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

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ABSTRACT

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

1. Introduction

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

The saxitoxin (STX) group of toxins, commonly referred to as paralytic shellfish toxins (PST), incorporate a suite of water soluble analogues that includes the following sub-groups: carbamates, gonyautoxins, sulfocarbamoyls, decarbamoyls and hydroxylated saxitoxins (Anonoymous., 2009; Shumway, 1990). In the marine environment, PST are mainly produced by the dinoflagellates *Gymnodinium catenatum* and *Pyrodinium bahamense* and members of the genus *Alexandrium* (Anonoymous., 2009). Symptoms of illness in humans from these toxins include tingling of the lips, gums and tongue and in more serious cases, numbness in the fingers and toes that spreads up the arms, legs and neck within four to 6 h (Toyofuku, 2006). In severe cases, mortality due to respiratory paralysis can occur within two to 12 h of consumption if there is no medical intervention (Toyofuku, 2006). Due to the serious public health risk posed by these toxins, they are regulated internationally. The Codex Alimentarius Commission has set a maximum level

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of 0.8 mg STX.2HCl eq/kg for bivalve shellfish (CODEX STAN 292-2008). Although there is no regulatory level set for PST in crustaceans, the Tasmanian regulatory authorities use the bivalve regulatory level to protect public health and market access.

In October 2012, PST were detected in mussels (*Mytilus galloprovincialis*) harvested from Spring Bay on the east coast of Tasmania, Australia at levels above the Australian regulatory limit of 0.8 mg STX eq/kg (Campbell et al., 2013). Subsequent investigation identified a bloom of the toxic alga *Alexandrium tamarense* complex group 1 as the most likely source of PST. Testing also identified violative levels of PST in other bivalve shellfish (scallops, clams and oysters). Levels of toxins of up to 2.8 mg STX eq/kg were also identified in the hepatopancreas (also commonly called 'liver', 'tomalley' or 'lobster mustard') of Southern Rock Lobster, *Jasus edwardsii* (Campbell et al., 2013; McLeod et al., 2012). The identification of toxins in the hepatopancreas of lobsters resulted in a commercial closure for this species being implemented in November 2012 along the majority of the east coast of Tasmania. Although there have been several reports of PST in lobsters internationally (Desbiens and Cembella, 1997; Grindley and Sapeika, 1969; Lawrence et al., 1994; Sang and Ming, 1984), there has only been one previous report of PST in Australian lobsters, with a maximum level of 1.8 mg STX eq/kg measured in gut tissues (Arnott, 1998). Internationally, there have been several reports of PST-related illness associated with lobsters (Garth and Alcala, 1977; Halstead, 1965; Hashimoto et al., 1967; Todd et al., 1993). However, although the reported symptoms in these cases were typical of PST-related illness, none of these reports provide direct association as the implicated food samples were not tested. The identification of PST in Southern Rock Lobster highlighted a number of uncertainties and data gaps for the Australian industry, including the mechanism by which lobsters accumulate toxins, the amount of time it would take for PST to depurate to compliant levels, and whether PST in lobster hepatopancreas pose a risk to human health.

Studies conducted in various regions (principally in North America) have shown that trophic transfer of PST is an important exposure route, and is likely to represent the principal uptake mechanism for PST in crustaceans (Haya et al., 1994; Oikawa et al., 2005). However, this had not previously been demonstrated experimentally in Australian lobsters. The aim of this study was to investigate whether PST-contaminated mussels are a potential vector leading to PST accumulation in *J. edwardsii,* and to better understand PST uptake and depuration rates under controlled experimental settings. These data may be useful in future consideration of risk mitigation strategies for *J. edwardsii* during PST-producing algal blooms.

2. Methods

2.1. Animal ethics

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

2.2. Experimental system

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

2.3. Lobsters, treatments and sampling regime

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

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

For all other experimental lobsters, an uptake phase of 28 days was used where lobsters were fed two PST-contaminated mussels (in the shell) daily (*M. galloprovincialis*). These mussels were sourced in 2012 from the east coast of Tasmania during a bloom of *A. tamarense*. Mussels were stored at approximately −20 °C in vacuum packaged bags. PST levels in the mussels were determined prior to the experiment by analysing three replicate samples of pooled mussels ($n = 16$ in each pool). Ten mussels were also analysed to establish mussel-to-mussel variability of PST. The shell length, total weight (meat and shell) and meat weight of 162 toxic mussels were recorded for use in later modelling to estimate meat weight consumed by the lobsters. Each day, a record was made of the length and weight of the mussels fed to the lobsters, and a record made of whether the mussels supplied the previous day had been consumed (as either a yes or no result).

As consumption of toxic mussels was variable between lobsters and between days during the uptake phase, a selective sampling strategy was used to ensure the maximum PST level was achieved in test animals at the end of the uptake phase. After 16 days of uptake, four animals that had consumed the least mussels were selected for testing, to increase the likelihood that there would be detectable PST levels in remaining animals at the end of the uptake phase. After 28 days of uptake, four animals that had fed consistently, consuming 50-52 mussels each, were selected for testing, to