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5 Total mercury concentrations in invasive lionfish (*Pterois volitans/miles*) from the Atlantic
6 coast of Florida

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31 ABSTRACT

32 Invasive lionfish (*Pterois volitans/miles*) pose a serious threat to marine ecosystems
33 throughout the western Atlantic Ocean and Caribbean Sea. The development of a fishery
34 for lionfish has been proposed as a strategy for controlling populations; however, there is
35 concern about consumption of this species by humans due to its high trophic position and
36 potential for bioaccumulation of mercury. We analyzed total mercury (THg) in tissues of
37 lionfish from two locations on the east coast of Florida. THg in lionfish increased with size
38 and differed by location and sex. THg was highest in muscle tissue and was strongly
39 positively correlated among tissues. THg in lionfish was lower than other commonly
40 consumed marine fishes, and falls into Florida's least restrictive advisory level.
41 Consumption of lionfish poses a low risk and concerns over mercury bioaccumulation
42 should not present a significant barrier to lionfish harvest.

43

44 *Keywords:* invasive species, marine, direct mercury analysis, bioaccumulation,
45 biomagnification

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48 **1. Introduction**

49 Biological invasions are a significant and growing threat to global ecosystems
50 causing both ecological harm and staggering economic costs (Pimentel et al. 2005;
51 Simberloff et al., 2013). A marine invader of particular concern in the western Atlantic
52 Ocean is the Indo-Pacific lionfish (two morphologically indistinct species, *Pterois miles* and
53 *P. volitans*). Following introduction into the coastal waters of south Florida beginning over
54 three decades ago (Hamner et al. 2007; Freshwater et al., 2010; Schofield et al., 2016), a
55 combination of favorable life history traits has enabled this species to become among the
56 most conspicuous and abundant residents in coastal ecosystems throughout the region
57 (Schofield, 2009; Morris, 2012; Dahl and Patterson, 2014).

58 Lionfish have long venomous spines that deter predation by native predators
59 (Mumby et al., 2011) and are resistant to common parasites (Sikkel et al., 2014) making
60 biological control of this species limited. Lionfish also inhabit a wide range of habitats and
61 environmental conditions (Claydon et al., 2012, Jud et al., 2013; Cure et al., 2014), and are
62 capable of long distance dispersal during pelagic egg and larval phases (Ahrenholz and
63 Morris, 2010; Johnston et al. 2017) facilitating range expansion and colonization. Lionfish
64 consume a generalist diet and are voracious predators of an array of reef fishes (Côté et al.
65 2014; Peake et al. 2018), and can reduce native fish recruitment by nearly 80% (Albins and
66 Hixon, 2008) and overall native species biomass by 65% (Green et al., 2012). Lionfish
67 directly impact recreationally and commercially important fishes by preying on them as
68 juveniles (Swenarton, 2016; Chagaris et al. 2017; Peake et al., 2018) and impact adults of
69 these same species indirectly through competition for food resources (Layman and
70 Allgeier, 2012; Chagaris et al., 2017).

71 To mitigate the impacts of lionfish on native ecosystems numerous removal
72 strategies have been proposed (Morris and Whitfield, 2009). Among these, harvest of
73 lionfish by recreational and commercial spearfishers has shown promise as one method to
74 help control populations of this invasive species (Barbour et al., 2011; Frazer et al., 2012;
75 Morris et al., 2012; Gomez-Lozano et al., 2013; Côté et al. 2014; Dahl et al. 2016; Pfieffer et
76 al., 2017; Harms-Touhy et al., 2019; Harris et al., 2019), and is actively promoted by
77 management agencies throughout the western Atlantic Ocean and Caribbean Sea.
78 However, although lionfish have a white flesh with flavor and texture similar to highly-
79 valued species such as snapper and grouper; efforts to develop a viable fishery for lionfish
80 have been hampered by concerns that the concentration of biologically derived toxins,
81 pollutants and heavy metals in this predator may be high enough to present an exposure
82 risk for humans (Buddo, 2011). In Florida, mercury contamination is of particular concern,
83 with high levels of this toxin found in many species of fishes (Adams et al., 2003; Korouna-
84 Renier et al., 2011; Adams and Sonne, 2013).

