Invasive Caribbean lionfish: ecological impacts, behavioural interactions and habitat use



Christina L. Hunt

St Anne's College

University of Oxford

A thesis submitted for the degree of Doctor of Philosophy

October 2020

Abstract

Lionfish (*Pterois volitans* and *P. miles*) have become invasive across the western Atlantic and are considered one of the most heavily impacting invasive marine vertebrates. Most lionfish research focuses on consumptive effects in small-scale patch reef studies, with little research on their behaviour, non-consumptive effects or regional impacts. Using binary choice laboratory experiments, I found that lionfish were not attracted to conspecific cues. Instead, habitat preference likely drives aggregation formation, as *in situ* observations revealed that lionfish avoid fine-scale complexity but are attracted to broad-scale complexity, such as caves and overhangs. Shelter use by lionfish may impact native shelter-using species, such as commercially important spiny lobsters and ecologically important long-spined sea urchins. I found that lionfish did not affect shelter use by either native species in laboratory experiments, indicating that negative impacts of lionfish from competition for shelter may not be as widespread as feared. Interestingly, lionfish reduced their shelter use in the presence of the native species. I also investigated herbivorous fish grazing as a potential non-consumptive effect of lionfish. By comparing the same small areas of reef with and without lionfish, I found that responses to lionfish varied even within a small reef system, indicating that lionfish presence is not the only driver of the observed changes. The highly variable results of small-scale studies like these highlight the urgent need for regional-scale studies to determine the true extent of lionfish impacts in their invaded range. Prior to this thesis, no research had been conducted into the impacts of lionfish on native fish communities that simultaneously considered sites with different invasion timelines across the western Atlantic. I took a novel approach to understanding large-scale impacts using temporally offset additive mixed models centred on year of first invasion, combining 11,000 transects from eight countries with 14 contextual variables known to affect reef fish communities. I found clear shifts in native fish communities between pre-invasion and two or more years post-invasion, indicating that lionfish invasion rapidly results in a stable change in native fish communities. I found pre-invasion increases in native fish abundance, species richness and diversity that continued in the initial years of the invasion, however, I detected simultaneous declines in all metrics at five years post lionfish invasion. This thesis provides new insights into invasive lionfish

behaviour and non-consumptive effects and provides the first suggestion that lionfish exert region-wide impacts by using a novel approach for studying the large-scale impacts of invasive species.

Declaration of authorship

I declare that the work presented in this thesis is my own, conducted under the supervision of Professor Alex Rogers (University of Oxford), Professor Owen Lewis (University of Oxford), Dr Dan Exton (Operation Wallacea) and Dr Dominic Andradi-Brown (WWF-US). I lead-authored all four data chapters in this thesis and have acknowledged the contributions of all co-authors using CRediT taxonomy in the author contributions section of each chapter. I designed and ran the studies, analysed the data and wrote the manuscripts, whilst co-authors assisted with data collection, statistical advice, supervision and review of the manuscripts. Chapters two, three and four use primary data that I collected during fieldwork whereas chapter five uses secondary data from existing sources. Where I have used existing data sources or quoted and discussed others' work, this has been clearly indicated and referenced.

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Oxford. No part of this thesis has already been accepted, or is concurrently being submitted, for any degree, diploma, certificate or other qualification in this University or elsewhere.

Christina L. Hunt

Student no: 652033

October 2020

Covid-19 impact statement

My fourth chapter (Non-consumptive effects of invasive lionfish on herbivorous fish grazing vary within a reef system) was impacted by the Covid-19 pandemic as my final fieldwork season in Honduras was cancelled due to lockdown and travel restrictions. The work I have presented in chapter four was intended to be a pilot study, prior to the full study being conducted during June-August 2020. Full details of the improved data collection methods that I had planned to implement are in the Covid-19 impact statement in chapter four.

Formatting notes

This thesis is presented as an integrated thesis, with each data chapter prepared as a manuscript. Chapters two and three have already been published and are included as journal offprints in the appendices. Chapter four is presented in the form of a manuscript but is not publishable in its current form because the Covid-19 pandemic prevented data collection in summer 2020. I plan to conduct the full study of chapter four in summer 2021, after which I will update the manuscript and prepare it for submission. Chapter five will be edited and prepared for submission after completion of this thesis.

Funding

This thesis was made possible by funding from the Natural Environment Research Council (Grant number: NE/L002612/1), Operation Wallacea (CASE partner) and St Anne's College, Oxford. I would also like to thank the Fisheries Society of the British Isles for funding chapter three and Somerville College, Oxford for funding my PADI Divemaster training to enable me to collect data during my fieldwork.

Acknowledgements

Firstly, I would like to thank my supervisors. Thanks to Professor Alex Rogers for supervising the beginning of my DPhil and Professor Owen Lewis for supervising the end. Thanks to Dr Dan Exton for sticking with me the whole way and providing me with endless advice and encouragement! I also want to say a special thank you to Dom Andradi-Brown ─ thanks for always being there for me and offering to help even when you didn't need to- I really appreciate it!

Secondly, I would like to say a massive thank you to all the Operation Wallacea students and staff who assisted with my data collection in Tela, Honduras in 2017-2019. I'd like to say a particularly big thank you to Master's students George Kelly and Eilidh McCracken from 2017, the 'lionfish ladies' (Charley Clubley, Emma Cox, Georgia Northway, Kate Foster, Nia Jones, Nicola Cooke, Rachel Stirling) from 2018 and my lab buddies Callum Hudson and Frankie Noades from 2019. One more massive thank you goes to Josh Bennett-Williams for conducting all the scuba diving data collection in 2019 when I wasn't able to dive. A big thanks also to Antal Borcsok for allowing me to conduct my fieldwork at the Tela Marine Research Centre. To everyone I spent time with in Tela, thank you so much for the most amazing time of my life!

Last but by no means least, I want to say a huge thank you to my Mum Helen and my sister Annabel. Thank you for your support, encouragement, laughter and positivity throughout my DPhil and always.

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1 Introduction

1.1 Invasive species

Terms such as non-native, alien, exotic and non-indigenous are often used interchangeably with the term invasive species, but these are not synonymous (Colautti and MacIsaac, 2004). Only a sub-set of non-native species meet the criteria to be defined as invasive species. In some studies, a species is referred to as invasive if it spreads beyond the area of introduction, becomes locally abundant or spreads into natural areas (Lodge et al., 2006). Similarly, Colautti and MacIsaac (2004) describe a non-native species as an invasive when it becomes widespread, numerically abundant, or both. Other definitions include criteria such as ecological impact (McGeoch et al., 2012), economic impact (Mooney, 2005) or public health impact (Beck et al., 2008). Some definitions consider both the positive and negative impacts of non-native species and only define a species as invasive if the negative impacts outweigh the positive impacts (Beck et al., 2008). It has been argued that the definition of invasive can also apply to native species that have undergone population increases and spread because of anthropogenic changes to their environment (Valery et al., 2009).

Invasive species have frequently been reported as emerging issues for global conservation and biological diversity (Sutherland et al., 2017, Sutherland et al., 2010, Sutherland et al., 2020). Despite increasing awareness of invasive species both in scientific publications (Geraldi et al., 2019) and in the media (Colautti and MacIsaac, 2004), the rate of new invasive species records is continuing to increase, with little sign of slowing down (Seebens et al., 2017). Closer examination of invasive species records shows a continuing increase in the rate of new records for species that are introduced accidentally, but a recent reduction in the rate for mammals, likely due to stricter regulations preventing intentional releases (Seebens et al., 2017). There has also been a reduction in the rate of new invasive fish records globally, however, the rate of new fish records is continuously increasing in Europe, which may be partially driven by Lessepsian migration through the Suez canal (Seebens et al., 2017). A study on a subset of countries found a range of 9-222 invasive species per country

(McGeoch et al., 2012), however, uncertainty in invasive species recording often leads to underestimates of invasive species numbers (McGeoch et al., 2012).

The negative impacts of invasive species are varied and can include impacts on the environment, economy and public health (Beck et al., 2008). Environmental effects can be partitioned into consumptive (direct) effects and non-consumptive (indirect) effects. Consumptive effects are where a predator feeds on a prey item, whilst non-consumptive effects include competition for resources such as food (Human and Gordon, 1996) and shelter (Gherardi and Daniels, 2004), changes in prey behaviour due to fear effects (Kindinger and Albins, 2017), hybridisation (Huxel, 1999) and changes in ecological processes such as fire regimes (Knapp, 1996). These consumptive and non-consumptive effects can exert knock-on effects on other species, with the intermediate species referred to as the transmitter species (Preston et al., 2012). Changes that are mediated through a transmitter species are also called non-consumptive effects and are classified as density-mediated or trait-mediated. Density-mediated effects occur when the invasive species changes the density of the transmitter species through consumptive effects, whilst trait-mediated effects occur when the invasive species changes any other character of the transmitter species through non-consumptive effects (Abrams et al., 1996). Economic effects of invasive species are varied and include reduced stocks of native species for harvesting (Oguz et al., 2008), reduced agricultural yields (Eagle et al., 2007), reduced house prices (Zhang and Boyle, 2010) and costs of invasive species management (Pimentel et al., 2005). The final type of effect ─ public health effects ─ are caused by invasive pathogens such as West Nile Virus, a neuropathogen that was introduced to North America in 1999 (Campbell et al., 2002). Although the impacts of each invasive species are often considered in isolation, the invasion of one species may facilitate the invasion of another species, in a process termed invasional meltdown (Simberloff and Von Holle, 1999).

However, invasive species can also have positive impacts (Rodriguez, 2006). Invasive species can facilitate native species by creating or beneficially modifying habitat (Bially and MacIsaac, 2000), acting as a food source (Cadée, 2001), pollinating native species (Cox, 1983), releasing native species

from competition by predating a native competitor species (Grosholz et al., 2000) and releasing native species from predation by predating a native predator (Crossland, 2000). Invasive species can also benefit humans by providing a food source for harvesting (Chapman et al., 2016) and increased employment in invasive species management (Bax et al., 2003).

Invasive species have been documented in 84% of the world's marine ecoregions (Molnar et al., 2008). Marine species can be introduced to new areas through a variety of vectors, including transport in ship ballast water, intentional release for aquaculture or fisheries, accidental or intentional release of aquarium species and transport on floating anthropogenic debris (Bax et al., 2003). To date, the only successful eradications of marine invasive species have involved species with a small invaded range (Anderson, 2005, Culver and Kuris, 2000, Ferguson, 1999). For invasive species with larger invaded ranges, complete eradication is considered unlikely (Barbour et al., 2011, Ruesink and Collado-Vides, 2006).

In a global study of marine invasive species, 57% of species included in the study were considered ecologically harmful because they disrupt multiple species or entire ecosystems (Molnar et al., 2008). Bax et al. (2003) suggested that marine invasive species can engineer their habitat in three ways: predate native species, smother existing habitat or create new habitat. However, these three categories are only a sub-set of possible impacts, as marine invasive species have the potential to exert any of the negative or positive effects discussed previously.

1.2 Invasive lionfish (*Pterois* spp.)

The Scorpaenidae family contains 65 genera and 454 species (Nelson et al., 2016). Some characteristics of the family include a compressed body, ridges or spines on the head, and spines on the dorsal, pelvic and anal fins (Nelson et al., 2016). The genus of concern in this thesis is *Pterois*, which contains 12 species (Wilcox et al., 2018). Within the *Pterois* genus are two species that have become invasive: *Pterois volitans* and *Pterois miles* (Betancur-R et al., 2011). Although it was

initially suggested that these two species could be distinguished meristically (Schultz, 1986), these meristic counts are not definitive and individuals with mixed meristics have been found (Freshwater et al., 2009). A recent genetic study suggested that *P. volitans* is a hybrid of *P. miles* and a Pacific *Pterois* lineage comprised of *P. lunulata/russelii* (Wilcox et al., 2018), however, other genetic studies recognise *P. volitans* and *P. miles* as two distinct species (Betancur-R et al., 2011, Freshwater et al., 2009). In their native range, *P. volitans* is restricted to the Pacific ocean and *P. miles* is restricted to the Indian ocean and Red sea, but the ranges of the two species overlap in the Indonesian region (Kulbicki et al., 2012). Most studies on invasive lionfish in the western Atlantic do not identify the individuals to species level, thus the two species are collectively referred to as lionfish in the literature and will be referred to as lionfish throughout this thesis.

Lionfish were first detected in the western Atlantic off the coast of Florida in the 1980s, but there were no further sightings until 1992 (Schofield, 2009). From the year 2000 onwards, lionfish began to spread more rapidly with sightings as distant as North Carolina and Costa Rica by 2009 (Schofield, 2009). Following this rapid spread, lionfish were recognised as a global conservation issue in 2010 (Sutherland et al., 2010). Lionfish are now widespread throughout the western Atlantic (Schofield, 2010) and a single individual has even been recorded from southern Brazil (Ferreira et al., 2015). Genetic studies have shown that *P. miles* is restricted to Bermuda and the east cost of the USA whilst *P. volitans* is found throughout the invaded range (Betancur-R et al., 2011). Lionfish are thought to have been introduced to the western Atlantic through aquarium releases (Betancur-R et al., 2011, Whitfield et al., 2002), the suggested cause of the numerous sightings of non-native ornamental fish species off the coast of Florida (Semmens et al., 2004).

Lionfish have also become invasive in the Mediterranean, with the first sighting occurring in Israel 1991 (Golani and Sonin, 1992). No more sightings were recorded until 2012, when two lionfish, genetically confirmed as *P. miles*, were collected off the coast of Lebanon (Bariche et al., 2013). Although a modelling study in 2014 suggested that a Mediterranean invasion was unlikely to occur (Johnston and Purkis, 2014), lionfish are now found across the eastern Mediterranean (Yapici, 2018)

and have been found as far west as Tunisia (Dailianis et al., 2016). The Mediterranean invasion is likely to have resulted from Lessepsian migration from the Red Sea through the Suez canal (Bariche et al., 2017).

There are many potential explanations for the success of invasive lionfish. Some relate to lionfish biology, including a high reproductive rate (Cote et al., 2013, Gardner et al., 2015) and low susceptibility to parasites (Sellers et al., 2015, Sikkel et al., 2014), although recent evidence suggests that lionfish may be susceptible to an ulcerative skin disease (Harris et al., 2020a). Lionfish are generalist predators (Peake et al., 2018) and can alter their diet based on prey availability (Malpica-Cruz et al., 2019). Lionfish are also habitat generalists (Barbour et al., 2010, Biggs and Olden, 2011, Dahl and Patterson, 2014) and can even tolerate low salinity (Jud et al., 2015), which allows them to colonise most marine and estuarine habitats and may allow them to cross dispersal barriers, such as the Amazon-Orinoco plume (Jud et al., 2015). Naivety of native fish towards lionfish (Anton et al., 2016, Kindinger, 2015) may contribute to lionfish success by enabling higher prey consumption rates than native predators (Albins, 2013). Lionfish are a novel predator in relation to both their colouration and hunting method. Their colouration and long fin rays are thought to either provide camouflage or masquerade mimicry, where native species may misidentify them as algae or crinoids (Albins and Hixon, 2013). Lionfish flare their pectoral fins, undulate their dorsal spines and sometimes blow jets of water at their prey whilst hunting (Albins and Lyons, 2012), all of which are unlike any other native predator. Another factor contributing to the success of lionfish is the lack of predators in the invasive range (Cote et al., 2013), with the exception of humans (Cote et al., 2014). More recently, the behaviourally-mediated learning ability of lionfish has been suggested as contributing to their invasion success (DeRoy et al., 2020).

Current lionfish management relies predominantly on culling by volunteer divers and snorkellers using hand nets and pole spears (Green et al., 2017) and is thus restricted to shallow water (<30 m, Andradi-Brown et al., 2017a). Although lionfish culling is effective at reducing lionfish densities (de Leon et al., 2013, Green et al., 2017) and allowing native fish populations to recover (Green et al.,

2014), it does have some limitations. Culling by humans can induce fear effects in lionfish, causing them to hide deeper in the reef and become more wary of humans (Andradi-Brown et al., 2017a, Cote et al., 2014), which may make finding and culling lionfish more difficult (Cote et al., 2014). An additional limitation of culling is that it only targets a narrow band of lionfish habitat, as lionfish have been observed down to a maximum depth of 300 m (Gress et al., 2017). In Honduras, mesophotic lionfish populations contain larger individuals and more actively spawning females than shallow populations (Andradi-Brown et al., 2017a), suggesting that the most reproductively mature individuals are not being removed by standard culling programmes. Alternatives to culling, including lionfish traps (Harris et al., 2020b) and lionfish removal robots (Sutherland et al., 2017), are being trialled to expand the depth limits of culling and reduce the time and effort required to remove lionfish. Although lionfish management currently relies on volunteers, there is increasing interest in the development of commercial lionfish fisheries (Bogdanoff et al., 2021, Chapman et al., 2016).

1.3 Ecological impacts of invasive lionfish

The ecological impacts of lionfish have, to date, been studied using a combination of *in situ* experiments (Ellis and Faletti, 2016, Ingeman, 2016, Layman et al., 2014), laboratory experiments (Eaton et al., 2016, Marsh-Hunkin et al., 2013, Trehern et al., 2020) and field observations (Elise et al., 2015, Hackerott et al., 2017, Lesser and Slattery, 2011). Most *in situ* experiments focus on small patch reefs (Albins, 2013, Albins and Hixon, 2008, Ingeman, 2016) or artificial reefs (Dahl and Patterson, 2014, Harris et al., 2019), however, some observational studies have been conducted at the scale of whole reefs (Elise et al., 2015, Hackerott et al., 2017) or sub-regions (Ballew et al., 2016). The consumptive effects of lionfish on native fish are well studied (Albins and Hixon, 2008, Albins and Hixon, 2013, Green et al., 2012) but the consumptive effects on invertebrates and the non-consumptive effects on fish and invertebrates are not well studied (but see Eaton et al., 2016, Kindinger and Albins, 2017, Layman et al., 2014 and Trehern et al., 2020 for exceptions).

The consumptive effects of lionfish are widespread across prey species due to the generalist diet of lionfish, with 128 fish species recorded from lionfish stomach contents (Peake et al., 2018) and a range of invertebrates including crustaceans (Eddy et al., 2016), ophiuroid echinoderms (Munoz et al., 2011) and cephalopod (Dahl and Patterson, 2014), gastropod (Sancho et al., 2018) and bivalve molluscs (Aguilar-Medrano and Vega-Cendejas, 2020). Lionfish have even been observed predating a previously undescribed species of goby (Tornabene and Baldwin, 2017). Teleost fish generally comprise the bulk of lionfish diet (Layman and Allgeier, 2012, Morris and Akins, 2009) and the importance of teleost fish in the diet increases with lionfish size (Eddy et al., 2016, Sancho et al., 2018).

Lionfish cause greater reductions in native prey than a similarly-sized native predator (Albins, 2013). This may be driven by prey naivety, where prey display ineffective anti-predator behaviour because they have not evolved with the invasive predator (Sih et al., 2010). Prey naivety has been recorded in damselfish, parrotfish, wrasse, grunts and gobies (Anton et al., 2016, Kindinger, 2015, Marsh-Hunkin et al., 2013), all of which are found in lionfish diets (Peake et al., 2018). However, some species do respond to lionfish as a threat (Eaton et al., 2016, Kindinger and Albins, 2017). This may be because they have learnt to recognise invasive lionfish, or because they display generalised predator avoidance behaviour (Berchtold and Cote, 2020).

Studies investigating the effects of lionfish on individual fish species have observed reductions in abundance of fairy basslet (*Gramma loreto*; Ingeman, 2016), tomtate grunt (*Haemulon aurolineatum*; Ballew et al., 2016) and bluehead wrasse (*Thalassoma bifasciatum*; Tuttle, 2017). Lionfish also reduce the abundance of specific families such as parrotfish (Kindinger and Albins, 2017), and feeding guilds such as small herbivorous fish (Kindinger and Albins, 2017). Other studies have found reductions in abundance (Ellis and Faletti, 2016, Peiffer et al., 2017, Tuttle, 2017) and recruitment (Albins and Hixon, 2008) of entire prey fish communities. Lionfish also reduce prey fish biomass (Albins, 2015, Benkwitt, 2015), however, the observed effect is dependent on lionfish density. In one study, prey fish biomass increased when lionfish were below a reef-specific threshold density but

decreased when lionfish were above the threshold density (Green et al., 2014). In addition to effects on fish abundance and biomass, lionfish cause reductions in native fish species richness (Albins, 2013, Albins, 2015, Ellis and Faletti, 2016). Lionfish consume some species more than expected based on their local abundance (Albins, 2015, Chappell and Smith, 2016), which is a particular problem for rare species and has been shown to cause local extinction of some fairy basslet (*Gramma loreto*) populations (Ingeman, 2016).

