

OXIDATIVE STRESS RESPONSE OF FORSTER'S TERNS (*STERNA FORSTERI*) AND CASPIAN TERNS (*HYDROPROGNE CASPIA*) TO MERCURY AND SELENIUM BIOACCUMULATION IN LIVER, KIDNEY, AND BRAIN

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Abstract—Bioindicators of oxidative stress were examined in prebreeding and breeding adult and chick Forster's terns (*Sterna forsteri*) and in prebreeding adult Caspian terns (*Hydroprogne caspia*) in San Francisco Bay, California. Highest total mercury (THg) concentrations (mean \pm standard error; $\mu\text{g/g}$ dry wt) in liver (17.7 ± 1.7), kidney (20.5 ± 1.9), and brain (3.0 ± 0.3) occurred in breeding adult Forster's terns. The THg concentrations in liver were significantly correlated with hepatic depletion of reduced glutathione (GSH), increased oxidized glutathione (GSSG):GSH ratio, and decreased hepatic gamma-glutamyl transferase (GGT) activity in adults of both tern species. Prefledging Forster's tern chicks with one-fourth the hepatic THg concentration of breeding adults exhibited effects similar to adults. Total mercury-related renal GSSG increased in adults and chicks. In brains of prebreeding adults, THg was correlated with a small increase in glucose-6-phosphate dehydrogenase (G-6-PDH) activity, suggestive of a compensatory response. Brain THg concentrations were highest in breeding adult Forster's terns and brain tissue exhibited increased lipid peroxidation as thiobarbituric acid-reactive substances, loss of protein bound thiols (PBSH), and decreased activity of antioxidant enzymes, GSSG reductase (GSSGr), and G-6-PDH. In brains of Forster's tern chicks there was a decrease in total reduced thiols and PBSH. Multiple indicator responses also pointed to greater oxidative stress in breeding Forster's terns relative to prebreeding terns, attributable to the physiological stress of reproduction. Some bioindicators also were related to age and species, including thiol concentrations. Enzymes GGT, G-6-PDH, and GSSGr activities were related to species. Our results indicate that THg concentrations induced oxidative stress in terns, and suggest that histopathological, immunological, and behavioral effects may occur in terns as reported in other species. Environ. Toxicol. Chem. 2011;30:920–929. © 2011 SETAC

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INTRODUCTION

Mercury (Hg) and selenium (Se) are widely distributed contaminants that often co-occur in the environment, and have been shown to cause adverse effects in wildlife. Methylmercury (MeHg) bioaccumulates in aquatic ecosystems with adverse effects to piscivorous birds [1–7]. Methylmercury is thought to be one of the possible causes for reduced reproduction of long-legged wading birds (Ciconiiformes) in the Florida Everglades in recent decades [8–10] and toxic responses to MeHg have been documented for piscivorous birds within the lower Carson River system of Nevada, USA, where evidence of cellular damage (i.e., histopathologic and physiologic, including oxidative stress) in the nervous, immune, hepatic, and renal systems of young snowy egrets (*Egretta thula*), black-crowned night-herons (*Nycticorax nycticorax*), and double-crested cormorants (*Phalacrocorax auritus*) were reported [11–13]. Additionally, common loons (*Gavia immer*) in northeast North America have been shown to exhibit impaired reproduction, due in part to MeHg [14–15]. Selenium in wild birds has been associated with mortality, impaired reproduction, teratogenesis, and histopathological lesions with alterations in glutathione status [16–20].

Mercury and Se contamination in the tributaries of San Francisco Bay (CA, USA) has resulted in elevated concentrations of both contaminants in abiotic and biotic components across the estuary [21,22]. Specifically, recent work has identified levels of Hg [23,24] and Se [25] in waterbird tissues that approach or exceed toxicity thresholds for reproductive impairment. Additionally, previous studies of waterfowl wintering on San Francisco Bay have shown that diving ducks may contain potentially toxic concentrations of both Hg and Se [26,27]. For example, livers of some diving ducks contained concentrations of Se similar to those in dabbling ducks (*Anas* spp.) from the San Joaquin Valley (CA, USA), where severely impaired reproduction was correlated with Se [16,18]. At the same time, concentrations of Hg in livers of diving ducks were higher than concentrations in livers that affected overall reproductive success over multiple generations in mallards (*A. platyrhynchos*) [28]. Recent work has shown that THg concentrations in diving duck livers increased with time spent in the estuary, before migrating to their breeding grounds [24]. Similar time trends have been documented in breeding migratory birds in the region. For example, 13 and 22% of Forster's terns sample during prebreeding (March to May) in San Francisco Bay had blood ($>3.0 \mu\text{g/g}$ wet wt) and feather Hg concentrations ($>20.0 \mu\text{g/g}$ fresh wt), respectively, indicating that they may be at high risk for deleterious effects of mercury [29], whereas 48% of breeding adult Forster's terns exceeded high-risk blood THg concentrations of $3 \mu\text{g/g}$ wet weight during

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the breeding season [24]. Selenium may also pose an issue for terns, but at the same time may help detoxify Hg in the liver [27]. In San Francisco Bay, Se concentrations ($\mu\text{g/g}$ dry wt) in livers ranged from 3.7 to 14.5 in adult Forster's terns (7.1 ± 0.4) and 4.8 to 14.4 in adult Caspian terns (6.7 ± 0.8), and body condition was negatively related to liver Se concentrations in adult Forster's terns [25]. However, it is unclear if Hg or Se are causing toxic responses in Forster's terns or Caspian terns.

Oxidative stress and altered glutathione metabolism have been associated with exposure to Hg and to Se, providing an index of potential cellular toxicity in wildlife. Oxidative stress (disturbance of the prooxidant-antioxidant balance) is recognized as one cause of contaminant toxicity in wildlife that may ultimately result in cellular dysfunction and damage. Reduced glutathione (GSH) and associated antioxidant enzymes are major combatants of oxidative stress that influence redox status. Several studies with mallards and other species of aquatic birds have confirmed the utility of such measurements for Hg and Se exposure both in the laboratory and the field [20,30]. Therefore, in the present study we examined a number of bioindicators of altered glutathione metabolism and oxidative stress in prebreeding and breeding adults and pre fledging chicks in two tern species, Forster's tern and Caspian tern, in San Francisco Bay. Our goal was to evaluate whether current exposure to Hg and/or Se may be inducing sublethal toxicity in these bird species and to identify the life-history stages that may be most at risk to contaminant-related stress.

MATERIALS AND METHODS

Sample collection

Sampling details have been previously described [23]. Birds were captured, collected, and marked under California Department of Fish and Game scientific collection permits, federal U.S. Fish and Wildlife Service permits, and U.S. Geological Survey Bird Banding Laboratory permits. Research was conducted under the guidelines of the U.S. Geological Survey, Western Ecological Research Center, Animal Care and Use Committee.

