The palatability of stonefish (*Synanceia* spp.) ichthyocrinotoxins to potential predators

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ABSTRACT

Numerous fish species are known to utilise ichthyocrinotoxins to defend themselves against predators, often by deterring the predator from feeding on them. This study investigated whether stonefish ichthyocrinotoxins act as antifeedants by assessing its effect on the feeding behaviours of two potential predators of stonefish: lionfish and morays. Food laced with either *Synanceia horrida* (Estuarine Stonefish) or *Synanceia verrucosa* (Reef Stonefish) ichthyocrinotoxin were highly palatable (>85% food acceptance) to both predator types, demonstrating similar acceptance rates as untreated food (>95% food acceptance). In contrast, food coated in *Diploprion bifasciatum* (Barred Soapfish) ichthyocrinotoxin, a known antifeedant used experimentally as a positive control, prompted high rejection rates (<10% food acceptance). These results suggest that stonefish ichthyocrinotoxins may not play a significant role in antifeedant defence. Stonefish ichthyocrinotoxins also exhibited a greater adhesion to prawn fillets (>45% retention), compared to soapfish ichthyocrinotoxin (27% retention), which may reflect differences in their ecological functions and/or application mechanisms. The findings of this study have expanded our understanding of stonefish toxin ecology.

Stonefish are one of only a few known animals that produce both a venom and a secreted poison (ichthyocrinotoxin). Stonefish venom, which is contained within twin glands associated with each of their 13 dorsal spines, is used exclusively for defence against predators (Endean 1961). Due to its deleterious nature to people, stonefish venom is also considered a medically significant toxin (Saggiomo et al. 2021). Consequently, most of the research on stonefish toxins is focused on the venom, particularly around improving outcomes in cases of envenomation (Saggiomo et al. 2021). However, very little is known about the ichthyocrinotoxins that these animals secrete onto their skin.

Ichthyocrinotoxins from various fish species, including soapfish, flatfish and boxfish, are widely evidenced to serve in predator defence, and are often rapidly dispensed when the user is provoked (see review by Lennox-Bulow et al. 2023a). The behavioural responses of predators to the toxin reportedly include avoiding, regurgitating or rejecting the ichthyocrinotoxic fish, jerking away, swimming erratically, mouth gaping, and increased opercular movements (Clark 1983, Randall et al. 1971). In early studies where brevity-stricken researchers physically tasted ichthyocrinotoxic secretions, it was noted that many, including ichthyocrinotoxins secreted by stonefish, harbour a distinctive bitter taste (Cameron & Endean 1973, Maretzki & Del Castillo 1967). As such, it was hypothesised that ichthyocrinotoxins may deter predation by reducing the palatability of the user, thus functioning as an antifeedant.

Later studies determined that the active components within the ichthyocrinotoxins of numerous fish species, such as grammistin from soapfish, pardaxin from flatfish, and pahutoxin from boxfish, are amphipathic surfactants (Primor et al. 1978, Shiomi et al. 2000). Furthermore, the gill membranes of predatory species, such as *Squalus acanthias* (Dogfish Shark), reportedly exhibited increased permeability to solutes following exposure to ichthyocrinotoxin from *Pardachirus marmoratus* (Red Sea Moses Sole) (Primor et al. 1980, Primor et al. 1984). As such, it was hypothesised that ichthyocrinotoxins may also serve a defensive role by disrupting the physiological processes of their target. Specifically, the surfactant components likely function by forming pores in gill membranes and incapacitating the osmoregulatory capabilities of the target (Primor et al. 1980, Shiomi et al. 2000).

