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1 Experimental uptake and depuration of paralytic shellfish toxins in Southern Rock Lobster, *Jasus*
2 *edwardsii*

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19 **Abstract**

20 In October 2012, paralytic shellfish toxins (PST) were detected in the hepatopancreas of Southern
21 Rock Lobsters (*Jasus edwardsii)* collected from the east coast of Tasmania, Australia. This resulted
22 in the first commercial closure in Australia for this species. Questions were raised on how the toxins
23 were transferred to the lobsters, how long the toxins would persist, whether PST-contaminated
24 hepatopancreas posed a risk to human health, and what management strategies could be applied. The
25 aim of this study was to investigate whether PST-contaminated mussels are a potential vector
26 enabling toxin accumulation in *J. edwardsii* and to collect information on toxin uptake, distribution
27 and depuration rates and toxin profiles under controlled experimental settings. Lobsters were fed
28 mussels naturally contaminated with PST for a period of 28 days in an experimental setting;
29 following this, lobsters were allocated to either fed or starved treatment groups. PST were not
30 detected in the tail tissue of lobsters at any stage of the experiment. Lobster hepatopancreas
31 contained mean levels of 2.4 mg STX.2HCl eq/kg after 28 days of uptake, although substantial
32 variability in total toxicity was observed. The PST profile of the hepatopancreas was similar to that
33 of the contaminated mussels used as feed. Significant differences were noted in the PST depuration
34 rates between fed and starved treatment groups. The daily depuration rate for total PST was
35 estimated to be 0.019 and 0.013 mg STX.2HCl eq/kg for the fed and starved treatment groups
36 respectively using a constant-rate decay model. After 42 days of depuration, total PST (STX
37 equivalents) levels in the hepatopancreas of all lobsters were below 0.8 mg STX.2HCl eq/kg, which
38 represents the regulatory level applied to bivalves. This result indicates that long-term holding to
39 depurate PST may potentially be used as a risk management tool.

40

41 **Keywords**

42 saxitoxin, crustacean, biotoxin accumulation, tomalley, liver

43

44 **Introduction**

45 Some types of marine microalgae are capable of producing potent toxins and, under favourable
46 environmental conditions, these organisms can multiply rapidly. The toxins produced may
47 accumulate in marine biota (especially in bivalve shellfish due to their filter feeding action) at levels
48 that can cause illness in humans (Shumway, 1990).

49 The saxitoxin (STX) group of toxins, commonly referred to as paralytic shellfish toxins (PST),
50 incorporate a suite of water soluble analogues that includes the following sub-groups: carbamates,
51 gonyautoxins, sulfocarbamoyls, decarbamoyls and hydroxylated saxitoxins (Anonoymous., 2009;
52 Shumway, 1990). In the marine environment, PST are mainly produced by the dinoflagellates
53 *Gymnodinium catenatum* and *Pyrodinium bahamense* and members of the genus *Alexandrium*
54 (Anonoymous., 2009). Symptoms of illness in humans from these toxins include tingling of the lips,
55 gums and tongue and in more serious cases, numbness in the fingers and toes that spreads up the
56 arms, legs and neck within four to six hours (Toyofuku, 2006). In severe cases, mortality due to
57 respiratory paralysis can occur within two to twelve hours of consumption if there is no medical
58 intervention (Toyofuku, 2006). Due to the serious public health risk posed by these toxins, they are
59 regulated internationally. The Codex Alimentarius Commission has set a maximum level of 0.8 mg
60 STX.2HCl eq/kg for bivalve shellfish (CODEX STAN 292-2008). Although there is no regulatory
61 level set for PST in crustaceans, the Tasmanian regulatory authorities use the bivalve regulatory
62 level to protect public health and market access.

63 In October 2012, PST were detected in mussels (*Mytilus galloprovincialis*) harvested from Spring
64 Bay on the east coast of Tasmania, Australia at levels above the Australian regulatory limit of 0.8
65 mg STX eq/kg (Campbell et al., 2013). Subsequent investigation identified a bloom of the toxic alga
66 *Alexandrium tamarense* complex group 1 as the most likely source of PST. Testing also identified

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67 violative levels of PST in other bivalve shellfish (scallops, clams and oysters). Levels of toxins of up
68 to 2.8 mg STX eq/kg were also identified in the hepatopancreas (also commonly called 'liver',
69 'tomalley' or 'lobster mustard') of Southern Rock Lobster, *Jasus edwardsii* (Campbell et al., 2013;
70 McLeod et al., 2012). The identification of toxins in the hepatopancreas of lobsters resulted in a
71 commercial closure for this species being implemented in November 2012 along the majority of the
72 east coast of Tasmania. Although there have been several reports of PST in lobsters internationally
73 (Desbiens and Cembella, 1997; Grindley and Sapeika, 1969; Lawrence et al., 1994; Sang and Ming,
74 1984), there has only been one previous report of PST in Australian lobsters, with a maximum level
75 of 1.8 mg STX eq/kg measured in gut tissues (Arnott, 1998). Internationally, there have been several
76 reports of PST-related illness associated with lobsters (Garth and Alcala, 1977; Halstead, 1965;
77 Hashimoto et al., 1967; Todd et al., 1993). However, although the reported symptoms in these cases
78 were typical of PST-related illness, none of these reports provide direct association as the implicated
79 food samples were not tested. The identification of PST in Southern Rock Lobster highlighted a
80 number of uncertainties and data gaps for the Australian industry, including the mechanism by
81 which lobsters accumulate toxins, the amount of time it would take for PST to depurate to compliant
82 levels, and whether PST in lobster hepatopancreas pose a risk to human health.
83 Studies conducted in various regions (principally in North America) have shown that trophic transfer
84 of PST is an important exposure route, and is likely to represent the principal uptake mechanism for
85 PST in crustaceans (Haya et al., 1994; Oikawa et al., 2005). However, this had not previously been
86 demonstrated experimentally in Australian lobsters. The aim of this study was to investigate whether
87 PST-contaminated mussels are a potential vector leading to PST accumulation in *J. edwardsii,* and
88 to better understand PST uptake and depuration rates under controlled experimental settings. These
89 data may be useful in future consideration of risk mitigation strategies for *J. edwardsii* during PST-
90 producing algal blooms.

