Influence of sex and maturity state on trace elements content in liver and muscle of the Scianidae *Totoaba macdonaldi*

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Abstract

Background. The fish *Totoaba macdonaldi* is endemic to the Upper Gulf of California. Its migratory movements involve sites with high levels of trace elements in the environment that can accumulate in tissues. In this study, lead (Pb), copper (Cu), cadmium (Cd), zinc (Zn) and iron (Fe) concentrations in male and female totoaba liver and muscle were quantified at various sexual maturity stages along the species' geographic distribution.

Methods. Generalized linear models were used to explore associations between trace element concentrations and season of the year, sex/maturity stage, and total fish length.

Results. No detectable Pb concentrations were recorded in liver or muscle; Cu, Cd, Zn and Fe contents in totoaba liver and muscle were typical of fish inhabiting areas with no contamination issues and are within international maximum permissible levels for human consumption. Variations in the content of Cd, Cu, Zn and Fe in liver of totoaba seem to be more related to the feeding and reproductive physiology of this species than as result of environmental exposure. Results suggest that consumption of totoaba muscle does not pose a public health risk. Furthermore, depending on the sex/maturity stage of totoaba, this fish's muscle may provide approximately 70% Cu, 60% Zn and 100% Fe of the recommended dietary reference intake.

Introduction

39 *Totoaba macdonaldi* (*Gilbert, 1890*), commonly known as totoaba (*Page et al., 2013*), is an
40 endemic fish found in the Gulf of California. Totoaba has a long life of up to 25 years (*Román*
41 *Rodríguez & Hammann, 1997*) and late sexual maturity with an average size at first reproduction
42 of 1,240 mm (*De Anda-Montañez et al., 2013*). It is a highly selective piscivorous predator that
43 feeds primarily on anchoveta (*Cetengraulis mysticetus*) and, to a lesser extent, on the silverside
44 of the genus *Colpichthys* as well as several species of mollusks (*De Anda-Montañez et al., 2013*).
45 Among the Sciaenidae inhabiting this region, *T. macdonaldi* displays the greatest size and
46 weight, with up to two meters in length and weighing over 100 kg *(Guevara, 1990; Cisneros-*
47 *Mata et al., 1997; Román Rodríguez & Hammann, 1997)*. These attributes make it attractive for
48 fishing. In fact, totoaba fishery was important in the first half of last century in the Gulf of
49 California, yielding over 2000 tons in 1942; however, in the 1970's catches decreased to a
50 minimum and the Mexican government declared a permanent ban since 1975 *(Arvizu Martínez,*
51 *1987; Flanagan & Hendrickson, 1976)*. In the last decade, techniques for the aquaculture
52 (mariculture) of totoaba have been developed (*Rueda-López et al., 2011; True et al., 1997*) and,
53 due to its organoleptic properties, totoaba has become a highly sought-after item in restaurants.
54 Establishing base line trace elements concentration in muscle of totoaba is relevant to
55 characterize and improve the knowledge about their biology.
56   Trace element content in organisms depends on the species' capacity to regulate metal
57 (*e.g.*, copper (Cu), zinc (Zn)) concentration and, within tissues, is associated to the specific
58 tissue's function and enzymatic makeup (*Hamza-Chaffai et al., 1995*). Sex and reproductive
59 stage have a significant effect in the concentrations of trace elements in marine organisms. In
60 marine and freshwater female fish, liver Zn content linked to metallothionein increases during
61 reproduction, potentially aiding vitellogenesis and the synthesis of estradiol-17β (*Thompson et*
62 *al., 2001*). The demand for Zn and Cu increases during embryonic and neonatal development
63 (*Olsson et al., 1987; Thompson et al., 2002*). During the annual reproductive cycle of the
64 rainbow trout, *Salmo gairdneri* Cu levels in livers from male fishes were higher than those
65 observed in the females and the Cu levels in males also showed larger fluctuations than females
66 (*Olsson et al. 1987*). The liver is the primary organ where trace elements are stored and
67 physiologically regulated in fish (*Hamza-Chaffai et al., 1995; Roméo et al., 1999*). Variability in
68 trace element concentrations is associated to increased accumulation with age, to the geographic
69 location, and to the species' feeding ecology.
70   Marine organisms found near the coasts of industrial and agricultural centers, as well as
71 those that feed in the upper levels of the food chain, have higher concentrations of trace elements
72 and other contaminants *(O'Shea & Brownell, 1994)*, although there are alternative suggestions
73 (i.e., *Mathews & Fisher, 2009*). In the Gulf of California, trace element concentrations change
74 due to natural phenomena, such as upwelling, and anthropogenic activities, including mining and
75 agriculture. Cadmium is abundant in the Gulf of California and its concentrations are associated
76 to upwelling processes (*Ruelas-Inzunza & Páez-Osuna, 2008*). Naturally occurring Cu deposits
77 in Santa Rosalia, that also contain Zn, have been intermittently exploited since the 18th century
78 (*Shumilin et al., 2000*). Sediment lead (Pb) concentrations in some urbanized areas along the

79   coasts are relatively high (up to 89 ppm) and it has been suggested that these reflect
80   anthropogenic activities (*Shumilin et al., 2001*).   Adult totoabas reach the Upper Gulf of
81   California to spawn from late winter to early spring in areas adjacent to the Colorado River delta,
82   where they remain until late May-early June; then, they migrate southwards to the waters
83   surrounding the large islands (Ángel de la Guarda and Tiburón Islands) (Figure 1). Totoaba
84   remain in this area until late fall (October), when they travel north along the coast of Sinaloa and
85   Sonora to subsequently return to the Colorado River delta in January-February *(Arvizu and*
86   *Chavez, 1972; Cisneros-Mata et al., 1995*). Therefore, this species' migration includes sites
87   enriched by trace elements as a result of either natural conditions or anthropogenic inputs and
88   areas where human activities, mostly agriculture, influence water quality (*Cadena-Cárdenas*
89   *et al., 2009*).
90   Trace metals tend to accumulate in marine organisms that are included in the human diet.
91   Some trace elements such as Zn, Cu, and iron (Fe) are essential elements involved in different
92   metabolic processes; but others, such as Pb and cadmium (Cd) are non-essential elements that
93   compete with essential elements for enzyme sites. Although less than 20% of Pb and Cd are
94   assimilated in the human body, their half-life in human tissues is around 30 years (*EFSA, 2009;*
95   *EFSA, 2010*). Even essential trace elements in excess can cause serious effects to both human
96   and animal health. For example, high intake levels of Cu, Zn, and Pb have been related to
97   Alzheimer's disease; Zn and Fe, with Parkinson's disease; Cd may induce kidney dysfunctions,
98   osteomalacia, and reproductive deficiencies, among others (*Han et al. 2013; Manea et al. 2020*).
99   Due to fish being an essential source of nutrients for humans, but potentially also a source of
100   metals in concentrations that can cause health problems to the consumers *(Ruelas-Inzunza et al.,*
101   *2010; Zamaora-Arellano et al., 2018*), it is necessary to perform health risk assessments to
102   identify the adequacy of fish species for human consumption.
103   The objective of this study was to assess changes on the concentrations of essential
104   elements (Cu, Zn, Fe) and toxic elements for humans (such as Cd and Pb) in relation to the
105   sex/maturity stage, season, and habitat in liver and in the edible tissue (muscle) of totoaba. This
106   information can provide support for totoaba aquaculture which can contribute to the protection of
107   this species if it is no longer fished.
108

Material & Methods

Overview of experimental program

111   An overview of the experimental program is shown in Figure 2. Totoaba individuals (n = 144)
112   were collected from the Upper Gulf of California in three types of habitats, 1) estuarine, 2)
113   rocky, and 3) continental. Field trips were conducted to collect samples during all seasons
114   (spring, summer, autumn, winter) along the species' distribution; however, fish were not found
115   in summer in any of the sampled sites; in winter and spring totoaba were found in the spawning
116   grounds (estuarine habitat), while specimens sampled in autumn were collected in non-spawning
117   areas (coastal and rocky habitats) (*De Anda-Montañez et al., 2013*). From each totoaba caught,
118   total length and weight were recorded, and liver (n = 142) and muscle (n = 144) samples, as well