Despite limited research, stonefish ichthyocrinotoxins present an intriguing case. Unlike ichthyocrinotoxins secreted by other fish taxa, stonefish ichthyocrinotoxins reportedly exhibit limited toxicity to fish (i.e. blennies and mosquito fish) and vertebrate cell lines (i.e. red blood cell erythrocytes) (Cameron et al. 1981). Conversely, it immobilised ciliated protozoans and mussel gill cilia, as well as increased the tone of barnacle scutorum rostralis muscle (Cameron et al. 1981). This suggests that stonefish ichthyocrinotoxins likely serve a different function than disrupting the physiology of vertebrate predators. However, whether they perform an antifeedant function is currently unknown. Therefore, the aim of this study was to determine whether stonefish ichthyocrinotoxins play a role in predator defence by reducing the animal's palatability to predators. Although limited data exists on the natural predators of stonefish across all life stages, potential predators reportedly include sharks, stingrays and eels (Harris et al. 2021), and likely also encompass octopus, sea snakes, morays and opportunistic predatory fish (such as lionfish). This study quantified the effect of ichthyocrinotoxins from *Synanceia horrida* (Estuarine Stonefish) and *Synanceia verrucosa* (Reef Stonefish) on the feeding behaviours of two potential predators of stonefish: lionfish and morays.

MATERIALS AND METHODS

Animal husbandry

Thirty specimens, comprising five *S. verrucosa*, five *S. horrida*, and five *Diploprion bifasciatum* (Barred Soapfish), as well as three *Dendrochirus zebra* (Zebra Lionfish) and twelve *Gymnothorax pseudothyrsoideus* (Highfin Moray) were collected from the wild by local commercial fish suppliers — Cairns Marine and Monsoon Aquatics — within the Cairns region (Queensland, Australia) under James Cook University Animal Ethics Committee approval (#A2848). While limited data on the natural predators of stonefish are currently available, a documented incidence revealed predation by morays (National Geographic 2018). Lionfish are

also well known to predate on a wide range of fish species (Batjakas et al. 2023, Eddy et al. 2016, Morris & Akins 2009). In addition, the predators *D. zebra* and *G. pseudothyrsoideus* were selected for this study due to their generalist, opportunistic and voracious feeding behaviours, as well as their overlapping distribution with both stonefish species. All species used in this study will now be referred to by their common names to improve readability.

Animals were relocated to the eduQuarium at James Cook University (JCU) Nguma-bada campus in Cairns, Queensland. All specimen aquariums were connected to a 65,000 L recirculating saltwater system maintained with the following water quality parameters: pH 8.3 ± 0.1; salinity 35‰ ± 0.5‰; temperature 27ºC ± 1ºC; with nitrate and nitrite levels between ~0 to 0.1 mg/L; and dissolved oxygen above 90% saturation. Stonefish were housed in small groups (three animals) in 350 L tanks with an exchange rate of 500 L/hour. Soapfish were housed individually in 130 L tanks with an exchange rate of 220 L/hour. Lionfish were housed in groups of three in 150 L tanks with an exchange rate of 500 L/hour. Morays were housed individually in 200 L tanks with an exchange rate of 600 L/hour. The photoperiod within each tank was maintained as a 12:12 day:night cycle, turning on at 06:00 and off at 18:00. Animal welfare was monitored daily and tank conditions were regularly maintained by aquarium staff. Stonefish and soapfish were acclimated to the aquarium conditions for a minimum of two weeks before undergoing toxin extraction. Lionfish and morays were acclimated to the aquarium conditions for one month prior to commencing the experiment.

Toxin extraction

Extracting stonefish ichthyocrinotoxin

Stonefish ichthyocrinotoxins were collected from three to five mature individuals under James Cook University Animal Ethics Committee approval (approval #A2848). The sex of each individual was unable to be determined. The extraction of stonefish ichthyocrinotoxins followed the protocol outlined by Lennox-Bulow et al. (2023b). In brief, an individual stonefish was transferred to a saltwater-dampened surface and secured by gently holding either side of the pectoral fins. A saltwater-dampened cotton cloth was placed over the animal's eyes to minimise stress throughout the process. Ichthyocrinotoxin was then extracted from the skin tubercles using targeted mechanical massage under a controlled vacuum. This vacuum was generated by a purpose-built extraction apparatus comprised of a 100 mm segment of clear vinyl tubing affixed to a 50 mL syringe. To account for potential individual variation in toxin composition and activity, stonefish ichthyocrinotoxin was extracted and pooled from three to five mature individuals of each species. Pooled ichthyocrinotoxin samples were stored in 1.5 mL Eppendorf tubes at -80°C until required.