91

92 **Methods**

93 *Animal Ethics*

94 This study was conducted under Animal Ethics Permit 2/14 granted by the Primary Industries and
95 Regions South Australia Animal Ethics Committee. Permit 2/14 also covered a pilot study (data not
96 shown) that was used to refine the experimental system design, feeding regime and sampling
97 framework.

98

99 *Experimental system*

100 Lobsters (*n*=58) were held in a flow-through experimental system where each animal was housed
101 separately in a 30 L plastic tank lined with a mesh basket to minimise physical handling. Seawater
102 was supplied to each tank independently at a flow rate of approximately 1.7 L/h in a manner that
103 prevented cross-contamination between tanks. A pre-conditioned sponge filter was situated in the
104 corner of each tank to maintain water quality through biological filtration. Faeces and surplus food
105 were removed from each tank daily via siphoning and each tank was re-filled with seawater,
106 resulting in a bulk water exchange per day of approximately 20%. Regular monitoring of water
107 quality (ammonia, nitrite and nitrate) was undertaken to ensure wellbeing of the animals. Water
108 temperature was monitored daily, and maintained at 13 to 16°C through control of ambient
109 temperature; average surface seawater temperature in Tasmanian lobster harvest regions ranged
110 from 13.09 to 16.2°C in 2005 (Pecl et al. 2009). Ambient red light was provided to replicate a light
111 period of 11 hours per day.

112

113 *Lobsters, treatments and sampling regime*

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114 Live Southern Rock Lobster, *J. edwardsii,* weighing between 600 and 800 grams were sourced from
115 a commercial lobster processor in South Australia and translocated to the experimental system
116 within 24 hours. All lobsters were added to the experimental tanks in sequential order and
117 immediately allocated to either control or contaminated treatment groups using random sequences
118 generated at www.random.org to remove any effect of the experimental system. Lobsters were
119 allowed to acclimate to the experimental system for three weeks to ensure that animals were
120 regularly consuming food. During this time, two non-toxic blue mussels in the shell (*Mytilus*
121 *galloprovincialis*) were given to the lobsters daily to encourage feeding. These mussels were
122 purchased from a commercial mussel producer in South Australia as a bulk lot and stored frozen
123 prior to use. They were demonstrated to be free of PST by randomly selecting three pooled samples
124 of 12 mussels and subjecting these to chemical analysis by the Lawrence screen method (as
125 described below).

126 Control lobsters (*n*=12) were used to demonstrate that the experimental animals were free of PST
127 prior to entering the system, and that PST were not transferred between tanks during the experiment.
128 These lobsters were fed one non-toxic mussel each day (in the shell) whilst in the experimental
129 system. Four control lobsters were harvested on Day 0, Day 28 and Day 98 (*n*=12) and their
130 hepatopancreas tested for PST using the Lawrence confirmation method (as described below). Tail
131 meat samples from these control lobsters at Day 0, 28 and 98 were tested for PST using a Lawrence
132 screen method as composite samples (as described below).

133 For all other experimental lobsters, an uptake phase of 28 days was used where lobsters were fed
134 two PST-contaminated mussels (in the shell) daily (*M. galloprovincialis*). These mussels were
135 sourced in 2012 from the east coast of Tasmania during a bloom of *A. tamarense*. Mussels were
136 stored at approximately –20°C in vacuum packaged bags. PST levels in the mussels were
137 determined prior to the experiment by analysing three replicate samples of pooled mussels (*n*=16 in

138 each pool). Ten mussels were also analysed to establish mussel-to-mussel variability of PST. The
139 shell length, total weight (meat and shell) and meat weight of 162 toxic mussels were recorded for
140 use in later modelling to estimate meat weight consumed by the lobsters. Each day, a record was
141 made of the length and weight of the mussels fed to the lobsters, and a record made of whether the
142 mussels supplied the previous day had been consumed (as either a yes or no result).
143 As consumption of toxic mussels was variable between lobsters and between days during the uptake
144 phase, a selective sampling strategy was used to ensure the maximum PST level was achieved in test
145 animals at the end of the uptake phase. After 16 days of uptake, four animals that had consumed the
146 least mussels were selected for testing, to increase the likelihood that there would be detectable PST
147 levels in remaining animals at the end of the uptake phase. After 28 days of uptake, four animals that
148 had fed consistently, consuming 50-52 mussels each, were selected for testing, to give an indication
149 of the potential maximum PST uptake.
150 A depuration phase was initiated on Day 28. Contaminated lobsters were randomly separated into
151 fed and starved treatments groups using sequences obtained at www.random.org to establish if
152 subsequent feeding would change the rate of toxin depuration from the lobsters. Fed lobsters were
153 given two non-contaminated mussels daily (in the shell), whilst the starved animals were not
154 provided with any food source. Animals from both treatment groups were tested in replicates of four
155 on each of the following occasions: after 21, 42 and 70 days (Days 49, 70 and 98 respectively) of
156 depuration. Animals from both treatment groups were randomly selected at each time point using
157 sequences obtained at www.random.org.
158
159 *Preparation of animals for testing*