Therefore, we will only briefly describe the approach here. During 2005 we collected 68 terns (Forster's tern adults, $n = 46$; Forster's tern chicks, $n = 12$; and Caspian tern adults, $n = 10$) using either a shotgun (with steel shot), bow net, or by hand at three regions within the San Francisco Bay: North Bay ($n = 31$; Napa Marsh Restoration Area; $38^\circ 08' \text{ N } 122^\circ 18' \text{ W}$), Central South Bay ($n = 5$; Eden Landing Ecological Reserve; $37^\circ 36' \text{ N } 122^\circ 08' \text{ W}$), and Lower South Bay ($n = 32$; Don Edwards San Francisco Bay National Wildlife Refuge) as shown by map previously [24]. Sexes were determined via necropsy and confirmed via DNA analysis (Zoogen Services). We dissected internal tissues (liver, kidney, and brain) in the field, immediately after collection using stainless steel tools that were cleaned with nitric acid and hexane prior to use. Small aliquots (≈ 1 g wet wt) were subsampled from the tip of the larger lobe of each liver for oxidative stress and P450 measurements and placed in sterile, polypropylene cryovials. The remainder of each liver sample was stored in chemically cleaned I-CHEM glass vials (Chase Scientific Glass). All samples were immediately placed on dry ice, where they were stored until return to the laboratory, within 48 h. In the laboratory, samples were stored at -80°C until analysis and the remaining livers were stored at -20°C until processing and contaminant analyses.

Mercury and selenium determination

Total Hg concentrations were determined at the U.S. Geological Survey, Davis Field Station Mercury Lab (Davis, CA) and Se was determined by the Trace Element Research Lab, Texas A&M University (College Station, TX, USA), respectively, according to published methods [27]. A subset of livers was also analyzed for MeHg, but since the correlation between MeHg and THg was strong ($R^2 = 0.97$), as reported [23], we used THg concentrations in all subsequent analyses. Methylmercury was found to be approximately 88% of THg when THg concentrations were less than $8 \mu\text{g/g}$, but when above $8 \mu\text{g/g}$ the percent MeHg declined by approximately 10% per order of magnitude [27]. For kidneys, percent MeHg was approximately 80%. Brains were not analyzed for MeHg but in loons and eagles (*Haliaeetus leucocephalus*) with THg concentrations in the brain of less than $4 \mu\text{g/g}$ dry weight over 80% of Hg is MeHg [7].

The Hg:Se molar ratio increased linearly with log liver THg concentrations with a slope of 1.35 until $8 \mu\text{g/g}$ THg, at which point the slope declined to 0.75. Also, liver Se was correlated with liver inorganic Hg when liver THg concentrations exceeded $8 \mu\text{g/g}$, but there was no correlation when liver THg concentrations were below $8 \mu\text{g/g}$. This suggested that MeHg was demethylated in Forster's tern livers once the concentration of THg reached about $8 \mu\text{g/g}$, and that Se played a role in sequestering the inorganic Hg to facilitate removal. However, the rate of detoxification did not keep up with accumulation, so although demethylation reduced the overall toxicity, it was not completely effective [27].

Oxidative stress bioindicators

Basic methods and assay conditions are described [30]. Oxidative stress indicator assays of potential Hg or Se-related effects included: liver: glutathione peroxidase (GSHpx) including total glutathione peroxidase (T-GSHpx) and Se-dependent glutathione peroxidase (S-GSHpx), glutathione reductase (GSSGrd), glutathione-S-transferase (GSH-T), glucose-6-phosphate dehydrogenase (G-6-PDH), gamma-glutamyl transferase (GGT), reduced glutathione (GSH), oxidized glutathione (GSSG), total sulfhydryl concentration (TSH), protein bound sulfhydryl concentration (PBSH), thiobarbituric acid reactive substances (TBARS); kidney: blood urea nitrogen (BUN), uric acid (UA), and enzymes related to glutathione metabolism, oxidative stress, and TBARS per liver assays; brain: acetylcholinesterase (ACHE), plus all liver variables.

Portions of the liver, kidney, and brain were homogenized (1:10 wt/volume [w/v]) in ice-cold 1.15% KCl-0.01MNa, K-phosphate buffer (pH 7.4). Whole homogenate was used as the starting basis for determining GSH, TSH, PBSH, GSSG, and TBARS concentrations. The 10,000 g supernatant was used for all above assays of enzymes related to glutathione metabolism and antioxidant activity as previously described [30].

Statistical analysis

We used analysis of covariance (ANCOVA) to assess the influence of THg, Se, lifestage (prebreeding adult, breeding adult, or chick), and sex on oxidative stress biomarkers in liver, kidney, and brain tissues. We conducted separate ANCOVA models for each tissue biomarker. We included THg \times lifestage, THg \times species, THg \times sex, and THg \times Se interactions in all initial models and removed them from the final models if $p > 0.10$. For brain tissues, there was a significant

THg \times lifestage interaction for some of the biomarkers (see *Results*); therefore, we evaluated the responses of each lifestage separately. We used Tukey's post-hoc pairwise comparisons to evaluate the differences in biomarker response among factor categories. We considered the result for statistical significance $p < 0.10$.

RESULTS

Hepatic oxidative stress

The highest THg concentrations in liver were found in adult breeding terns. In fact, Forster's tern chicks had only half the hepatic THg concentration as prebreeding adults and less than one-fourth that of breeding adults.

After controlling for the effects of Se, species, lifestage, and sex, we found a significant negative relationship between liver THg concentrations and both GSH and GGT (Table 1, Fig. 1), as well as a marginally significant positive relationship between liver THg concentrations and GSSG:GSH ratios (Table 1, Fig. 1).

Liver biomarker activity was also related to lifestage for TSH, TBARS, PBSH, GSSG, and GSSGred (Table 1). Total sulfhydryl concentration activity did not differ between prebreeding and breeding adults, but adult TSH activity was higher than in chicks. Thiobarbituric acid reactive substances levels did not differ between chicks and either prebreeding or breeding adults, but levels were higher in breeding adults than prebreeding adults. Conversely, GSSGred was higher in chicks than either adult category. Protein bound sulfhydryl concentration activity was higher in prebreeding adults than chicks, but breeding adults did not differ from prebreeding adults or chicks. In contrast, GSSGred activity was higher in

chicks than prebreeding adults, and did not differ between breeding adults and either chicks or prebreeding adults.

We found significant species and sex effects only for GSSG:GSH (species effect), GSSGred (species effect), and G-6-PDH (sex effect), and selenium concentrations were not related to any of the biomarker responses (Table 1). GSSG:GSH was approximately twice as high in Forster's terns than in Caspian terns, whereas GSSGred was higher in Caspian terns than Forster's terns. Reduced glutathione was marginally significantly higher in Caspian terns than in Forster's terns. For G-6-PDH, males had higher levels than females.