Extracting soapfish ichthyocrinotoxin

Soapfish ichthyocrinotoxins were collected from two to three mature individuals under James Cook University Animal Ethics Committee approval (approval #A2848). The sex of each individual was unable to be determined. To induce toxin release, each soapfish was removed from its holding tank and gently massaged unidirectionally along its lateral line from head to caudal fin for a maximum of 30 seconds. The expelled ichthyocrinotoxin was drip-collected in a clean plastic weigh boat (150 x 150 mm) that was placed underneath the animal. To account for potential individual variation in toxin composition and activity, soapfish ichthyocrinotoxin was extracted and pooled from two to three individuals. Pooled ichthyocrinotoxin samples were stored in 1.5 mL Eppendorf tubes at -80°C until required.

Reversed Phase-High Performance Liquid Chromatography Analysis of ichthyocrinotoxin adhesion to prawn

To ensure that the predators would be exposed to ichthyocrinotoxin during the feeding experiment, toxin adhesion to deshelled prawn fillet following a simulated feeding event was quantified using Reversed Phase-High Performance Liquid Chromatography (RP-HPLC). In brief, deshelled prawn fillets (~5 x 5 mm) were pat dry with a piece of paper towel and then immersed in the crudest obtainable samples of Estuarine Stonefish, Reef Stonefish, or Barred Soapfish ichthyocrinotoxin for five minutes (n = 1 prawn fillet per toxin). This was performed in a Ziploc bag containing enough crude ichthyocrinotoxin to completely submerge each prawn fillet. To simulate a feeding event

(treatment conditions), each toxin-laced prawn fillet was submerged in an animal-free 250 L saltwater aquarium tank for 30 seconds. After treatment, fillets were placed in a 1:1 buffer mixture of solvent A (250 µL H_2O/0.05% trifluoroacetic acid (TFA; Auspep): solvent B (250 µL 90% ACN (Merck)/10% H_2O/0.045% TFA). An additional prawn fillet was soaked in seawater (prawn-only control) which was later used for prawn contaminant identification. Furthermore, three 'native toxin' controls (10 µL aliquots of each ichthyocrinotoxin in 500 µL 1:1 A:B buffer solution) were prepared. All samples were then refrigerated (4°C) overnight. The following day, prawn pieces were removed, and samples were centrifuged at 4°C, 15,000 rpm for 10 min to remove debris. The resulting supernatants were then transferred to new 1.5 mL Eppendorf tubes. Sample supernatants (10 µL) were analysed using a C18 Phenomenex Aeris® PEPTIDE XB liquid chromatography column (250 x 10 mm, 5 µm, 100 Å). The separation used a gradient of 0–60% solvent B in 60 min, 60–90% solvent B in 5 min, 90% solvent B for 10 min, and 90–0% solvent B in 5 min. The flow rate remained constant at 0.250 mL/min. The absorbance at 214 nm was continuously monitored over 80 mL of the eluant.

Raw absorbance data (collected by OpenLAB CDS ChemStation v1.8.1) was processed to remove prawn component contaminants from the toxin-laced treatment conditions groups. This was achieved by subtracting the absorbance values from the 'prawn-only control' at each elution volume from the corresponding values in the 'treatment conditions' data. Chromatograms were generated in GraphPad Prism 10. To quantify the retention of ichthyocrinotoxin under the treatment conditions against the native toxin, the area under the curve was calculated for each species. The area under the curve was quantified for each 'treatment conditions' and 'native toxin' trace by multiplying the difference between each consecutive x-value by the average of the respective y-values and summing the products. The percentage of toxin retained following exposure to the treatment conditions was calculated by dividing the total area of the 'treatment conditions' trace for each species by the total area of the corresponding 'native toxin' trace and multiplying this by 100.

