Environmental Science & Technology

Article

pubs.acs.org/est

Mercury Sources and Trophic Ecology for Hawaiian Bottomfish

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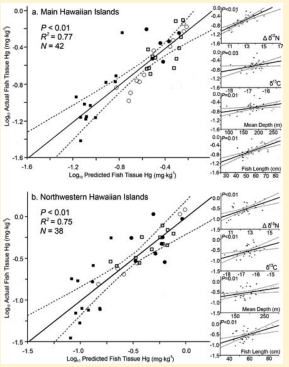
ABSTRACT: In Hawaii, some of the most important commercial and recreational fishes comprise an assemblage of lutjanids and carangids called bottomfish. Despite their importance, we know little about their trophic ecology or where the mercury (Hg) that ultimately resides in their tissue originates. Here we investigated these topics, by analyzing muscle samples for mercury content, nitrogen, carbon, and amino acid specific nitrogen isotope ratios in six species distributed across different depths from the Northwestern Hawaiian Islands (NWHI) and the Main Hawaiian Islands (MHI). Fishes had different sources of nitrogen and carbon, with isotopic values suggesting benthic food sources for shallow nearshore species. High trophic position lutjanids that foraged in deeper water, benthic environments generally had higher Hg levels. Model results also suggested that benthic Hg methylation was an important source of Hg for shallow benthic feeders, while deepwater sources of mercury may be important for those with a diet that derives, at least in part, from the pelagic environment. Further, despite the lack of freshwater sources of Hg in the NWHI, statistical models explaining the variation in tissue Hg in the MHI and NWHI were nearly identical, suggesting freshwater Hg inputs were not a major source of Hg in fish tissue.



INTRODUCTION

An extensive amount of research has been directed at understanding mercury (Hg), a highly toxic heavy metal that accumulates in fish tissue and is distributed throughout global marine, terrestrial, and atmospheric environments.[1,2] Much of this research is directed at aquatic ecosystems because consumption of contaminated fish is the most direct pathway for Hg to affect the health of humans and wildlife.[1−3] Consequently, a number of important predictors have been linked to fish tissue Hg concentrations such as trophic level, fish size, depth of forage, geographic region, dissolved oxygen, pH, and dissolved organic carbon (DOC).[4−8] Despite this knowledge the source or relative contribution of different sources of methylmercury (MeHg), the organic neurotoxic form of Hg, which ultimately resides in fishes, can rarely be identified. Additionally, because MeHg enters at the base of the food web and bioaccumulates through diet,[4,9,10] understanding sources of MeHg to fishes, provides information on foraging ecology and food web dynamics; information largely lacking for our study species, the economically important bottomfishes of Hawaii.[11] Nitrogen and carbon isotopic compositions (δ^{15}N and δ^{13}C values) have also been used in ecology to improve our understanding of trophic ecology, dietary partitioning and energy flow.[12,13] For instance, δ^{15}N values can be used to estimate relative trophic position[6,14] while δ^{13}C values can be useful indicators of the sources of dietary carbon (e.g., benthic vs pelagic).[15−17] Here we aim to use stable isotopic data to link feeding ecology with the trophic transfer of the bioaccumulative contaminant, Hg.[17,18]

While studies have examined Hg inputs to the open ocean on a global scale to provide insight into Hg in marine biota,[19,20] the scale of these studies often do not take the regional and local influence of benthic and coastal sources of Hg into account. For example, understanding that freshwater inputs of Hg to the open ocean are comparatively low to other oceanic sources does not account for the larger impact freshwater Hg input has on coastal ecosystems where a large number of marine species frequent or reside.[21] In addition, though many studies have examined trophic position and mercury values in fishes,[19,20] few have combined carbon, amino acid, and bulk nitrogen isotope values with Hg concentrations to determine the primary sources of Hg in fish tissue.[21−23] This information is particularly important as research on Hg sources in fish tissue can be equivocal. For instance, some studies examining pelagic fishes have suggested that marine fish derive tissue Hg from freshwater and coastal benthic bacterial methylation and subsequent advection to the open ocean,[24] while other more recent studies have indicated water column methylation in the oxygen minimum zone (OMZ) as the primary source of Hg in pelagic fish tissue, suggesting no necessary connection to benthic or coastal food webs.[19,20,25,26] A recent study by Choy et al.[27] found that Pacific pelagic fishes that primarily foraged at depths below the surface mixed layer in the open ocean had

Received: August 5, 2014
Revised: April 30, 2015
Accepted: May 2, 2015

DOI: 10.1021/acs.est.5b01009
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

higher tissue Hg levels, suggesting a deep-ocean source of Hg methylation.[28] Mercury isotopic analysis confirmed that up to 80% of Hg in these pelagic fish was methylated below the surface mixed layer of the ocean.[28] However, Senn et al.[21] demonstrated that while open ocean pelagic species showed similar results in the Gulf of Mexico, coastal pelagic species derived Hg from freshwater and coastal sources of Hg methylation. Here we aimed to examine the generality of these finding for demersal fishes. For bottomfish, or fish that live in association with the seafloor and may forage on the bottom or in the water column just above it,[29−33] we are unable to predict sources of Hg exposure because close association with the benthos, both in coastal and deep-ocean habitats, may expose them to different sources of MeHg with different bioaccumulation pathways. For instance, shallow species associated with the benthos will often by-default be close to a landmass and potentially exposed to high levels of freshwater, coastal and benthic sources of Hg methylation. Unlike pelagic fish species, deepwater benthic species have two potential sources of MeHg exposure, that from the benthos in addition to in situ methylation from the OMZ. As such, the primary sources of MeHg in fish tissue, particularly in fish that reside near the seafloor, are still unresolved. This lack of understanding hampers effective natural resource management and development of human health policy pertaining to bottomfish.[29] In addition, few studies have examined bottomfish Hg levels[34] and none to our knowledge have examined Hawaiian bottomfish; a surprise considering how highly valued bottomfish are as a food item in Hawaii[35,36] and how widespread this group of fish is across the greater IndoPacific region. Determining Hg sources and obtaining detailed information on feeding ecology will improve our knowledge of trophic pathways and dietary partitioning. This information is also critical to understanding Hg biogeochemical cycling in ecosystems, to parametrize ecosystem models,[37] and to guide future attempts at contaminant mitigation or remediation.

