Environ Biol Fish (2015) 98:961–969
DOI 10.1007/s10641-014-0313-0

False positive tests for ciguatera may derail efforts to control invasive lionfish

Christie L. Wilcox · Mark A. Hixon

Received: 4 December 2013 / Accepted: 9 July 2014 / Published online: 26 July 2014

Abstract There has been a recent push for human consumption of the invasive Pacific lionfishes *Pterois miles/volitans* as a management strategy throughout the greater western tropical Atlantic region, where lionfish have become a significant ecological problem. Recent tests have indicated that invasive lionfishes may be ciguatoxic, threatening the viability of a fishery-based management strategy. However, if innate scorpaenitoxins in the flesh of lionfish are mimicking ciguatoxin, consumption may be safe after all. There have been no confirmed cases of ciguatoxin poisoning from eating lionfish, indicating that false positive tests may be occurring. Based on the high degree of similarity in the biochemical effects of ciguatoxin and scorpaenitoxins, it is possible that bioassays for ciguatoxin are inaccurate in scorpaeniform fishes. Preliminary results suggest that scorpaenitoxins or other venom components are capable of contaminating ciguatoxin assays, and thus we urge caution regarding interpretation of ciguatoxin assays in invasive lionfishes. We recommend that ciguatera tests of lionfish be done after cooking the flesh, which denatures the scorpaenitoxins yet leaves ciguatoxin intact.

Keywords Lionfish invasion · Lionfish management · Ciguatera tests · Ciguatoxin · Scorpaenitoxins

C. L. Wilcox
Department of Cell and Molecular Biology, University of Hawai'i at Mānoa,
651 Ilalo St, Honolulu, HI 96813, USA

C. L. Wilcox (✉)
Hawai'i Institute of Marine Biology,
PO Box 1346, Kāne'ohe, HI 96744, USA
e-mail: wilcoxcl@hawaii.edu

M. A. Hixon
Department of Biology, University of Hawai'i at Mānoa,
2538 McCarthy Mall−Edmondson 216, Honolulu, HI 96822, USA

Introduction

Invasive species are one of the most economically damaging threats to ecosystems worldwide, costing an estimated $1.4 trillion annually (Pimental et al. 2001). Invaders may directly consume or outcompete native species, alter habitats and species interactions, and ultimately disrupt ecosystem structure and function (Lovell and Stone 2005). Invasions often exacerbate other stresses on ecosystems, such as overfishing, climate change, and pollution (Ehrenfield 2010), and further threaten already declining species (Wilcove et al. 1998). While the best management strategies are prevention, early detection, and rapid removal of invasives, the species that evade these measures may become established pests that require drastic management actions. Marine ecosystems now contain hundreds of non-native species, with more than 80 % of systems possessing unwelcome invaders (Molnar et al. 2008). Successful marine fish introductions were once considered rare, but have increased in prevalence over time (Ruiz et al. 1997).

In the 1980s, two sister species of Indo-Pacific lionfish, *P. volitans* and *P. miles* (hereafter *P. volitans/miles*)

were introduced to the Atlantic Ocean in the vicinity of Florida (Morris and Whitfield 2009), likely via the aquarium trade (Semmens et al. 2004). Lionfishes are well suited to invasion, being fast-growing predators that breed year-round and consume a wide variety of small prey, predominantly of fishes, while having no substantial natural predators in their non-endemic range (Morris and Whitfield 2009; Albins and Hixon 2013; Côté et al. 2013; Hackerott et al. 2013; but see Mumby et al. 2011). The introduced lionfishes have spread rapidly and are now observed as far south as Venezuela, as far north as Rhode Island, and throughout the greater Caribbean region and the Gulf of Mexico (Morris 2012). They are predicted to expand their range even further along the Atlantic coast of South America (Kimball et al. 2004; Morris 2012). On small patch reefs, invasive lionfish can reduce native fish recruitment by about 80 % in 5 weeks (Albins and Hixon 2008), and by over 90 % in 8 weeks (Albins 2013). This mortality causes steep population declines in native prey species across broader spatial scales (Green et al. 2012). There is also concern that lionfish, through predation on herbivorous fishes, may cause phase shifts from coral-dominated to algal-dominated reefs (Lesser and Slattery 2011; Albins and Hixon 2013). Therefore, the invasion of lionfishes is considered one of the top threats to global biodiversity (Sutherland et al. 2010).

Reductions in lionfish population sizes mitigate some of their ecological impacts, therefore removal via targeted derbies and directed fisheries is a primary management strategy (Morris and Whitfield 2009; Barbour et al. 2011; Morris 2012). Removal strategies for pest fishes in general are difficult and often hindered by a lack of selective methods that target the invasive species (Britton et al. 2010). A specific fishery for the nuisance species is thus the most effective means of targeted removal, though it relies on the manpower of local community members to maintain efforts. The Reef Environmental Education Foundation (REEF) was one of the first organizations to support local lionfish round-ups or 'derbies' in heavily infested places like the Bahamas (Morris and Whitfield 2009), which collect thousands of lionfish in a single day. The organization went on to release a lionfish cookbook, hoping to appeal to restaurant owners and the public alike (Ferguson and Akins 2010). In 2010, the National Oceanographic and Atmospheric Administration (NOAA) joined REEF, launching its "Eat Lionfish" campaign promoting the nutritional value and taste of lionfish (Morris et al. 2011). Local communities have enthusiastically accepted these campaigns, turning local fishermen acting as conservation advocates (Moore 2012).

