Paralytic Shellfish Poisoning Toxins in Butter Clams (*Saxidomus gigantea*) from the Kodiak Archipelago, Alaska.

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Abstract

Consumption of toxic butter clams (*Saxidomus gigantea*) is the most frequent cause of paralytic shellfish poisoning (PSP) in Alaskan coastal communities. This study examines seasonal variation in total paralytic shellfish toxin concentrations and congener distribution in tissues of butter clams collected in three communities in the Kodiak Islands, Alaska: the City of Kodiak, Ouzinkie and Old Harbor. In response to questions from local harvesters, the efficacy of removing particular clam tissues on total toxin levels was also assessed. Butter clam samples were collected ~monthly during 2015-2020 in each community to monitor shellfish toxin levels. Results were combined with clam monitoring data collected previously (2013-2015) to document the seasonal distribution of saxitoxin (STX) and its congeners (neosaxitoxin, gonyautoxin) in clam tissues. Seasonally, paralytic shellfish toxin levels in butter clams were highest in summer, declined in winter, but often remained above regulatory limits throughout the year in the three Kodiak communities. Butter clams collected from Ouzinkie (2013-2020) averaged 165±87 µg STX equivalents (Eq.) 100 g^{-1}, compared to Kodiak 73±54 µg STX Eq. 100 g^{-1} and Old Harbor 143±103 µg STX Eq. 100 g^{-1}. STX accounted for 59-71% of the total toxin concentration in clams at Ouzinkie, Kodiak, and Old Harbor, while neosaxitoxin (neoSTX) accounted for 12-18%. Gonyautoxins (GTXs) represented 31-60% of the total toxin concentration during the seasonal *Alexandrium catenella* bloom in June-July, with lower percentages in other months. The fraction of total toxin varied among clam tissues: the siphon tip (2-29%), the neck (3-56%), the gut (3-65%) and the body (6-85%). Removal of the siphon tip reduced total toxin content substantially in some samples but had little effect in others. Saxitoxin congeners varied greatly and somewhat unpredictably among clam tissues, and the results indicate removal of specific tissues was not an effective strategy for reducing paralytic shellfish toxin levels in butter clams for safe consumption.

1. Introduction

In North America, paralytic shellfish poisoning (PSP) occurs most commonly through consumption of bivalves that have ingested microalgae in the genus *Alexandrium*. Many *Alexandrium* species produce potent neurotoxins (saxitoxins, STXs) which can accumulate to high concentrations in shellfish (Wiese et al., 2010). STXs act by blocking voltage-gated sodium channels and inhibiting mammalian nerve cell depolarization, resulting in adverse gastrointestinal, neurological and cardiovascular symptoms (Noda et al., 1990; Cusick and Sayler, 2013). The potencies of the individual saxitoxin congeners vary by more than 100-fold, the most toxic being STX, neosaxitoxin (neoSTX) and some of the gonyautoxins (GTXs) (Etheridge, 2010; FAO/WHO, 2016). After ingestion of *Alexandrium* cells, shellfish can metabolically convert less toxic congeners found in the microalgae into more toxic compounds (Botelho et al., 2012; Suarez-Isla, 2015). Most notably, GTXs and the less potent N-sulfocarbamoyl toxins C1-C4 often predominate in *Alexandrium* cells and may be converted to the more toxic congeners STX and neoSTX in shellfish tissue (Etheridge, 2010; Laabir et al., 2013). Such congener conversion has been documented in shellfish before, where STX, neoSTX and the GTXs represented up to 15%, 58%, and 38% of the total toxin load (Reis Costa et al., 2009; Trainer et al., 2014). However, specific congeners can be ephemeral, changing seasonally in response to localized *Alexandrium* bloom progression and metabolic processes within the shellfish (Beitler, 1988).

Paralytic shellfish toxins (PSTs) may also be distributed unequally among shellfish tissues following assimilation. For example, Cembella et al. (1993) found toxin concentrations in the organs of Atlantic surf clams (*Spisula*) and sea scallops (*Placopecten*) varied greatly among the adductor muscle, gill, mantle, siphon and foot. Research with Pacific butter clams

(*Saxidomus gigantea* Deshayes, 1839) indicated a similar level of inter-tissue variability, with the highest toxin levels often associated with the siphon (Beitler, 1988). As a result, removal of all or part of the siphon has been adopted previously by commercial and noncommercial harvesters to reduce clam toxicity (Waskiewiez et al., 1955); and regulatory agencies have recommended recreational butter clam harvesters remove the siphon tip to reduce PSP risk (Nishitani et al. 2004; BCCDC, 2021).

In the U.S., the danger to human health from PSTs is most severe in Alaska due to recurrent toxic *Alexandrium* blooms, the relative remoteness of many coastal communities, and historical reliance on shellfish resources for subsistence and cultural practices (RaLonde, 2001). PSP is a continuing threat to shellfish harvesters in Alaska, and more than 100 cases of PSP have been recorded by the State since the early 1990s, with recent fatalities recorded in 1994 (Kodiak), 1997 (Kodiak), 2010 (SE Alaska), and most recently in July 2020 (Unalaska) (Gessner et al., 1997; Trainer et al., 2014; ADHSS, 2020). To prevent outbreaks of PSP in the U.S., State public health agencies (through the U.S. Food and Drug Administration [FDA] and Interstate Shellfish Sanitation Conference) have adopted a safe, regulatory limit of 80 µg STX Eq. 100 g^{-1} of shellfish tissue (FDA, 2019; https://dec.alaska.gov/eh/fss/shellfish/). Historical data indicate toxin levels in shellfish along the Gulf of Alaska and the Aleutians commonly exceed 1,000 µg STX Eq. 100 g^{-1}, with extreme toxin levels over 10,000 µg STX Eq. 100 g^{-1} observed periodically (Castrodale, 2015). Mitigation of Alaskan PSP risk generally hinges upon direct surveillance of PST concentrations in shellfish rather than *Alexandrium* cell abundance. Past studies have shown shellfish toxin levels provide a reasonable measure of *Alexandrium* cell abundance and bloom severity (Matweyou, 2003; Vandersea et al., 2018) relative to the greater time and manpower costs and difficulties associated with reliable

identification and counting of *Alexandrium* cells in plankton samples via microscopy (Anderson et al., 2005; Godhe et al., 2007; Litaker et al., 2018). *Alexandrium* cell-based monitoring is also limited by the capacity for molecular methods within the State. Because of limited State toxin testing capacity and the enormous Alaskan coastline, only commercially harvested shellfish are routinely tested for PSTs by the Alaska Dept. Environmental Conservation. The management strategy for non-commercial harvesting has been to alert residents that all shellfish are potentially unsafe and their consumption should be avoided (ADPH, 2018; ADHSS, 2020).

Subsistence harvesting of shellfish resources is common in coastal Alaska despite PSP risks. In the Kodiak Archipelago (Fig. 1A) for instance, harvested shellfish are often dominated by butter clams due to both their large size and their wide distribution and abundance. Survey data from the Kodiak communities of Ouzinkie, the City of Kodiak, and Old Harbor (Figs. 1B-D) indicated Native residents exhibit higher shellfish consumption rates than elsewhere in the state (Wolfe, 2004; Lance et al., 2019). Clam harvesting is also an important part of the cultural identity of Kodiak Native communities, where group harvesting and consumption of butter clams have long been important social activities. Harvested items are commonly shared among family members, friends and other households statewide, and may even be shipped to other North American communities (Mishler, 2001; Reedy-Maschner and Maschner, 2012). In combination with shellfish toxin concentrations frequently greater than the regulatory limit (Ralonde, 1996; Matweyou and Bartz, 2015), these social factors result in very high PSP risks to Kodiak shellfish consumers.

Here are described the results of a 5-year, community-based shellfish monitoring project in the Kodiak Archipelago. In part, this work represents an extension of a 2013-2015 pilot

126 project by the State of Alaska (Matweyou and Bartz, 2015). Specifically, we report PST data
127 from butter clams collected in Ouzinkie, the City of Kodiak and Old Harbor during 2013-2020
128 (Fig. 1). Seasonal trends in toxin levels and the contribution of major PST congeners to the clam
129 toxin pool are examined. Although butter clams are not ideal for shellfish toxicity monitoring, in
130 that individuals often retain PSTs for months after an *Alexandrium* bloom (Chambers and
131 Magnusson, 1950), this species is target by subsistence harvesters in the Kodiak Islands, and was
132 identified as the preferred species by participating communities. Although it was not possible to
133 monitor phytoplankton cell abundances during this study, plankton surveys conducted by the
134 authors before and after the study period indicated blooms of *A. catenella* were responsible for
135 the observed toxin levels in butter clams. An investigation into toxin distribution in butter clam
136 tissues was added in response to inquiries by Kodiak shellfish harvesters regarding the effect of
137 local preparation methods on toxin levels in clam meats. The distribution of PSTs among the
138 clam siphon tip, neck, gut and body was determined and the effect of removing the siphon tip on
139 potential toxin exposure was evaluated.
140
141 **2. Methods**
142 *2.1 Study sites*
143 The Kodiak Archipelago includes 16 major islands located 40-50 km off the southeast
144 coast of the Alaska Peninsula. The sampling sites were located on traditional clam harvesting
145 beaches in the communities of Ouzinkie, Kodiak, and Old Harbor (Fig. 1A). Ouzinkie is an
146 Alutiiq community located on Spruce Island; here, butter clams were collected from Sourdough
147 Flats (Fig. 1B). The City of Kodiak (hereafter Kodiak) is the largest population center in the
148 archipelago and is located on Chiniak Bay at the east end of Kodiak Island. Here, butter clams
149 were collected from Mission Beach (MB) and the east side of Near Island (NI; Fig. 1C). Old

150 Harbor is an Alutiiq community on the southeast coast of Kodiak Island. Butter clams from this
151 location were collected at Shipwreck Beach (Fig. 2D).