Although many studies have found negative effects of lionfish, some studies have found no effect of lionfish on prey fish abundance (Hackerott et al., 2017) or species richness (Hackerott et al., 2017, Peiffer et al., 2017), with one study even reporting higher prey fish abundance and species richness on reefs with lionfish than on reefs without lionfish (Elise et al., 2015). The studies showing no change or an increase in abundance were conducted at the scale of whole reefs (Elise et al., 2015, Hackerott et al., 2017), rather than at the scale of individual patch or artificial reefs (Albins, 2013, Albins and Hixon, 2008, Ingeman, 2016), suggesting that the effects detected in small, isolated habitats may not scale up to larger areas. Temporal scale of the study may also affect the observed impacts of lionfish, as impacts may be delayed until lionfish have reached a critical density or until prey fish have transitioned into the studied size category. A sub-regional scale study of lionfish impacts on tomtate grunt (*Haemulon aurolineatum*) abundance found that a negative impact of lionfish was only detectable 7-12 years after lionfish invaded (Ballew et al., 2016).

The impacts of lionfish are dependent on many other variables, which may lead to effects being detected in some studies but not others. For example, lionfish prey items are often less than 5 cm in total length (Green and Cote, 2014, Morris and Akins, 2009, Munoz et al., 2011), therefore studies that focus on larger prey items (e.g Hackerott et al., 2017) may be less likely to detect effects. Lionfish density can influence observed effects of lionfish, with reductions in prey biomass only observed when lionfish exceed a reef-specific threshold density (Green et al., 2014). Lionfish body size may also influence results because larger lionfish consume larger prey (Morris and Akins, 2009) and a greater proportion of teleost fish (Eddy et al., 2016, Morris and Akins, 2009, Sancho et al.,

2018), thus studies on smaller lionfish may be more likely to detect effects on invertebrates than on fish. Morphological and behavioural traits of prey can influence their vulnerability to predation by lionfish (Green and Cote, 2014), therefore lionfish impacts will only be detected when studying prey that are vulnerable to lionfish predation.

In contrast to the extensive literature on the consumptive effects of lionfish on native fish, and despite invertebrates often being an important component of lionfish diet (Eddy et al., 2016, Peake et al., 2018), only one study has investigated consumptive effects on invertebrates. Lionfish were found to significantly change benthic invertebrate assemblages by reducing the abundance and biomass of the three most common taxa (Layman et al., 2014).

Lionfish can also exert non-consumptive effects on native species. One form of non-consumptive effects are 'fear effects', whereby prey change their behaviour to reduce the risk of predation in the presence of a predator (Catano et al., 2016). Herbivorous fish have been shown to reduce their grazing rate (Eaton et al., 2016, Kindinger and Albins, 2017) and cleaner shrimp move closer to their anemones (Ellis and Faletti, 2016) in the presence of lionfish. Bicolour damselfish (*Stegastes partitus*) fecundity is reduced in the presence of lionfish (Davis, 2018), however, the mechanism for this is unclear. Lionfish also exert non-consumptive effects on native competitor species; graysby grouper (*Cephalopholis cruentata*) shift their diet (Curtis et al., 2017), schoolmaster snapper (*Lutjanus apodus*) reduce their shelter use (Trehern et al., 2020) and Nassau grouper (*Epinephelus striatus*) avoid shelter (Raymond et al., 2015) in the presence of lionfish.

Another form of non-consumptive effects are density- or trait-mediated indirect effects. For example, lionfish predation on fairy basslet (*Gramma loreto*) releases blackcap basslet (*Gramma melacara)* from competition and allows them to occupy prime feeding locations on the reef (Kindinger, 2018). Density-mediated indirect effects are thought to have caused a phase-shift from coral-dominated to algal-dominated reefs in the Bahamas; the invasion of lionfish coincided with a reduction in

herbivorous fish abundance, which is thought to have caused the phase-shift through a reduction in grazing pressure (Lesser and Slattery, 2011).

1.4 Habitat use of invasive lionfish

Lionfish have been recorded from a diverse range of habitats in the invaded western Atlantic range, including shallow patch and continuous coral reefs (Tamburello and Cote, 2015), mesophotic reefs (Andradi-Brown et al., 2017a), seamounts (Quattrini et al., 2017), artificial reefs (Dahl and Patterson, 2014), deep sea habitats (Gress et al., 2017), seagrass (Claydon et al., 2012), mangroves (Claydon et al., 2012), estuaries (Jud and Layman, 2012), algal sand plains (Munoz et al., 2011), gorgonian fields (Arredondo-Chavez et al., 2016) and rhodolith reefs (Goodbody-Gringley et al., 2019).

Densities of lionfish are often higher on artificial reefs than natural reefs (Dahl and Patterson, 2014, Harris et al., 2019). It has also been noted anecdotally that lionfish are especially abundant around artificial structures on mesophotic and deep sea habitats (Tornabene and Baldwin, 2017). Lionfish are observed around a range of artificial habitats including wrecks (Whitfield et al., 2002), jetties (Schofield, 2009), artificial reefs (Dahl and Patterson, 2014) and fishing gear including fish traps (Butkowski et al., 2015), lobster traps (Akins et al., 2012) and lobster condos (non-enclosed shelter traps, Henderson and Cotê, 2011).

Lionfish inhabit a wide depth range, from inter-tidal pools (Whitfield et al., 2002) to mesophotic reefs (Andradi-Brown et al., 2017b, Eddy et al., 2016, Lesser and Slattery, 2011, Whitfield et al., 2002) and deep sea habitats (Albins and Hixon, 2013, Gress et al., 2017, Tornabene and Baldwin, 2017). The maximum recorded depth of lionfish is 304 m in Bermuda (Gress et al., 2017), however, this was the maximum survey depth, thus it is likely that lionfish are also found in greater depths.

Regardless of the habitat that lionfish are observed in, they often shelter around biotic or abiotic structures (Claydon et al., 2012). For example, in less structurally complex habitats such as seagrass

meadows, lionfish are often associated with sponges, wooden logs and artificial structures (Trégarot et al., 2015). Lionfish are almost always associated with artificial structures when natural hard substrate is lacking (Smith and Shurin, 2010, Jud and Layman, 2012). On shallow coral reefs, lionfish are often associated with structures such as caves (Garcia-Rivas et al., 2017), crevices (Biggs and Olden, 2011), overhangs (Whitfield et al., 2014) and coral heads (Tilley et al., 2016). On low structural complexity mesophotic reefs in the Gulf of Mexico, lionfish associate with grouper pits (depressions in the sediment that are excavated by groupers), which provide higher complexity habitat than the surroundings (Harter et al., 2017).

Aggregations of lionfish are often found around complex structures on both shallow (de Leon et al., 2013, Garcia-Rivas et al., 2017, Jud and Layman, 2012) and mesophotic (Andradi-Brown, 2019, Harter et al., 2017, Sherman et al., 2013) reefs, with larger group sizes found on tall structures than on flatter structures (Cardoso et al., 2015). In a study from Venezuela, almost half of all lionfish observed were in aggregations inside caves (Agudo and Salas, 2014). Lionfish are often found in pairs or small groups (de Leon et al., 2013, Garcia-Rivas et al., 2017, Jud and Layman, 2012), but group sizes of over 100 have been recorded (Sherman et al., 2013). It is unknown why lionfish are attracted to and often form aggregations around complex structures. Lionfish that were actively hunting in Mexico were mostly found around complex structures (Garcia-Rivas et al., 2018) and the presence of native fish is positively associated with the number of large lionfish present (Garcia-Rivas et al., 2017), suggesting that complex structure may benefit hunting. *Pterois miles* hunt cooperatively in the native range (Kendall Jr, 1990, McTee and Grubich, 2014), thus aggregations may form at complex structures to maximise feeding success. Aggregating lionfish are often larger than solitary lionfish (Garcia-Rivas et al., 2017, Jud and Layman, 2012) and small lionfish do not aggregate with large lionfish (Garcia-Rivas et al., 2017), suggesting that larger individuals may aggregate to spawn (Domeier and Colin, 1997). Prior to this thesis, no research had been conducted on the drivers of lionfish aggregations.

When considering reef complexity at the transect or whole reef scale, rather than at the scale of specific habitat features, the relationship between lionfish and complexity is less clear. No relationship between rugosity and lionfish abundance or biomass has been detected, whether rugosity is measured using the chain and tape method (Anton et al., 2014, Cardoso et al., 2015) or visually estimated (Cure et al., 2014, de Leon et al., 2013, Valdivia et al., 2014). When substratum height was used as a proxy for complexity, a positive relationship between complexity and lionfish density was found on the reef drop-off, but not on the inner edge or middle of the reef (Bejarano et al., 2015). When assessing lionfish presence, rather than abundance or biomass, lionfish were found on transects with a significantly higher percentage cover of hard coral and overhanging structures (Biggs and Olden, 2011).

1.5 Study site and statistical methods

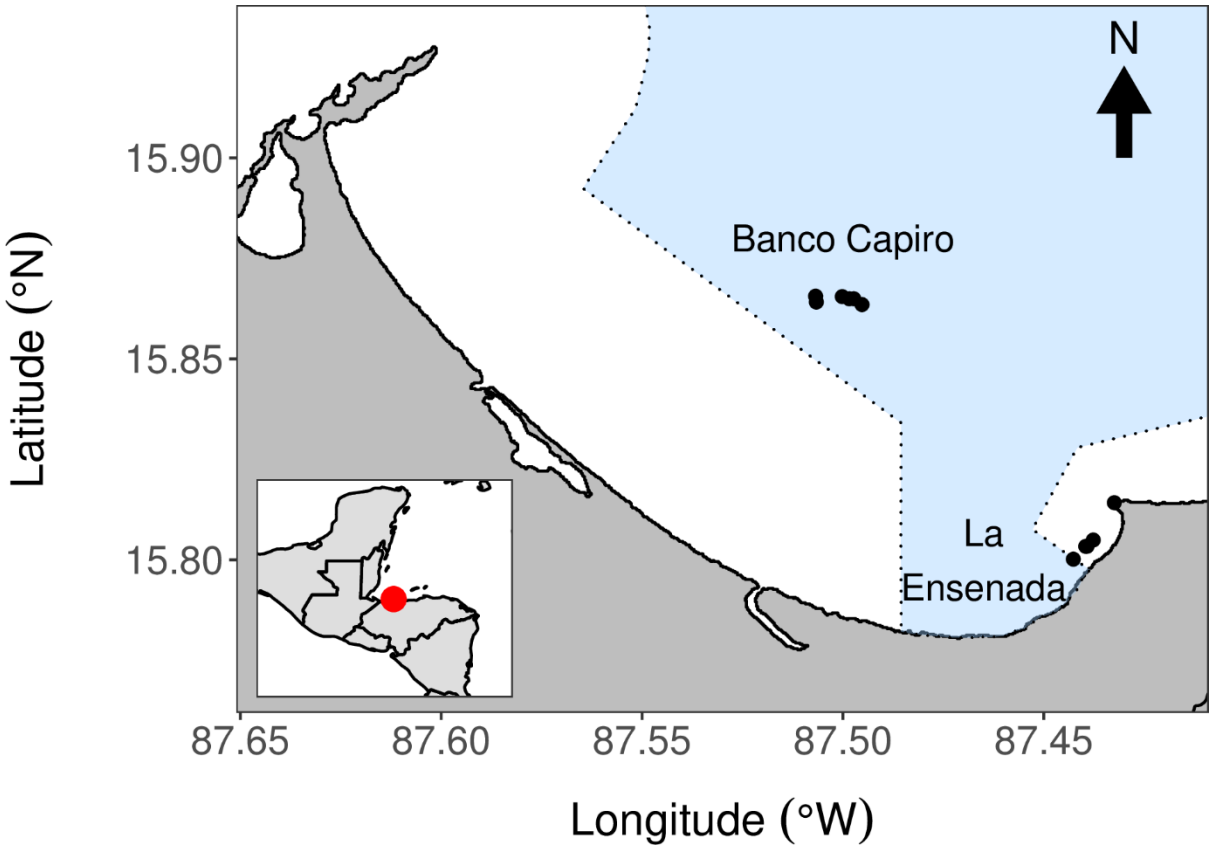
The overall aim of this thesis was to increase understanding of invasive lionfish aggregation behaviour, non-consumptive effects and regional-scale ecological impacts in the western Atlantic. In chapter two I studied the drivers of lionfish aggregation behaviour by conducting binary choice laboratory experiments and recording habitat use of lionfish *in situ*. In chapter three I investigated the impact of lionfish on shelter use of native invertebrates by recording *in situ* habitat preference of lionfish, native spiny lobsters and native long-spined sea urchins and observing shelter use in laboratory experiments. In chapter four I studied the impacts of lionfish on grazing behaviour of herbivorous fish using remote cameras to film *in situ* grazing behaviour in the presence and absence of lionfish. In chapter five I studied the impacts of lionfish across the invaded western Atlantic range using data from before and after lionfish invaded each sub-region, whilst controlling for contextual variables that are known to influence native fish communities.

1.5.1 Study site: Tela Bay, Honduras

The study site for chapters two, three and four is Tela Bay, Honduras (Figure 1). Honduras is situated on the Mesoamerican Barrier Reef, which extends from Yucatan, Mexico to the Bay Islands,

Honduras (Almada-Villela et al., 2002). In 2017 a new marine protected area (MPA) covering 863 km^2 was designated in Tela Bay (ProtectedPlanet, 2020). The MPA was assigned an IUCN category four, indicating that it is a habitat or species management area (Dudley, 2008).

Figure 1. Map of my study sites in Tela Bay (main map) and the location of Tela Bay within Central America (inset map). Study sites are shown as black points and are distributed between two reef systems: Banco Capiro and La Ensenada. The newly designated MPA is shown in blue.



I studied two reef systems in Tela bay: Banco Capiro and La Ensenada. Banco Capiro is a newly recognised reef system located approximately 8 km offshore (Bodmer et al., 2015) that begins at 10 m and descends to over 30 m (Titus et al., 2019). Although the best reefs in Honduras were originally thought to be on the Bay Islands and Cayos Cochinos (Almada-Villela et al., 2002), Banco Capiro rivals other Honduran and Caribbean reefs for its high scleractinian coral cover of 62% (Bodmer et al., 2015). La Ensenada is an inshore patch reef system that extends from 3-10 m depth (Bodmer,

2019). The shallow depth of La Ensenada makes it a suitable site for collections of live lionfish for laboratory experiments, as barotrauma injuries to the lionfish are minimised when collecting them from shallow water. Banco Capiro and La Ensenada have populations of Caribbean spiny lobster (*Panulirus argus*) and both reefs have higher densities of long-spined sea urchins (*Diadema antillarum*) than other Honduran reefs (Bodmer, 2019, Bodmer et al., 2015), making these suitable sites for studying shelter competition between lionfish and native invertebrates. In contrast to Banco Capiro, La Ensenada is considered degraded due to low scleractinian coral cover of just 9% and high macroalgal percentage cover of 21% (Bodmer, 2019). The new MPA encompasses all my Banco Capiro sites and one of my La Ensenada sites.

Lionfish were first observed in Honduras off the Bay Island of Roatan in May 2009 and were observed on neighbouring island Utila by August 2009 (Schofield, 2009). By 2010 lionfish had been observed in Cayos Cochinos, a group of islands and coral cays located between the Bay Islands and the mainland, and down to 120 m in Roatan (Schofield, 2010). The first lionfish sighting from mainland Honduras was in 2011 (USGS, 2020). Several studies have focused on lionfish in Honduras, including studies of ontogenetic migrations (Andradi-Brown et al., 2017a, Andradi-Brown et al., 2017b), effectiveness of culling (Peiffer et al., 2017), habitat preference (Biggs and Olden, 2011) and depth limits (Gress et al., 2017), however, the majority were conducted on the Bay Islands, with less research conducted on the mainland. My research from the mainland will thus help to better understand the lionfish invasion in Honduras.

Diving pressure and lionfish culling pressure are both high on the Bay Islands, leading to low lionfish densities on the regularly dived sites. However, lionfish culling pressure in Tela Bay is low due to low tourist numbers and weather conditions preventing diving for much of the year. The low culling pressure has led to high lionfish densities on both Banco Capiro and La Ensenada, making Tela bay an ideal location to observe lionfish *in situ* and collect sufficient specimens for laboratory research. On Banco Capiro, lionfish are usually found resting under ledges and overhangs on the reef wall and around coral bommies on the reef flat and in the reef channels but are occasionally observed

swimming or hunting above *Agaricia tenufolia* corals. On La Ensenada, lionfish are always observed around areas of high complexity, such as small outcrops of coral and rock, which are surrounded by areas of sand and algae. Lionfish aggregations are common at both Banco Capiro and La Ensenada.

1.5.2 Statistical methods

Throughout this thesis I have used a variety of statistical methods to suit each data type being analysed. Normality assumptions of parametric tests were checked and where possible, non-normally distributed data were transformed to meet the normality assumption. Where transformations were unsuccessful at fixing the violation of normality or other assumptions, non-parametric or robust tests were used. When more complex models were used, model simplification followed the recommended method for each statistical test. Five broad categories of statistical method were used in this thesis: (i) visualisation and testing of multivariate differences between groups, (ii) comparison of the means between two or more groups, (iii) regression using non-count-based proportions, (iv) robust linear mixed models, (v) generalised additive mixed models.

Multivariate differences between groups were visualised using non-metric multidimensional scaling (nMDS). A benefit of nMDS is that all the variance in the dataset is used to create a two-dimensional ordination, whereas the first two dimensions of other methods only use part of the variance (Paliy and Shankar, 2016). Although nMDS is useful for visualisation, it is not a statistical test. I used analysis of similarities (ANOSIM) to test for significant differences between groups (Ramette, 2007). When the ANOSIM was significant, I used similarity percentages (SIMPER) to determine which species contributed the most to the dissimilarity between groups (Clarke et al., 2014).

Comparisons of the means between groups were conducted using two-sample *t*-tests, Mann-Witney *U*-tests and Welch's *t*-tests for two groups and Kruskal-Wallis tests for more than two groups. The Welch's *t*-test was used for groups that violated the assumption of equal variance between groups, whilst the Mann-Whitney *U*-test and Kruskal-Wallis test were used for groups that violated the normality assumption (Whitlock and Schluter, 2009).

I used beta regression to analyse changes in proportions. Although logistic regression is often used for proportion data, my proportions could take a large range of values and thus were considered non-count-based proportion data, which is best analysed using beta regression (Douma and Weedon, 2019). The use of beta regression accounts for the heterogeneity and skewness that are common with proportion data (Cribari-Neto and Zeileis, 2010). An additional benefit of beta regression is the ability to include terms for variable precision, which can account for differences in variance between treatments (Douma and Weedon, 2019). Model selection followed a two-step approach, whereby I selected the best model from the candidate set when assuming constant precision, then selected the best model from a new candidate set that incorporated variable precision (Bayer and Cribari-Neto, 2017). I compared models using AICc (the small sample size version of AIC) and selected the model with the lowest AICc as the best model (Anderson and Burnham, 2004).

Linear mixed models are common in the analysis of biological data , but I replaced these with robust linear mixed models to reduce the influence of outliers that skewed the data (Koller, 2016). Each replicate in my study was tested twice, each time with a different treatment, therefore I included replicate as a random effect. To prevent over-parameterization, the ratio of data points to estimated parameters should be at least three (Crawley, 2012). I used a model with 11 parameters but only 20 data points (because my final fieldwork season was cancelled due to the Covid-19 pandemic), resulting in a data points to estimated parameters ratio of 1.8, meaning that my model was over-parameterised. Had I been able to conduct my final fieldwork season, I would have ensured that I collected at least 33 data points so that my model would not have been over-parameterised.

The final statistical method used in this thesis is Generalised Additive Mixed Models (GAMM), which estimate smooth trends between predictor and response variables (Pedersen et al., 2019). GAMMs were chosen because they can model non-linearity and they require no prior assumptions about the shape of the trends (Simpson, 2018). GAMMs are similar to linear mixed models in that they allow both fixed and random effects to be included in the models. Model selection was

conducted using the double penalty method (Marra and Wood, 2011), which allows uninformative smooth terms to be removed from the model. As the double penalty method only affects smooth terms, to decide whether to include the factor variable I ran the best model with and without the factor variable and selected the model with the lowest AIC value as the final model (Anderson and Burnham, 2004).

