stock, Fig. 4), and a further 6-fold increase with an additional week of incubation time (comparing the light gray and dark gray bars for Port Orchard/Madison’s residential bay only, Fig. 4). Early-mid stage embryos from Port Orchard/Madison stock spawning along the residential open shoreline (Pt. Bollin in Fig. 1, and “residential, open” in Fig. 4) exhibited a slight increase in \( \Sigma_{31} \) PAH compared to ovarian eggs, while a decrease (and lower concentration overall) was observed with an additional week of incubation time (comparing light gray and dark gray bars for residential, open for Port Orchard/Madison in Fig. 4).

All three of the late-stage embryo samples from Hidden Cove exhibited substantial mortality from >50% to near total mortality. Embryos from these three samples exhibited a range of conditions from normal, translucent (Fig. 2a) to fully opaque (Fig. 2b) and engulfed by fungus (not shown). Some embryos exhibited patently necrotic tissue, apparent from small to large patches of tissue with higher contrast visible by stereomicroscope. Opaque eggs or embryos such as these were rare in all other samples from all locations.

One of five subsites within the Hidden Cove spawning area showed high mortality in February 2003. Embryos at this site were first identified at the late blastula stage, and their development proceeded normally through gastrulation and early segmentation over a five day observation period. High mortality (61 \( \pm \) 13%) was first noted at the 30-somite stage (mid-segmentation; \( n = 236 \) embryos in 7 samples). Re-sampling the same field location three days later showed embryos with 40+ somites, some delayed at 30 somites, with 69 \( \pm \) 7% mortality (\( n = 199 \) embryos in 6 samples). Segmentation stage embryos that were in the process of dying generally appeared normal except for variable degrees of necrosis (tissue opacity) that seemed to originate from the region where the tailbud extended from the yolk sac, often involving Kupffer’s vesicle (see arrow in Fig. 2d).

3.3. Comparison of PAH patterns in sediments, ovarian eggs, and embryos

Thirty of 31 PAH compounds analyzed in 41 ovarian or spawned-egg samples were detected at least once, with frequencies ranging from 10 to 100% (Table 2). Forty-four percent of all individual PAH measurements were reported at less than the limit of quantitation, and so were censored as not-detected. Acenaphthylene, acenaphthene, fluorene, C1- and C2-fluorene, dibenzothiophene, C2-, and C3-dibenzothiophene, phenanthrene, and C1-, C2-, and C3-phenanthrenes/anthracenes were the most commonly observed 2- and 3-ring HMW compounds, detected in more than 50% of samples. Of the 4- and 5-ring HMW compounds, fluoranthene, pyrene, C1-fluoranthenes/pyrenes, benz[ghi]fluoranthene and benz[g,h,i]perylene were detected in more than 50% of samples. The rarest PAHs were C2- and C3-chrysenes and dibenz[a,h]anthracene, each detected in less than 20% of samples. C4-chrysenene was never detected in any sample.

A comparison of all embryo samples from all locations showed a positive correlation between the HMW:\( \Sigma_{31} \) PAH ratio and \( \Sigma_{31} \) PAH concentration (Fig. 5). Whereas the PAH mix was dominated by low molecular weight compounds in ovary (open circle) and some early/mid-stage embryo (gray-filled circle) samples exhibiting low \( \Sigma_{31} \) PAH,
this ratio was reversed in embryos exhibiting higher concentrations of \( \Sigma_{31} PAH \), including those from bay shore types, and those with greater incubation time (dark circles). A closer inspection of the relative abundance of individual PAH compounds in embryos from two Hidden Cove locations shows that LMW compounds remained constant from ovaries to 10-day-old embryos, whereas HMW compounds increased as embryos incubated (Fig. 6a,b).