Monitoring the feeding behaviour of predators

The palatability of stonefish ichthyocrinotoxins to potential predators was assessed by monitoring the feeding behaviours of lionfish and morays following exposure to toxin-laced food. Predators (n = 3 lionfish and n = 12 morays) were acclimated to a regular feeding regime (daily for lionfish and every three days for morays) of deshelled prawn fillets, which constituted a normal diet, for one month to establish baseline feeding behaviours. Following acclimation, predators were presented with four food types: 1) untreated prawn fillet (no toxin, used as an indicator for high palatability); 2) prawn fillet laced with Barred Soapfish ichthyocrinotoxin (a known defensive toxin, used as an indicator for low palatability); 3) prawn fillet laced with Estuarine Stonefish ichthyocrinotoxin; and 4) prawn fillet laced with Reef Stonefish ichthyocrinotoxin. Each food type was offered once per feeding event. Toxin-lacing the food involved patting the prawn fillets dry with a paper towel, then soaking them in the crudest obtainable samples of ichthyocrinotoxin for five minutes. The soaking was carried out in a Ziploc bag containing enough crude ichthyocrinotoxin to completely submerge the prawn fillet. During feeding events, appropriately sized prawn pieces (5 x 5 mm ± 2 mm for lionfish and ~10 mm length pieces ± 5 mm for morays) were skewered onto a 60 cm long ± 3 mm diameter dowel rod (feeding stick) and presented to a predator for 30 seconds.

Predators that completely consumed the prawn fillet within 30 seconds were recorded as 'ate'. Predators that did not attempt to consume the food (full food refusal) or expelled the food after tasting (partial food refusal) within 30 seconds were recorded as 'did not eat'. Following toxin exposure, predators were returned to their regular diet (untreated prawn fillet) for at least two feedings, or until feeding behaviours returned to baseline. Additionally, predators were monitored closely for 24 hours after each toxin-laced feeding event to ensure there were no adverse physical and/or behavioural effects. This process was repeated until each predator had been offered each food type at least three times.

The proportion of feeding attempts where each individual predator ate the food offering was calculated across all feeding attempts for each food treatment. These proportions were arcsine square root transformed to normalise the data prior to analysis. A two-way Analysis of Variance (ANOVA) was conducted to determine the effect of food treatment (no toxin, or food laced with ichthyocrinotoxin from Estuarine Stonefish, Reef Stonefish and Barred Soapfish) and predator species (Zebra Lionfish, Highfin Moray) on food acceptance rates. Following a significant ANOVA result, a post-hoc least significant difference (LSD) test was used to identify specific food treatments that significantly influenced food acceptance rates. Statistical analyses were performed in IBM SPSS version 27, and figures were generated using Graph Pad Prism version 10.4.0.

RESULTS

Toxin present on prawn fillet following a simulated feeding event

RP-HPLC revealed a high degree of similarity between the absorbance profiles of native toxin (ichthyocrinotoxin extracted directly from the animal) and treatment conditions (ichthyocrinotoxin coated on prawn fillets and submerged in an aquarium for 30 seconds) for Barred Soapfish, Estuarine Stonefish, and Reef Stonefish ichthyocrinotoxin (Figure 1). For Barred Soapfish, the treatment conditions profile showed good consistency with the native toxin for peaks eluting around 1 minute, 45 minutes, and between 55–60 minutes retention time (Figure 1A). However, lower absorbances were observed in the treatment conditions profile for peaks between 40–44 minutes, 46–54 minutes, and 61–70 minutes, potentially suggesting a weaker adherence of these components onto the prawn fillet. Similarly, both Estuarine Stonefish (Figure 1B) and Reef Stonefish (Figure 1C) treatment conditions profiles displayed a high fidelity to the native toxin up to approximately 50 minutes retention time. After 50 minutes, peaks present in the native toxin were absent in the treatment conditions profile of Reef Stonefish, and less consistent in the treatment conditions profile for Estuarine Stonefish, indicating that highly hydrophobic components may have a weaker adherence to the prawn fillet. Furthermore, area under the curve calculations revealed that ichthyocrinotoxins from Estuarine Stonefish (65.5% adhesion) and Reef Stonefish (45% adhesion) exhibited a greater adhesion to prawn fillet following exposure to treatment conditions than Barred Soapfish ichthyocrinotoxin (27% adhesion). However, the adhesion of Reef Stonefish ichthyocrinotoxin on prawn fillet following immersion was approximately 20% less than Estuarine Stonefish ichthyocrinotoxin.