1.6 Aims and objectives

The overall aim of this thesis was to increase understanding of invasive lionfish aggregation behaviour, non-consumptive effects and regional-scale ecological impacts in the western Atlantic. Most research on lionfish focuses on their consumptive effects in small-scale patch reef studies, with little research on their behaviour, non-consumptive effects or region-wide impacts. This thesis aims to fill these knowledge gaps by providing evidence of the drivers of invasive lionfish aggregations (chapter two), testing for non-consumptive effects of invasive lionfish on native invertebrates (chapter three), investigating the non-consumptive effects of invasive lionfish on native herbivorous fish grazing (chapter four) and quantifying the regional impacts of invasive lionfish on native fish communities using an ambitious analytical approach based on one of the largest global coral reef monitoring datasets (chapter five). For chapters two, three and four I use the reefs of Tela Bay, Honduras as an invaded reef system on which to study interactions between lionfish and native species and to collect specimens for laboratory experiments. I take a novel regional approach for chapter five, using data from eight countries across the invaded western Atlantic region including the Bahamas, Jamaica, Central America and the West Indies. Chapter five uses invasive lionfish as a model species, providing a case study to illustrate a new approach for the detailed investigation of region-wide invasive species impacts.

Chapter two: Aggregating behaviour in invasive Caribbean lionfish is driven by habitat complexity

Lionfish are often found in aggregations, but until this study, nothing was known about the drivers of this behaviour. If invasive lionfish aggregate to spawn or cooperatively hunt as they do in the native

range, then aggregation behaviour may result in greater negative impacts on invaded reefs through faster population growth or higher prey consumption rates. Aggregation behaviour could potentially be harnessed to improve lionfish management, either through targeted culling or artificially stimulating aggregations, which could then be culled or trapped. It is therefore important to understand why aggregations form from both a behavioural ecology and a conservation management perspective. I conducted binary choice laboratory experiments to test whether lionfish were attracted to visual, olfactory, or a combination of cues from conspecifics. I also compared the habitat complexity of areas inhabited by solitary and aggregating lionfish and compared areas inhabited by lionfish with background levels of habitat complexity, to test whether lionfish demonstrated habitat preference. The hypotheses I tested were:

1. Lionfish will be attracted to visual and/or olfactory cues of conspecifics because lionfish in the native range have been observed to aggregate for spawning and cooperative hunting
2. Lionfish will be attracted to high complexity habitat because they are cryptic species and high complexity habitat provides structures such as caves and crevices to shelter in
3. Aggregating lionfish will be found in more complex habitat than solitary lionfish because high complexity habitat provides more shelters and can thus support more lionfish

Chapter three: Shelter use interactions of invasive lionfish with commercially and ecologically important native invertebrates on Caribbean coral reefs

Although the preference of lionfish for shelter, such as caves, crevices and overhangs, is frequently acknowledged, little research has been conducted into the interactions of lionfish with native shelter-using species. Lower commercial catches of spiny lobster (*Panulirus argus*) have been reported when lionfish are present in lobster traps, suggesting that lionfish may reduce lobster catches and negatively affect the commercial fishery. Long-spined sea urchins (*Diadema antillarum*) are another species that may compete with lionfish for shelter. Long-spined sea urchins are an important algal grazer on reefs, but lack of available shelters is suggested as a barrier to recovery of the species following their mass mortality in 1983-1984. Occupation of shelters by lionfish may further reduce the availability of shelters and thus contribute to the lack of recovery of long-spined sea urchins. I recorded *in situ*

habitat use of lionfish, native spiny lobsters and native long-spined sea urchins and combined this with laboratory experiments comparing activity and shelter use of each species when alone and when lionfish were paired with each native species. The hypotheses tested were:

1. Lionfish, spiny lobster and long-spined sea urchin will use habitats of similar structural complexity *in situ* because all three species inhabit shelters on the reef and are of broadly similar body size
2. Spiny lobster and long-spined sea urchin will change their activity and shelter use in the presence of invasive lionfish because invasive species are often superior competitors and lionfish have been shown to impact the behaviour and shelter use of other native species

Chapter four: Non-consumptive effects of invasive lionfish on herbivorous fish grazing vary within a reef system

Consumption of herbivorous fish by invasive lionfish has been suggested to cause phase-shifts on reefs because herbivorous fish populations are crucial for grazing algae and thus maintaining reefs in a coral-dominated state. In addition to consumptive effects, lionfish may also exert non-consumptive effects on herbivorous fish, such as reductions in grazing due to fear effects. In comparison to consumptive effects, which only affect one individual at a time, non-consumptive effects such as fear effects can affect multiple individuals simultaneously. I used remote cameras to film grazing behaviour of herbivorous fish and record fish communities in small areas of reef with lionfish present, then I repeated the measurements in the same areas after lionfish had been removed. This methodology allowed me to test whether the presence of lionfish affected herbivorous fish grazing behaviour and whole fish communities. The hypotheses tested were:

1. Herbivorous fish will reduce their grazing behaviour in the presence of lionfish as an anti-predator response, which may be caused by recognition of lionfish as a predator or generalised anti-predator behaviour
2. The fish communities that are observed in the video will differ in the presence of lionfish because prey fish may hide deeper in the reef or avoid areas with predators as an anti-predator response

Chapter five: Temporally offset additive mixed models show invasive lionfish drive fish community shifts across the western Atlantic

Many studies of invasive species compare pre- and post-invasion with little consideration for time since invasion, while few studies consider multiple sites that were invaded at different times. But to gain a full understanding of invasive species impacts, studies must account for changes that occur over both time and space. Prior to this thesis, no research had been conducted into the ecological impacts of invasive lionfish on native fish communities that simultaneously considered sites with different invasion timelines across the western Atlantic invaded range. I used invasive lionfish as a model species and data covering large spatial and temporal scales, whilst accounting for a comprehensive set of contextual variables in order to disentangle the impact of lionfish from other potential biophysical, social and management factors. Lionfish invasion occurred asynchronously across the western Atlantic, covering six years in my dataset, so I centred the data on year of lionfish arrival to reduce the influence of large-scale point impact events on my results. Using data from 11,000 transects in eight countries across an 18-year period, combined with data on 14 biophysical, social and management confounding variables, I investigated the impacts of lionfish on native fish communities over the course of the lionfish invasion. The hypotheses tested were:

1. Lionfish will cause regional reductions in fish abundance and biomass because their prey consumption rates are higher than native predators and small-scale studies show that lionfish reduce native fish abundance and biomass
2. Lionfish will exert greater effects on fish that are a suitable size to be predated because lionfish are gape-limited predators
3. Lionfish will cause regional reductions in fish species richness and diversity because lionfish preferentially consume certain species and have been shown to drive some local populations of species extinct

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2 Aggregating behaviour in invasive Caribbean lionfish is driven by habitat complexity

Christina L. Hunt, George R. Kelly, Hannah Windmill, Jocelyn Curtis-Quick, Helen Conlon, Max D.V. Bodmer, Alex D. Rogers, Dan A. Exton

This chapter is published as:

Hunt, C.L., Kelly, G.R., Windmill, H., Curtis-Quick, J., Conlon, H., Bodmer, M.D.V., Rogers, A.D. & Exton, D.A. 2019. Aggregating behaviour in invasive Caribbean lionfish is driven by habitat complexity. *Scientific Reports,* 9**,** 9.

2.1 Abstract

Caribbean lionfish (*Pterois* spp.) are considered the most heavily impacting invasive marine vertebrate ever recorded. However, current management is largely inadequate, relying on opportunistic culling by recreational SCUBA divers. Culling efficiency could be greatly improved by exploiting natural aggregations, but to date this behaviour has only been recorded anecdotally, and the drivers are unknown. We found aggregations to be common *in situ*, but detected no conspecific attraction through visual or olfactory cues in laboratory experiments. Aggregating individuals were on average larger, but showed no further differences in morphology or life history. However, using visual assessments and 3D modelling we show lionfish prefer broad-scale, but avoid fine-scale, habitat complexity. We therefore suggest that lionfish aggregations are coincidental based on individuals' mutual attraction to similar reef structure to maximise hunting efficiency. Using this knowledge, artificial aggregation devices might be developed to concentrate lionfish densities and thus improve culling efficiency.

2.2 Introduction

Since their introduction in the early 1980s (Schofield, 2009), lionfish (*Pterois volitans* and *P. miles*) have spread throughout the western Atlantic with devastating impacts on the region's coral reefs (Albins and Hixon, 2013). Successful eradications of marine invasive species are rare and generally involve species with a small invaded range (Anderson, 2005, Culver and Kuris, 2000). Complete eradication of lionfish is considered unlikely (Barbour et al., 2010), as they are now well established with a large geographical distribution (Schofield, 2010) and have the potential for long distance dispersal (Cote et al., 2013). Rather than eradication, management should therefore be focused on population reduction through culling (Green et al., 2017).

Lionfish culling is typically performed by recreational SCUBA divers and snorkelers using hand nets and pole spears (Green et al., 2017). This can be opportunistic or as part of organised events involving competing teams of divers and fishermen (Malpica-Cruz et al., 2016). On shallow reefs, culling has been successful at reducing lionfish densities (de Leon et al., 2013, Green et al., 2017) and allowing native species to recover (Green et al., 2014). However, whilst culling is largely restricted to depths <30m (Andradi-Brown et al., 2017a), lionfish are known to have invaded deeper mesophotic and sub-mesophotic habitats to at least 304m (Andradi-Brown et al., 2017a, Gress et al., 2017). Technical diving provides a partial solution, although cost, expertise and safety concerns will limit the extent of its use (Andradi-Brown et al., 2017b), and lionfish traps to collect lionfish from deeper water are still being tested (Gittings et al., 2017).

Groups of lionfish have been observed throughout the invaded range (de Leon et al., 2013, Garcia-Rivas et al., 2017, Jud and Layman, 2012) and it has been noted that aggregating individuals often appear larger than solitary individuals (Garcia-Rivas et al., 2017, Jud and Layman, 2012). By exploiting this natural behaviour and artificially stimulating aggregations, culling or trapping efficiency could be improved. Mass trapping of invasive and pest species has been successful in both aquatic and terrestrial ecosystems (Alpizar et al., 2002, Brown et al., 2017, Muller and Schwarzkopf,

2017) but requires knowledge of the species' aggregation drivers to inform the design of effective lures to trap them. Although previous work has focused on many aspects of the lionfish invasion (Cote and Smith, 2018), nothing is known about the drivers of lionfish aggregations, and quantitative data on their prevalence is lacking.

One possible driver of lionfish aggregations is social attraction, which may be for cooperative hunting (as observed in *P. miles* in its native range; McTee and Grubich, 2014), spawning (Domeier and Colin, 1997), minimising the chance of predation (Magurran and Pitcher, 1987) or pooling experiences to solve problems (Webster et al., 2017). Social attraction in fish can be initiated by visual (Lecchini et al., 2005), olfactory (Ward et al., 2002), acoustic (Lecchini et al., 2005) or a combination of cues (Johnston and Dixson, 2017). Zebrafish will even aggregate with robotic fish that mimic their shape and movement (Kim et al., 2018). Another possible aggregation driver is habitat preference. Fish may seek out specific habitat because it provides a source of prey, good hunting conditions or shelter from predators (Bohnsack, 1989). Marine habitat complexity can be measured via a range of metrics (e.g Gratwicke and Speight, 2005, Young et al., 2017) and is known to be a strong predictor of fish abundance (Darling et al., 2017, Gonzalez-Rivero et al., 2017). Although lionfish have been anecdotally reported to be found in more complex habitat (de Leon et al., 2013, Garcia-Rivas et al., 2017, Jud and Layman, 2012), this has not been determined quantitatively.

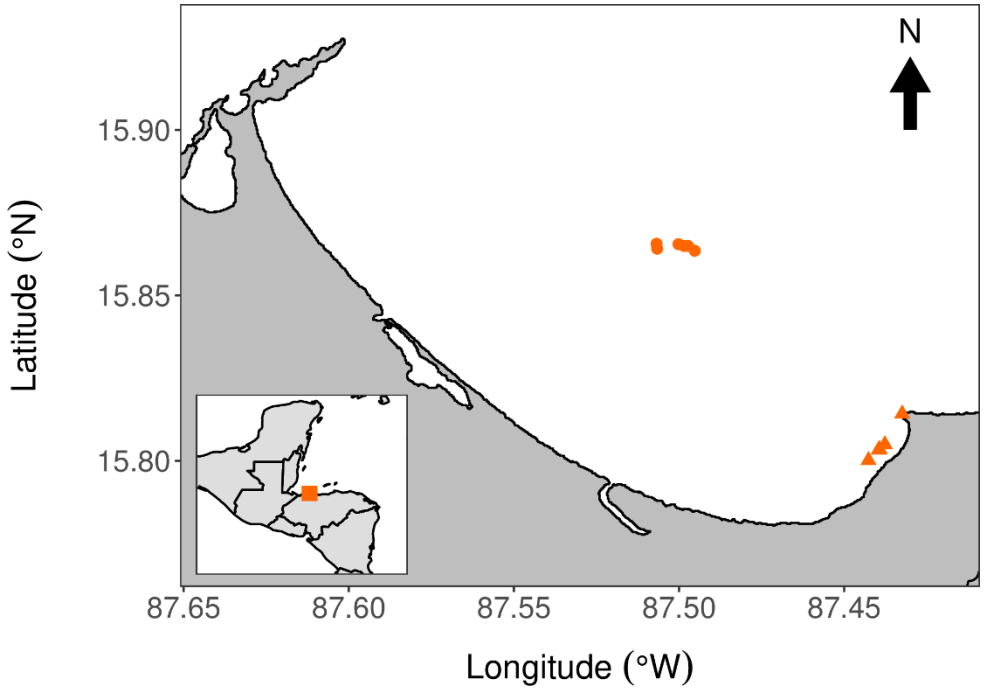
Here, we provide the first quantitative assessment of lionfish aggregations and their drivers, specifically exploring: (i) morphology and life history traits of aggregating and solitary individuals via *in situ* population surveys and *ex situ* dissections, (ii) social attraction using laboratory binary choice tests focusing on visual and olfactory cues, and (iii) habitat preference and the role of habitat complexity using 3D modelling and visual scores.

2.3 Methods

Study sites

All data were collected from reefs in the mainland Tela Bay, Honduras (Figure 1; Supplementary Table 1) between June and August 2017 using open-circuit SCUBA. Lionfish were culled for dissection at six sites on the offshore reef system of Banco Capiro at depths between 10-18 m (see Bodmer et al. (2015) for a general site description). Lionfish were collected alive for use in laboratory social attraction experiments from four sites on the shallow nearshore reef system of La Ensenada at depths <10 m. A recent genetic study suggests invasive lionfish in Honduras are likely to all be *P. volitans* (Betancur-R et al., 2011), and so we assumed this to be the case. However, we did not identify them to species level and so will refer to them simply as lionfish hereafter.

Figure 1. Map of our study sites in Honduras. Population structure data and habitat assessment scores were recorded from all 10 sites. Triangles mark the four sites on the La Ensenada reef system where lionfish were collected live for laboratory social attraction experiments. Circles mark the six sites on the Banco Capiro reef system where lionfish were culled and 3D modelling was carried out. The location of Tela bay, Honduras within central America is shown on the inset map.



In situ population structure surveys and *ex situ* dissections

Roving diver surveys were conducted, and any lionfish encountered deeper than 10 m were culled using pole spears before being transferred into a ZooKeeper container (ZooKeeper LLC, USA) for transport back to the laboratory. Approximate size, supra-ocular tentacle type and distinguishing features (e.g. unusual patterning) were noted *in situ* to enable later identification of individuals. Depth of capture and, if aggregating, the number of individuals within the aggregation were also recorded. An aggregation was defined here as two or more individual lionfish being found within approximately four body lengths of one another (Magurran and Pitcher, 1987).

On return to the laboratory, culled lionfish were refrigerated and dissected within 12 hours. Total length was recorded to the nearest mm from the tip of the snout to the longest point of the tail. Percentage body fat, which was used as a measure of feeding success, was calculated as fat weight (to the nearest 0.1 g) divided by body weight (to the nearest g), then multiplied by 100. Fat weight was obtained by removing all fat deposits from within the gut cavity and weighing these using an electronic balance. Fulton's condition factor, which was also used as a measure of feeding success, was calculated as body weight divided by total length cubed (Ricker, 1987, Villumsen et al., 2017), with higher values indicating that fish were heavier for their size. Macroscopic analysis of the gonads was used to determine sex (Green et al., 2012). Lionfish reproduce throughout the year (Gardner et al., 2015), which enabled us to measure reproductive maturity during our study. Reproductive maturity of males and females was measured using the gonadosomatic index (Gardner et al., 2015), which was calculated as gonad weight (to the nearest 0.1 g) divided by body weight and then multiplied by 100. Dissection methods followed Green et al. (2012).

Laboratory visual and olfactory attraction experiments

Lionfish capture and experimental setup

A total of 58 lionfish were captured alive using hand nets and transferred to large plastic dry-bags, before being slowly brought to the surface. Lionfish were transported inside the dry-bags to the laboratory by 15-minute boat ride. On arrival at the laboratory lionfish were immediately transferred

to the holding tank. Live collections were restricted to <10 m to avoid barotrauma injuries. Lionfish ranged in total length from 4.7-29.5 cm with a mean (± 1 SE) of 17.0 (± 0.88) cm. Each lionfish was used once to explore one of (i) visual cues (n = 15), (ii) olfactory cues (n = 13), (iii) visual+olfactory cues (n = 18) or (iv) artificial model cues (n = 12). Post-trial, some lionfish were used as stimulus fish for up to two trials. Stimulus fish were never re-used as test fish.

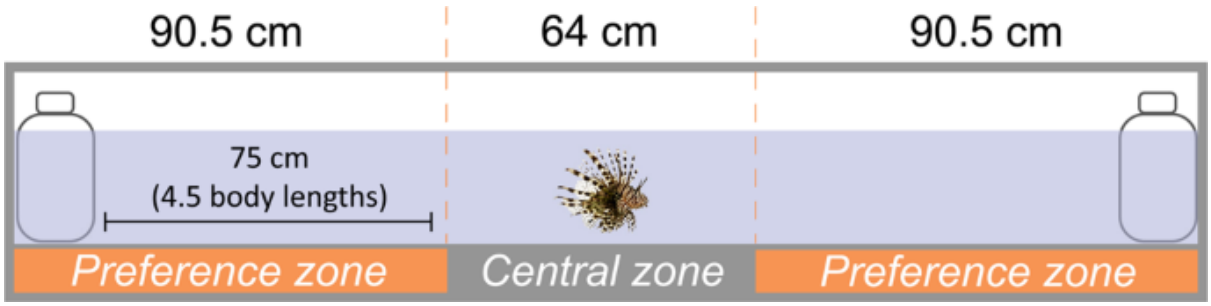
The holding and trial tanks measured 245 x 56 x 24 cm (length x width x depth) and were filled with unfiltered natural seawater to a depth of 16 cm. Water in the tanks was maintained at ambient temperature (29°C). Water changes were conducted at 100% every 24 hours in the holding tank, and 100% between each trial in the trial tank using freshly collected seawater (which was assumed to be constant in aeration and quality). No lionfish were present in the tanks during water changes. Tanks were naturally lit by large windows and thus followed the ambient light:dark cycle. All experiments were conducted during daylight hours between 6:00am and 6:00pm. During the trials two artificial lights (10 watt LED lamps) were used above the trial tank to account for shading by the building structure and ensure standardisation of lighting between all trials.

Lionfish were kept in the holding tank for an acclimatisation period of 24 hours during which they were starved to ensure similar hunger levels between individuals (Webster et al., 2008). A maximum of six lionfish were maintained in the holding tank at any time. The holding tank was fitted with a Tetra whisper power filter (Spectrum Brands Inc., USA) and Aqua Culture air pump (Walmart Stores Inc., USA) to ensure the water remained clean and aerated.

We used one trial tank that was divided into two preference zones of 90.5 cm (one at either end of the tank) and a central zone of 64 cm, by placing tape across the top of the tank (Figure 2). The preference zones encompassed a 15.5 cm diameter stimulus container plus approximately 4.5 body lengths distance from the stimulus container, based on the average total length of 17.0 cm for lionfish in this study. This distance encompassed the four body length distance used to define fish aggregations (Magurran and Pitcher, 1987) to ensure that, if present, aggregation behaviour was

detected. The tank was surrounded by opaque white plastic to prevent interference from external stimuli (Gomez-Laplaza et al., 2017).