Renal oxidative stress

The highest THg concentrations in kidney were found in adult breeding terns. After controlling for species, lifestage, and sex effects, renal THg concentrations were related only with GSSG activity, which was positively correlated with THg concentrations (Table 1, Fig. 2). We also found species differences in oxidative stress response in GSH, TSH, PBSH, GSSGred, and GGT (Table 1). Total sulfhydryl concentration, PBSH, and GGT activity was higher in Forster's terns than Caspian terns, whereas GSH and GSSGred activity was higher in Caspian terns than Forster's terns. Lifestage influenced oxidative stress activity for TSH, TBARS, and PBSH (Table 1). Prebreeding adult and breeding adult TSH and PBSH activity did not differ from one another, but were higher in both adult lifestages than in chicks. Conversely, TBARS were higher in breeding adults than prebreeding adults, but did not differ between chicks and either breeding or prebreeding adults.

Brain tissue oxidative stress

The highest THg concentrations in brain were found in adult breeding terns. In brain tissue there were significant

Table 1. Analysis of covariance model for biomarker response in liver and kidney tissue of Forster's terns and Caspian terns in San Francisco Bay, CA, USA

Biomarker		Liver biomarker response					Kidney biomarker response			
		THg	Se	Species	Lifestage	Sex	THg	Species	Lifestage	Sex
GSH	<i>df</i>	1,61	1,61	1,61	2,61	1,61	1,61	2,61	1,61	1,61
	<i>F</i> value	3.80	0.64	3.10	1.05	0.02	0.003	11.09	0.86	0.22
	<i>p</i> value	0.05	0.43	0.08	0.35	0.90	0.95	0.001	0.43	0.64
TSH	<i>df</i>	1,61	1,61	1,61	2,61	1,61	1,61	2,61	1,61	1,61
	<i>F</i> value	0.01	0.01	1.76	4.59	0.01	0.10	55.6	12.83	0.003
	<i>p</i> value	0.93	0.75	0.19	0.01	0.94	0.75	<0.0001	<0.0001	0.95
TBARS	<i>df</i>	1,61	1,61	1,61	2,61	1,61	1,61	2,61	1,61	1,61
	<i>F</i> value	0.07	0.10	0.01	5.00	0.01	1.27	0.17	7.76	2.72
	<i>p</i> value	0.80	0.75	0.92	0.01	0.94	0.26	0.68	0.001	0.10
PBSH	<i>df</i>	1,61	1,61	1,61	2,61	1,61	1,61	2,61	1,61	1,61
	<i>F</i> value	0.18	0.01	0.87	4.95	0.01	0.14	84.88	14.81	0.003
	<i>p</i> value	0.67	0.90	0.35	0.01	0.91	0.71	<0.0001	<0.0001	0.95
GSSG	<i>df</i>	1,61	1,61	1,61	2,61	1,61	1,61	2,61	1,61	1,61
	<i>F</i> value	0.85	0.03	2.16	6.27	1.01	4.80	2.39	1.31	0.63
	<i>p</i> value	0.36	0.85	0.15	0.003	0.32	0.03	0.13	0.28	0.43
GSSG:GSH	<i>df</i>	1,61	1,61	1,61	2,61	1,61	1,61	2,61	1,61	1,61
	<i>F</i> value	3.28	0.25	5.93	2.97	0.05	2.09	0.11	0.92	0.61
	<i>p</i> value	0.07	0.61	0.02	0.06	0.82	0.15	0.74	0.40	0.44
GSSGred	<i>df</i>	1,61	1,61	1,61	2,61	1,61	1,61	2,61	1,61	1,61
	<i>F</i> value	0.63	0.11	31.12	6.41	0.02	0.19	10.95	1.00	0.01
	<i>p</i> value	0.43	0.74	<0.0001	0.003	0.88	0.66	0.002	0.37	0.93
G-6-PDH	<i>df</i>	1,61	1,61	1,61	2,61	1,61	1,61	2,61	1,61	1,61
	<i>F</i> value	1.89	0.07	1.50	0.81	10.29	0.15	0.01	1.28	0.06
	<i>p</i> value	0.17	0.79	0.23	0.45	0.002	0.70	0.95	0.29	0.81
GGT	<i>df</i>	1,61	1,61	1,61	2,61	1,61	1,61	2,61	1,61	1,61
	<i>F</i> value	12.20	0.01	NA	1.51	0.17	0.02	6.94	1.12	2.63
	<i>p</i> value	0.001	0.95	NA	0.23	0.68	0.87	0.01	0.33	0.11

Model effects include tissue-specific total mercury (THg) and selenium (Se) concentrations, species (Forster's tern, Caspian tern), lifestage (prebreeding adult, breeding adult, chick), and sex (male, female). Italicized values indicate $p \leq 0.10$. See figure legends for biomarker abbreviations.