The effect of ichthyocrinotoxin on the feeding behaviours of predators

There was no significant interaction between food treatment (no toxin, or food laced with ichthyocrinotoxin from stonefish or soapfish) and predator species (Highfin Moray and Zebra Lionfish) on food acceptance rates (Figure 2, $F_{3, 52} = 1.08$, $p = 0.36$). There was also no significant difference in food acceptance rates between the two predator species ($F_{1, 52} = 1.99$, $p = 0.16$). However, food treatment was found to have a significant effect on the rate of food acceptance ($F_{3, 52} = 65.86$, $p < 0.001$). Both predator species exhibited a strong aversion to food laced with Barred Soapfish ichthyocrinotoxins (less than 10% acceptance). In contrast, predators readily accepted food laced with ichthyocrinotoxins from Reef and Estuarine Stonefish (over 85% acceptance), which was comparable to the no toxin control group (over 95% acceptance).

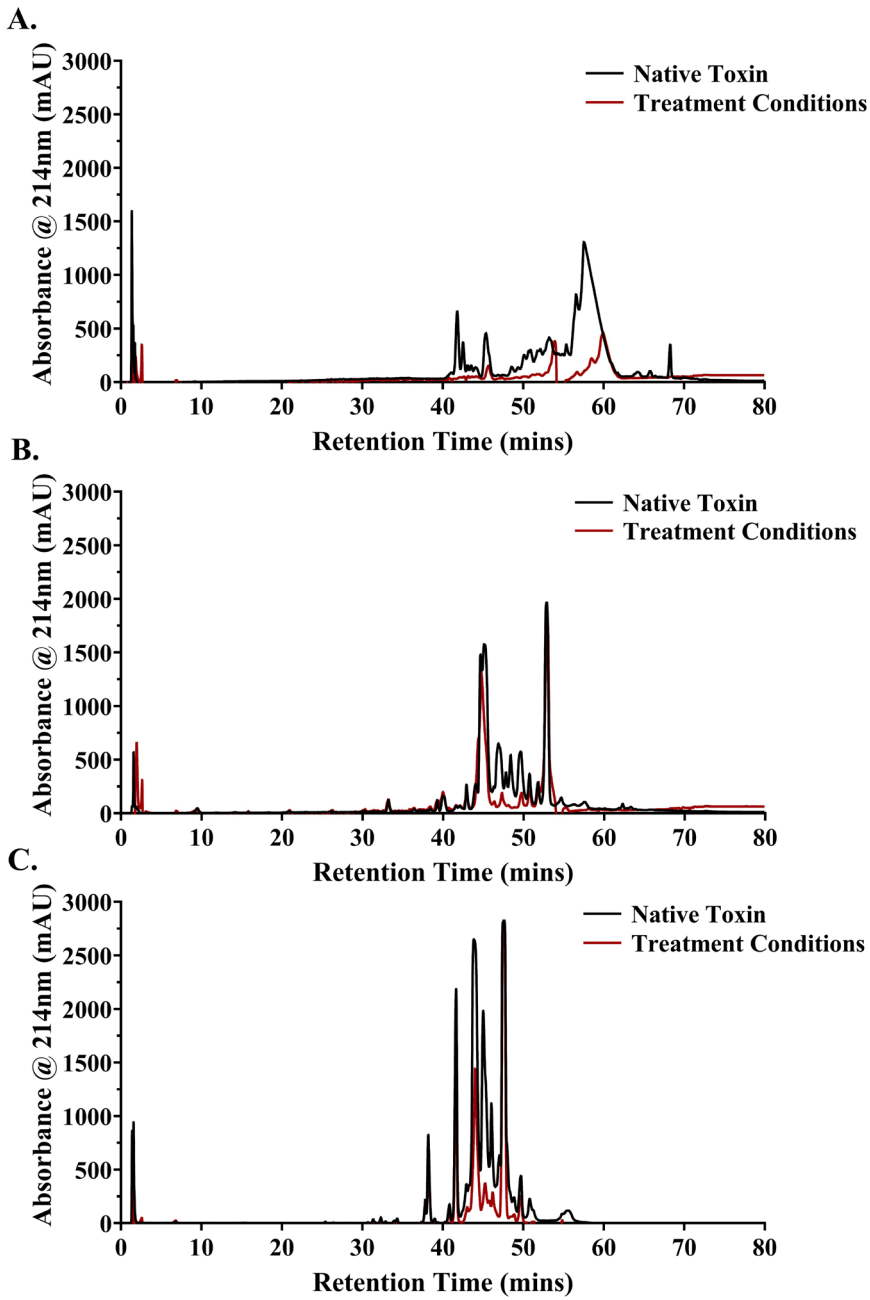

Figure 1. The adhesion of ichthyocrinotoxins on prawn fillet following a simulated feeding event. RP-HPLC was used to profile ichthyocrinotoxin samples from Barred Soapfish (*Diploprion bifasciatum*, Panel A), Estuarine Stonefish (*Synanceia horrida*, Panel B), and Reef Stonefish (*Synanceia verrucosa*, Panel C). For each species toxin, the absorbance profiles of two sample types were compared: 1) native toxin (—), unprocessed ichthyocrinotoxin extracted directly from the animal; and 2) treatment conditions (—), ichthyocrinotoxin that had been coated on a prawn fillet and submerged in an aquarium for 30 seconds (a simulated feeding event). Peaks corresponding to prawn components were removed from the treatment conditions profiles for better visualisation of ichthyocrinotoxin components. Absorbance was monitored at 214 nm.