85 Mercury is a naturally occurring toxic metal that is known to bioaccumulate in the
86 tissue of fishes. As mercury is primarily obtained from the diet, mercury can be magnified
87 in the tissues of aquatic and marine predators, like lionfish, that feed at high trophic levels
88 (Mergler et al., 2007; Layman and Allgeier, 2012; Dahl and Patterson, 2014). The
89 accumulation of mercury can not only directly impact fish health (Adams et al., 2010) and
90 adversely affect many aspects of reproduction (Weis, 2009), but also poses a serious
91 exposure risk for humans consuming them, particularly for young or pregnant individuals
92 (NRC, 2000; U.S. EPA, 2001; Karagas et al., 2012). Further, most of the mercury present in
93 fish muscle is present as organic methylmercury (MeHg; Bloom, 1992), the most highly

94 toxic and bioactive form. MeHg exposure in humans is almost exclusively from the
95 consumption of fish (Ratcliffe et al., 1996; Sweet and Zelikoff, 2001). To limit human
96 exposure to mercury, dietary guidelines for fish consumption have been established (U.S.
97 EPA, Florida DOH) and because rates of mercury bioaccumulation vary as a function of
98 biological, environmental, and temporal factors, recommended levels of consumption often
99 differ across species, sizes and locations.

100 The goal of this study was to quantify total mercury (THg) in lionfish as a function of
101 capture location, sex, size and tissue type; information that may be useful for (1) assessing
102 the value of lionfish in biomonitoring, (2) understanding the ecophysiological processes
103 underlying mercury distribution, detoxification and sequestration in marine fishes, (3)
104 determining whether THg may adversely affect health and reproduction in this invasive
105 species, and most importantly (4) evaluating the potential risk to consumers of this species
106 in a rapidly developing fishery. Previous studies have provided assessments for mercury
107 risk in lionfish (Hoo Fung et al., 2013; Huge et al., 2014; Tremain and O'Donnell, 2014;
108 Ritger et al., 2018); however, none of these occurred in our study region only one examined
109 mercury in lionfish across the entire range of sizes currently being harvested. This study
110 builds on earlier work by (1) expanding the spatial coverage to include and assessment of
111 mercury in lionfish from unstudied regions with high coastal human population density,
112 (2) expanding the range of sizes examined (particularly large individuals which have the
113 highest potential for mercury bioaccumulation), and (3) assessing mercury levels in
114 different lionfish tissues to assess potential impacts on health and reproduction.

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117 **2. Methods**

118 *2.1 Ethics Statement*

119 All lionfish used in this study were handled in strict accordance with a UNF IACUC protocol
120 (IACUC#13-004) and tissues of opportunity waivers approved by the University of North
121 Florida. UNF IACUC defines tissues of opportunity as samples collected: (1) during the
122 course of another project with an approved IACUC protocol from another institution; (2)
123 during normal veterinary care by appropriately permitted facilities; or (3) from free-
124 ranging animals by appropriately permitted facilities. Lionfish removals are encouraged by
125 the State of Florida and sample collection locations did not require any specific
126 permissions. No endangered or protected species were harmed during the course of this
127 study.

128

129 *2.2. Field collections*

130 Lionfish were collected in coordination with four recreational fishing tournaments
131 (also termed derbies): three in northeast Florida (NEF; August 2013, April 2014 and
132 August 2014) and one in southeast Florida (SEF; August 2014; Fig 1). During each derby,
133 teams of recreational divers captured lionfish from local sites and then returned to
134 tournament headquarters where fish were counted, measured and weighed. Lionfish were
135 then separated by location of capture and placed on ice for transport to the laboratory.
136 Lionfish were either dissected fresh or frozen whole and stored in freezers at -20 °C until
137 later processing and mercury analysis.

138

139 *2.3. Sample processing*

140 Lionfish were processed following standard protocols for mercury analysis to minimize the
141 potential for cross-contamination among samples (U.S. EPA, 2000). Each fish was
142 measured for standard length (SL, mm) and total length (TL, mm), weighed (g), and sexed
143 (Table 1). Sex determination was not possible for many smaller immature individuals
144 (Morris et al., 2012), and not available for some tissue samples collected in the field. To
145 represent the portion of the fish most often consumed by humans, muscle was collected
146 from the fillet of the left side of each fish, just above the lateral line (Adams et al., 2003).
147 For a subset of male (n=26) and female fish (n=31), we collected additional samples of
148 liver, adipose and ovarian tissue (female only) for analysis. All samples were dried in a
149 60°C oven for 48 hours, then homogenized using a mortar and pestle prior to analysis of
150 total mercury concentrations (hereafter THg). The proportion of methylmercury (CH_3Hg^+)
151 was not quantified; however, THg provides a reasonable approximation for finfish muscle
152 in which greater than 95% of total mercury is methylated (Grieb et al., 1990; Bloom, 1992).
153 Unfortunately, the relationship between methylmercury and THg in other tissues is more
154 variable (Wagemann et al., 1997; Joiris 2000; Houserova et al., 2006), thus THg may not be
155 a good proxy for methylmercury and the concentrations for other tissues in this study are
156 best considered as maximum possible values.