At the same time, Florida Sea Grant, in conjunction with the Food and Drug Administration (FDA), caution that REEF and NOAA's campaigns are premature, based on results of a preliminary test indicating that lionfish are can contain the dreaded ciguatera toxin. The high prevalence of ciguatera reported in the Florida Sea Grant unpublished report is surprising given that no confirmed poisonings by lionfish have been documented in 5 years of local lionfish round-ups and associated barbeques (Gill 2012). While the lead researcher from the FDA initially stated that the administration does not support REEF and NOAA's campaigns, the administration has since waivered, declining to condemn the consumption of lionfish (Silk 2012). Yet the report caused great concern in regions where ciguatera is a known problem, such as the Virgin Islands, thus threatening the viability of controlling the lionfish invasion via a fishery-based management strategy.

Ciguatera fish poisoning ("CFP") is the most common marine poisoning worldwide, with more than 50,000 cases reported annually (Ting and Brown 2001), though estimates place the actual frequency of CFP as high as 500,000 cases per year (Arena et al. 2004). The responsible agents are ciguatoxin and its close congeners ("CTXs"), all of which are bioaccumulating lipophilic toxins produced by reef-associated dinoflagellates. The toxins are colorless and odorless, and are thus impossible to detect without biochemical assays. Because the toxins are heat-stable, not inactivated through any normal means of fish preparation, prior detection of fish with high toxin concentration is the only way to prevent poisoning (Juranovic and Park 1991). In communities where CFP is endemic, large, predatory reef fish are avoided by humans, reducing the number of fish available for sustenance and increasing fishing intensity on other species.

The dinoflagellates that produce CTXs are found globally in tropical and subtropical latitudes, and are endemic to American island states and territories, including Hawai'i, Florida, Puerto Rico, Guam, the US Virgin Islands, American Samoa, and the Commonwealth of the Northern Mariana Islands. In Puerto Rico, it is estimated that almost one tenth of the residents have experienced CFP, whereas across the Pacific islands as a whole, 70 % of the population has been poisoned (Fleming et al. 1998).

Although *P. volitans/miles* are only mid-sized predators (mesopredators), they are typically piscivorous, and so have the potential to be ciguatoxic. Their relatively long lifespan (decades) increases their bioaccumulation potential, and thus careful study of CTX prevalence in these species is warranted and necessary to ensure safe consumption. There have been no published peer-reviewed studies to date on the prevalence of CTXs in lionfishes, either in their native or invasive ranges. However, results from the 2012 Florida Sea Grant/FDA investigation concluded that among 194 fish tested, 42 % showed detectable levels of CTXs and 26 % were above the FDA's illness threshold of 0.1 parts per billion (Gill 2012). The method of testing was not reported. Here, we present evidence that the venom ubiquitous to lionfish species may mimic ciguatoxin in bioassays, potentially causing false positives.

Scorpaenitoxins vs. ciguatoxin

Most scorpaeniform fishes are known to be toxic, especially in the form of fin spine venoms, and it has been suggested that similar toxins occur across different lineages within this order. For example, antivenoms developed for stonefishes of the Family Synanceiidae ("SFAV") have been shown to interact with toxin extracts from members of several scorpaeniform families (Shiomi et al. 1989; Church and Hodgson 2001; Church and Hodgson 2003; Andrich et al. 2010). The isolation of similar protein toxins across distant lineages (Kiriake and Shiomi 2011) demonstrates that a unique toxin family (scorpaenitoxins) is highly conserved in this taxonomic group.

Clinically, ciguatera poisoning and lionfish envenomation generate overlapping symptoms, indicating that similar effects may be occurring at the cellular level. The predominant symptom of lionfish envenomation is intense, throbbing pain at the sting site, which may radiate from the site of injury and persist up to 12 h (Halstead 1988; Trestrail and Al-Mahasneh 1989). However, anesthesia, paresthesia, and hypesthesia have all been reported, and all are symptoms of ciguatera poisoning (Kizer et al. 1985; Kasdan et al. 1987; Trestrail and Al-Mahasneh 1989; Patel and Wells 1993). While systemic effects of envenomations are less common, they are similar to clinical presentations of CFP. These include headache, nausea, vomiting, abdominal pain, delirium, seizures, limb paralysis, hypertension and hypotension, respiratory distress, heart problems, muscle weakness, chills and death (Kizer et al. 1985; Kasdan et al. 1987; Trestrail and Al-Mahasneh 1989). Intravenous introduction of scorpaeniform venom extracts in mice yield similar effects to injection of ciguatoxin, including ataxia, limb paralysis, muscle weakness, and death, with muscular effects more pronounced for lionfish venom than stonefish venom (Saunders and Taylor 1959).

Most methods of ciguatera detection depend on physiological effects in test animals, using in vitro bioassays to determine presence and concentration of CTXs. Intraperitoneal injection of mice with crude fish extracts has been used by the Hawai'i Department of Health to detect CTX (Hokama 2004), with key indicators of toxicity being weakness, paralysis and death. However, these effects are also seen with intraperitoneal injections of scorpaeniform extracts (Saunders and Taylor 1959; Saunders 1960; Shiomi et al. 1989; Khoo et al. 1992; Khoo 2002). In guinea pig atria, both CTX and stonustoxin cause negative inotropy associated with cell depolarization and calcium overload (Austin et al. 1965; Lewis 1988). CTX is also a highly potent sodium channel activator, and a number of assays, like the rapid hemolysis assay and the neuroblastoma assay (Shimojo and Iwaoka 2000), assess sodium channel activation as an indicator of CTX. Yet stonustoxin from scorpaeniform fishes has also been shown to activate sodium channels, and like CTX, activating effects are blocked by sodium channel blockers such as tetrodotoxin (Hopkins et al. 1996).