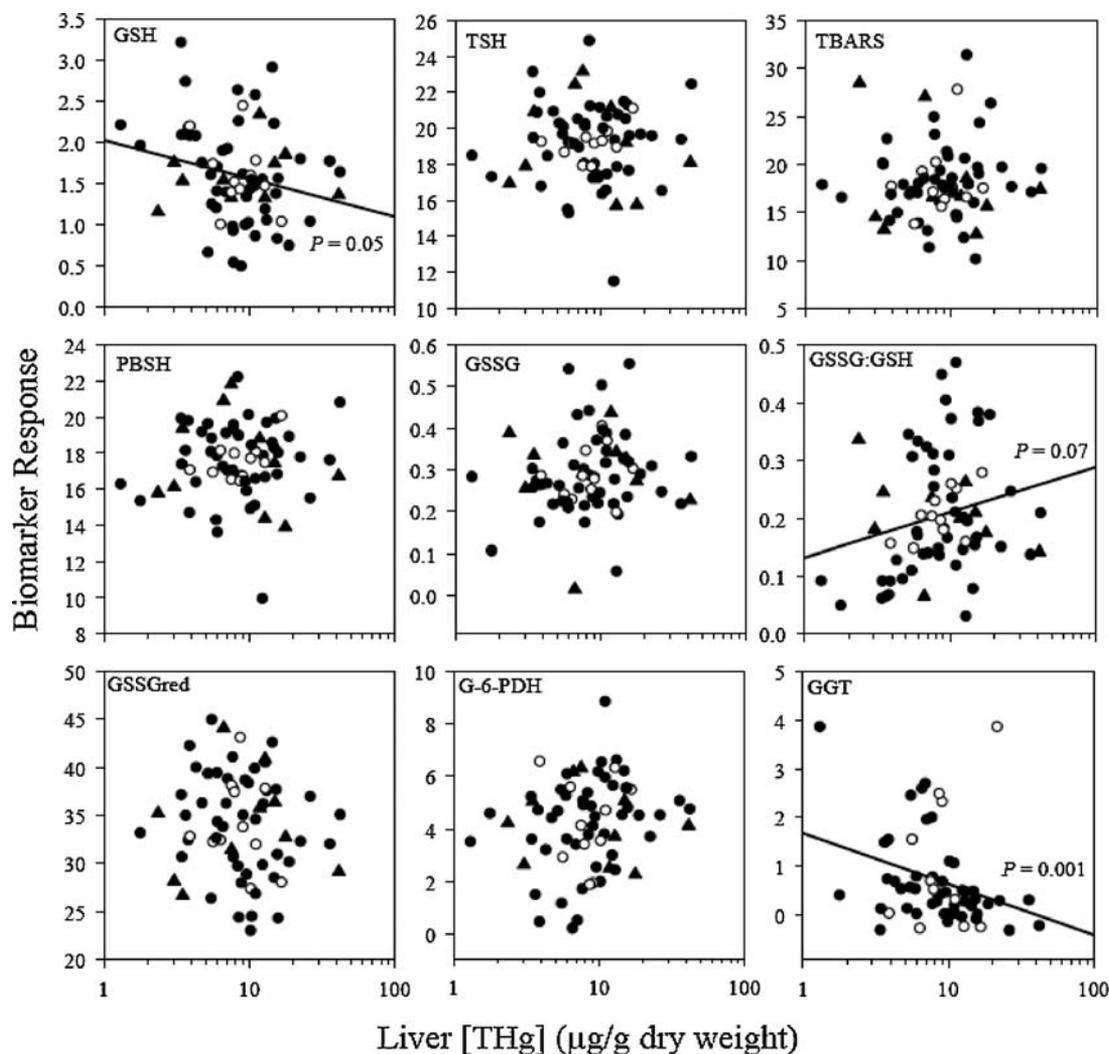


Fig. 1. Leverage plots for the relationship between total mercury (THg) concentrations ($\mu\text{g/g}$ dry wt) and a suite of biomarkers in livers of Forster's tern and Caspian tern adults (closed circles and closed triangles, respectively), and Forster's tern chicks (open circles) from San Francisco Bay, California, USA. Reduced glutathione (GSH), total thiols (TSH), protein bound thiols (PBBSH), and oxidized glutathione (GSSG) are expressed as $\mu\text{mol/g}$ of tissue; GSSG reductase (GSSGred) as nmoles of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidized/min/mg of 10,000 g supernatant protein; and glucose-6-phosphate dehydrogenase (G-6-PDH) and gamma-glutamyl transferase (GGT) as nmoles/min/mg of 10,000 g supernatant protein. Leverage plots control for the effects of selenium, species, lifestage, and sex. Regression lines are included for relationships where $p \leq 0.10$. Total mercury concentrations for livers expressed as geometric means and range were prebreeding adult Forster's terns (6.8, 1–27), breeding adult Forster's terns (15.6, 6–42), prebreeding adult Caspian terns (8.9, 2–69), and Forster's tern chicks (3.4, 1–9). Selenium concentrations ($\mu\text{g/g}$ dry wt) for the above were respectively (8.1, 4–15), (6.3, 4–13), (6.8, 5–14), and (3.2, 3–4). Further details on Hg and Se concentrations in liver can be found as previously reported [23–25].

lifestage \times THg interactions for TSH, TBARS, PBBSH, GSSGred, and G-6-PDH, indicating that oxidative stress response to brain THg concentrations differed among lifestages. Therefore, we conducted separate analyses for prebreeding adults (models included THg, species, and sex), breeding adults, (models included THg and sex), and chicks (models included only THg). In prebreeding adults, brain THg concentrations influenced only G-6-PDH activity (Table 2, Fig. 3), where there was a slight increase in G-6-PDH activity with increasing THg concentrations. Conversely, after controlling for sex effects THg concentrations in breeding adult brains were correlated with TBARS, PBBSH, GSSGred, and G-6-PDH (Table 2, Fig. 3). Thiobarbituric acid reactive substances increased with increasing THg concentrations, whereas PBBSH, GSSGred, and G-6-PDH activities decreased with increasing THg concentrations. In chicks, both TSH and PBBSH decreased significantly with increasing THg concentrations, but no other biomarkers were correlated with brain THg concentrations. Species

effects revealed slightly higher TSH in prebreeding Forster's terns than prebreeding Caspian terns but G-6-PDH activity was almost twice as high in Forster's terns than in Caspian terns. Sex effects revealed only slightly higher brain GSSGred activity in breeding males than breeding females.

No lifestage \times THg interactions were noted for GSH, GSSG, or GSSG:GSH, indicating that responses did not differ among lifestages. Therefore, we used a global model to simultaneously evaluate the influence of THg, species, lifestage, and sex on those biomarkers of stress. No significant THg, species, or sex effects were found on biomarker response (Table 3), but activity of all three biomarkers differed among lifestage (Table 3). Reduced glutathione activity differed among all three lifestages, and was highest in prebreeding adults, followed by breeding adults, then chicks. Oxidized glutathione was higher in chicks than prebreeding adults, and activity in breeding adults did not differ from prebreeding adults or chicks. Oxidized glutathione:reduced glutathione values did not differ between chicks