The goals of our research were to quantify the sources and pathways of MeHg bioaccumulation in six common, economically important, and frequently consumed Hawaiian bottomfish species and to use isotopic data to better understand their foraging ecology. We hypothesized that the depth of occurrence for four lutjanid and two carangid bottomfish species would influence Hg accumulation similarly to pelagic fish despite accumulating Hg, in part, from the benthos.[27] Further, we aim to determine how Hg sources and pathways compare between the Main Hawaiian Islands (MHI) and Northwestern Hawaiian Islands (NWHI). Contrary to the MHI (~200 to 10 000 km^2 in size), the NWHI lack freshwater ecosystems and areas where large volumes of freshwater and saltwater mix because the islands are submerged or have minimal atmospheric exposure (<4 km^2), which would presumably limit freshwater and coastal sites of Hg methylation.[38−41] This is an important distinction as Hg concentrations in freshwater runoff from the MHI have exceeded the United States Environmental Protection Agency (USEPA) recommended criteria for aquatic life and have been seen to concentrate in coastal aquatic species.[42,43] Additionally, groundwater discharges in the MHI are a significant source of Hg to coastal areas compared to other sites across the globe, have been seen to exceed water concentrations of Hg in surface ocean waters (groundwater Hg ~4 pM, surface ocean water ~1 pM), and have caused Hg concentrations to double in coastal waters (1.2−2.4 pM).[26,44] We, therefore, hypothesized that shallow bottomfish in the MHI would have higher Hg levels than those same species in the NWHI. We also hypothesized that the variation in Hg levels with depth of occurrence for fish would differ between the MHI and NWHI as a result of coastal Hg inputs in the MHI. We also used $\delta^{15}N$ and $\delta^{13}C$ values measured in bottomfish tissue to quantify relative trophic position[1,45] and the primary sources of nitrogen and carbon (e.g., benthic versus pelagic) for individual fish.[16] Lastly relative trophic position estimated from amino acid compound specific isotope analysis were compared to reported trophic position from diet studies to further evaluate bottomfish feeding ecology.

MATERIALS AND METHODS

Sample Collection. We analyzed fish muscle tissue from four lutjanids (Lutjanidae: uku *Aprion virescens*, opakapaka *Pristipomoides filamentosus*, onaga *Etelis coruscans;* ehu *Etelis carbunculus*) collected by the National Oceanographic Atmospheric Administration (NOAA) Pacific Islands Fisheries Science Center from 2007 to 2011 in the MHI and NWHI. To evaluate whether our results were specific to lutjanids or general among other taxa we also targeted and analyzed muscle tissue from two carangids or jacks (giant trevally *Caranx ignobilis*, greater amberjack *Seriola dumerili*) collected in 2013 from the MHI by local anglers. These species were chosen because they are closely associated with and forage near the seafloor and have distinctly different depth distributions (Figure 1, with cited literature).[29−33] In addition, we targeted 30 samples for each species from each area. Sample sizes for onaga, a deepwater lutjanid, however, were limited. We therefore included a second deepwater lutjanid, ehu *Etelis carbunculus*, with a similar depth distribution to onaga, thus increasing the number of individuals that occurred at a mean depth of 250m (Figure 1). This inclusion provided the statistical power necessary to determine the effect of "depth of occurrence" on the level of tissue Hg in individual fish (Table 1; Figure 1).

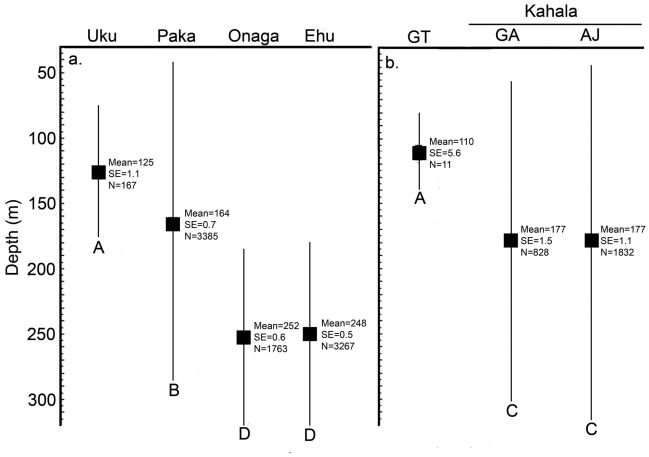


Figure 1. Mean, standard error (SE; encompassed by black square markers), range (vertical lines), and sample size of depth distributions for four lutjanids (a; uku *Aprion virescens*, paka = opakapaka *Pristipomoides filamentosus*, onaga *Etelis coruscans*, ehu *E. carbunculus*) and three carangid (b; GT = giant trevally *Caranx ignobilis*, kahala = GA = greater amberjack *Seriola dumerili* and AJ = almaco jack *S. rivoliana*) species using data collected from a baited camera system among the Main Hawaiian Islands (data from Sackett et al.[33]). Capital letters represent significant differences among species ($P < 0.01$). .

DOI: 10.1021/acs.est.5b01009
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Table 1. Summary Statistics of Fish Length and Fish Tissue Hg for Six Species of Bottomfish Collected in the Main Hawaiian Islands (a. MHI) and Northwestern Hawaiian Islands (b. NWHI)[a]

			length (cm)		fish tissue Hg (mg·kg^{-1} ww)		
location	species	N	mean	SE	mean	SE	range
a. MHI	Uku	30	59.64	1.06	0.39	0.03	0.17−0.79
	Paka	30	58.00	0.88	0.13	0.02	0.04−0.57
	Onaga	30	63.09	1.80	0.30	0.04	0.10−0.80
	Ehu	14	35.70	0.96	0.57	0.06	0.28−1.13
	GT	15	76.62	4.51	0.45	0.15	0.08−2.41
	Kahala	8	77.95	2.97	0.76	0.07	0.51−1.12
b. NWHI	Uku	30	60.19	0.97	0.51	0.05	0.17−1.65
	Paka	30	58.77	0.72	0.17	0.03	0.04−0.78
	Onaga	12	63.69	3.25	0.47	0.12	0.16−1.25
	Ehu	19	43.20	1.44	0.66	0.07	0.29−1.42

[a]*N* = sample sizes. Lutjanid species: uku = *Aprion virescens*, paka = opakapaka *Pristipomoides filamentosus*, onaga = *Etelis coruscans*, ehu = *E. carbunculus*. Carangid species: GT = giant trevally *Caranx ignobilis*, kahala = greater amberjack *Seriola dumerili* and almaco jack *S. rivoliana*..

