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HABITAT-SPECIFIC FORAGING AND SEX DETERMINE MERCURY CONCENTRATIONS IN SYMPATRIC BENTHIC AND LIMNETIC ECOTYPES OF THE THREESPINE STICKLEBACK

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Abstract

Mercury (Hg) is a widespread environmental contaminant known for the neurotoxicity of its methylated forms, especially monomethylmercury, which bioaccumulates and biomagnifies in aquatic food webs. Mercury bioaccumulation and biomagnification rates are known to vary among species utilizing different food webs (benthic vs limnetic) within and between systems. The authors assessed whether carbon and nitrogen stable isotope values and total Hg (THg) concentrations differed between sympatric benthic and limnetic ecotypes and sexes of threespine stickleback fish (*Gasterosteus aculeatus*) from Benka Lake, Alaska, USA. The mean THg concentration in the limnetic ecotype was significantly higher (26 mg/kg dry wt, 16.1%) than that of the benthic ecotype. Trophic position and benthic carbon percentage utilized were both important determinants of THg concentration; however, the 2 variables were of approximately equal importance in females, whereas trophic position clearly explained more of the variance than benthic carbon percentage in males. Additionally, strong sex effects (45 mg/kg dry wt, 29.4%) were observed in both ecotypes, with female fish having lower THg concentrations than males. These results indicate that trophic ecology and sex are both important determinants of Hg contamination even within a single species and lake and likely play a role in governing Hg concentrations in higher trophic levels.

Keywords

Benthic carbon; Bioaccumulation; Biomagnification; *Gasterosteus aculeatus*; Trophic position

INTRODUCTION

Considerable variation has been observed in the bioaccumulation and biomagnification of mercury (Hg) in fishes from different systems. This variation has been attributed variously to differences in lake and watershed level factors, the biology of individuals, and the

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All Supplemental Data may be found in the online version of this article.

SUPPLEMENTAL DATA

Table S1.

Figure S1. (156 KB PDF).

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structure and function of food webs. Lake- and watershed-dependent factors such as watershed morphology, lake chemistry, and proximity to Hg sources affect Hg methylation and demethylation reactions and, thus, determine the bioavailability of Hg in aquatic systems [1,2]. Differences in the age, growth rate, body condition, and sex of individuals composing a population also influence Hg accumulation in various compartments of a system. In many systems, older animals exhibit higher concentrations and body burdens [3], and the concentrations of Hg in tissues are often influenced by the composition of the tissue and the process of growth dilution [4]. Further, females often have lower concentrations of Hg in their tissues, though the mechanism responsible is not clear [5–9].

The structure of food webs largely establishes the pathways for bioavailable Hg through aquatic systems [10,11]. While both trophic position (TP; i.e., the vertical position of an individual within a food web [12,13]) and underlying dietary pathways of consumers (i.e., horizontal position in a food web, such as pelagic vs benthic feeders [11,14]) influence the concentration and biomagnification of contaminants in aquatic ecosystems [10,15], these factors may or may not act independently [11]. For example, Kidd et al. [16] found that in Lake Malawi, East Africa, concentrations of Hg were higher in biota connected to pelagic food webs than in species of a similar TP relying on benthic food sources, despite similar biomagnification rates in the 2 habitats.

While the importance of TP and foraging habitat has been used to explain differences in contaminant concentrations between species in the same system [16–22] and between populations in separate systems [1,2,23,24], the possibility of the same factors explaining intrapopulation differences has only recently received attention. Many species of freshwater fish display ecological divergence between individuals feeding on macroinvertebrates associated with littoral habitats (benthic ecotypes) and individuals feeding on zooplankton in the limnetic zone (limnetic ecotypes) [25]. Though these individuals of the same species inhabit the same system, they utilize largely separate food webs and likely have different pathways of dietary contaminant exposure.

The threespine stickleback (*Gasterosteus aculeatus*, hereafter “stickleback”) fish model provides an excellent system for examining the role of habitat-specific foraging in determining Hg concentrations. The stickleback is a widespread generalist consumer found throughout much of the Northern Hemisphere [26]. In Alaska, stickleback are the primary (sometimes the only) fish in many lakes and the principal prey of many sport-fish and bird species [26]. Stickleback display differentiation between benthic and limnetic ecotypes, both allopatrically in thousands of lakes around the Northern Hemisphere and sympatrically in several lakes along the Pacific coast of North America [25–27]. Sympatric ecotypes of stickleback provide an opportunity to examine the effects of habitat-specific foraging on Hg concentrations in a single species of fish from the same lake.