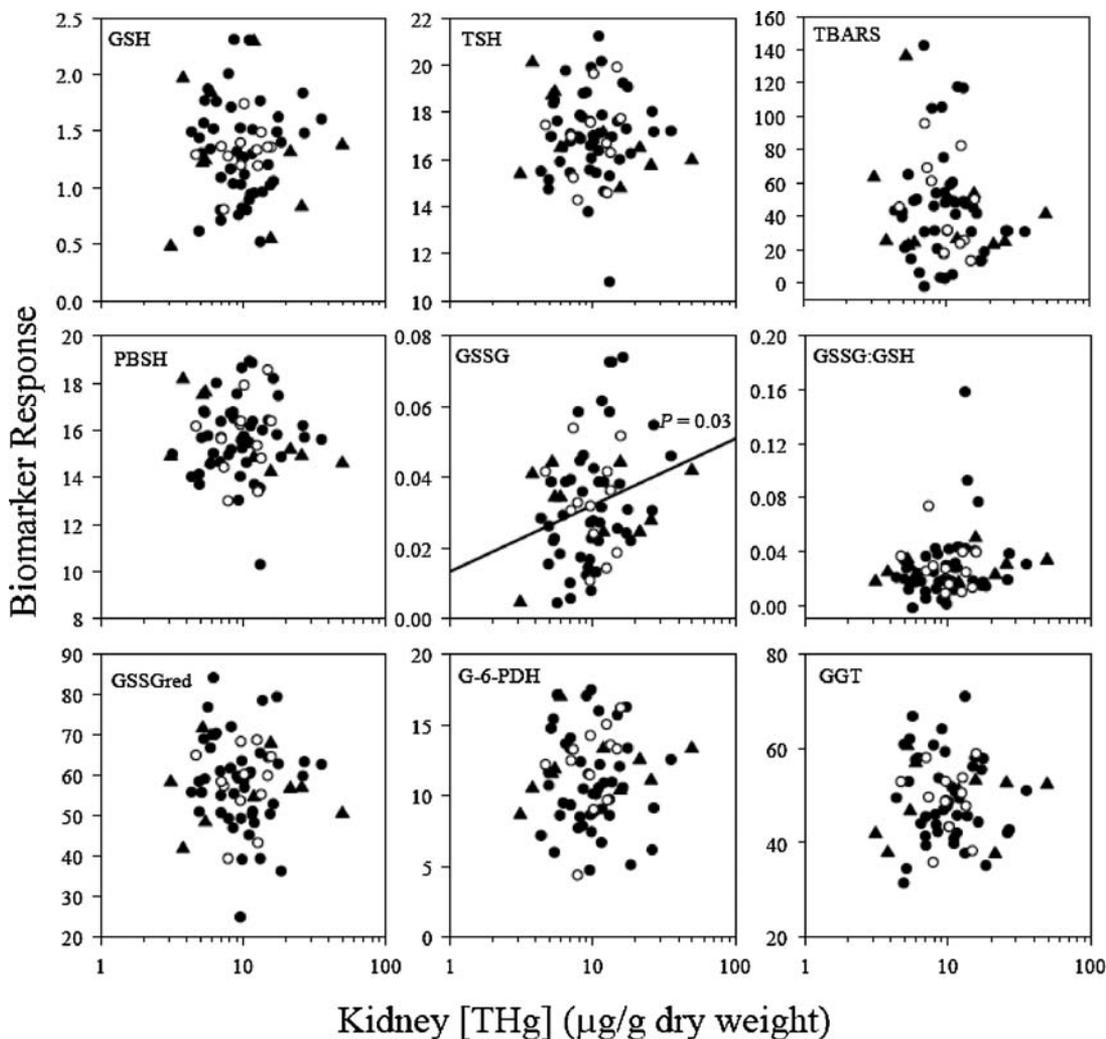


Fig. 2. Leverage plots for the relationship between total mercury (THg) concentrations ($\mu\text{g/g}$ dry wt) and a suite of biomarkers in kidneys of Forster's tern and Caspian tern adults (closed circles and closed triangles, respectively), and Forster's tern chicks (open circles) from San Francisco Bay, California, USA. Reduced glutathione (GSH), total thiols (TSH), protein bound thiols (PBBSH), and oxidized glutathione (GSSG) are expressed as $\mu\text{mol/g}$ of tissue; GSSG reductase (GSSGred) as nmoles of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidized/min/mg of 10,000 g supernatant protein; and glucose-6-phosphate dehydrogenase (G-6-PDH) and gamma-glutamyl transferase (GGT) as nmoles/min/mg of 10,000 g supernatant protein. Leverage plots control for the effects of species, lifestage, and sex. Regression lines are included for relationships where $p \leq 0.05$. Total mercury concentrations for kidneys expressed as geometric means and range were prebreeding adult Forster's terns (8.1, 2–25), breeding adult Forster's terns (19.1, 9–42), prebreeding adult Caspian terns (9.3, 3–66) and Forster's tern chicks (3.3, 2–5). Further detail on Hg and Se concentrations in kidney can be found as previously reported [23–25].

and breeding adults, and both lifestages had higher GSSG:GSH ratios than prebreeding adults. A statistically marginal species effect was found in which GSH in Forster's terns was slightly higher than in Caspian terns.

DISCUSSION

Glutathione is considered the primary soluble intracellular, low molecular weight compound involved in maintaining the integrity of living cells. Its actions are catalyzed by a system of enzymes that utilize the properties of glutathione to react with potentially harmful endogenous compounds and xenobiotics including Hg and excess Se [31,32]. Changes in glutathione status, and, in particular, depletion of the reduced form of glutathione (GSH) and increase in the oxidized form (GSSG) are thought to increase the likelihood of injury to liver and other tissues. Therefore, the replenishment of GSH is an important strategy for meeting the challenge of sustained or acute oxidative stress [20]. In the present study, manifestations of

oxidative stress in terns were related to Hg concentrations in liver, kidney, and brain tissue as well as reproductive status of birds and are discussed in the following subsections.

Hepatic oxidative stress

Significant relationships were observed between both hepatic GSH concentration and GGT activity and tern liver THg concentrations, as well as a marginally significant relationship between GSSG:GSH ratio and liver THg concentration. Both GSH concentration and GGT activity declined with increasing THg concentration in livers of adults and chicks, whereas GSSG:GSH ratio increased with THg concentrations. Hepatic depletion of GSH and concomitant increase in GSSG:GSH ratio is consistent with the findings of many other studies. Hepatic GSH concentrations were negatively correlated to mercury levels in double-crested cormorant nestlings, snowy egret nestlings, great blue heron (*Ardea herodias*) embryos, adult surf scoters (*Melanitta perspicillata*), ruddy ducks (*Oxyura jamaicensis*), and greater scaup (*Aythya marila*) [11,13,19,33].

Table 2. Analysis of covariance model for biomarker response in brain tissue of Forster's terns and Caspian terns in San Francisco Bay, CA, USA

Biomarker		Prebreeding adults			Breeding adults		Chick
		THg	Species	Sex	THg	Sex	
TSH	<i>df</i>	1,24	<i>1,24</i>	1,24	1,25	1,25	<i>1,10</i>
	<i>F</i> value	0.95	<i>4.18</i>	0.09	2.48	0.09	<i>5.29</i>
	<i>p</i> value	0.34	<i>0.05</i>	0.77	0.13	0.76	<i>0.04</i>
TBARS	<i>df</i>	1,24	<i>1,24</i>	1,24	<i>1,25</i>	1,25	<i>1,10</i>
	<i>F</i> value	0.45	<i>3.26</i>	0.09	<i>6.05</i>	0.03	<i>2.09</i>
	<i>p</i> value	0.51	<i>0.08</i>	0.76	<i>0.02</i>	0.86	<i>0.18</i>
PBSH	<i>df</i>	1,24	<i>1,24</i>	1,24	<i>1,25</i>	1,25	<i>1,10</i>
	<i>F</i> value	0.54	2.62	0.30	<i>2.87</i>	0.50	<i>5.37</i>
	<i>p</i> value	0.47	0.12	0.59	<i>0.10</i>	0.48	<i>0.04</i>
GSSGred	<i>df</i>	1,24	<i>1,24</i>	1,24	<i>1,25</i>	1,25	<i>1,10</i>
	<i>F</i> value	0.51	0.04	0.51	<i>5.88</i>	<i>4.57</i>	0.76
	<i>p</i> value	0.48	0.85	0.48	<i>0.02</i>	<i>0.04</i>	0.47
G-6-PDH	<i>df</i>	1,24	<i>1,24</i>	1,24	<i>1,25</i>	1,25	<i>1,10</i>
	<i>F</i> value	5.56	<i>24.37</i>	2.33	7.76	0.83	0.55
	<i>p</i> value	0.03	<i><0.0001</i>	0.14	<i>0.01</i>	0.37	0.48

Separate model were run for each lifestage (prebreeding adult, breeding adult, and chick). Model effects include tissue-specific total mercury (THg) concentrations, species (Forster's tern, Caspian tern), and sex (male, female). Italicized values indicate $p \leq 0.10$. See figure legends for biomarker abbreviations.