157
158 Fig1. Location of lionfish collection sites from northeast (NEF) and southeast (SEF) Atlantic
159 coasts of Florida.
160

161 Table 1. Mean (\pm sd) values for sample size (n), fish morphometrics: (Standard length (SL), Total length (TL) and Mass (g)),
 162 and raw total mercury concentration ($\mu\text{g g}^{-1}$) on a dry weight and wet weight basis for lionfish collected in two regions along
 163 Florida's Atlantic coast. THg dry weight was converted to wet weight based on a simple linear regression model (WW =
 164 $0.1972 \times \text{DW}$); only mean, minimum and maximum values are shown. For each region, data are summarized for all individuals
 165 and by sex.

166

Location	n	Morphometrics			THg dw ($\mu\text{g g}^{-1}$)			THg ww ($\mu\text{g g}^{-1}$)		
		SL (mm)	TL (mm)	Mass (g)	THg (dw)	Min	Max	THg (ww)	Min	Max
A. Northeast Florida										
Male	50	206 \pm 45	281 \pm 57	382 \pm 231	0.27 \pm 0.16	0.06	1.05	0.05	0.01	0.21
Female	31	167 \pm 38	245 \pm 38	227 \pm 115	0.26 \pm 0.16	0.12	0.81	0.05	0.02	0.16
Unknown	33	148 \pm 67	246 \pm 38	148 \pm 176	0.21 \pm 0.17	0.09	0.99	0.04	0.02	0.20
All	114	178 \pm 56	250 \pm 72	250 \pm 72	0.25 \pm 0.16	0.06	1.05	0.05	0.01	0.21
B. Southeast Florida										
Male	38	178 \pm 40	267 \pm 62	285 \pm 188	0.24 \pm 0.26	0.11	1.30	0.05	0.02	0.26
Female	22	165 \pm 27	247 \pm 38	260 \pm 148	0.32 \pm 0.53	0.11	3.28	0.06	0.02	0.65
Unknown	1	65	110	12	0.17	0.17	0.17	0.03	0.03	0.03
All	61	167 \pm 35	167 \pm 35	167 \pm 35	0.29 \pm 0.44	0.11	3.28	0.06	0.02	0.65
Total	175	174 \pm 50	250 \pm 66	271 \pm 202	0.26 \pm 0.29	0.06	3.28	0.05	0.01	0.65

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168 2.3. *Mercury analyses*

169 THg in lionfish tissues were measured using a Direct Mercury Analyzer (DMA-80;
170 Milestone Inc., Shelton, CT, USA); a method recognized by the U.S. EPA (EPA Method 7473).
171 The DMA-80 was calibrated weekly using serial dilutions from a liquid standard (1000
172 mg/L \pm 5 mg/L, Certified Reference Material (CRM), ASSURANCE, SPEX CertiPrep, Inc.).
173 To facilitate comparisons with previous work and to evaluate mercury levels relative to
174 national (U.S. EPA, 2001) and local (Florida DOH, 2014) guidelines for consumption, we
175 converted THg in muscle tissue from a dry weight to wet basis using a simple linear
176 regression model fit to a subsample of fish for which sample weights were available both
177 before and after drying. The slope of the line allowed for percent moisture to be calculated
178 and THg dry weight to be converted to a wet weight basis for unknown samples.

179 Quality assurance and control (QA/QC) protocols were rigidly followed to ensure
180 acceptable levels of accuracy and precision in the data. Weekly calibration of the DMA-80
181 with liquid mercury standard yielded standard curves with r^2 values > 0.99 . Method blanks
182 returned THg concentrations well below the lowest recorded value from any fish sample in
183 the study (<10% of the lowest value recorded in the study; EPA method 7473) confirming
184 that samples were not substantially affected by contamination. Standard blanks using a
185 solid standard (CRM, NIST Standard Ref 2709a) were all within expected values, 0.9 ± 0.2
186 $\mu\text{g g}^{-1}$. Duplicate ($n = 66$) and triplicate ($n=34$) tissue samples from the same individual
187 were run a minimum of every 10 samples over the course of the study and yielded
188 coefficients of variation (CVs) averaging 2.17% indicating acceptable levels of precision.
189
190

