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Human risk associated with palytoxin exposure

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ABSTRACT

Palytoxin (PTX) was first isolated from the zoanthid *Palythoa toxica*. Evaluation of PTX toxicity using various animal models determined that PTX was extremely potent through intravenous, intraperitoneal, and intratracheal exposure. PTX was less potent by direct intragastric exposure. PTX also caused significant, non-lethal effects through dermal and ocular exposure. PTX and PTX-like compounds have now been found in additional zoanthid species, red alga, a sea anemone, and several dinoflagellates. PTXs are found throughout certain reef associated food webs, including in fish and crabs responsible for human illness and death. Many of the organisms found to contain PTXs in the environment are also sold in the home aquarium trade, and recent evidence suggests poisonings have occurred through exposure to these organisms. Due to co-occurrence with other seafood toxins, such as ciguatoxins, saxitoxins, and tetrodotoxin, it has been difficult to assess the true risk of PTX poisoning through seafood consumption in humans, but limited cases have been well documented, some involving human fatalities. Recent evidence also suggests that humans are negatively impacted through PTX exposure by inhalation and dermal routes. Continued research into the distribution and occurrence of PTX and PTX-like compounds both in seafood and marine organisms sold in the aquarium trade appears warranted.

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1. Introduction

Palytoxins (PTXs) are a group of complex, extremely potent, marine natural products first described from tropical Cnidarian zoanthids (a type of colonial anemone). PTXs have been found throughout the food web including in fish and crabs responsible for human illnesses. PTXs target membrane sodium–potassium pumps responsible for maintaining ionic gradients critical to cellular function (Na^+/K^+-ATPase), essentially converting these ion-specific pumps into non-selective cationic pores (Artigas and Gadsby, 2003; Hilgemann, 2003). Disruption of these pumps results in a myriad of organism level effects, some life threatening. Characteristic aspects of PTX activity include delayed hemolysis with a large pre-lytic loss of potassium (Habermann et al., 1981). The cardiac glycoside ouabain specifically inhibits the action of PTX on certain, but not all, Na^+/K^+-ATPases (Habermann et al., 1989). For example, rat and mouse erythrocytes are the most sensitive to PTX induced hemolysis but are the least sensitive to the inhibitory effects of ouabain (Habermann et al., 1989; unpublished data).

Several recent reviews have described in detail the occurrence (Mundy, 2008), chemistry (Katikou, 2008; Kita and Uemura, 2008), and pharmacology (Vale, 2008) of PTXs. Provided here is a brief review of the toxins involved in the syndrome palytoxicosis, including their biological origin and symptoms reported in exposed animals and humans. The primary focus of this article are the potential routes and risks of human exposure to PTXs including two new accounts of poisonings following exposure to marine aquarium zoanthids; one involving dermal exposure and the other detailing an unusual case of inhalational toxin exposure.

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2. Toxins

In Moore and Scheuer's (1971) initial description of the marine toxin palytoxin, they recount the ancient Hawaiian legend of the "Limu Make o Hana" (deadly seaweed of Hana) in which a creature with a shark's mouth on its back is burned and its ashes cast into a tidal pool after it terrorizes a local village in Muolea, in the district of Hana on the island of Maui. As the legend goes, after this episode the Limu in the pool became toxic and the pool itself became kapu (taboo) to the Hawaiians who believed that an ill fate would befall anyone who attempted to gather the toxic limu. As part of ongoing research to determine the biological origins of ciguatoxin, researchers at the Hawaii Institute of Marine Biology set out to determine the location of this fabled pool where warriors were once said to smear the limu on spear points to make their wounds fatal. On December 31, 1961 the pool was finally visited and found to contain no limu (seaweed) but a previously undescribed species of coelenterate zoanthid eventually named *Palythoa toxica* (Walsh and Bowers, 1971). Coincidentally, a fire destroyed the main building of the Hawaii Marine Laboratory in Oahu that very afternoon. After two subsequent collection trips to the pool (one each in 1963 and 1964), purified ethanolic extracts of approximately 0.75 kg of *P. toxica* yielded a non-protein compound of ca. 3300 molecular weight with an i.v. LD_{50} of 0.15 μg/kg in mice making it one of the most toxic substances known to this day.

Nearly a decade later, the structure of palytoxin (PTX) was reported independently by two groups (Moore and Bartolini, 1981; Uemura et al., 1981). Moore and Bartolini (1981) described a PTX of molecular formula $C_{129}H_{221}N_3O_{54}$ (M_r 2659)[1] from a Tahitian *Palythoa* sp. and 2 isomeric hemiketals of the molecular formula $C_{129}H_{223}N_3O_{54}$ (M_r 2677) from Hawaiian *P. toxica*. Uemura et al. (1981) described a PTX of molecular weight (2680 Da)[2] from Japanese *Palythoa tuberculosa* with the same chemical formula and structure as one of the isomeric hemiketals from Moore and Bartolini (1981). PTX from *P. toxica* (chemical formula $C_{129}H_{223}N_3O_{54}$) possesses 115 carbons in a continuous chain, making it the longest continuous chain of carbons known in a natural product. Subsequently, numerous PTX-like substances have been described from various marine organisms (for detailed review, see Mundy, 2008). Homo-PTX, bishomo-PTX, neo-PTX, and deoxy-PTX were isolated and characterized from *P. tuberculosa* (Uemura et al., 1985). A compound termed Caribbean palytoxin (C-PTX) (structure not described) was isolated from Puerto Rican *Palythoa caribaeorum* (Beress et al., 1983). A compound with the same molecular weight and HPLC retention time as PTX from *P. toxica* was described from Japanese *Palythoa* aff *margaritae* (Oku et al., 2004). In addition to large (>5000 Da) polypeptide toxins, a PTX-like toxin indistinguishable by U.V., I.R., and NMR spectroscopy from PTX from *P. toxica* was described from the sea anemone *Radianthus macrodactylus* from the Seychelle Islands (Mahnir et al., 1992). Two PTX analogs were isolated from the red alga, *Chondria armata*, which was also reported to produce domoic acid (Maeda et al., 1985, as reported in Yasumoto and Murata, 1990). A PTX analog named ostreosin-D (42-hydroxy-3,26-didemethyl-19,44-dideoxypalytoxin) from the dinoflagellate *Ostreopsis siamensis* has been described to have a molecular weight of 2636 Da and a chemical formula of $C_{127}H_{220}N_3O_{53}$ (Usami et al., 1995; Ukena et al., 2001). Additional putative palytoxin analogs were later identified from *Ostreopsis* spp.; mascarenotoxins A and B from Indian Ocean *O. mascarenensis* (Lenoir et al., 2004), molecular weights between 2500 and 2535 Da, and ovatoxin-a, from Mediterranean *Ostreopsis ovata* (Ciminiello et al., 2008), molecular weight 2647 Da and molecular formula of $C_{129}H_{223}N_3O_{52}$ (both inferred from mass spectral data only). Lastly, a compound with biological activity consistent with PTX was described from an *Ostreopsis* sp. from Japan (Taniyama et al., 2003) while two compounds, ostreotoxin-1 and 3, have been described from Caribbean *Ostreopsis lenticularis* (Mercado et al., 1994). Unlike PTX, ostreotoxin 3 was later shown to be active against voltage dependent sodium channels (Menuier et al., 1997). Structural similarity to PTX has not been determined for the ostreotoxins.

3. Toxicity

In 1964, long before the structure of palytoxin was elucidated, Dr. Friedrich Hoffman brought a sample of Moore and Scheuer's semi-purified material from Hawaiian *Palythoa vestitus* (although this appears to be the same material described to be from *Palythoa* sp. in Moore and Scheuer (1971) and *P. toxica* in Moore and Bartolini (1981)) to the US Army Toxicology Division located at the Edgewood Arsenal of the Aberdeen Proving Grounds, Maryland, USA for further toxicological evaluation (Wiles et al., 1974). During these studies, a combination of monkeys, dogs, rabbits, guinea pigs, rats, and mice was evaluated by various routes of exposure including intramuscular, subcutaneous, intraperitoneal, intratracheal, intragastric, intrarectal, percutaneous, and ocular. Although this work was done with semi-purified material, as evidenced by the fact that the authors admit that in earlier work different batches of toxic extracts gave different animal lethality scores, this work remains the most thorough evaluation to date of the potential risks of palytoxin exposure in mammals. The average 24-h LD_{50} results for the various routes of exposure for the animal species tested in these studies are summarized in Table 1. A summary of toxic effects in these animal species are provided below.

[1] The chemical formula of $C_{129}H_{221}N_3O_{54}$ (M_r 2659) given for palytoxin from Tahitian *Palythoa* sp. is most likely an error and should be $C_{129}H_{221}N_3O_{53}$ which would be consistent with both the given relative molecular weight and the figure of this compound on page 2494 of Moore and Bartolini (1981).

[2] Both Moore and Bartolini (1981) and Uemura et al. (1981) report a palytoxin of the chemical formula $C_{129}H_{223}N_3O_{54}$ from Hawaiian *P. toxica* and Japanese *P. tuberculosa*, respectively, but Moore and Bartolini (1981) report a relative molecular weight (M_r) of 2677 while Uemura et al. (1981) report a molecular mass (MW) of 2680.168 Da. The difference in the units used explains this apparent inconsistency.

Table 1
Summary of toxicity, exposure routes, and exposure concentrations for palytoxin in various animal models from Wiles et al. (1974).