Increased hepatic GSSG:GSH ratio was related to Hg in mallards, greater scaup, and double-crested cormorants. However, in two studies where birds were dosed with MeHg at various concentrations, hepatic GSH was not affected in common loons and increased only slightly in great egrets (*Ardea alba*) [34,35]. Pied flycatcher (*Ficedula hypoleuca*) nestlings exposed to lower Hg than the present study, in combination with other metals at contaminated field sites exhibited increased hepatic GSH [36]. The decrease in hepatic GGT activity in terns in the present study is somewhat unusual since GGT activity is generally quite low in avian liver tissue including terns, but considerably higher in kidneys of birds. Few effects if any of Hg on hepatic GGT activity of other avian species have been reported, whereas there have been reported effects on kidney GGT [11,13]. Gamma-glutamyl transferase is produced primarily by the liver in mammals and catalyzes GSH, so it plays a key role in the synthesis and degradation of GSH. Therefore, decreased GGT activity as seen in the present study would be expected to contribute to oxidative stress.

In other studies, Hg-induced hepatic G-6-PDH activity has been reported in young snowy egrets and in adult black-crowned night-herons with generally similar THg concentrations as the present study [11] and is considered a compensatory mechanism that would increase the supply of NADPH as a cofactor for the enzyme GSSGred, which in turn converts GSSG to the reduced form GSH in order to maintain the status of reduced to oxidized glutathione. However, even higher hepatic mercury concentrations than in the present study were found to decrease G-6-PDH activity in adult double-crested cormorants and mallards, possibly due to inhibition of the enzyme by Hg [11,30].

Increased hepatic lipid peroxidation as TBARS related to Hg exposure has been reported in young black-crowned night-herons, young and adult double-crested cormorants, and great egret nestlings [11,34]. Other evidence of hepatotoxicity linked to Hg in birds includes increased activities of plasma enzymes in young snowy egrets and double-crested cormorants where elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), and lactic dehydro-

genase (LDH) activities appeared to reflect hepatocellular disruption, as supported by histopathological findings that included increased vacuolar changes in hepatocytes as well as correlations of increased liver inflammation with blood and tissue Hg concentrations in snowy egrets [13]. In double-crested cormorant fledglings, which had much higher mean blood THg concentrations (over 5 $\mu\text{g/g}$ wet wt) than snowy egrets, greater elevations of plasma enzymes and other manifestations of hepatic injury were apparent; these included severe inflammation of the liver and a large increase in hepatic thiobarbituric acid-reactive substances indicative of lipid peroxidation and cellular damage [11]. In the present study, although plasma chemistries were not recorded, 13% of Forster's terns sampled during prebreeding (March to May) in San Francisco Bay had blood THg concentrations $>3.0 \mu\text{g/g}$ wet weight [29], and 48% of adult breeding Forster's terns had blood THg concentrations $>3.0 \mu\text{g/g}$ wet weight during the breeding season [24]. These blood Hg concentrations most likely would result in altered plasma chemistries.

Renal oxidative stress

As in liver, the highest kidney THg concentrations were found in adult breeding terns. After statistically accounting for species, lifestage, and sex effects, renal THg concentrations were related only to renal GSSG concentration, which was positively correlated with THg concentrations. Similar effects were reported in young snowy egrets and great egret chicks [13,34]. Increases in both renal GSSG and GSSG:GSH ratio were reported in young snowy egrets and in young black-crowned night-herons that were associated with renal Hg [13,37]. In contrast, GSSG decreased in young cormorants [11]. Other evidence of renal toxicity linked to Hg in birds includes alterations [11,13] in renal G-6-PDH activity which was depressed in adult and young snowy egrets but elevated in loon chicks and great egret nestlings, which may account for lack of renal glutathione oxidative shifts in those two species [34,35].

Further manifestations of renal toxicity linked to Hg in birds include positive correlations between blood THg concentration and plasma uric acid (UA) concentration and sometimes blood urea nitrogen (BUN) concentration as reported in young snowy egrets and night-herons [11,13]. Uric acid is the primary catabolic end product of protein, nonprotein nitrogen, and purine metabolism in birds, representing much of the total nitrogen excreted by the kidneys. Hyperuricemia is indicated by UA values greater than 15 mg/dl in birds and may be associated with renal disturbances ([38,39]; <http://www.ivis.org/proceedings/ACVP/2004/campbell/IVIS.pdf>) as reported in several contaminant exposure studies with mallards and bobwhite (*Colinus virginianus*) [40,41]. Mean plasma UA levels as high as 23 to 27 mg/dl for snowy egrets and night-herons were related to plasma Hg. In the present study, although plasma chemistries were not recorded, 13% of Forster's terns sampled during prebreeding (March to May) in San Francisco Bay had blood THg concentrations $>3.0 \mu\text{g/g}$ wet weight [29], and 48% of adult breeding Forster's terns had blood THg concentrations $>3.0 \mu\text{g/g}$ wet weight during the breeding season [24]. These blood THg concentrations most likely would have resulted in elevated plasma UA values. In addition, plasma inorganic phosphorus (IP) was found to correlate positively with increasing blood THg in snowy egrets, also suggestive of some renal disturbance [38,42]. In support of the above, significant histopathological effects were found in kidney tissue and included increased extramedullary hematopoiesis,