160 A humane killing method was used for harvest of lobsters whereby animals were removed from the
161 experimental tanks and immediately placed at −20°C until rendered senseless, followed by piercing
162 with a sharp knife through the carapace in a position immediately posterior to the eyes.
163 Prior to knifing, lobster weights, carapace length and sex (based on morphology of the swimmerets
164 under the tail) were recorded. Following knifing, animals were immediately dissected and the
165 hepatopancreas and tail meat removed. The digestive tract was carefully separated from surrounding
166 muscle tissues in order to avoid spilling gut contents that might otherwise compromise PST analysis
167 of tail meat. Hepatopancreas samples from individual lobsters were homogenized using a WiseTis®
168 Homogenizer (Wisd Laboratory Instruments, Wertheim, Germany) at 1000 rpm and 5 g subsamples
169 were taken for PST analysis. Tail tissues from each animal were finely diced and mixed to achieve a
170 homogenous sample, then 50 g from each of four replicate lobsters was used to form a composite
171 sample and blended using a Waring blender (Torrington, Connecticut, USA) at 1000 rpm. The
172 composite sample was tested for PST. All samples were stored at −20°C until transported to the
173 laboratory for analysis as blind samples.

174

175 *Analytical methods*

176 All PST analyses were conducted at the Cawthron Institute in New Zealand. Each sample was
177 thawed and a 5 g sub-sample subjected to PST analysis using a pre-column oxidation HPLC-FLD
178 method (Harwood et al., 2013) based on that described by Lawrence et al. (AOAC 2005.06)
179 (Lawrence et al., 2005). Briefly, PST present in samples were quantified by comparison with
180 certified reference materials. Recovery, dilution and toxicity equivalents factors were applied to give
181 the contribution of each analogue to sample toxicity. These were then summed to give total sample
182 toxicity in STX equivalents. Testing consisted of a rapid periodate screen (PST screen) and/or a

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183 more complex confirmation test (PST confirmation) to obtain a more accurate measure of sample
184 toxicity. PST screen analysis was used for non-contaminated mussels used as feed and lobster tail
185 samples. PST confirmation analysis was used for all contaminated mussel samples and all lobster
186 hepatopancreas samples. The performance of the PST confirmation test was assessed by several
187 spike recovery experiments performed on blank lobster tail meat and hepatopancreas tissue. As
188 comparable recovery and inter-day precision results were observed between lobster matrix and
189 shellfish matrices that had been previously assessed in detail (Holland et. al. 2010; Lawrence et al
190 2004; Lawrence et. al. 2005), the methodology was deemed suitable for use in this study. Several
191 PST analogues are known to give multiple fluorescent oxidation products, some of which co-elute
192 when using the pre-column oxidation method (Harwood et al., 2013). The PST screen analysis uses
193 the cleaned-up sample extract prior to ion exchange fractionation, and typically overestimates
194 sample toxicity as it is assumed that chromatographic peaks generated from co-eluting PST
195 oxidation products are due entirely to the most toxic analogue. The PST confirmation analysis
196 procedure involves fractionation of the initial pH-adjusted extract using an ion exchange SPE
197 cartridge, followed by separate oxidations with periodate and peroxide.
198
199 *Statistical Analysis*
200 All statistical analyses were performed using the R software (R Core Development Team, version
201 3.1.3, 2015). The depuration rates of total PST (mg STX.2HCl eq/kg) in lobster hepatopancreas
202 were modelled using constant-rate decay models of the form $C=C_0 10^{kt}$ where C represents the toxin
203 concentration, C_0 is the initial concentration, k is the constant rate and t is time. This model was
204 obtained by fitting a linear regression to the $\log_{10}$-transformed data, similar to the approach
205 described by Hubbart et al. (2012) and McLeod et al. (2017).

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206 To enable prediction of PST uptake by lobsters through consumption of contaminated mussels, it
207 was necessary to determine how much mussel meat was consumed and how much toxin was present
208 within the mussels. As the actual amount of meat consumed was not measured (because animals
209 were fed toxic mussels in shell), a linear regression model for predicting mussel meat weight was
210 developed based on shell length and total weight. Another linear regression model was fitted to the
211 results of measured mussel meat weight and PST levels from ten individually analysed mussels to
212 enable estimates of PST concentration from mussel meat weight. The mussels consumed (number,
213 shell length and total weight) and the depuration rates of PST in fed lobster were used to predict
214 lobster PST levels at the end of the uptake phase.
215 The comparison and significance of PST loss rates between treatments and analogues were
216 statistically tested using Analysis of Variance (ANOVA). Results from all statistical analyses were
217 assessed against a significance level of 0.05 and deemed to be statistically significant if the p-value
218 was < 0.05.
219