Species (weight kg)	Rat (0.2–0.25)	Mouse (0.025–0.035)	Monkey (2.0–4.0)	Dog (6.0–11.0)	Rabbit (2.0–3.3)	Guinea pig (0.40–0.60)
Route of exposure	*24-h LD_{50} (μg/kg)*					
Intravenous	0.089 ($n=50$)	0.45 ($n=96$)	0.078 ($n=24$)	0.033 ($n=32$)	0.025 ($n=46$)	0.11 ($n=38$)
Intramuscular	0.24 ($n=30$)			0.080 ($n=17$)		
Intraperitoneal	0.63 ($n=34$)					
Intratracheal	0.36 ($n=21$)					
Subcutaneous	0.40 ($n=30$)	1.39 ($n=34$)				
Intrarectal	>10.0 ($n=12$)					
Intragastric	>40.0 ($n=42$)					
Histology (exposure concentration μg)						
Intradermal		0.11–0.55 ($n=2$)			0.11–0.55 ($n=2$)	0.11–0.55 ($n=2$)
Percutaneous					0.25–0.5[a] ($n=1$)	
Ocular					0.3–1 ($n=6$)	

[a] Applied to 2 different areas on the same animal.

3.1. Acute toxicity

3.1.1. Intravenous (i.v.)

For rats, guinea pigs, and mice, exposed animals became drowsy and inactive initially with prostration, dyspnea, and convulsions occurring 30–60 min prior to death. Early signs in monkeys were ataxia, drowsiness, and limb weakness, followed by collapse and death. Vomiting occurred in some animals. In dogs, early signs of toxicity included defecation and vomiting followed by ataxia, weakness, collapse, and death. In some dogs where death was delayed, a shock-like state was observed which included decreased body temperature, extensive hemorrhaging of the digestive tract, with some animals experiencing bloody vomitus and diarrhea. These effects were only seen in dogs. For all species, histological damage was observed in various organs including liver, lungs, kidneys, brain, and gastrointestinal tract.

3.1.2. Intramuscular (i.m.)

LD_{50}s in 17 dogs and 30 rats were approximately 2.5-fold higher compared to i.v. exposure. Onset of symptoms was delayed compared to i.v. exposure and local irritation and swelling occurred at the site of injection.

3.1.3. Subcutaneous (s.c.)

LD_{50}s in 32 mice and 30 rats were 4.5- and 30-fold higher, respectively, compared to i.v. exposure. Onset of symptoms was delayed compared both to i.v. and i.m. exposure.

3.1.4. Intraperitoneal (i.p.)

Toxic signs and speed of symptom onset were similar to those for i.m. and s.c.

3.1.5. Intratracheal (i.t.)

Toxic symptoms were similar to those for i.p. administration except with more pronounced respiratory difficulty and wheezing.

3.1.6. Intragastric (i.g.)

Potency reduced by >200-fold compared to i.v. little to no toxic symptoms.

3.1.7. Intrarectal (i.r.)

No deaths or toxic signs observed.

3.2. Morphologic and histologic effects

3.2.1. Intradermal

Blanching of the skin at the site of injection with swelling, edema, and erythema surrounding blanched area. Focal necrosis at site of injection with surrounding inflammation. Kidney necrosis and damage to pulmonary vessels. No deaths or other signs of toxicity.

3.2.2. Percutaneous

Blanched and slightly raised area at site of application. Some swelling and necrosis of blanched and surrounding area.

3.2.3. Ocular

Within 4 h, slight to moderate tearing, irritation, swelling, edema, and conjunctivitis. By 24 h, affected eyes were completely closed with an exudate of pus and blood, as well as severe conjunctivitis, edema, corneal ulceration and opacity. Ocular lesions at a dose of 0.40 μg/kg were irreversible. When healed, eyes had scarred cornea and adhesions between iris and anterior synechia. Rinsing with isotonic saline post-exposure lessened effects and increased rate of healing but did not inhibit toxic effects entirely.

4. Reports of human exposure

In the 35 years that have passed since the initial reports on the toxicity of palytoxin, many of the observations made by Wiles et al. (1974) have now been documented through incidental exposure to humans. Some of these human exposures occurred through the consumption of PTX contaminated seafood but others were through dermal, ocular, and even inhalation exposure from either marine aerosols or substances associated with aquarium kept zoanthids. Unfortunately, only some of this data is well documented in the scientific literature. Much if it exists in the form of anecdotal reports of exposure to non-confirmed PTX-like substances. Still others are found in the form of

internet postings shared by aquarium zoanthid keeping hobbyists.

4.1. Seafood consumption

4.1.1. Fish

Palytoxin presence in edible fish was first suggested by Hashimoto et al. (1969) (as reported in Yasumoto and Murata, 1990) from gut extracts of the filefish *Altera scripta* shown to contain fragments of a *Palythoa* sp. Toxins indistinguishable from PTX by mouse bioassay and HPLC were later confirmed in viscera (31 MU/g) and flesh (0.27 MU/g) of the trigger fish *Melichtys vidua* (Fukui et al., 1987), a species historically associated with a severe form of Ciguatera Fish Poisoning in Japan, but unlike the previous example, this species was reported to feed on filamentous algae and not known to feed on *Palythoa* spp. Taniyama et al. (2003) found PTX-like activity, based on delayed hemolytic activity inhibitable by an anti-PTX antibody and ouabain, in both parrotfish (*Scarus ovifrons*) and the dinoflagellate *Ostreopsis* sp., found both in the environment and in gut contents during a toxic outbreak in Tokushima Prefecture, Japan in 1997. PTX-like activity was also found in freshwater puffer fish (*Tetraodon* sp.) in Bangladesh (Taniyama et al., 2001). Puffers in Bangladesh have been associated with numerous poisonings, some fatal, and have also been shown to contain high concentrations of paralytic shellfish poisoning (PSP) toxins (Zaman et al., 1997), but being freshwater, the origin of the toxins, both PSP and PTX-like, are currently unknown. A species of marine puffer fish (*Sphoeroides spengleri*) from the Caribbean Sea was observed to feed on *Palythoa* sp. and was found to contain high concentrations of PTX in addition to the traditional puffer toxin tetrodotoxin (TTX) (Mebs, 1998).

PTX has now been suggested to be the cause of another, poorly understood, toxic syndrome associated with the consumption of fish, clupeotoxism. In 1994, a woman in Madagascar died 15 h after eating one of four locally caught sardines (*Herklotsichthys quadrimaculatus*) (Onuma et al., 1999). Due to an unusual bitter taste, the woman only consumed a small portion of the fish. A cat that consumed the remainder of the fish died within 15 min. A child who consumed another of the 4 fish showed no symptoms. Prior to death, the woman's symptoms included malaise, followed by uncontrollable vomiting and diarrhea, followed by tingling of the extremities, and finally delirium. The 2 fish heads were obtained and analyzed (after remaining frozen for nearly 2 years), and were shown to contain a PTX-like compound based on mouse assay, delayed hemolysis inhibitable by an anti-PTX antibody, and chromatographic and mass spectral similarity to a PTX standard. Based on this data, the authors concluded that PTX was the probable cause of clupeotoxism, a rare but often fatal syndrome associated with the consumption of certain tropical clupeid fishes first described in 1770 from present day Dominican Republic (Halstead, 1967). It is interesting to note that in a review of clupeotoxism, it was reported that in 1877, New Caledonian sardines would become seasonally toxic after consuming a "green monad" which discolored the sea and also caused conjunctivitis, coryza (inflammation of the mucus membranes), and erythema (redness of the skin) in persons coming in contact with them (Randall, 2005). Alcala (1983) reported on several separate poisoning events due to the consumption of *Sardinella* sp. on southern Negros Island, Philippines. These poisoning events had a mortality rate of about 10%.

4.1.2. Crustaceans

In addition to fish, PTX has caused human poisonings and has been implicated in human fatalities due to the consumption of several species of xanthid crabs in the Philippines. Implicated species include *Lophozozymus pictor*, *Demania alcalai*, and *Demania reynaudii*, which have all been shown to contain PTX-like compounds using chromatographic, and/or mass spectral techniques (Alcala et al., 1988; Yasumoto et al., 1986). Yasumoto et al. (1986) reported toxicity in all tissues tested for *L. pictor* and *D. alcalai*, with highest levels in viscera and gills. Lau et al. (1995) isolated a structural isomer of PTX from *L. pictor* that was recognized by an anti-PTX antibody but, unlike PTX from *P. tuberculosa* and *P. caribaeorum*, this compound is fluorescent. A fatality rate of 80–100% was reported for several poisoning events on southern Negros Island, Philippines due to the consumption of *L. pictor* (Alcala, 1983; Gonzales and Alcala, 1977). The poisoning due to *D. reynaudii* reported by Alcala et al. (1988) also resulted in a fatality. Symptoms associated with these various poisoning events included a bitter/metallic taste, vomiting, diarrhea, muscle cramps, abdominal pain, numbness of the extremities, bradycardia, difficulty breathing, and renal failure (Alcala et al., 1988; Gonzales and Alcala, 1977).

4.2. Inhalation exposure

In the summer of 2005 approximately 200 people experienced symptoms of rhinorrhea, cough, mild dyspnea, bronchoconstriction, and fever that coincided with an unusual bloom of *O. ovata* along the rocky Mediterranean coast of Liguria, near Genoa, Italy (Ciminiello et al., 2006). Some people also experienced conjunctivitis and 20 people required extended hospitalization. *O. ovata* was the dominant species in a surface water sample, a concentrated plankton sample of the water column, and in washed macroalgal (epiphytic) samples. Symptoms peaked in association with bloom climax, and ended in association with bloom dissipation. A PTX-like compound with similar potency in mouse assay, HPLC retention time, and mass spectral fragmentation pattern to standard PTX was found in all samples (Ciminiello et al., 2006). Okadaic acid, spirolides, azaspiracids, yessotoxins, saxitoxins, and domoic acid were not detected. Further analysis of cultured *O. ovata*, isolated during a similar event in 2006, was shown to contain a putative palytoxin and a new compound ovatoxin-a (Ciminiello et al., 2008). Using various mass spectral techniques, ovatoxin-a (the dominant toxin present during the 2006 bloom) was estimated to have a molecular weight of 2648.5 Da and a molecular formula of $C_{129}H_{225}N_3NaO_{52}$; structurally similar to PTX but containing 2 fewer oxygen atoms (Ciminiello et al., 2008).