191 *2.4. Statistical analysis*

192 All statistical analyses were run within SAS 9.4 (SAS Institute), SPSS Statistics 25 for
193 Windows (IBM) or SigmaPlot (Systat Software) software packages. When necessary THg
194 data were log-transformed (\ln THg) prior to analyses to satisfy the assumptions of
195 normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test)
196 required for parametric statistical tests. Temporal differences in THg in lionfish from NEF
197 were assessed using a one-way analysis of covariance (ANCOVA) with collection date as a
198 main factor and standard length (SL) as a covariate. SL was chosen as the best covariate for
199 all analyses because body mass was not available for all fish and TL is more likely to be
200 biased by potential damage to the caudal fin occurring naturally or during capture, storage
201 and processing. Potential differences in THg between locations (NEF and SEF) and sexes
202 (male and female) were tested using a two-way ANCOVA model with SL as a covariate. In
203 cases where interactions were present between model main effects and covariates, we
204 applied the Johnson-Neyman procedure as suggested by Wilcox (1987).

205 Differences in THg between sexes and among tissues were assessed using a two-way
206 repeated measures ANCOVA (RM ANCOVA) for three tissues (muscle, liver, adipose)
207 excluding ovarian tissue. A one-way RM ANCOVA model was also run for females
208 separately to include ovary tissue in the analysis. In all cases, we accounted for violations
209 of the assumption of sphericity by adjusting model degrees of freedom (Greenhouse and
210 Geisser, 1959). Following significant results from ANCOVA, a post-hoc multiple
211 comparison test (Dunn-Šidák; Day and Quinn, 1987) was applied to determine significant
212 differences among tissues. The relationship among THg in various tissues was assessed
213 using correlation (Pearson's r) for females and males independently. To maintain

214 experimentwise error rate (nine total comparisons), we applied the sequential Bonferroni
215 correction when assessing significance (Holm, 1979).

216

217 **3. Results**

218 A total of 175 samples of lionfish muscle tissue were analyzed for THg. The samples
219 included 88 males, 53 females and 34 individuals for which sex could not be determined.
220 Overall, fish ranged widely in length (SL: 57 to 306 mm, $\bar{x} = 175$ mm, TL: 83 to 411mm, $\bar{x} =$
221 250 mm), weight (4.5 to over 1000g, $\bar{x} = 271$ g) and THg (0.01 to 3.28 $\mu\text{g g}^{-1}$ dw; $\bar{x} = 0.26$;
222 Table 1).

223 To compare our results to earlier work and to recommended guidelines for
224 consumption, we converted THg in muscle tissue from a dry weight to wet weight basis
225 using a simple linear regression model. The linear relationship between THg wet weight
226 and dry weight (WW = 0.19*DW) was highly significant ($r^2 = 0.99$, $p < 0.0001$); no intercept
227 was included in the final linear model since it was not significantly different from 0 ($t =$
228 0.002, $p = 0.998$). The percent moisture in lionfish muscle averaged $80.4 \pm 1.8\%$ with high
229 precision ($CV = 0.02$) and was independent of fish size, sex and location. Converted THg on
230 a wet weight basis is provided in Table 1.

231 Collection time had no significant effect on THg ($F_{[2,57]} = 1.05$, $p = 0.37$), thus data for
232 NEF were pooled across time for subsequent analyses. Our initial two-way ANCOVA model
233 revealed several significant interactions ($p < 0.05$) among location and SL (Table 2A). To
234 aid with interpretation of the data and to remove potentially confounding interactions, we
235 ran ANCOVA models for each location separately (Table 2B, C). In these reduced models,
236 ANCOVA revealed no significant differences in THg by sex at either site (Table 2B, C, Fig 2),

237 although females had generally higher THg for a given size. A one-way ANCOVA model
238 with sexes pooled revealed a highly significant positive relationship between SL and THg
239 and a significant effect of location (Table 2D, Fig 3). Additionally, a significant interaction
240 indicated that the regression slopes for the two locations were not the same (Table 2B).
241 Further analysis (Johnson-Neyman procedure; Wilcox, 1975) determined that individuals
242 greater than 196 mm SL were significantly higher in THg in SEF than in NEF, while smaller
243 individuals were not significantly different between locations (Fig 3).
244 THg varied significantly among tissues ($F_{1,2,66.7} = 9.36$, $n = 78$, $p = 0.002$), but did not vary
245 between sexes ($F_{1,53} = 1.81$, $n = 78$, $p = 0.18$; Fig 4), nor was an interaction present. For
246 both sexes, muscle had significantly higher THg than other tissues (muscle > liver >
247 adipose; Dunn-Šidák, Fig 4). In females, THg in ovaries was significantly different from
248 muscle and adipose, but not liver (Dunn-Šidák, Fig 4). THg in all tissues from females were
249 highly correlated (r from 0.66 to 0.88, $p < 0.001$, Fig 5A-F); however only THg in muscle
250 and liver tissues were correlated in male lionfish ($r = 0.95$, $p < 0.001$, Fig 5I).