Unlike CTX, scorpaenitoxins are readily degraded when heated or ingested (Saunders and Taylor 1959; Saunders 1960; Glaziou and Legrand 1994; Chun 2005). Thus while scorpaenitoxins might throw off a ciguatoxin test, they pose no threat to the consumer. Based on the high degree of similarity in the effects of CTX and scorpaeniform venoms, it is possible that bioassays for CTX are inaccurate in scorpaeniform species. The production of venom components could explain the putative prevalence of CTX in invasive lionfishes despite the complete lack of poisoning incidents. If this effect occurs, then it has likely gone undocumented because there are few commonly consumed ciguatoxic fish that might produce such toxins. Our preliminary results suggests that scorpaenitoxins (a) are present in the tissues commonly used for ciguatera testing, and (b) can be detected after extraction protocols commonly used in ciguatera testing. These data indicate caution and skepticism when interpreting results from

ciguatera testing in scorpaeniform fishes, including invasive lionfishes. The ultimate solution is more reliable and specific testing for ciguatera, allowing for improved management of these highly invasive species.

Materials and methods

Protein extractions

Crude tissue extracts of skin, muscle, liver and spines were taken from adult invasive *P. volitans/miles* (15–30 cm in length, $n=14$), and as a positive control, the confamilial *Scorpaenopsis diabolus* ($n=3$). *P. volitans/miles* were spear-fished off the coast of Beaufort, North Carolina, and the specimens of *S. diabolus* were spearfished from locations around Oahu. All animals were frozen on dry ice immediately after capture and preserved at −80°C until analyzed. Spine, skin, muscle and liver samples were collected from frozen individuals and homogenized in one of four extraction buffers: phosphate buffered saline (PBS) with 10 mM EDTA and 1 mM PMSF (Stable Salt Buffer, SSB), a 70 % methanol/30 % PBS, 100 % methanol, and 100 % acetone. Homogenized tissues were shaken overnight at 4°C. Samples were centrifuged at 14,000×g for 10 min and the supernatant was transferred to a clean vial. Methanol and acetone extractions were evaporated in a rotary evaporator until nearly-dry, and were resuspended in freezer storage buffer (SSB+20 % glycerol), while the saline supernatants were dialyzed into the same storage buffer. Total protein concentration was determined using a modified Lowry assay (Bio-Rad). All samples were stored at −20°C until use.

Protein size sorting and Immunoblotting

Relative scorpaenitoxin levels in each sample were analyzed using a western blotting protocol as employed by previous scorpaenitoxin investigations (Shiomi et al. 1989; Church and Hodgson 2001, 2003; Andrich et al. 2010). Studies have shown that scorpaenitoxins from distantly related fishes, including lionfish, scorpionfish and soldierfish, all cross-react with stonefish antivenin (SFAV) (Shiomi et al. 1989; Church and Hodgson 2001, 2003; Andrich et al. 2010), with scorpaenitoxin subunits around 75 kDa in size. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to separate venom proteins by size, using the method developed by Laemmli (1970) on 7.5 % TGX gels (Bio-Rad). Samples were pre-treated using Laemmli sample buffer (Bio-Rad) at 100°C for 5 min. Native PAGE was also performed to resolve intact scorpaenitoxins, with samples prepared in Native buffer (Bio-Rad) and run under non-reducing conditions in Tris/Glycine buffer (Bio-Rad). The molecular masses were estimated by a relative mobility method, comparing the migration of the obtained bands with that from a mixture of protein molecular markers. After running, proteins were visualized by staining with Coomassie Brilliant Blue R-250.

For immunoblotting, proteins were electrotransferred (4°C, overnight) onto a PVDF membrane, which was then blocked with PBS–Casein (1 % w/v, 1 h). After washing the membrane with PBS–Tween 20 (0.05 % v/v), it was probed overnight at 25°C with SFAV diluted in PBS (1:500) and another washing step was performed. The bound antibodies were probed (1 h, 25°C) with a diluted peroxidase-labeled anti-horse IgG preparation (1:1000 in PBS), and resolved using the Clarity Western ECL Substrate (Bio-Rad). Blots were visualized using GeneSnap (SynGene) with relative expression levels calculated using GeneTools (SynGene).

Results

SDS-PAGE investigation of the proteins in the skin, muscle, spine and liver tissues of *P. volitans/miles* revealed the presence of scorpaenitoxin and other venom proteins in all tissue types. While clear differences in the types of proteins expressed in each tissue type were visualized with SDS-PAGE (Fig. 1a), when analyzed with western blotting, stonefish antivenom reacted to a number of these proteins ranging in size, most of whose functions are unknown. The strong reactivity by two proteins roughly 75 kDa in size is consistent with the alpha and beta subunits of PvTx identified by Kiriake and Shiomi (2011) (Fig. 1b). These scorpaenitoxin bands (Fig. 2a) were strongest in spine tissues (67.2± 46.3 times more optically dense than in liver tissues), but were also present in skin and muscle tissues at around 1/10th the concentration (Fig. 2c). Intact scorpaenitoxins of ~150 kDa were also detected in spine, muscle and skin (Fig. 2b). Thus, while the spines expressed higher levels of scorpaenitoxins, they were also readily detected in the skin and muscle tissues, and

therefore could potentially contaminate ciguatoxin assays, which use those tissues for analysis.