4.3. Dermal exposure

While numerous anecdotal stories describing numbness of the hands and arms from handling aquarium zoanthids can be found in on-line marine aquarium keeping forums, Hoffmann et al. (2008) provided the first data to substantiate these accounts. A man in Germany collapsed 16 h after receiving minor cuts to 3 fingers while handling several zoanthid colonies in a home aquarium. Initial symptoms started 2 h after contact which included shivering, myalgias, and general weakness of the extremities, progressing to dizziness and speech disturbance at the time of collapse. At the time of admission to the hospital (20 h post-exposure) the patient's speech was impaired and swelling and erythmea were noted at the site of the finger cuts with the numbness and paresthesias of the fingers progressing to involve the whole arm over the next 20 h. Most clinical testing was within normal ranges with the exception of an abnormal electrocardiogram (ECG) demonstrating a right bundle branch block pattern with widened QRS complex in leads V1 and V2 and an inverted S wave in V6. Serum biochemistries demonstrated slightly elevated levels of creatine kinase (CK 198 U/l), lactate dehydrogenase (LDH, 304 U/l), and C-reactive protein (CRP, 13.8 mg/l). Treatment consisted of infusion of intravenous fluids. Cardiac function returned to normal over the next 24 h, but paraesthias, weakness, and muscle pain persisted until the patient was discharged 48 h. later. Colonies of *Palythoa* sp. and *Parazoanthus* sp. were tested for PTX-like activity using a hemolysis assay with inhibition using ouabain. *Palythoa* sp. showed no activity while the *Parazoanthus* sp. colony possessed 7700 hemolytic units/g, which was estimated to be equivalent to 2–3 mg PTX eq./g wet weight.

5. Clinical symptoms

As originally observed by Wiles et al. (1974), symptoms associated with palytoxin exposure vary greatly depending upon the route of exposure. Mortalities have only occurred due to PTX injection (in test animals) or ingestion (in humans), but a variety of additional non-lethal symptoms have been observed due to ingestion, dermal, ocular, and inhalational exposure in humans. Some of these symptoms have been detailed in Section 4 above. Due to the rarity of diagnosed PTX poisonings and the fact that most poisonings occur in tropical communities without access to specialized health care, detailed clinical symptomology is lacking. Updated animal studies with well characterized materials are necessary for both PTX and many of the recently described PTX-like compounds to aid health care providers and epidemiologists in the clinical diagnoses of this toxic syndrome.

5.1. Rhabdomyolysis

Based on the limited clinical data collected from PTX poisoning cases thus far, one of the most commonly reported complications of PTX poisoning appears to be rhabdomyolysis (Kodama et al., 1989; Okano et al., 1998; Taniyama et al., 2002). Rhabdomyolysis is a syndrome caused by injury to skeletal muscle, muscle breakdown, and leakage of large quantities of intracellular (myocyte) contents into blood plasma (for review, see Muscal et al., 2007). According to Muscal et al. (2007), life-threatening renal failure and disseminated intravascular coagulation (DIC) are the complications of severe rhabdomyolysis. The root cause of this syndrome appears to be disruption of the sarcolemma with concomitant release of intracellular myocyte components such as calcium. Increases in intracellular calcium levels results in hyperactivity of proteases and proteolytic enzymes and causes the generation of free oxygen radicals. These enzymes and free radicals degrade myofilaments and membrane phospholipids causing leakage of additional intracellular contents into plasma such as potassium, phosphate, creatine kinase, uric acid, and myoglobin. Excessive myoglobin can precipitate in glomerular filtrate, causing renal tubular obstructions, direct nephrotoxicity, and ultimately renal failure. Common symptoms of rhabdomyolysis include myalgias and generalized weakness; additional nonspecific symptoms include fever, nausea, and vomiting. Affected persons may also experience muscle pain and tenderness, with the most commonly involved muscle groups including the calves and lower back. Common clinical chemistry findings including elevated serum creatine kinase (CK), hyperkalemia, and myoglobinuria (indicated in severe cases by darkened/black urine). In Taniyama et al.'s (2003) study on the origin of PTX in parrotfish (*S. ovifrons*), sublethal injections of crude toxin extracts from *S. ovifrons*, the dinoflagellate *Ostreopsis* sp., and a PTX standard induced rhabdomyolysis, as evidenced by elevated serum CK levels, in mice. In humans, CK levels typically rise within 12 h of muscle injury, peak in 24–36 h, and decrease at a rate of 40% per day. The serum half-life of CK is approximately 36 h. CK levels decline 3–5 days after resolution of muscle injury. Peak CK levels more than 5000 U/L are predictive of renal failure. Aggressive hydration is important for the prevention of this life-threatening condition.

5.2. Reports of illness

Several well documented cases of rhabdomyolysis have been reported due to the consumption of PTX contaminated fish.

5.2.1. Report 1

Okano et al. (1998) described a case of fish poisoning involving rhabdomyolysis and myocardial damage due to the consumption of blue humphead parrotfish (*S. ovifrons*) in Japan. The patient experienced weakness and myalgia of all four extremities five hours after consuming raw meat and liver. Others consuming only cooked meat developed no symptoms. Clinical chemistry examination revealed elevated serum CK, white blood cell (WBC) count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), C-reactive protein (CRP), and myoglobin in the urine. Gastric lavage with activated charcoal and forced mannitol-alkaline diuresis therapy were performed to prevent renal failure. Serum CK concentration peaked at 40,000 U/L on day 3 and recovered to normal by day 18. The weakness and myalgias of the extremities gradually resolved by 4 weeks post-exposure.

Toxin analysis was not reported. *S. ovifrons* feeds on a variety of benthic organisms including shellfish, crabs, prawns, and seaweed.

5.2.2. Report 2

Taniyama et al. (2002) reported eleven people out of thirty-three became ill after consuming the boiled muscle, viscera and/or liver of a single large grouper (*Epinephelus* sp.) caught near Kashiwa Island in southern Japan in 2000. Symptoms, originally diagnosed as ciguatera fish poisoning (CFP), included lower back and shoulder pain and discharge of black urine. Symptoms began 3–36 h post-ingestion. Serum CK levels in five patients (A–E) that required hospitalization ranged from (3300–22,500 U/L),(1100–12,400 U/L),(700–23,800 U/L),(2500–10,600 U/L), and (1100–2100 U/L), respectively. After approximately one month, serum CK and urine color returned to normal for all patients. After testing for ciguatoxins was negative, uncooked flesh samples were shown to contain a PTX-like compound based on mouse assay and hemolysis neutralization assay using ouabain. It is interesting to note that the species of grouper in this case was carnivorous, suggesting that, like ciguatoxins, PTX can reach levels that can potentially cause illness in higher trophic level fish species.

5.2.3. Report 3

Kodama et al. (1989) reported a near fatal case of fish poisoning in 1986 on the island of Kauai, Hawaii due to the consumption of smoked mackerel (*Decapterus macrosoma*) imported from the Philippines. The affected individual consumed two fish, including the viscera from one, and within several hours experienced weakness, sweating, abdominal cramps, nausea, diarrhea, circumoral paresthesia, paresthesias of the extremities, dysesthesia (temperature reversal), muscle spasms, and tremors. Forty eight hours post-consumption, the muscle spasms progressed to painful, uncontrollable tonic contractions of all muscle groups, which caused respiratory distress and required endotracheal intubation. Serum CK levels were 41,000 U/L (normal range: 45–235), lactate dehydrogenase (LDH) was 673 U/L (normal range: 99–215), and serum glutamic oxaloacetic transaminase (SGOT) was 774 U/L (normal range: 13–47). Patient's urine color was dark brown. The individual recovered and was discharged from the hospital 9 days later. Methanol extracts of both flesh and viscera were toxic to mice. It was reported that chromatographic and radioimmunoassay methods indicated the presence of PTX-like compounds, although no data were provided in the publication. Ten samples from the suspect Hawaiian lot, as well as 159 of 179 (90%) of additional samples from the distributor also tested positive for ciguatoxin (CTX) using a stick enzyme immunoassay.[3] Based on the clinical and laboratory findings, it was likely that PTX, and possibly additional polyether toxins such as CTX, were responsible for this severe illness. *D. macrosoma* is a pelagic schooling species that feeds mainly on zooplankton and small invertebrates.

6. Prevalence in seafood

Quantitative studies on the prevalence of PTX in potential seafood species in different tropical areas are lacking, but some qualitative examples do exist. Using hemolysis neutralization assays and HPLC, PTX-like compounds were shown to be extensively distributed in tropical reef communities in the Caribbean Sea (Columbia, South America) and the Pacific Ocean (Lizard Island, Australia) (Gleibs and Mebs, 1999; Gleibs et al., 1995; Mebs, 1998). In these studies, PTX-like compounds and activities were found throughout the food chain including zoanthids (*Palythoa* sp. and *Zoanthus* sp.) various sponges and corals, echinoderms, shellfish, polychaete worms, crustaceans, and fish.