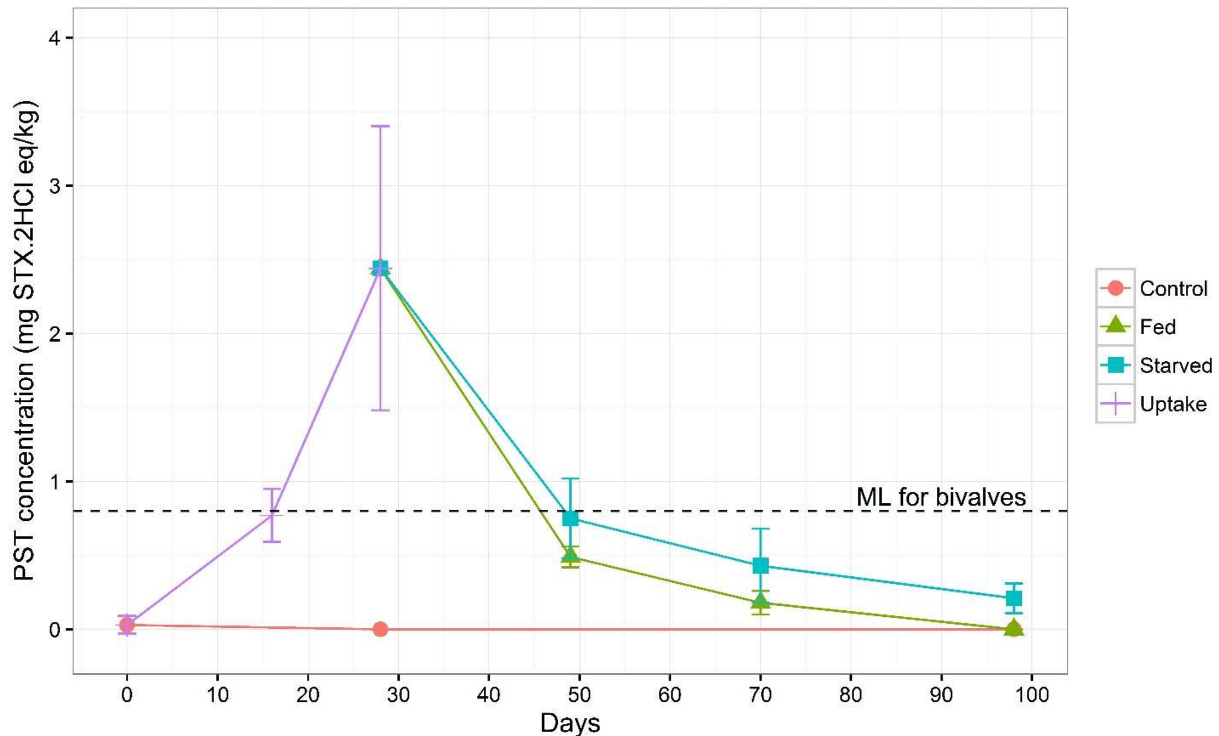
220 **Results**

221 *PST-contaminated mussels*

222 Testing of mussels harvested from Tasmania in 2012 during an *A. tamarense* bloom demonstrated
223 that these shellfish were contaminated with PST and that levels were well above the regulatory level
224 applied to bivalve shellfish. The results for each of the three pooled samples were 2.5, 1.7 and 2.0
225 mg STX.2HCl eq/kg. Analysis of the individual mussels showed a high level of variation between
226 mussels, with a mean concentration of 1.51 ± 0.77 mg STX.2HCl eq/kg (range 0.57 - 2.83 mg
227 STX.2HCl eq/kg). The data on shell length and total weight of mussels were used to develop a
228 model to estimate the mussel meat weight (in grams). Individual models to estimate mussel meat

229 weight from shell length and/or total weight were assessed; however, the model that combined both
230 provided the best prediction with an R^2 value of 0.83.
231
232 *Lobster feeding*
233 Initially, lobsters fed consistently on the PST-contaminated mussels during the initial stages of the
234 experiment. Over time, some lobsters reduced their intake. It was observed that some lobsters would
235 consistently consume one or two mussels per day, others would abstain from feeding for several
236 days before starting again and some lobsters consumed all mussels that were offered. All except one
237 of the eight remaining control lobsters fed consistently. All test lobsters consumed between 35 and
238 52 mussels during the 28 days of the uptake stage of the experiment.
239
240 *Lobster PST results*
241 Of the four control hepatopancreas samples tested at Day 0 of the experimental trial, one was found
242 to contain PST at a level of 0.12 mg STX.2HCl eq/kg, with only STX detected. All other control
243 hepatopancreas samples (*n*=8), across all time points, were negative for the presence of PST.
244 PST was not detected in any tail tissue samples tested throughout the study. Analysis of
245 hepatopancreas samples during the uptake phase showed PST were present in the lobsters (Figure 1).
246 After the uptake phase, the mean PST concentration in the hepatopancreas samples was 2.4 ± 0.96
247 mg STX.2HCl eq/kg. The maximum PST level detected in the lobster hepatopancreas at the end of
248 the uptake phase was 3.7 mg STX.2HCl eq/kg. During uptake, no statistically significant difference
249 in PST levels in lobster hepatopancreas was observed between male and female lobsters (P=0.89).
250 At the end of the depuration period (70 days), all samples were below the regulatory level for
251 bivalves of 0.8 mg STX.2HCl eq/kg. The mean PST levels for starved animals at the end of the
252 depuration period was 0.21 ± 0.1 mg STX.2HCl eq/kg and all the fed animals were below the level

253 of reporting for the method. Linear regression analysis showed a significant difference between the
254 treatment groups (P=0.002).
255



256

257 **Figure 1: Total PST concentrations (mg STX.2HCl eq/kg) observed from Southern Rock**
258 **Lobster hepatopancreas during a controlled feeding study. Error bars are standard deviations**
259 **about the mean (*n*=4 at each time point during uptake and *n*=4 for each treatment and time**
260 **point during the depuration phase). Note: a targeted sampling framework was used in the**
261 **uptake phase based on feeding amount.**

262

263 *Lobster consumption of PST in mussels*

264 To allow prediction of PST levels in lobsters throughout the experiment, data generated by the
265 model for estimating mussel tissue weight were used as an input into an additional model for PST
266 uptake in lobster hepatopancreas that considered amount of tissue consumed, the PST concentration
267 in the mussels and the daily depuration rate of fed animals (reported below). The model showed a