Further, greater amberjack and almaco jack (*Seriola rivoliana*) are both called kahala among the Hawaiian Islands because they are morphologically similar, have similar depth distributions and similar diets (Figure 1).[46−48] As such, although we targeted greater amberjack, our samples included both carangid species called kahala. These data were pooled for analyses due to small sample size (greater amberjack, n = 3; almaco jack, n = 5), similarity between species, and because isotopic and Hg data were not significantly different between kahala species (Kruskal−Wallis test, P-value range = 0.63−0.88). In addition, mean depth of occurrence for giant trevally (110m) were estimated using data collected from a baited camera system with an upper limitation of 90m (Figure 1).[33] Because these data likely overestimated the mean depth of occurrence of this shallow water species, we used mean depth of occurrence estimates determined by Kelley and Moriwake[32] for giant trevally in our statistical analyses (80 m), as this data set was more robust and did not have an upper depth limit to the sampling design.

Tissue Analyses. Because MeHg generally constitutes >95% of total mercury in fish tissue,[10,49] all samples were analyzed for total Hg, a much more cost-effective approach than analyzing MeHg. However, a subset of samples (four samples of each species, n = 24) was analyzed for MeHg to validate this assumption for bottomfish. Tissue samples were freeze-dried, ground into a homogeneous powder and measured for total Hg using a Direct Mercury Analyzer (DMA-80; Milestone Inc., Monroe, CT) according to USEPA Method 7437.[50] Following analysis, total Hg (hereafter referred to as Hg) concentrations were converted from dry to wet weight using percent moisture values obtained from lyophilizing each sample. Analyses were performed with appropriate quality assurance/quality control (QA/QC) protocols, including four National Research Council (NRC) certified reference materials (CRMs; CRM-TMF 100; CRM-TMF 1000, DORM-3 fish protein, DOLT-4 dogfish liver), and 28 randomized replicate samples (∼10% of all samples). On each day of analysis, two calibration standards were used for verification of the current nine point calibration curve (SPEX-CertiPrep, NIST traceable) for analysis of the samples (n = 222) and QC samples. Fresh calibration standards were prepared monthly. All CRM results (n = 103) were within acceptable limits and had a mean recovery of 101% ± 4% SD. The mean relative standard deviation (RSD) for 28 replicate samples was 2.60%. For MeHg analysis, 80−120 mg of freeze-dried tissue from each sample was digested with protease XIV and analyzed for MeHg and inorganic Hg (iHg) by high performance liquid chromatography and inductively coupled plasma mass spectrometry (HPLC-ICPMS).[51] This method has a lower limit of quantification of 0.005 ppm for Hg. Digestion with protease has also been reported to provide the most accurate ratio of MeHg to inorganic Hg (iHg) as it results in a very high extraction efficiency while preventing transformation of MeHg to iHg.[51] The mean percent of MeHg to total Hg (MeHg+iHg) for all 24 samples tested was 99% ± 0.46% SE. The mean RSD for six replicate samples was 0.07%.

Bulk tissue nitrogen and carbon isotopic compositions (hereafter δ^{15}N and δ^{13}C values) of a subset of freeze-dried fish tissue samples (≥40% for each species) were determined by combustion using a Costech ECS 4010 Elemental Combustion System (Costech Analytical Technologies Inc., Valencia, CA) coupled with a ConFlo IV interface to introduce samples to a Delta XP Isotope Ratio Mass Spectrometer

Table 2. Mean and Standard Error (SE) of the δ^{15}N Values of Three Source Amino Acids (AA_{source}; Glycine, Lysine, Phenylalanine[a]

		AA_{source}		$TP_{Nielsen}$			Δ δ15N[b]		$TP_{FishBase}$	
species	N	mean	SE	mean	SE	N	mean	SE	mean	SE
uku	5	−2.18^{B}	1.12	3.98^{B}	0.08	24	13.19^{B}	0.10	4.50	0.80
paka	9	−1.25^{B}	0.29	3.99^{B}	0.07	24	10.69^{D}	0.09	3.80	0.50
onaga	5	−1.05^{B}	0.51	3.95^{B}	0.05	18	11.99^{C}	0.10	4.50	0.80
ehu	5	−2.26^{B}	0.89	4.37^{A}	0.10	14	14.67^{A}	0.18	4.50	0.80
GT	4	1.26^{A}	0.81	3.56^{C}	0.13	8	10.19^{E}	0.50	4.20	0.70
kahala	4	−0.88^{AB}	0.8	4.11^{B}	0.03	8	12.11^{C}	0.07	4.50	0.80

[a]See eq 2), trophic position (TP) estimated using the equation developed by Nielsen et al.,[54] a proxy for relative trophic position (Δ δ^{15}N) for six species of bottomfish collected in the Main Hawaiian Islands and Northwestern Hawaiian Islands. Mean and SE of trophic position estimates from FishBase are also indicated in the table.[54,57,58]. [b]Data used in models as a proxy for relative trophic position (see Table 3). Superscript capital letters indicate significant differences among species (P < 0.05). N = sample sizes for the columns following the N column. Lutjanid species: uku = *Aprion virescens*, paka = opakapaka *Pristipomoides filamentosus*, onaga = *Etelis coruscans*, ehu = *E. carbunculus*. Carangid species: GT = giant trevally *Caranx ignobilis*, kahala = greater amberjack *Seriola dumerili* and almaco jack *S. rivoliana*

DOI: 10.1021/acs.est.5b01009
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