152
153 *2.2 Sample collection*
154 Butter clam samples were collected approximately monthly in Ouzinkie and Old Harbor
155 during 2013 - 2020, and in the City of Kodiak during (2016 - 2020). For butter clam monitoring,
156 at least 12 clams were collected within a two-meter radius (if possible); small specimens (<4 cm
157 length) were avoided because they were considered too small for subsistence harvesting. Clams
158 were scrubbed, rinsed with tap water to remove sediment and debris, and were then shucked and
159 drained (ASEHL, 2020). The meats were pooled and frozen at -20 °C pending shipment to the
160 National Oceanic and Atmospheric Administration (NOAA) Laboratory in Beaufort, North
161 Carolina for analysis.
162 To examine the distribution and seasonality of PSTs in clam tissues, 3-6 groups of butter
163 clams (at least 12 clams per group) were collected from Mission Beach and Shipwreck Beach
164 during 2015-2018 (Table 1). Clams were shucked as above. Meats were then dissected and
165 pooled into samples of the black siphon tip (tip), the remainder of the siphon (neck), the gut
166 contents (gut), and the remaining tissue (body), reflecting the tissue types typically discarded or
167 retained prior to consumption (see Fig. 4). The tissue types from each clam were then pooled,
168 weighed and frozen at -20 °C pending toxin analysis.

169
170 *2.3 Toxin analysis*
171 Shellfish samples were analyzed via high performance liquid chromatography (HPLC)
172 with pre column oxidation using the standard methods of Lawrence et al. (2005) and refined by
173 Ben-Gigirey (2012) and Harwood et al. (2013). Briefly, samples were processed using a
174 Kinematica Polytron model PT-MR 2500E homogenizer fitted with a 12 mm dispersing head

(Kinematica, Inc., New York, USA). A five g subsample of homogenized tissue was extracted with 3 mL of 1% acetic acid in a 100 °C water bath for 5 min. After cooling at 4 °C, the sample was centrifuged at 4,500 rpm for 10 min, and the supernatant was collected. The remaining pellet was re-extracted and the supernatants combined. One mL of the combined extract was passed through a conditioned SPE C18 cartridge (Milford, Massachusetts, USA), pH-adjusted to 6.5, and diluted to 4 mL for oxidation with periodate and peroxide. PSTs were quantified using Agilent 1100 (Santa Clara, California, USA) or Waters Aquity Arc HPLC systems equipped with fluorescence detection and 5 µm C18 columns (150×4.6 mm, Phenomenex, Inc., Torrance, California, USA). Concentrations of STX, neoSTX, decarbamoyl saxitoxin (dcSTX), gonyautoxins 2 and 3 (GTX2, GTX3), decarbamoyl gonyautoxins 2 and 3 (dcGTX2, dcGTX3), gonyautoxins 1 and 4 (GTX1, GTX4), gonyautoxin 5 (GTX5), and the di-sulfated toxins C1 and C2 were quantified using standards purchased from the National Research Council Canada (Halifax, Nova Scotia, Canada). Isomers GTX 1 and 4, GTX 2 and 3, and C1 and C2 toxins could not be resolved with pre-column oxidation (Lawrence et al., 2005) and are reported as pairs (GTX1/4, GTX2/3, C1/C2). In keeping with the Alaska Department of Environmental Conservation's protocols, toxicity equivalency factors (TEFs) from the European Food Safety Authority 2009 (ESFA, 2009) were used to convert congener concentrations to STX Eq., with the higher TEF used for unresolved congener pairs. Throughout this study toxin concentrations in clams and tissue components are reported in total STX Eq. The contribution of individual congeners to the clam toxin pool was calculated by weight based on STX Eq. The fraction of clam toxin concentrations associated with specific tissues (% toxin) was calculated as the STX Eq. in each tissue relative total toxin pool in that tissue component.

197 Quality assurance of toxin data was completed by instrument validation using
198 homogenates of butter clams and mussels analyzed previously by the Alaska Department of
199 Environmental Conservation via post-column oxidation. Linear regression was used to compare
200 results using pre- and post-column oxidation methods ($y = 0.94x-6.04$, $r^2 = 0.985$). Daily quality
201 assurance was performed by analyzing toxin standards pre- and post-analysis. In each case
202 instrument response was within 97% of original standard curve results. Positive controls were
203 also included during each run.
204
205 *2.4 Data analysis*
206 Interannual and seasonal trends in total toxin levels for 2013-2020 samples collected
207 from Ouzinkie, Kodiak and Old Harbor were analyzed graphically using SigmaPlot 14.0
208 software (Systat Software, Inc., San Jose, California, USA). Comparisons of toxin concentrations
209 and % toxin content in butter clams among sites were performed using Kruskal-Wallis one-way
210 analyses of variance on ranks, as the data were highly variable and did not meet the assumptions
211 of a parametric ANOVA. Seasonal trends were examined by binning toxin levels by month and
212 calculating the mean and standard deviation. The contribution of major toxin congeners (STX,
213 neoSTX, total GTXs [i.e., GTX2/3 + GTX1/4 + GTX5]) to butter clam toxin levels was
214 determined as the STX equivalents for each congener relative to the total toxin level (%). This
215 approach provided a measure of relative importance of STX, neoSTX and the GTXs (2/3 and
216 1/4) to the total clam toxicity in STX equivalents. Congeners having very low toxicity (e.g.,
217 C1/C2) were present in very low quantities and contributed little to the total toxicity in STX
218 equivalents. Similarly, congeners that could not be quantified (e.g., GTX6) were not considered
219 in the analysis.

220 To better understand how toxins were distributed among butter clam tissues and how
221 distribution of these compounds changed over time, variation in toxin levels among the tip, neck,
222 gut and body tissues was examined as both toxin concentrations (µg STX Eq. 100 g^{-1}) in each
223 tissue component and as % toxin content relative to total toxin content in the whole clam. The
224 contribution of dominant toxin congeners (STX, neoSTX, total GTXs) in each type of clam
225 tissue was calculated using the concentration of each congener relative to the total toxins in that
226 tissue component (%).
227
228 **3. Results**
229 *3.1 Total Toxin concentrations in butter clams*
230 Total toxin concentrations in butter clams from the three Kodiak monitoring sites show a
231 pattern of increasing levels in summer and decreasing levels in winter. Distinct peaks in total
232 toxin levels were evident at all three sites in May-August, consistent with the annual occurrence
233 of *Alexandrium* blooms (Fig. 2). Following the bloom period, toxin concentrations typically
234 declined, reaching minimum levels in the winter months (December- February).
235 At Ouzinkie, total toxin levels ranged from 46-578 µg STX Eq. 100 g^{-1} among the 71
236 samples collected, averaging 165±87 µg STX Eq. 100 g^{-1} (Fig. 2A). Though toxin concentrations
237 subsequently declined in the fall and winter, they remained above the FDA regulatory limit of 80
238 µg STX Eq. 100 g^{-1} after January 2014 except for samples collected in June and September of
239 2015 (62.3, 61.8 µg STX Eq. 100 g^{-1}, respectively).
240 In comparison, data from Near Island, Kodiak showed toxin levels ranged from 16 µg
241 STX Eq. 100 g^{-1} in May 2018 to 385 µg STX Eq. 100 g^{-1} in June 2018 (Fig.2B). In contrast to
242 Ouzinkie, total toxin concentrations in Kodiak were generally low through the entire study
243 period with a few periods when concentrations exceeded 80 µg STX Eq. 100 g^{-1}, including April,

August and December of 2017, June-July of 2018, and May-June of 2019. It is worth noting that both the minimum and maximum toxin concentrations observed at Near Island (16 and 385 µg STX Eq. 100 g^{-1}) occurred between May and June of the same year. The reason for this rapid increase in butter clam toxin concentrations is not known, but is consistent with the ephemeral nature of the early summer *Alexandrium* blooms in Kodiak (May-June; Matweyou, 2003). The peak toxin concentration was followed by a rapid decline to 129 µg STX Eq. 100 g^{-1} in July followed by relatively low levels for the remainder of the winter season (36-70 µg STX Eq. 100 g^{-1}, Fig. 2B).

At Old Harbor, butter clam toxin concentrations ranged between a high of 672 µg STX Eq. 100 g^{-1} in June 2016 and a low of 41 µg STX Eq. 100 g^{-1} and in January 2020 (Fig. 2C). Despite occasional gaps in data collection, distinct peaks in toxin concentrations were evident at Old Harbor in summer of 2014 (340-421 µg STX Eq. 100 g^{-1}), 2015 (399 µg STX Eq. 100 g^{-1}), 2016 (672 µg STX Eq. 100 g^{-1}), 2017 (276 µg STX Eq. 100 g^{-1}) and 2018 (273-276 µg STX Eq. 100 g^{-1}, Fig. 2C). In contrast, it seems the summer of 2019 was characterized by an early, more moderate bloom in March-May (142-167 µg STX Eq. 100 g^{-1}) followed by lower butter clam toxin levels < 80 µg STX Eq. 100 g^{-1} through the following summer and until the end of the study in February 2020.