Analysis of extracts from *P. volitans/miles* tissues using four commonly used methods for ciguatoxin revealed that scorpaenitoxins showed remarkable levels of intact proteins. While it was expected that proteins would be isolated using a saline extraction buffer, they were equally detected in 70 % methanol extractions (Fig. 3). Acetone extractions (100 %), the most commonly used in CTX analysis, also contained detectable levels of scorpaenitoxins, albeit at much lower levels (Fig. 3b). No scorpaenitoxins could be detected in the methanol extracts. The presence of scorpaenitoxins in the three extracts further suggests that venom proteins could currently contaminate assays for ciguatoxin activity.

Discussion

The analyses reported here indicate that, while scorpaenitoxin venom components are highly concentrated in the spines of lionfishes responsible for the Atlantic invasion (*P. volitans/miles*), they can also be found throughout the body, including tissues commonly tested for the presence of ciguatoxin. Given that scorpaenitoxins and ciguatoxin cause similar biochemical reactions, it is possible that standard ciguatera tests of lionfish result in false positives. If so, the falsehood that lionfish are unsafe to eat would hinder directed fisheries that could help mitigate the impact of this invasive (Morris 2012). A simple solution is to cook lionfish before conducting a ciguatera test, which denatures scorpaenitoxins, leaving only ciguatoxin, if present (Saunders and Taylor 1959; Saunders 1960).

Our findings confirmed the presence of scorpaenitoxins in multiple tissues. To date, seven lethal scorpaenitoxin proteins have been isolated from three species of stonefish, two species of lionfish and one species of scorpionfish: stonustoxin (SNTX *Synanceja horrida*, Poh et al. 1991; Ghadessy et al. 1996), verrucotoxin (VTX, *Synanceja verrucosa*, Garnier et al. 1995, 1997), neoverrucotoxin (neoVTX, *Synanceja verrucosa*, Ueda et al. 2006), trachynilysin (TLY, *Synanceja trachynis*, Kreger 1991), PvTx (*P. volitans*, Kiriake and Shiomi 2011), PaTX (*P. antennata*, Kiriake and Shiomi 2011), and Sp-CTX (*Scorpaena plumieri*, Andrich et al. 2010). All are large, multi-subunit proteins that are thought to cause cytotoxicity through pore formation and sodium channel activation. Of the seven, the complementary DNA (cDNA) of five toxin mRNA sequences have been generated. Subunits within the same taxonomic family are>87 % homologous (Ueda et al. 2006), while those between families are>45 % homologous (Kiriake and Shiomi 2011). This family of proteins, the scorpaenitoxins, is unknown in any other animal lineage.

It is unclear why scorpaenitoxins are present in tissues other than spine venom glands, though there are several possible explanations. While scorpaenitoxins are viewed as venom proteins, it is possible that they serve other functions, such as immunological defense. Many venom proteins are recruited from key regulatory processes (see Fry et al. 2009). Currently, the evolutionary history of the scorpaenitoxins is unknown, and only one terminal domain is homologous to any known protein. If the venoms of fish evolved through similar pathways involving the duplication and modification of encoding genes, it would not be surprising if endogenous proteins similar to the venom scorpaenitoxins are present elsewhere. While western blotting is able to detect the presence of similarly sized proteins, it is unable to determine whether the proteins detected in the skin and muscles are identical to the one present in the spines. Therefore, the proteins detected in body tissues may not be the venom, though they must contain enough similarities to interact with the stonefish venom antibodies. It is also possible that the detectable levels represent incomplete sequestration of venom components. Previous research has indicated that lionfish are resistant to their own venom (Allen and Eschmeyer 1973), a trait not universal in venomous organisms. It is possible such resistance is a necessity if venom sequestration is incomplete. Whatever the ultimate cause of scorpaenitoxins in body tissues, the presence of these proteins may result in the contamination of ciguatoxin assays.

The lipophilic nature of scorpaenitoxins further contributes to concerns about contamination of ciguatera tests. Though methanol- and acetone-based extraction methods are designed to extract lipids, it is well established that other highly lipophilic compounds, including proteins, can be solubilized in these organic solvents (Erickson 1993). The presence of scorpaenitoxins in 100 % acetone extracts is particularly troubling, as this is a common first step for CTX detection. These results stress the importance of clean-up