6.1. Fish

In response to reports that up to 15% of Ciguatera cases reported to the Hawaiian Department of Health implicated PTX or a mixture of polyether toxins in addition to ciguatoxins as responsible for Ciguatera Fish Poisoning (CFP), Wachi et al. (2000) used a hemolysis neutralization assay with both ouabain and an anti-PTX antibody to screen several herbivorous and carnivorous reef species for the presence of PTX-like hemolytic activity. Their findings were that a moderate percentage of gut extracts from herbivorous reef species and flesh extracts from carnivorous species did exhibit PTX-like activities. As a follow-up study, using a combination of mouse bioassay and hemolysis neutralization assays, Wachi and Hokama (2001) screened 45 separately pooled flesh and gut samples, representing 10 herbivorous and 12 carnivorous reef species collected from Barber's Point Harbor, Oahu, Hawaii for the presence of PTX-like activity. For pooled gut extracts, 74% of the samples showed significant hemolytic activity (>50% lysis from a 100 μg/ml extract), while 39% of the samples caused death in mice. For flesh samples, 35% of the samples caused death in mice, while 26% caused significant hemolytic activity. Only a moderate correlation was found between samples causing death in mice and samples with PTX-like hemolytic activity. Results for flesh toxicity are summarized in Table 2. Conclusions from this study were that several toxins appear to be present in Hawaiian reef species, both carnivorous and herbivorous, including PTX-like compounds and (most likely) CTXs, but, while due to their lipophilic nature, CTXs are known to bioaccumulate in higher trophic level fish flesh (FAO, 2004), it was surprising to find a number of fish species with high PTX-like activity also in their flesh given the water soluble nature of PTX. Maitotoxins (MTXs), extremely potent marine toxins first described from the viscera of CFP associated herbivorous surgeonfish (*Ctenochaetus striatus* and *Acanthurus lineatus*) (Yasumoto et al., 1971, 1976) and produced by the same benthic dinoflagellates (*Gambierdiscus* spp.) that produce the CTXs have not been reported to occur in high concentrations in fish flesh due (presumably) to their hydrophilic

[3] The CTX stick enzyme immunoassay commercially available at that time was later shown to also cross-react with other polyether toxins such as maitotoxin and okadaic acid (Van Dolah and Ramsdell, 2001); therefore is not confirmatory for the presence of CTX.

Table 2
Total toxicity, as determined by mouse assay, and palytoxin-like activity, as determined by hemolysis neutralization assay, in pooled flesh extracts of Hawaiian reef fish. Adapted from Wachi and Hokama (2001).

Species	Common name	Mouse toxicity	Hemolytic activity	Blockable with anti-PTX
Herbivores				
Acanthurus olivaceus	Orangespot surgeonfish	1–3	+++	Y
Acanthurus nigroris	Bluelined surgeonfish	1–2	–	
Acanthurus sandvicensis	Convict surgeonfish	2–4	+++/+++	Y/Y
Zanclus cornutus	Moorish Idol	1–1	+++	Y
Thallasoma ballieui	Blacktail wrasse	0–1	–	
Forcipiger flavissumus	Longnose butterfly fish	0–1	–	
Abudeffuf abdominalis	Green damselfish	0–2	+	NT
Carnivores				
Lutjanus kasmira	Bluestripe snapper	1–1	–	
Cirrhitus pinnulatus	Stocky hawkfish	0–1	–	
Parupeneus multifasciatus	Manybar goatfish	0–1	–	
Myripristis berndti	Blotcheye soldierfish	1–1	+++	Y
Gymnothorax flavimarginatus	Yellow-edged moray	0–1	–	
Kuhlia sandvicensis	Hawaiian flagtail	1–3	+++	Y
Rinecanthus rectangulus	Wedge-tail triggerfish	0–1	–	

– No hemolysis.
+ <50% hemolysis at 100 μg tissue eq./ml.
++ >50% hemolysis at 100 μg tissue eq./ml.
+++ >50% hemolysis at 50 μg tissue eq./ml.

nature. Like PTXs, MTXs are also less potent orally compared to i.p.(Wiles et al., 1974; FAO, 2004). It should be noted that these studies detected the presence of PTX-like compounds using only hemolysis neutralization assays which are indicative, but not confirmatory, for the presence of PTX.

6.2. Shellfish

The risks of PTX exposure through shellfish consumption are still unknown. High PTX-like activity was found in Caribbean mussels (*Arca imbricate*, *Artina* sp., *Barbatia candida*, and *Chama macerophylla*) (Gleibs and Mebs, 1999). Further, high PTX-like activity (33–97 μg putative-PTX/kg) was detected in mussels (*Mytilus galloprovincialis* and *Modiolus barbatus*) and clams (*Venus verrucosa*) in the North Aegean Sea where toxic *Ostreopsis* spp. are now known to occur (Aligizaki et al., 2008). However, in short term (3 day) feeding trials at bloom densities of toxic *O. siamensis*, Rhodes et al. (2002) saw limited uptake and/or retention of PTX-like activity in New Zealand Greenshell™ mussels (*Perna canaliculus*), scallops (*Pecten novaezealandiae*) and oysters (*Crassostrea gigas*). No outbreaks of PTX poisoning have been confirmed from the consumption of shellfish.

7. Palytoxin exposure risks from aquarium trade zoanthids

Human exposure risks to palytoxin and palytoxin-like compounds go beyond the consumption of contaminated seafood. The roots of PTX research go back to tribal mythology of toxic "limu" and poison spears. Due to the potential for acute exposure to high concentrations of toxin, the proper handling of PTX-containing marine zoanthids sold in the home aquarium trade must be acknowledged. We report here for the first time two recent cases of putative-PTX exposure from incidental contact with marine aquarium zoanthids, including an unusual case of inhalation exposure.

7.1. Case Report 1

In October 2006, a marine aquarium hobbyist was admitted to a hospital in Atlanta, Georgia, USA with symptoms of chest pain, lightheadedness, and weakness and numbness of the left arm. The patient's left hand had contacted what was described by the patient as a red and pink zoanthid recently purchased from a local fish store. Upon admission, the patient's heart rate and blood pressure were elevated (116 bpm and 184/96 mmHg, respectively). The patient was given O_2 by nasal cannula and blood chemistry was examined. The patient showed elevated creatine kinase (CK) levels (patient 508; normal range 22–269) suggestive of mild rhabdomyolysis. Additional testing demonstrated normal serum electrolytes, complete blood count, urinalysis, and coagulation factors. The ECG demonstrated sinus tachycardia without ischemia. Left arm numbness and chest pain subsided over the next 4 h after admission. Eight hours after admission, the repeat CPK level was 425 with all other serum biomarkers remaining normal. After discussion with the patient – who was knowledgeable about zoanthid poisoning from his own on-line research – and consultation with a medical toxicologist at the Georgia Poison Center, palytoxin exposure was considered a cause of the patient's presenting toxidrome. After an additional 8 h, CPK levels were still elevated (415) but continuing to drop. Despite his resolving symptoms, the decision was made to admit and observe the patient in the telemetry unit overnight. The patient was discharged the following day.

A serum sample collected 20 min after admission (approximately 1 h after exposure) was found to contain a component which caused hemolysis in mouse red blood cells but the activity was not inhibited by incubation with

an anti-PTX antibody (Fig. 1A). Anti-PTX antibody (73D3) was a gift from Hawaii Biotech (Honolulu, USA) and assays were performed according to Bignami (1993) with measurement of released hemoglobin in supernatants at 540 nm as described in Deeds et al. (2002). A PTX standard (from *P. tuberculosa* purchased from Wako Pure Chemicals, Ltd., Japan) spiked into human control serum (purchased from Sigma Chemicals, St. Louis, USA) behaved as expected with activity inhibited by the addition of 25 μg/ml anti-PTX antibody (Fig. 1B). Both High Performance Liquid Chromatography (HPLC) and Liquid Chromatography Mass Spectrometry (LC/MS) analyses of the serum sample (method details provided in the following section) did not reveal any detectable compounds consistent with a *P. tuberculosa* palytoxin standard. Due to small sample size, sample clean up or concentration were not possible. Based on the fact that the hemolytic activity was not inhibited by an anti-palytoxin antibody, this suggests that if this activity was due to a PTX-like compound, it was a metabolite not recognized by the anti-PTX. Further studies are required to confirm this possibility. During a follow-up investigation, it was learned that the suspect zoanthid was disposed of by the patient and the aquarium dealer claimed to no longer carry the variety sold to the affected person; therefore palytoxin exposure could not be confirmed and can only be considered a presumptive cause in this case.