3.2 Seasonal toxin variation and major STX congeners

When the concentrations of major toxin congeners in butter clams were expressed as a percentage of the total toxin pool, STX was the most important congener overall, with a lesser contribution by neoSTX and a marked increase in the GTXs during the summer months (Fig. 3). Over the 2013-2020 study period, saxitoxin accounted for an average of 67±17% of the total toxin concentrations at Ouzinkie, 71±16% at Kodiak and 59±18% at Old Harbor (Fig. 3B). In

contrast, neoSTX accounted for only 18±13%, 13±12% and 12±10% of the total concentrations at the three sites, respectively (Figs. 3C).

On a seasonal basis, the contribution of STX to total toxin levels generally remained above 50% at all sites during most of the year, but declined during the summer *Alexandrium* bloom, when GTXs contributed a greater portion of toxin levels (Fig. 3B, D). This pattern was paralleled by a decline in the average contribution of neoSTX, which represented ~15-25% of toxin levels during late summer through spring months, but declined to <15% during the *Alexandrium* bloom in May-July (Fig. 3C). The relative importance of the GTXs to clam toxin levels was evident by the increasing contribution of these congeners during the spring-summer bloom (Fig. 3D). Seasonality of GTXs was most pronounced in Old Harbor clams, where GTXs represented 12-16% of clam toxicity during the winter months (Nov-Feb), 27-41% in March-May, and ~60% of clam toxicity in June (Fig. 3D).

Averaged over the year, GTXs contributed to 15±18% of the clam toxin levels at Ouzinkie, 15±17% at Kodiak and 28±21% at Old Harbor. The results of a non-parametric ANOVA indicated the contribution of STX to clam toxicity was significantly lower ($H = 10.1$, $p<0.05$) and GTX significantly higher ($H = 19.3$, $p<0.05$) at Old Harbor compared to clams from the other two sites.

3.3 Distribution of PSTs in clam tissues

Dissection of butter clam tissues enabled analysis of toxin distribution patterns relevant to preparation methods used by Kodiak subsistence harvesters (Fig. 4). Overall, the distribution of PSTs among butter clam tissues was highly variable among sampling sites and different times of year although total PST concentrations generally followed the same seasonal trend (i.e., Fig. 3). When all groups of samples from both locations were pooled, the toxin concentrations within the

butter clam gut were higher on average than all other tissues (mean 579±760 µg STX Eq. 100 g^{-1}). Average toxin concentrations in the siphon tip averaged 305±152 µg STX Eq. 100 g^{-1}, those in the neck were 194±182 µg STX Eq. 100 g^{-1}, and those in the body averaged 157±205 µg STX Eq. 100 g^{-1} (Fig. 5A). In contrast, when toxin levels in each tissue were expressed as percentages of the toxin pool present in the whole clam, the highest average toxin amounts were found in the body (42%), followed by the neck (26%), the gut (21%) and the siphon tip (11%, Fig. 5B). The greater % toxin in the clam body reflects the greater relative mass of this tissue component.

Seasonal changes in toxin levels accounted for the bulk of variation in toxin concentrations among tissue types and was driven by the clams' ingestion of *Alexandrium* cells during the summer bloom. The greatest degree of toxin variation occurred in the gut 10-2,492 µg STX Eq. 100 g^{-1} and the body 11-789 µg STX Eq. 100 g^{-1}, with smaller variation in the neck 71-1,184 µg STX Eq. 100 g^{-1} and siphon tip 106-706 µg STX Eq. 100 g^{-1} (Fig. 5A, Table 1). This variability was largely attributable to changes in *Alexandrium* abundance during yearly blooms. These seasonal changes in toxin levels among clam tissues is exemplified by samples collected in May 2017 and Jun 2018 (Figs. 6, 7). On 25 May 2017, the highest toxin concentrations were observed in the clam siphon tip (212-470 µg STX Eq. 100 g^{-1}) and neck (81-368 µg STX Eq. 100 g^{-1}), which accounted for 18-29% and 32-56% (respectively) of the total clam toxin pool (Fig. 6, Table 1). Toxin concentrations were much lower in the gut (10-19 µg STX Eq. 100 g^{-1}), representing only 3-4% of the total toxin pool. Toxins in the body of the clams ranged between 11 and 24 µg STX Eq. 100 g^{-1} (17-35% of total).

In contrast, monitoring samples from the same site on 18 June 2018 showed a much higher toxin level (273 µg STX Eq. 100 g^{-1}, Figs. 2B, Fig. 7). Although fewer groups of clams were collected in June 2018 (n = 3), tissue data showed high toxin concentrations in the gut

315 (1,937-2,491 µg STX Eq. 100 g^{-1}), representing 57-64% of the total toxin pool (Figs. 7A-C,
316 Table 1). On the same day, toxin levels in the siphon tip and neck were 212-258 and 132-163 µg
317 STX Eq. 100 g^{-1} (respectively), representing only 2-3% and 5-7% (respectively) of the total toxin
318 pool.
319
320 *3.4 Saxitoxin congeners in butter clam tissues*
321 The distribution of the dominant saxitoxin congeners (STX, neoSTX, GTXs), and their
322 relative contribution to tissue toxin pools was assessed in greater detail using 2016-2018 samples
323 collected at Mission Beach, Kodiak (Fig. 8). Here, STX and neoSTX were the dominant
324 congeners in the siphon tip and neck (Figs. 8A-F). STX and neoSTX were also prominent in the
325 clam gut and body, but GTXs contributed a much greater percentage of overall clam toxicity. On
326 average GTXs represented the bulk of the toxin load in the clam gut in June 2016 (82%, Fig.
327 8A), June 2017 (54%, Fig. 8C), June 2018 (85%, Fig. 8E) and July 2018 (82%, Fig. 8F),
328 although STX was prominent on the other two dates (May 2017, Apr 2018, Figs. 8B, D). The
329 clam body was marked by elevated GTX percentages in June 2016 (53%) and Jun-Jul 2018 (75-
330 62%, Figs 8A, E, F), with prominent STX levels in May-Jun 2017 and April 2018 (Figs. 8B, C,
331 D).
332
333 **4. Discussion**
334
335 *4.1 Patterns of toxin levels in shellfish*
336 Butter clams are the most common shellfish species responsible for PSP incidence in
337 Alaska. A 20-year retrospective analysis of Alaskan PSP cases indicated butter clams caused
338 58% of PSP incidences, with the remainder of the illnesses involving mussels (22% *Mytilus*

339 spp.), cockles (13% *Clinocardium* spp.), razor clams (2% *Siliqua patula*) and littleneck clams
340 (2% *Leukoma staminea*; Gessner and Middaugh, 1995). A more recent report by Castrodale
341 (2015) showed a similar pattern, where butter clams caused 34% of 70 PSP incidents (26% of
342 117 cases). Despite their predominance in PSP outbreaks, butter clams remain a preferred
343 shellfish species among Native residents in the Kodiak Archipelago. This preference was evident
344 during the design of both the current study and the preceding ADEC pilot study (Matweyou and
345 Bartz, 2015), and reflects an enduring Kodiak tradition of butter clam harvesting. A survey of
346 Kodiak Archipelago residents by the Alaska Division of Public Health indicated PSP risk was 12
347 times higher among long term Kodiak residents (>20 years) than in those emigrating to Kodiak
348 more recently (Ralonde, 1996). Furthermore, PSP risk was not uniform across the Islands.
349 Residents of Old Harbor were three times more likely to report symptoms of PSP than those in
350 Kodiak. This pattern is supported by toxin data from the current study as well (Fig. 2). The
351 apparent difference in PSP incidence among the two communities is attributable to higher butter
352 clam toxin concentrations at Old Harbor, greater access to commercially sourced foods in the
353 City of Kodiak (restaurants, grocery stores), more frequent exposure to PSP advisory information
354 in the more urban location, and the greater proportion of Native residents in Old Harbor relative
355 to the city. The current study also confirmed traditional knowledge that butter clams are less
356 toxic in winter, but showed winter toxin levels often remained above the recommended safety
357 level of 80 µg STX Eq. 100 g^{-1} at Ouzinkie and Old Harbor (Figs. 2, 3). This validates previous
358 work in Alaska confirming that harvesting in winter months does not guarantee butter clams are
359 safe to eat (Gessner and Middaugh, 1995; Castrodale, 2015).
360
361 *4.2 Efficacy of removing tissues to reduce butter clam toxin levels*