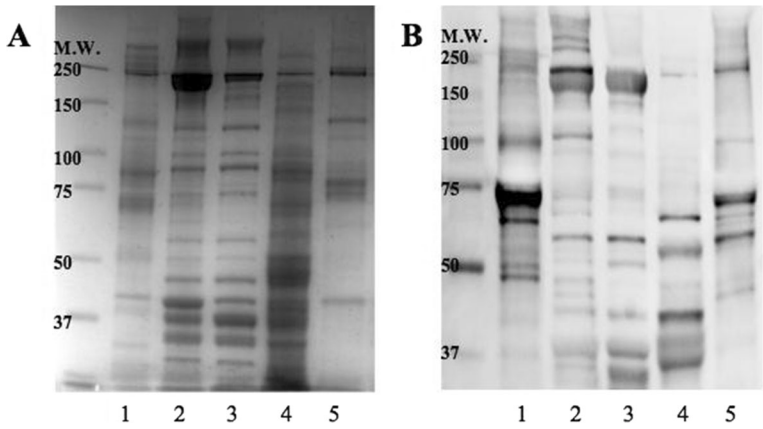

Fig. 1 SDS PAGE analysis of *P. volitans/miles* tissue extracts on TGX protein gels, showing the presence of venom proteins in all tissues tested. Darker bands indicate increased amounts of protein. **a** Coomassie stain of total protein, revealing the similarities and differences in the concentration of different proteins in different tissue types. **b** Western blot with stonefish antivenin, which highlights the proteins similar to those in stonefish venom. Scorpaenitoxin subunits are ~75 kDa in size. Lanes: 1. spine; 2. skin; 3. muscle; 4. liver; 5; *S. diabolus* spine (positive control)

protocols and multiple extraction steps to ensure the purity of any samples tested for CTX. Again, the best precaution is to cook a lionfish before testing for the presence of CTX.

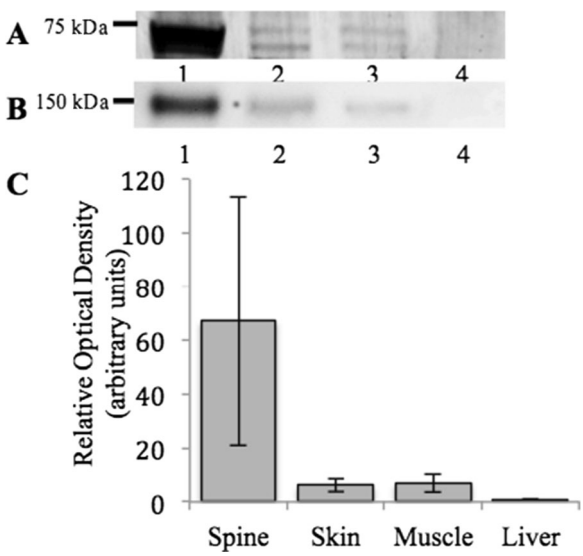

Fig. 2 Relative scorpaenitoxin content in different tissues from invasive lionfish *P. volitans/miles*, showing that scorpaenitoxins are present in the spines, skin, and muscle. **a** Representative western blot after denaturing SDS-PAGE showing the detection of the alpha and beta subunits of PvTx in the various tissue types. Lanes: 1. spine; 2. skin; 3. muscle; 4. liver. **b** Representative western blot after Native-PAGE showing the detection of complete PvTx in the various tissue types. Lanes: 1. spine; 2. skin; 3. muscle; 4. liver. **c** Relative scorpaenitoxin levels across tissue types. Values represent mean densitometry values of protein bands (±1 standard error of the mean) detected by western blotting of SDS-PAGE; band intensities standardized by the liver sample from each fish, which we use as a reference; n=4 for each bar

Though scorpaenitoxins are the most lethal component of scorpaeniform venoms, they are not the only toxic constituents that may play a role in the in vitro similarities with ciguatoxins. Venoms are complex chemical cocktails with multiple compounds contributing to toxicity (Casewell et al. 2012). Biologically active peptides have been isolated from multiple scorpaeniform fishes. Juzans et al. (1995) isolated a

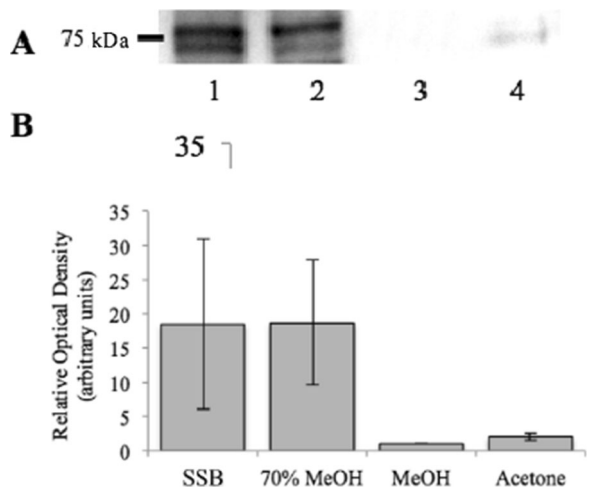

Fig. 3 Relative scorpaenitoxin content in different extraction methods from invasive lionfish *P. volitans/miles*, showing that scorpaenitoxins survive extraction protocols designed for ciguatoxin assays. **a** Representative western blot showing the detection of the alpha and beta subunits of PvTx in the various extraction methods. Lanes: 1. SSB buffer; 2. 70 % Methanol; 3. 100 % Methanol; 4. 100 % Acetone. **b** Relative scorpaenitoxin levels across extraction methods. Values represent mean densitometry values of protein bands (±1 standard error of the mean) detected by western blotting; band intensities standardized by the methanol sample, which we use as a reference; n=3 for each bar

peptide from *Synanceia trachynis* that, like CTX, causes spontaneous release and depletion of acetylcholine from motor nerve terminals. Balasubashini et al. (2006) isolated an antiproliferative peptide (7.6 kDa) from *P. volitans* venom. In *P. volitans*, several proteins of various sizes cross-react with stonefish antivenom and may indicate venomous use (Fig. 1b). In other investigations of lionfish venom, proteolytic enzymes weighing roughly 45 kDa have been detected though not purified (Balasubashini et al. 2006), and other proteins weighing 29 kDa, 66 kDa, 97 kDa and 116 kDa have been separated using SDS-PAGE, though their functions are unknown (Choromanski 1985). Non-proteinaceous components have also been found in all scorpaeniform venoms examined. Additionally, there is strong evidence for the presence of neurotransmitters in the venoms of lionfish (Cohen and Olek 1989; Church and Hodgson 2002) and other Scorpaeniformes (Garnier et al. 1996), including acetylcholine and noradrenaline. Although little is known about lipid toxins in these species, Nair et al. (1985) isolated an unknown lipophilic ichthyotoxin from *P. volitans* spines.