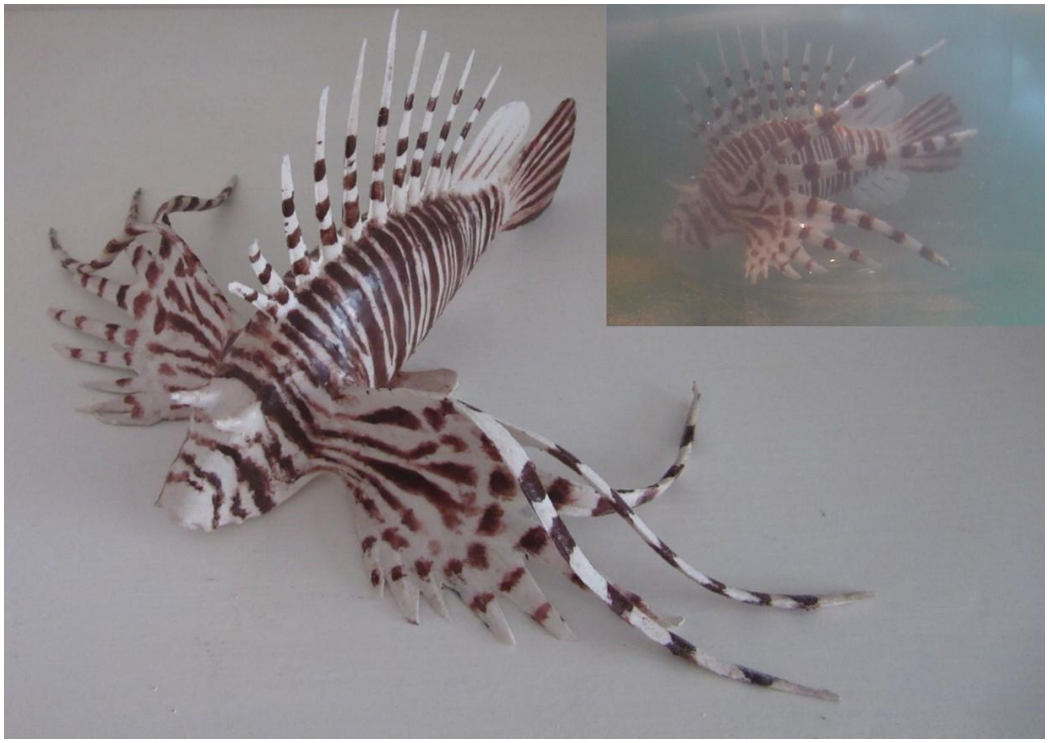
Figure 2. Tank set-up for laboratory social attraction experiments. The tank was divided into two preference zones (stimulus and control) and a central zone. Dotted lines mark boundaries between zones, which were marked by placing tape across the top of the tank. A GoPro Hero5 camera (GoPro Inc., USA) was mounted on the ceiling 1 m above the centre of the tank. Diagram is not drawn to scale. Lionfish photo ©iStock.com/GlobalP.



Experimental design

Stimuli were added to one end of the trial tank based on the following: (i) live lionfish and water from the trial tank added to a submerged transparent plastic container (visual cue), (ii) water from the holding tank added to a submerged transparent plastic container with 30 evenly spaced 4 mm diameter holes (olfactory cue), (iii) live lionfish and water from the trial tank added to a submerged transparent plastic container with 30 evenly spaced 4 mm diameter holes (visual+olfactory cue), and (iv) 3D printed lionfish model (Figure 3) and water from the trial tank added to a submerged transparent plastic container (artificial model cue). A control was placed at the opposite end of the tank, comprising water from the trial tank added to a submerged transparent plastic container without holes (visual and artificial model controls) or with 30 evenly spaced 4 mm diameter holes (olfactory and visual+olfactory controls). The end of the tank containing the stimulus/control was randomised between trials and the order of trials was randomised between days.

Figure 3. Artificial model lionfish used in the laboratory social attraction experiments. The model was moulded from aquarium grade silicone using a custom-made mould created photogrammetrically using video footage of a live lionfish. The model measures 15 cm total length. Underwater the pectoral fins spread out to give a more realistic appearance (inset photograph).



A randomly chosen lionfish was placed in a 30 cm diameter mesh acclimatisation cylinder in the centre of the tank. After a ten minute acclimatisation period ((Kim et al., 2018); which allowed the lionfish to acclimatise to the water, stimuli and artificial lighting), the cylinder was lifted and the lionfish was filmed for a trial period of ten minutes (Kim et al., 2018) using a GoPro Hero5 camera (GoPro Inc., USA) mounted 1 m above the centre of the tank. Subsequently, the videos were analysed and the position of the lionfish (stimulus, control or central zone) recorded every second for the duration of the trial, as well as which preference zone (stimulus or control) was entered first. Position was determined as the zone that the tip of the lionfish's snout was in. Overall preference was calculated as time spent in the stimulus zone minus time spent in the control zone.

Once lionfish had been used in a trial they were humanely culled by cervical transection, followed by pithing to destroy the brain tissue (Leary et al., 2013). These lionfish were subsequently dissected as described earlier, except that fat weight was not recorded.

Habitat preference and habitat complexity

Whenever lionfish were encountered on the reef, a 1 x 1 m quadrat was placed over the reef centred on the position of the individual lionfish or the centre of the aggregation. Habitat assessment scores were then performed following Gratwicke and Speight (2005), whereby five categories of structure were estimated visually on a five-point scale. Maximum substratum height, refuge size and variety of growth forms were recorded, along with average live cover (percentage cover of sessile organisms such as live corals, macroalgae and sponges) and rugosity scores for each quadrat. Over the course of the study 153 quadrats were assessed. Only one data point for complexity was taken per aggregation.

In addition, 30 large quadrats (2 x 2 m) were placed on reef areas where lionfish were encountered (23 solitary and 7 aggregations). These quadrats were filmed in a lawnmower pattern, approximately 0.5 m above the benthos, using a GoPro Hero3 camera (GoPro Inc., USA). Areas of reef without lionfish present were also sampled to give a background average for the reef system. Transects were laid at randomly chosen directions from each mooring line and quadrats were placed at regular intervals along each transect to give a total of 44 background quadrats. The resulting footage was converted into stills and used to render 3D models in Agisoft Photoscan (Agisoft LLC, Russia), before being analysed for habitat complexity using Rhinoceros 3D (Robert McNeel and Associates, USA), following the method of Young et al. (2017). Habitat complexity was measured using three metrics: linear rugosity (2 cm resolution), vector dispersion (1 cm resolution) and fractal dimension. Fractal dimension was measured at five spatial resolutions (1-5 cm, 5-15 cm, 15-30 cm, 30-60 cm and 60-120 cm) to explore patterns between lionfish habitat preference and habitat complexity at varying spatial scales.

Statistical analysis

All tests were two-tailed with an a-priori significance level of 0.05 and were conducted in R version 3.4.2 (R Core Team, 2017). Prior to analysis, datasets were checked for normality using a Shapiro-Wilk test, and groups were checked for homogeneity of variance using Fisher's *F* test. When the assumption of normality was violated, transformation was attempted. When data transformation did not normalise the data, a non-parametric test was conducted on the original data.

For the social attraction experiments, initial preference was tested using binomial tests and overall preference was tested using one-sample *t*-tests or sign tests. For tests between two groups (lionfish quadrats and background quadrats or solitary lionfish and aggregations), two-sample *t*-tests were used for continuous, normally distributed data, whilst two-proportions *Z*-tests were used for proportions. For continuous data that violated the normality assumptions, and for ordinal data, Mann-Whitney *U*-tests were conducted. Data that violated both normality and homogeneity of variance assumptions were analysed using a ranked Welch's *t*-test (Ruxton, 2006, Zimmerman and Zumbo, 1993).

Ethics statement

Lionfish culling was carried out in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (Leary et al., 2013). All experimental protocols were approved by the University of Oxford Animal Welfare and Ethical Review Body. No permits were required to collect lionfish, however, a research permit to allow this work to be conducted in Honduras was obtained from the Instituto de Conservacion Forestal Honduras (permit number: DE-MP-081-2017).

2.4 Results

Aggregating lionfish are larger and heavier, but otherwise similar to solitary individuals

A total of 283 lionfish were caught and dissected for this study, comprising 178 solitary and 105 aggregating individuals. We recorded 33 aggregations of varying sizes from 2 to 7 individuals, with a

mean (± SE) of 3 (± 0.34) and a median of 2. Lionfish were collected from depths of 3.0 – 18.4 m, with similar mean depths (± SE) for solitary lionfish (10.7 ± 0.28 m) and aggregations (10.6 ± 0.52 m).

Lionfish found in aggregations were significantly larger than solitary individuals ($p<0.001$) and were also heavier for their size ($p<0.001$). Mean total lengths (± 1 SE) were 22.2 cm (± 0.55) for aggregating lionfish and 18.4 cm (± 0.54) for solitary lionfish. However, there was no difference in sex ratio ($p>0.05$), the gonadosomatic indices of males and females ($p>0.05$ for both) or percentage body fat ($p>0.05$) between solitary and aggregating individuals.

Lionfish are not attracted to conspecific visual or olfactory cues

The proportion of trials where lionfish entered the stimulus zone first did not differ significantly from chance levels for any of the stimuli tested ($p>0.05$ for all stimuli; Table 1). Lionfish in the visual, visual+olfactory and artificial model treatments showed no significant preference for either stimulus or control ($p>0.05$ for each; Table 1). Lionfish in the olfactory treatment showed a significant preference for the control, or avoidance of the stimulus, with a mean (± 1 SE) time difference of -229 (± 62) seconds.

Lionfish prefer broad-scale, rather than fine-scale, habitat complexity

Habitat assessment scores found that aggregating lionfish inhabited areas with significantly larger refuge sizes ($p<0.001$), higher percentage live cover ($p<0.05$) and a greater variety of growth forms ($p<0.05$) than the areas inhabited by solitary lionfish (Figure 4). There were no significant differences for rugosity or substratum height ($p>0.05$ for both).

We also used 3D modelling to test for lionfish preferences towards habitat complexity. As there were no significant differences in the three complexity metrics (linear rugosity, vector dispersion and fractal dimension) between reef areas harbouring solitary lionfish and aggregations, these data sets

were pooled to represent complexity with lionfish (lionfish quadrats) and compared with randomly selected areas of reef without lionfish (background quadrats).

Although there was no significant difference in linear rugosity between lionfish quadrats and background quadrats (p>0.05; Table 2), lionfish were found in areas with significantly lower vector dispersion values (mean ± 1 SE; 0.144 ±0.009 compared with 0.239 ±0.007), indicating an association with low complexity at fine spatial scales (p<0.001; Table 2). This is supported by assessment of fractal dimension across five spatial scale ranges (Table 2; Figure 5). Lionfish quadrats were significantly less complex than background quadrats at the finest spatial scale (1-5 cm; p<0.001). Conversely, lionfish quadrats were significantly more complex than background quadrats at the broadest spatial scales (30-60 cm and 60-120 cm; p<0.05 for both). At intermediate spatial scales (5-15 cm and 15-30 cm) there was no significant difference. These results demonstrate an association with low complexity at fine spatial scales but high complexity at broad spatial scales.

Table 1. Results of laboratory social attraction experiments. Initial preference *p*-values were calculated using binomial tests with a null hypothesis of 0.5. Overall preference *p*-values were calculated using one-sample *t*-tests (olfactory, visual+olfactory and artificial model cues) and sign tests (visual cue) with a null hypothesis of 0. * indicates p<0.05.

Stimulus	Sample size	Initial preference		Overall preference	
		Proportion of trials where stimulus zone was entered first	*p*-value	Test statistic	*p*-value
Visual	15	0.6	0.6072	NA	0.6072
Olfactory	13	0.62	0.5811	-3.6764	0.00317*
Visual+olfactory	18	0.28	0.09625	-0.35812	0.7247
Artificial model	12	0.67	0.3877	1.2259	0.2458

Figure 4. Habitat assessment scores for solitary lionfish and aggregations. The percentage frequency of lionfish in each habitat assessment score category plotted for solitary lionfish (grey bars; *n*=120) and aggregations (orange bars; *n*=33).

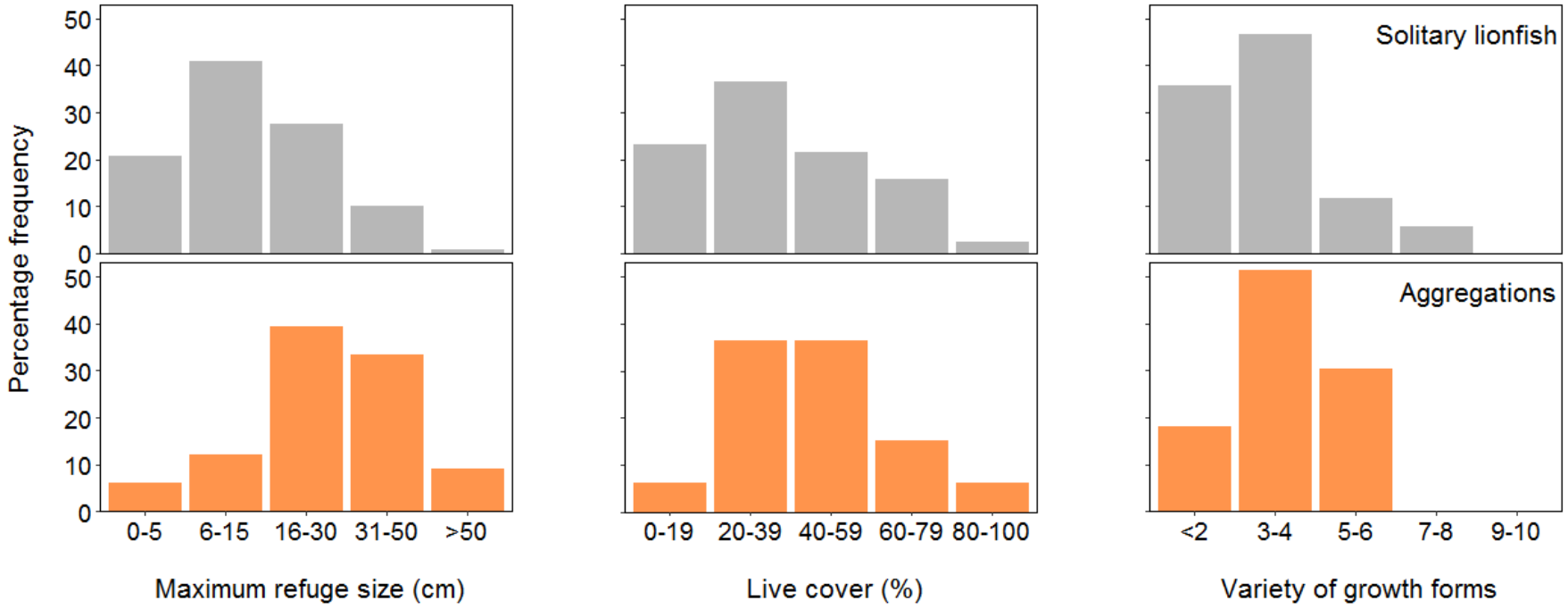
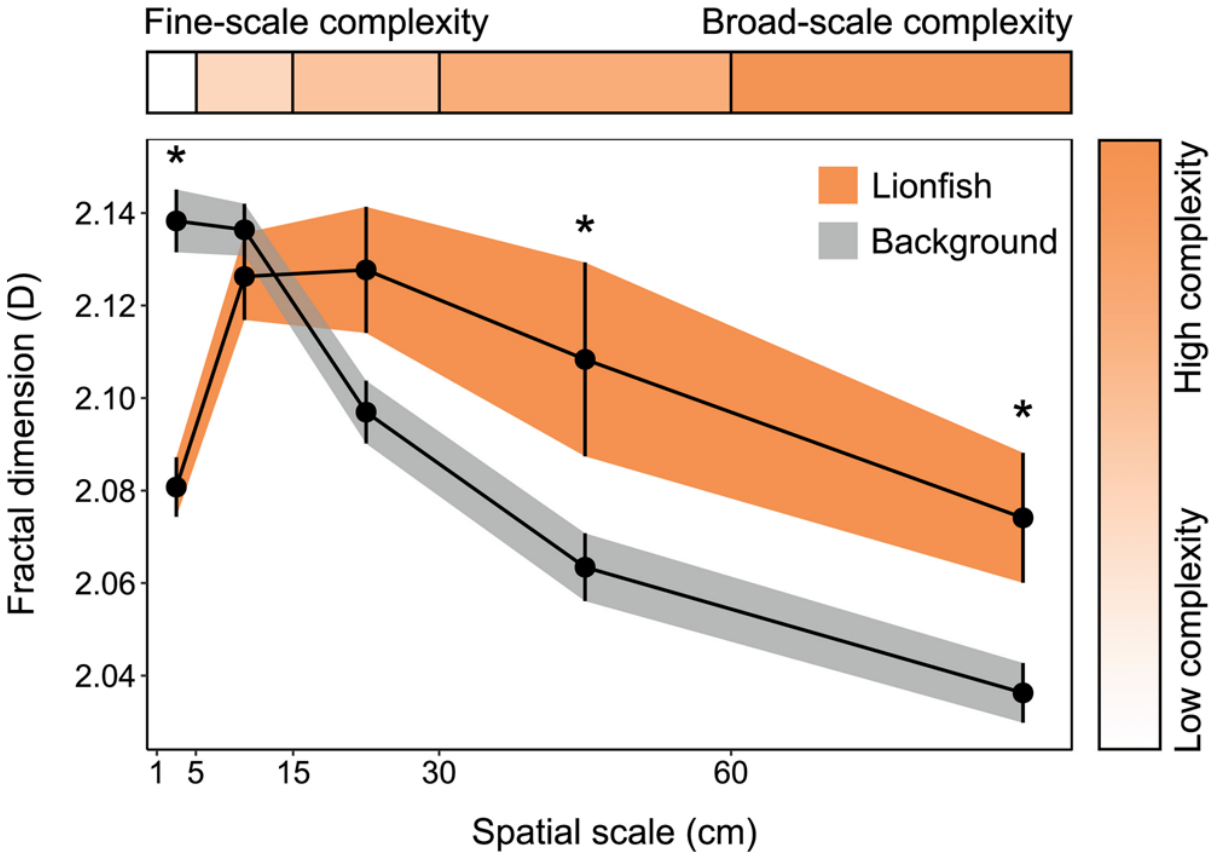


Table 2. Tests of complexity between lionfish and background quadrats. Complexity was compared between lionfish quadrats (n=30) and background quadrats (n=44). Rugosity was tested using a Welch's t-test, vector dispersion was tested using a two-sample t-test and all fractal dimension spatial scales were tested using ranked Welch's t-tests. * indicates $p<0.05$

Complexity measure		Test statistic	p-value
Rugosity		-1.8179	0.07534
Vector dispersion		8.7092	<0.001 *
Fractal dimension	1-5 cm	6.3722	<0.001 *
	5-15 cm	0.9375	0.3528
	15-30 cm	-1.7142	0.09248
	30-60 cm	-2.2406	0.02899 *
	60-120 cm	-2.5757	0.01255 *

Figure 5. Fractal dimension at varying spatial scales. Fractal dimension values were calculated at five spatial scales: 1-5 cm, 5-15 cm, 15-30 cm, 30-60 cm and 60-120 cm. Means (± 1 SE) are plotted at the midpoint of each spatial scale range for both lionfish quadrats (orange; *n*=30) and background quadrats (grey; *n*=44). * indicates $p<0.05$.



2.5 Discussion

Prior to this study, lionfish aggregation behaviour had only been observed anecdotally and little was known about its drivers. Through a combination of approaches and both *in situ* and *ex situ* data collection we have made a substantial contribution to this aspect of invasive lionfish research.

We found that aggregating individuals were larger than solitary individuals. Although larger female fish are often more fecund (Hixon et al., 2014), we found no difference in reproductive maturity between solitary and aggregating lionfish. This suggests that the aggregations observed during our

study period were not for the purpose of spawning, unlike in other reef fish species (Erisman et al., 2007, Farmer et al., 2017). Alternatively, aggregating lionfish may be larger because of higher growth rates, which may occur through cooperative hunting (which has been demonstrated to improve hunting success; Lonnstedt et al., 2014) or because lionfish choose areas that maximise their individual hunting success. Cooperative hunting has been observed in another lionfish species, *Dendrochirus zebra*, in the laboratory (Lonnstedt et al., 2014) and in *P. miles* in its native range (McTee and Grubich, 2014). However, in Mexico lionfish were more likely to be seen hunting when solitary than when in aggregations (Garcia-Rivas et al., 2018). Although we found no difference in percentage body fat between aggregating and solitary individuals, we did find a difference in the value of Fulton's condition factor, with aggregating individuals being heavier for their size. This difference may result from the benefits of cooperative hunting, or because aggregations form in areas where hunting success is higher.