Benthic and limnetic ecotypes of stickleback have been studied extensively, and differences in their morphologies, ecologies, and life histories are well established [25–28]. The morphological differences between these 2 ecotypes consist of numerous traits that influence the foraging efficiency of fish feeding in different habitats and on different types of prey [25,26]. Benthic fish typically have deeper heads and bodies, fewer and more widely spaced gill rakers, more robust cranial morphology, and smaller eyes. Limnetic ecotypes have traits that are well suited for feeding on planktonic prey, including a more fusiform body shape, a longer and narrower snout, numerous fine gill rakers, and relatively large eyes. These morphological differences are correlated with several ecological traits, including diet, TP, and habitat use [14,25,29]. Additionally, in sympatric populations, benthic and limnetic stickleback differ in their reproductive allocations [28], behavior, and nesting sites [26,27]. The major differences between benthic and limnetic stickleback are maintained under

common conditions, and hybrids are intermediate, indicating that these traits are heritable [26,29]. Further, there is differential fitness of the ecotypes in their respective habitats [26,29], indicating that the distinction between ecotypes is ecologically and evolutionarily relevant.

Benka Lake is the only lake in Alaska (USA) known to have both benthic and limnetic ecotypes of stickleback. The Benka ecotypes have distinct morphologies, diets, and life histories similar to those seen in other sympatric populations of benthic and limnetic stickleback, though the Benka ecotypes are less differentiated [27–29]. To understand the role of habitat-specific foraging in determining Hg concentrations of stickleback from Benka Lake, we measured total Hg (THg) and stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in reproductive (age 2) females and males of both ecotypes. Specifically, isotope data were used to estimate TP and percentage of benthic carbon utilized (α). Levels of THg, TP, and α were used to address 3 questions: 1) Do THg concentrations vary between ecotypes? 2) Do THg concentrations vary between sexes? 3) How important is α relative to TP? The present study provides a means of examining the relative importance of α and TP in the accumulation of THg in a single population and establishes the foundation for future studies examining the relative importance of these factors in comparison to interpopulation variation.

MATERIALS AND METHODS

Study site

Benka Lake (62.1875 °N, 150.0040 °W) is a relatively small (<0.5 km²), landlocked lake located approximately 125 km north of Anchorage in the Cook Inlet basin of Alaska, USA (Figure 1). Benka Lake has several shallow bays, which provide benthic habitats, and a central basin, which provides limnetic habitats. The central basin is deep (maximum depth = 23 m) compared with similar-sized lakes in the region, and there are many areas where the shoreline descends steeply into deeper waters [27,30].

Sample collection

Stickleback were collected in July 2010 from 8 areas of Benka Lake that had previously been identified as breeding areas for either benthic or limnetic ecotypes (Figure 1) [27]. Stickleback were collected using unbaited 0.64-cm wire mesh minnow traps set near shore. While all traps were on the bottom and approximately the same distance from the shore, traps in benthic areas were set at depths <0.5 m, whereas traps in limnetic sites were often 1 to 2 m deep. Fish were designated as benthic or limnetic depending on collection site [27–29] and differences in cranial morphology (J.J. Willacker, unpublished data). Fish were killed with an overdose of buffered MS-222 anesthetic (Argent Laboratories), rinsed in lake water, and stored on crushed dry ice while in the field (2–8 h) and then at –80 °C in the laboratory. To account for differences in the isotopic baselines of benthic and limnetic food webs, 8 gastropods (*Helisoma anceps*) and 8 mussels (*Anodonta beringiana*) were collected from the same areas as stickleback and preserved in the same manner.

Sample preparation

Analyses utilized 40 adult fish of each sex from each ecotype except for limnetic males, which had a sample size of 39 (total $n = 159$). To minimize errors in sex assignment, only reproductively mature fish (based on gonad dissections and secondary sexual characteristics) were used. Use of reproductive fish also reduced variation in age since the vast majority of reproductive stickleback are age 2 [26,28]. For each fish, standard length (anterior tip of premaxilla to posterior border of hypural plate) was measured to the nearest millimeter, and a unique specimen identification number was assigned. The head of each specimen was then

removed with a cut directly behind the operculum for a separate study of cranial morphological divergence in Benka Lake stickleback. The exclusion of heads did not alter isotope ($t = -0.53$; degrees of freedom [df] = 58, $p = 0.591$) or Hg ($t = 0.68$, $df = 58$, $p = 0.496$) values compared with those of whole (head-on) homogenates of stickleback ($n = 30$). Thus, all further preparation procedures and analyses are for the headless carcasses. In addition, all macroparasites (*Schistocephalus solidus* and Anisakidae), eggs, and stomach contents were removed to prevent biases due to the presence of unassimilated materials and the high lipid content of eggs (mean lipid content of eggs = 10.5% dry weight, $n = 36$).