The detection of all of these components from different tissues and extraction methods was outside the scope of this work, but it is important to remember that with our limited knowledge of the relative contribution of venom components to in vitro activities, any of these components could cause false positives in CTX activity assays. Some, like the unidentified toxin isolated by Nair et al. (1985), may be even more likely to survive lipid-specific extraction methods. Thus, further research into the diversity of toxins in lionfish tissues is necessary to completely understand the potential for contamination of CTX assays.

There are severe consequences to inaccurate ciguatoxins tests. Poisonings have direct and indirect negative social and economic impacts. Even a few cases of CFP can drastically alter the use of reef resources, and fish avoidance can have an adverse economic impact (Lewis 1986). In French Polynesia, for example, CFP costs over $1 million dollars annually in lost productivity due to illness and more than $1 million in lost earnings due to banned fish (Glaziou and Legrand 1994). If innate lionfish toxins are causing false positives on ciguatoxin tests, there is little hope of establishing a stable fishery that could otherwise help to control this worst of marine invasions.

Though the data reported here do not provide conclusive evidence of venom contamination, they provide sufficient evidence for the re-evaluation of ciguatera testing methods in lionfish and other venomous species. Certainly caution is indicated in interpreting positive results from CTX bioassays of invasive lionfish. While there is likely no doubt that lionfish in areas with high levels of ciguatera prevalence possess the same potential danger as similar mesopredators, there is no evidence that lionfish in ciguatera-free areas are a threat to public health.

Acknowledgments We gratefully acknowledge the four anonymous reviewers for helpful comments that much improved the manuscript. CLW thanks Sigma Xi's Grants-In-Aid of Research and Achievement Rewards for College Scientists Honolulu Chapter for their support. CLW also thanks K. Winkel and B. Fry for their contributions of SFAV, M. Gerschenson and D. Libutti for their advice and generous assistance in the laboratory, Discovery Diving in Beaufort, NC for their help in specimen collection, and Stumpy for his personal donations to this work. MAH thanks the National Science Foundation for supporting his lab's research on the ecological effects of the lionfish invasion (08–51162 and 12–33027). This is contribution no. 1594 from the Hawai'i Institute of Marine Biology, no. 9174 from the School of Ocean and Earth Science and Technology, and no. 2014-13 from the Department of Biology at the University of Hawaii at Manoa. This study complied with current laws in the United States and was conducted in accordance with the regulations of the University of Hawai'i Institutional Animal Care and Use Committee (IACUC).

References

Albins MA (2013) Effects of invasive Pacific red lionfish *Pterois volitans* versus a native predator on Bahamian coral-reef fish communities. Biol Invasions 15:29–43

Albins MA, Hixon MA (2008) Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. Mar Ecol Prog Ser 367:233–238

Albins MA, Hixon MA (2013) Worst-case scenario: potential long-term effects of invasive predatory lionfish (*Pterois volitans*) on Atlantic and Caribbean coral-reef communities. Environ Biol Fish 96:1151–1157

Allen GR, Eschmeyer WN (1973) Turkeyfishes at Eniwetok. Pac Discov 26:3–11

Andrich F, Carnielli J, Cassoli J, Lautner R, Santos RA, Pimenta AM, de Lima ME, Figueiredo SG (2010) A potent vasoactive cytolysin isolated from *Scorpaena plumieri* scorpionfish venom. Toxicon 56:487–496

Arena P, Levin B, Fleming L, Friedman M, Blythe D (2004) A pilot study of the cognitive and psychological correlates of chronic ciguatera poisoning. Harmful Algae 3:51–60

Austin L, Gillis R, Youatt G (1965) Stonefish venom: some biochemical and chemical observations. Aust J Exp Biol Med Sci 43:79–90

Balasubashini SR, Karthigayan S, Somasundaram ST, Balasubramanian T, Rukkumani R, Menon VP (2006) FV

peptide induces apoptosis in HEp 2 and HeLa cells: an insight into the mechanism of induction. J Carcinog 5:27

Barbour AB, Allen MS, Frazer TK, Sherman KD (2011) Evaluating the potential efficacy of invasive lionfish (*Pterois volitans*) removals. PLoS ONE 6(5):e19666. doi:10.1371/journal.pone.0019666

Britton JR, Gozlan RE, Copp GH (2010) Managing non-native fish in the environment. Fish Fish 12:256–274

Casewell NR, Wüster W, Vonk FJ, Harrison R, Fry BG (2012) Complex cocktails: the evolutionary novelty of venoms. Trends Ecol Evol 28:219–229

Choromanski J (1985) Chemical stabilization and pharmacological characterization of the venom of the lionfish, *Pterois volitans*. Dissertation, Oregon State University