251

252 Fig 2. Linear regression relationships between size (Standard length) and total mercury
253 concentration for female (solid circles, solid line) and male (open circles, dotted line)
254 lionfish in northeast Florida (A) and southeast Florida (B).
255

256 Fig 3. Linear regression relationships between size (Standard length) and total mercury
257 concentration in lionfish from NEF (solid circles, solid line) and SEF (open circles, dotted
258 line). The shaded region indicates the sizes (SL > 196 SL) for which lionfish THg was
259 significantly higher ($\alpha = 0.05$) in SEF than in NEF.
260

261 Fig 4. Total mercury concentrations in lionfish tissues in female (solid black bars) and male
262 (solid grey bars) lionfish. Male testes were not sampled (nd). No significant differences
263 were observed for tissues as a function of sex; letters (capitals = females; lower case =
264 males) above tissues represent significant differences from total mercury in tissue types
265 from a Ryan's Q post-hoc test (see text for details) following ANCOVA.
266

267 Fig 5. Relationship among THg (all axes are shown in $\mu\text{g g}^{-1} \text{dw}$) in various tissues in female
268 (Panels A-F, n = 31) and male lionfish (Panels G-I, n = 26). Coefficients from Pearson
269 canonical correlation analysis (r) are inset within each Panel, significant correlations at $\alpha =$
270 0.05 with sequential Bonferroni correction are noted by asterisks (**).

271 Table 2. Output from Analyses of covariance (ANCOVA) models of ln total mercury ($\mu\text{g g}^{-1}$)
272 concentrations in lionfish muscle tissue from A. Both locations, B. Northeast Florida (NEF),
273 and C. Southeast Florida (SEF) D. Sexes combined.
274

A. Full model					
Source	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>p</i>
Location	1	0.94	0.94	6.97	0.009
Sex	1	0.09	0.09	0.66	0.417
SL	1	21.64	27.69	149.22	0.000
Location \times Sex	1	0.44	0.44	5.51	0.074
Location \times SL	1	1.08	1.08	10.57	0.005
Sex \times SL	1	0.97	0.97	5.83	0.058
Location \times Sex \times SL	1	0.44	0.44	5.41	0.073
Error	132	17.73	0.13		
B. Northeast Florida					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>p</i>
Sex	1	0.09	0.09	0.89	0.349
SL	1	9.94	9.94	95.94	0.000
Sex \times SL	1	0.00	0.00	0.01	0.922
Error	77	7.98	0.10		
C. Southeast Florida					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>p</i>
Sex	1	0.36	0.36	2.01	0.162
SL	1	12.05	12.05	119.68	0.000
Sex \times SL	1	0.71	0.71	3.94	0.052
Error	55	9.76	0.18		
D. Sexes combined					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>p</i>
Location	1	2.31	2.31	12.72	0.000
SL	1	16.54	16.54	91.02	0.000
Location \times SL	1	2.47	2.47	13.57	0.000
Error	171	22.01	0.17		

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277

278 **4. DISCUSSION**

279

280 The main findings of this study were: (1) mercury concentrations in invasive
281 lionfish from northeast and southeast FL were similar to previously reported values and
282 generally below comparable values for commonly consumed reef species, (2) muscle had
283 significantly higher levels of mercury than other tissues or organs, (3) mercury levels
284 increased with size and rates of accumulation differed by location, (4) females were
285 generally higher in mercury than males, and (5) mercury concentrations in all lionfish
286 tissues examined in this study were highly correlated for females, but only between liver
287 and muscle for males. Collectively, our findings indicate that THg in lionfish is low and
288 lionfish are safe for human consumption.

289

290 *4.1. THg in lionfish from northeast and southeast FL*

291 Mean THg in lionfish (0.05 – 0.06 $\mu\text{g g}^{-1}$ ww; Table 1) in our study were within the
292 range previously reported from Florida (0.02 – 0.15 $\mu\text{g g}^{-1}$ ww; Huge et al., 2013; Tremain
293 and O'Donnell, 2014), Jamaica (0.037 $\mu\text{g g}^{-1}$ ww; Hoo Fung et al., 2013) and Curaçao
294 (Range: 0.008 – 0.016 $\mu\text{g g}^{-1}$ ww; Ritger et al. 2018).