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268 significant relationship (R^2=0.75, P=0.005) between the predicted lobster PST concentration and the
269 analytical results at the end of the uptake phase. However, the model overestimated the
270 concentration by 25% on average.
271
272 *Mussel and lobster PST profile*
273 Percentage profiles of PST analogues on a molar concentration basis for mussels and lobster
274 hepatopancreas are presented in Figure 2. Mussels were dominated by N-sulfocarbamoyl-
275 gonyautoxin-2/3 (C-1,2) and carbamate toxins STX and gonyautoxin-2/3 (GTX-2,3) with low levels
276 of gonyautoxin-5 (GTX-5) and N-sulfocarbamoyl-gonyautoxin-1/4 (C-3,4) above the analytical
277 level of reporting. Lobster hepatopancreas tissues were also dominated by C-1,2, STX and GTX-2,3
278 during uptake; although the proportion of STX and GTX-2,3 had increased in comparison to the
279 mussel samples. GTX-5 appeared in the hepatopancreas samples after 28 days of uptake.
280 During the depuration phase, the toxin profile of fed lobsters remained dominated by C-1,2, STX
281 and GTX-2,3, with C-1,2 no longer detected at Day 70. Similarly, the toxin profile of starved
282 lobsters was also dominated by these analogues, however, C-1,2 was slower to depurate, being
283 detected on Day 70, but not on Day 98. The analogue dcSTX was detected in one of the four starved
284 lobsters harvested at Day 49. As this analogue was not found in any other lobster or mussel samples,
285 we consider this result to be an outlier.

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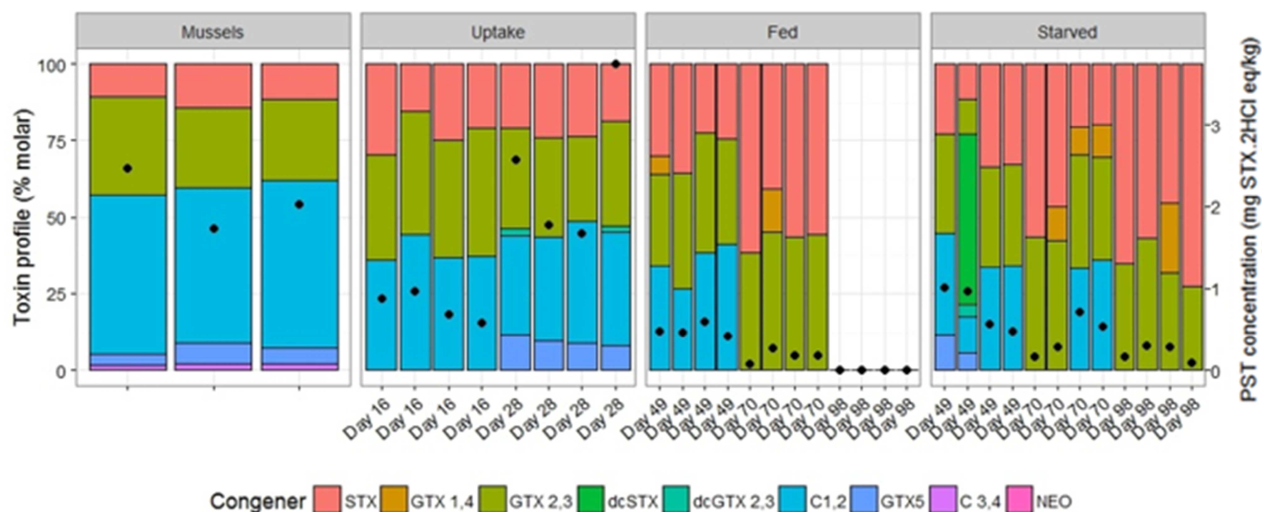

286

287 **Figure 2: Total PST concentration (black dots) and percentage profiles of PST analogues on a**
288 **molar concentration basis for three pooled mussel samples and lobster hepatopancreas during**
289 **the common uptake phase and during the depuration phase for fed and starved animals.**

290

291 *Lobster PST depuration model*

292 The total PST depuration rates were highest in the fed animals (0.019 mg STX.2HCl eq/kg/day) as
293 compared to the starved animals (0.013 mg STX.2HCl eq/kg/day). A reasonable fit of the depuration
294 models for total PST (STX.2HCl eq/kg) was observed for the fed and starved animals, with R^2
295 values of 0.84 and 0.78 respectively.

296

297 **Discussion**

298 This study demonstrates that feeding PST-contaminated mussels to Southern Rock Lobster
299 (*J. edwardsii*) results in toxin accumulation in the hepatopancreas. This provides strong evidence
300 that PST-contaminated prey organisms are a likely vector in the natural environment. However,
301 some lobsters reduced their intake of contaminated mussels in the uptake phase and it is unclear if

302 this would also occur in the wild; one of the control animals also reduced feed intake, so the feeding
303 changes may be reflective of the artificial system used. The maximum concentration of toxins
304 detected in the hepatopancreas of lobsters at the end of the uptake phase (3.7 mg STX.2HCl eq/kg)
305 is similar to levels measured by regulatory authorities in *J. edwardsii* during a bloom of *A.*
306 *tamarense* in Tasmania in 2012 by Madigan et al. (unpublished data) of up to 4.03 mg STX.2HCl
307 eq/kg. However, this is lower than levels reported in *Homarus americanus* where levels up to 15.1
308 mg STX eq/kg have been found in naturally harvested animals during *Alexandrium excavatum*
309 blooms (Desbiens and Cembella, 1995). Haya et al. (1992) fed the digestive glands of scallops
310 containing 40 mg STX eq/kg to *H. americanus* twice weekly for 16 weeks, which resulted in lobster
311 hepatopancreas levels ranging from 27.5 to 32 mg STX eq/kg across the course of the uptake phase.
312 Haya et al. (1992) noted a large variation in the level of PST in the hepatopancreas of lobsters at a
313 single time point. This variation was also noted in the current study, particularly at the end of the
314 uptake phase (Day 28) where there was more variability in PST concentration between lobsters than
315 at any other time point. Each of these lobsters had consumed a similar amount of toxic feed. Each
316 animal consumed between 50 and 52 mussels, resulting in a calculated weight of 366-393 g mussel
317 meat using the mussel tissue consumption model. In addition, lobsters were similar in size (11-11.9
318 cm carapace length) and total weight (730-790 g). The differences in PST concentration were
319 unrelated to the sex of test lobsters.
320 Given the foregoing, other factors must also influence the uptake rate. It should be noted that the
321 sampling approach that was used in the uptake phase would have impacted the uptake phase results.
322 Lobsters that had consumed the least quantity of toxic mussels were selectively sampled on Day 16
323 in the middle of the uptake phase to ensure the maximum PST level was achieved in remaining test
324 animals at the end of the uptake phase. Thus, it was expected that the level of PST measured in
325 hepatopancreas at Day 16 would be lower than if these animals had consumed toxic mussels at a