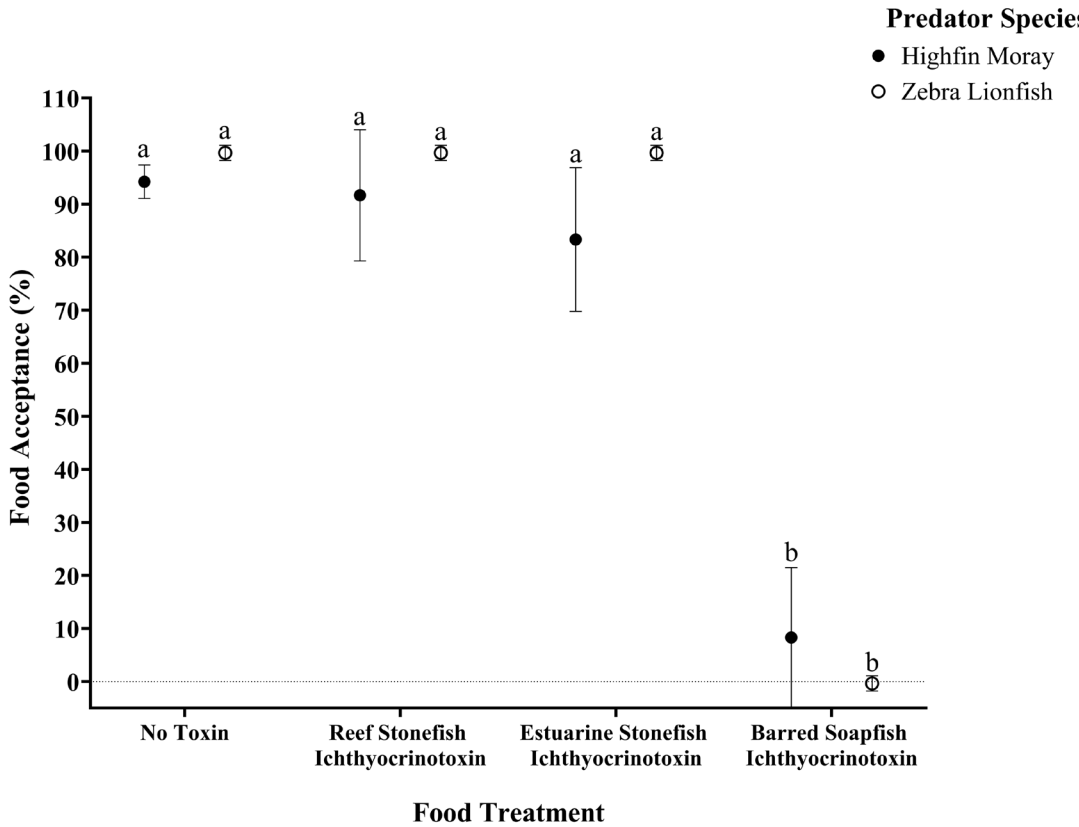

Figure 2. The palatability of ichthyocrinotoxins to predators. Highfin Morays (*Gymnothorax pseudothyrsoideus*, •, *n* = 12) and Zebra Lionfish (*Dendrochirus zebra*, ◦, *n* = 3) were offered untreated prawn fillet (no toxin), or prawn fillet laced with ichthyocrinotoxin from Reef Stonefish (*Synanceia verrucosa*), Estuarine Stonefish (*Synanceia horrida*), or Barred Soapfish (*Diploprion bifasciatum*). Food acceptance (%) was determined based on proportion of feeding events where predators consumed the food offering within 30 seconds. Letters (a–b) represent statistically significant post-hoc LSD groupings. Error bars represent 95% CI.

DISCUSSION

Previous studies have shown that ichthyocrinotoxin from *S. horrida* exhibited minimal ichthyotoxic activity against blennies, *Petroscirtes japonicus* (synonym for *Omobranchus punctatus*) and mosquito fish (*Gambusia affinis*) (Cameron et al. 1981). This led to the suggestion that stonefish ichthyocrinotoxins are unlikely to function as a predator deterrent or repellent, unless their bitter taste renders them unpalatable (Cameron et al. 1981). The present study found that the feeding behaviours of Zebra Lionfish (*D. zebra*) and Highfin Moray (*G. pseudothyrsoideus*) were unchanged by ichthyocrinotoxin from either Reef (*S. verrucosa*) or Estuarine Stonefish (*S. horrida*). Ichthyocrinotoxins from both *S. horrida* and *S. verrucosa* were highly palatable (>85% food acceptance) to both predator species, comparable to untreated food (>94% food acceptance). Additionally, no adverse effects on the predators were observed following exposure to stonefish ichthyocrinotoxins, indicating that they may be non-toxic to these predator species. In contrast, food coated in Barred Soapfish (*D. bifasciatum*) ichthyocrinotoxin, a known antifeedant, prompted high rejection rates (>90% rejection) from both predators. These findings demonstrate that stonefish ichthyocrinotoxins do not function as an antifeedant against these