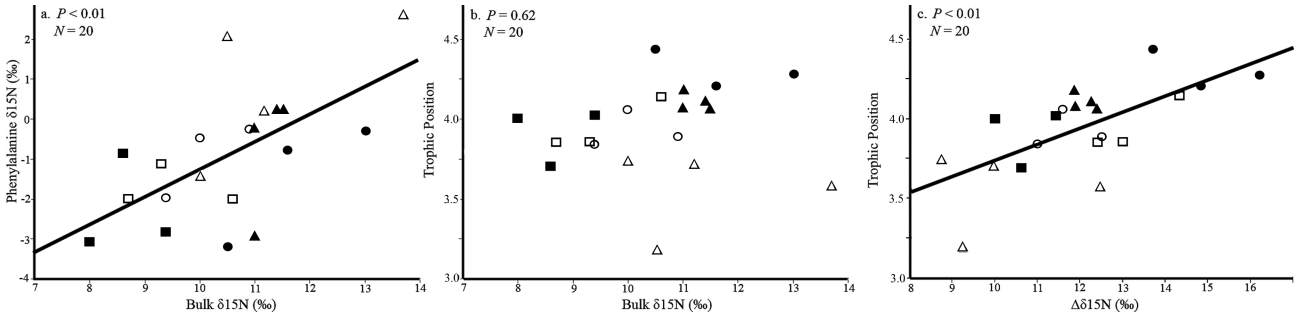

Figure 2. Baseline $\delta^{15}N$ values (a; using the source amino acid, phenylalanine) and trophic position (b; based on AA-CSIA using constants derived by Nielsen et al.[54]) versus bulk $\delta^{15}N$ values. Trophic position estimates using AA-CSIA were also regressed against a proxy for trophic position (c; $\Delta\delta^{15}N$). Different symbols represent different species. Uku *Aprion virescens* = open rectangles, opakapaka *Pristipomoides filamentosus* = closed rectangles, onaga *Etelis coruscans* = open circles, ehu *Etelis carbunculus* = closed circles, giant trevally *Caranx ignobilis* = open triangles, kahala, amberjack *Seriola dumerili* and almaco jack *S. rivoliana* = closed triangles.

(Thermo Finnigan, Bremen, Germany). Because all tissue samples presented low lipid content (C:N molar ratio <4.0), lipid extractions were unnecessary.[52] We also analyzed a subset of tissue samples for amino acid compound specific isotopic composition (AA-CSIA) to characterize baseline $\delta^{15}N$ values and to determine relative trophic position of individual fish (Table 2). The methods and instrumentation used have previously been described in Dale et al.[14] Briefly, dried tissue samples were subjected to acid hydrolysis, esterification of the carboxyl terminus and trifluoracetylation of the amine group prior to being introduced into a Delta V or MAT 253 mass spectrometer interfaced with a Trace GC gas chromatograph through a GC-C III combustion furnace (980 °C), reduction furnace (650 °C) and liquid N cold trap. All 20 samples were analyzed in triplicate. Reproducibility of amino acids used in our analyses (alanine, leucine, glutamic acid, glycine, lysine, phenylalanine) averaged 0.31‰ SD and ranged from 0.01‰ to 0.85‰ SD. Instrument accuracy was determined using known $\delta^{15}N$ values of aminoadipic acid and norleucine coinjected with all samples. The mean difference between known and measured $\delta^{15}N$ values of aminoadipic acid and norleucine was 0.64‰ ± 0.49‰SD (n = 20). Due to the high cost of AA-CSIA only three samples per lutjanid species and area and four samples per carangid species were analyzed. Individuals chosen for AA-CSIA encompassed the size and geographic ranges of our data for each species being tested. In addition, previous research at the University of Hawaii also conducted amino acid nitrogen isotope analysis, providing 12 additional data records for our lutjanid species. These data supplemented our estimates of trophic position although tissue Hg was not measured in these specimens.

Trophic Position and Statistical Analyses. We calculated trophic position for those samples where AA-CSIA data were available using glutamic acid and phenylalanine as described by Chikaraishi et al.[53] and Nielsen et al.[54]

$$TP = ((\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - \beta)/TEF) + 1 \quad (1)$$

where TP is the trophic position, $\delta^{15}N_{Glu}$ is the $\delta^{15}N$ value for the trophic amino acid glutamic acid (Glu), $\delta^{15}N_{Phe}$ is the $\delta^{15}N$ value for the source amino acid phenylalanine (Phe), β is the difference between $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ in marine primary producers, and TEF is the trophic enrichment factor or the relative change in $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values with each trophic transfer. The values suggested by Chikaraishi et al.[53] for β and TEF are 3.4 ± 0.9 SD and 7.6 ± 1.2 SD. However, not all studies agree with the values of these constants[14,55,56] because they were developed using 17 primary producers, a few zooplankton and two fish species in the larval stage of development in controlled laboratory feeding experiments. Conversely, Nielsen et al.[54] used 359 different marine species with diets spanning four trophic levels. It is therefore reasonable to expect that the equation developed using a large compendium of marine organisms[54] would be best in providing an estimate of trophic position for large ocean predators such as bottomfish. We therefore estimated trophic position using the β and TEF values suggested by Nielsen et al.[54] (β = 2.8 ± 2.0SD, TEF = 6.6 ± 1.7SD). We compared estimates of trophic position based on AA-CSIA to reported trophic position estimates published online by FishBase.[57,58] For bulk $\delta^{15}N$ values, which were analyzed for a much larger portion of the data set compared to AA-CSIA (Table 2), β and TEF are unknown and thus these data could not be used to determine precise trophic positions. However, a proxy for trophic position ($\Delta\delta^{15}N$) was estimated by subtracting a weighted mean $\delta^{15}N$ value of source amino acids ($\delta^{15}N_{source}$)[59] for each species from bulk tissue $\delta^{15}N$ values measured for each individual ($\Delta\delta^{15}N$) to use in our models. This process normalized bulk $\delta^{15}N$ values so different baseline $\delta^{15}N$ values did not confound our analyses or interpretation of relative trophic position and made no assumption about the magnitude of the TEF for bulk data. The necessity of this normalization was evident because baseline isotopic variations strongly influenced bulk $\delta^{15}N$ values (Figure 2).[13] This approach is further justified because trophic position estimates using AA-CSIA data did not correlate with bulk $\delta^{15}N$ data, but did correlate with our proxy for trophic position, $\Delta\delta^{15}N$ (Figure 2). Only individuals with Hg data were used to create our $\Delta\delta^{15}N$ proxies. We calculated weighted mean $\delta^{15}N$ values of source amino acids using glycine, lysine, and phenylalanine

$$\delta^{15}N_{source} = \frac{\Sigma\frac{\delta^{15}N_x}{\sqrt{\sigma_x^2}}}{\Sigma\frac{10}{\sqrt{\sigma_x^2}}} \quad (2)$$

where $\delta^{15}N_x$ is the $\delta^{15}N$ value of a specific source amino acid and σ_x is the standard deviation of triplicate isotopic analysis of the specific amino acid.[59]

Mercury data were log transformed for statistical tests to meet assumptions of normality and equal variance. Differences in fish size, depth distributions, tissue Hg and C and N isotope data among species and sites were determined using a one-way analysis of variance (ANOVA). Posthoc comparisons among