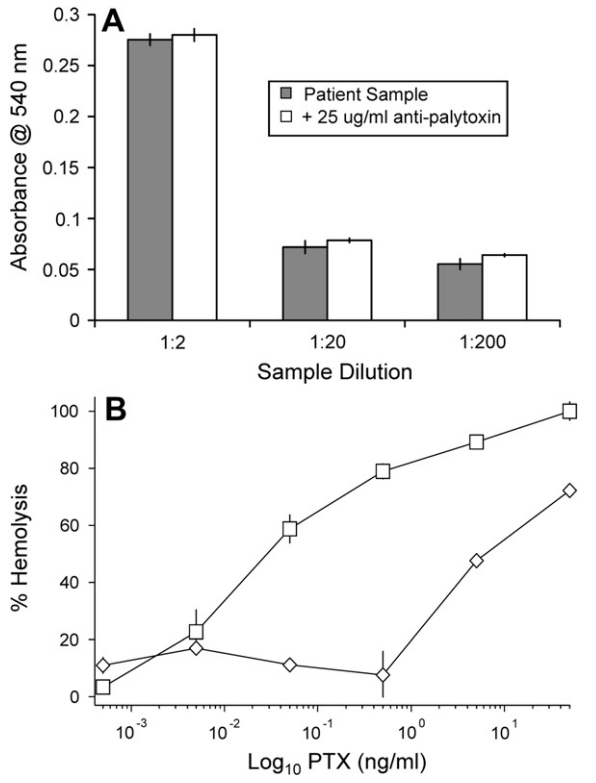

Fig. 1. Analysis of patient serum sample from 2006 suspected palytoxin dermal exposure from a marine aquarium zoanthid.(A) ×1, ×1/10, or ×1/100 serum samples mixed 1:1 with either mouse red blood cells or mouse red blood cells pre-incubated with 25 μg/ml anti-palytoxin antibody, incubated for 4 h, and released hemoglobin measured in supernatants at 540 nm. $n = 3$; lines represent ± 1 SD. (B) Hemolysis neutralization assay using standard palytoxin from *P. tuberculosa* spiked into human control serum. [□] Diluted palytoxin mixed 1:1 with mouse red blood cells. [◇] Diluted palytoxin mixed 1:1 with mouse red blood cells pre-incubated with 25 μg/ml anti-palytoxin antibody. $n = 3$; lines represent ± 1 SD (lines not visible are within the size of the marker).

7.2. Case Report 2

In February 2007, a marine aquarium hobbyist in Virginia, USA was attempting to remove a colony of medium sized green/brown zoanthids from a rock by pouring boiling water over the infested portion. The zoanthids had been growing in the aquarium for 3 years and had arrived as a contaminant with live rock. During this process, the patient inhaled steam and immediately stopped after noticing a foul odor. The zoanthid containing rock was retained in a separate aquarium. Within 20 min the patient experienced rhinorrhea and coughing at which time the patient took an antihistamine believing the symptoms to be caused by seasonal allergies. Within 4 h post-exposure, the patient experienced difficulty breathing and lightheadedness which progressed to severe fits of coughing and chest pain. At this point the patient was admitted to a local hospital. Upon admission, ECG was normal. The patient was administered an anti-inflammatory corticosteroid and pain medication. It is not known if CK levels were tested. After 15 h of observation the patient was released and prescribed an inhaled steroid treatment and cough suppressant. A follow-up examination by a pulmonary specialist 2 weeks post-exposure diagnosed the patient with asthma-like symptoms of bronchial inflammation and bronchoconstriction. The inhaled steroid treatment was continued until symptoms subsided, approximately 1 month post-exposure.

Upon inspection, the zoanthids from the saved rock appeared to be consistent with descriptions of *Palythoa*/*Protopalythoa* sp. Approximately 10 individual polyps (0.26 g wet weight total) were carefully removed and placed in 10 ml of 80% EtOH at 4 °C for 24 h.(polyps were weighted after EtOH extraction). Crude ethanolic extracts were analyzed by Hemolysis Neutralization Assay, High Performance Liquid Chromatography (HPLC) and Electrospray Ionization Mass Spectrometry (ESI-MS).

7.2.1. Hemolysis neutralization assay

The hemolysis assay used was similar to those described by Bignami (1993) and Deeds et al. (2002). Briefly, 100 μl of the 80% EtOH zoanthid extract, serially diluted into hemolysis assay buffer [phosphate buffered saline, plus 1 mM $CaCl_2$ and 0.5 mM H_3BO_4, pH 7.4] was mixed in triplicate with either 100 μl of a 5% washed mouse red blood cell (RBC) suspension, or the same RBC suspension that had been pre-incubated for 30 min with 25 μg/ml anti-PTX antibody. For this set of experiments, anti-PTX antibody was a gift from Dr. Mark Poli (USAMRIID, Frederick, MD, USA) and was produced in August 2007 from the same mouse hybridoma that was used to produce the 73D3 anti-PTX from Hawaii Biotech used for the previous case study investigation. Samples were mixed in 96 well V-bottom plates, sealed, and incubated for 4 h at 37 °C, after which time plates were centrifuged at 2500 rpm, and 100 μl of

supernatant was transferred to a new flat bottom 96 well plate. Absorbance of released hemoglobin was measured at 540 nm using a 96 well plate reader and % hemolysis was calculated through comparison to wells lysed with 10 μg saponin (from Quillaja bark; Sigma Chemical Co., St. Louis, MO). A standard curve (50, 5, 0.5, 0.05, 0.005, 0.0005 ng/ml) using PTX from *P. tuberculosa* purchased from Wako Pure Chemicals Industries, Ltd.(Japan) was used to calculate PTX eq. concentrations by using a sigmoidal regression of log transformed data using GraphPad Prism software (ver. 4.03, GraphPad Software, Inc., San Diego, CA). Results are shown in Fig. 2. Using this method, a concentration of 309.1 μg/g PTX eq. was calculated for the zoanthid sample recovered from the patient's home aquarium.

7.2.2. High performance liquid chromatography

HPLC analysis was performed using an Agilent 1200 series HPLC system (Agilent, Wilmington, DE) with UV detection following Ciminiello et al. (2006). Samples were diluted 1:10 using a solution of 80% acetonitrile in HPLC grade H_2O containing 30 mM acetic acid and 30 μl was injected onto a Gemini C18 column (5 μm, 110 Å, 150 mm × 2 mm). The sample was eluted using a gradient of 20% solvent A/80% solvent B to 100% solvent A over 10 min at 0.25 ml/min at 30 °C [Solvent A: 95% acetonitrile in HPLC grade water, Solvent B: HPLC grade water, 30 mM acetic acid added to both]. Beginning and ending gradient conditions were maintained for 5 min before and after the gradient run, respectively. PTX-like compounds were detected at 263 nm and quantified through linear regression of a PTX standard (2680 Da from *P. tuberculosa* purchased from Wako Pure Chemical Industries, Ltd., Japan) at concentrations of 10, 5, 2.5, 1.25, 0.625, 0.3125 μg/ml. The zoanthid extract from the Virginia inhalation exposure event contained a peak consistent with the PTX standard based on retention time and characteristic UV spectra (maxima at 233 and 263 nm) (Fig. 3). Linear regression analysis performed using GraphPad Prism software (ver. 4.03, GraphPad Software, Inc., San Diego, CA) resulted in a calculated concentration of 613 μg/g PTX eq. Samples of this zoanthid are currently being examined using histologic and molecular techniques to attempt a species level identification. These results will be reported separately.

7.2.3. Electrospray ionization mass spectrometry

Initial separations were performed using an Agilent 1100 HPLC system (Agilent, Wilmington, DE) connected via electrospray interface to an ABI 4000 Q Trap (Applied Biosystems, Beverly, MA) linear ion trap quadrupole mass spectrometer operated with version 1.4.2 Analyst software (Applied Biosystems, Beverly, MA). Toxins were separated using a Phenomenex 2 × 150 mm Luna 3 μm C8(2) LC column (Phenomenex, Torrance, CA). Column oven temperature was held at 40 °C. Injections were 5 μl. All solvents were of HPLC grade. A mobile phase consisting of 0.1% formic acid in water for solvent A and 0.1% formic acid in acetonitrile for solvent B was pumped at a flow rate of 0.25 ml/min. A 10 min linear gradient from 20% solvent B/80% solvent A to 100% B then held at 100% B for 5 more minutes was used for the chromatographic separation. MS

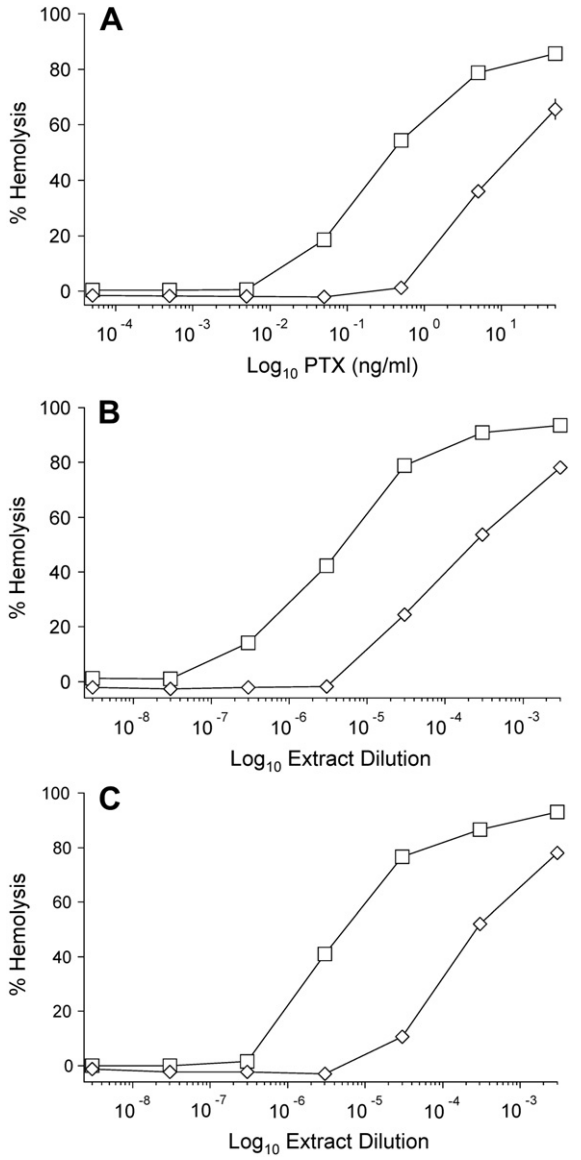

Fig. 2. Hemolysis neutralization assay for (A) palytoxin standard,(B) diluted 80% ethanol extract of zoanthid (*Palythoa* sp.) sample from Virginia inhalation exposure event (calculated PTX eq. concentration 309.1 μg/g),(C) diluted 80% ethanol extract from zoanthid (*Palythoa* sp.) purchased from local aquarium store (calculated PTX eq. concentration 90.8 μg/g). For all: [□] sample mixed 1:1 with mouse red blood cells, [◇] sample mixed 1:1 with mouse red blood cells pre-incubated with 25 μg/ml anti-palytoxin antibody. $n = 3$; lines represent ± 1 SD (lines not visible are within the size of the marker).