Chun, LH (2005) Functional and structural studies of Stonustoxin (SNTX), a lethal factor from Stonefish (*Synanceja horrida*) venom. Master's Thesis, National University of Singapore

Church J, Hodgson W (2001) Stonefish (*Synanceia* spp.) antivenom neutralizes the in vitro and in vivo cardiovascular activity of soldierfish (*Gymnapistes marmoratus*) venom. Toxicon 39:319–324

Church J, Hodgson W (2002) Adrenergic and cholinergic activity contributes to the cardiovascular effects of lionfish (*Pterois volitans*) venom. Toxicon 40:787–796

Church J, Hodgson W (2003) Stonefish (*Synanceia trachynis*) antivenom: in vitro efficacy and clinical use. Toxin Rev 22: 69–76

Cohen AS, Olek AJ (1989) An extract of lionfish (*Pterois volitans*) spine tissue contains acetylcholine and a toxin that affects neuromuscular transmission. Toxicon 27:1367–1376

Côté IM, Green SJ, Hixon MA (2013) Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. Biol Conserv 164:50–61. doi:10.1016/j.biocon.2013.04.014

Ehrenfield JG (2010) Ecosystem consequences of biological invasions. Annu Rev Ecol Evol Syst 41:59–80

Erickson MC (1993) Lipid extraction from channel catfish muscle: comparison of solvent systems. J Food Sci 58:84–89

Ferguson T, Akins L (2010) The lionfish cookbook. REEF Environmental Education Foundation, Key Largo

Fleming LE, Baden DG, Bean JA, Weisman R, Blythe DG (1998) Marine seafood toxin diseases: Issues in epidemiology and community outreach. In: Reguera B, Blanco J, Fernandez ML, Wyatt T (eds) Harmal Algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Vigo and Paris, pp 245–248

Fry BG, Roelants K, Champagne DE, Scheib H, Tyndall JD, King GF, Nevalainen TJ, Norman JA, Lewis RJ, Norton RS, Renjifo C, de la Vega RC (2009) The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. Annu Rev Genomics Hum Genet 10:483–511

Garnier P, Goudey-Perriere F, Breton P, Dewulf C, Petek F, Perriere C (1995) Enzymatic properties of the stonefish (*Synanceia verrucosa* Bloch and Schneider, 1801) venom and purification of a lethal, hypotensive and cytolytic factor. Toxicon 33:143–155

Garnier P, Grosclaude JM, Goudey-Perrière F, Gervat V, Gayral P, Jacquot C, Perrière C (1996) Presence of norepinephrine and other biogenic amines in stonefish venom. J Chromatogr B Biomed Appl 685:364–369

Garnier P, Ducancel F, Ogawa T, Boulain J, Goudey-Perrière F, Perrière C, Ménez A (1997) Complete amino-acid sequence of the [beta]-subunit of VTX from venom of the stonefish (*Synanceia verrucosa*) as identified from cDNA cloning experiments. Biochim Biophys Acta 1337:1–5

Ghadessy F, Chen D, Kini R, Chung M, Jeyaseelan K, Khoo H, Yuen R (1996) Stonustoxin is a novel lethal factor from stonefish (*Synanceja horrida*) venom. J Biol Chem 271: 25575–22581

Gill E (2012) Florida Sea Grant says not to eat lionfish. TCPalm. http://www.tcpalm.com/news/2012/jul/02/florida-sea-grant-says-not-to-eat-lionfish-23815/. Accessed 02 July 2012

Glaziou P, Legrand A-M (1994) The epidemiology of ciguatera fish poisoning. Toxicon 32:863–873

Green SJ, Akins JL, Maljković A, Côté IM (2012) Invasive lionfish drive Atlantic coral reef fish declines. PLoS ONE 7:e32596. doi:10.1371/journal.pone.0032596

Hackerott S, Valdivia A, Green SJ, Côté IM, Cox CE, Akins L, Layman CA, Precht WF, Bruno JF (2013) Native predators do not influence invasion success of Pacific lionfish on Caribbean reefs. PLoS ONE 8(7):e68259. doi:10.1371/journal.pone.0068259

Halstead B (1988) Poisonous and venomous marine animals of the world. Darwin Press, Princeton

Hokama Y (2004) Ciguatera fish poisoning: Features tissue and body effects. In: Watson RR, Preedy VR (eds) Reviews in food and nutrition toxicity. CRC Press, Boca Raton, pp 43–83

Hopkins BJ, Hodgson WC, Sutherland SK (1996) Evidence for adrenergic and tachykinin activity in venom of the stonefish (*Synanceja trachynis*). Toxicon 34:541–554

Juranovic LR, Park DL (1991) Foodborne toxins of marine origin: ciguatera. Rev Environ Contam T 117:51–94

Juzans P, Colasante C, Kreger AS, Molgo J (1995) Increase in spontaneous quantal ACh release and alterations of motor nerve terminals induced by an isolated peptide of stonefish (*Synanceia trachinis*) venom. Toxicon 33:1125

Kasdan ML, Kasdan AS, Hamilton DL (1987) Lionfish envenomation. Plast Reconstr Surg 80:613–614

Khoo H (2002) Bioactive proteins from stonefish venom. Clin Exp Pharmacol Physiol 29:802–806

Khoo H, Yuen R, Poh C, Tan C (1992) Biological activities of *Synanceja horrida* (stonefish) venom. Nat Toxins 1:54–60