DOI: 10.1021/acs.est.5b01009
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Table 3. Standard Least Squares Regression Models to Predict Lutjanid (uku *Aprion virescens*, opakapaka *Pristipomoides filamentosus*, onaga *Etelis coruscans*, ehu *E. carbunculus*), and Carangid (giant trevally *Caranx ignobilis*, kahala = greater amberjack *Seriola dumerili* and almaco jack *S. rivoliana*) Fish Tissue Hg, Ranked with Akaike'S Information Criterion (AIC_c)[61] in the Main Hawaiian Islands (a. MHI) and Northwestern Hawaiian Islands (b. NWHI)[a]

location	group	model	N	P	R^2	AIC_c	W_i
a. MHI	Carangids	$\Delta\ \delta^{15}N$	16	<0.01	0.54	−17.26	0.45
		length	16	<0.01	0.50	−16.71	0.34
		$\Delta\ \delta^{15}N$, $\delta^{13}C$, depth, length	16	<0.01	0.81	−14.17	0.10
		$\delta^{13}C$	16	0.07	0.22	−13.54	0.07
		depth	16	0.01	0.30	−12.86	0.05
	Lutjanids	$\Delta\ \delta^{15}N$, $\delta^{13}C$, depth, length	42	<0.01	0.77	−57.36	0.99
		$\Delta\ \delta^{15}N$	42	<0.01	0.43	−47.89	0.01
		$\delta^{13}C$	42	<0.01	0.19	−41.35	0.00
		length	42	0.31	0.03	−38.00	0.00
		depth	42	0.35	0.02	−37.93	0.00
b. NWHI	Lutjanids	$\Delta\ \delta^{15}N$, $\delta^{13}C$, depth, length	38	<0.01	0.75	−44.57	0.91
		$\Delta\ \delta^{15}N$	38	<0.01	0.47	−39.57	0.07
		$\delta^{13}C$	38	<0.01	0.35	−36.15	0.01
		depth	38	0.29	0.03	−29.55	0.00
		length	38	0.43	0.02	−29.31	0.00

[a]$\Delta\ \delta^{15}N$ = proxy for relative trophic position calculated by subtracting bulk $\delta^{15}N$ from $\delta^{15}N_{source}$ (see eq 2). Depth = mean depth of occurrence (see Figure 1). W_i = probability that the model was the best of those tested..

means were conducted using an each-pair student t test. In addition, standard least-squares models including each factor separately (fish length, trophic position proxy $\Delta\delta^{15}N$, $\delta^{13}C$ values, mean depth of occurrence) and all factors together were tested to reveal which model was the best in explaining the variation in fish tissue Hg for lutjanid species in the MHI and NWHI and carangid species in the MHI separately (Table 3). Variance inflation factors (VIF) were calculated for models that included all factors together as a test of collinearity. All VIFs were well below 10 (range = 1.3−4.7) indicating little to no collinearity of independent factors.[60] All models were ranked and the best model was determined using Akaike's Information Criterion adjusted for small sample size (AIC_c).[61] Model weights were also calculated to determine the probability that the top model was the best of those tested (W_i).[61] Further, if the model with all four factors was ranked the best, parameter estimates and leverage plots were evaluated to determine the significance of each factor. However, because AIC_c penalizes models (raises the AIC_c value) for each additional parameter added to a model it would be unlikely for the model with the most factors to be ranked the best unless those parameters significantly explained additional variation in fish tissue Hg data.[61] Further, to ensure ages (i.e., variable growth rates) among species did not affect our results, age was estimated using the von Bertalanffy growth equation ($L(t) = L_{inf} \times (1 - \exp(-k \times (t - t_0)))$)[62] for those species where reliable growth parameters were available (ehu, onaga, opakapaka, and giant trevally).[57,63] The ages of lutjanid species were not significantly different in the MHI ($P = 0.22$) or NWHI ($P = 0.53$). The carangids, however, could not be compared as ages could not be estimated for kahala. All analyses were conducted using JMP Pro 9.0.2 (2010 SAS Institute Inc.).

RESULTS AND DISCUSSION

Food Web Dynamics. Trophic position varied among species ($P < 0.01$), though were generally consistent with published values from stomach content analyses on FishBase.[57] For instance, giant trevally were reported to have a trophic position of 4.2 ± 0.7 SE by FishBase and a diet consisting of fishes, cephalopods, and benthic crustaceans.[57,58] Our estimates were slightly, though not significantly, lower than FishBase estimates with a mean of 3.6 ± 0.13 SE, the lowest of the species we examined ($P < 0.01$; Table 2), suggesting their diet may consist of more benthic crustaceans and/or herbivorous fishes and less carnivorous fishes than previously stated (Table 2). The fish in Sudekum et al.'s[58] study were comparable in size to fish in our study. Papastamatiou et al.[64] found that the trophic positions of giant trevally were bimodal and related to two contingents of behavior, one similar to Galapagos sharks and another with a lower trophic position of approximately 3.8.[64] The trophic position of the fish in our study were consistent with the latter contingent which is perhaps the result of sampling the population captured largely by shore fishers. These results were further supported by relatively high $\delta^{13}C$ and $\delta^{15}N_{source}$ values which indicated that the diets of giant trevally from this study were primarily derived from the benthos (Table 2; Figure 3).[15,65] Conversely, opakapaka have been estimated to have a trophic level of 3.8 ± 0.5 SE and diet that consists of fishes and planktivorous invertebrates that reside in or come into close proximity with the seafloor (e.g., filter feeding tunicates).[11,57,66] Our mean estimate was slightly, though not significantly, higher at 4.0 ± 0.07 SE suggesting that opakapaka sampled in our study were slightly more piscivorous than those in Haight et al.[11] Opakapaka in our study were also generally larger (50.7−67.6 cm) than those in Haight et al.[11] (26.7−65.4 cm). Low $\delta^{13}C$ values also supported the reliance, at least in part, of opakapaka on the pelagic food web (Figure 3). Ehu had the highest trophic position compared to all other species ($P < 0.05$). Although this species attains smaller maximum sizes than many other Hawaiian bottomfish and was the smallest on average of the fish we sampled (Table 1), studies have suggested a diet of mostly benthic fishes.[11] FishBase estimates for ehu, onaga, uku, and kahala were primarily piscivorous, with similar trophic levels of approximately 4.5 ± 0.8 SE.[47,48,57] Likewise, the trophic positions of onaga, uku, kahala, and opakapaka were not significantly