119   as gonads (n = 144), were collected. Based on the histological analysis of gonads and on gamete
120   development and tissue characteristics sex and maturity stage were defined.
121
122   **Sample Collection**
123   Totoaba individuals were collected from the Upper Gulf of California (Figure 1) under scientific
124   collection permits issued by the Mexican government (SGPA/DGVS/02913/10,
125   SGPA/DGVS/05508/11 and SGPA/DGVS/00039/13) based on the research protocol described
126   in the project entitled "Health and Conservation status of the totoaba population (*Totoaba*
127   *macdonaldi*) in the Gulf of California: a critically endangered species" (CONABIO:
128   FB1508/HK050/10; CONACYT 2011-01/165376; CIBNOR:137C/EP0.04). This study was
129   performed following the demands, requirements and protocols that are required by the various
130   institutions involved (SGPA/DGVS, CONABIO, CONACYT, CIBNOR). A total of 144
131   individuals were collected between May 2010 and April 2012. The organisms were collected in
132   three types of habitats; 1) estuarine, which correspond to the area of the Upper Gulf of California
133   and Colorado River Delta Biosphere Reserve, 2) rocky, refers to the area around the Islands, and
134   3) continental, which refers to the east coast of the Gulf of California in different seasons of the
135   year along the species' distribution across the Gulf of California. Fishing was carried out on
136   board 26-foot vessels with outboard motors. The fishing gears used included gillnets measuring
137   120 m long with a mesh size of 10 inches, and fishing rods with no. 5 and 6 hooks; shrimp heads,
138   squid and/or comber were used as bait. The total length and weight of each totoaba caught were
139   recorded. Liver (142) and muscle (144) samples were collected from each fish; samples were
140   separately placed in plastic bags, stored on ice, and transported to the laboratory at CIBNOR for
141   storage at -80°C until analyzed. Gonads (n = 144) were also sampled and stored in Davidson´s
142   fixative for sex identification and maturity stage determination. Each individual fish was
143   classified as follows, based on the histological analysis of gonads, immature female (IF), mature
144   female (MF), immature male (IM), and mature male (MM). This classification was based on
145   gamete development and the characteristics of the reproductive structures, such as follicles,
146   germinal epithelium, and interfollicular connective tissue (*Tyler & Sumpter, 1996*). Fish in which
147   gonads lacked characteristics allowing for the differentiation of ovaries or testicles were
148   classified as undifferentiated (UN).
149
150   **Trace Elements Analysis**
151   From each totoaba collected, 5 g of muscle and 5 g of liver were oven-dried separately at 70°C
152   and subsequently digested with nitric acid (HNO_3) and H_2O_2 in a microwave (Mars 5x, CEM,
153   Matthew, NC, USA). After acid digestion, 1 mL of hydrochloric acid (HCl) was added to each
154   sample and the volume was brought to 50 mL with deionized water (*Cadena-Cárdenas et al.,*
155   *2009; Barrera-García et al., 2012*). Copper, Cd, Zn, Fe, and Pb concentrations were determined
156   by atomic absorption spectrophotometry (GBS Scientific AVANTA, Dandenong, Australia)
157   using an air/acetylene flame. Each sample was tested in triplicate. High-purity reagents were
158   used in all cases and blanks were analyzed in parallel to validate the efficiency of the method

159 (*Barrera-García et al., 2012*). A quality control sample was analyzed at an interval of every ten
160 samples to ensure the quality of analyses in addition to blanks and calibration standard solutions.
161 Certificate reference material (DORM-2, National Research Council of Canada) was also
162 analyzed in each ten samples run as a quality control *(Jara-Marini et al., 2009)*; a 93% - 106%
163 recovery rate was calculated. The detection limits and quantification limits ($\mu g\ g^{-1}$) were as
164 follows, respectively: Cd: 0.01 and 0.02; Pb: 0.07 and 0.10; Cu: 0.017 and 0.020; Zn: 0.021 and
165 0.060; Fe: 0.65 and1.35. All the results are expressed in dry weight.
166

Nutritional and risk assessment

168 The estimated daily intake (EDI, mg trace element kg^{-1} BW day^{-1}) of Cu, Cd, Zn and Fe when
169 totoaba muscle is consumed was calculated as follows in Equation 1 (*Bilandžić et al., 2014*):
170 $$EDI = (Cm * CR)/BW \qquad \text{(Equation 1)}$$
171 where:
172 Cm = mean concentration of trace metals in the muscle of totoaba, expressed in fresh weight (μg
173 g^{-1}, fw).
174 CR = Mean per capita daily consumption rate of fish muscle (in this study, fishermen
175 populations living on the coast of north-western Mexico (130 g day^{-1}) *(Zamora-Arellano et al.,*
176 *2018*).
177 BW = mean body weight in the general population or subpopulation (74 kg; *Zamora-Arellano*
178 *et al., 2018*).
179 The contribution of the mean daily consumption of totoaba muscle to the recommended daily
180 intake (RDI) was assessed for the essential elements addressed in this study, including Cu: 0.9
181 mg day^{-1}, Zn: 11 mg day^{-1} and Fe: 8 mg day^{-1} person *(Trumbo et al., 2001)* and RfD (*National*
182 *Academies of Sciences, Engineering, and Medicine, 2017*).
183 The hazard index (HI) for Cu, Cd, Zn and Fe based on daily ingestion of muscle of totoaba was
184 achieved by deterministic and probabilistic (Monte Carlo simulations) approaches (*Sanaei et al.,*
185 *2020*). With the deterministic approach, the contribution of the geometric mean of Cu, Cd, Zn
186 and Fe concentration found in muscle of totoaba was compared to their respective metal
187 reference dose (RfD). The RfD is an estimate (with uncertainty spanning perhaps an order of
188 magnitude) of a daily oral exposure for an acute duration (24 hours or less) to the human
189 population (including sensitive subgroups) that is likely to be without an appreciable risk of
190 deleterious effects during a lifetime (*USEPA, 2020*). The Environmental Protection Agency
191 (EPA) recommends for Cu 0.40 mg $kg^{-1}day^{-1}$; Cd 0.001 mg $kg^{-1}day^{-1}$; Zn 0.30 mg $kg^{-1}day^{-1}$;
192 and Fe 0.7 mg $kg^{-1}day^{-1}$ (*USEPA, 2020*). The EPA has not established an RfD for Pb because a
193 "safe" exposure limit for Pb toxicity is still uncertain (*EFSA Panel on Contaminants in the Food*
194 *Chain (CONTAM), 2010*; *Korkmaz et al., 2019*).
195 As a probabilistic approach of risk assessment, Monte Carlo simulations (MCS) were
196 performed. MCS recently have been used for environmental health and safety risk assessments
197 associated to metal exposure through water and food (*Jia et al., 2018*; *Chen et al., 2019*; *Sanaei*
198 *et al., 2020*). MCS reduce the probability of uncertainty caused when only a single point *value* is

199 used for each variable during the risk assessment (as was calculated deterministically using
200 Equation 1. The distributions of parameters for MCS are listed in Table 1. MCS mainly consist
201 of three steps, (1) introduction and definition of the distribution functions of the variables; (2)
202 inclusion of the formula that will be used to calculate the risk considering also the RfD, and (3)
203 carrying out several simulations generating random numbers of the unknown parameter from a
204 specific probability density function. Each random number simulates a scenario independently.
205 The process is iterative and the results are displayed as a probability curve, where the X axis
206 represents the simulated variable and the Y axis represents the probability of occurrence (*Chen et*
207 *al., 2019*). In this study, Cristal Ball (11.1.2.4) software from Oracle was used to perform MCS.
208 Iterations for every run were set to 10,000 (*Jia et al. 2018; Sanaei et al. 2020; Chen 2019*) and
209 Log-normal probability distribution functions for the parameters (Table 1) were employed to
210 generate the cumulative hazard index (*Chen et al., 2019*) of Cu, Zn, Cd and Fe associated to the
211 consumption of muscle of totoaba.
212   Based on the results of the MCS, a sensitivity analysis was conducted by introducing
213 variation in some parameters and their effect over hazard index. The level of sensitivity is shown
214 as contribution to variance (%) and correlation. This analysis is employed to judge the
215 significance of the input parameters to the risk estimation (*Chen et al., 2019; Sanaei et al.,*
216 *2020*).
217   Aiming to identify potential factors that can influence the concentration of Cu, Cd, Zn
218 and Fe in liver and muscle, and therefore in the health risk for consumption of totoaba muscle, a
219 principal component analysis (PCA) was performed using TIBCO STATISTICA software ver.
220 13.3.
221
Statistical Analysis
223 The Shapiro-Wilk's normality and Bartlett's homoscedasticity tests were run. Differences
224 between groups (IF, MF, IM, MM, UN) were examined by means of analysis of variance
225 (ANOVA) followed by *post-hoc* Tukey's honest significance test (*Zar, 1998*). Differences were
226 considered significant when $p < 0.05$. Statistica 8.0 (StatSoft Inc. Tulsa, OK, USA) software was
227 used to perform all statistical analyses. Results are expressed as mean ± SE.
228 Generalized linear models (GLM) were used to explore the variables that may affect muscle and
229 liver Cd levels in the totoaba (dependent variables, Table 2) through simultaneous effects of the
230 independent variables (season of the year, sex/maturity stage, total length, and concentrations of
231 trace elements in liver and muscle of totoaba) (Tables 2 and 3). Two models were fitted to the
232 data; the variables selected as continuous dependent (response) variable in each model were liver
233 Cd concentration in model 1, and muscle Cd in model 2. In both cases, the dependent variables
234 were modeled assuming a gamma distribution error since no significant differences were
235 obtained from the Chi square test ($p > 0.05$). In the GLM, the season and sex/maturity stage were
236 included as categorical explanatory variables; length and metal concentrations in liver and
237 muscle were included as continuous explanatory variables (Tables 2 and 3). In all cases, the log-
238 type link function was used to relate the dependent variable (liver Cd, muscle Cd) with
239 categorical and continuous independent variables. Finally, the models were visually validated