362 The results of this study indicate removal of the siphon tip or other tissues from butter
363 clam meats during preparation is not an effective approach for reducing toxicity to safe levels.
364 This finding is significant, given the widespread belief among Kodiak residents that it is possible
365 to prepare shellfish in such a way that PSP could be prevented (Ralonde, 1996; this study). In the
366 current study, the observed distribution of toxins among clam tissues was not consistent, and the
367 data indicate removal of specific tissues, such as the siphon tip, is often insufficient to reduce the
368 overall toxin load to safe levels (Figs. 5-7, Table 1). For instance, in butter clams collected from
369 Kodiak and Old Harbor, the siphon tip often exhibited the highest concentration of toxins (106-
370 706 µg STX Eq. 100 g^{-1}). But since the siphon tip represents only a small fraction of the whole
371 clam, it contained <30% on average of the total clam toxin pool (Figs. 5B, 6, 7, Table 1). Given
372 this information, removal of the siphon tip is not adequate to reduce clam toxin loads to safe
373 levels, especially at sites like Ouzinkie and Old Harbor where toxin concentrations are often
374 several times the regulatory limit (Fig. 2A, C). Although toxin levels were generally lower at
375 Kodiak sites (Fig. 2B), the efficacy of siphon tip removal in reducing toxin levels was still highly
376 variable, yielding a reduction in clam toxin levels of 18-29% in May 2017, but only 2-3 % in
377 June of 2018 (Figs. 6 & 7, Table 1). These data underscore the risks to harvesters in the Kodiak
378 Archipelago and elsewhere in Alaska who consume untested shellfish.
379 The data from this study mirror those reported in early efforts to measure toxin levels in
380 Alaskan butter clams more than 60 years ago. These early efforts used mouse bioassays and
381 focused on environmental factors governing shellfish toxicity, identification of sites with high
382 and low toxin levels, development of screening capacity and methods for reducing butter clam
383 toxicity (Chambers and Magnusson, 1950; Chambers et al., 1955; Magnusson et al., 1955;
384 Waskiewiez et al., 1955; Page and Meyers, 1957). Data from a retrospective by Brown (1960)

385 indicated the siphon of butter clams collected at sites in southeast Alaska during 1946-1960
386 exhibited toxin concentrations between two and 13-fold higher than those in the clam bodies. On
387 average, siphons contained 5,886 mouse units (MU) STX Eq. 100 g^{-1} (~1,177 µg STX Eq. 100 g^{-1})
388 versus 869 MU STX Eq. 100g^{-1} (~174 µg STX Eq. 100 g^{-1}) in the clam bodies (0.2 µg STX
389 Eq. MU^{-1}, Wekell et al., 2004). More detailed follow-up work showed 24% of butter clam toxins
390 were associated with the black tip of the siphon and 54% with the neck (middle+inner siphon),
391 with the remaining 22% distributed among the various organs (Quayle, 1967; Quayle and
392 Bourne, 1972).
393 Given the similarity between the current results (siphon tip 2-30%, neck 3-56% of the
394 butter clam toxin levels, Table 1) and those from previous studies about the relative
395 ineffectiveness of siphon removal, it is reasonable to question the need to revisit the same issue.
396 In this study, HPLC was used to track distribution of STX, neoSTX and GTX among clam
397 tissues (Figs. 5-8), an analytical method that was unavailable to earlier researchers. These
398 congener distribution data are highly relevant to shellfish safety in Alaska as exemplified by the
399 recent PSP-related death that occurred in southwest Alaska in July 2020 (ADHSS, 2020). A
400 sample collected from Unalaska showed STX and neoSTX concentrations of 313 and 472 µg
401 100 g^{-1} (respectively), while the concentration of GTXs was nearly 9,800 µg 100 g^{-1} (pers.
402 comm., Alaska DEC Environmental Health Laboratory). The elevated GTX levels in the
403 Unalaska shellfish are indicative of *Alexandrium* blooms very similar to those in Kodiak during
404 the current study. Taken together, the congener data from Kodiak and Unalaska exemplify the
405 importance of quantifying GTXs in addition to STX. In particular, this is a vital concern for PSP
406 field test kits used for screening Alaskan shellfish. To date, available field tests have been
407 designed primarily to detect STX, which is the most toxic of the PST congeners (McCall et al.,

2019). The data from Kodiak indicate quantification of GTXs may be just as relevant to PSP risk in Alaskan shellfish tissue.

4.3 Seasonal differences in toxin distribution among tissues

The distribution of PSTs in clam tissues varies through the bloom season. When saxitoxin-producing dinoflagellate cells are present, toxins are generally apparent first in the bivalve digestive gland and stomach as the cells are ingested (Bricelj and Shumway, 1998). Toxins are then transferred sequentially to the digestive and excretory organs before reaching the muscular tissues such as the siphon, foot and adductor muscles (Cembella et al., 1993; Kwong et al., 2006; Medina-Elizalde et al., 2018). A similar progression occurs in butter clams, where previous data showed toxin levels increase rapidly in the visceral mass (digestive organs) over several days, followed by a more gradual increase in siphon toxin levels over several weeks (Beitler, 1988). The clam tissue data collected in Kodiak and Old Harbor in this study indicate a similar pattern. In samples from Kodiak, the fraction of toxin in the gut averaged only 5% during April 2018, increased to 60% during the *Alexandrium* bloom in June, and then declined to 27% as the bloom subsided in July (Table 1). During the same period, toxins in the siphon represented 55% of the total toxin pool in April, declined to only 9% in June, and then increased to 23% in July as the bloom subsided.

Given this seasonal pattern of toxin distribution in the gut relative to the siphon, the relative effectiveness of removing these tissues depends on the time of year when clams are harvested and which tissues are removed. For example, this study indicates removal of the gut contents might have little effect on overall toxin levels when *Alexandrium* cells are sparse during March, but might substantially reduce toxin levels during peak bloom levels in June. Conversely, elimination of the clam siphon during June would reduce toxin levels by <10% compared to

other times of the year. Because no butter clam data were available from the winter months, it was not possible to assess potential reduction in toxin levels during that season.

It is noteworthy that data from this study and from Beitler (1988) each indicate the shift in the bulk of clam toxins from the gut to the siphon may occur very quickly as a bloom develops. Butter clam monitoring data from Kodiak showed clams were devoid of GTXs on 08 April 2018, for instance, but these toxins were detected in the clam gut just nine days later (17 April, congener data not shown). This pattern of rapid toxin accumulation was mirrored in laboratory data where GTXs accumulated in the butter clam gut within a few days after exposure to *Alexandrium* cells (Beitler, 1988). Such rapid toxin accumulation poses a danger to shellfish harvesters given the apparent shift in bloom timing expected during regional warming events in Alaska. If higher water temperatures prompt an *Alexandrium* bloom in March-April instead of June-July, for instance, the degree of PSP risk is likely to shift as well. Earlier spring blooms due to the widening thermal window for *Alexandrium* growth have been identified elsewhere in Alaska (Gobler et al., 2017; Vandersea et al., 2018). Shifts in the timing of *Alexandrium* blooms and ensuing changes in shellfish toxin levels in Kodiak may have serious consequences for butter clam harvesters seeking to lessen toxin levels by removing the siphon or other tissues.

5. Conclusions

With exposure to some of the highest levels of PSTs in the state and strong cultural dependence on butter clam resources, residents of the Kodiak Archipelago are particularly vulnerable to PSP risks. This study was designed in direct response to inquiries from the subsistence harvesters in Ouzinkie, Kodiak and Old Harbor to help address these risks by application of community-based butter clam monitoring. Such monitoring was intended to better define seasonality of butter clam toxin levels, to examine differences among traditional

456 harvesting beaches, and to test the efficacy of traditional preparation methods in reducing toxin
457 exposure. Monitoring data collected in 2013-2020 indicated butter clam toxin levels were 2 to
458 2.5-fold higher on average in Ouzinkie and Old Harbor than at collection sites in Kodiak. While
459 STX was the predominant toxin in butter clams, seasonal patterns indicated the congener GTX
460 accounted for 27-55% of toxins during the spring and summer, but only 3-8% before the summer
461 *Alexandrium* bloom. Toxin distribution in clam tissues showed wide variability among the
462 siphon tip (2-29% of total), the neck (3-56%), the gut (3-65%) and the body (6-85%). As a result,
463 preparation methods such as removal of the siphon tip or gut contents could reduce clam toxin
464 levels substantially in some instances but have little effect in others. Taken together, these data
465 indicate tissue removal is not a reliable strategy for reducing PSP risk in butter clams.
466

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475

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688 **Figure Captions**
689
690 Fig. 1. Study sites in the Kodiak Archipelago, Alaska. A. Regional map of southwest Alaska
691 showing location of Kodiak Islands off the Alaska Peninsula. B. Enlargement of the City of
692 Kodiak with Butter clam collection sites (red dots): Near Island (NI) and Mission Beach (MB).
693 C. Enlargement of Ouzinkie with collection site at Sourdough Flats (SF). D. Enlargement of
694 Old Harbor with collection site at Shipwreck Beach (SB).
695
696 Fig. 2. 2013-2020 butter clam toxin concentrations at A. Sourdough Flats, Ouzinkie, B. Trident
697 Basin (bars) & Mission Beach (dots), Kodiak, and C. Shipwreck Beach, Old Harbor. The red
698 dashed line denotes the action limit for saxitoxins in shellfish established by the U.S. Food and
699 Drug Administration (80 µg STX Eq. 100 g^{-1}).
700
701 Fig. 3. Average 2013-2020 butter clam toxin levels (µg STX Eq. 100 g^{-1}) ± Std Dev and
702 contributions from toxin congeners in butter clams from Ouzinkie, Kodiak and Old Harbor. A.
703 Total toxin concentrations (µg STX Eq. 100 g^{-1}). The red dashed line in panel A denotes the
704 action limit for saxitoxins in shellfish established by the U.S. Food and Drug Administration (80
705 µg STX Eq. 100 g^{-1}). B. Percentage of total toxin level due to saxitoxin (STX), C. Percent due
706 to neosaxitoxin (neoSTX), D. Percent due to gonyautoxins (GTX2/3, 1/4 and 5).
707
708 Fig. 4. Butter clam tissues for this study. A. Diagram of butter clam anatomy from Quayle and
709 Bourne (1972) showing location of the major tissues: Siphon Tip, Neck, Body and Gut
710 (digestive gland, stomach, gonads, style and intestine). B. Example of butter clam meats
711 prepared for dissection with higher magnification inset showing the prominent siphon and black
712 tip. Panel A from Quayle and Bourne (1972) re-printed courtesy of Canadian Science