Headless stickleback carcasses were freeze-dried for 72 h and then ground into a fine powder using a Beadbeater tissue mill (Bio Spec Products) with 3.2-mm stainless steel beads. Gastropod and mussel samples were removed from their shells and freeze-dried for 144 h (due to their larger volumes) before homogenization in the tissue mill.

Stable isotope analysis

For each sample, approximately 0.5 mg of dried homogenate was sealed in a tin capsule and analyzed for stable carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) isotope ratios at the University of Alaska Anchorage Environmental and Natural Resources Institute Stable Isotope Laboratory (Anchorage, AK, USA), using a Thermo-Finnigan Delta Plus XP Isotope Ratio Mass Spectrometer coupled to a Costech 4010 Elemental Combustion System. Isotope ratios are presented in standard δ notation as parts per thousand (‰) differences between the isotope ratio of the sample and that of a standard (Vienna Pee Dee Belemnite for carbon and air for nitrogen). Samples depleted in the heavier isotope (^{13}C or ^{15}N) in comparison with the standard have lower δ values. All instruments are regularly calibrated using a suite of International Atomic Energy Agency standards to ensure that precision, accuracy, and tuning parameters are to specification. At least 6 replicates each of a stable isotope reference material (SRM 1547: peach leaves, available from the National Institute of Standards and Technology; $\delta^{13}\text{C} = -25.89 \pm 0.3\text{‰}$, $\delta^{15}\text{N} = 1.90 \pm 0.3\text{‰}$) and laboratory working standard (Cheney Lake stickleback; $\delta^{13}\text{C} = -29.50 \pm 0.2\text{‰}$, $\delta^{15}\text{N} = 10.13 \pm 0.1\text{‰}$) were analyzed with every batch of 40 samples. External instrument reproducibility for both carbon and nitrogen isotope analysis was $\pm 0.2\text{‰}$. Isotope values were lipid-normalized for a subset of samples ($n = 40$); however, because lipid content was uniformly low in carcasses (lipid of males and females with eggs removed = $2.1 \pm 0.7\%$, C:N = 3.34 ± 0.3), normalization had minimal impact and nonnormalized values are presented.

Hg analysis

Concentrations of THg in stickleback were measured in approximately 26 ± 4 mg of dried headless carcass homogenate at the Wildlife Toxicology Laboratory, University of Alaska Fairbanks, using the DMA-80 Direct Mercury Analyzer (Milestone). Quality assurance/quality control followed standard laboratory procedures and included duplicate method blanks, spiked blanks, and standard reference materials run with each batch of 20 samples. The standard reference material consisted of fish protein homogenate (DORM-3; THg = 0.382 ± 0.060 mg/kg) obtained from the National Research Council of Canada. Recovery rates for spiked blanks ranged from 88% to 97%. All samples were initially analyzed in duplicate, and when the relative standard deviation between 2 replicates was greater than 10%, additional replicates were run. Mercury data are presented on a dry weight basis but can be converted to wet weight estimates using the formula

$$[\text{THg}]_{\text{w}} = [\text{THg}]_{\text{d}} \times [1 - (0.01 \times \text{PM})]$$

where $[\text{THg}]_{\text{w}}$ is the estimated wet weight THg concentration, $[\text{THg}]_{\text{d}}$ is the measured dry weight THg concentration, and PM is the mean moisture percentage of stickleback used in the present study ($75.7\% \pm 0.02\%$, $n = 68$).