240   through the residual deviation of observed and predicted values selected based on the proportion
241   of the explained deviance *(Murillo-Cisneros et al., 2018)*, and Akaike's and Bayesian
242   information criteria (AIC and BIC, respectively) *(Burnham & Anderson, 2004; Hobbs &*
243   *Hilborn, 2006; Johnson & Omland, 2004; Zuur et al., 2009)*.
244
245   **Results**
246   Table 3 shows trace elements concentrations in muscle and liver of totoaba, grouped by habitat
247   (estuaries, rocky and continental), season (winter, spring and autumn), and sex/maturity stage. In
248   liver, Cu levels were significantly higher in MF in winter in estuarine habitat than IF, IM, and
249   UN individuals collected in autumn in continental habitats ($p < 0.05$). Female individuals
250   (mature and immature) in spring had about 3 times lower liver Cd concentrations compared to
251   IF, IM, and UN specimens collected in autumn in rocky habitat; there were no significant
252   differences between the individuals collected in winter and the totoabas collected in spring (both
253   estuarine and rocky habitats) and autumn (continental habitats) ($p > 0.05$). Female totoabas
254   collected in winter showed significantly higher liver Zn levels in comparison with their
255   counterparts collected in spring ($p < 0.05$). Mature females in spring from rocky habitat had
256   significantly lower liver Fe as compared to IF and UN collected in autumn from the same habitat
257   ($p < 0.05$) (Table 3). Also, in autumn, significantly higher concentrations of Fe were observed in
258   organisms from rocky *versus* continental habitats ($p < 0.05$). No detectable Pb levels (< 0.07 µg
259   g^{-1}) were observed in any of the analyzed liver samples.
260   In totoaba muscle, Pb levels were below the detection limit (< 0.07 µg g^{-1}), and only 10%
261   of the samples showed detectable Cd levels (> 0.01 µg g^{-1}) (Table 3). No significant differences
262   were observed in muscle Cu and Cd levels ($p > 0.05$). Mature males and MF had significantly
263   higher muscle Zn levels in winter as compared to organisms collected both in spring and autumn
264   in rocky and continental habitats ($p < 0.05$).
265   Copper, Zn, and Fe concentrations were significantly higher in liver than in muscle ($p =$
266   0.001) (Table 3). While 100% of liver samples showed detectable Cu and Cd levels, these
267   elements were undetectable in muscle in 40% and 90% of samples, respectively. Liver Cu, Zn,
268   and Fe concentrations were approximately up to 40-, 7- and 80-fold higher, respectively, than in
269   muscle.
270
271   **Health Risk**
272   Figure 3 shows the Cu, Cd, Zn and Fe content in muscle of *Totoaba macdonaldi* relative to the
273   RDI, by sex/maturity stage groups and season (winter, spring, autumn). Consumption of totoaba
274   muscle can provide the human diet with approximately 70% of Cu, 60% of Zn, and more than
275   100% of Fe of the RDI. In addition, the concentrations of Cu, Zn and Fe in muscle of totoaba are
276   from one to several orders of magnitude below the RfD. Cadmium input from totoaba muscle to
277   the human diet can be from zero to approximately 20% of the RfD, depending on the fish's
278   sex/maturity stage. No detectable concentrations of Pb were recorded in muscle of totoaba;
279   therefore, no comparisons to RfD are available.

280   The potential health risk to local inhabitants from long-term exposure for Cu, Cd, Zn and
281   Fe in fish muscles of totoaba was assessed deterministically (*Sanaei et al., 2020*) and through the
282   hazard index (HI) obtained by the probabilistic approach using MCS in adult population. Monte-
283   Carlo simulation was introduced to conduct uncertainty through 10,000 iterations for each trace
284   element simulation. The probabilistic distributions of health risk as hazard index (HI) obtained
285   from MCS are demonstrated in Figure 4, including mean, 5th percentile and 95th percentile for
286   each metal. The HI values obtained from probabilistic values (MCS) are lower than those
287   calculated by deterministic approach; Cu: 0.08 vs 0.10; Cd: 0.06 vs 0.07; Zinc: 0.17 vs 0.21 for
288   Fe 0.10 vs 0.13, respectively. The percentile values for the HI for all the metals are lower than 1.
289   In Figure 5 the associated risk given by the HI of Cu, Cd, Zn and Fe from totoaba muscle
290   consumption is shown. The contribution of each HI can be arranged in decreasing order as
291   follows $HI_{Zn} > HI_{Fe} > HI_{Cu} > HI_{Cd}$. The sensitivity analysis (Table 4) showed that the
292   consumption rate of muscle of totoaba is the parameter that contributes with the highest
293   explanation of the variance of each metal HI, 75.4% in HI_{Cu}; 82.7% in HI_{Cd}, 82.7 en HI_{Zn} and
294   83% in HI_{Fe}. Otherwise, the body index has a negative relationship and in the case of totoaba
295   muscle the influence of metal content has the lowest importance in the contribution of the
296   variance.
297   For qualitative evaluation of clustering behavior of Cu, Cd, Zn and Fe in liver and muscle
298   of totoaba regarding season, habitat and gonadic state a principal component analysis (PCA)
299   when varimax normalization was applied. The results are shown in Table 5. The PCA of these
300   data indicates their association and grouping in three factors that explain 52.28% of the variance.
301   Factor 1 contributed 24.60% of the total variance with the highest loadings of Cu_{muscle}, Cu_{liver},
302   and Zn_{muscle}, that are directly related to winter, estuarine, mature females and mature male, but
303   inversely related to spring, and in less magnitude, to immature organism. Factor 2 contributed
304   15.88% of the total variance with the highest loading of Cd_{liver}, Zn_{liver}, and Fe_{liver} that are only
305   associated to autumn and to a lesser extent spring. Factor 3 has the highest loading of Fe_{muscle} that
306   is inversely to autumn and to rocky habitat but directly related to continental habitat.

307
Generalized Linear Models (GLM)
308
309   Due to Cd being an important element in relation to health risk, GLM were used to explore the
310   variables that may affect the content of Cd in muscle and liver in totoaba. The first GLM model
311   explains 46% of the variance (Table 6), fitting the data reasonably well. The ratios of the
312   deviance and Pearson Chi square over the degree of freedom close to 1.0, in particular model 2,
313   does not show evidence of overdispersion. The model suggests that liver Cd concentration varies
314   inversely with liver Zn concentration. Other elements measured in liver or muscle samples were
315   not identified as significantly affecting liver Cd content. However, the winter season and
316   sex/maturity stage and two interactions were significant. The second GLM model (Table 6),
317   which explains 71% of the variance, suggests that liver Cd is the only element that significantly
318   affects muscle Cd concentrations in totoaba. Furthermore, sex/maturity stages and an interaction
319   were significant. Both models were validated using the analysis of residuals (Figure 6),