Many fish that are found in groups exhibit social attraction (Hiermes et al., 2015, Ward and Mehner, 2010) but lionfish showed no preference for any of the cues tested here, both in initial reaction or overall preference. This is not unique; a lack of response to visual cues has been observed in other aggregating fish (Ward et al., 2002). The lack of response might have been caused by differing personalities between individuals, resulting in differing motivation to aggregate. Several fish species show personality (e.g Houslay et al., 2018, Jolles et al., 2015) and personalities can influence social attraction (Jolles et al., 2015). Although we did not control for personality differences, the lionfish we caught may have been the boldest individuals (Cote et al., 2014), thus minimising personality differences between individuals.

Lionfish did show a significant response in the olfactory treatment, however, their preference was for the control. This may have been due to the presence of waste products (e.g. ammonia; Ip and Chew, 2010) in the olfactory cue, which are known to stimulate avoidance behaviour in other fish species (Richardson et al., 2001). Based on this result we suggest that future studies should use alternative

methods of introducing olfactory cues, such as a lionfish behind an opaque, but water permeable, barrier.

Sound has a well-established role in reef fish behaviour (Simpson et al., 2005, van Oosterom et al., 2016) and lionfish are known to produce sound (Beattie et al., 2017), therefore the use of acoustic cues for social attraction is possible. Acoustic cues were not standardised in our experiment therefore we cannot deduce whether lionfish use acoustic cues for social attraction. However, our findings do suggest that aggregating behaviour in invasive lionfish is not driven by visual or olfactory cues between conspecifics.

Previous studies provide mixed results on the association of lionfish with habitat complexity. Lionfish have been anecdotally reported from more complex habitat (de Leon et al., 2013, Garcia-Rivas et al., 2017, Jud and Layman, 2012) and shown to be associated with areas of greater hard coral cover and overhanging structures (Biggs and Olden, 2011). However, other studies have reported no relationship between habitat complexity and lionfish density or biomass (Anton et al., 2014, de Leon et al., 2013, Valdivia et al., 2014). By using more rigorous methods and quantifying multiple metrics of reef structure, we found a clear link between lionfish, aggregations and habitat complexity.

Firstly, our data show aggregations were associated with larger refuge sizes, higher live cover and a greater variety of growth forms. Preference of lionfish for these habitats may increase food availability; larger refuges can be associated with greater fish abundance (Friedlander and Parrish, 1998), increased coral cover may provide preferred habitat for larval settlement of prey species (Jones et al., 2004) and a greater variety of growth forms can be associated with increased species richness (Gratwicke and Speight, 2005).

Lionfish habitat preference varied across different spatial scales. At finer scales (1-5 cm for fractal dimension and 1 cm for vector dispersion) lionfish were associated with lower complexity. Predatory fish may hunt less effectively in habitats that are complex at fine scales (Gotceitas and Colgan, 1989),

therefore lionfish may avoid high complexity at this scale to maximise hunting success. If this is indeed the case, lionfish hunting success may be increased because of reef flattening which is occurring throughout the Caribbean (Alvarez-Filip et al., 2009). At broader scales (30-60 cm and 60-120 cm for fractal dimension) lionfish were associated with higher complexity that is often created by large crevices and overhangs. The erosion of broad-scale complexity as part of reef flattening may be less important to lionfish as artificial reefs such as shipwrecks and concrete structures can provide this type of complexity. This may explain why lionfish were found at higher density on artificial, rather than natural, reefs in the Gulf of Mexico (Dahl and Patterson, 2014). Our study highlights the importance of considering spatial scale in assessments of species associations with habitat complexity. By creating "complexity signatures" using fractal dimension (Figure 5) a much more detailed picture can be gained of how a species associates with both the quality and quantity of complexity (Young et al., 2017).

Caves and overhangs provide low light environments that may increase hunting success, given that lionfish prey capture rates have been shown to be higher on overcast than clear days (Cote and Maljkovic, 2010). The physical structure of caves and overhangs may also benefit lionfish hunting; lionfish have been observed herding prey towards vertical and concave surfaces before striking (Cote and Maljkovic, 2010). The inverse relationship between substratum height and availability of prey refugia (Bejarano et al., 2015) suggests that these habitats often lack refuges for prey species. However, these habitats may provide shelter for certain prey and mutualist species. For example, overhangs provide shelter for *Gramma loreto* (Ingeman, 2016), a fish that has been found in lionfish stomachs (Harms-Tuohy et al., 2016, Valdez-Moreno et al., 2012) but that is also a potential lionfish cleaner (Garcia-Rivas et al., 2017).

Based on these findings, we propose that aggregations of invasive lionfish are coincidental and form when multiple lionfish are attracted to the same area of reef, rather than through visual or olfactory attraction to conspecifics. This attraction to broad-scale over fine-scale complexity is likely to be

driven by a search for maximised hunting efficiency, characterised by reduced refugia for prey items, leading multiple lionfish to congregate on areas of reef where feeding is easiest.

This knowledge could be used to significantly improve the efficiency of culling efforts. Our results can be used to inform the design of artificial aggregation devices or to improve existing lionfish traps that already involve some complexity (Gittings et al., 2017), but which could benefit from incorporating broad-scale crevices and minimising fine-scale refugia. Artificial aggregation devices and traps could in turn extend the depth limit of culling efforts to exploit the significant populations found on mesophotic reefs (Andradi-Brown et al., 2017a, Gress et al., 2017), which are believed to include the most reproductively active individuals (Andradi-Brown et al., 2017a). Only through improved understanding of the behaviour and ecology of this widespread invader will managers have the upper hand. By expanding and improving culling efforts using the findings of this study, a long-term solution will be one step closer.

2.6 Acknowledgements

We thank all the Operation Wallacea volunteers and staff who assisted with data collection. We also thank Steve Dey and colleagues at ThinkSee3D Ltd. (UK) for producing the 3D printed lionfish model. This work was financially supported by the Natural Environment Research Council (Grant number: NE/L002612/1) and Operation Wallacea.

2.7 Author contributions

Conceptualisation: Christina L. Hunt, Alex D. Rogers, Dan A. Exton

Formal analysis: Christina L. Hunt

Investigation: Christina L. Hunt, George R. Kelly, Hannah Windmill, Max D.V Bodmer

Methodology: Jocelyn Curtis-Quick, Helen Conlon

Supervision: Alex D. Rogers, Dan A. Exton

Visualisation: Christina L. Hunt, Dan A. Exton

Writing- original draft: Christina L. Hunt

Writing- review and editing: Christina L. Hunt, George R. Kelly, Hannah Windmill, Jocelyn Curtis-Quick, Helen Conlon, Max D.V Bodmer, Alex D. Rogers, Dan A. Exton

2.8 Supplementary information

Supplementary Table 1. Study site co-ordinates. Co-ordinates of our 10 study sites within Tela bay, Honduras.

Reef system	Site name	Latitude (°N)	Longitude (°W)
Banco Capiro	Aldrid's	15.86496	87.49841
	Butterfingers	15.86351	87.49525
	Canyon	15.86414	87.50662
	Kisci's garden	15.86550	87.50017
	Mushroom mountain	15.86497	87.4973
	Rotunda	15.86561	87.50680
La Ensenada	Acuario	15.81423	87.43244
	Becky's choice	15.80494	87.43768
	Nelly's island	15.80017	87.44264
	Palm view one	15.80339	87.43922

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3 Shelter use interactions of invasive lionfish with commercially and ecologically important native invertebrates on Caribbean coral reefs

Christina L. Hunt, Dominic A. Andradi-Brown, Callum J. Hudson, Joshua Bennett-Williams, Frankie Noades, Jocelyn Curtis-Quick, Owen T. Lewis, Dan A. Exton

This chapter is published as:

Hunt, C.L., Andradi-Brown, D.A., Hudson, C.J., Bennett-Williams, J., Noades, F., Curtis-Quick, J., Lewis, O.T. & Exton, D.A. 2020. Shelter use interactions of invasive lionfish with commercially and ecologically important native invertebrates on Caribbean coral reefs. *PloS one,* 15**,** e0236200.

3.1 Abstract

Indo-Pacific lionfish have become invasive throughout the western Atlantic. Their predatory effects have been the focus of much research and are suggested to cause declines in native fish abundance and diversity across the invaded range. However, little is known about their non-consumptive effects, or their effects on invertebrates. Lionfish use shelters on the reef, thus there is potential for competition with other shelter-dwelling organisms. We demonstrate similar habitat associations between invasive lionfish, native spiny lobsters (*Panulirus argus*) and native long-spined sea urchins (*Diadema antillarum*), indicating the potential for competition. We then used a laboratory experiment to compare activity and shelter use of each species when alone and when lionfish were paired with each native species. Spiny lobsters increased their activity but did not change their shelter use in the presence of a lionfish, whilst long-spined sea urchins changed neither their activity nor shelter use. However, lionfish reduced their shelter use in the presence of spiny lobsters and long-spined sea urchins. This study highlights the importance not only of testing for the non-consumptive effects of invasive species, but also exploring whether native species exert non-consumptive effects on the invasive.

3.2 Introduction

Invasive predatory species are well known to cause consumptive effects on native species (Albins and Hixon, 2008, Doherty et al., 2016, Wanless et al., 2007). Yet, invasive species may also exert non-consumptive effects, such as competition for food (Kakareko et al., 2013) and space (Gittenberger and Moons, 2011). Competition for space occurs in terrestrial (Clark et al., 2019), freshwater (Lauer and Spacie, 2004) and marine environments (Lopez-Victoria et al., 2006) and in both plants (Kellner and Asner, 2014) and sessile and mobile animals (Clark et al., 2019, Lopez-Victoria et al., 2006). Prior residency may confer an advantage in preventing displacement by a competitor species (Kellner and Asner, 2014), whereas other factors such as body size may be more important determinants of which species gains access to the shelter (McCormick and Weaver, 2012).

Coral reef ecosystems are in decline around the globe as a result of stressors including climate change (Hoegh-Guldberg et al., 2018, Hughes et al., 2017), unsustainable fishing practises (Exton et al., 2019), pollution (Edinger et al., 1998) and invasive species (Molnar et al., 2008). Invasive lionfish have colonised both the western Atlantic (Schofield, 2010) and, more recently, the Mediterranean (Kletou et al., 2016). Lionfish are habitat generalists found on shallow and mesophotic reefs (Andradi-Brown, 2019, Andradi-Brown et al., 2017b), the deep sea (Gress et al., 2017), mangroves (Barbour et al., 2010), and seagrass beds (Claydon et al., 2012). Two species of lionfish have invaded the western Atlantic: *Pterois volitans* and *Pterois miles* (Schofield, 2009), hereafter collectively referred to as lionfish. The consumptive effects of lionfish on native fish are well known (Cote and Smith, 2018), with numerous studies reporting declines in reef fish abundance (Ballew et al., 2016), species richness (Albins, 2015) and recruitment (Albins and Hixon, 2008). However, fish are only one component of lionfish diets. Shrimps are also an important prey item (Eddy et al., 2016), particularly in the diet of smaller lionfish (Morris and Akins, 2009). Despite the importance of shrimps in the diet, the consumptive effects of lionfish on shrimps and other crustaceans have been investigated to a lesser extent (Layman et al., 2014, South et al., 2017).

In addition to consumptive effects, lionfish are subject to and can exert non-consumptive effects, for example 'fear effects', whereby prey change their behaviour to reduce the risk of predation in the presence of a predator (Catano et al., 2016). In the western Atlantic, some (but not all) native prey species recognise invasive lionfish as predators (Kindinger, 2015, Marsh-Hunkin et al., 2013). For example, some herbivorous fish show reduced grazing (Eaton et al., 2016, Kindinger and Albins, 2017) and cleaner shrimp stay closer to their anemones (Ellis and Faletti, 2016) in the presence of lionfish. In addition to exerting non-consumptive effects, lionfish can also be subjected to these effects by humans; lionfish hide deeper in the reef and are more wary of humans as a consequence of lionfish culling programs (Andradi-Brown et al., 2017a, Cote et al., 2014). Lionfish also exert non-consumptive effects through 'transmitter' species, for example lionfish predation on fairy basslet releases blackcap basslet from competition and allows them to occupy prime feeding locations on the reef (Kindinger, 2018). Another type of non-consumptive effect is competition. Previous studies have identified competition for prey between invasive lionfish and native grouper (Albins, 2013), potentially affecting native grouper populations. Studies using artificial reefs have demonstrated that limited shelter availability can constrain the abundance of organisms (Hixon and Beets, 1989), therefore if lionfish occupy shelters on the reef they may exclude native species. This has been suggested by previous studies, indicating that native groupers avoid shelters that are occupied by a lionfish (Raymond et al., 2015) and native snappers reduce their shelter use in the presence of a lionfish (Trehern et al., 2020).

Studies investigating shelter use interactions between lionfish and native invertebrates are lacking. Lionfish show preference for broad-scale complexity such as caves and overhangs (Hunt et al., 2019), which they use as shelter (Cote et al., 2014), but they avoid fine-scale complexity such as intricate branching corals (Hunt et al., 2019). Spiny lobsters (*Panulirus argus;* hereafter referred to as lobsters) and long-spined sea urchins (*Diadema antillarum;* hereafter referred to as *Diadema*) are both native invertebrates that use reef shelters. Neither species has been the focus of research on lionfish impacts because spiny lobsters are rarely consumed by lionfish (Peake et al., 2018) and there are no reports of *Diadema* being consumed by lionfish. Lobster is an economically important species exploited for

food across much of its native range (Cochrane and Chakalall, 2001), whilst *Diadema* is an ecologically important species for its keystone role in macroalgal grazing (Edmunds and Carpenter, 2001). Both lobsters and *Diadema* use shelters on the reef to avoid predation (Eggleston et al., 1990, Levitan and Genovese, 1989) and along with lionfish, all three species spend more time using shelter during the day than during the night (Benkwitt, 2016, Briones-Fourzan et al., 2006, Ogden et al., 1973). The availability of reef shelters decreases with increasing shelter size (Menard et al., 2012), suggesting that shelters at a suitable scale for these three species may be limiting. Research indicating that spiny lobsters and *Diadema* will recruit to artificial shelters that are placed on natural reefs (Arce et al., 1997, Bodmer, 2019) further strengthens our hypothesis that suitable shelters are often limited on coral reefs.

If lionfish show similar shelter preferences as native lobsters and *Diadema*, as seen in grouper-lionfish interactions (Biggs and Olden, 2011, Bejarano et al., 2015), they may compete for the limited shelters that are available. This could lead to lionfish displacing lobsters and *Diadema* from shelters, thus exposing them to greater predation risk. Similar shelter preferences between lionfish, spiny lobsters and *Diadema* have been suggested (Davis, 2019), and shelter use interactions between lionfish and lobsters are believed to occur. Lionfish are highly abundant by-catch species in lobster traps (Akins et al., 2012) and the presence of lionfish is associated with lower lobster abundance in both traps (Lazarre et al., 2013) and condos (non-enclosed shelter traps; Henderson and Cotê, 2011, Henderson, 2012). Competition for shelter between invasive lionfish and native species could therefore have significant impacts on lobster and *Diadema* populations, which may affect economic benefits from the lobster fishery and ecological functions provided by *Diadema*.

In this study we investigate whether invasive lionfish compete with native lobsters and *Diadema* for shelter on Caribbean coral reefs. We first tested whether lionfish, lobster and *Diadema* occupy similar habitats on the reef, by conducting *in situ* assessments of habitat complexity. We then tested whether the presence of lionfish influences the behaviour of lobsters and *Diadema*, using a controlled laboratory experiment with limited shelter availability. Our study clearly identifies the overlap in

shelter preferences and illustrates the behavioural responses caused by interactions between native invertebrate species and invasive lionfish, highlighting the importance of non-consumptive effects of invasive species on the ecology of native species.

3.3 Methods

Study species and area

Lionfish (assumed to be *Pterois volitans* based on Betancur-R et al., 2011), lobsters (*Panulirus argus*) and long-spined sea urchins (*Diadema antillarum;* hereafter referred to as *Diadema*) were studied in the shallow (3-12 m) near-shore reef system of La Ensenada in Tela Bay, Honduras, at the southern end of the Mesoamerican Reef (Supplementary table 1) from June-August 2019. La Ensenada has a mean coral cover of 8.5% and mean macroalgal cover of 21.4% (Bodmer, 2019).

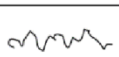
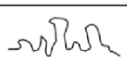
In situ habitat associations

Habitat associations of lionfish (*n*=35), lobsters (*n*=28) and *Diadema* (*n*=22) were recorded during roving SCUBA dives at three sites on the La Ensenada reef system (Supplementary table 1). SCUBA dives (*n*=43) took place between 8:00 am and 2:00 pm across June, July and August. All three species are generally inactive and inside shelter during the day (Benkwitt, 2016, Bertelsen, 2013, Ogden et al., 1973), so we assume that the habitat associations observed represent daytime shelters. Habitat was assessed within a 1 x 1 m quadrat that was placed with the centre directly over the individual of interest. Habitat associations were only recorded from individuals that were removed from the reef, thus preventing pseudoreplication, which could have occurred if the same individual was sampled on multiple occasions. We aimed to select animals with body sizes of approximately 16 cm to match the size of the experimental shelter, since many marine species choose shelters that scale with their body size (Bodmer, 2019, Eggleston et al., 1990). However, it was not always possible to find animals of this size. Mean sizes (± standard error) of the specimens used for our habitat association study were 21.5 (± 0.5) cm total length for lionfish, 5.5 (± 0.1) cm carapace length for lobsters and 4.8 (± 0.1) cm test diameter for *Diadema*. Details on the conversion from carapace length or test diameter to overall

body size are provided in the experimental design section of the methods. These habitat associations were compared to randomly sampled background areas of reef (*n*=36), obtained by laying three 50 m transects in random directions from the mooring line at approximately 5 m depth at each of the three collection sites (Supplementary table 1). A 1 x 1 m quadrat was placed every 12.5 m along the transects with the measurement as the centre point, omitting the quadrat at 0 m to remove bias in the placement of the start of the transect tape.

In all quadrats placed, Habitat Assessment Scores (HAS) for six complexity categories were recorded on a scale from 1-5, with larger values indicating higher complexity, following Gratwicke and Speight (2005). Maximum refuge size and substratum height were measured, variety of growth forms was counted (this applies to all sessile organisms and includes categories such as encrusting, branching, filamentous, and massive; see Gratwicke and Speight, 2005 for a full list of growth forms) and average live cover (percentage cover of sessile organisms such as live corals, macroalgae and sponges), average hard substratum (percentage cover that was not sand, silt or rubble) and rugosity (the topography of the reef) were estimated (Gratwicke and Speight, 2005; Figure 1). When aggregations of lionfish or lobsters were encountered, only one quadrat was recorded at the centre of the aggregation to prevent pseudoreplication. No aggregations of *Diadema* were observed.

Figure 1. Habitat Assessment Score (HAS) table. This is a modified version of the HAS table (Gratwicke and Speight, 2005) that indicates the criteria for each HAS value. Higher HAS values represent greater complexity.

HAS variable	Habitat Assessment Score (HAS)				
	1	2	3	4	5
Rugosity					
Variety of growth forms	<2	3-4	5-6	7-8	9-10
Height (cm)	0-9	10-19	20-39	40-79	≥80
Maximum refuge size (cm)	0-5	6-15	16-30	31-50	>50
Live Cover (%)	0-19	20-39	40-59	60-79	80-100
Hard substratum (%)	0-19	20-39	40-59	60-79	80-100

Specimen collection

Specimen collections were conducted by recreational SCUBA divers during roving dives in depths of 4.2 – 12.1 m. The individuals used in our laboratory experiment are a subset of those individuals that were collected during the habitat association aspect of our study. Not all collected individuals were used because the cameras failed during some of the laboratory trials. Lionfish (*n*=35) were collected from the reef using hand-nets, whilst lobsters (*n*=28) were caught by hand. Following capture, lionfish and lobsters were transferred to separate 57-litre drybags filled with seawater. *Diadema* (*n*=22) were collected and transported in a large bucket of seawater. All specimens were slowly brought to the surface and then transported to the laboratory by 15-minute boat ride within the drybag or bucket they were collected in.