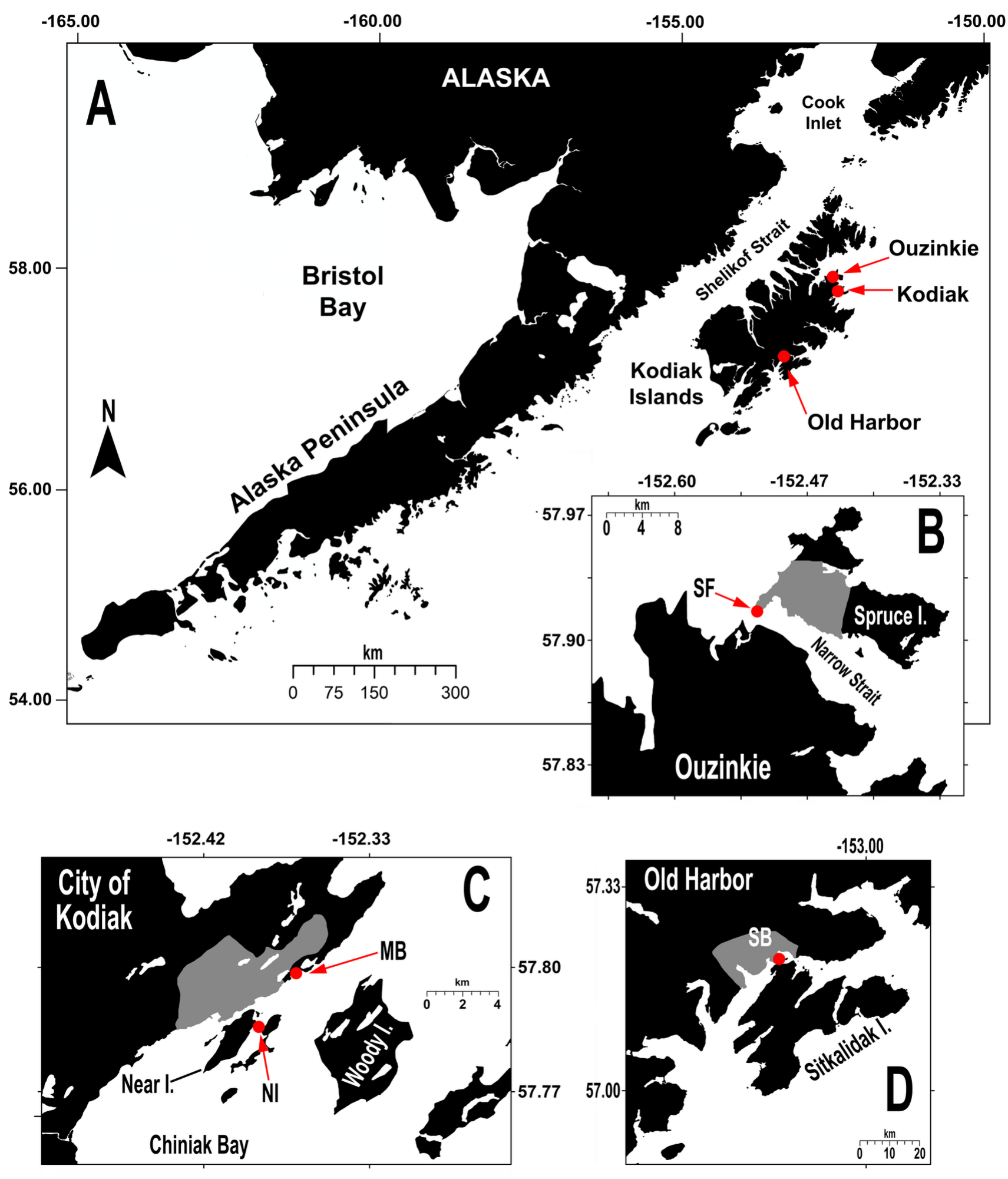
713 Publishing (https://www.nrcresearchpress.com/page/authors/services/reprints). Panel B by J.
714 Matweyou.
715
716 Fig. 5. Box plots of PST levels in butter clam tissues from the Kodiak Islands during 2015-2018.
717 Data from clam tissue components: the black tip of the siphon (Tip), the remainder of the siphon
718 (Neck), the gut contents (Gut), and the remaining tissue (Body). A. Toxin concentrations (µg
719 STX Eq. 100 g^{-1}) in clam tissue components. B. % of total toxin pool in each component. Box
720 plot: solid line denotes the median, dotted line indicates the mean, box bounds represent the
721 25th - 75th percentiles, error bars denote the 10th - 90th percentiles, and dots represent outliers.
722
723 Fig. 6. PST distribution in tissues of six groups of butter clams (panels A-F, n ≥12 clams per
724 group) collected at Mission Beach, Kodiak on 25 May 2017. Bars illustrate toxin concentrations
725 (µg STX Eq. 100 g^{-1}) of four clam tissues: the black tip of the siphon (Tip), the remainder of the
726 siphon (Neck), the gut contents (Gut), and the remaining tissue (Body). Pies represent the
727 proportion of toxins (weight %) associated with each tissue. Tissue mass data were not available
728 to calculate % toxin data in panel D.
729
730 Fig. 7. PST distribution in tissues of three groups of butter clams (panels A-C, n ≥12 clams per
731 group) collected at Mission Beach, Kodiak on 18 June 2018. Bars illustrate toxin concentrations
732 (µg STX Eq. 100 g^{-1}) of four clam tissues: the black tip of the siphon (Tip), the remainder of the
733 siphon (Neck), the gut contents (Gut), and the remaining tissue (Body). Pies represent the
734 proportion of clam toxins (weight %) associated with each tissue.
735
736 Fig. 8. Contribution of saxitoxin congeners (% total toxin in each tissue) within butter clam tissue
737 components collected at Mission Beach, Kodiak in 2016-2018. Tissues: the black tip of the

738 siphon (Tip), the remainder of the siphon (Neck), the gut contents (Gut), and the remaining
739 tissue (Body). Bars represent mean percentages of saxitoxin (STX, black bars), neosaxitoxin
740 (neoSTX, blue bars) and total gonyautoxins (GTXs, white bars) among 3-6 groups of butter
741 clams (n ≥12 per group) collected on each date.
742

743 Table 1. Percentage of total toxin (STX Eq.) associated with four tissues, the black tip of the siphon (Tip), the
744 remainder of the siphon (Neck), the gut contents (Gut), and the remaining tissue (Body). "n/a" denotes samples
745 where the tissue sample or the weight was not available.

Date	Location	Replicate	Tip (%)	Neck (%)	Gut (%)	Body (%)
15 Jun 2015	Shipwreck Beach	1	8.4	20.0	65.2	6.4
		2	4.1	3.4	7.2	85.3
		3	1.7	4.4	35.3	58.6
		4	12.3	22.5	24.6	40.6
		5	n/a	n/a	n/a	n/a
		6	n/a	n/a	n/a	n/a
09 Jun 2016	Mission Beach	1	1.7	27.2	22.5	48.5
		2	6.7	9.8	34.2	49.3
		3	6.1	5.4	27.8	60.8
		4	2.7	3.6	26.1	67.6
		5	12.2	4.0	37.4	46.4
25 May 2017	Mission Beach	1	18.3	55.9	2.5	23.2
		2	29.3	32.3	3.6	34.8
		3	25.5	48.0	2.6	23.9
		4	n/a	n/a	n/a	n/a
		5	21.9	49.3	3.8	24.9
		6	24.1	55.7	3.4	16.8
22 Jun 2017	Mission Beach	1	11.7	38.5	12.5	37.3
		2	10.4	37.2	12.4	40.0
		3	13.0	27.2	12.8	47.0
		4	16.6	35.2	10.1	38.1
		5	13.4	44.7	6.6	35.3
		6	14.3	42.3	12.3	31.1
17 Apr 2018	Mission Beach	1	10.6	35.8	8.7	44.9
		2	21.8	47.7	3.5	27.0
		3	11.0	43.3	3.3	42.4
		4	12.9	38.0	3.3	45.8
19 Apr 2018	Shipwreck Beach	1	13.0	19.1	15.5	52.4
		2	14.9	20.5	14.4	50.1
		3	10.9	19.9	13.4	55.8
18 Jun 2018	Mission Beach	1	2.2	7.2	59.3	31.3
		2	2.9	6.9	57.0	32.9
		3	2.3	5.3	64.2	28.2
07 Jul 2018	Mission Beach	1	5.9	18.6	25.2	50.3
		2	5.4	13.4	28.0	52.0
		3	6.4	18.0	28.9	46.7