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326 similar rate to the animals harvested at Day 28. Other factors could be physiological differences
327 between lobsters or the water-soluble nature of PST, as it is possible that a lobster that consumes
328 feed more slowly could be exposed to a lesser amount of PST due to loss from the feed into the tank
329 water. Feeding rates were not assessed in this study, but different feeding behaviours were observed
330 between lobsters where some lobsters would feed vigorously and some would feed overnight.
331 The detection of PST in one of the four control lobsters tested at Day 0 at a level of 0.12 mg
332 STX.2HCl eq/kg was an unexpected result and the possible presence of PST in other lobsters at the
333 initiation of this experiment cannot be excluded. In the pilot study (data not shown), where lobsters
334 were also acclimated in the system for three weeks before testing, PST was not detected in any
335 lobsters tested at Day 0 (n=4) and at Day 28 of uptake (n=4). PST had not been tested in South
336 Australian lobsters prior to these samples and no algal blooms were known to have occurred prior to
337 sourcing the lobsters. Lobsters from different harvest locations within a fishery are commonly co-
338 mingled in lobster processing facilities, so it is likely that the lobsters used in this experiment did not
339 all originate from the same fishing location. Using the depuration model for PST developed in this
340 study for fed animals, the PST concentration in the hepatopancreas of this animal was estimated as
341 being 0.30 mg STX.2HCl eq/kg when purchased from the processor. This may have contributed to
342 the variability noted between lobsters at the same sampling point.
343 The model developed to assess uptake of PST toxins in lobster hepatopancreas overestimated the
344 concentration of PST in comparison to the analytical results by 25% on average. The disparity is
345 likely associated with portions of mussels not being consumed; lobsters shred and grind their food
346 and small amounts of discarded mussel tissues were always left behind in the tank following
347 consumption (Mikami and Takashima, 2008). Jester et al. (2009) tested the faecal matter of the
348 paddle crab, *Ovalipes catharus*, and detected PST analogues indicating that loss could also have
349 occurred via the faeces.

350 At the end of the uptake phase, the toxin profile in lobster hepatopancreas was similar to that of PST
351 contaminated mussels consumed by the lobsters. This contrasts with the results reported by Haya et
352 al. (1994) who evaluated the uptake of PST in *H. americanus* in an experimental setting. They did
353 not detect C1 and C2 toxins in hepatopancreas despite these toxins being measured in scallops fed to
354 the lobsters. In the current study, C-toxins were seen in both mussel and hepatopancreas matrices.
355 The concentration of PST decreased rapidly in the first 21 days of depuration with mean levels in
356 both fed and starved animals reduced by more than 50% compared to levels at the end of the uptake
357 stage (Day 28). This result is supported by the work of Oikawa et al. (2005) who examined the
358 depuration of PST in the shore crab *Telmessus acutidens* and reported a rapid initial depuration of
359 toxins followed by a more gradual loss. Differences were also reported between depuration rates in
360 the fed versus starved animals. It is likely that the movement of digested material within the ducts of
361 the hepatopancreas increases the depuration rate. In the present study, C-1,2 appeared to depurate
362 faster than STX and GTX-2,3 in both fed and starved lobsters. This observation supports the
363 findings of Haya et al. (1994) who suggested that lobster hepatopancreas may have a greater affinity
364 for retaining carbamoyl group toxins. The appearance of GTX-1,4 at trace levels in some fed
365 lobsters and low levels (maximum of 0.13 mg STX.2HCl eq/kg) in the starved animals is indicative
366 of biotransformation (N1-hydroxylation) occurring as these compounds were not detected in either
367 mussels or lobster hepatopancreas during the uptake phase.
368 Desbiens and Cembella (1995) concluded that long-term holding to facilitate PST depuration from
369 *H. americanus* was not possible due to the length of time that PST persisted in the hepatopancreas.
370 In that study, lobsters were not fed during the depuration period and PST levels in the
371 hepatopancreas remained high with no significant differences in mean PST concentration found
372 across 53 days of depuration. Furthermore, the authors alluded to an earlier study where lobster
373 hepatopancreas retained levels of PST over 3 mg STX eq/kg after five months of depuration. Haya