Statistical analysis

To assess the importance of trophic ecology as a determinant of THg concentration, we calculated the proportion of carbon from benthic sources (α) in the diet of individual stickleback using the equation in Post [12]

$$\alpha = (\delta^{13}\text{C}_{\text{stickleback}} - \delta^{13}\text{C}_{\text{lim}}) / (\delta^{13}\text{C}_{\text{ben}} - \delta^{13}\text{C}_{\text{lim}})$$

where $\delta^{13}\text{C}_{\text{lim}}$ is the $\delta^{13}\text{C}$ value of the limnetic baseline (mussels) and $\delta^{13}\text{C}_{\text{ben}}$ is the $\delta^{13}\text{C}$ value of the benthic baseline (gastropods). We also calculated the TP of stickleback following Post [12]

$$\text{TP}_{\text{stickleback}} = \lambda_{\text{base}} + (\delta^{15}\text{N}_{\text{stickleback}} - [\delta^{15}\text{N}_{\text{ben}} \times \alpha + \delta^{15}\text{N}_{\text{lim}} \times (1 - \alpha)]) / \Delta_{\text{N}}$$

where λ_{base} is the TP of the consumers that serve as the isotopic baselines ($\lambda_{\text{base}} = 2$), $\delta^{15}\text{N}_{\text{ben}}$ is the $\delta^{15}\text{N}$ value of the benthic baseline, $\delta^{15}\text{N}_{\text{lim}}$ is the $\delta^{15}\text{N}$ value of the limnetic baseline, and Δ_{N} is the trophic enrichment between consumers. In the present study a Δ_{N} of 2.63 was used based on observed trophic fractionation in controlled feeding experiments with threespine stickleback from 2 nearby populations (J.J. Willacker, unpublished data). When a generalized Δ_{N} of 3.4 is utilized, the results are qualitatively the same (J.J. Willacker, unpublished data).

Prior to analysis, data were examined to ensure that they met the underlying assumptions of parametric statistics. Mercury data were natural log-transformed to meet the assumption of homogeneity of variances, and geometric means are presented unless otherwise noted. Differences in standard length, $\delta^{13}\text{C}$, α , $\delta^{15}\text{N}$, TP, and THg between ecotypes were initially assessed using 2 sample *t* tests. After establishing that the ecotypes differ, we followed a more refined approach of using α as a measure of resource use because α is a continuous, quantitative variable that accounts for ecological variation within ecotypes as well as differences between ecotypes [14]. For analyses that could be conducted in common using either ecotype as a fixed factor or α as an independent variable, the results were qualitatively the same; therefore, we present model results that employed α as an independent variable rather than ecotype as a fixed factor. We used linear regression coupled with quantitative model selection techniques to examine the effects of sex, standard length, α , and TP on THg concentrations. Sample size-corrected Akaike's information criterion (AIC_C) was used to select the most parsimonious model for explaining THg concentrations from a set of a priori candidate models [31]. A systematic AIC_C approach was used to first compare 34 candidate models including all possible main-effects combinations of sex, α , TP, and standard length, as well as interactions between sex and each covariate (Supplemental Data, Table S1).

Differences between the AIC_C of the best model and the other candidate models (ΔAIC_C) and the Akaike weights (W) of candidate models were compared to assess each model's probability of being the best-fitting model [31]. Interpretation of main effects was complicated by interactions between sex and the covariates in the top model. Therefore, models were split by sex, and a set of 8 candidate models were compared for each sex. These second-order models were assessed in the same way as the first-order models. In addition, partial regressions were used to assess the relative importance of each variable in the top models. Analysis of covariance was not employed because collinearity between α and TP violates the assumption of independent covariates. All analyses were conducted in R version 2.9.2 [32].

RESULTS

Benthic and limnetic primary consumers in Benka Lake were distinct in their $\delta^{13}\text{C}$ values, with limnetic mussels isotopically depleted compared to benthic gastropods (-35.0‰ and $-$

20.1%, respectively; $t = 24.83$, $df = 14$, $p < 0.001$). The 2 ecotypes of stickleback were significantly differentiated in all variables measured (Table 1). Stickleback ranged in standard length from 44 mm to 67 mm, with benthic fish being larger on average than limnetic fish (Table 1; $t = 3.83$, $df = 157$, $p < 0.001$). The isotopic values of stickleback reflected those of the primary consumers of the habitat in which they were captured, with limnetic individuals having depleted $\delta^{13}\text{C}$ values compared to fish from the benthic habitat (Table 1; $t = 2.94$, $df = 157$, $p = 0.004$). Calculated α values for individuals ranged from 0.04 to 0.83, and again fish captured from the limnetic habitat had a lower mean α than fish from the benthic habitat (Table 1; $t = 2.94$, $df = 157$, $p = 0.004$). The TP of individuals ranged from 3.56 to 4.86, and fish of the limnetic ecotype had on average higher $\delta^{15}\text{N}$ ($t = -4.27$, $df = 157$, $p < 0.001$), and thus TP ($t = -4.50$, $df = 157$, $p < 0.001$), than fish of the benthic ecotype (Table 1).