320   suggesting that the variance is homogeneous for all the independent variables and that the
321   relationship between the observed and fitted values is nearly linear.
322
Discussion
323
324   Compared to muscle, liver accumulates a larger concentration of metals because of its
325   detoxifying function and specific metabolic rate, acting as a depot of chemical elements, some of
326   which function as enzyme cofactors (*Kalay et al., 1999*). This held true for totoaba analyzed in
327   the present study; liver concentrations of Cu, Zn and Fe were 40-, 7-, and 80-fold higher than in
328   muscle. The liver is highly active in fish metabolism and, therefore, the liver may accumulate
329   metals to higher levels than other tissues, such as the muscle (*Kalay & Canli, 2000*).
330   In this study, no detectable Pb levels were recorded in either liver or muscle of totoaba.
331   Other scianid fish, such as *Otolithes ruber* and *Johnius belangerii,* also show a low Pb content
332   relative to other fish species, including *Pampus argenteus* (Stromateidae*), Pomadasys sp.*
333   (Haemulidae*), Euryglossa orientalis* (Soleidae), and *Cynoglossus arel* (Cynoglossidae) collected
334   in the same sites from the Persian Gulf (*Agah et al., 2009; Monikh et al., 2012*). In biological
335   systems, trace elements may be either bound to molecules or found as chemical fractions
336   (speciation) that cannot be assimilated by the organism; in which case, trace elements are not
337   bioavailable, can be excreted, and are not bioaccumulated (*Bebianno and Langston, 1991*).
338   Although Pb has been reported at high concentrations in invertebrates such as clams (up to $9.2 \pm$
339    $1.4 \mu g$ g-1 dry weight) from the Upper Gulf of California (*Cadena-Cárdenas et al., 2009*), in
340   fishes of this region the levels are characteristic of areas not contaminated by this element
341   (*Ruelas-Inzunza & Paez-Osuna, 2008; Jara-Marini et al., 2009; Ruelas-Inzunza, et al., 2010*).
342   Apparently, Pb does not undergo biomagnification across some food chains in the Gulf of
343   California (*Jara-Marini et al., 2009*). This may be due to the functional (carboxyl, sulfhydryl)
344   groups to which Pb can bind in mollusk and fish species (*Ruelas-Inzunza & Páez-Osuna, 2008*)
345   that may be found as part of the totoaba diet (*De Anda-Montañez et al., 2013*).
346   The highest Cu levels in liver and muscle of totoaba quantified in this study were below
347    $70 \mu g$ g^{-1}. Natural Cu deposits are found along the Baja California peninsula, particularly in
348   Santa Rosalia, Baja California Sur. The Cu concentrations in totoaba in this study were found to
349   be similar to those recorded in other fish, such as *O. ruber* (7.5-19.5 μg g^{-1}), *J. belangerii* (0545-
350   1458 μg g^{-1}), *Menticirrhus americanus* (8.8-18 μg g^{-1}), *Micropogonias furneiri* (10-122 μg g^{-1}),
351   and *Cynoscion guatucupa* (22-119 μg g^{-1}) (*Monikh et al., 2012; Agah et al., 2009; Viana et al.,*
352   *2005*). These Cu concentrations are considered suitable for human consumption (approximately
353   120 μg g^{-1} dry weight; 30 μg g^{-1} ww, *Viana et al., 2005; Monick et al., 2012*) even though in
354   these studies, fish were sampled in areas influenced by wastewater from tanning, chemical, and
355   agricultural industries, or from urban effluents (*Agah et al., 2009; Viana et al., 2005*). Variations
356   in tissue Cu levels in sciaenids are attributed to the physiology of each species, rather than to the
357   influence of the environmental conditions in the area where they live (*Hamza-Chaffai et al.,*
358   *1995*). In totoaba in this study, significantly higher Cu content in MF as compared to IF is likely
359   due to Cu functioning also as a cofactor of cytochrome c oxidase (*Kirchgessner et al., 1977*); the

360 activity of this enzyme increases in the reproductive stage contributing to meet the additional
361 energy requirements during this process in fishes *(Olsson et al., 1987)*.
362   Mature female and male totoaba had higher Zn concentrations in winter than in spring. In
363 the squirrelfish (*Sargocentron spiniferum*), a reef associated fish, and the euryhaline rainbow
364 trout (*Oncorhynchus mykiss*), a freshwater fish, liver Zn content bound to metallothionein
365 increases, more so in MF than in MM, during reproduction, potentially aiding vitellogenesis and
366 the synthesis of estradiol-17β (*Thompson et al., 2001*). The demand for Zn increases during
367 embryonic and neonatal development (*Olsson et al., 1987; Thompson et al., 2002*). In spring, Fe
368 content in the liver of MF and MM was almost 50% the content observed in totoaba collected in
369 winter. This difference in Fe levels could be associated with fasting conditions reported for
370 totoaba in its spawning grounds in the north of the Upper Gulf of California over the winter (*De
371 Anda-Montañez et al., 2013*). The feeding rate of totoaba adults has been reported to decrease
372 during the reproductive season (*Román Rodríguez & Hammann, 1997*).
373   The Cd concentration in the liver of immature totoaba collected in autumn in rocky
374 habitat was more than three-fold the level in organisms collected in spring ($p < 0.05$). In a study
375 that compared Cd content between *J. belangerii* (Sciaenidae), *Liza abu* (Mugilidae), and
376 *Euryglossa orientalis* (Mugilidae), the sciaenid fish showed significantly higher Cd levels (3.80
377 $\pm$ 0.66 µg g^{-1}), which was attributed to its feeding habits, because *J. belangerii* lives in close
378 association with sediment, and feeds mainly upon crustaceans, mollusks, and shrimp (*Monikh
379 et al., 2012*). In autumn, totoaba feeds in the area adjacent to the large islands; the oceanographic
380 conditions in this area foster a high primary productivity, reflected as a high abundance of birds
381 and marine mammals *(Hidalgo-González & Alvarez-Borrego, 2004)*. This photosynthetic
382 activity is maintained in part by nutrient enrichment, including Cd (*Álvarez-Molina, 2013;
383 Delgadillo-Hinojosa et al., 2001*). In this area, totoaba feed mainly on anchoveta *Cetengraulis
384 mysticetus* and silverside *Colpichthys* spp (*De Anda-Montañez et al., 2013*), which in turn feed
385 on plankton known to contain around 17 µg g^{-1} Cd (*Lane et al., 2005*). The Cd assimilated by
386 fish accumulates in the liver; in which it is bound mainly to metallothionein, a protein of low
387 molecular weight with a high content of amino and sulfhydryl groups (*Hamza-Chaffai et al.,
388 1995*). These proteins play a central role in the regulation and detoxification of trace elements.
389 Divalent cations such as Cd, have high affinity for sulfhydryl groups in metallothionein; as a
390 result, Cd displaces essential elements such as Zn, which upon release boost the synthesis of
391 additional metallothionein (*Bebianno & Langston, 1991*). The factors obtained by the PCA
392 analysis showed that trace elements could be associated mainly to the reproductive physiology of
393 the organisms as well as the starving/feeding conditions during the different seasons and in the
394 different habitats. The reproduction process demands some metals that are required as co-factors
395 of several enzymatic reactions involved in metabolic pathways (*Olsson et al., 1987; Farkas et
396 al., 2002; Canli & Atl, 2003*). The combined results from this study suggest that the observed
397 variations in Cd, Cu, Zn and Fe concentrations in totoaba liver are related to the feeding and
398 reproductive physiology of this species rather than to environmental exposure.
399   In this study, Cu, Cd, Zn, Fe, and Pb contents in totoaba muscle were within the ranges
400 reported in fish from unpolluted areas of the Gulf of California *(Ruelas-Inzunza et al., 2010)*. No