predator species. Moreover, the findings support the hypothesis that stonefish ichthyocrinotoxins likely do not play a major role in predator defence, acting as neither a defensive toxin nor an antifeedant.

While the primary function of ichthyocrinotoxins secreted by other well-studied fish taxa (soapfish, boxfish and flatfish) is predator defence (Clark 1983, Maretzki & Del Castillo 1967, Thomson 1969), stonefish ichthyocrinotoxins appear to be an anomaly. Furthermore, although ichthyocrinotoxins typically exhibit proteolytic and/or haemolytic activity towards vertebrates (Abdul-Haqq & Shier 2008, Indumathi & Khora 2013, Maretzki & Del Castillo 1967), stonefish ichthyocrinotoxins were found to lack proteolytic action and possess only mild haemolytic properties (Cameron et al. 1981). Another markable distinction between stonefish and other ichthyocrinotoxin-producing taxa is stonefish being equipped with an injectable toxin (i.e. venom). In contrast to their ichthyocrinotoxin, stonefish venom is highly noxious to vertebrates and undeniably serves in predator defence (Endean 1961, Saggiomo et al. 2021). Some evolutionary theories suggest that fish venoms may have evolved from the ability to produce a toxic glandular secretion, such as ichthyocrinotoxin (Cameron & Endean 1973). The rationale is that it is likely a more effective defensive strategy to inject toxin directly into a threat, rather than secrete one into an environment of infinite dilution (Cameron & Endean 1973). This might suggest that the evolutionary trajectory of stonefish ichthyocrinotoxins has diverged from that of other fish species, leading to a unique function, and, though unlikely, a vestigial role in predator defence.