DOI: 10.1021/acs.est.5b01009
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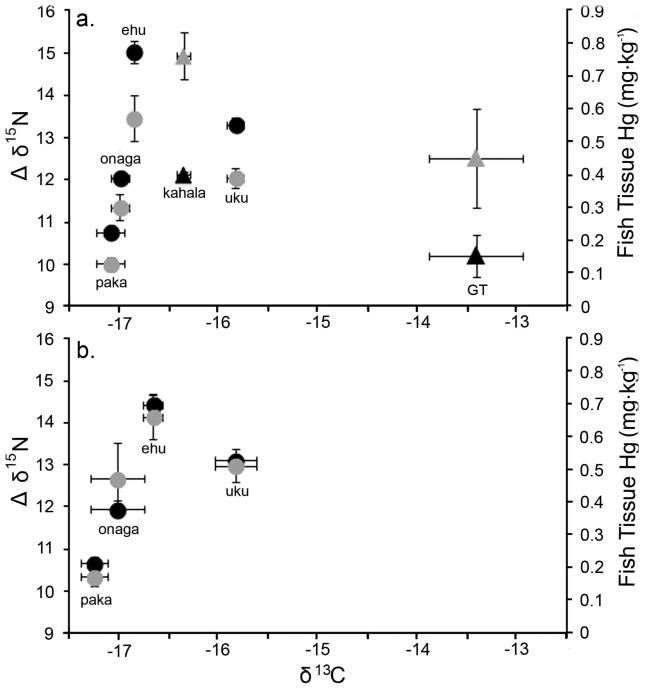

Figure 3. Proxy for relative trophic position ($\Delta\delta^{15}$N; black symbols) and fish tissue Hg (gray symbols) versus δ^{13}C values for six species of bottomfish in the Main Hawaiian Islands (a) and Northwestern Hawaiian Islands (b). $\Delta\delta^{15}$N values represent the difference between bulk δ^{15}N values and $\delta^{15}N_{source}$ values (see eq 2; Nielsen et al.[54]). Circles = lutjand species (uku = *Aprion virescens*, paka = opakapaka *Pristipomoides filamentosus*, onaga = *Etelis coruscans*, ehu = *E. carbunculus*). Triangles = carangid species (kahala = *Seriola dumerili* and *S. rivoliana*, GT = giant trevally *Caranx ignobilis*). Error bars = standard error.

different ($P < 0.05$; Table 2). Despite the small sample sizes used to calculate trophic position using AA-CSIA, the values here are in general agreement with the few previous studies that have used diet to determine trophic level for these species when the constants of Nielsen et al.[54] were adopted.[11,47,48,57,58] In addition, our proxy for relative trophic position ($\Delta\delta^{15}$N) was in general agreement with our trophic position estimates using only $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ values (Figure 2). For example, ehu had the highest mean relative trophic position estimated from $\Delta\delta^{15}$N values and giant trevally the lowest ($P < 0.05$; Table 2). However, where trophic position was calculated using AA-CSIA suggested that other species were similar, $\Delta\delta^{15}$N values suggested that there were significant differences in trophic position among other species. For instance, using $\Delta\delta^{15}$N, uku occupied the second highest trophic position, onaga and kahala occupied very similar intermediate trophic positions and opakapaka occupied the second lowest trophic position ($P < 0.05$; Table 2). These differences were likely more evident using $\Delta\delta^{15}$N because of the larger sample size for these data compared to AA-CSIA data (Table 2). Isotope and trophic position results were also consistent between species from both the MHI and NWHI (Figure 3).

While trophic position can be determined using isotopic values of nitrogen, dietary resources from different food webs can also be distinguished using δ^{13}C and $\delta^{15}N_{source}$ values. High $\delta^{15}N_{source}$ values indicate a nitrate-rich (e.g., coastal benthos or offshore upwelling) versus a nitrate-poor (e.g., atmospheric N) source of nitrogen.[14,15,65] Giant trevally had the highest $\delta^{15}N_{source}$ values of any species ($P < 0.05$; Table 2). While kahala, the other carangid, had the second highest $\delta^{15}N_{source}$ values, these were not significantly different from the $\delta^{15}N_{source}$ values of lutjanid species. In addition, the two species that inhabit the shallowest waters (giant trevally and uku) had the highest δ^{13}C values among species (δ^{13}C = −13.4 ± 0.47‰ SE and −15.8 ± 0.11‰ SE), with giant trevally having a significantly higher carbon isotopic composition than any other species ($P < 0.01$; Figure 3). These results imply that uku and especially giant trevally rely more on a nearshore benthic-based food web because global mean δ^{13}C values for marine benthic algae are −17‰, whereas δ^{13}C values for marine phytoplankton are approximately −22‰ and δ^{13}C values record the relative isotopic compositions of carbon at the base of the foodweb.[15,16] Other lutjanid and carangid species had more intermediate carbon isotope compositions with kahala and ehu having slightly higher values than onaga and opakapaka. These results suggest that the latter species relied more on pelagic sources of carbon than the others (Figure 3). However, the δ^{13}C values for bottomfish in this study were all greater than those of marine primary producers and although coarse, this relative comparison demonstrated that all bottomfish relied to some extent on the benthic food web. This result is further supported by carbon isotopic values in the Pacific[67] and at Station ALOHA in the MHI (pelagic particulate organic matter from 0 to 400 m = −22 ± 0.1‰ SE) that were consistent with global averages, and diet studies that show varying proportions of benthic and pelagic prey.[57] It is important to note that benthic species are not excluded from pelagic influences simply because they reside near the seafloor. For instance, the bottomfish with more pelagic δ^{13}C values in this study likely consume vertically migrating or advected pelagic prey that come into close proximity with the seafloor (e.g., *Thaliacea* or pelagic tunicates in the case of opakapaka).[11,57,66]

Sources of Hg. Mercury concentrations in fish tissue ranged from 0.04 to 2.41 mg·kg^{-1} ww, varied among species ($P < 0.01$), and followed $\Delta\delta^{15}$N estimates (Table 1; Figure 3). Models including only a single factor and ranked with AIC_c also indicated that the variation in fish tissue Hg was best explained by $\Delta\delta^{15}$N values; demonstrating that relative trophic position was the primary driver of tissue Hg for both carangids and lutjands (Table 3). However, for lutjanids in both the MHI and NWHI the best overall model included all factors ($\Delta\delta^{15}$N, δ^{13}C values, fish length, and mean depth of occurrence; Table 3). These models explained ≥75% of the variation in fish tissue Hg, had model weights >90% and suggested that larger, higher trophic level lutjanids that foraged in deeper water, benthic environments generally had higher Hg levels (Table 3; Figure 4).