401 significant variations in Cu and Cd content were observed in muscle neither significant loadings
402 were obtained in the PCA indicating that the content of these elements in muscle is unaffected
403 by season (potentially, migration) or by the maturity stage of the totoaba *(Agah et al., 2009;*
404 *Ruelas-Inzunza et al., 2010)*, In this study, in the factor 1 in the PCA, the content of Zn in
405 muscle is associated especially with mature organisms in winter. Zinc is an essential component
406 of metalloenzymes involved in the energy metabolism that contributes with muscle strength and
407 resistance to fatigue (*Krotkiewski et al., 1982*).
408   In the PCA, in factor 2 the highest loadings of Cd_{liver}, Zn_{liver} and Fe_{liver} that can be
409 associated to the nutrition status of the totoaba, due to their starving/feeding periods, are also
410 influenced by changes in their diet composition throughout the life cycle of these organisms
411 (*Farkas et al., 2002*). Factor 3 could be associated with environmental conditions in the rocky
412 and continental habitats that influence the Fe content in muscle of totoaba. All the HI obtained
413 by MCS were HI < 1; this suggests a negligible health effect (*Sanaei et al., 2020, USEPA, 1989*)
414 of Cu, Cd, Zn, Fe or Pb content in muscle of totoaba consumed in human diet. The sensitivity
415 analysis agrees with several previous studies reporting that the ingestion rate, rather than the
416 concentration of the element in food, is more sensitive; therefore, small changes in the
417 consumption rate would affect HI to a greater extent (*Sanaei, et al., 2020; Chen et al., 2019; Guo*
418 *et al., 2019*).
419   Muscle Cu, Cd, Zn, Fe, and Pb concentrations were below the maximum permissible
420 levels for human consumption established by the World Health Organization (WHO) and the
421 European Community for seafood; i.e., 10 µg g^{-1} ww for Cu (Australian legislation; *(Nauen,*
422 *1983)*, 50 µg g^{-1} ww for Zn (*Collings et al., 1996*), 0.05 and 0.2 µg g^{-1} ww for Cd and Pb,
423 respectively (European Community, *(EC), 2005*;(*WHO, 1996*). Thus, consumption of totoaba
424 does not pose a public health risk. Furthermore, depending on the sex/maturity stage of totoaba,
425 consumption of this fish's muscle may provide approximately 70% Cu, 60% Zn and 100% Fe of
426 the recommended DRI. Both RDA and RfD are comparable to the values obtained in muscle of
427 several fish species from the Mediterranean Sea and the Gulf de California *(Bilandžić et al.,*
428 *2014; Korkmaz et al., 2019; Zamora-Arellano et al., 2018*). It is calculated that 130 g of totoaba
429 muscle can provide approximately 20% of the daily tolerable dose of Cd.
430   In a previous study, liver superoxide dismutase (SOD) activity was found to be related to
431 totoaba's maturity stage *(Hernández-Aguilar et al., 2018)*; thus, it was used in this study to
432 further discriminate between environmental and physiologic effects on trace element content in
433 totoaba. This third GLM explains 55% of the variance and suggests that in liver, SOD activity is
434 inversely related to Cd concentration in winter and spring ($p < 0.05$) but not in autumn ($p >$
435 0.05). The GLMs constructed in this study suggest that liver Cd concentration varies inversely
436 with liver Zn concentration, that liver Cd content affects muscle Cd concentrations, and that in
437 the liver, SOD activity is inversely related to Cd concentration and length of the organisms. The
438 totoaba collected in autumn have the highest liver Cd concentrations, and the highest SOD
439 activity (*Hernández-Aguilar et al., 2018*). In autumn, immature and undifferentiated totoabas
440 feed on sardines and crustaceans *(Cisneros-Mata et al., 1995)*. In the Upper Gulf of California,
441 in winter and spring, the feeding rate of totoaba is reduced as a result of the reproductive activity
442 *(Román Rodríguez & Hammann, 1997)*. The liver is the primary organ where trace elements are
443 stored and physiologically regulated in fish (*Hamza-Chaffai et al., 1995; Roméo et al., 1999*); it
444 also participates in antioxidant defense mechanisms (including SOD) that protect tissues from

445 the deleterious effects of free radicals (*Basha & Rani, 2003; Halliwell & Gutteridge, 2007*). In
446 the present study, Cd content in totoaba liver may be indicative of the presence of this metal in
447 the environment, but the content of Cu, Zn, and Fe may be associated primarily with the
448 physiology of this fish rather than with the presence of these elements in the environment, as has
449 been reported for other marine fish species (*Hamza-Chaffai et al., 1995*).
450

451 **Conclusions**
452 In summary, although totoaba migrates throughout the Gulf of California, where sites enriched
453 by trace elements are common, Cd, Cu, Zn, Fe and Pb content in liver and muscle of totoaba
454 were within the levels considered as indicators of sites without contamination. Significantly
455 higher Cu content in mature as compared to immature female totoaba was observed. The highest
456 liver Cd concentrations were recorded at the time that totoaba are feeding around the large
457 islands and the lowest Cd levels in liver were found during the reproductive stage. In muscle,
458 significant variations were observed in Zn content associated with the maturity stage of the
459 female totoaba. Therefore, variations in the content of Cd, Cu, Zn and Fe in liver of totoaba seem
460 to be more related to the feeding and reproductive physiology of this species than as result of
461 environmental exposure; further totoaba feeding ecology studies are suggested to confirm this.
462 Results suggest that consumption of totoaba muscle does not pose a public health risk.
463 Furthermore, depending on the sex/maturity stage of totoaba, this fish's muscle may provide
464 approximately 70% Cu, 60% Zn and 100% Fe of the recommended DRI.

465

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472

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707

Monte Carlo parameter distributions and parameters used in the health risk assessment.

EDI= estimated daily intake; Bw= body weight; CR= consumption rate; HI= hazard index;

C_{metal}= metal concentration in muscle of totoaba; RfD= reference dosis

Parameters	Unit	Description	Distribution	Value	Ref
EDI	$(mg\ kg^{-1})$ day^{-1}	Estimated Daily Intake through ingestion	-	Equation 1	Bilandžić et al., 2014
Bw	Kg	Body weight adult	Log normal	74±10 kg	Zamora-Arellano et al., 2018
CR	$Kg\ day^{-1}$	Consumption Rate	Log normal	0.100±0.030	Zamora-Arellano et al., 2018
HI		Hazard Index	-		Sanaei et al. 2020
C_{metal}	$mg\ kg^{-1}$		Log normal		
RfD	$(mg\ kg^{-1})$ day^{-1}	Reference dose of heavy metals	Fixed values	Varied depending on the specific metal	USEPA, 2020; Chen et al. 2029

Factors hypothesized to affect cadmium (Cd) in liver and muscle of totoaba (*Totoaba macdonaldi*) sampled in the Gulf of California, Mexico.

Variable	Type	Description
Season	Categorical	Autumn
		Winter
		Spring
Sex/maturity stage	Categorical	MF; mature females
		MM; mature males
		IF; immature females
		IM; immature males
Length	Continuous	Length of the organism (g)
Cadmium in liver and muscle	Continuous	Metal concentration ($\mu g \cdot g^{-1}$)
Copper in liver and muscle	Continuous	Metal concentration ($\mu g \cdot g^{-1}$)
Zinc in liver and muscle	Continuous	Metal concentration ($\mu g \cdot g^{-1}$)
Iron in liver and muscle	Continuous	Metal concentration ($\mu g \cdot g^{-1}$)

Trace elements concentration ($\mu g \cdot g^{-1}$) in liver and muscle of *Totoaba macdonaldi* by season and sex/maturity stage groups.

Totoaba groups: MF, mature females; MM, mature males; IF, immature females; IM, immature males; UN, undifferentiated. Different letters indicate significant differences (p<0.05); lowercase letters denote differences within a habitat and season between maturity stages for each sex in liver; uppercase letters denote differences within a habitat and season between maturity stages for each sex in muscle. E= estuarine; R=rocky; C= continental. Values are shown as means ± standard error.

Tissue	Habitat	n	Season	Sex/maturity Stage	Copper	Cadmium	Zinc	Iron	Lead
Liver									
			Winter						
	E	15		MF	44.04±5.02[a]	1.82±0.30[a]	116.13±6.26 [a]	1,284±103[abcd]	<0.07
	E	18		MM	35.84±2.39[ab]	3.18±1.13[abc]	107.98±4.14 [ab]	1,187±151[abcd]	<0.07
			Spring						
	E	8		MF	36.02±8.05[ab]	0.89±0.38 [a]	80.68±6.81 [ab]	623±137 [ab]	<0.07
	E	3		MM	22.78±5.83 [ab]	0.89±0.73 [ab]	72.26±6.58 [ab]	628±46[abcd]	<0.07
	R	7		MF	24.23±2.20 [ab]	1.64±0.89 [ab]	69.41±1.52 [b]	556±93 [a]	<0.07
	R	8		MM	20.07±4.43 [b]	1.24±0.37 [ab]	79.86±6.29 [ab]	813±173[abd]	<0.07
	R	11		IF	21.80±3.34 [b]	0.47±0.36 [a]	86.66±5.95 [ab]	560±59 [ab]	<0.07
	R	6		IM	23.40±6.21 [ab]	1.06±0.70 [ab]	76.55±11.15 [ab]	787±283 [abc]	<0.07
			Autumn						
	R	12		IF	28.87±4.02 [ab]	5.53±0.96[c]	93.89±11.70 [ab]	1,521±189 [bcd]	<0.07
	R	11		IM	31.34±4.32 [ab]	6.7±1.01[c]	97.82±10.29 [ab]	1961±336[d]	<0.07
	R	21		UN	25.89±2.96 [b]	5.33±0.61[c]	96.00±5.34 [ab]	1,792±181 [cd]	<0.07
	C	**9**		IF	16.30±2.93 [b]	1.32±0.22 [a]	89.58±8.37 [ab]	400±63[a]	<0.07
	C	7		IM	11.80±2.64[b]	1.06±0.09 [ab]	75.74±4.26 [ab]	359±49[a]	<0.07
	C	6		UN	20.90±4.58[b]	2.38±0.50 [abc]	113.62±12.84 [ab]	470±92[ab]	<0.07
Muscle									