Sediments may have been a source of PAHs to developing embryos. In a comparison of the relative abundance of PAH compounds in ovaries to early- to late-stage embryos from two Hidden Cove locations, the pattern of PAH compounds increasingly resembled the pattern of PAHs in sediments as the embryos developed (Fig. 6a,b). Ovarian samples were characterized by low concentrations of primarily LMW, 3-ring compounds such as acenaphthene, acenaphthylene, fluorenes and phenanthrenes. These compounds were also present in early-stage spawned eggs, however the LMW compounds remained relatively constant in later stages. Late-stage embryos exhibited a number of HMW compounds that increased in concentration two- to four-fold, including fluoranthene, pyrene, chrysenes, benzo[a]fluoranthene and benzo[k] fluoranthene. As the relative abundance of these HMW compounds increased in late-stage embryos, their PAH patterns came to roughly resemble the PAH pattern in sediments. Concentrations of parent compounds for three of the most abundant compounds in both samples of late-stage embryos, phenanthrene, fluoranthene, and chrysene, were consistently greater than their alkylated compounds.

4. Discussion

This study assessed the extent and magnitude of PAH accumulation in naturally spawned herring embryos from a wide range of shore types, demonstrating that herring populations spawning in residential and industrial bays are exposed to and accumulate PAHs at levels that could affect embryo survival. Embryos deposited along open shorelines, whether industrial or not, accumulated relatively low levels of PAHs. Maternal transfer of PAHs did not appear to account for elevated PAHs in embryos, although it appeared to be the dominant source for one stock, Cherry Point, wherein PAH concentrations were low overall. In
evaluating potential causes for the decline in abundance of herring from the Cherry Point stock (which spawn along shore types we described as industrial, open and rural, open), it is notable from this study that PAHs were low in Cherry Point embryos relative to stocks spawning along residential bay shore types in the central Puget Sound Basin (Fig. 4). It is possible that open shorelines such as Cherry Point may dilute or disperse local PAH sources, whereas embayments with restricted circulation (such as Quartermaster Harbor and Port Orchard/Madison’s Hidden Cove) may entrain pollutants such as PAHs.

Other PAH-embryo field studies to date have focused on oil spill exposures in embryos of salmon (Carls et al., 1996) and herring (Incardona et al., 2012a, 2012b), and the physiological effects of exposure to low aqueous PAH concentrations (Carls et al., 2002; Carls and Meador, 2009; Incardona et al., 2004; Incardona et al., 2009). Incardona et al. (2012a) illustrated the difficulty in interpreting post-oil-spill tissue PAH concentrations and effects on developing herring embryos in an urban setting (San Francisco Bay), without knowledge of pre-spill background PAH conditions in embryos.

Herring do not typically spawn along highly urbanized shorelines in Puget Sound where the greatest PAH inputs occur. Among the various shore-types we sampled, embryos from residential and industrial embayments exhibited the greatest PAH tissue residues, with samples similar to or greatly exceeding summed PAH (Σ31PAH) levels in embryos taken from oiled shorelines in San Francisco Bay after the Cosco Busan spill. Mean Σ3PAH concentration in the oiled Cosco Busan embryos ranged from 19 to 81 ng/g wet weight, compared with 5.2 to 140, 15 to 26, and 14 to 18 ng/g wet weight, from Port Orchard/Madison (residential, bay and residential, open shore types), Quartermaster Harbor (residential, bay) and Fidalgo Bay (industrial, bay), respectively.

A number of our samples, including all the late-stage embryos from Port Orchard/Madison’s Hidden Cove fell within a health effects threshold range of 22 to 108 ng/g wet weight, for herring embryos exposed to weathered crude oil (Carls et al., 1999). That study reported sublethal effects such as yolk sac edema and premature hatching at the low end of this threshold, and malformations, genetic damage, decreased body size, inhibited swimming for hatched larvae, and mortality, at the upper end. The highest mortality observed in our study was in embryos with the greatest Σ3PAH concentrations, at concentrations in the upper end or exceeding the Carls et al. (1999) threshold, suggesting a link between PAHs and embryo health in Port Orchard/Madison’s Hidden Cove.

The increase of PAH concentration in the early- to late-stage embryos from Port Orchard/Madison’s Hidden Cove, and the similarity in PAH pattern with sediment PAHs suggest accumulation of exogenous PAHs after eggs were laid. Eggs from that location were deposited on Gracilariopsis sp., a fleshy red alga, which grows in large mats close to the seafloor, typically less than 30 cm tall. Sabin et al. (2010) noted the importance of sediment-PAHs as a source of PAHs in overlying water, which suggests sediment-derived PAHs may be available to organisms such as herring embryos spawned close to the substrate.