Interestingly, our best lutjanid models also suggested that fish tissue Hg increased with δ^{13}C values and mean depth of occurrence, despite the species with the highest δ^{13}C values having the shallowest mean depth of occurrence (uku; Figures 3 and 4). Although these results seem contradictory, the relationship with mean depth of occurrence was largely driven by a deeper water lutjanid, onaga, which had a more pelagic carbon isotopic composition or lower δ^{13}C values (Figures 3 and 4). Indeed, onaga have been documented to eat mesopelagic prey that migrate from deeper depths.[69] As such, these results support the previously documented theory that migrating members of the mesopelagic boundary community are a trophic vector connecting offshore pelagic and shallower benthic habitats.[69,70] In addition, these results indicate that

DOI: 10.1021/acs.est.5b01009
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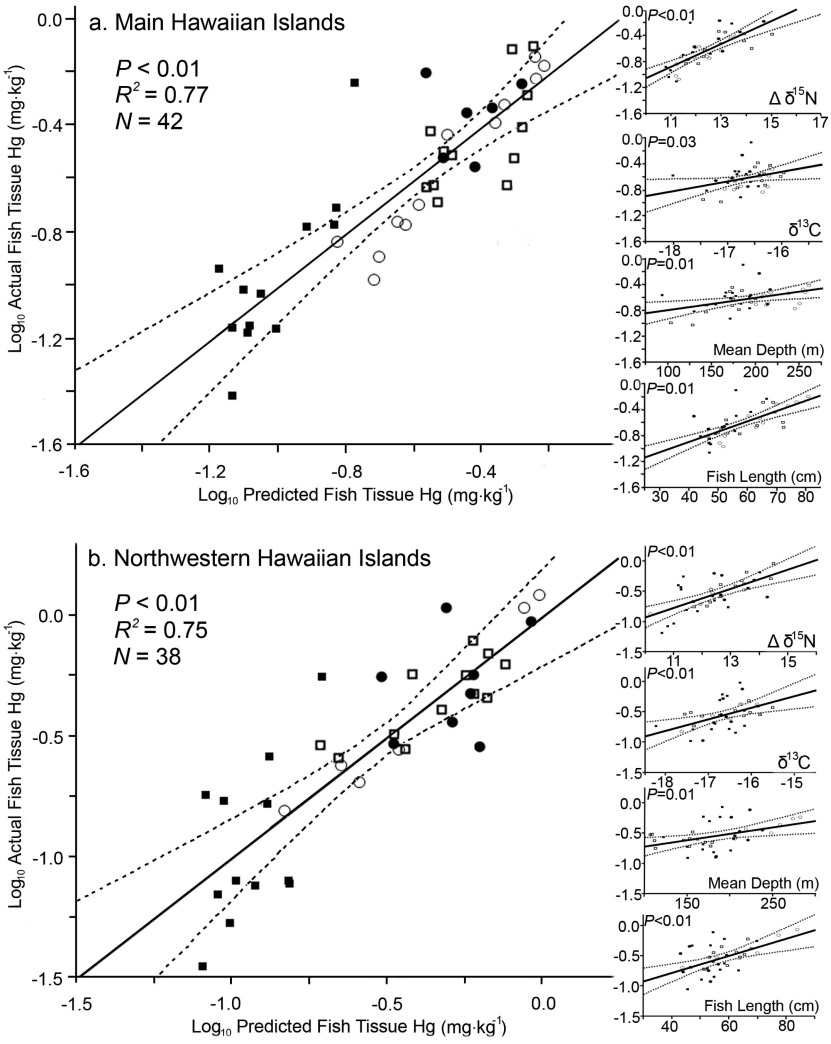

Figure 4. Actual fish tissue Hg concentrations versus those predicted by the best model for lutjanids collected in the Main Hawaiian Islands (a) and Northwestern Hawaiian Islands (b), which included a proxy for trophic position ($\Delta\delta^{15}$N), δ^{13}C values, mean depth of occurrence (see Figure 1), and fish length. Leverage plots for each of these factors, which show the relationship between fish tissue Hg and each variable while controlling for other factors, are depicted on the right side of the figure (Sall 1990). Different symbols represent different species. Uku *Aprion virescens* = open rectangles, opakapaka *Pristipomoides filamentosus* = closed rectangles, onaga *Etelis coruscans* = open circles, ehu *Etelis carbunculus* = closed circles.

benthic Hg methylation is an important source of Hg for shallow coastal feeders, as higher δ^{13}C values indicate a more benthic diet,[15,16] while in situ deepwater sources of MeHg may be important for those with food derived, even partially, from the pelagic environment. Higher concentrations of fish tissue Hg at deeper depths in the open ocean have been attributed to the high levels of MeHg found in deeper low-oxygen water compared to surface waters (e.g., OMZ).[26−28] The mechanisms that govern higher concentrations of MeHg in deeper ocean water could be due to a number of processes. First, the low oxygen conditions (<1 mL L^{-1}) of the OMZ (~400−1000m)[68] may foster methylation because anaerobic sulfur- and iron-reducing bacteria that thrive in these conditions are often responsible for the methylation of Hg into the organic, neurotoxic and bioavailable form MeHg.[5−9] Further, inorganic Hg often binds to and is transported by dissolved organic matter[71,72] until remineralized in deeper ocean waters, which could release and supply freely available inorganic Hg to methylating bacteria in the OMZ.[3] Lastly, solar radiation, which penetrates the euphotic zone can photodegrade MeHg and photoreduce the resultant inorganic form of Hg into the insoluble, elemental form that is subsequently re-emitted into the atmosphere.[28,73,74] This degradation process would likely occur with less frequency in deeper, light-limited waters, leaving a larger net concentration of bioavailable MeHg (e.g., $MeHg_{surface}$ = ~20−50 fM, $MeHg_{600m}$ = ~40−400 fM).[28] Our results suggest that even for bottomfish, which are closely associated with the seafloor, those individuals with a diet that relies more on the pelagic food web show an increase in tissue Hg with depth, supporting recent studies that there is a primary source of MeHg in deeper water that is likely independent of coastal benthic Hg methylation.[22,23,25−28]

Comparing lutjanid model results between the MHI and NWHI indicated that freshwater and coastal (where large volumes of freshwater and saltwater mix) Hg inputs were not a major source of Hg in fish tissue. The NWHI lacks freshwater and coastal sources of Hg because there is little to no land above the ocean's surface (<4 km^2) and thus no area for freshwater to gather in any significant amount, or estuaries where large volumes of freshwater and saltwater can mix.[40,41] Furthermore, freshwater aquifers and groundwater discharges that are prevalent in the MHI are absent in the NWHI. Because