was operated in single quad (Q1 MS) positive ion mode scanned from 300 to 2800 Da in 3 s. Turbo spray source conditions were: all gases were nitrogen; ion spray voltage (IS) 5500 V; nebulizing gas (GS1) 50 L/h; desolvation gas (GS2) 50 L/h; turbospray heater temperature (TEM) 500 °C; curtain gas (CUR) 10 L/h; declustering potential (DP) 125 V; exit potential (EP) 10 V. Using this method, the full scan mass chromatograms for a PTX standard from *P. tuberculosa* and the hemolytic toxin from the contaminant zoanthid from the Virginia investigation were nearly identical; both

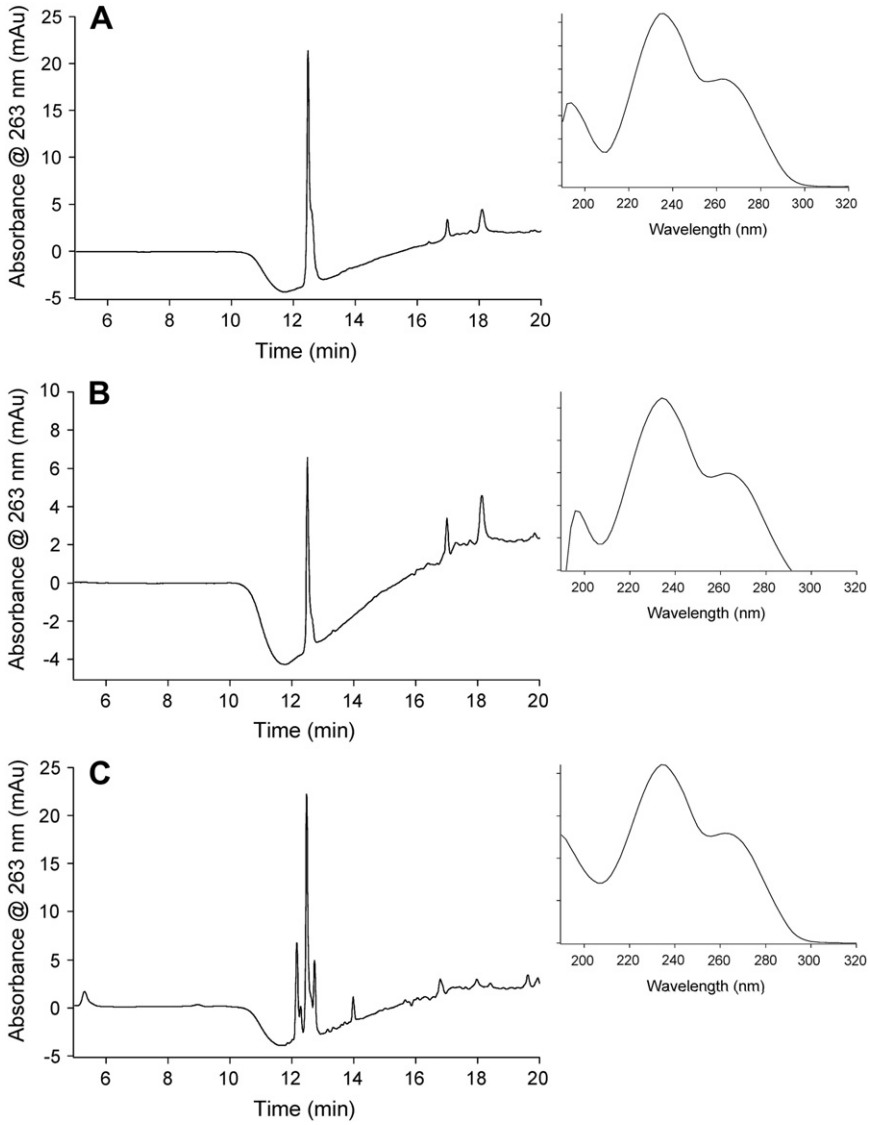

Fig. 3. HPLC analysis of 30 μl injections of (A) 2.5 μg/ml palytoxin standard,(B) 1:10 dilution of 80% ethanol extract of zoanthid (*Palythoa* sp.) sample from Virginia inhalation exposure event (calculated PTX eq. concentration 613 μg/g),(C) 1:10 dilution of 80% ethanol extract from zoanthid (*Palythoa* sp.) purchased from local aquarium store (calculated PTX eq. concentration 515 μg/g). For all: inset figure on right represents UV spectra of 12.4 min (largest) peak.

contained identical doubly charged and triply charged ion clusters (centered at 907 Da and 1315 Da respectively) as well as the characteristic fragment at 328 Da reported in Ciminiello et al. (2006) (Fig. 4). A few of the ion ratios were slightly different for the two compounds, but this data, combined with the HPLC and hemolysis neutralization data, strongly suggests that the compound from the contaminant zoanthid involved in this unusual inhalation exposure is a putative-PTX.

7.3. Prevalence in aquarium trade zoanthids

There is a great deal of conflicting information available to people attempting to assess the risks of PTX exposure from store bought aquarium zoanthids. Literature on the maintenance of aquarium zoanthids such as Borneman (2004) warn that all *Palythoa*/*Protopalythoa* spp. are toxic while other public resources such as CBWInfo.com, a website for information about chemical and biological weapons for emergency, safety, and security personal, states that "several species of *Palythoa* are used in aquariums, but do not produce the toxin". Numerous unconfirmed anecdotal stories can be found posted by affected individuals in online coral reef hobbyist forums such as Reef Central (www.reefcentral.com). In two such examples a pet dog died 12 h after believed ingestion of an aquarium zoanthid (http://reefcentral.com/forums/showthread.php?s=7c204fc9683238ee6546c579279b734b&threadid=158663&perpage=25&pagenumber=1 posted 3/2/2003, accessed 8/11/2008) and a hobbyist experienced severe eye irritation with apparent corneal damage after accidentally being squirted in the eye while lifting a zoanthid colony from a tank

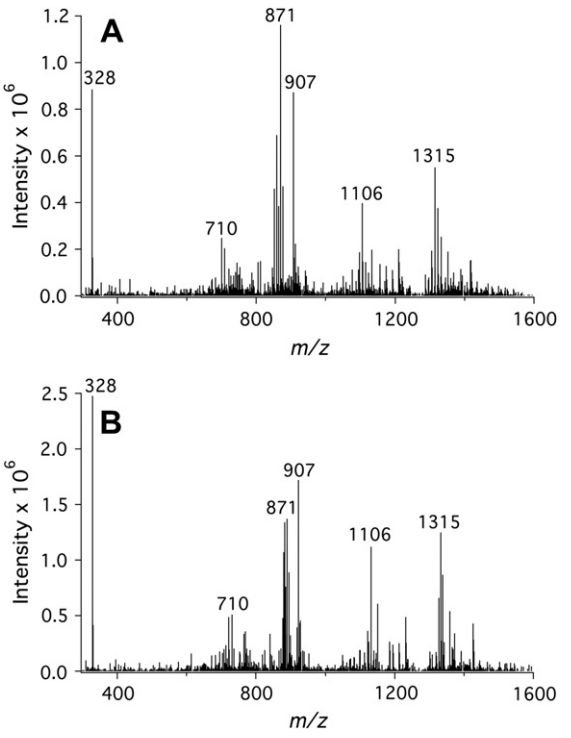

Fig. 4. Full scan mass spectra generated using electrospray ionization mass spectrometry of a 5 μl injection of (A) 10 μg/ml palytoxin standard, and (B) 80% ethanol extract of zoanthid (*Palythoa* sp.) sample from Virginia inhalation exposure event. Note for both, double charged ion cluster centered at 1315 Da, triple charged ion cluster centered at 907 Da, and characteristic 328 Da fragment.

(http://www.reefcentral.com/forums/showthread.php?s=6b504d5a6e1e5cbc29e9d433b6cded2c&threadid=1214868&perpage=25&pagenumber=1 posted 9/25/2007, accessed 8/11/2008).

As a follow-up to the investigations described above, 7 zoanthid colonies were purchased from a local Maryland fish store and analyzed for the presence of PTX-like compounds to assess the prevalence of PTX in commercially available zoanthid species. Five of the purchased samples were morphologically consistent with *Zoanthus* sp. while the other two were consistent with *Palythoa*/*Protopalythoa* sp. Six of the purchased zoanthids (all of the presumptive *Zoanthus* sp. and one of the presumptive *Palythoa*/*Protopalythoa* sp.) were reported by the store owner to be of tropical Indo-Pacific origin, while the last *Palythoa*/*Protopalythoa* sp. was purchased by the store owner from a Florida dealer and (presumably) came from the Caribbean Sea. Approximately 1 g of polyps (wet weight) from each sample were extracted for 24 h in 10 ml of 80% EtOH at 4 °C. Crude extracts were analyzed for PTX-like compounds as described above. Only the presumptive *Palythoa* sp. of reported Indo-Pacific origin contained concentrations of PTX-like compounds in the range of the zoanthid from the aquarium inhalation poisoning event in Virginia (515 μg/g PTX eq. as measured by HPLC and 90.8 μg/g as measured by hemolysis assay) (Figs. 2 and 3). Three additional zoanthid samples (the *Palythoa* sp. reported to be from the Caribbean Sea and two of the presumptive *Zoanthus* spp.) showed delayed hemolytic activity that was inhibited by an anti-PTX antibody in crude extracts but all were below the PTX detection limit by HPLC or ESI-MS. Histological and molecular analyses are in progress to attempt species identity. These results as well as additional toxin analyses will be reported separately.