374 et al. (1994) also reported that *H. americanus* took 70 days for levels of PST in the hepatopancreas
375 to fall below the regulatory level for bivalves. In the Haya et al. (1994) study, lobsters were fed non-
376 contaminated feed during the depuration phase. In contrast, in the current study of PST depuration in
377 *J. edwardsii*, toxin concentrations in the hepatopancreas of two out of the four starved animals were
378 marginally above 0.8 mg STX.2HCl eq/kg after 21 days of depuration, while toxins in the
379 hepatopancreas from all other animals on this day (*n*=6; 2 starved, 4 fed) were below this level, as
380 were all hepatopancreas toxin levels after 42 days of depuration. It is unknown whether the longer
381 depuration times found in Desbiens and Cembella (1995) and Haya et al. (1994) are due to
382 differences in clearance rates between species, higher initial loads of PST or other factors, as data on
383 the initial toxin levels for the studies on *H. americanus* were not provided.
384 Using the depuration models for total PST developed here, and taking the maximum level of PST
385 found thus far during bloom sampling of wild *J. edwarsdii* (4.03 mg STX.2HCl eq/kg), fed lobsters
386 would need to be held for 37 days without additional PST uptake to reduce PST concentration to
387 below the regulatory level. If the hepatopancreas PST concentration was 2.0 mg STX.2HCl eq/kg,
388 animals would need to be held for 21 days. Similarly, if the same animals were starved, it would
389 require 54 or 31 days of depuration to reduce from 4.03 or 2.0 mg STX.2HCl eq/kg respectively to
390 below the regulatory level. This species is commonly held in tanks during distribution and such
391 withholding periods in a processing facility are possible from an animal health perspective,
392 particularly considering that lobsters do not significantly lose weight even when starved for periods
393 up to 35 weeks (Stewart et al., 1972). Whilst depuration may not be commercially viable as a routine
394 measure, this information could be beneficial if harvest closures resulted in significant volumes of
395 contaminated lobsters being held in the supply chain. Included in this consideration is that either the
396 holding water would need to be free of toxin producing algae, or it would have to be established that

397 exposure to PST through the gills does not result in significant PST accumulation, as other studies
398 have detected low levels of PST in crustacean gills (Jester et al. (2009); Sephton et al. (2007).
399 Using a similar depuration calculation as above but applied to *J. edwarsdii* in the wild, the estimated
400 depuration rates can be used to inform timing of sampling after a bloom event. This will minimise
401 unnecessary/excessive sampling and provide industry with significant cost savings in sampling and
402 testing.
403
404 **Conclusions**
405 This feeding study has demonstrated experimental uptake of PST into the hepatopancreas of
406 *J. edwardsii* from PST-contaminated mussels. This provides strong evidence that consumption of
407 toxic food is a likely route for uptake of PST in *J. edwardsii*. No toxins were detected in tail tissue,
408 which is the most commonly consumed part of the lobster.
409 In contrast to studies on other lobster species, this work has calculated depuration rates for PST in
410 *J. edswardsii* hepatopancreas, and demonstrated that active feeding can increase the depuration rate.
411 These results can help risk managers determine appropriate sampling time points, improving the cost
412 effectiveness of a sampling program. The results also suggest that long-term holding to depurate
413 PST from the hepatopancreas of *J. edwardsii* may be an option for risk management during bloom
414 events.
415
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422

423 **References**

424

425 Anonoymous., 2009. Scientific opinion of the panel on contaminants in the food chain on a request from the
426 european commission on marine biotoxins in shellfish – saxitoxin group. The EFSA Journal 1019, 3-76.
427 Arnott, G.H., 1998. Toxic marine microalgae: a worldwide problem with major implications for seafood
428 safety. Advancing Food Safety 1, 24-34.
429 Campbell, A., Hudson, D., McLeod, C., Nicholls, C., Pointon, A., 2013. Tactical Research Fund: Review of the
430 2012 paralytic shellfish toxin event in Tasmania associated with the dinoflagellate alga, *Alexandrium*
431 *tamarense*, in: Corporation, F.R.a.D. (Ed.). FRDC Project 2012/060 Appendix to the final report, Adelaide,
432 Australia.
433 Desbiens, M., Cembella, A.D., 1995. Occurence and elimination kinetics of PSP toxins in the America Lobster
434 (*Homarus americanus*). Lavosier, Paris.
435 Desbiens, M., Cembella, A.D., 1997. Retention and possible transformation of paralytic shellfish toxins in
436 lobster (Homarus americanus): Proceedings of the Contributed Papers, Aquaculture Canada '97. Bulletin of
437 the Aquaculture Association of Canada 97, pp. 75-77.
438 Garth, J.S., Alcala, A., 1977. Poisonous crabs of Indo-West Pacific coral reefs, with special reference to the
439 genus Demania Laurie, Proceedings, Third International Coral Reef Symposium, Rosenstiel School of Marine
440 and Atmospheric Science, University of Miami, Miami, Florida, pp. 645-651.
441 Grindley, J.R., Sapeika, N., 1969. The cause of mussel poisoning in South Africa. South African Medical
442 Journal 43, 275-279.

443 Halstead, B., 1965. Poisonous and venomous marine animals of the world. Volume 1 - invertebrates. US
444 Department of Defense, Washington DC.
445 Harwood, D.T., Boundy, M., Selwood, A.I., van Ginkel, R., MacKenzie, L., McNabb, P.S., 2013. Refinement
446 and implementation of the Lawrence method (AOAC 2005.06) in a commercial laboratory: Assay
447 performance during an *Alexandrium catenella* bloom event. Harmful Algae 24, 20-31.
448 Hashimoto, Y., Konosu, S., Yasumoto, T., Inoue, A., Noguchi, T., 1967. Occurrence of toxic crabs in Ryukyu
449 and Amami Islands. Toxicon 5, 85-90.
450 Haya, K., Oshima, Y., Young-Lai, W.W., 1994. Profile of paralytic shellfish poisoning toxins in lobsters during
451 uptake and depuration, in: Forbes, J.R. (Ed.), Fourth Canadian Workshop on Harmful Marine Algae. Canadian
452 Technical Report of Fisheries and Aquatic Sciences Sidney.
453 Haya, K., Young-Lai, W.W., Stewart, J.E., Jellett, J.F., 1992. Uptake and excretion of paralytic shellfish toxins
454 by lobster fed scallop digestive glands. Canadian Technical Report of Fisheries and Aquatic Sciences 0, 38-38.
455 Holland, P., McNabb, P., Van Ginkel, R., Selwood, A., 2010. Validation of the Lawrence method for screening
456 saxitoxins in shellfish. Prepared for New Zealand Food Safety Authority. Cawthron Report No. 1738, 12p plus
457 appendices.
458 Hubbart, B., Pitcher, G.C., Krock, B., Cembella, A.D., 2012. Toxigenic phytoplankton and concomitant toxicity
459 in the mussel *Choromytilus meridionalis* off the west coast of South Africa. Harmful Algae 20, 30-41.
460 Jester, R., Rhodes, L., Beuzenberg, V., 2009. Uptake of paralytic shellfish poisoning and spirolide toxins by
461 paddle crabs (*Ovalipes catharus*) via a bivalve vector. Harmful Algae 8, 369-376.
462 Lawrence, J.F., Maher, M., Watson-Wright, W., 1994. Effect of cooking on the concentration of toxins
463 associated with paralytic shellfish poison in lobster hepatopancreas. Toxicon 32, 57-64.
464 Lawrence, J.F., Niedzwiadek, B., Menard, C., 2004. Quantitative Determination of Paralytic Shellfish
465 Poisoning Toxins in Shellfish Using Prechromatographic Oxidation and Liquid Chromatography with
466 Fluorescence Detection: Interlaboratory Study. Journal of AOAC International 87, 83–100.