746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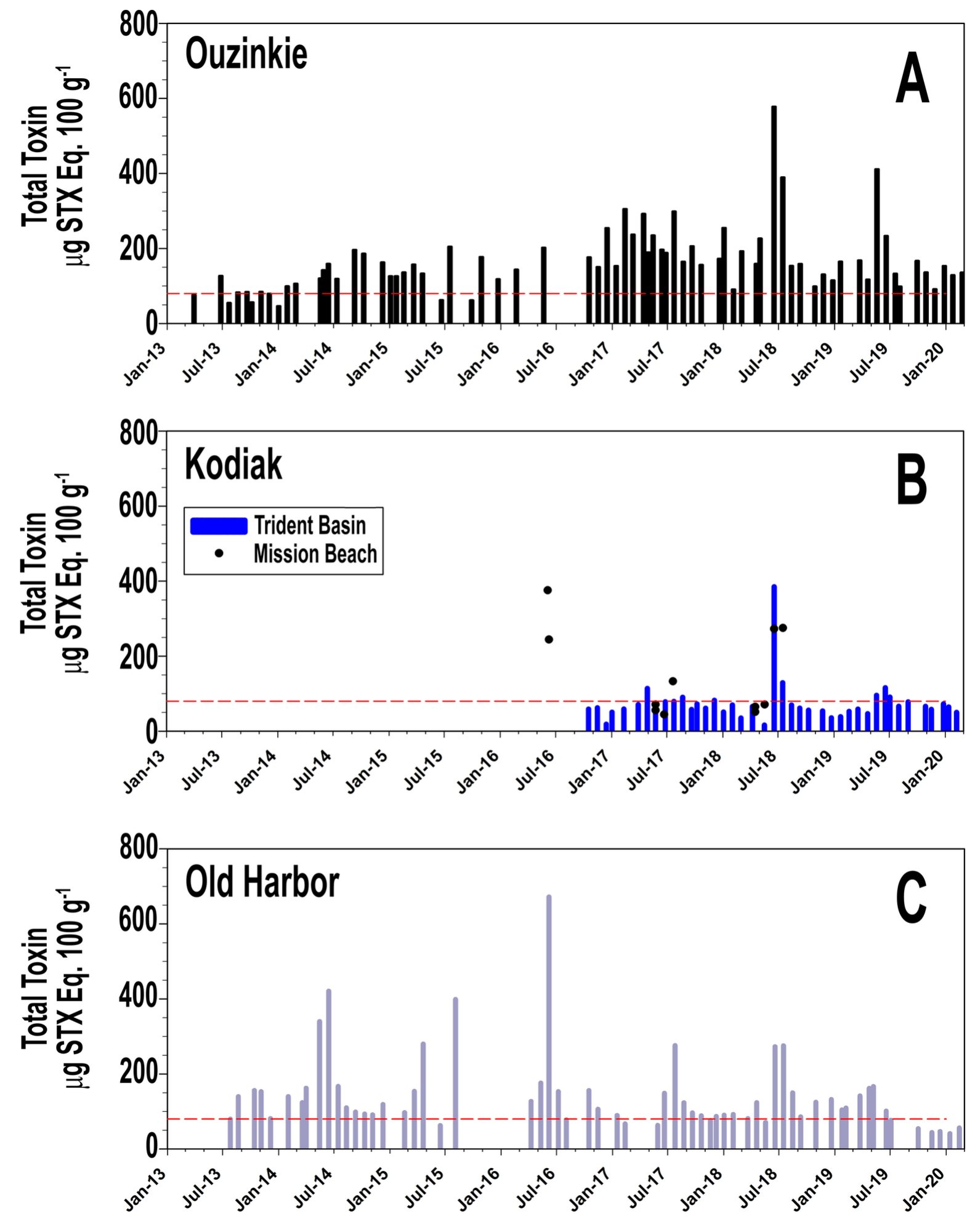
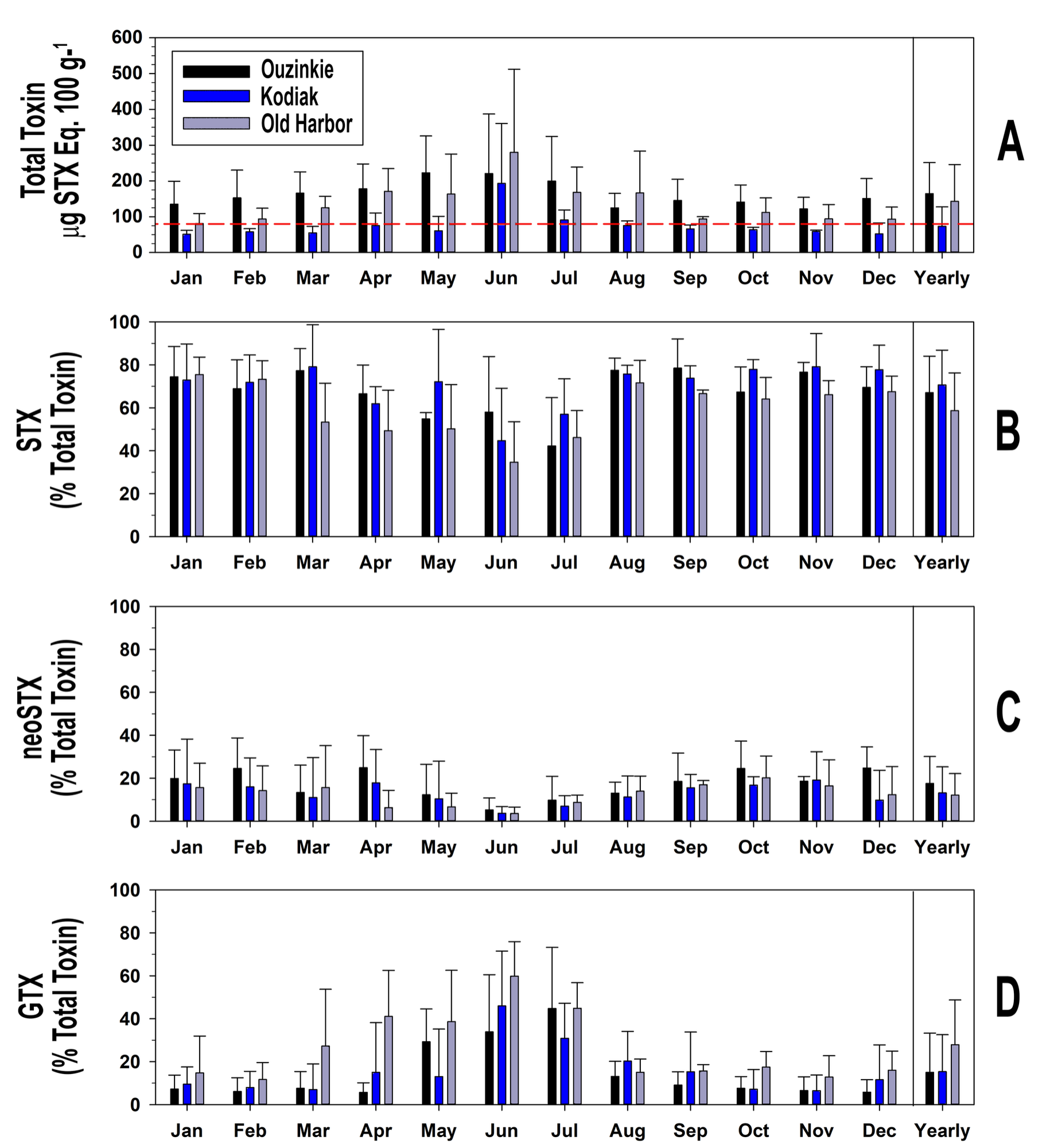
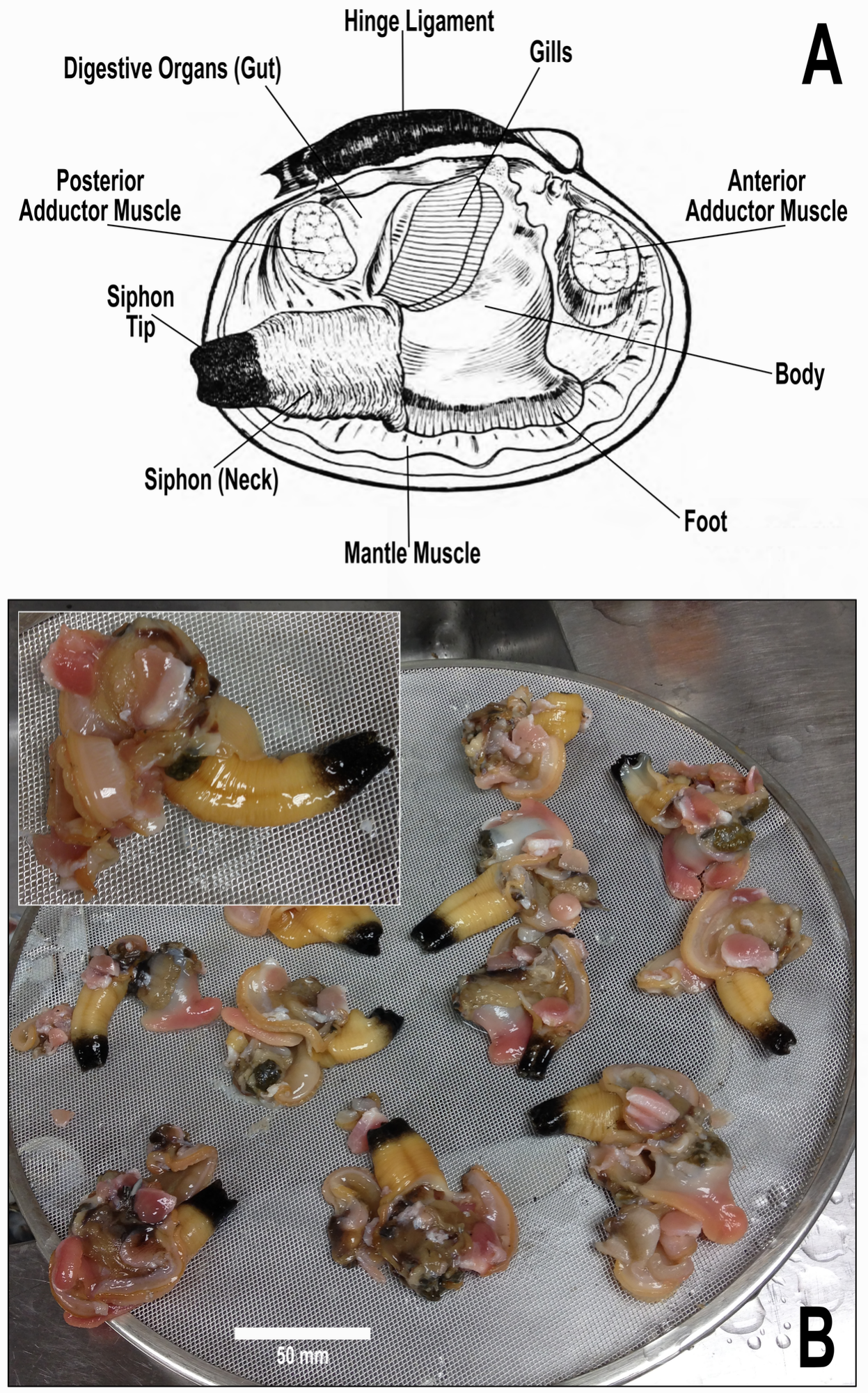
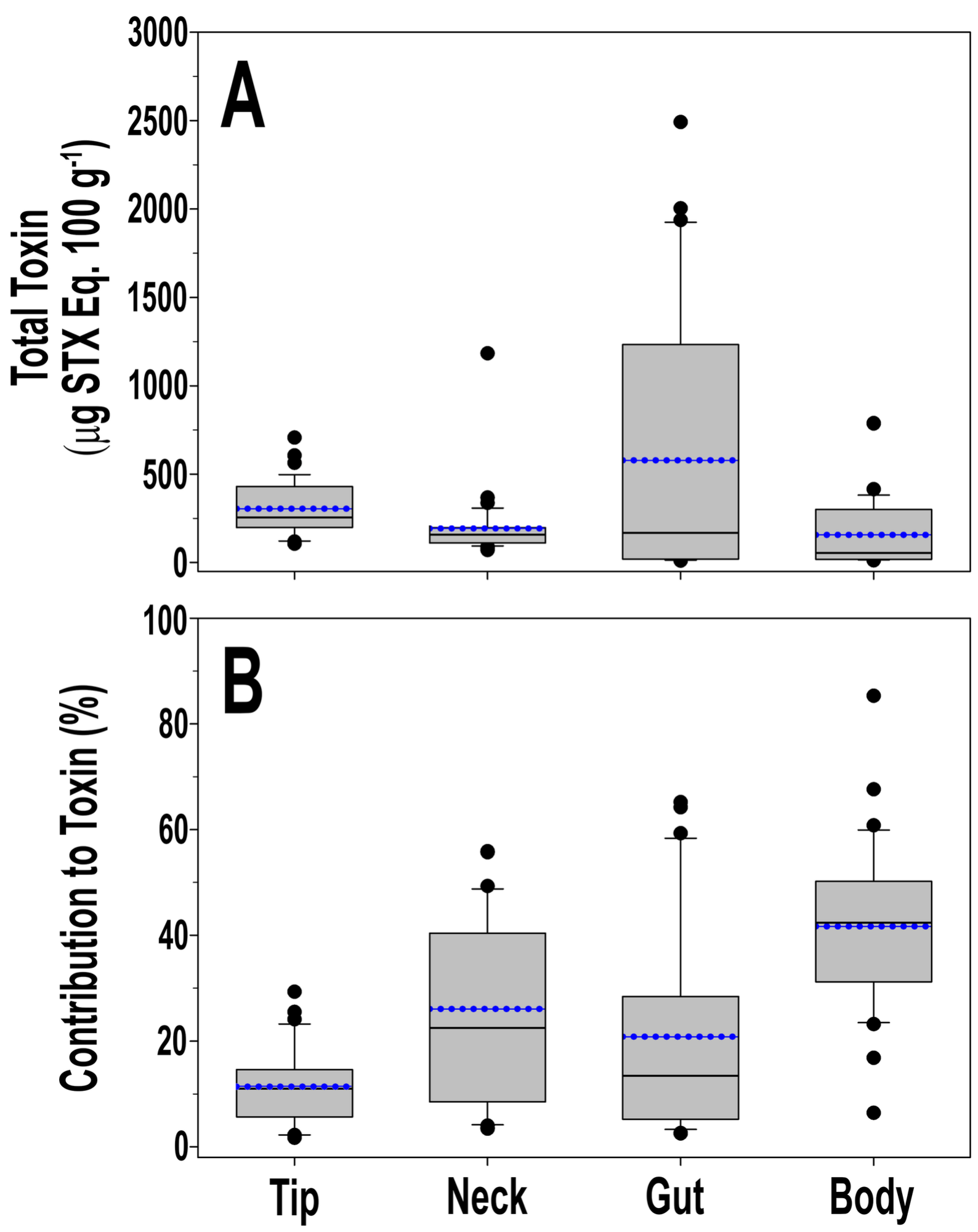
km
0 75 150 300
-152.60
-152.47
-152.33
57.97
57.90
57.83
B
km
0 4 8
SF
Spruce I.
Narrow Strait
Ouzinkie
-152.42
-152.33
City of Kodiak
C
MB
km
0 2 4
Woody I.
Near I.
NI
Chiniak Bay
57.80
57.77
-153.00
57.33
57.00
Old Harbor
SB
Sitkalidak I.
D
km
0 10 20

