Depuration and transformation of PSP toxins in the South African abalone *Haliotis midae*

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Abstract
Abalone were grown: on diets of 1) artificial feed, 2) kelp, their *in situ* food source, which contained saxitoxin (STX) derivatives, and 3) in filtered seawater without a food source, to investigate the depuration and transformation of toxins under feeding and starving conditions. The abalone were toxic at the start of each treatment (~160 µg STX eq 100 g^{-1} tissue). They depurated at a rate of 6.27 µg STX eq 100 g^{-1} tissue d^{-1} over the 2 week incubation period when fed artificial feed; however, no depuration was observed in starved or kelp-fed animals. Toxin transformations occurred in abalone for each treatment. Toxin derivatives decreased in organisms fed artificial feed, and no B1 was detected during this treatment. Toxin composition was not significantly different between starved and kelp-fed treatments. For these abalone, neoxanthin (NEO) and B1 derivatives increased, whereas gonyautoxin (GTX)-2+3 decreased. The need for including non-traditional vectors such as abalone in routine monitoring programs is clearly evident.

Introduction
Dinoflagellates of the genus *Alexandrium* are the most common sources of paralytic shellfish poisoning (PSP) toxins, and suspension-feeding bivalves are the usual vectors. However, in 1999 saxitoxin was detected in the abalone *Haliotis midae* off the coast of South Africa and was retained in the organism for > 7months. The toxin profile was very different from that of the local *Alexandrium* population, a potential toxin source. Toxins have been detected, however, in association with the kelp *Eklonia maxima*, the primary *in situ* food source for the abalone (Fig. 1; Etheridge 2002); thus, kelp is presumed to be the source of the abalone toxicity. The goals of this study were 1) to determine the toxin depuration rate in abalone under feeding and starving conditions and 2) to quantify toxin transformations in the abalone.

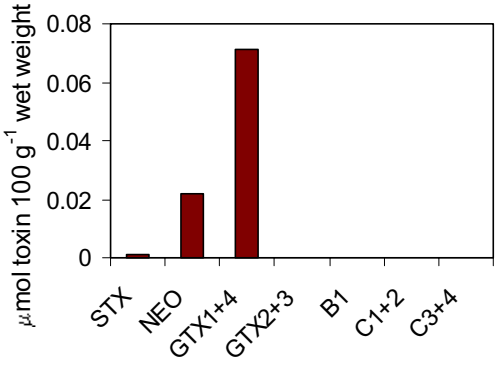

Figure 1. Toxin composition for the kelp *E. maxima*, in µmol toxin 100 g^{-1} wet weight.

Methods
Abalone were obtained from an abalone farm (I&J, LTD) on the south coast of South Africa where they are fed a diet of the kelp *E. maxima*. The animals used in experiments were approximately 2 cm in length, with an average wet tissue weight of 0.58 ± 0.25 g. The abalone (n = 7 or 8, depending on the treatment) were incubated in aerated 500 ml flasks containing 300 ml of 0.7 µm filtered seawater, and they were exposed to one of the following conditions in the laboratory for two weeks: 1) commercial artificial feed obtained from the abalone farm (control), 2) the kelp *E. maxima*, and (3) filtered seawater, representing non-toxic feeding, toxic feeding and starving conditions, respectively. Samples were extracted and analyzed for toxins using the methods described in Etheridge (2002). Toxin concentrations are reported as µmol toxin 100 g^{-1} wet weight of tissue. Toxicity values in µg STX eq 100 g^{-1} tissue are also estimated from HPLC-based concentrations using the conversion factors of Oshima (1995). Given the facile epimerization of GTX1 and GTX4, GTX2 and GTX3, C1 and C2, and C3 and C4 (Hall et al. 1990), the toxin compositions are reported with the epimer pairs combined. Departures from sample means are represented with standard error values, and differences between treatments were assessed using the Student's t-test.

Results

The abalone were initially toxic (160.15 ± 37.57 µg STX eq 100 g^{-1} tissue), exceeding the legal limit for commercial markets (80 µg STX eq 100 g^{-1} tissue, Fig. 2). The animal-to-animal variation was very high (cv = 0.52). When abalone were fed artificial feed only for the two week period, they became less toxic (72.30 ± 12.50 µg STX eq 100 g^{-1} tissue), suggesting that depuration occurred once the toxin source was removed under actively feeding conditions. The average depuration rate over the 2-week period was 6.27 µg STX eq 100 g^{-1} tissue d^{-1}. There was no significant difference in estimated toxicity between the abalone at initial conditions and those in the kelp feeding or starving treatments due to the high initial cv although the mean values were 40% lower. The average coefficient of variation for all treatments (cv = 0.42) was less than that for the initial conditions.

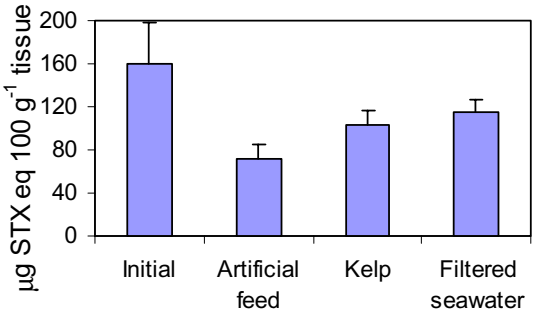

Figure 2. Mean abalone toxicity and standard error in µg STX eq 100 g^{-1} tissue for initial conditions and all treatments. The artificial feed treatment was significantly different from the initial conditions and other treatments ($p<0.1$).