295 Overall, THg in lionfish was lower than native predatory reef fishes that occupy
296 similar trophic positions in offshore hard bottom habitats like those sampled in our study.
297 THg in carnivorous fishes of similar size (e.g., red grouper, *Epinephelus morio*; black sea
298 bass, *Centropristes striata*) are 3-4 times higher than the values observed in our study
299 (Adams et al., 2003; Tremain and Adams, 2013) and more the 8-10 times higher in larger,
300 long-lived species such as gag (*Mycteroperca microlepis*) and red snapper (*Lutjanus*
301 *campechanus*, Zapp Sluis et al., 2012; Tremain and Adams, 2012). The presence of low THg

302 in lionfish is somewhat surprising based on studies of diet (Muñoz et al., 2011; Dahl and
303 Patterson, 2014; Swenarton, 2016; Peake et al., 2018) which indicate that lionfish are
304 carnivores throughout their life history and almost exclusively piscivorous as adults.
305 Indeed, stable isotope analysis paired with diet analyses suggests that both small
306 (presumably young) and larger lionfish are feeding at the same trophic level (Ritger et al.,
307 2018). Food web modeling and stable isotope analysis also demonstrates a high trophic
308 position for lionfish within the invaded food web and a large degree of overlap with native
309 top predators (Arias-Gonzalez et al., 2011; Layman and Allgeier, 2012; Chagaris et al.,
310 2017). Thus, low levels of THg in lionfish do not appear to be related to differences in
311 feeding ecology as has been demonstrated in groupers and sea basses from this region
312 (Tremain and Adams, 2012). Low levels of THg in lionfish could reflect species-specific
313 physiological attributes that favor either reduced uptake or increased rates of elimination.
314 Although lionfish physiology is poorly understood; these rates are highly variable across
315 taxa (Luoma and Rainbow, 2005) and in fishes (Wang, 2012).

316 Low THg in lionfish may be at least partially explained by the extremely rapid
317 growth of lionfish relative to native predators. Lionfish grow to nearly 300 mm TL by age
318 two (Barbour et al., 2011; Edwards et al., 2014; Johnson and Swenarton, 2016; Fogg et al.,
319 2019); while important fishery species from Florida waters such as red grouper
320 (Lombardi-Carlson et al., 2008), black sea bass (Hood et al., 2008), and grey snapper
321 achieve sizes of 200, 127, and 204 mm TL at age two, respectively. As a result, young
322 lionfish (1-3 years old) of similar size to these species have been accumulating THg for as
323 little as half the time. This rationale is supported by empirical studies for many fishes for

324 which age is a better predictor of THg than body size or mass (Verdouw et al., 2011; Fincel
325 et al., 2013).

326

327 *4.2. Relationship between THg, size and location*

328 Because THg is not readily depurated, it accumulates within tissues over time and
329 the amount of THg in fish tissues is expected to increase as fish age (Tremain and Adams,
330 2012; Wang et al., 2014). Our data are in close agreement with this commonly observed
331 pattern; THg in lionfish was significantly positively correlated with fish length (a proxy for
332 age) accounting for between 50 and 60% of the variation ($r^2 = 0.55 - 0.61$) in THg
333 depending on sex and location of capture (Fig 2A, B). Rates of THg bioaccumulation were
334 site-specific and significantly higher in SEF than in NEF resulting in significantly mercury
335 levels for the largest fish (SL > 196mm; Table 2D, Fig 3). Previous work has noted much
336 larger differences among regions in Florida (Tremain and O'Donnell, 2014); however that
337 study examined mercury at a larger spatial scale and sampled in locations with known
338 processes affecting mercury methylation and bioavailability (Chen et al., 2009; Sparling,
339 2009; Driscoll et al., 2012) distinct from our offshore collection sites. In particular, lionfish
340 from the Florida Keys have been shown to be high in mercury (Tremain and O'Donnell,
341 2014), a pattern consistent with elevated mercury in other studies (Huge et al., 2014) and
342 in other piscivorous fishes from that region (Adams et al., 2003, 2010; Adams and Onorato,
343 2005).

344 Both the positive relationship between THg and size in lionfish and site-specific
345 bioaccumulation rates have been reported previously (Tremain and O'Donnell, 2014), but
346 the strengths of the relationships were weaker and more variable ($r^2 = 0.07 - 0.35$) than

347 reported here. The reduced variability observed in our study may result from our regional
348 spatial scale and similar habitats (offshore hard bottom) in comparison with the earlier
349 study which sampled a larger geographic area, at nearshore and offshore locations, and
350 sampled in the Florida Keys which has unique biogeochemical properties due to the nearby
351 Everglades watershed (Evans and Crumley, 2005; Tremain and O'Donnell, 2014).