Laboratory behaviour experiment

Aquarium setup

Three tanks measuring 100 x 55 x 55 cm (length x width x depth) were used for acclimatisation and two tanks measuring 245 × 56 × 24 cm were used to run the trials. We used the largest tanks available to minimise the impact of confinement on interspecific competition (Gurevitch et al., 1992). Trial tanks were the same as those used in a lionfish behaviour experiment (Hunt et al., 2019), which are similar or larger than tanks used in published experiments on spiny lobsters (Horner et al., 2006) and *Diadema* (Bodmer et al., 2017). Tanks were filled with unfiltered natural seawater to a depth of 17 cm. The air conditioning in the laboratory was controlled to ensure that water temperature in the tanks remained at 29°C, matching the ambient sea temperature at the collection sites. Tanks were illuminated from above by sunlight from large windows and thus followed the natural light-dark cycle. Tanks were surrounded by black plastic on four sides to prevent visual disturbance from neighbouring tanks. All acclimatisation and trial tanks contained a shelter that measured 18 x 16 x 15 cm internally (width x height x depth) and was constructed from six concrete bricks (Figure 2). The water level in the tanks extended 0.5 cm above the ceiling of the shelter to ensure that the internal space of the shelter was completely submerged. An internal shelter diameter of approximately 15 cm was chosen to match the previously reported preference of invasive lionfish in Honduras; solitary lionfish most often use 6-15 cm diameter shelters whilst aggregating lionfish most often use 16-30 cm diameter shelters (Hunt et al., 2019). We aimed to collect animals that matched the internal shelter dimensions to limit shelter sharing and thus create a situation of limited shelter availability.

The shelter was placed at one end of the tank, with the opening facing towards the centre of the tank. Gaps on either side of the shelter were blocked off using extra concrete bricks to prevent animals from sheltering between the edge of the tank and the shelter. Each acclimatisation tank was fitted with a Tetra whisper air pump (Spectrum Brands Inc., Virginia, USA) to maintain oxygenation of the water, however, air pumps were not used in the trial tanks because the much greater surface area would have allowed sufficient oxygen transfer and we did not want the air pumps to interfere with natural diffusion of chemical cues. At the end of every acclimatisation period and every trial, water quality

(pH, nitrate, nitrite and ammonium) was tested using a saltwater master test kit and all values were found to be within acceptable water quality guidelines (API Fishcare, Pennsylvania, USA).

Figure 2. Lobster inside the shelter in the trial tank.



Specimen acclimatisation

On arrival at the laboratory, each species was placed into a separate acclimatisation tank, with a maximum of two conspecific individuals per tank. Animals were collected between 8:00am and 2:00pm and tested the following morning, resulting in acclimatisation times of 16-24 hours. This was deemed to be enough time because at the beginning of each trial, all individuals were displaying normal day-time behaviour of resting inside shelter (Cure et al., 2012, Kanciruk and Herrnkind, 1972, Ogden et al., 1973). Although shorter acclimatisation times have been used for studies on *Diadema* (Bodmer et al., 2017), we chose to standardise the acclimatisation time across species. Animals were not fed during the acclimatisation period to standardise hunger levels between individuals (Webster et al., 2008, Kaiser et al., 1990).

Experimental design

Single individuals of each species were tested alone (lionfish alone *n*=12, lobster alone *n*=12, *Diadema* alone *n*=12) to determine their activity and shelter use in the absence of other, potentially competing, species. Lobsters and *Diadema* were also tested in the presence of a lionfish (lionfish-lobster together *n*=12, lionfish-*Diadema* together *n*=7) to determine whether behaviour differed between these 'together' trials and the 'alone' trials. Each animal was only used once. The individuals used in our laboratory experiment are a sub-set of those used in the habitat association study, thus we now report the range of sizes used for our laboratory experiment. Lionfish ranged in total length (TL) from 14.6-26.6 cm, with a mean (± standard error) of 21.2 (± 0.6) cm. Lionfish size at sexual maturity varies between the sexes and between locations but on average is 10 cm for males and 17.5 cm for females (Morris Jr, 2009), indicating that the majority of our lionfish were likely to be adults. Lobsters ranged in carapace length (CL) from 4.1-7.1 cm, with a mean of 5.4 (± 0.1) cm, and thus were classed as small juveniles (Eggleston et al., 1990). The minimum carapace size at which lobsters can legally be fished is 7.8 cm (Henderson, 2012), thus the lobsters in our experiments were below the legal size. Using a CL to TL conversion for *Panulirus argus* (Creaser, 1952), we calculated that the lobsters ranged from 10-18 cm in TL. We only used lobsters with hard exoskeletons, indicative of intermoult condition, because crustaceans can be more vulnerable to predation when they have recently moulted (Marshall et al., 2005), potentially influencing their sheltering behaviour in a way that would confuse interpretation of the results. *Diadema* ranged in test diameter from 4.0-5.6 cm, with a mean of 4.9 (± 0.1) cm, and were classified as adults because they had no banded spines (Randall et al., 1964). We only used adults of the more common black phenotype because behaviour is known to vary between phenotypes (Bodmer et al., 2017). Using a test diameter to spine length conversion for *Diadema* spp. (Coppard and Campbell, 2004), we calculated that the *Diadema* individuals used in our experiment ranged from 20-28 cm in total diameter.

To begin the trial, lionfish and/or lobsters were placed at the opposite end of the tank to the shelter, but *Diadema* always began the trial inside the shelter due to a lack of vision. The artificial shelter

would not have provided natural olfactory cues for *Diadema*, thus if they had been placed at the opposite end of the tank, they may not have found the shelter. As we were using two identical trial tanks, we randomised which tank was used for each trial.

Trials were filmed using a Bushnell Trophy Cam HD Aggressor (Bushnell Corporation, Missouri, USA) positioned 1 m from the end of the tank containing the shelter and angled down at 45 degrees. This positioning of the camera allowed us to see into the back of the shelter and 17 cm in front of the shelter. The camera filmed using infrared (850 nm wavelength) during both day and night because preliminary trials found that videos were often underexposed when filmed in daylight without the use of flash. Lionfish, lobsters and *Diadema* show low sensitivity to wavelengths above 600 nm (Carroll, 2018, Cummins et al., 1984, Millott and Yoshida, 1957) so the light emitted by these cameras should have been non-detectable. Trials ran for 24 hours and video recordings were made for 60 seconds every hour.

Following the trials, we telson-clipped the lobsters to ensure that individuals were not re-used in subsequent experiments. *Diadema* were not marked because current tagging methods are invasive and can impact survival (Rodriguez-Barreras and Sabat, 2015). Instead, *Diadema* were released by the mooring line at the collection site and subsequent individuals were collected a minimum of 20 m away. Average movement each night by *Diadema* is less than 5 m (Tuya et al., 2004) and individuals often return to the same crevice repeatedly (Carpenter, 1984), thus we assume that 20 m was sufficient to prevent individuals being recaptured. All lobsters and *Diadema* were released back onto the reef within two hours of the trial ending. Immediately after the trial, lionfish were humanely culled using a two-step method for finfish euthanasia, whereby cervical transection was followed by pithing to destroy the brain tissue (Leary et al., 2013). All acclimatisation and trial tanks were emptied, cleaned, and refilled with new seawater between trials.

For each 60 second video, the time spent: (i) active vs resting (activity), and (ii) inside vs outside the shelter (shelter use) were recorded. Individuals were recorded as inside the shelter if at least 25% of

their body length was inside and were recorded as active if they were moving across the tank. Lionfish often moved their fins and lobsters moved their legs whilst stationary, but we categorised these behaviours as inactive because the individual remained in one location.

For our analyses, we calculated the time spent in each behaviour over the course of the day, but we removed the first hour of each trial to ensure that all animals had acclimatised to the trial tank for a minimum of one hour. Analysed trial data thus spanned 23 hours– comprised of 23 videos of 60 seconds. We calculated activity as the proportion of time spent active out of the time spent in view of the camera, since when animals were out of view we did not know whether they were active or inactive. We calculated shelter use as the proportion of time spent inside shelter out of the total trial time, since there was only one shelter in the tank so even when not in view of the camera we knew that animals were not inside the shelter. In addition to our formal analyses, we recorded the individual that entered the shelter first and the individual that spent the longest length of time inside the shelter (over the 23-hour trial). In the lionfish-*Diadema* together trials, the *Diadema* always started the trial inside the shelter, as previously explained.

Statistical analysis

All tests were two-tailed with an *a priori* significance level of 0.05 and were conducted in R version 3.6.2 (R Core Team, 2019). All graphs were plotted using the ggplot2 package (Wickham, 2016) with the colour-blind friendly viridis colour palette from the viridis package (Garnier, 2018).

To visualise *in situ* habitat associations, we performed non-metric multidimensional scaling (nMDS) with a Bray-Curtis dissimilarity matrix using the vegan package in R (Oksanen et al., 2019), and plotted vectors of the HAS categories to aid interpretation of the visual output. We then conducted an analysis of similarities (ANOSIM) test to determine whether complexity differed between quadrats (background, lionfish, lobster and *Diadema*).

To examine *in situ* habitat associations for specific HAS categories, we conducted Kruskal-Wallis tests, which were chosen because the HAS data were ordinal, rather than continuous. We conducted one Kruskal-Wallis test for each of the six HAS categories: rugosity, variety of growth forms, substratum height, maximum refuge size, percentage live cover and percentage hard substratum. Each Kruskal-Wallis test compared HAS between background, lionfish, lobster and *Diadema* quadrats. When the results were significant- indicating a difference in HAS value between quadrat types- we ran post-hoc Dunn tests (Dinno, 2015) to compare all treatment combinations: background-lionfish, background-lobster, background-*Diadema*, lionfish-lobster, lionfish-*Diadema* and lobster-*Diadema*. Our post-hoc Dunn tests were run using the Benjamini-Hochberg method to correct for multiple testing (by controlling the type I error rate) whilst retaining greater power than the Bonferroni method (Waite and Campbell, 2006).

Our data for activity and shelter use were proportions. The denominator of the fractions was large (up to 1380), thus allowing the proportions to take a wide range of values. For this reason our data can be described as non-count-based proportions and thus were analysed using beta regression (Douma and Weedon, 2019), which can account for the heteroskedasticity and skewness that are common with proportion data (Cribari-Neto and Zeileis, 2010). Beta regression does not work with values of exactly 0 or 1, so the response variable must be transformed prior to analysis (Cribari-Neto and Zeileis, 2010, Douma and Weedon, 2019). We transformed our response variables using equation 1 (modified from Cribari-Neto and Zeileis, 2010) to scale our response between 0.05 and 0.95. We used the default logit link function for the beta regression analyses because the choice of link function will only affect model fit when one or more of the predictors is continuous (Douma and Weedon, 2019).

Equation 1. This equation, modified from Cribari-Neto and Zeileis (2010), converts the response variable y, which is a proportion that can range from 0-1, to a new response variable y' that can range from 0.05-0.95.

$$y' = \frac{(y \times 9) + 0.5}{10}$$

Model selection followed the two-step process detailed in Bayer and Cribari-Neto (2017), whereby we first selected the best model from our candidate models assuming fixed precision. We then created a new series of candidate models that incorporated variable precision, which can account for differences in variance between treatments (Douma and Weedon, 2019) and thus improve model fit. The best model was deemed to be the one with the lowest AICc value (Anderson and Burnham, 2004). Most of our models differed from the next best model by more than two AICc units and thus our 'best' models have substantial support relative to the next best model (Anderson and Burnham, 2004). However, two of our models (lobster activity and *Diadema* shelter use) differed from the next best model by less than two AICc units, with the next best model containing one extra parameter. These extra parameters can be considered uninformative (Arnold, 2010), thus we selected the simpler models, which had the lowest AICc. We used AICc rather than AIC because of our small sample sizes (Anderson and Burnham, 2004).

Beta regression analyses (hereafter referred to as BR) were conducted using the betareg package in R (Cribari-Neto and Zeileis, 2010) and AICc values were calculated using the AICcmodavg package in R (Mazerolle, 2019). Trial type (lionfish alone, lobster alone, *Diadema* alone, lionfish-lobster together, lionfish-*Diadema* together) was always included in the mean models but we also tested whether to include trial tank (left, right) in the mean and precision models. We tested the difference in behaviour (activity and shelter use) between trial types in the 23-hour dataset. Trial tank was never included in the best fitting mean or precision models and thus will not be discussed further.

Ethics statement

Lionfish culling was carried out in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (Leary et al., 2013). All experimental protocols were approved by the University of Oxford Department of Zoology Animal Welfare and Ethical Review Body. No specific permits were required to collect lionfish, lobsters, or urchins. A research permit for this work was obtained from the Instituto de Conservacion Forestal, Honduras (ICF-508-2019).

3.4 Results

Lionfish, lobsters and *Diadema* have similar habitat preferences

There was a significant difference in habitat complexity between the species quadrats and the background quadrats (ANOSIM, R=0.440, p=0.001; Figure 3), with all three species generally found in more complex areas of the reef when compared with average reef complexity (Dunn tests, p<0.05 for all; Table 1; Figure 4). All three species were found in areas with significantly higher values for rugosity, variety of growth forms, percentage live cover and percentage hard substrate (Dunn tests, p<0.05 for all; Table 1), but none of the species differed significantly from one another (Dunn tests, p>0.05 for all; Table 1). For height, values for all three species were significantly higher than the background, whilst both lionfish and lobsters were found in significantly more complex habitat than *Diadema* (Dunn tests, p<0.05 for all; Table 1).

Maximum refuge size showed the greatest difference between species. Lionfish were found in areas with greater maximum refuge sizes than lobsters or *Diadema*, and greater than the background (Dunn tests, p<0.05 for all; Table 1). Lobsters were found in significantly more complex habitat than *Diadema* or the background (Dunn tests, p<0.05 for both; Table 1). *Diadema* did not differ significantly from the background (Dunn test, Z=-0.937, p=0.349). The median refuge size HAS were: background (6-15 cm), *Diadema* (16-30 cm), lobsters (16-30 cm) and lionfish (31-50 cm). Despite these differences between species, there was still considerable overlap in HAS for maximum refuge size between species (Figure 4).

Figure 3. Non-metric multidimensional scaling ordination of reef quadrat complexity. Each point represents a 1 x 1 m quadrat that is either a randomly sampled background quadrat (*n*=36), or contains a lionfish (*n*=35), lobster (*n*=28) or *Diadema* (*n*=22). The plot was constructed using values of the six HAS categories: rugosity, variety of growth forms, substratum height, maximum refuge size, percentage live cover and percentage hard substratum. Quadrats with more similar complexity are closer together on the plot. Vectors indicate the direction of the gradient for each HAS category.

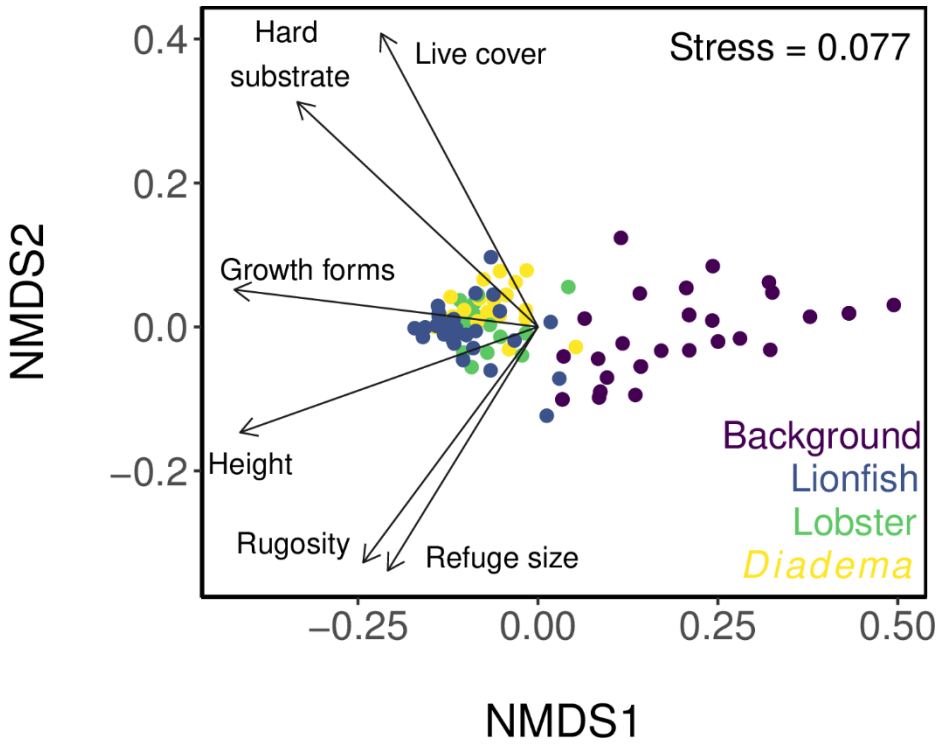


Figure 4. Habitat Assessment Score (HAS) joyplot. HAS are plotted for background (*n*=36), lionfish (*n*=35), lobster (*n*=28) and *Diadema* (*n*=22) quadrats. Larger HAS represents greater complexity. HAS are recorded on an ordinal scale from 1-5, thus it is the peaks in this joyplot that highlight the overlap, or lack of overlap, between the four groups. H-values and *p*-values represent the test statistic and significance of each Kruskal-Wallis test.

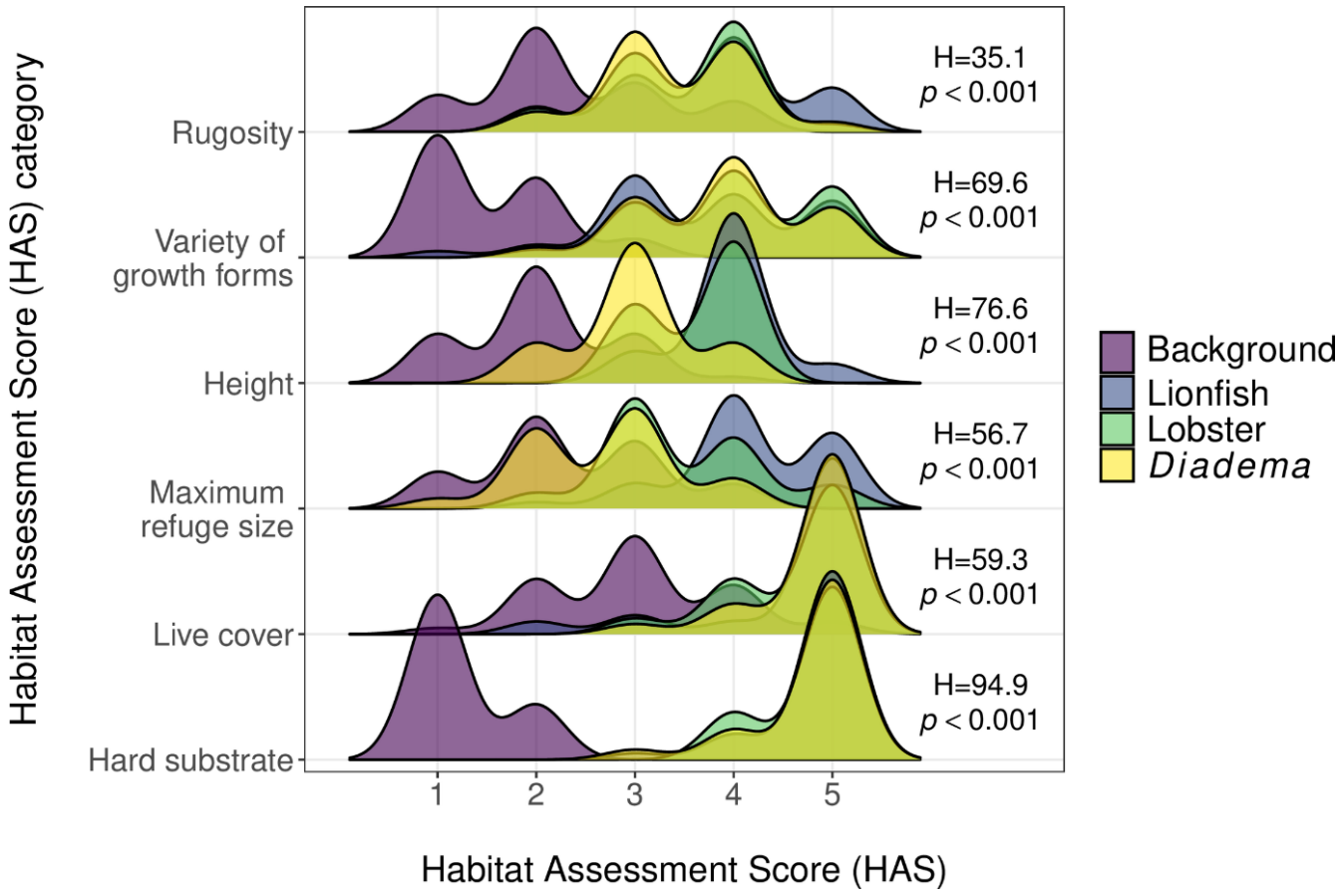


Table 1. Dunn tests results. Z-values and Benjamini-Hochberg adjusted *p*-values of post-hoc Dunn tests are displayed for all Habitat Assessment Score (HAS) categories. Highlighted cells represent significant results ($p<0.05$). Z-values are rounded to three significant figures and *p*-values are rounded to three decimal places (except for $p<0.001$).