Mean Hg concentrations were 16.1% higher in limnetic stickleback than in benthic stickleback (Table 1; $t = -3.15$, $df = 157$, $p = 0.002$). Overall, male stickleback had 29.4% higher mean THg concentrations than female stickleback (mean THg = 0.198 mg/kg and 0.153 mg/kg dry wt for males and females, respectively; $t = -6.38$, $df = 157$, $p < 0.001$). The difference between sexes was maintained in both benthic ($t = -4.90$, $df = 78$, $p < 0.001$) and limnetic ($t = -4.50$, $df = 77$, $p < 0.001$) ecotypes (Figure 2). Total Hg concentrations were not associated with fish size (standard length; $r = 0.12$, $n = 159$, $p = 0.158$).

The most parsimonious model explaining THg concentrations in stickleback included sex, α , and TP as main effects (ΔAIC_C for all other models >2 ; Supplemental Data, Table S1). The best-fitting model also included interactions between sex and both covariates, suggesting that both covariates differed in their relationship with THg for female and male fish. Subsequent assessment of ΔAIC_C and Akaike's weights in sex-specific models indicated a high probability that the top model was the most parsimonious ($W = 0.649$ and $W = 0.708$ for females and males, respectively); both the female and male models included both α and TP as main effects (Table 2). However, the treatment of α in the models differed by sex. In particular, males demonstrated a slightly positive relationship between THg and α , while females demonstrated a negative relationship. In both sexes, TP was positively correlated with THg, though the coefficients differed.

Due to a high degree of covariation between α and TP, partial regressions were used to assess the relative importance of each variable to the models (Figure 3). Partial regressions indicated that TP was only slightly more important than α in determining THg concentration (partial regression coefficient, $\eta^2 = 0.101$ and 0.057 for TP and α , respectively) in females. In contrast to females, the partial regressions of the male model demonstrate that TP is clearly more important than α ($\eta^2 = 0.217$ and 0.053 for TP and α , respectively). Variable weights supported these conclusions and showed that α and TP were approximately equal in importance ($V = 0.858$ and 0.903 , respectively) in females, while TP ($V = 1.000$) was a more important factor than α ($V = 0.706$) in males.

DISCUSSION

Stickleback occupy middle TPs in most food webs and are important prey for many piscivorous fishes and birds. Thus, additional Hg biomagnification will occur at TPs above that of stickleback. Canada [33] and California [34] both have set an Hg advisory level of 0.033 mg/kg wet weight for forage fish. This value incorporates the potential for additional biomagnification in piscivorous wildlife and is thus a conservative guide for assessing Hg levels in forage fish. In Benka Lake, the overall mean THg concentration in stickleback was over this advisory level; however, segregated by sex and ecotype, benthic females had THg concentrations that were not significantly different from the advisory level (single sample t

test, $t = -0.51$, $df = 39$, $p = 0.615$), while THg concentrations in males of both ecotypes and in limnetic females were significantly higher than the advisory level ($p = 0.002$). Thus, while the differences in Hg concentrations reported in the present study are small, they may be large enough to impact the vulnerability of some sensitive wildlife species, especially long-lived, high-trophic level consumers. These results underscore the importance of both sex and food-web utilization in determining Hg accumulation in higher trophic levels.

The limnetic ecotype of stickleback in Benka Lake had a significantly higher (16.1%) concentration of THg than the benthic ecotype. Differentiation in the Hg concentrations of trophically divergent species and populations has been examined in a variety of systems [1,2,16–24]; to date, however, few studies have examined the role of habitat-specific foraging in a single species from a single system (but see Chumchal et al. [17]). As in the present study, Hg concentrations in limnetic feeding fish are typically higher than corresponding concentrations in fish utilizing benthic habitats.

Two explanations for this trend have been proposed. First, limnetic food webs may have more bioavailable Hg at low TPs or Hg may be more efficiently accumulated from limnetic habitats. Phytoplankton are known to bioconcentrate Hg at a much higher rate than benthic algae [35] and often have Hg concentrations thousands of times greater than the surrounding water [22,23]. In addition, zooplankton and littoral macroinvertebrates differ in their feeding specificities. While zooplankton primarily consume bacteria and phytoplankton of autochthonous origin, inputs of terrestrial and lake detritus are a major source of energy for littoral macroinvertebrates. Because detrital materials do not actively incorporate new Hg from the water, use of these resources could reduce the incorporation of environmental Hg into the littoral food web [24]. As a result of these factors, differences in Hg concentration have also been observed between limnetic zooplankton and littoral associated macroinvertebrates [24] and between limnetic and littoral-associated zooplankton [36]. Furthermore, Paterson et al. [37] found that methylmercury concentrations in zooplankton increased more rapidly and to a greater extent than in littoral macroinvertebrates following experimental increases in the production of methylmercury. These data support the hypothesis that higher THg concentrations in limnetic feeding fish are driven by increased uptake of Hg into limnetic food webs compared with benthic food webs.