352

353 *4.3. Comparison of THg in lionfish tissues*

354 The differential uptake and accumulation of THg within the body tissues of fishes is
355 not well resolved. While numerous studies have reported low levels of THg in muscle
356 relative to lipophilic tissues such as liver (Agusa et al., 2007), others have found no
357 difference (Alvarez et al., 2012; Burger et al., 2013) or elevated THg in muscle (Martins et
358 al., 2006; Alvarez et al., 2012; Khoshnamvand et al., 2013; Squadrone et al., 2013). In this
359 study, THg in lionfish muscle was significantly higher than in other tissues. This finding is
360 in agreement with earlier work (Berg et al., 2000; Havelková et al., 2008; Kružíkova et al.,
361 2013) which supports a general pattern of higher THg in muscle relative to internal organs
362 in fish from lightly contaminated localities which is likely the case with our fish collected in
363 offshore marine waters (>20 km from land). A key limitation of the current study was that
364 methylmercury was not quantified. While THg is a good proxy for methylmercury in fish
365 muscle (Grieb, 1990), the fraction of the THg pool present as methylmercury is highly
366 variable in other tissue types (Houserova et al., 2006), can vary as a function fish size (Joiris
367 et al., 2000) and among species (Mieiro et al., 2009; Berzas Nevado et al., 2011). Future
368 work on Hg speciation in lionfish organ tissues is needed to assess the value of using
369 lionfish as an indicator species to assess ecosystem health and risk (e.g., Havelková et al.,

370 2008), and for better understanding ecophysiological mechanisms underlying Hg
371 distribution, detoxification, sequestration in this species (Cizdziel et al., 2003). Despite
372 these limitations, THg represents the maximum possible concentration for methylmercury
373 and observed THg in all tissues were below accepted thresholds for negative health effects
374 (Depew et al., 2012) and reproductive impairment (Crump and Trudeau, 2008). Thus,
375 lionfish appear unlikely to be substantially affected by mercury toxicity.

376 THg in lionfish were generally positively correlated among tissues as is commonly
377 seen in fishes (Cizdziel et al., 2003). However, while this relationship was particularly
378 strong for females; only liver and muscle tissue were found to be correlated in males. This
379 finding could reflect real sex-specific differences in physiology leading to differential
380 accumulation and sequestration of THg in fat tissue. Alternatively, this result could be an
381 artifact of the data. Further study will be required to determine if this trend is real and, if
382 so, the physiological processes that underlie it.

383

384 4.4. Implications for human health

385 In the present study, THg in lionfish captured from the east coast of Florida were
386 low ($0.05 \mu\text{g g}^{-1}$), placing them in Florida's least restrictive consumption advisory level
387 (FDOH 2014) and within the range of fishes (e.g., salmon, tilapia, cod) promoted for safe
388 consumption by the EPA-FDA (U.S. EPA 2014). Because lionfish are a marine species found
389 in the southeastern U.S. and Caribbean, they are most likely to replace similar regional
390 species such as grouper and snapper in the diet. THg in lionfish is lower than other
391 commonly consumed reef fishes of similar size such as red grouper, (*Epinephelus morio*,
392 $0.17 \mu\text{g g}^{-1}$ ww), grey snapper (*Lutjanus griseus*, $0.18 - 0.21 \mu\text{g g}^{-1}$ ww), graysby

393 (Cephalopholis crenata, 0.16 µg g⁻¹ ww), and black sea bass (*Centropristes striatus*, 0.14 µg
394 g⁻¹ ww; Adams et al., 2003; Tremain and Adams, 2013) and much lower than fishery legal-
395 sized individuals of larger species such as gag (*Mycteroperca microlepis*, 0.40 µg g⁻¹ ww),
396 black grouper (*M. bonaci*, 0.91 µg g⁻¹ ww, and red snapper (*L. campechanus*, 0.49 µg g⁻¹ ww;
397 Zapp Sluis et al., 2012; Tremain and Adams, 2013). Thus, lionfish would appear to
398 represent a low mercury alternative to these species, many of which have been severely
399 depleted by commercial and recreational fishing pressure.

400 THg in lionfish increased with size, a general pattern consistently observed in fishes.
401 One of the largest fish (SL > 300) in our study had THg concentrations that would fall into
402 the FDOH limited consumption (0.5-1.5 ppm) category which calls for restricting
403 consumption to once a month for women of childbearing age and children and weekly for
404 all others (FDOH 2014). However, these large fish are exceptionally rare; lionfish
405 harvested in Florida are predominantly young fish (SL < 250 mm); larger fish (SL > 250)
406 comprised only 5% of individuals in northeast Florida (Johnson and Swenarton, 2016).