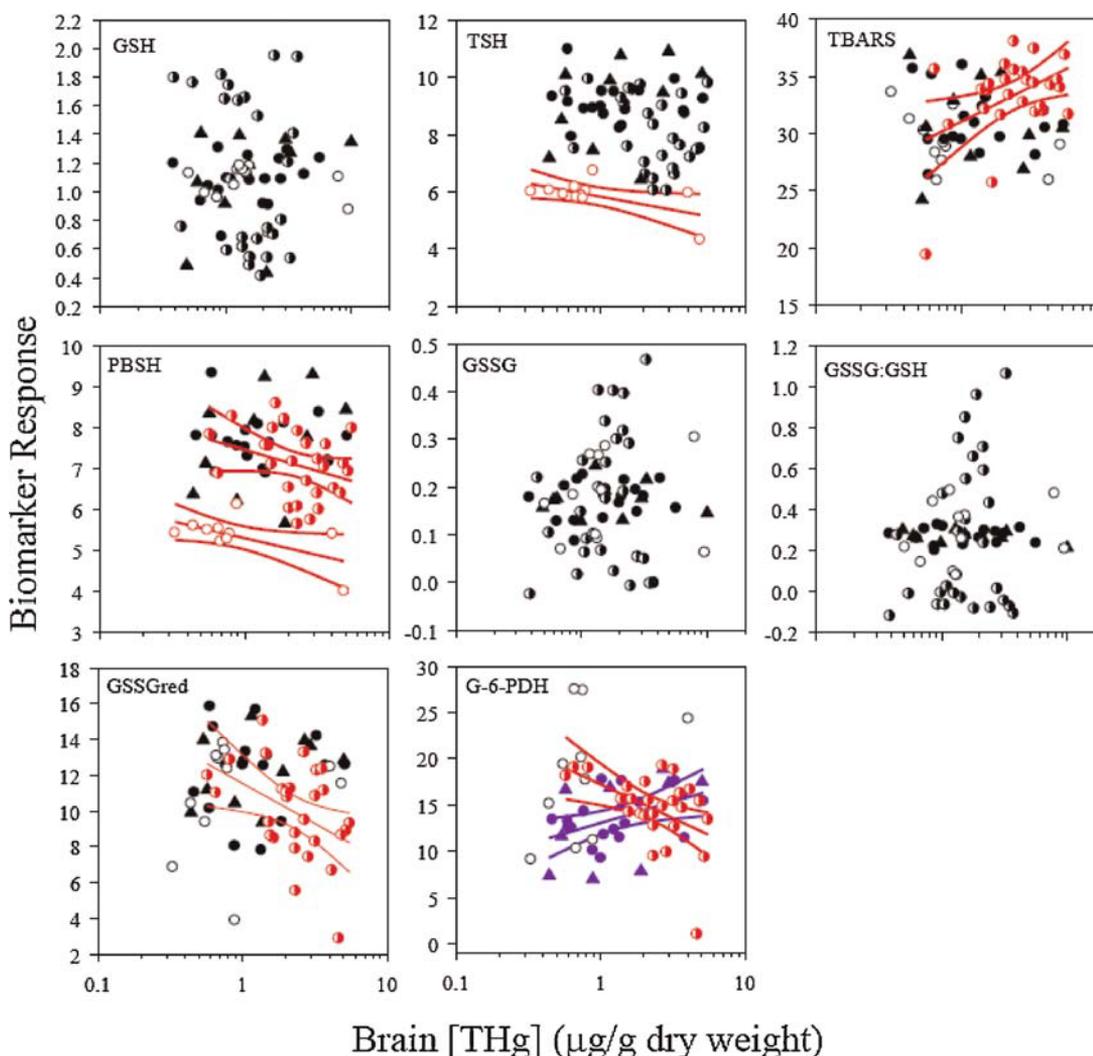


Fig. 3. Leverage plots for the relationship between total mercury (THg) concentrations ($\mu\text{g/g}$ dry wt) and a suite of biomarkers in brains of prebreeding Forster's tern and Caspian tern adults (closed circles and closed triangles, respectively), breeding adult Forster's terns (semiclosed circles), and Forster's tern chicks (open circles) from San Francisco Bay, California, USA. Reduced glutathione (GSH), total thiols (TSH), protein bound thiols (PBSH), and oxidized glutathione (GSSG) are expressed as $\mu\text{mol/g}$ of tissue; GSSG reductase (GSSGred) as nmoles of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidized/min/mg of 10,000 g supernatant protein; and glucose-6-phosphate dehydrogenase (G-6-PDH) and gamma-glutamyl transferase (GGT) as nmoles/min/mg of 10,000 g supernatant protein. Symbols and lines in color indicate a significant correlation ($p \leq 0.05$) between biomarkers and brain THg concentrations. Black symbols indicate no relationship between variables. Total mercury concentrations for brains expressed as geometric means and range were prebreeding adult Forster's terns (1.4, 0.2–4.5), breeding adult Forster's terns (2.7, 1.2–6.3), prebreeding adult Caspian terns (1.7, 0.5–13.8) and Forster's tern chicks (0.7, 0.3–0.9).

pyelonephritis with increasing lymphocyte infiltrate associated with ureter and collecting ducts, and increased distension of Bowman's space in Hg exposed snowy egrets [13]. Furthermore, significant positive correlations were apparent between kidney extramedullary hematopoiesis, pyelonephritis, distension of Bowman's capsule, interstitial inflammation, and blood and tissue Hg.

Brain oxidative stress

In brain tissue the highest mercury concentrations also were found in adult terns and levels were higher in breeding than prebreeding terns. In prebreeding adults, brain THg concentrations influenced only G-6-PDH activity where a slight increase was observed in G-6-PDH activity with increasing THg concentrations. An increase in G-6-PDH activity is considered a compensatory mechanism that would increase the supply of NADPH as a cofactor for the enzyme GSSGred, which in turn converts GSSG to the reduced form GSH in order to maintain the status of reduced to oxidized glutathione and other thiols. Conversely, after accounting for sex effects,

THg concentrations in brains of breeding adults were correlated positively with TBARS but negatively with PBSH concentration and GSSGred and G-6-PDH activities. These findings indicate the most severe oxidative stress was apparent in breeding adult terns that also had the highest levels of brain Hg. This was manifested by the combination of increased TBARS (lipid peroxidation), decreased thiols as PBSH, and decreased protective antioxidant enzyme activities, GSSGred and G-6-PDH. Both of these enzymes are linked to maintaining the status of NADPH, GSH, and other reduced thiols such as PBSH. In Forster's tern chicks, both TSH and PBSH decreased with THg concentrations. Similar negative correlations between brain THg and brain TSH and PBSH concentrations also were found, similar to reduced thiol depletion in young snowy egrets [13]. Other studies have reported depletion of brain GSH linked to Hg in young double-crested cormorants, adult and young snowy egrets [11,37]. Increase in the ratio of brain GSSG:GSH associated with increased brain Hg also has been reported in loon chicks [35], whereas a decrease was reported in young snowy egrets [11,13]. Impacts on neural tissue associated with

Table 3. Analysis of covariance model for biomarker response in brain tissue of Forster's terns and Caspian terns in San Francisco Bay, CA, USA