Kimball ME, Miller JM, Whitfield PE, Hare JA (2004) Thermal tolerance and potential distribution of invasive lionfish (*Pterois volitans/miles* complex) on the east coast of the United States. Mar Ecol Prog Ser 283:269–278

Kiriake A, Shiomi K (2011) Some properties and cDNA cloning of proteinaceous toxins from two species of lionfish (*Pterois antennata* and *Pterois volitans*). Toxicon 58:494–501

Kizer KW, McKinney HE, Auerbach PS (1985) Scorpaenidae envenomation: a 5-year poison center experience. JAMA 8: 807–810

Kreger A (1991) Detection of a cytolytic toxin in the venom of the stonefish (*Synanceia trachynis*). Toxicon 29:733–743

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lesser MP, Slattery M (2011) Phase shift to algal dominated communities at mesophotic depths associated with lionfish

(*Pterois volitans*) invasion on a Bahamian coral reef. Biol Invasions 13:1855–1868

Lewis ND (1986) Epidemiology and impact of ciguatera in the Pacific: a review. Mar Fish Rev 48:6–13

Lewis R (1988) Negative inotropic and arrhythmic effects of high doses of ciguatoxin on guinea-pig atria and papillary muscle. Toxicon 26:639–649

Lovell SJ, Stone SF (2005) The economic impacts of aquatic invasive species: a review of the literature. US Environmental Protection Agency Working Paper #05-02, 64

Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. Front Ecol Environ 6:485–492

Moore AM (2012) The aquatic invaders: marine management figuring fishermen, fisheries, and lionfish in the Bahamas. Cult Anthropol 27:667–688

Morris JA (2012) Invasive lionfish: a guide to control and management, vol 1. Gulf and Caribbean Fisheries Institute Special Publication, Marathon

Morris JA, Whitfield PE (2009) Biology, ecology, control and management of the invasive Indo-Pacific lionfish: an updated integrated assessment. NOAA Technical Memorandum NOS NCCOS 99

Morris JA, Thomas A, Rhyne AL, Breen N, Akins L, Nash B (2011) Nutritional properties of the invasive lionfish: a delicious and nutritious approach for controlling the invasion. AACL Bioflux 4:21–26

Mumby PJ, Harborne AR, Brumbaugh DR (2011) Grouper as a natural biocontrol of invasive lionfish. PLoS ONE 6:e21510. doi:10.1371/journal.pone.0021510

Nair MSR, Cheung P, Leong I, Ruggieri GD (1985) Short communications: a non-proteinaceous toxin from the venomous spines of the lionfish Pterois volitans (*Linnaeus*). Toxicon 23: 525–527

Patel MR, Wells S (1993) Lionfish envenomation of the hand. J Hand Surg [Am] 18:523–525

Pimental D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T (2001) Economic and environmental threats of alien plant, animal and microbe invasions. Agric Ecosyst Environ 84:1–20

Poh C, Yuen R, Khoo H, Chung M, Gwee M, Gopalakrishnakone P (1991) Purification and partial characterization of stonustoxin (lethal factor) from *Synanceja horrida* venom. Comp Biochem Physiol B 99:793–798

Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. Am Zool 37:621–632

Saunders PR (1960) Pharmacological and chemical studies of the venom of the stonefish (genus *Synanceja*) and other scorpion fishes. Ann N Y Acad Sci 90:798–804

Saunders P, Taylor P (1959) Venom of the lionfish *Pterois volitans*. Am J Physiol 197:437

Semmens BX, Buhle ER, Salomon AK, Pattengill-Semmens CV (2004) A hotspot of non-native marine fishes: evidence for the aquarium trade as an invasion pathway. Mar Ecol Prog Ser 266:239–244

Shimojo RY, Iwaoka WT (2000) A rapid hemolysis assay for the detection of sodium channel-specific marine toxins. Toxicol 154:1–7

Shiomi K, Hosaka M, Fujita S, Yamanaka H, Kikuchi T (1989) Venoms from six species of marine fish: lethal and hemolytic activities and their neutralization by commercial stonefish antivenom. Mar Biol 103:285–289

Silk R (2012) FDA wavers on ciguatera warning. Florida Keys News. http://keysnews.com/node/40782. Accessed 30 June 2012

Sutherland WJ, Clout M, Côté IM, Daszak P, Depledge MH, Fellman L, Fleishman E, Garthwaite R, Gibbons DW, De Lurio J, Impey AJ, Lickorish F, Lindenmayer D, Madgwick J, Margerison C, Maynard T, Peck LS, Pretty J, Prior S, Redford KH, Scharlemann JPW, Spalding M, Watkinson AR (2010) A horizon scan of global conservation issues for 2010. Trends Ecol Evol 25:1–7

Ting JY, Brown AF (2001) Ciguatera poisoning: a global issue with common management problems. Eur J Emerg Med 8:295–300

Trestrail JH, Al-Mahasneh QM (1989) Lionfish sting experiences of an inland poison center: a retrospective study of 23 cases. Vet Hum Toxicol 31:173–175

Ueda A, Suzuki M, Honma T, Nagai H, Nagashima Y, Shiomi K (2006) Purification, properties and cDNA cloning of neoverrucotoxin (neoVTX), a hemolytic lethal factor from the stonefish *Synanceia verrucosa* venom. Biochim Biophys Acta 1760:1713–1722

Wilcove DS, Rothstein D, Dubow J, Phillips J, Losos E (1998) Quantifying threats to imperiled species in the United States. Biosci 48:607–615