	Winter						
E	14	MF	1.80±0.41	<0.01	17.74±1.00^A	22.99±2.84 ^{ABC}	<0.07
E	18	MM	1.54±0.32	<0.01	17.78±0.72^A	20.43±4.00 ^{ABC}	<0.07
	Spring						
E	8	MF	0.86±0.43	<0.01	15.15±0.61^{AB}	21.67±1.95 ^{ABC}	<0.07
E	3	MM	1.01±0.54	<0.01	15.73±1.78^{AB}	22.49±3.63 ^{ABC}	<0.07
R	7	MF	2.00±0.80	0.03±0.02	13.86±3.20^{AB}	21.90±5.88 ^{ABC}	<0.07
R	8	MM	0.39±0.19	<0.01	11.79±1.16 ^{B}	12.74±1.55^{ABC}	<0.07
R	12	IF	<0.017	<0.01	12.42±0.42 ^{B}	11.73±2.13 ^{AB}	<0.07
R	6	IM	0.32±0.21	<0.01	13.84±0.49 ^{AB}	21.90±4.47 ^{ABC}	<0.07
	Autumn						
R	12	IF	0.48±0.19	<0.01	12.23±1.44 ^{B}	7.96±2.73^B	<0.07
R	11	IM	1.68±1.06	<0.01	11.91±1.04 ^{B}	15.03±4.79^{ABC}	<0.07
R	21	UN	0.04±0.02	<0.01	11.74±0.59 ^{B}	10.84±2.54 ^{AB}	<0.07
C	**9**	IF	1.37±0.62	<0.01	11.35±0.65^B	18.18±1.58^{ABC}	<0.07
C	8	IM	0.48±0.35	<0.01	10.95±0.50^B	33.17±6.50^C	<0.07
C	7	UN	0.69±0.46	0.15±0.14	13.07±0.66 ^{AB}	31.19±5.36^{AC}	<0.07

Sensitivity analyses of input parameters for the effect in the hazard index (HI) as estimation of the health risk for consumption of muscle of totoaba. The level of sensitivity is shown as contribution to the variance (%) and rank correlation (10,000 tria

	Parameters	Contribution to Variance (%)	Rank correlation
Sensitivity: HI$_{copper}$	Consumption Rate	7.54E-01	8.53E-01
	Body weight	1.60E-01	-3.92E-01
	Copper $_{muscle}$	8.56E-02	2.87E-01
Sensitivity: HI$_{cadmium}$	Consumption Rate	8.27E-01	8.98E-01
	Body weight	1.72E-01	-4.10E-01
	Cadmium $_{muscle}$	1.34E-05	3.62E-03
Sensitivity: HI$_{zinc}$	Consumption Rate	8.27E-01	8.98E-01
	Body weight	1.72E-01	-4.10E-01
	Zinc $_{muscle}$	1.74E-05	4.12E-03
Sensitivity: HI$_{iron}$	Consumption Rate	8.30E-01	9.01E-01
	Body weight	1.70E-01	-4.07E-01
	Iron $_{muscle}$	6.95E-05	8.24E-03

Loadings for season (winter, spring and autumn), habitat (estuarine, rocky, continental), sex/maturity stage (male, female, mature, immature, undifferentiated) and estimated dairy intake (EDI) of trace metal content in muscle in totoaba on VARIMAX-rotated

Variable	Factor 1	Factor 2	Factor 3
Winter	-0.864*	0.201	0.114
Spring	0.726*	0.496	0.380
Autumn	0.001	-0.724*	-0.518
Estuarine	-0.932*	-0.002	0.049
Rocky	0.632	0.074	-0.664*
Continental	0.316	-0.099	0.848*
Mature females	-0.541*	-0.219	-0.095
Mature male	-0.505*	-0.084	-0.112
Immature females	0.389*	-0.146	-0.040
Immature males	0.294*	0.006	0.146
Undifferentiated	0.401	0.445*	0.144

Copper muscle	-0.332*	-0.111	0.225
Cadmium muscle	0.069	0.018	0.251*
Zinc muscle	-0.700*	0.100	0.070
Iron muscle	-0.281	-0.206	0.524*
Copper liver	-0.511*	0.434	-0.248
Cadmium liver	0.149	0.788*	-0.224
Zinc liver	-0.322	0.663*	-0.006
Iron liver	-0.011	0.780*	-0.318
Eigenvalue	4.67	3.02	2.24
Explained variance (%)	24.60	15.88	11.81
Cumulative variance	24.60	40.48	52.28

Generalized linear models explaining the variability of cadmium (Cd) in liver and muscle of *Totoaba macdonaldi*. Values marked with * were significantly different ($p < 0.05$) from the intercept.

MF, mature females; MM, mature males; IM, immature males; IF, immature females; [a]Akaike

information criterion, [b]Bayesian information criterion; s. e., standard error.

Statistics	Models	
	1; Cd in liver	2; Cd in muscle
Error	Gamma	Gamma
Link	Log	Log
Deviance	172	73
Explained deviance	46%	71%
Pearson Chi^2	103	76
Log likelihood	-167	505
Df	100	74
Deviance/Df	1.72	0.98
Pearson's Chi^2/Df	1.03	1.03

Level of Effect	Estimate	s. e.	Estimate	s. e.
Intercept	-4.04*	0.92	-5.93*	0.73
Copper in muscle	-0.06	0.08	-0.07	0.06
Cadmium in muscle	0.70	1.18	--	--
Zinc in muscle	-0.02	0.03	-0.04	0.03
Iron in muscle	0.004	0.01	0.01	0.008
Length	0.00006*	0.00002	0.00002	0.46
Copper in liver	0.02	0.009	-0.008	0.007
Cadmium in liver	--	--	0.14*	0.05
Zinc in liver	0.01*	0.006	-0.001	0.005
Iron in liver	0.0003	0.0002	0.0001	0.0002
Season 1 (winter)	-2.98*	0.58	0.33	0.40
Season 2 (spring)	0.31	0.45	0.14	0.24
Sex/maturity stage (MF)	1.28*	0.49	2.16*	0.44
Sex/maturity stage (MM)	1.79*	0.41	1.08*	0.37
Sex/maturity stage (IM)	0.45	0.70	1.35*	0.45
Sex/maturity stage (IF)	0.83*	0.39	-1.18*	0.27
Season 1*Sex/maturity stage	3.50*	0.95	2.21*	0.58
Season 2*Sex/maturity stage	1.88*	0.96	--	--
AIC[a]	386		-964	
BIC[b]	459		-905	

Figure 1

Totoaba macdonaldi study area in the Gulf of California.