Biomarker		THg	Species	Lifestage	Sex
GSH	<i>df</i>	1,61	1,61	2,61	1,61
	<i>F</i> value	0.06	2.99	23.05	0.01
	<i>p</i> value	0.81	0.09	<0.0001	0.93
GSSG	<i>df</i>	1,61	1,61	2,61	1,61
	<i>F</i> value	0.62	1.01	5.79	1.16
	<i>p</i> value	0.43	0.32	0.005	0.28
GSSG:GSH	<i>df</i>	1,61	1,61	2,61	1,61
	<i>F</i> value	0.87	0.04	10.17	0.39
	<i>p</i> value	0.35	0.84	0.0002	0.54

Model effects include tissue-specific total mercury (THg) concentrations, species (Forster's tern, Caspian tern), lifestage (prebreeding adult, breeding adult, and chick), and sex (male, female). Italicized values indicate $p \leq 0.10$. See figure legends for biomarker abbreviations.

oxidative stress have included vacuolar change and inflammation of peripheral nerves in young snowy egrets that were found to correlate with blood and tissue Hg [13]. More severe vacuolar degeneration and inflammation of peripheral nerves occurred in young double-crested cormorants, which had higher blood Hg concentrations than snowy egrets [11]. Some peripheral nerve damage also was evident in night-herons. A similar response to high doses of MeHg was found for great egrets [43].

The evidence of neurological cellular oxidative stress (increased lipid peroxidation, decreased protective thiols, and decreased protective antioxidant enzyme activities) with increasing brain THg concentrations in breeding Forster's terns suggests the potential for impaired breeding behavior as a result of mercury exposure. Other studies have reported behavioral effects of methylmercury in birds. Heinz [28] reported that mallard hens with brain Hg of approximately 0.5 $\mu\text{g/g}$ wet weight laid a greater percentage of eggs outside their nest boxes and fewer eggs than controls, and ducklings were less responsive to maternal calls but exhibited hypersensitivity to a fright stimulus. In great egret chicks, low-dosed birds with brain Hg of 3.4 $\mu\text{g/g}$ wet weight exhibited decreased activity, greater tendency to seek shade, and less motivation to hunt prey [44]. Significant oxidative stress was not apparent at this level but was at a higher dose which resulted in ataxia [34]. In loon chicks with blood Hg of approximately 3 $\mu\text{g/g}$, oxidative stress in brain tissue was apparent and included increased GSSG and ratio of GSSG to GSH [35]. These same loon chicks were found less likely or able to right themselves when placed in a supine position on their backs [45]. Young loon chicks in the field demonstrated increased time spent preening and decreased time riding on the backs of their parents with increasing blood Hg concentrations [46], and adults showed altered breeding behaviors with blood Hg >3 $\mu\text{g/g}$ wet weight [14]. Although oxidative stress indices were not part of these field studies, collectively they suggest that when oxidative stress is apparent in brain tissue the likelihood of behavioral effects increases substantially. Forty-eight percent of breeding Forster's terns in San Francisco Bay contained blood mercury concentrations exceeding 3 $\mu\text{g/g}$ wet weight [24], further supporting the likelihood of potentially impaired behavior. Additionally, a relationship between egg THg concentrations and the prevalence of embryo malpositions in terns has been reported [47]. Embryo malpositions are commonly associated with inadequate parental egg turning and other nesting behaviors that may be influenced by adult mercury exposure. Future research should focus on these behavioral relationships to better understand the mechanisms involved.

Breeding status, age, and species-related differences

Multiple indicator responses also pointed to greater oxidative stress in breeding compared to prebreeding Forster's terns, most likely attributable to the physiological stress associated with breeding. These included higher hepatic and renal TBARS concentrations, lower brain GSH, and higher brain GSSG:GSH ratio in breeding than prebreeding adults. Because reproduction is an energetically demanding activity which increases both basal and field metabolic rates, breeding efforts in birds have been reported to result in increased oxidative stress with a consequent decrease in total antioxidant defenses [48–50] as observed in the present study in breeding terns. All bioindicators of oxidative stress due to THg in liver and kidney were affected in tern chicks as well as adults even though THg levels in tissue of the chicks were about one-fourth the concentration of breeding adults. In brain, fewer variables were related to THg in chicks than adults. Overall, these findings would suggest a greater level of sensitivity in chicks relative to adults, which has been the case for several other species including snowy egrets, black-crowned night-herons, and double-crested cormorants [11]. Some bioindicator measurements also were age-related, where reduced thiol concentrations in tissues were generally lower in chicks than adults, which could contribute to a greater Hg sensitivity in chicks than adults. Species bioindicator differences between Forster's terns and Caspian terns may be related to differences in metabolic rates given the smaller size of Forster's terns relative to Caspian terns. For example, adult P450 activity was found to be higher in Forster's terns than in Caspian terns [51].

CONCLUSION

The highest THg concentrations were found in liver, kidney, and brain of adult breeding Forster's terns, and total and methyl Hg tissue concentrations were related to at least several manifestations of oxidative stress in adults of both species and in Forster's tern chicks. Hepatic oxidative stress included depletion of liver GSH with an increase in the ratio of hepatic GSSG:GSH, as reported in other avian Hg studies, and decreased activity of an antioxidant enzyme, GGT. In kidneys of all terns, oxidative stress was manifested as increased renal GSSG concentration with increasing THg. Tissue Se concentrations were not high enough to result in any observable effects. Although pre fledging Forster's tern chicks contained one-fourth the hepatic THg concentration of breeding adults, they still exhibited the above hepatic and renal effects. In brain tissue of prebreeding adult terns, THg was associated with a small increase in G-6-PDH activity, suggestive of a compensatory response that would lessen the extent of oxidative stress. In breeding adult Forster's terns, in which brain THg concentrations were the highest, the most oxidative stress was evident and included brain lipid peroxidation manifested as increased TBARS with a loss of protein bound thiols (PBSH) and decreased activity of two antioxidant enzymes, GSSGred and G-6-PDH. In brain tissue of Forster's tern chicks, a significant decrease was noted in two protective reduced thiols, TSH and PBSH. Similar liver, kidney, and brain THg concentrations in other avian species that cause oxidative stress have been associated with histopathological, immunological, and behavioral effects, suggesting there is potential for these effects in terns as well.

Multiple indicator responses also indicated greater oxidative stress in breeding Forster's terns than in prebreeding terns, most likely attributable to the physiological stress associated with

reproduction. These included higher hepatic and renal TBARS concentrations, lower brain GSH but higher brain GSSG:GSH ratio in breeding than prebreeding adults. Other bioindicator measurements were age-related. In tern chicks, reduced thiols including hepatic and renal TSH and PSSH and brain GSH concentrations were lower than in adults, but hepatic GSSGred activity higher than in adults. Species-related differences were also apparent and included higher hepatic GSSG:GSH; renal TSH, PSSH, and GGT activity, brain TSH and G-6-PDH activity in Forster's terns than Caspian terns, but higher hepatic and renal GSSGred activity and renal GSH in Caspian terns than Forster's terns.

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