Another explanation for higher Hg concentrations in limnetic food webs relates to differing biological conditions in the 2 food webs. Specifically, differences in size, age, productivity, growth rates, and TP are known to alter the concentration of Hg in fish. Many studies have demonstrated that Hg biomagnifies through food webs such that individuals at higher TPs often have substantially higher Hg concentrations than individuals at lower TPs. The biomagnification of contaminants in a food web is related to the number of trophic transfers in the web (i.e., food chain length) [15] as well as the assimilation efficiency of the consumers. The rate of Hg assimilation in consumers does not vary widely between lake food webs [2,10,16]; however, limnetic food webs typically have more trophic transfers and a greater degree of omnivory (i.e., consumers feeding on multiple trophic levels) than littoral food webs [13,15]. Thus, higher Hg concentrations in limnetic foraging fish could result from higher TPs in limnetic habitats compared with benthic habitats.

While this mechanism may be an important determinant of biomagnification for other contaminants [11], few studies have found support for TP driving benthic–limnetic differentiation in Hg concentrations. In most studies, differences in benthic and limnetic Hg concentrations were associated with differing Hg availability to low trophic levels rather than differences in the TP of consumers or biomagnification of Hg through a food web [1,16–19, 22, 24]. For example, Ethier et al. [1] found that limnetic feeding yellow perch

(*Perca flavescens*) had higher Hg concentrations than benthic feeding pumpkinseed sunfish (*Lepomis gibbosus*) despite the similarity in their TPs.

In the present study, benthic and limnetic stickleback differed in their TPs, with the limnetic ecotype having a higher mean TP than the benthic ecotype. Multiple regression and AIC indicate that both α and TP are important determinants of THg concentrations in Benka Lake stickleback; however, the 2 variables were strongly colinear. Thus, we used variable weights and partial regressions to examine the relative importance of the 2 variables. While results differed between the sexes, TP was a more important determinant of THg concentration than α in both female and male stickleback. The higher relative importance of TP compared to α is consistent with the results of the only other study of habitat-specific Hg accumulation in the sub-Arctic [21] but differs from studies conducted in more temperate to tropical regions [1,16–19, 22, 24]. Power et al. [21] suggested that the increased importance of TP (approximated as $\delta^{15}\text{N}$) observed in Stewart Lake, Nunavik, Canada, compared with more temperate lakes might be due to reduced growth rates. Growth rate is known to influence Hg concentration by regulating the dilution of Hg in tissue [4,35]. Differences in the growth rates of benthic and limnetic foraging fish could provide a mechanism to explain increased biomagnification rates in the limnetic versus benthic food webs of Benka Lake, where shallow benthic habitats warm faster in the spring and are typically 4 °C to 6 °C warmer than deep limnetic habitats (Supplemental Data, Figure S1). We found that adult benthic stickleback in Benka Lake are significantly larger than limnetic stickleback of the same age, consistent with temperature differences in the 2 habitats but also possibly due to foraging differences. Cresko [29] found that stickleback confined to benthic habitats of Benka Lake grew faster than those confined to limnetic habitats. Baker et al. [28] also suggest that temperature and resulting differences in growth rates are responsible for divergent reproductive strategies in benthic and limnetic females from Benka Lake. In sum, the lower observed Hg concentration in benthic stickleback from Benka Lake could be due to growth dilution resulting from either temperature or foraging effects.