		Rugosity		Variety of growth forms		Height		Maximum refuge size		% Live cover		% Hard substrate	
		Z-value	*p*-value	Z-value	*p*-value	Z-value	*p*-value	Z-value	*p*-value	Z-value	*p*-value	Z-value	*p*-value
Comparisons with the background	Background-lionfish	-5.52	<0.001	-6.44	<0.001	-8.17	<0.001	-7.02	<0.001	-6.42	<0.001	-8.34	<0.001
	Background-lobster	-4.28	<0.001	-7.06	<0.001	-6.31	<0.001	-3.94	<0.001	-5.69	<0.001	-7.51	<0.001
	Background-*Diadema*	-3.71	<0.001	-6.17	<0.001	-3.03	0.004	-0.937	0.349	-6.07	<0.001	-7.11	<0.001
Comparisons between species	Lionfish-lobster	0.912	0.434	-0.987	0.486	1.38	0.168	2.66	0.012	0.353	0.724	0.347	1.00
	Lionfish-*Diadema*	1.12	0.393	-0.518	0.725	4.11	<0.001	5.19	<0.001	-0.438	0.794	0.205	1.00
	Lobster-*Diadema*	0.260	0.795	0.383	0.702	2.70	0.008	2.59	0.011	-0.732	0.696	-0.113	0.910

Lionfish exert non-consumptive effects, but so do native species

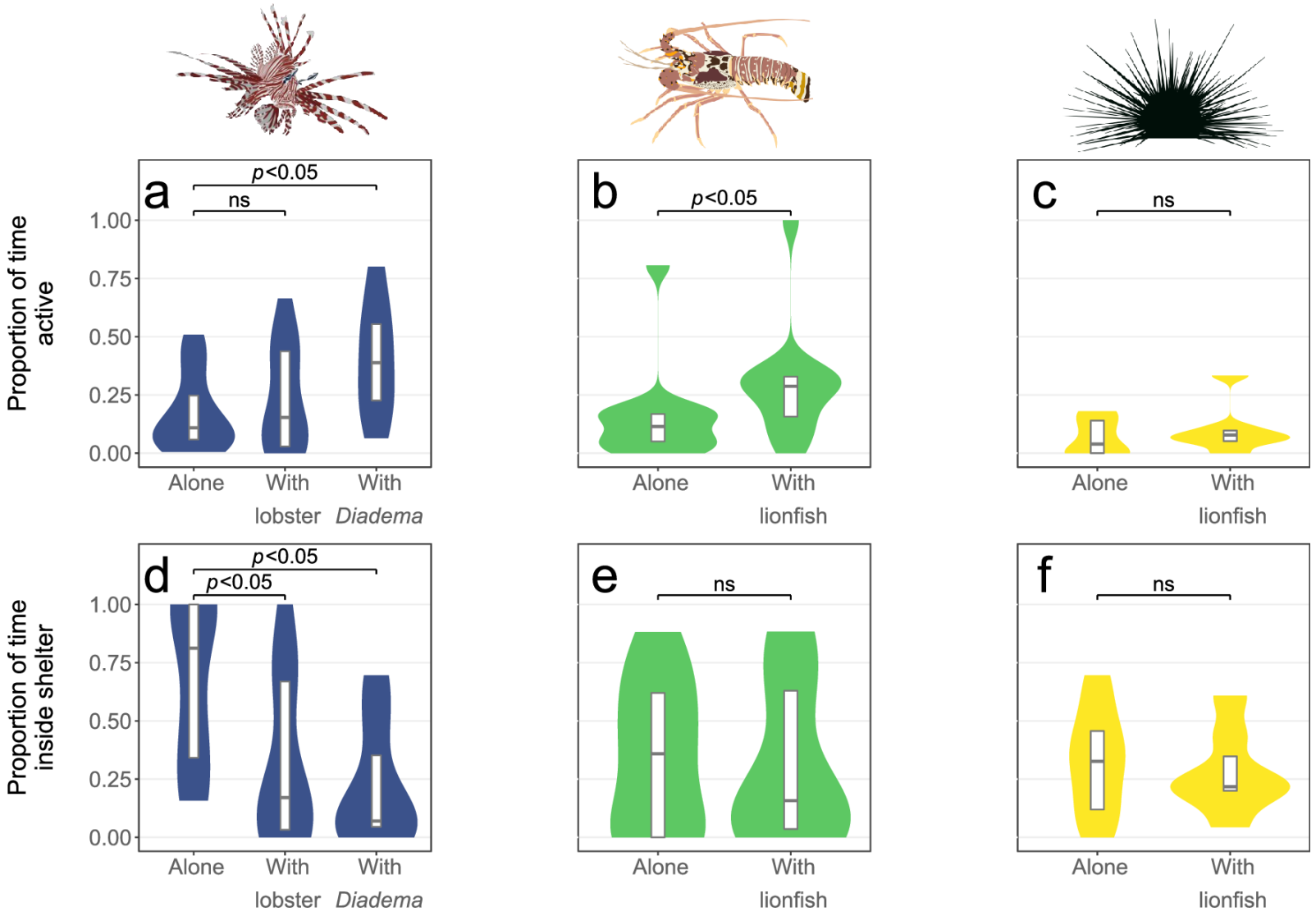
Lobsters were 1.8 times more active (BR, Z=2.43, p=0.015; Figure 5), but did not change their shelter use (BR, Z=-0.119, p=0.906), in the presence of a lionfish during the 23-hour trial. The presence of lionfish had no effect on activity (BR, Z=1.77, p=0.077; Figure 5) or shelter use (BR, Z=0.302, p=0.763) of *Diadema*. Lionfish and lobsters shared the shelter in one out of twelve trials (8% trials), whilst lionfish and *Diadema* shared the shelter in two out of seven trials (29% of trials). In ten of the twelve lionfish-lobster together trials (83% trials), the species that entered the shelter first was also the species that spent the most time inside the shelter (Table 2). However, in the lionfish-*Diadema* together trials, lionfish and *Diadema* appeared equally likely to occupy the shelter (dominating the shelter in n=3 and n=4 trials respectively), even though *Diadema* always began the trial inside the shelter.

Lionfish were 1.9 times more active in the presence of *Diadema* than when alone (BR, Z=2.39, p=0.017; Figure 5) during the 23-hour trial. In contrast, the presence of lobsters had no effect on lionfish activity (BR, Z=0.487, p=0.626). Lionfish used shelter 1.9 times more when alone than when in the presence of lobsters (BR, Z=-2.75, p=0.006) and 2.2 times more when alone than when in the presence of *Diadema* (BR, Z=-2.85, p=0.004).

Table 2. Shelter occupancy data from lionfish-lobster together trials. Trials (n=12) are categorised based on which species entered the shelter first and which species spent the most time inside the shelter.

Species that entered the shelter first	Species that spent the most time inside the shelter	
	Lionfish	Lobster
Lionfish	6	1
Lobster	1	4

Figure 5. Activity and shelter use of lionfish, lobsters and *Diadema*. The proportion of time that lionfish, lobsters and *Diadema* spent active (a,b,c) and inside shelter (d,e,f) in the 'alone' and 'together' treatments during the 23-hour trial. Each plot represents how the behaviour of the focal species (indicated by the creature image) varies between the 'alone' and 'together' trials. For the lionfish graphs (a and d), the 'alone' bar represents the behaviour of lionfish when alone, the 'with lobster' bar represents the behaviour of lionfish in the presence of a lobster, and the 'with *Diadema*' bar represents the behaviour of lionfish in the presence of a *Diadema*. For the lobster (b and e) and *Diadema* (c and f) graphs, the 'alone' bar represents their behaviour when alone, whilst the 'with lionfish' bar represents their behaviour in the presence of a lionfish. Violin plots represent the spread of the data and are scaled so that the violins on each plot have the same area. Each violin plot is overlaid with a boxplot showing the median and inter-quartile range. All treatments have n=12, except the 'lionfish-*Diadema* together' treatment, for which n=7.


a
b
c
d
e
f
p<0.05
ns
Proportion of time active
Proportion of time inside shelter
1.00
0.75
0.50
0.25
0.00
Alone
With lobster
With Diadema
With lionfish

3.5 Discussion

We found invasive lionfish have similar *in situ* habitat preferences as native lobsters and *Diadema* (Figure 4), thus creating the potential for competition when shelter availability is limited. Our experimental trials then indicated that lionfish presence led to increased activity in lobsters but had no effect on *Diadema* (Figure 5). We found the presence of lionfish did not affect shelter use by lobsters or *Diadema*. Lionfish, however, had reduced shelter use when in the presence of lobsters or *Diadema*.

Lionfish, lobsters and *Diadema* preferred high complexity habitats

All three species preferred habitat that was more complex than the background average. For lobsters and *Diadema*, higher complexity habitat provides protection from predation (Eggleston et al., 1990, Levitan and Genovese, 1989). The preference of lionfish for complex habitat applies across the invaded western Atlantic range (Agudo and Salas, 2014, Davis, 2019, Garcia-Rivas et al., 2017). In areas with frequent culling, lionfish use complex habitat to escape culling by humans (Cote et al., 2014). However, lionfish have few natural predators in the invaded range (Cote et al., 2013), so in areas where they are not culled by divers, habitat preference may instead be related to hunting efficiency (Hunt et al., 2019). For four of the HAS metrics (rugosity, variety of growth forms, percentage live cover and percentage hard substrate) all three species showed similar habitat preference. This suggests lionfish, lobsters and *Diadema* are likely to compete for reef shelters in the Caribbean. Reef flattening, a reduction in hard coral cover and subsequent decline in architectural complexity, is occurring across the region (Alvarez-Filip et al., 2009), reducing the availability of shelters and so leading to increased shelter competition.

We found significant differences in maximum refuge size between species. A previous study from Honduras found that lionfish preferred shelter sizes of 6-15 cm and 16-30 cm (Hunt et al., 2019). However, we found that lionfish preferred shelter sizes of 31-50 cm. This disparity may reflect differences between the availability of shelters on different reefs, with lionfish habitat associations reflecting their preferred habitat based on local habitat availability. Spiny lobsters use shelters that

scale to their body size when alone (Eggleston et al., 1990) but they will use larger shelters when aggregating with conspecifics (Eggleston and Lipcius, 1992). Most of the lobsters we found were solitary, which may explain their preference for smaller shelters of the size 16-30 cm. When suitably sized shelters are unavailable, spiny lobsters will use any crevice that is large enough to protect their abdomen (Briones-Fourzan et al., 2006). Although we attempted to collect similar sized individuals, the lionfish we collected were larger on average than the lobsters, which may explain the observed difference in preferred shelter size. We used lobsters that were below the minimum legal size, which suggests that the shelter size preference of larger, legal-sized, lobsters may overlap more with that of lionfish. A study on *Diadema* in Utila, Honduras, found that they preferred refuges of the size 5-15 cm (Bodmer, 2019), which is smaller than the median refuge size of 16-30 cm found in our study. *Diadema* select shelters that scale with their test size to avoid predation (Bodmer, 2019), therefore this difference in shelter size preference may be related to the high proportion of juvenile urchins present at the sites surveyed by Bodmer (2019), whereas we only included adults in our study. By converting lobster carapace size and *Diadema* test diameter to total body size, we found that the *Diadema* were larger in overall size than the lobsters and lionfish. Although *Diadema* were larger, it is likely that they chose smaller shelters than lionfish or lobsters because they choose shelters that scale with their test diameter (Bodmer, 2019), rather than with their total body size. This indicates that *Diadema* may only be subject to shelter competition from small lionfish.

The relationship between lionfish and native invertebrates

Lobsters became more active in the presence of lionfish. This increase in activity may be related to aggression, as spiny lobsters display several aggressive behaviours (Shabani et al., 2009). American lobsters (*Homarus americanus*) spend more time active in the presence of other lobsters than when alone and this activity is related to fighting and defending a shelter (Spanier et al., 1998), so the increased activity in our study may be related to shelter defence. Increased activity can reduce the scope for growth in other crustaceans (Parslow-Williams et al., 2002), and so could result in reduced growth rates of lobsters. In the absence of shelter, spiny lobsters will remain motionless in the presence of a predator (Weis et al., 2008), suggesting that increased activity may attract predators and

increase predation risk. Lobsters sense their environment using olfactory (Horner et al., 2006), visual (Herrnkind.W, 1969) and tactile (Wilkens et al., 1996) cues and can detect predator olfactory cues from at least 2 m away (Berger and Butler, 2001). Our experiment cannot distinguish which cues might have been used by the lobsters to detect lionfish, but if they used olfactory cues then the results we observed may be widespread on reefs with invasive lionfish populations, even when lionfish are out of visual range of the lobster. We did not conduct trials with two lobsters, or trials where a lobster was paired with another species, thus we cannot conclusively show that the increased activity of lobsters is driven by the presence of lionfish. Even if the observed response is not specific to lionfish presence, the increase in activity is still cause for concern because as lionfish populations increase, the frequency and duration of lionfish-lobster encounters is likely to increase.

Despite the increase in lobster activity, there was no change in shelter use in the presence of a lionfish, suggesting that the lobsters may be dominant and able to displace lionfish from the shelter, or prevent displacement by lionfish once they are inside the shelter. The lack of change in shelter use suggests that the increased activity observed in the presence of a lionfish is unlikely to be related to submissive behaviour and movement away from the shelter. Lionfish and lobsters shared the shelter in one of our trials, suggesting that even on reefs with limited, and already occupied, shelters, lobsters may still be able to access the shelters. However, we found that prior occupancy influenced shelter use, as in other fish and crustaceans (Nakata and Goshima, 2003, Braddock, 1949). We found that the first species to enter the shelter was generally the species that spent the most time in the shelter during the trial. This suggests that if shelters are not large enough for lionfish and lobsters to share, then prior occupancy of a lionfish may prevent shelter use by lobsters.

Lionfish had no effect on activity or shelter use of *Diadema*. Several fish families are known predators of *Diadema* (Randall et al., 1964) and predator cues trigger increased movement (Kintzing and Butler, 2014), so the lack of activity change suggests that lionfish were not considered to be a predator. Alternatively, the lack of a change in activity or shelter use may be because the experimental shelter was considered high quality, as *Diadema* are less likely to flee from a high-quality crevice

(Carpenter, 1984). In the absence of predators, *Diadema* will avoid shelters containing conspecific odours, which is thought to be a method of avoiding shelter competition (Kintzing and Butler, 2014). Lionfish and *Diadema* shared the shelter in two of our seven trials, suggesting that lionfish are not considered a shelter competitor and thus the presence of a lionfish inside the shelter does not impact *Diadema* shelter use. However, we found that prior occupancy had no effect on which species used the shelter the most, suggesting that in some cases lionfish may be able to exclude *Diadema* from their shelters.

Impacts of lobsters and *Diadema* on lionfish

Lionfish did not change their activity in the presence of lobsters but they became more active in the presence of *Diadema*. Lionfish increase their activity in response to intraspecific competition (Benkwitt, 2016), thus we suggest that lionfish may become more active in the presence of *Diadema* because they perceive them as a shelter competitor. Lionfish may not have increased their activity in response to lobsters because the lobsters may have been perceived as a dominant competitor, as is suggested by other studies on shelter use interactions between fish and crustaceans (Griffiths et al., 2004, Bubb et al., 2009). The differential response of lionfish to lobsters and *Diadema* indicates that lionfish are not simply responding to over-crowding (there are two individuals in the 'together' trials but only a single individual in the 'alone' trials). Instead, lionfish behavioural responses are determined by the identity of the competitor, as is observed in other fish species (Desiardins et al., 2008).

Increased activity in lionfish may lead to less energy being available for other functions such as growth. In areas where lionfish are culled by divers, increased activity of lionfish may make them more conspicuous and easier to capture, thus aiding culling efforts. But an increase in lionfish activity may not always be beneficial for the native community. Increased activity may increase the likelihood of interactions with native species, potentially leading to other non-consumptive effects, such as reduced grazing by herbivorous fish (Eaton et al., 2016, Kindinger and Albins, 2017).

In contrast to other invasive species, where shelter use of the invasive is unchanged in the presence of native species (Griffiths et al., 2004, Raymond et al., 2015), we found that lionfish reduced their shelter use in the presence of lobsters or *Diadema*. Our findings lend support to anecdotal evidence that reefs with high urchin densities support few lionfish (Davis, 2019), potentially because urchins occupy all available shelters. In our lionfish-lobster together trials we found an effect of prior residency, where lionfish were less likely to dominate a shelter if a lobster occupied the shelter first. However, the shelter we used in our laboratory experiment (16 cm) was smaller than the preferred shelter size of the lionfish used in this study (31-50 cm), thus the motivation of the lionfish to use the shelter may have been less than it would be had the shelter been larger. Nevertheless, a reduction in shelter use by lionfish may mean that if shelter is limiting, it is the natives that gain access to the shelters. Reduced shelter use may be detrimental to lionfish health and survival, meaning that lionfish may be less successful invaders on reefs with limited shelter. Feeding success may be reduced if use of shelters enhances feeding success, as suggested by Hunt et al (2019), thus leading to reduced consumptive effects of lionfish on reef communities. Larger and heavier female lionfish produce more eggs (Gardner et al., 2015), so any subsequent loss in body condition may reduce fecundity and thus reduce the population growth rate. Reduced shelter use of lionfish may therefore be beneficial to reef communities.

Management implications

Studies on the commercial spiny lobster fishery have shown that lionfish are often caught as by-catch in traps (Akins et al., 2012) and the presence of lionfish is associated with reduced lobster abundance in traps (Lazarre et al., 2013) and condos (non-enclosed shelter traps; Henderson and Cotê, 2011, Henderson, 2012). These results have led to the suggestion that lionfish may deter or out-compete lobsters (Lazarre et al., 2013). Competition between lionfish and lobster may be particularly strong around lobster condos because these are often placed in areas of otherwise low habitat complexity (Henderson and Cotê, 2011). In our experiments we found that lionfish, rather than lobster, reduced their shelter use when the two species were together, suggesting that lobsters may instead out-compete lionfish. We hypothesise that lobsters exclude lionfish from traps and condos and that the strength of

the behavioural change in lionfish may be related to lobster abundance in the trap. This would explain why lionfish are found in traps with small, but not large, numbers of lobsters. The lobsters used in our experiments were below the legal harvesting limit of 7.8 cm carapace length (Henderson, 2012) and so our results may not be applicable to larger lobsters. However, if larger lobsters are stronger shelter competitors, as in other lobster species (Oneill and Cobb, 1979), then the effects on lionfish shelter use that we observed may be even stronger when lionfish compete with larger lobsters.

Although our results suggest that lionfish do not affect lobster shelter use, and therefore may not reduce lobster catches, this does not mean that lionfish have no effect on spiny lobster fisheries. The presence of lionfish in lobster condos increases handling time by fishers as they work more cautiously around lionfish to avoid envenomation and this may lead to reduced earnings as fishers are unable to visit as many condos each day (Henderson, 2012). However, these reduced earnings could be partially compensated by increasing lionfish fisheries and selling the lionfish to restaurants or developing value-added lionfish products – such as jewellery (Chapman et al., 2016, Henderson, 2012).

A lack of structural complexity and the subsequent lack of predation refugia has been suggested as a barrier to recovery of *Diadema* following the 1983-1984 mass mortality (Bodmer et al., 2015). One restoration effort has thus been to provide artificial shelters (Bodmer, 2019). Lionfish often use and aggregate around artificial structures (Dahl and Patterson, 2014, Jud and Layman, 2012) and could potentially hinder restoration efforts if they affect *Diadema* shelter use. We initially hypothesised that lionfish would enter the shelter and exclude the *Diadema*, but our results showed that lionfish had no effect on *Diadema* activity or shelter use. This lack of response by *Diadema* suggests that lionfish may not interfere with restoration efforts and that the presence of lionfish on a reef may not a barrier to recovery of *Diadema*.