Upper Gulf of California
and Colorado River Delta
Biosphere Reserve

Puerto Peñasco

San
Felipe

Piedra
Consag

Islas Las
Encantadas

Bahía San
Luis Gonzaga

Isla Ángel
de la Guarda

El Desemboque

Isla
Tiburón

Bahía de
los Angeles

N

28°

Santa
Rosalía

Bahía de Lobos

Bahía
Concepción

Gulf of California

Pacific Ocean

Las Grullas

24°

Bahía
de La Paz

Mazatlán

111°

107°
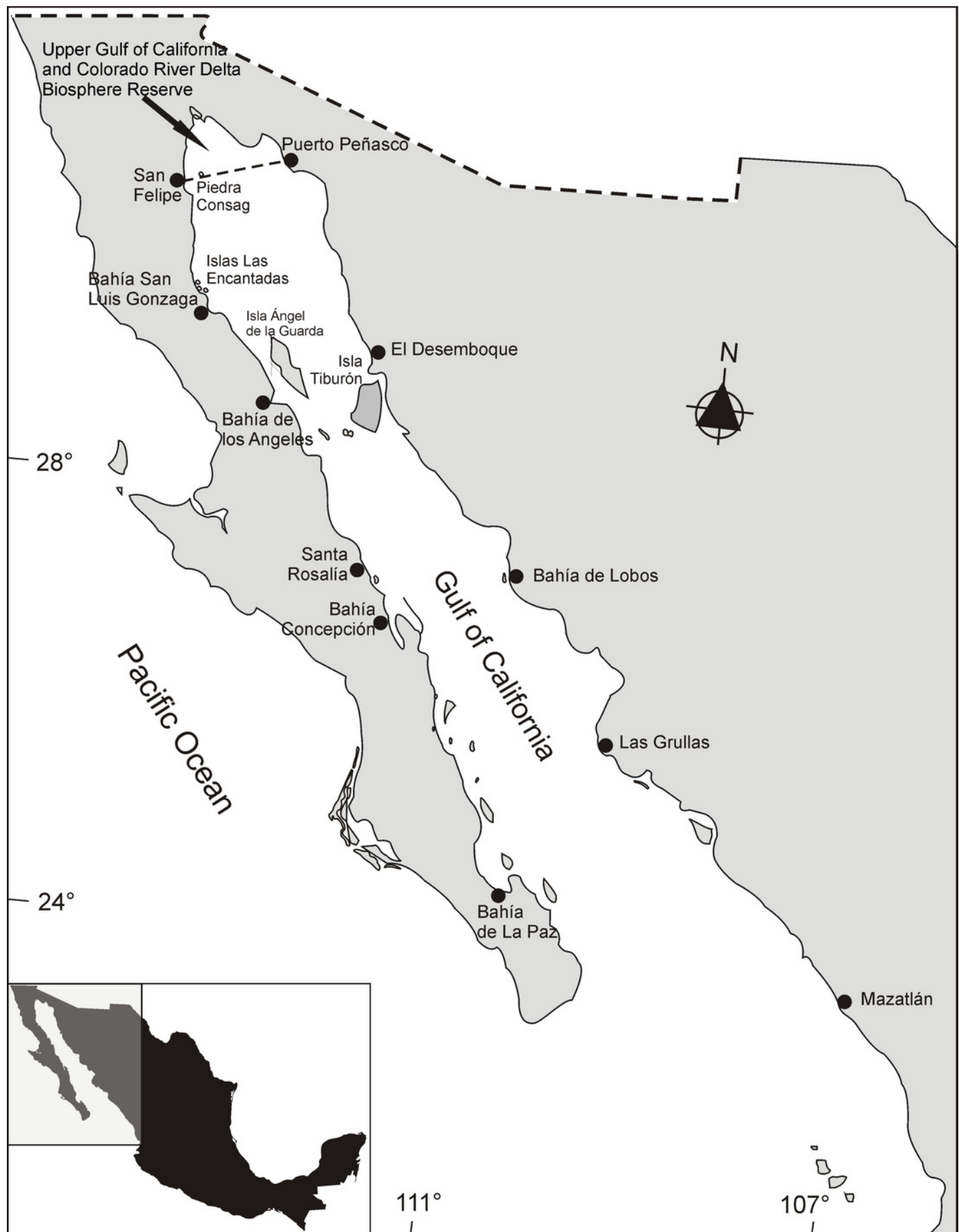
Figure 2

Overview of experimental program.
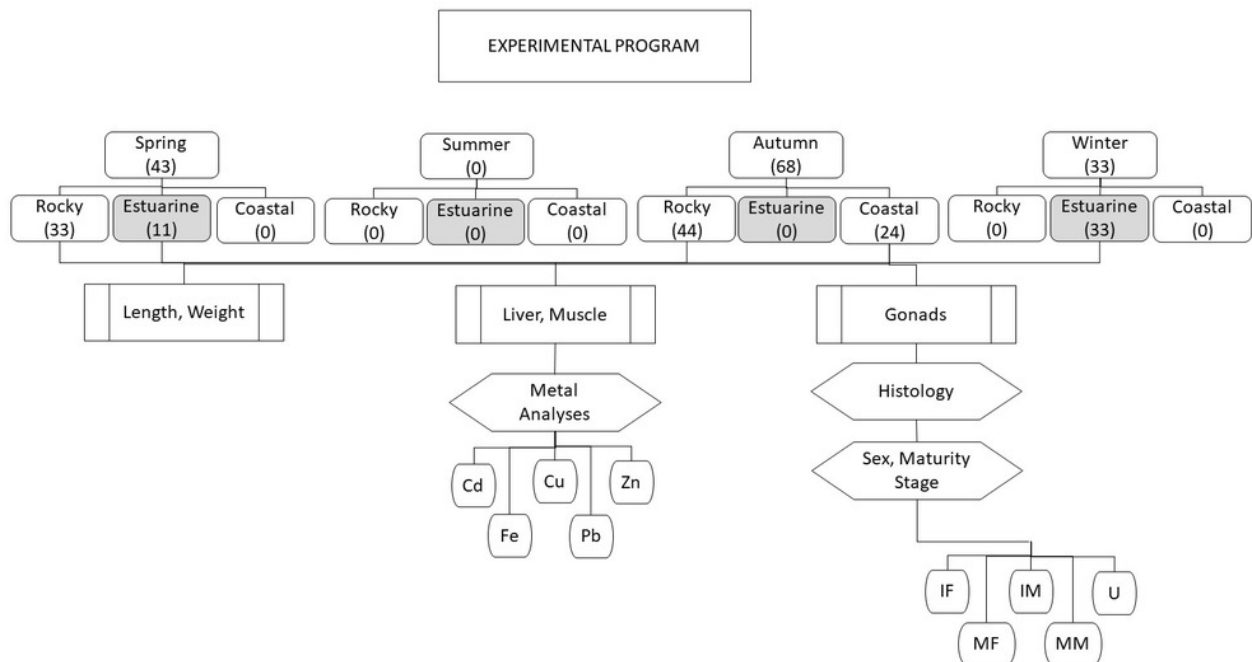
Figure 3

Contribution of copper, cadmium, zinc and iron by consumption of muscle of *Totoaba macdonaldi* (μg day^{-1}) to the dietary reference intakes (DRI, *National Academies of Sciences, Engineering and Medicine, 2017*; dotted line) and referenc
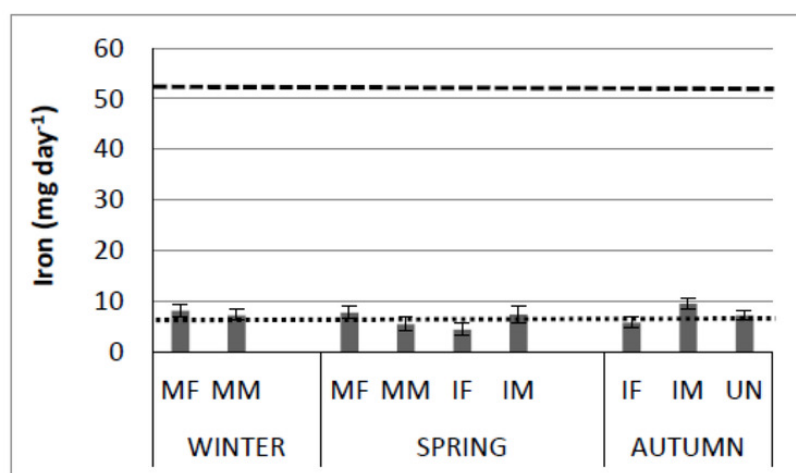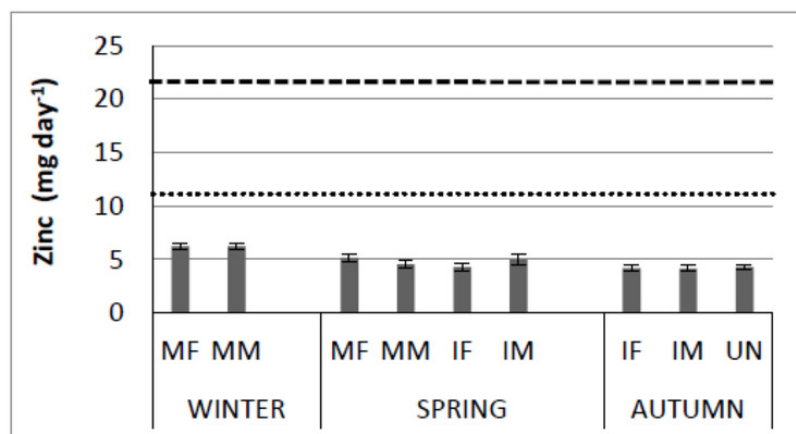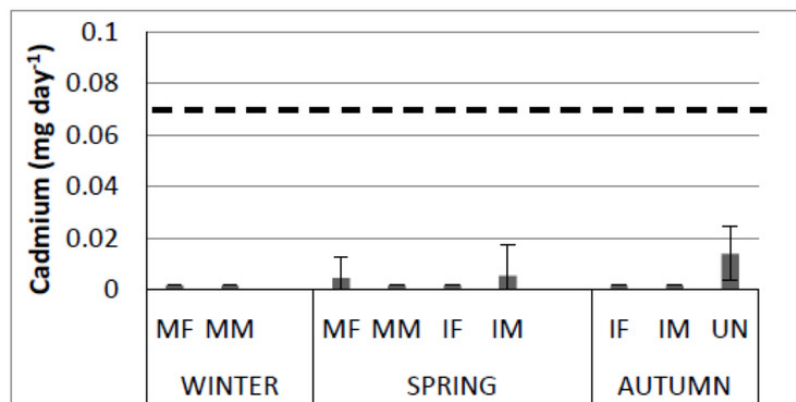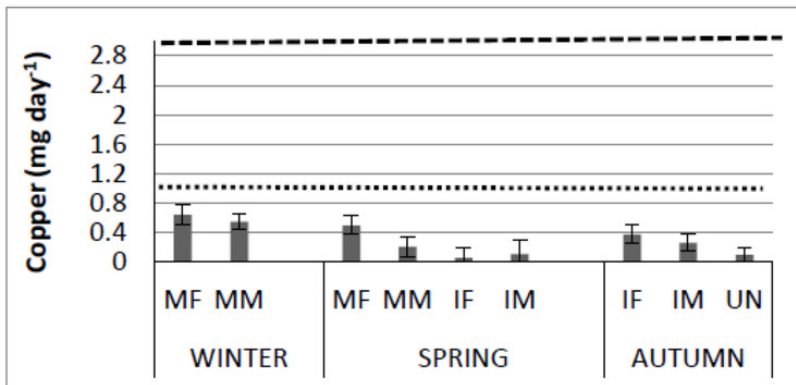
Figure 4