While α was a significant determinant of THg concentrations in Benka Lake stickleback, we did not find as strong of a correlation as has been observed in other systems [1,16–22,24]. This is expected, considering that we parsed variation between TP and α rather than relying on simple regressions between THg and each covariate, which overestimates the variance explained [31]. Additionally, we examined a single species within a single lake, while most studies have examined multiple species in a single lake or a single species across multiple lakes. Although the 2 ecotypes in Benka Lake are morphologically and ecologically divergent, they represent modal phenotypes across a relatively narrow continuum of differentiation and thus overlap in their morphological and ecological distributions [27–29]. The mean level of benthic carbon differed significantly between the ecotypes, but the difference was modest (36% vs 29% for benthic and limnetic fish, respectively; Table 1). Thus, both ecotypes in Benka Lake heavily utilize limnetic resources. This small but significant difference in diet mirrors differences in the morphologies of the 2 ecotypes and supports the conclusion that the Benka Lake ecotypes are less differentiated than sympatric benthic–limnetic species pairs of stickleback in British Columbia or some allopatric populations throughout the Cook Inlet basin [27–29]. Considering the modest extent of dietary differences in Benka Lake stickleback, it is not surprising that the relationship between THg concentration and resource utilization is reduced compared to this relationship for allopatric populations or different species.

In both ecotypes, females had lower THg concentrations than males. Sex differences were greater than differences between ecotypes. The relationship between THg concentration, TP, and α differed in the 2 sexes. Partial regressions and variable weights indicate that, in males, TP is a more important determinant of THg concentration than α . In females, although TP is

more important than α , the difference is modest and both variables displayed low partial regression coefficients. The seemingly low explained variance of the 2 variables in females is likely a reflection of the similar but opposite partial regression coefficients, which result in underestimation of the variance assigned to each variable [38].

Similar trends have been observed in several other species, and in some species, sex is the primary factor governing Hg bioaccumulation [6,7]. However, little agreement exists on the mechanisms underlying these differences. In many species of fish, including threespine stickleback, females and males are often ecologically differentiated [39]. In some populations of stickleback, the sexes are known to differ in their habitat use, feeding behavior, and trophic morphology [26]. In the present study, the sexes were ecologically distinct, with females of both ecotypes utilizing more benthic carbon than males. Furthermore, the pattern of isotopic divergence between the sexes mirrored differences in THg concentrations, suggesting that trophic differences may account for the observed differences in THg between the sexes. However, the different relationships between THg concentration, TP, and α in males and females indicate that sexual differences in THg are not a simple matter of the sexes occupying different positions along the benthic–limnetic axis. Thus, additional factors likely contribute to sexual differences in THg concentration.

It has been suggested that lower Hg concentrations in female fish are due to the loss of Hg in eggs [6,9,40]. However, Hg concentration in females of several fish species increases following egg deposition [8]. Furthermore, Niimi [8] and others [6,7,40] showed that the Hg concentration of eggs is only 0.3% to 2.3% of an individual's body concentration and concluded that spawning is not sufficient to explain differences in Hg concentration between the sexes. However, in Benka Lake stickleback, egg THg ranged from 0.034 mg/kg to 0.045 mg/kg dry weight ($n = 60$), which is 21.7% to 24.7% of the mean concentration in the body in both ecotypes (J.J. Willacker, unpublished data). Additionally, stickleback produce multiple egg clutches during the breeding season [26], which may result in high net loss despite relatively low loss in each clutch. Therefore, our Benka Lake data suggest that egg sequestration could be a more important driver of female–male Hg differences than in other species. In addition, these studies have examined only changes in tissue concentrations rather than burdens. Thus, changes in the total amounts of Hg in female tissues across the breeding season have not been examined. Furthermore, these studies generally treated fish as a single compartment and did not separately examine Hg burdens in the somatic and reproductive tissues. Such differentiation could provide important insights on the distribution of Hg in fish tissues and the mechanisms generating sexual differences.

Higher THg concentrations in males could also be the result of slower growth rates and, thus, reduced growth dilution of Hg. Differential growth rates have been implicated as a potential cause of sex-based differences in the Hg concentrations of several fish taxa, including northern pike (*Esox lucius*) [3], sharks [5], sunfish [9], and lake trout (*Salvelinus namaycush*) [7]. In stickleback, parental care is provided by males, and this care is energetically expensive [26]. Additionally, male stickleback are restricted to foraging near their nest during the breeding season, while females forage over large areas and prey on energetically productive eggs [26]. Therefore, it is plausible that growth rates differ between the sexes, contributing to differences in THg concentration.

Our results demonstrate that both sex and habitat-specific foraging can be important determinants of THg concentrations in a single threespine stickleback population. Further, we found that the relationships between THg concentration, TP, and α differed between the sexes such that TP and α were of approximately equal importance in female fish but TP was more important than α in male fish. Our data suggest that sex may play a critical role in determining Hg concentrations and modifying the roles of ecological factors and, therefore,

that the sexes should be examined separately in fish Hg studies. Additionally, the mechanisms underlying sexual differentiation of Hg levels need to be further examined.