Based on information available in the scientific literature and the limited new data provided here, it appears that the truth about the risks of PTX exposure from aquarium kept zoanthids is somewhere in between the two commonly reported beliefs that either all zoanthids are toxic or that none of the varieties sold to the public contain toxin. PTXs are not found in all commercially available zoanthid species but do clearly occur in potentially dangerous concentrations in a select few. Correct species identification, if species information is provided at all, for zoanthids sold in the aquarium trade is very rare. Complicating this is the fact that there is currently debate among traditional morphologic taxonomists and molecular geneticists as to the taxonomy of the Zoanthidea as a whole, especially considering the phenotypic plasticity observed within individuals of even the same species under different environmental conditions (Reimer et al., 2006a, 2006b, 2004; Ryland and Lancaster, 2003). Most aquarium store zoanthids are wild caught from tropical Indo-Pacific waters, with little record of collection information, and there are currently no US restrictions on the importation of any non-consumed marine organism based in potential toxicity. Also, many aquarium kept zoanthids are non-commercially exchanged and traded making their original origin difficult to trace. In the Virginia case reported here, the zoanthid responsible for toxicity was not purchased at all but arrived as a tank contaminant with live rock. Given all of these uncertainties, it is nearly impossible for the average aquarium hobbyist to make an informed decision as to the potential risks of keeping this group of organisms. Therefore all should be handled with appropriate care.

8. Conclusions

Due to its distribution mainly in tropical waters, and its prevalence in non-commercially harvested reef species, palytoxin (PTX) has been largely overlooked as a seafood-associated risk to consumer safety. Compounding this, is the fact that PTXs occur in species that also are known to occasionally harbor additional, more well known, toxins such as ciguatoxins, saxitoxins, and tetrodotoxin. But particularly in tropical Indo-Pacific coastal communities, PTX poisonings, some fatal, have been well documented. If PTXs are in fact responsible for clupeotoxism, which has been suggested but more data is required to prove conclusively, then the number of historical PTX-related illnesses and mortalities would greatly increase. Due to the knowledge that PTX-like compounds are also produced by certain dinoflagellates, it is now being realized that additional vectors (e.g. shellfish, marine aerosols) should also be considered potential threats to human health in areas where these species are prevalent.

With the exception of consuming the viscera of fish and crustaceans harvested from areas known to contain PTX-producing organisms, the risks of human exposure to PTX from processed seafood (i.e. fish flesh) remains an open question. It is often cited that PTX is far less potent orally compared to i.v. or i.p. administration. These reports are based primarily on Wiles et al. (1974) experiments using direct intragastric administration in rats. In that same study, Wiles et al. showed lethality in rats comparable to i.p. administration through intratracheal administration. It is possible that PTXs can be absorbed during ingestion prior to acid decomposition in the stomach, which may help to explain some of the severe effects, including mortalities, documented due to the consumption of PTX contaminated seafood. Systemic PTX effects have been reported through dermal contact with (presumably) absorption through small cuts in the skin (Hoffmann et al., 2008; this report). Further experimentation is required to confirm this possibility.

In conclusion, though relatively rare, due to their extreme potency PTX and PTX-like compounds do pose a threat to human safety through various routes of exposure including seafood consumption, exposure to certain marine aerosols, and even through dermal contact with select aquarium zoanthids. Continued research into the distribution and occurrence of PTX and PTX-like compounds, both in seafood and additional marine organisms, as well as updated risk assessment studies for various routes of exposure using well characterized, purified materials appears to be warranted.

Acknowledgements

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The authors attest that the mouse blood used in this study was acquired according to all appropriate approved animal care and use regulations established by the United States Army Medical Research Institute for Infectious Diseases (USAMRIID).

Conflicts of interest

There are no conflicts of interest.

References

Alcala, A.C., 1983. Recent cases of crab, cone shell, and fish intoxication on Southern Negros Island, Philippines. Toxicon (Suppl. 3), 1–3.

Alcala, A.C., Alcala, L.C., Garth, J.S., Yasumura, D., Yasumoto, T., 1988. Human fatality due to ingestion of the crab *Demania reynaudii* that contained a palytoxin-like toxin. Toxicon 26 (1), 105–107.

Aligizaki, K., Katikou, P., Nikolaidis, G., Panou, A., 2008. First episode of shellfish contamination by palytoxin-like compounds from *Ostreopsis* species (Aegean Sea, Greece). Toxicon 51 (3), 418–427.

Artigas, P., Gadsby, D.C., 2003. Na^+/K^+-pump ligands gating of palytoxin-induced ion channels. Proc. Natl. Acad. Sci. U.S.A. 100 (2), 501–505.

Beress, L., Zwick, J., Kolkenbrock, J., Kaul, P.N., Wassermann, O., 1983. A method for the isolation of the Caribbean palytoxin (C-PTX) from the coelenterate (zoanthid) *Palythoa caribaeorum*. Toxicon 21 (2), 285–290.

Bignami, G.S., 1993. A rapid and sensitive hemolysis neutralization assay for palytoxin. Toxicon 31 (6), 817–820.

Borneman, E.H., 2004. The Zoanthids: Colonial Anemones and Button Polyps. In: Aquarium Corals: Selection, Husbandry, and Natural Selection. F.F.H. Publications, Inc., Neptune City, NJ, pp. 177–191.

Ciminiello, P., Dell'Aversano, C., Fattorusso, E., Forino, M., Magno, G.S., Tartaglione, L., Grillo, C., Melchiorre, N., 2008. Putative palytoxin and its new analogue, ovatoxin-a, in *Ostreopsis ovata* collected along the Ligurian coasts during the 2006 toxic outbreak. J. Am. Soc. Mass Spectrom. 19, 111–120.

Ciminiello, P., Dell'Aversano, C., Fattorusso, E., Forino, M., Magno, G.S., Tartaglione, L., Grillo, C., Melchiorre, N., 2006. The Genoa 2005 outbreak. Determination of putative palytoxin in Mediterranean *Ostreopsis ovata* by a new liquid chromatography tandem mass spectrometry method. Anal. Chem. 78, 6153–6159.

Deeds, J.R., Terlizzi, D.E., Adolf, J.E., Stoecker, D.K., Place, A.R., 2002. Toxic activity from cultures of *Karlodinium micrum* (=*Gyrodinium galatheanum*) (Dinophyceae) – a dinoflagellate associated with fish mortalities in an estuarine aquaculture facility. Harmful Algae 1, 169–189.

FAO (Food and Agriculture Organization of the United Nations), 2004. Ciguatera Fish Poisoning. In: Marine Biotoxins, FAO Food and Nutrition Paper 80. Food and Agriculture Organization of the United Nations, Rome, pp. 185–217 (Chapter 7).

Fukui, M., Murata, M., Inoue, A., Gawel, M., Yasumoto, T., 1987. Occurrence of palytoxins in the trigger fish *Melichtys vidua*. Toxicon 25 (10), 1121–1124.

Gleibs, S., Mebs, D., 1999. Distribution and sequestration of palytoxin in coral reef animals. Toxicon 37 (11), 1521–1527.

Gleibs, S., Mebs, D., Werding, B., 1995. Studies on the origin and distribution of palytoxin in a Caribbean coral reef. Toxicon 33 (11), 1531–1537.

Gonzales, R.B., Alcala, A.C., 1977. Fatalities from crab poisoning on Negros Island, Philippines. Toxicon 15 (2), 169–170.

Habermann, E., Ahnert-Hilger, G., Chhatwal, G.S., Beress, L., 1981. Delayed haemolytic action of palytoxin: general characteristics. Biochim. Biophys. Acta 649, 481–486.

Habermann, E., Hudel, M., Dauzenroth, M.-E., 1989. Palytoxin promotes potassium outflow from erythrocytes, hela and bovine adrenomedullary cells through its interaction with Na^+, K^+-ATPase. Toxicon 27 (4), 419–430.

Halstead, B.W., 1967. Poisonous and Venomous Marine Animals of the World. In: Vertebrates, vol. 2. U.S. Government Printing Office, Washington D.C., USA.

Hashimoto, Y., Fusetani, N., Kimura, S., 1969. Aluterin: a toxin of filefish, *Alutera scipta*, probably originating from a Zoantharian, *Palythoa tuberculosa*. Bull. Jap. Soc. Sci. Fish. 35 (11), 1086–1093.

Hilgemann, D.W., 2003. From a pump to a pore: how palytoxin opens the gates. Proc. Natl. Acad. Sci. U.S.A. 100 (2), 386–388.

Hoffmann, K., Hermanns-Clausen, M., Buhl, C., Buchler, M.W., Schemmer, P., Mebs, D., Kauferstein, S., 2008. A case of palytoxin poisoning due to contact with zoanthid corals through skin injury. Toxicon 51 (8), 1535–1537.

Katikou, P., 2008. Palytoxin and analogs: ecobiology and origin, chemistry, metabolism, and chemical analysis. In: Botana, L.M. (Ed.), Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection. CRC Press, Boca Raton, FL, pp. 631–663.