DOI: 10.1021/acs.est.5b01009
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of this large difference between the MHI and NWHI we expected shallow water fish species in the MHI to have higher Hg concentrations as they are purportedly exposed to much higher freshwater sources of Hg through runoff, freshwater aquifers, and submarine groundwater discharges.[5,24,42−44] Surprisingly, Hg levels in our shallow water species (uku) were higher in the NWHI compared to the MHI (P = 0.03). Further, the models and individual relationships with fish tissue Hg in the MHI and NWHI were nearly identical (Figures 4a, $\log_{10}$ (Fish tissue Hg) = 0.18 × $\Delta\delta^{15}$N + 0.15 × δ^{13}C + 0.0019 × mean depth + 0.016 × fish length − 1.67; and 4b, $\log_{10}$(fish tissue Hg) = 0.16 × $\Delta\delta^{15}$N + 0.19 × δ^{13}C + 0.0021 × mean depth + 0.014 × fish length − 0.51). The largest difference in these equations was the intercept, which suggested the equation for the NWHI was shifted slightly up the y-axis to include slightly higher tissue Hg concentrations (Figure 4). Indeed, there were slightly larger fish in the NWHI (Table 1) and larger fish have been linked with higher tissue Hg in many studies.[6] Regardless, the higher tissue Hg in shallow fish from the NWHI and the nearly identical relationships of each factor with fish tissue Hg, particularly mean depth of occurrence, seen in the MHI and NWHI signified that freshwater and coastal Hg inputs were not an important source of Hg that ultimately resided in the fish tissue studied. These results are unexpected because Hg linked to freshwater runoff in the MHI has been seen to concentrate in coastal aquatic species and exceeded the USEPA recommended criteria for aquatic life.[42,43] In addition, groundwater discharges in the MHI are a significant source of Hg to coastal areas compared to other sites across the globe, were elevated in total Hg (∼4pM) compared to concentrations in surface ocean water (∼1 pM),[26] and have caused a direct increase in coastal total Hg concentrations (1.2− 2.4 pM).[44] Others have also suggested that marine fish derive tissue Hg from freshwater and coastal benthic bacterial methylation that is physically and biologically advected to the open ocean.[24,75] Furthermore, in a volcanic island chain such as Hawaii where 100 m depths can be reached just half a km from shore, runoff and groundwater Hg inputs were expected to influence slightly deeper waters. Our results contradict these studies and expectations for at least one group of bottomfish. One explanation for these results may be that freshwater inputs to the marine environment are initially less dense, remaining close to the sea surface where strong and constant sunlight would likely photodegrade those freshwater sources of MeHg and iHg. Alternatively, as part of an island group, freshwater sources of Hg could be quickly diffused to background levels. Another possibility is that conditions often cited to contribute most to freshwater Hg methylation, low lying flat plains with acidic and often hypoxic or anoxic waters,[5−9] are seldom found on volcanic island chains due to the steep terrain.

Akaike's information criterion results for our models differed for carangids and lutjanids, suggesting that results may not be generalized among other fish species (Table 3). In addition, the carbon isotopic composition of giant trevally in particular may suggest more terrestrial freshwater sources of Hg, contrary to all other species tested. However, because we were unable to compare giant trevally results between the MHI and NWHI we are unable to determine whether coastal sources of Hg influenced carangid Hg levels. Our samples sizes for carangids were also much lower than for lutjanids. Consequently, we did not have as much power to detect differences in each factor for carangids (i.e., mean depth of occurrence, δ^{13}C values). In addition, others have demonstrated similar results for open ocean pelagic species as for the more pelagic lutjanids in this study.[26,27] As such, our results are consistent with other fish taxa from other studies[26,27] and the low sample sizes for carangids in this study likely contributed to the lack of significance in model results.

Distinguishing primary sources of fish tissue Hg for any species is challenging but doing so in an open system such as the marine environment presents multiple potential difficulties (e.g., additional sources of Hg and MeHg).[3,27] Here blending isotopic data on foraging ecology with tissue Hg concentrations for six species of fish within two taxa, distributed at distinctly different depths with potentially different sources of Hg (e.g., MHI versus the NWHI) allowed for primary sources of Hg in fish tissue to be identified. Accordingly, our results suggest shallow coastal feeders derived much of the Hg in their tissue from benthic Hg methylation that, for lutjanids, did not originate from freshwater or coastal sources of Hg. Results also showed that deepwater bottomfish derived much of the Hg in their tissue from a combination of benthic and deepwater pelagic sources. These results are contrary to what a few others have suggested.[24,65] Detailed information on feeding ecology improves our knowledge of trophic pathways, dietary partitioning and is important to the sustainable management of these species. Further, understanding where fish tissue Hg originates is critical to comprehending the consequences of the changing environment on Hg cycles and what those changes mean for human health policy and fisheries management.[3] For instance, pH has been identified as an important driver of Hg methylation with more acidic water promoting higher rates of Hg methylation.[8,71] Thus, knowing that ocean sources of Hg methylation, independent of freshwater and coastal Hg, exist and govern tissue Hg levels in deeper water bottomfishes may portend that tissue Hg will increase in those species in the near future as ocean pH continues to decline and OMZs continue to strengthen and expand.[76]

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Notes

The authors declare no competing financial interest

ACKNOWLEDGMENTS

We thank the Joint Institute for Marine and Atmospheric Research (JE123368) and University of Hawaii Sea Grant College Program (Program Development Grant No. R/SS-7PD) for providing the funds for this project. Additional thanks goes to the State of Hawaii Division of Aquatic Resources for providing funds via the Federal Aid in Sport Fish Restoration program (F17R35-study IX). We also thank L. Dodie Lau of the Pelagic Fisheries Research Program and Dr. Mark Merrifield of the Joint Institute for Marine and Atmospheric Research for making this project possible. Special thanks go to Danielle Hoen for additional isotope work and data, and Natalie Wallsgrove, Steven Bailey, and Cassie Kàapu-Lyons for advice and lab assistance. Thanks also to three anonymous reviewers that helped to improve this paper. This research was also partially supported by the National Science Foundation under Grant Number OCE-1041329 (to B.N.P. and J.C.D.). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not

DOI: 10.1021/acs.est.5b01009
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necessarily reflect the views of the National Science Foundation. This is SOEST contribution number 9331.

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