The two dominant derivatives for the initial conditions were GTX2+3 and B1 (Fig. 3). However, NEO and GTX2+3 were dominant for abalone fed artificial feed, with no evidence of B1 (Fig. 3). Toxin compositions for the starved or kelp-fed abalone were not significantly different from each other, but were dominated by B1 and NEO (Fig. 3).

Discussion

The initial toxicity of the abalone was much higher than expected based on earlier reports for abalone from the south coast of South Africa. However, the abalone in this study were smaller (approximately 2 cm) than those measured previously (Pitcher et al. 2001). The work by Bravo et al. (1999) on Galician abalone demonstrated increased toxicity with decreasing body length. Results also demonstrated that there is considerable variability in toxicity among individual organisms, consistent with other abalone results (Bravo et al. 1999; Pitcher et al. 2001) and for other shellfish in general (e.g. Cembella et al. 1993; White et al 1993). The observed variability has significant implications for monitoring procedures and highlights the importance of designing protocols that allow for reliable sampling strategies for monitoring shellfish toxicity.

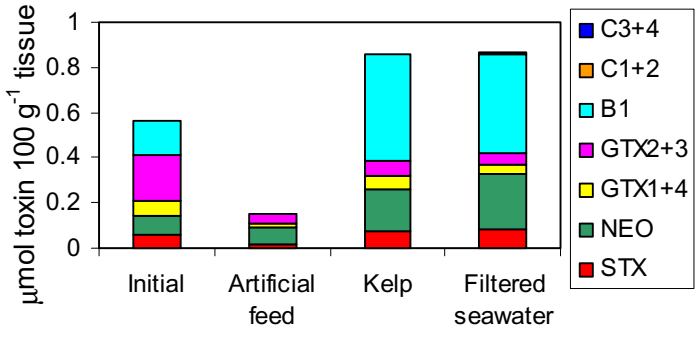

Figure 3. . Toxin composition expressed as µmol toxin 100 g^{-1} tissue for abalone at initial conditions and exposed to artificial feed, kelp, and filtered seawater treatments.

The only treatment where depuration was observed was for abalone fed non-toxic artificial feed. That the starved animals did not depurate suggests that toxin may be retained in the digestive tract or that depuration is dependent upon healthy metabolism. Based on our results, incubating abalone with artificial feed before making them commercially available may allow sufficient depuration. Although it is notable that abalone farmers have reported toxins in abalone raised on artificial feed. Thus, the use of artificial feed as a management strategy requires further investigation. Other strategies for making abalone safe for consumption include scrubbing or removing the epithelium (high in toxins) from the foot muscle to remove enough of the toxins to lower the concentration to below the legal limit for harvesting (Pitcher et al. 2001). Future investigations of the fate of toxins in the abalone will allow for improved fisheries management and shellfishing practices that will lead to safer seafood products.

Unlike previous studies in which only STX and dc-STX were detected, we observed a suite of toxins present in the abalone, including GTX and B derivatives. Biotransformations may be the cause of these discrepancies in toxin profiles. During the 2 weeks biotransformations were observed with the most obvious changes associated with the derivatives GTX2+3 and B1. In the presence of kelp only and during the non-feeding control (filtered seawater), GTX2+3 decreased, while B1 was synthesized. Regardless of the biochemical pathways for these conversions, the production of B1 is notable because this derivative is very similar to STX and dc-STX, the three differing in only one functional group. In addition to possible transformation between these three derivatives, it has been demonstrated in scallop homogenates that GTX1, 2, and 3 and NEO may transform into STX (Shimizu and Yoshioka 1981); therefore, over time the derivatives present in the abalone during our study could transform into STX, creating the profile observed by Pitcher et al. (2001) and thereby increasing the overall toxicity.

It is noteworthy that other possibilities may have contributed to the observed differences in toxin composition between this and previous studies. Different toxin compositions may arise from different extraction procedures that may influence the resulting toxin derivatives. Procedures involving sample extraction using the heated, hydrochloric acid method (AOAC 1990), are known to convert the N-sulfocarbamoyl toxins into carbamate derivatives increasing the overall toxicity (Hall 1982). While this procedure is designed to err on the side of caution for monitoring purposes, it does not necessarily represent the actual toxicity and toxin composition originally present. The samples in this study were extracted with a mild acetic acid procedure that maintains the integrity of the original toxin composition.

The detection of PSP toxins in abalone provides a new and additional risk to consumers. Abalone toxicity will add further to the existing damage on the economy caused by HABs (Anderson et al. 2000). It is necessary to understand the possibility of toxin depuration and transformation in these organisms in order to protect public health and ensure the safety of commercial seafood products. Results to date support the need for including non-traditional vectors such as these gastropods in routine monitoring programs for PSP.

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