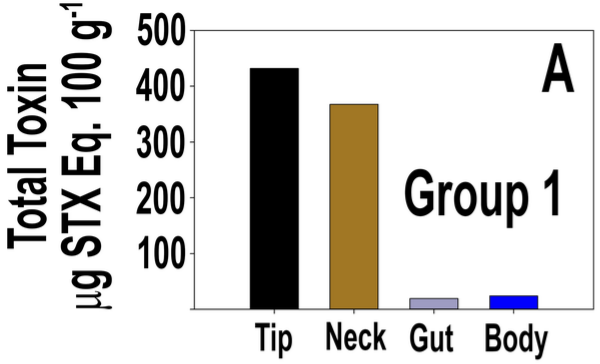

Ouzinkie
A
Kodiak
B
Trident Basin
Mission Beach
Old Harbor
C
Total Toxin
µg STX Eq. 100 g-1
800
600
400
200
0
Jan-13
Jul-13
Jan-14
Jul-14
Jan-15
Jul-15
Jan-16
Jul-16
Jan-17
Jul-17
Jan-18
Jul-18
Jan-19
Jul-19
Jan-20

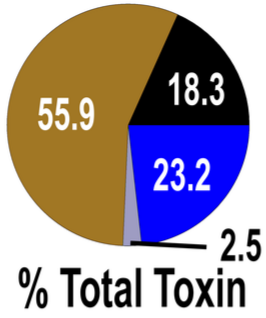

Total Toxin
µg STX Eq. 100 g-1
Ouzinkie
Kodiak
Old Harbor
A
STX
(% Total Toxin)
B
neoSTX
(% Total Toxin)
C
GTX
(% Total Toxin)
D
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Yearly

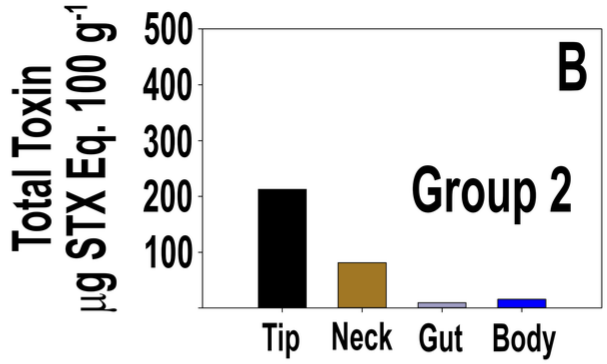

A
Hinge Ligament
Gills
Digestive Organs (Gut)
Posterior Adductor Muscle
Anterior Adductor Muscle
Siphon Tip
Body
Siphon (Neck)
Foot
Mantle Muscle
B
50 mm

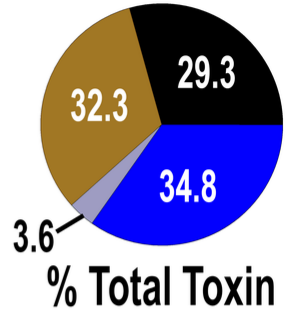

A
B
Total Toxin
(μg STX Eq. 100 g-1)
Contribution to Toxin (%)
3000
2500
2000
1500
1000
500
0
100
80
60
40
20
0
Tip
Neck
Gut
Body