467 Lawrence, J.F., Niedzwiadek, B., Menard, C., De Rojas Astudillo, L., Biré, R., Burdaspal, P.A., Ceredi, A., Davis,
468 B., Dias, E., Eaglesham, G., Franca, S., Gallacher, S., Graham, D., Hald, B., Heinze, L., Hellwig, E., Jonker, K.M.,
469 Kapp, K., Krys, S., Kurz, K., Lacaze, J.P., Gago Martinez, A., McNabb, P., Ménard, C., Milandri, A.,
470 Nsengiyumva, C., Pereira, P., Pineiro, N., Poletti, R., Riddell, G., Selwood, A., Stern, A., Tiebach, R., Van Den
471 Top, H., Wezenbeek, P., Yen, I.C., 2005. Quantitative determination of paralytic shellfish poisoning toxins in
472 shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection:
473 Collaborative study. Journal of AOAC International 88, 1714-1732.

474 McLeod, C., Stewart, I., Kiermeier, A., 2012. Paralytic Shellfish Toxins in Southern Rocklobsters (*Jasus*
475 *edwardsii*) Interim Food Safety Exposure Assessment. South Australian Research and Develoment Institute,
476 Australia.

477 McLeod, C., Dowsett, N., Hallegraeff, G., Harwood, D.T., Hay, B., Ibbott, S., Malhi, N., Murray, S., Smith, K.,
478 Tan, J., Turnbull, A., 2017. Accumulation and depuration of paralytic shellfish toxins by Australian abalone
479 *Haliotis rubra*: Conclusive association with *Gymnodinium catenatum* dinoflagellate blooms. Food Control 73,
480 971-980.

481 Mikami, S., Takashima, F., 2008. Functional morphology of the digestive system, Spiny Lobsters, 2 ed.
482 Blackwell Science Ltd, pp. 601-610.

483 Oikawa, H., Satomi, M., Watabe, S., Yano, Y., 2005. Accumulation and depuration rates of paralytic shellfish
484 poisoning toxins in the shore crab *Telmessus acutidens* by feeding toxic mussels under laboratory controlled
485 conditions. Toxicon 45, 163-169.

486 Pecl G, Frusher S, Gardner C, Haward M, Hobday A, Jennings S, Nursey-Bray M, Punt A, Revill H, van Putten I
487 (2009). The east coast Tasmanian rock lobster fishery – vulnerability to climate change impacts and
488 adaptation response options. Report to the Department of Climate Change, Australia.

489 Sang, J.W.T., Ming, T.T., 1984. Red tide and paralytic shellfish poisoning in Sabah, Malaysia. in Proceedings
490 of a consultative meeting held in Singapore, in: Centre, S.A.F.D.C.a.I.D.R. (Ed.), pp. 35-42.

491 Sephton, D.H., Haya, K., Martin, J.L., LeGresley, M.M., Page, F.H., 2007. Paralytic shellfish toxins in
492 zooplankton, mussels, lobsters and caged Atlantic salmon, Salmo salar, during a bloom of Alexandrium
493 fundyense off Grand Manan Island, in the Bay of Fundy. Harmful Algae 6, 745-758.

494 Shumway, S.E., 1990. A review of the effects of algal blooms on shellfish and aquaculture. Journal - World
495 Aquaculture Society 21, 65-104.

496 Stewart, J.E., Horner, G.W., Arie, B., 1972. Etrects of temperature, food, and starvation on several
497 physiological patameten of the lobster *Homarus americanus*. Journal Fisheries Research Board of Canada 29,
498 439-442.

499 Todd, E., Kuiper-Goodman, T., Watson-Wright, W., Gilgan, M., Stephen, S., Marr, J., Pleasance, S., 1993.
500 Recent illnesses from seafood toxins in Canada: Paralytic, amnesic and diarrhetic shellfish poisoning. DEV.
501 MAR. BIOL. 1993.

502 Toyofuku, H., 2006. Joint FAO/WHO/IOC activities to provide scientific advice on marine biotoxins (research
503 report). Marine Pollution Bulletin 52, 1735-1745.

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Highlights

- Uptake and depuration of paralytic shellfish toxins (PST) by Southern Rocklobster *Jasus edwardsii* in an artificial system is described
- Consumption of PST contaminated mussels resulted in accumulation of toxins in the lobster hepatopancreas but not in tail meat
- Substantial variability in total toxicity between lobsters was found
- Depuration followed a constant decay curve
- Depuration rates were slower in starved lobsters than in lobsters fed non-toxic material

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