Although the results of this study suggest that it is unlikely that stonefish ichthyocrinotoxins serve in predator defence, they may still serve a defensive function. Stonefish are devoid of scales and readily bury themselves into the substratum (Endean 1961). Consequently, their skin is presumably highly susceptible to a variety of benthic fauna, particularly invertebrate taxa like parasites and fouling animals. Previous studies showed that ichthyocrinotoxin from *S. horrida* immobilised ciliated protozoans and mussel gill cilia, as well as increased the tone of barnacle scutorum rostralis muscle (i.e. caused muscle contraction) (Cameron et al. 1981). Additionally, very few internal parasite taxa have currently been identified within members of *Synanceia* (Miller et al. 2018). This suggests that one potential function of stonefish ichthyocrinotoxins may be to provide a defensive barrier against skin incursions from invertebrate taxa. Preliminary investigations suggest that another potential function of stonefish ichthyocrinotoxins may be to facilitate the growth and/or attachment of algae on to their skin (Barnett 2014). Algal recruitment onto the skin may serve to enhance the animal's camouflage, thereby reducing its likelihood of being discovered by predators and prey, while also potentially luring herbivorous prey into their ambush range. However, further research is required to determine whether stonefish ichthyocrinotoxins play a role in integumentary defence and/or aid in algal recruitment or growth.

Stonefish ichthyocrinotoxins also exhibited a greater adhesion to prawn fillets (65% retention for *S. horrida* and 45% retention for *S. verrucosa*), compared to soapfish ichthyocrinotoxin (27% retention). This differential adhesion may be further indicative of the distinct biological roles of these toxins. Soapfish are known to employ a rapid-release strategy, secreting copious amounts of their ichthyocrinotoxin when threatened (Randall et al. 1971). Previous studies report that the active component within soapfish (family Grammistidae) ichthyocrinotoxins, called grammistin, is a surfactant peptide (Onuki et al. 1993, Oshima 1974). Biological surfactants facilitate the mixing of different substances by reducing their interfacial tension (Otzen 2017). Therefore, the weaker adhesion of soapfish ichthyocrinotoxin may be attributed to the surfactant properties of one or more of its components. These properties likely support the function of soapfish ichthyocrinotoxin in predator defence by facilitating its rapid dispersal in seawater (and specifically within the buccal cavity of aquatic vertebrate predators). In contrast, stonefish do not secrete large quantities of ichthyocrinotoxin when threatened. This might suggest that stonefish ichthyocrinotoxins provide localised protection, making it advantageous to secrete a toxin with greater adhesive properties.

Interestingly, the adhesive strength of stonefish ichthyocrinotoxins to prawn fillet also appeared

to differ between species. Ichthyocrinotoxins from Estuarine Stonefish seemingly exhibited a greater adhesion to prawn fillet (65% retention) compared to those from Reef Stonefish (45% retention). Recent work elucidated that the composition of stonefish ichthyocrinotoxin varies between these two species (Lennox-Bulow et al. 2023b). As such, it is possible that the different adhesive properties of stonefish ichthyocrinotoxins between species may be due to variations in their chemical composition. However, further research is needed to support these observations, as well as determine whether interspecific differences in the physical properties of stonefish ichthyocrinotoxins might reflect functional and/or mechanistic adaptations.

In conclusion, the findings of this study suggest that stonefish ichthyocrinotoxins likely do not play a major role in predator defence. Furthermore, differences in the adhesive properties of ichthyocrinotoxins between stonefish and soapfish, as well as among stonefish species, potentially reflect distinct ecological functions or application mechanisms. Future research should focus on the potential defensive role of these toxins against invertebrate taxa that are likely to interact with the animal's skin, such as parasites and fouling animals. The findings of this study have expanded our understanding of the toxin ecology of stonefish and highlight potential avenues for future research that may lead to the discovery of novel therapeutics or industrial solutions for utilitarian purpose.

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