Histograms of the cumulative distribution of the 5%, mean and 95% hazard indexes (HI) of copper (Cu), cadmium (Cd), zinc (Zn) and iron (Fe) for consumption of muscle of *Totoaba macdonaldi*.
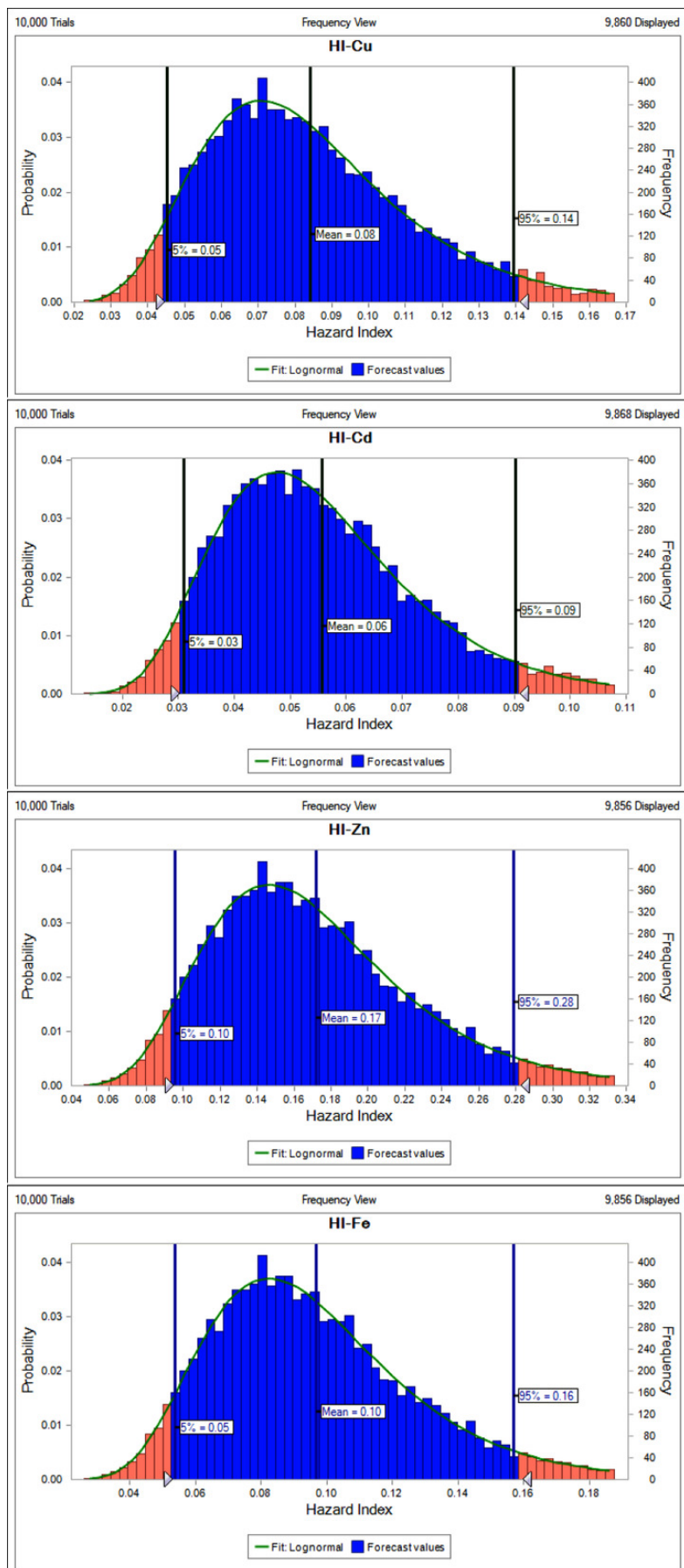
Figure 5

Cumulative probability distribution curve of hazard index for copper (Cu), cadmium (Cd), zinc (Zn) and iron (Fe) for consumption of *Totoaba macdonaldi* muscle.
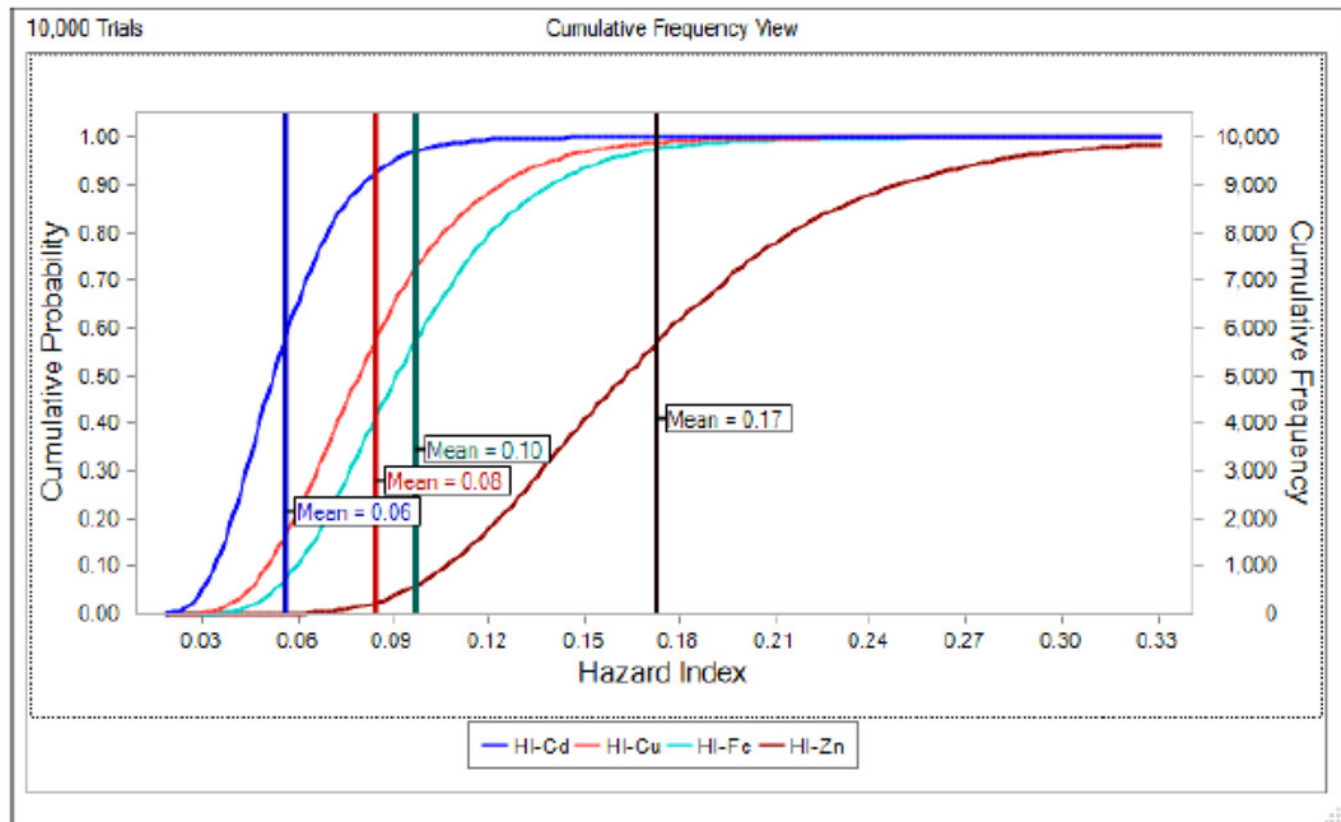
Figure 6

Residuals analysis of the generalized linear models, assuming a gamma error distribution, generated for cadmium (Cd) in liver and muscle of *Totoaba macdonaldi* in the Gulf of California, Mexico.

Model 1

a)

Model 2

a)

b)

b)

c)

c)