Kita, M., Uemura, D., 2008. Palytoxin and analogs: diverse chemical structures and bioactivities of marine toxin: palytoxin and symbiodinolide. In: Botana, L.M. (Ed.), Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection. CRC Press, Boca Raton, FL, pp. 665–674.

Kodama, A.M., Hokama, Y., Yasumoto, T., Fukui, M., Manea, S.J., Sutherland, N., 1989. Clinical and laboratory findings implicating palytoxin as cause of ciguatera poisoning due to *Decapterus macrosoma* (mackerel). Toxicon 27 (9), 1051–1053.

Lau, C.O., Tan, C.H., Khoo, H.E., Yuen, R., Lewis, R.J., Corpuz, G.P., Bignami, G.S., 1995. *Lophozozymus pictor* toxin: a fluorescent structural isomer of palytoxin. Toxicon 33 (10), 1373–1377.

Lenoir, S., Ten-Hage, L., Turquet, J., Quod, J.-P., Bernard, C., Hennion, M.-C., 2004. First evidence of palytoxin analogs from an *Ostreopsis mascarenensis* (Dinophyceae) benthic bloom in southwestern Indian Ocean. J. Phycol. 40, 1042–1051.

Mahnir, V.M., Kozlovskaya, E.P., Kalinovsky, A.I., 1992. Sea anemone *Radianthus macrodactylus* – a new source of palytoxin. Toxicon 30 (11), 1449–1456.

Mebs, D., 1998. Occurrence and sequestration of toxins in food chains. Toxicon 36 (11), 1519–1522.

Mercado, J.A., Rivera-Rentas, A.L., Gonzalez, I., Tosteson, T.R., Molgo, J., Escalona de Motta, G., 1994. Neuro- and myo-toxicity of extracts from the benthic dinoflagellate *Ostreopsis lenticularis* is sensitive to μ-conotoxin. Soc. Neurosci. Abstr. 20 (1), 718.

Menuier, F.A., Mercado, J.A., Molgo, J., Tosteson, T.R., Escalona de Motta, G., 1997. Selective depolarization of the muscle membrane in frog nerve-muscle preparations by a chromatographically purified extract of the dinoflagellate *Ostreopsis lenticularis*. Br. J. Pharmacol. 121, 1224–1230.

Moore, R.E., Bartolini, G., 1981. Structure of palytoxin. J. Am. Chem. Soc. 103, 2491–2494.

Moore, R.E., Scheuer, P.J., 1971. Palytoxin: a new marine toxin from a coelenterate. Science 172, 495–498.

Mundy, R., 2008. Occurrence and toxicology of palytoxins. In: Botana, L.M. (Ed.), Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection. CRC Press, Boca Raton, FL, pp. 693–713.

Muscal, E., de Guzman, M.M., Wilson, R., Shah, B.R., 2007. Rhabdomyolysis. In: Myones, B.L., Windle, M.L., Sherry, D.D., Rauch, D. (Eds.), eMedicine. http://www.emedicine.com/ped/TOPIC2003.HTM (accessed 27.08.08).

Okano, H., Masuoka, H., Kamei, S., Seko, T., Koyabu, S., Tsuneoka, K., Tamai, T., Ueda, K., Nakazawa, S., Sugawa, M., Sukuki, H., Watanabe, M., Yatani, R., Nakano, T., 1998. Rhabdomyolysis and myocardial damage induced by palytoxin, a toxin of blue humphead parrotfish. Intern. Med. 37 (3), 330–333.

Oku, N., Sata, N.U., Matsunaga, S., Uchida, H., Fusetani, N., 2004. Identification of palytoxin as a principle which causes morphological changes in rat 3Y1 cells in the zoanthid *Palythoa* aff. margaritae. Toxicon 43 (1), 21–25.

Onuma, Y., Satake, M., Ukena, T., Roux, J., Chanteau, S., Rasolofonirina, N., Ratsimaloto, M., Naoki, H., Yasumoto, T., 1999. Identification of putative palytoxin as the cause of clupeotoxism. Toxicon 37 (1), 55–65.

Randall, J.E., 2005. Review of clupeotoxism, an often fatal illness from the consumption of clupeoid fishes. Pacific Sci. 59 (1), 73–77.

Reimer, J.D., Ono, S., Iwama, A., Takishita, K., Tsukahara, J., Maruyama, T., 2006a. Morphological and molecular revision of *Zoanthus* (Anthozoa: Hexacorallia) from Southwestern Japan, with descriptions of two new species. Zool. Sci. 23, 261–275.

Reimer, J.D., Ono, O., Takishita, T., Tsukahara, J., Maruyama, T., 2006b. Molecular evidence suggesting species in the zoanthid genera *Palythoa* and *Protopalythoa* (Anthozoa: Hexacorallia) are congeneric. Zool. Sci. 23, 87–94.

Reimer, J.D., Ono, S., Fujiwara, Y., Takishita, K., Tsukahara, J., 2004. Reconsidering *Zoanthus* spp. diversity: Molecular evidence of conspecifity within four previously presumed species. Zool. Sci. 21, 517–525.

Rhodes, L., Towers, N., Briggs, L., Munday, R., Adamson, J., 2002. Uptake of palytoxin-like compounds by shellfish fed *Ostreopsis siamensis* (Dinophyceae). New Zealand J. Mar. Fresh. Res. 36, 631–636.

Ryland, J.S., Lancaster, J.E., 2003. Revision of methods for separating species of *Protopalythoa* (Hexacorallia: Zoanthidea) in the tropical West Pacific. Invert. Systemat. 17, 407–428.

Taniyama, S., Arakawa, O., Terada, M., Nishio, S., Takatani, T., Mahmud, Y., Noguchi, T., 2003. *Ostreopsis* sp., a possible origin of palytoxin (PTX) in parrotfish *Scarus ovifrons*. Toxicon 42 (1), 29–33.

Taniyama, S., Mahmud, Y., Tanu, M.B., Takatani, T., Arakawa, O., Noguchi, T., 2001. Delayed haemolytic activity by the freshwater puffer *Tetraodon* sp. toxin. Toxicon 39 (5), 725–727.

Taniyama, S., Mahmud, Y., Terada, M., Takatani, T., 2002. Occurrence of a food poisoning incident by palytoxin from a serranid *Epinephelus* sp. in Japan. J. Nat. Toxins 11 (4), 277–282.

Uemura, D., Hirata, Y., Iwashita, T., 1985. Studies on palytoxins. Tetrahedron 41, 1007–1017.

Uemura, D., Ueda, K., Hirata, Y., 1981. Further studies on palytoxin. II. Structure of palytoxin. Tetrahedron Lett. 22 (29), 2781–2784.

Ukena, T., Satake, M., Usami, M., Oshima, Y., Naoki, H., Fujita, T., Kan, Y., Yasumoto, T., 2001. Structure elucidation of Ostreocin D, a palytoxin analog isolated from the dinoflagellate *Ostreopsis siamensis*. Biosci. Biotechnol. Biochem. 65 (11), 2585–2588.

Usami, M., Satake, M., Ishida, S., Inoue, A., Kan, Y., Yasumoto, T., 1995. Palytoxin analogs from the dinoflagellate *Ostreopsis siamensis*. J. Am. Chem. Soc. 117, 5389–5390.

Vale, C., 2008. Palytoxins: pharmacology and biological detection methods. In: Botana, L.M. (Ed.), Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection. CRC Press, Boca Raton, FL, pp. 675–691.

Van Dolah, F.M., Ramsdell, J.S., 2001. Review and assessment of in vitro detection methods for algal toxins. J. AOAC Int. 84 (5), 1617–1625.

Wachi, K.M., Hokama, Y., 2001. Diversity of marine biotoxins in the near-shore ocean area: presence of a palytoxin-like entity at Barbers Point Harbor, Oahu. J. Nat. Toxins 10 (4), 317–333.

Wachi, K.M., Hokama, Y., Haga, L.S., Shiraka, A., Takenaka, W.E., Bignami, G.S., Levine, L., 2000. Evidence for palytoxin as one of the sheep erythrocyte lytic factors in crude extracts of ciguateric and non-ciguateric reef fish tissue. J. Nat. Toxins 9 (2), 139–146.

Walsh, G.E., Bowers, R.L., 1971. A review of Hawaiian zoanthids with descriptions of three new species. Zool. J. Linn. Soc. 50, 161–180.

Wiles, J.S., Vick, J.A., Christensen, M.K., 1974. Toxicological evaluation of palytoxin in several animal species. Toxicon 12 (4), 427–433.

Yasumoto, T., Bagnis, R., Vernoux, J.P., 1976. Toxicity of the surgeonfishes-II: properties of the principal water-soluble toxin. Bull. Jpn. Soc. Sci. Fish. 42 (3), 359–365.

Yasumoto, T., Hashimoto, Y., Bagnis, R., Randall, J.E., Banner, A.H., 1971. Toxicity of the surgeonfishes. Bull. Jpn. Soc. Sci. Fish. 37 (8), 724–734.

Yasumoto, T., Murata, M., 1990. Polyether toxins involved in seafood poisoning. In: Hall, S., Strichartz, G. (Eds.), Marine Toxins: Origin, Structure, and Molecular Pharmacology. American Chemical Society, Washington D.C., USA, pp. 120–132.

Yasumoto, T., Yasumura, D., Ohizumi, Y., Takahashi, M., Alcala, A.C., Alcala, L.C., 1986. Palytoxin in two species of xanthid crab from the Philippines. Agric. Biol. Chem. 50 (1), 163–167.

Zaman, L., Arakawa, O., Shimoso, A., Onoue, Y., 1997. Occurrence of paralytic shellfish poison in Bangladeshi freshwater puffers. Toxicon 35 (3), 423–431.