The presence of PAHs in herring ovaries complements previous monitoring work documenting PAHs in adult Puget Sound herring. O’Neill and West (2007) reported metabolites of PAHs in bile from adult, pre-spawning herring from Port Orchard/Madison, Quartermaster Harbor, and Semiahmoo (near Cherry Point), with concentrations similar to urban or near-urban benthic fish. Although PAH concentrations in embryos from maternal transfer appeared small compared to exogenous uptake from the current study, these studies illustrate a pathway from adult-to-egg for PAH chemicals that are otherwise considered metabolized by adults.

The pattern of PAHs in late stage embryos and sediments from Hidden Cove were consistent with a combustion rather than petroleum source of PAHs. Late-stage embryos were characterized by maximum concentration in parent (C0) compounds, with declining concentration in their alkylated homologues, a pattern consistent with a combustion source (Sporstol et al., 1983). This pattern was especially apparent for phenanthrene, fluoroanthene, and chrysene from these samples. Embryos from Port Orchard/Madison’s Hidden Cove spawning area also shifted from a pattern dominated by 2- and 3-ring compounds in ovary and early-stage embryos, to one dominated by 4- and 5-ring compounds in late-stage embryos, the latter which resembled the sediment pattern where eggs were spawned. Although the specific shape of the regression curve in Fig. 5 is leveraged by two or three highly influential points at high Σ3PAH concentrations, this overall trend of increasing dominance by high-molecular-weight compounds is unmistakable. Accumulation in older embryos of 4- (fluoroanthene, pyrene, chrysene) and 5-ring (benzo[b]fluoroanthene, benzo[k]fluoroanthene, and benzo[a]pyrene) compounds may increase their susceptibility to cytolytic damage related to the phototoxic nature of these compounds (Incardona et al., 2012b) and to cardiac damage from 5 ring compounds (Huang et al., 2012; Incardona et al., 2011).

The pyrogenic nature of PAHs in herring embryos is consistent with recent findings (Washington Department of Ecology, 2011) implicating woodstove, fireplace, and vehicle combustion emissions as primary sources of PAHs in Puget Sound waters. That study also implicated creosote-treated pilings as a PAH source, of which there are many in Puget Sound residential embayments, including Quartermaster Harbor and Port Orchard/Madison’s Hidden Cove. The PAH pattern in embryos from our study seemed inconsistent with a pure creosote source; Vines et al. (2000) reported 93% of diffusible creosote-derived compounds, to which herring embryos may be exposed in seawater, consisted of 3-ring compounds including flourene, which were rarely detected in our late-stage embryos. However it is possible that weathering of the PAH pattern in creosote from pilings may have hampered our ability to identify its pattern. Creosote-treated pilings have been used in these bays for over 100 years and it is likely that the relative abundance of PAHs from that source has been altered over time.

Effects of petrogenic PAH exposure have been characterized in detail for Pacific herring embryos; exposure to PAH mixtures dominated by bicyclic and tricyclic compounds (and in the absence of significant UV co-exposure) has been shown to produce morphological defects including spinal curvature and reduced jaw size, and cardiotoxicity characterized by edema (Incardona et al., 2004). These morphological abnormalities were not observed in herring embryos from Port Orchard/Madison’s Hidden Cove; however the tissue necrosis we reported in Hidden Cove herring embryos may have been related to co-exposure of PAHs with sunlight. Toxicity to herring embryos exposed to crude oil in Alaska increased 1.5 to 48 fold with a 5-hour exposure to sunlight (Baron et al., 2003), and photo-enhanced toxicity in zebrafish (Hatlen et al., 2010) and Pacific herring (Incardona et al., 2012b) following exposure to bunker fuel oil has been shown to produce a syndrome characterized by cytolyis or necrosis and deterioration of entire embryos. The necrosis we observed in dying embryos from Hidden Cove sampled in 2003 is consistent with these descriptions of photo-enhanced toxicity.