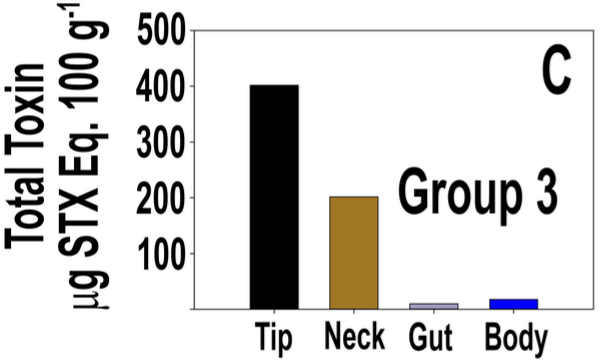
Mission Beach 25 May 2017

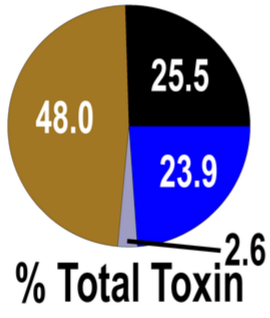


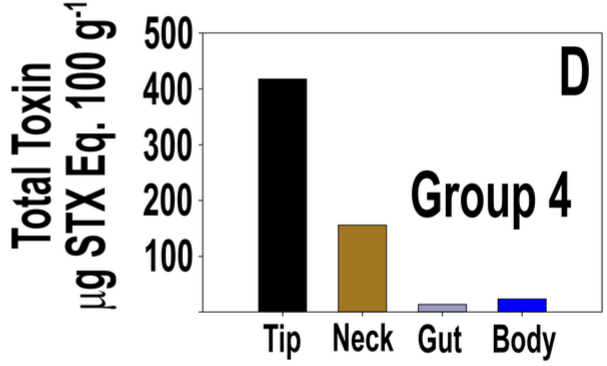


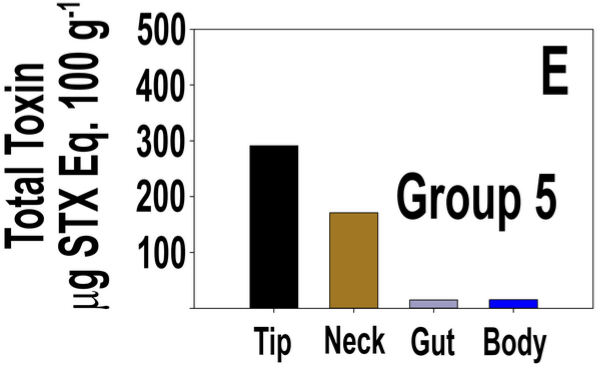


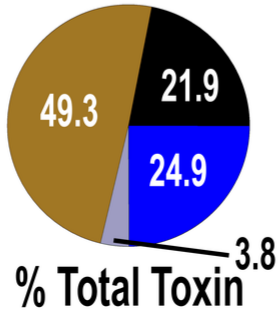


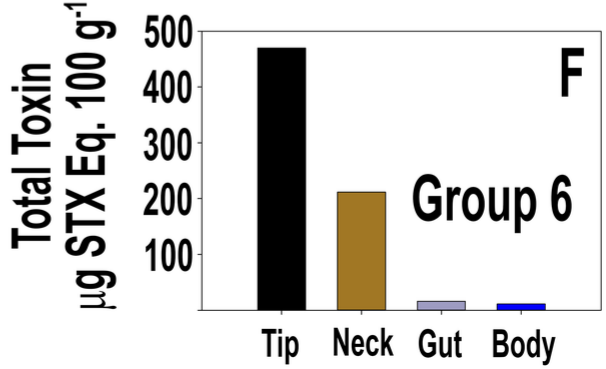


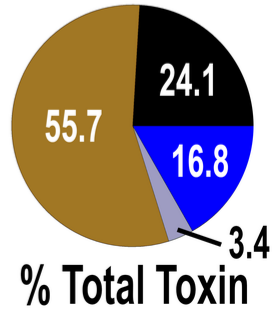




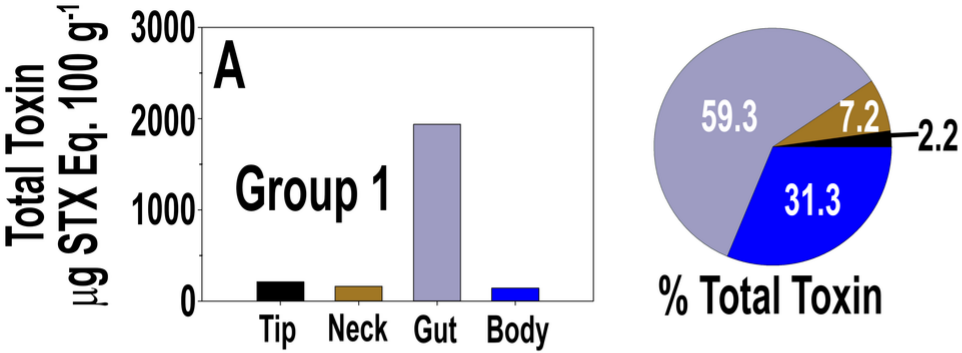


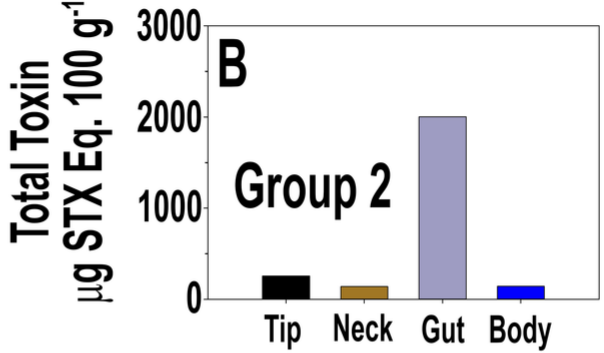


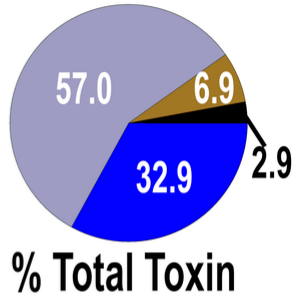


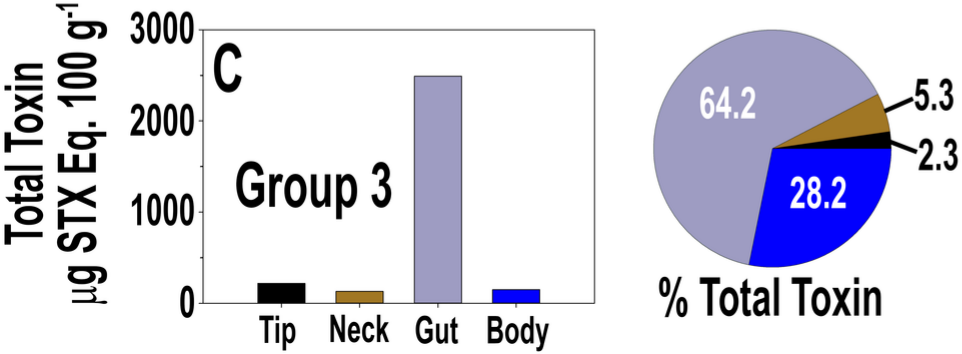


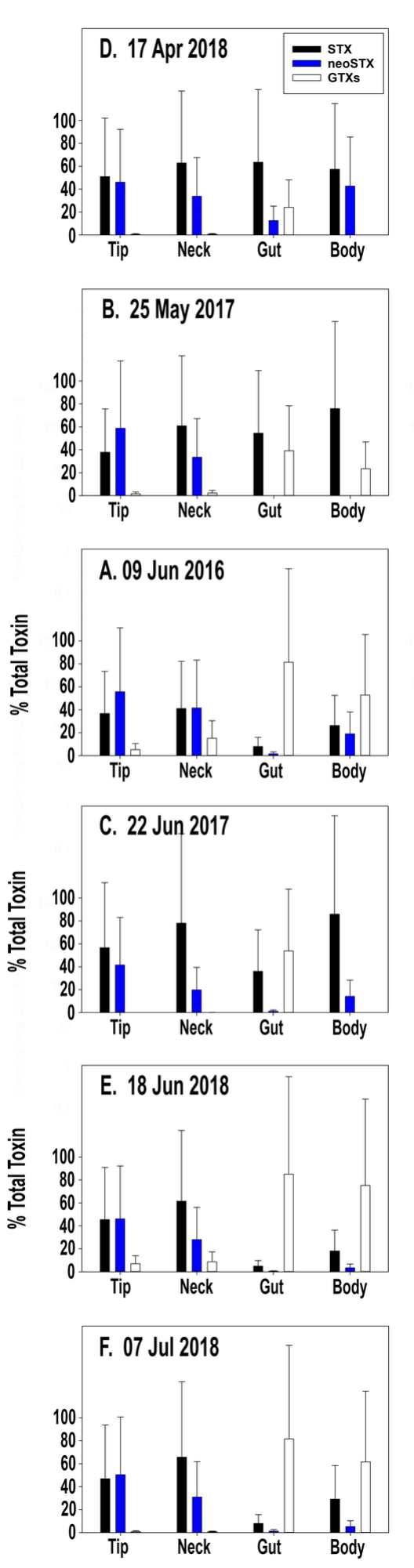
Mission Beach 18 Jun 2018










D. 17 Apr 2018
STX
neoSTX
GTXs
B. 25 May 2017
A. 09 Jun 2016
C. 22 Jun 2017
E. 18 Jun 2018
F. 07 Jul 2018
% Total Toxin
0
20
40
60
80
100
Tip
Neck
Gut
Body