The threespine stickleback species complex provides a valuable model for studying details of Hg ecology. In particular, the differentiation between benthic and limnetic feeding stickleback, both within and between lakes, provides an opportunity to examine the relative importance of habitat-specific foraging in relation to lake- and watershed-scale variables. Additionally, stickleback are easily reared under a variety of laboratory conditions and are thus amenable to captive studies examining the roles of individual environmental factors, their interactions, and the mechanisms by which these factors influence Hg accumulation in fish. Collectively, such field and laboratory studies can improve our understanding of Hg dynamics in aquatic systems and provide valuable insights into the factors governing Hg accumulation in higher trophic levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Map of Benka Lake, Alaska, USA, indicating benthic (circles) and limnetic (triangles) sampling sites. Depth contours are in 3.05-m increments. Grayed areas are islands.

Figure 2. Mean total mercury concentrations $\pm$ standard error (SE) in threespine stickleback from Benka Lake, Alaska, USA, by ecotype (benthic, limnetic) and sex (female, male). Bold letters designate group means that are statistically different ($p < 0.05$).

Figure 3. Relationships between log-transformed total mercury and (A) benthic carbon percentage (α) and (B) trophic position for female (solid points and lines) and male (open points and dashed lines) threespine stickleback from Benka Lake, Alaska, USA.

Table 1

Means (geometric and arithmetic for total mercury, arithmetic only for others) with standard deviation in parentheses for variables for benthic and limnetic ecotypes of threespine stickleback from Benka Lake, Alaska, USA

Parameter	Benthic (<i>n</i> = 80)	Limnetic (<i>n</i> = 79)	<i>p</i> ^a
Standard length (mm)	57.64 (3.9)	54.75 (5.1)	<0.001
δ ¹³ C (‰)	-29.56 (2.7)	-30.73 (2.2)	0.004
α ^b	0.36 (0.2)	0.29 (0.2)	0.004
δ ¹⁵ N (‰)	8.43 (1.2)	9.11 (0.8)	<0.001
TP	4.22 (0.3)	4.43 (0.2)	<0.001
THg (mg/kg dry wt)			
Geometric	0.156 (0.03)	0.181 (0.03)	0.002
Arithmetic	0.162 (0.05)	0.188 (0.05)	0.002

^a *p* values indicate results of *t* tests for significance between ecotypes.

^b The proportion of dietary carbon from benthic sources.

TP = trophic position; THg = total mercury.

Table 2

Structure and evaluation criteria for candidate models describing natural log-transformed total mercury concentrations in female and male threespine stickleback from Benka Lake, Alaska, USA

Model	Model structure	n	R ²	AIC _c ^a	ΔAIC _c ^b	w ^c
Females						
1	lnTHg ~ α + TP + SL	80	0.30	11.30	3.86	0.09
2 ^d	lnTHg ~ α + TP	80	0.31	7.43	0.00	0.65
3	lnTHg ~ α + SL	80	0.23	17.71	10.27	0.00
4	lnTHg ~ TP + SL	80	0.29	12.14	4.71	0.06
5	lnTHg ~ α	80	0.24	13.97	6.54	0.02
6	lnTHg ~ TP	80	0.28	10.15	2.72	0.17
7	lnTHg ~ SL	80	0.00	37.09	29.66	0.00
8	Null	80	0.00	35.08	27.65	0.00
Males						
1	lnTHg ~ α + TP + SL	79	0.22	-10.88	3.41	0.13
2 ^d	lnTHg ~ α + TP	79	0.20	-14.29	0.00	0.71
3	lnTHg ~ α + SL	79	0.02	3.53	17.82	0.00
4	lnTHg ~ TP + SL	79	0.15	-6.11	8.18	0.01
5	lnTHg ~ α	79	0.00	3.01	17.30	0.00
6	lnTHg ~ TP	79	0.15	-11.19	3.09	0.15
7	lnTHg ~ SL	79	0.03	1.56	15.85	0.00
8	Null	79	0.00	1.09	15.38	0.00

^aSample size-corrected Akaike's information criterion (AIC_c).

^bThe difference between the current model AIC_c and the AIC_c of the most parsimonious model.

^cAkaike's weight; the likelihood of the current model relative to others in the candidate set.

^dThe most parsimonious candidate model for each sex.

lnTHg = natural log-transformed total mercury; α = proportion benthic carbon; TP = trophic position; SL = standard length.