A particularly puzzling observation from our SCUBA sampling was that although herring embryo mortality was sometimes high and spatially widespread, we often found small patches of embryos which were not obviously necrotic, seemed healthy, and later hatched (as evidenced by empty chorions), adjacent to expanses of dead embryos. None of the spawning events observed in this study produced spawn densities that seemed thick enough to have caused mortality of interior eggs (e.g., Haegel and Schweigert, 1985). Egg thicknesses in our samples rarely exceeded two layers. Photo-enhanced toxicity could contribute to high variability in natural settings where embryos are spawned in shallow waters (exposed to UV light), but within a complex physical structure (e.g., algae) that may shade or expose embryos, depending on small-scale variability in algae movement and shape.

Although the scope of this study was insufficient to test the relationship between herring embryo mortality and their PAH body burden, there appears to be some relationship between the two. We confirmed
that embryos collected from one of the spawning areas that had exhibited chronic, extensive embryo mortality over the past 30 years (Hidden Cove, used by the Port Orchard/Madison spawning stock), also exhibited the highest levels of PAHs measured in our study. These concentrations were also within the range of the PAH effects threshold proposed by Carls et al. (1999). We did not observe embryo mortality from our sampled sites of the other chronically mortality-affected stock (Quartermaster Harbor), however Quartermaster Harbor embryos exhibited the second greatest PAH concentrations of the six stocks we studied. (We did not sample Port Gamble spawning areas). In addition, we focused solely on one class of chemicals, PAHs, whereas herring embryos are exposed to a wide range of other contaminants, including polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), polybrominated diphenyl ethers (PBDEs), chordanes, and others (Washington Department of Fish and Wildlife, Puget Sound Ecosystem Monitoring Program, unpublished data). These and other toxic contaminants have been detected in adult herring (West et al., 2008) and sediments and blue mussels from herring spawning habitats (Kimbrough et al., 2008, 2009).

Intertidal and shallow subtidal habitats are used for spawning by a wide range of fishes and invertebrates including ecologically important small, schooling pelagic planktivorous species. We have illustrated here conditions wherein spawnings of such a species, Pacific herring, appear to accumulate exogenous PAHs from local sources, at levels that may kill embryos or impair embryo health. Local sources in the protected bays where PAHs in embryos were the highest could include any combination of (1) nearshore creosote pilings, (2) historical local wood burning, (3) current combustion of fossil fuels, and (4) petroleum inputs from terrestrial and marine sources. In addition, restricted water circulation in embayments may exacerbate the risk of contaminant exposure to embryos.

This study raises important questions regarding the quality of marine nearshore spawning habitats related to chemical contamination. We observed PAH contamination of herring embryos spawned over lightly contaminated sediments along shores adjacent to residential areas. Future research should be focused on how survival might be affected for organisms that spawn along heavily urbanized shores which may contain or receive much greater PAH inputs. Moreover, survival of species whose embryos are spawned at higher tidal elevations may be exposed to greater risk related to photo-enhanced toxicity.

Acknowledgments

The authors gratefully acknowledge Kurt Stic and his WDFW Forage Fish crew for the guidance and advice on herring spawning history and patterns in Puget Sound, and for providing adult fish for the ovary analyses. Kurt Dobszinsky and his crew of the FV Chusina conducted the night-time midwater trawls to capture adult fish for ovaries. Stephen Quinnett, Jim Beam, Greg Lippert, Robert Pacunski, Michael Peterson CH, Sloan CA, Rice SD, Thomas RE, Carls MG, Heintz RA, Wertheimer AC, Murphy ML, et al. Impacts to marine nearshore spawning habitats related to chemical contamination. This manuscript benefited from several, thoughtful, anonymous reviews. This project was funded by the Washington State Department of Fish and Wildlife.

References

Sloan CA, Brown DW, Yitai GM, Buzijs J, Herman DP, Burrows DG, et al. Quality assur- dance of analytical samples for polycyclic aromatic compounds, persistent organic pollutants, fatty acids, stable isotope ratios, lipid classes, and

EX5105-000010-TRB