407 Overall, lionfish yields a comparable amount of flesh to similar-sized marine food
408 fishes, has high levels of fatty acids beneficial for health, and fares favorably in direct
409 comparisons with other high value marine species (Morris et al., 2011). Our findings
410 indicate that levels of THg in lionfish are low and lionfish are safe for human consumption.
411 As such, concerns over THg in lionfish should not present a significant roadblock to the
412 continued development of directed commercial and recreational fisheries for this invasive
413 species.

414

415

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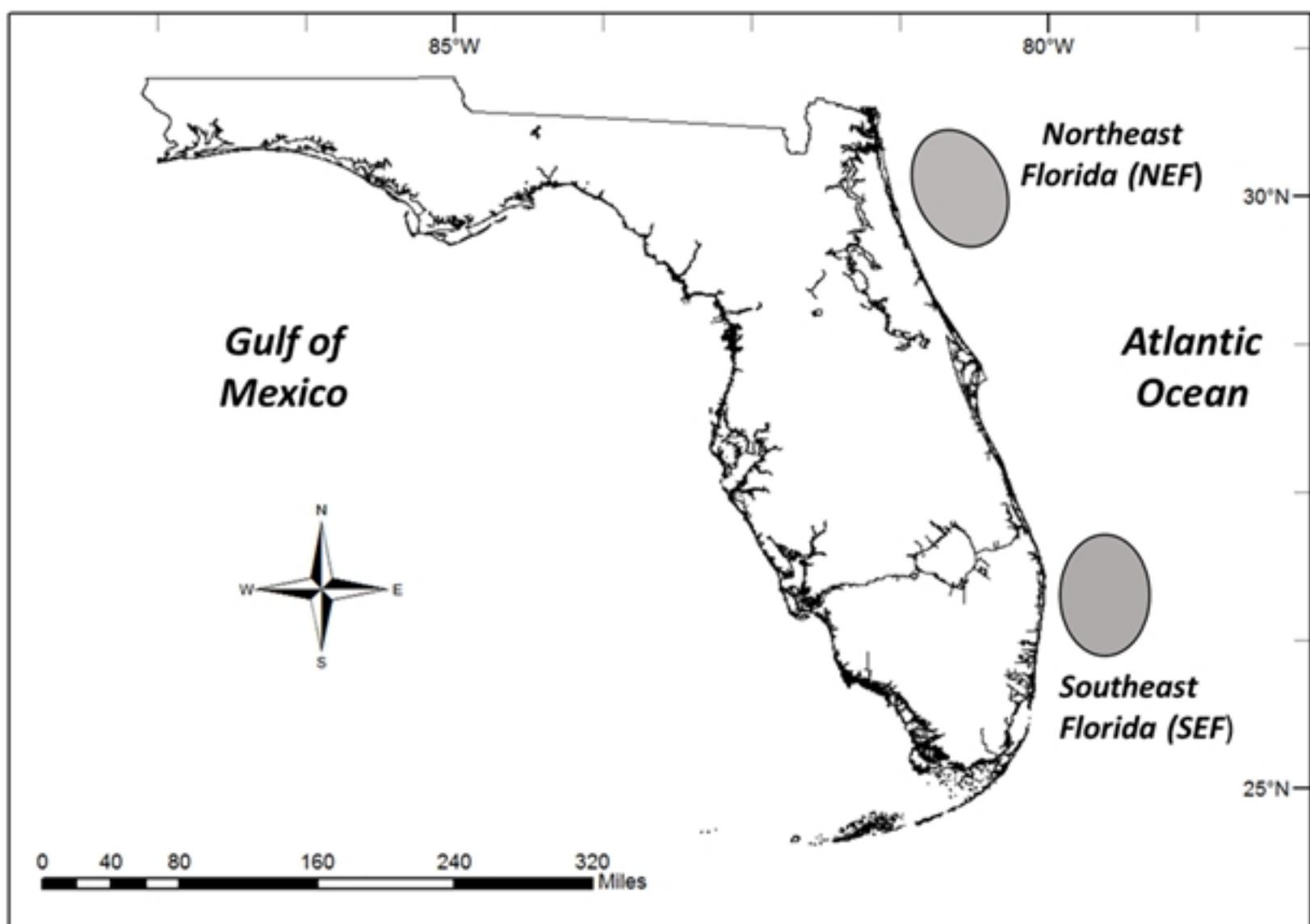
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