Lobster traps may prove useful for lionfish management, as studies have shown catches of almost 3000 lionfish in a single lobster fishing season (Akins et al., 2012). Trapping will complement current management methods because culling by recreational divers is restricted to 30 m (Andradi-Brown et

al., 2017a), but traps can be deployed much deeper to target the larger and more mature lionfish that are not currently culled (Andradi-Brown et al., 2017a). Our results have shown reduced shelter use of lionfish in the presence of lobsters, suggesting that lionfish catches would be maximised in the absence of lobsters. To develop lionfish-specific traps, current lobster traps need to be modified to increase lionfish catch but reduce lobster catch. Some work has been done in this area (Pitt and Trott, 2014), but lionfish-specific traps are still being tested (Harris et al., 2020) and are not yet commercially available. Current diver-based culling is insufficient to satisfy demand for international export of lionfish (Chapman et al., 2016), but the development of a lionfish trap fishery could provide a low-cost removal method that can be applied to depths inaccessible to divers (Andradi-Brown, 2019).

3.6 Conclusion

Our results have demonstrated the importance of testing for non-consumptive effects of invasive species. We found that lionfish are not detrimental to all native species, as there was no effect of lionfish on *Diadema* activity or shelter use. Invasive species research often focuses on the impacts on the invaded community or on trophic interactions exerted by native species on invasive species, however, we have shown the importance of investigating non-consumptive effects of the native species on the invasive. These effects may prove detrimental to the invader or may lead to additional negative effects on native species. By studying the interactions between lionfish and two different invertebrates, we have demonstrated that non-consumptive effects of native species on invasive species may be widespread. Our research suggests that we should look beyond the consumptive effects of invasive predators and consider how native species may induce changes in invasive species behaviour.

3.7 Acknowledgements

We thank all the Operation Wallacea volunteers and staff who assisted with the data collection, and special thanks goes to Antal Borcsok and the Tela Marine Research Centre. We also thank Olivia Farman for illustrating the lionfish, lobster and *Diadema* in Figure 5.

3.8 Author contributions

Conceptualization: Christina L. Hunt, Dominic A. Andradi-Brown, Jocelyn Curtis-Quick, Dan A. Exton.

Formal analysis: Christina L. Hunt, Dominic A. Andradi-Brown, Dan A. Exton.

Investigation: Christina L. Hunt, Callum J. Hudson, Joshua Bennett-Williams, Frankie Noades.

Supervision: Dominic A. Andradi-Brown, Dan A. Exton.

Visualization: Christina L. Hunt, Dominic A. Andradi-Brown, Callum J. Hudson, Owen T. Lewis, Dan A. Exton.

Writing – original draft: Christina L. Hunt.

Writing – review & editing: Christina L. Hunt, Dominic A. Andradi-Brown, Callum J. Hud- son, Joshua Bennett-Williams, Frankie Noades, Jocelyn Curtis-Quick, Owen T. Lewis, Dan A. Exton

3.9 Supplementary information

Supplementary table 1. Dive site co-ordinates. Co-ordinates of the mooring lines at each of our three study sites in Tela Bay, Honduras. Lionfish (*n*=35), lobsters (*n*=28) and *Diadema* (*n*=22) were collected from all three sites for use in the laboratory experiments. We recorded Habitat Assessment Scores (HAS) for 12 background quadrats at each site and for each individual that was collected. A total of 43 SCUBA dives took place between 8:00 am and 2:00 pm across June, July and August 2019. Coordinates are given in WGS84.

Dive site	Latitude (°N)	Longitude (°W)
Palm view 1	15.80339	87.4396
Palm view 2	15.80336	87.4392
Nelly's island	15.80017	87.4426

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4 Non-consumptive effects of invasive lionfish on herbivorous fish grazing vary within a reef system

Christina L. Hunt, Dominic A. Andradi-Brown, Joshua Bennett-Williams, Jess Sweeney, Dennis Duffin, Emma Taylor-Clark, Dan A. Exton

4.1 Abstract

Although the consumptive effects of invasive lionfish are well known, research on their non-consumptive effects is sparse. A potential non-consumptive effect is a reduction in herbivorous fish grazing, which may arise if herbivorous fish respond to lionfish as a predator. Reductions in grazing can have cascading effects on reef ecosystems including driving phase-shifts to algal-dominated states, thus it is important to quantify any effects of lionfish on herbivorous fish grazing. Using remote cameras, we observed grazing behaviour by herbivorous fish and recorded the fish communities observed in the same small areas of reef with and without lionfish. Our results suggest that although lionfish may reduce herbivorous fish grazing behaviour in some circumstances, these impacts are not uniform even within a small reef system. We suggest the observed differences in herbivorous fish response to lionfish may relate to differences in habitat complexity, predator density or the characteristics and behaviour of individual lionfish. This study highlights the importance of comparing invasive species impacts across multiple sites to detect variations in impacts.

4.2 Introduction

The effects of predatory species are often observed as consumptive effects, where predators reduce the abundance of their prey (Baum and Worm, 2009). However, predators can also exert non-consumptive effects, whereby they alter the morphology, physiology, life history or behaviour of their prey (Werner and Peacor, 2003). Non-consumptive effects can be as strong as, or stronger than, consumptive effects and are generally stronger in aquatic than in terrestrial systems (Preisser et al., 2005). In aquatic environments, changes in prey behaviour in response to predators can include differing shelter use, such as entering a refuge (Helfman, 1989) or spending more time inside shelter (Marsh-Hunkin et al., 2013), differing habitat use (Werner et al., 1983), reduced feeding intensity (Rizzari et al., 2014), increased group size (Hoare et al., 2004) and differing frequencies of species-specific behaviours, such as head-bobs in gobies (Marsh-Hunkin et al., 2013).

On coral reefs, herbivorous fish play an important role in maintaining benthic communities by grazing on algae (Hughes, 1994). The removal of herbivores leads to an increase in macroalgal percentage cover and biomass (Hughes et al., 2007, Lirman, 2001). Predatory fish consume herbivorous fish and can also induce 'fear effects', which may affect algal grazing (Catano et al., 2016). Site-attached herbivores reduce their foraging area in response to predators, creating a more heterogeneous distribution of algal patches on the reef (Madin et al., 2010a). For roving herbivores, the presence of predators leads to reduced macroalgal grazing, which leads to increased macroalgal biomass (Rasher et al., 2017). When algal grazing is reduced below a critical threshold, cascading effects can occur, leading to a phase-shift from a coral-dominated to an algal-dominated reef (Hughes, 1994).

The introduction of new predators may therefore impact herbivore-algal interactions. Lionfish (*Pterois volitans* and *P. miles*) were introduced off the coast of Florida in the 1980s (Schofield, 2009) and are now widespread across the western Atlantic (Schofield, 2010). Lionfish are generalist predators, consuming invertebrates and fish from many trophic guilds (Peake et al., 2018), including herbivorous fish such as parrotfish (Eddy et al., 2016, Mizrahi et al., 2017), damselfish (Layman and

Allgeier, 2012, Mizrahi et al., 2017, Sancho et al., 2018) and surgeonfish (Munoz et al., 2011). In one study, parrotfish were found in lionfish diet 67 times more frequently than expected based on parrotfish abundance in the area (Chappell and Smith, 2016). Lionfish predation has been shown to reduce the density (Kindinger and Albins, 2017) and biomass (Albins, 2015) of small herbivorous fish. Lionfish were the suggested cause of a phase-shift on mesophotic reefs in the Bahamas because the lionfish invasion coincided with a reduction in herbivorous fish abundance and a corresponding increase in macroalgal cover (Lesser and Slattery, 2011). In addition to consumptive effects, lionfish can also exert non-consumptive effects by reducing herbivorous fish grazing rates (Eaton et al., 2016, Kindinger and Albins, 2017).

Despite the large knowledge base on consumptive effects of lionfish, research on non-consumptive effects is lacking, with few published studies (Eaton et al., 2016, Hunt et al., 2020, Kindinger and Albins, 2017, Trehern et al., 2020). Only two studies have focused on the non-consumptive effects of lionfish on herbivorous fish (Eaton et al., 2016, Kindinger and Albins, 2017). Both studies found a reduction in macroalgal grazing by small, prey-sized, herbivorous fish in the presence of a lionfish (Eaton et al., 2016, Kindinger and Albins, 2017), but Kindinger and Albins (2017) also found a reduction in grazing by larger, non-prey-sized herbivorous fish on reefs with high lionfish density. Although prey naivety is common in marine systems (Anton et al., 2020) and some prey species have shown naivety towards invasive lionfish (Anton et al., 2016, Kindinger and Albins, 2017), the effects of lionfish on herbivorous fish grazing suggest that herbivorous fish may respond appropriately to, or perhaps even recognise, invasive lionfish. However, anti-predator responses may not be related to recognition of lionfish as a predator and may instead be caused by general risk avoidance (Berchtold and Cote, 2020). Reductions in grazing by several herbivore families (Kindinger and Albins, 2017) suggest that non-consumptive effects of lionfish on herbivorous fish may be widespread, thus more research needs to be conducted that investigates different herbivorous fish species and geographical areas.

In this study we investigate whether lionfish presence influences grazing behaviour by herbivorous fish and fine-scale fish community structure. Remote cameras were used to observe grazing behaviour by herbivorous fish and record fish communities present on small areas of reef. We initially sampled each location with lionfish present, then repeated the measurements at the same location after lionfish had been removed.

4.3 Methods

Study species and area

Lionfish (assumed to be *Pterois volitans* based on Betancur-R et al., 2011) were studied on the offshore reef system of Banco Capiro in Tela bay, Honduras at the southern end of the Mesoamerican reef (Figure 1). All data were collected between July and August 2019 using open-circuit SCUBA at depths of 12-15 m at five sites (Figures 1 & 2; Supplementary table 1). Banco Capiro is a healthy reef system with unusually high scleractinian coral cover, making it significantly healthier than other reefs in Honduras (Bodmer et al., 2015). However, diving pressure and lionfish culling are minimal in Tela Bay, leading to high lionfish densities.

Figure 1. Map of our study sites in Tela bay, Honduras (main map) and location of Tela Bay within Central America (inset map). Replicates were conducted on the Banco Capiro reef system at Aldrid's (*n*=3), Butterfingers (*n*=2), Canyon (*n*=2), Kisci's garden (*n*=1) and Mushroom mountain (*n*=2).

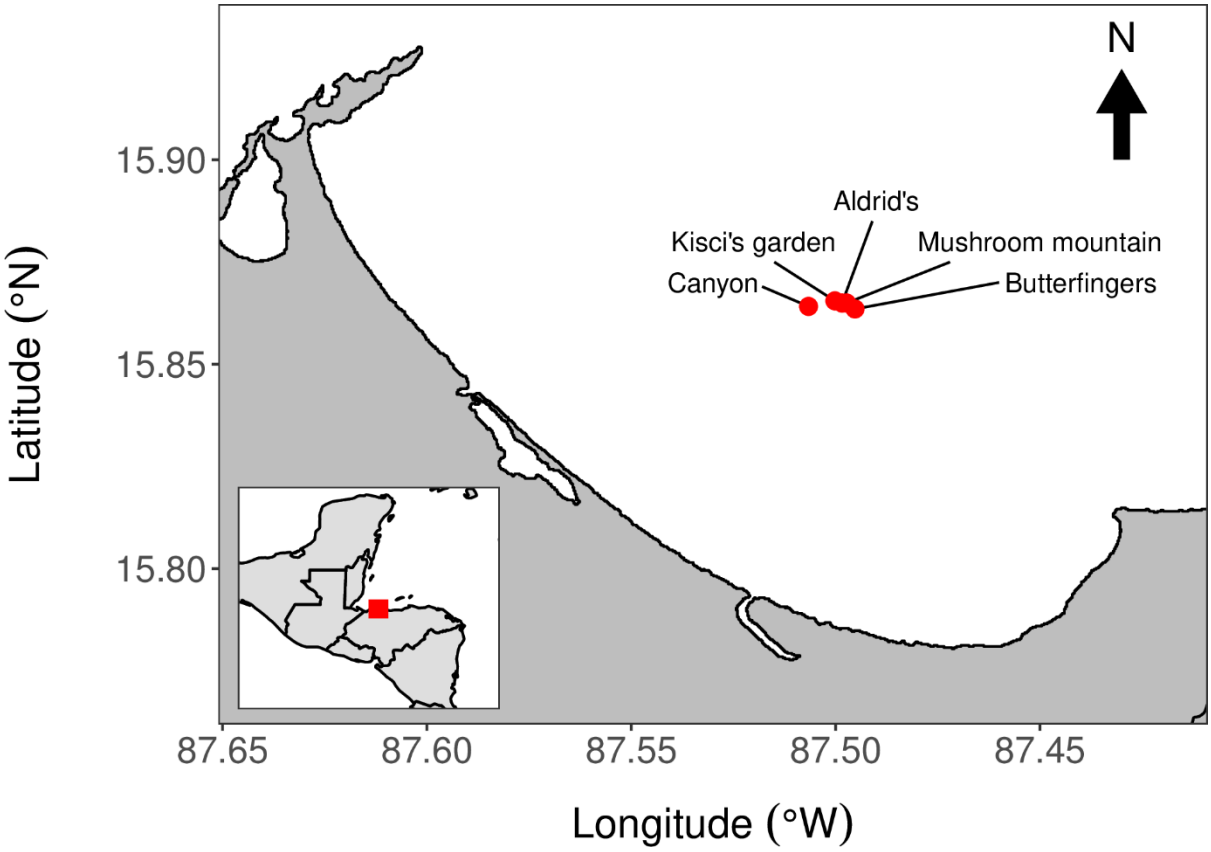
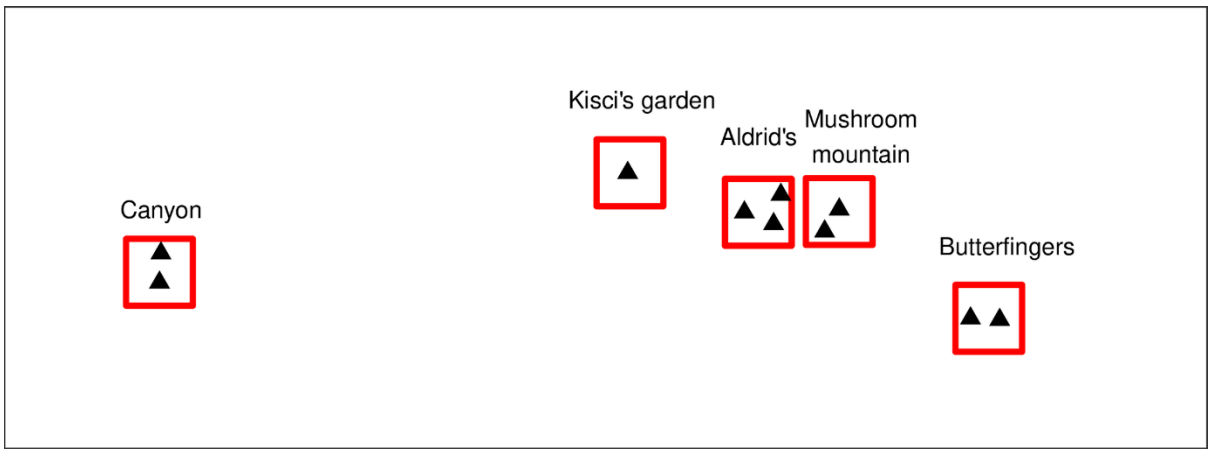
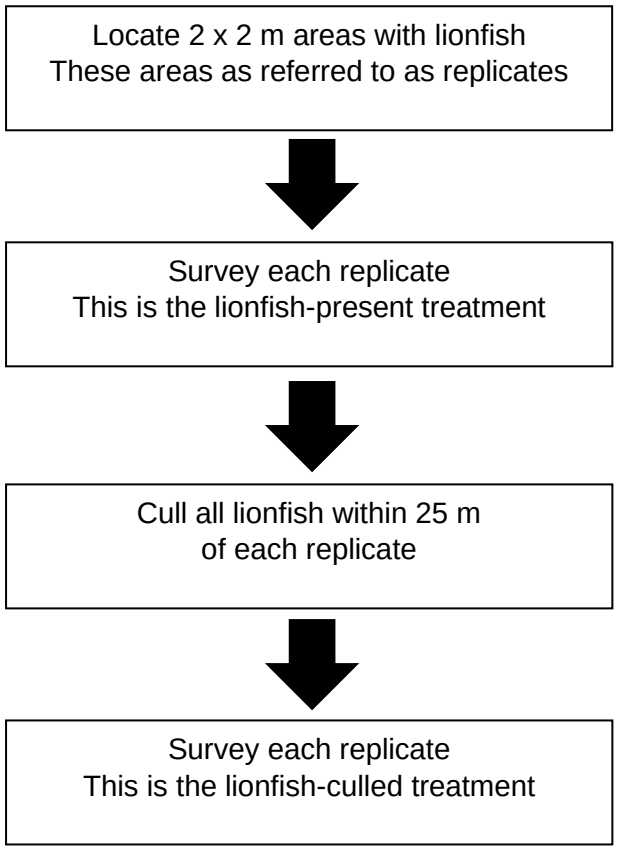


Figure 2. Visual representation of our sites and replicates. Our 2 x 2 m replicates (black triangles) were located within five sites (red boxes). Each replicate was tested as lionfish-present and as lionfish-culled. Diagram is not drawn to scale.



At each site we initially selected up to four areas that were inhabited by one or more lionfish, hereafter referred to as replicates (*n*=14; Figure 2). To ensure that lionfish were large enough to potentially be identified by native species as a predator, we only used sites with lionfish measuring at least 15 cm total length. Sample sizes at each site varied from two to four depending on the number of lionfish that were found during survey dives. Our replicates were approximately 2 x 2 m, centred on the lionfish. After conducting our initial observations of replicates with lionfish present, we culled the focal lionfish and all lionfish within a 25 m radius before conducting the lionfish-culled treatment (Figure 3). At the time of our study, lionfish daily movement estimates ranged from 3.9 m (Tamburello and Cote, 2015) to 23 m (Bacheler et al., 2015), therefore we denoted a 25 m radius surrounding our replicates to ensure that lionfish did not move into the replicates from the surrounding reef. Although more recent research found mean daily movements of 265 m (Dahl and Patterson, 2020), after culling we only observed lionfish in three of our 14 replicates, from which data were discarded, indicating that lionfish in our study area tend not to move such long distances.

Figure 3. Flow diagram of our research methodology.



Following analysis of the videos, four replicates were excluded from our analysis. Three replicates were excluded because a lionfish entered a replicate during the lionfish-culled treatment and one replicate was excluded because of camera malfunctions. Thus, our analysis consisted of ten replicates: one from Kisci's garden, two each from Butterfingers, Canyon and Mushroom Mountain, and three from Aldrid's (Figure 2). Each replicate was tested twice, first as lionfish-present and second as lionfish-culled.

Algal grazing assay

We conducted two algal grazing assays at each replicate: one for the lionfish-present treatment and one for the lionfish-culled treatment. All five sites were within a marine protected area (ProtectedPlanet, 2020), thus removing behavioural differences in native fish that may occur between protected and non-protected sites (Littler and Littler, 2007). Assays were performed using remote underwater video to avoid impacts of passive human presence caused by divers (Titus et al., 2015). Algal assays were placed approximately 50 cm away from lionfish during the lionfish-present treatment and were placed in the same position during the lionfish-culled treatment at each replicate. Paired assays were all conducted within 48 hours of one another, with the lionfish-present treatment being followed by the lionfish-culled treatment. Although using the same order of treatments could have confounded the results if fish were acclimatised to the camera when they saw it during the second but not during the first assay, this is unlikely to have occurred because fish can acclimatise to cameras in under ten minutes (Nanninga et al., 2017) and thus would have quickly acclimatised in both treatments.

The duration of grazing assays ranged from 132-185 minutes depending on camera battery life. All assays began at the same time each day (6:30 am) to ensure that feeding rates were consistent across trials, since herbivore feeding rates can vary throughout the day (Bruggemann et al., 1994). Herbivorous fish species often specialise on selected algal groups (Adam et al., 2015), so we used a variety of species and growth forms of algae. However, preliminary analyses indicated low grazing

rates on the algal assays (<1 bite per hour), thus we decided to also record grazing on naturally occurring algae seen within the video frame.

Algal assays comprised a piece of PVC pipe with three different macroalgae (*Dictyota* spp., *Lobophora* spp. and *Sargassum* spp.) attached at 12 cm intervals using plastic pegs. Macroalgae were always attached in the same order to remove any influence of positioning. The PVC pipe formed a T-shape, with a GoPro Hero4 (GoPro Inc, USA) mounted at the base of the T, 30 cm away from the macroalgae (Figure 4). A turf algal tile was attached to the top of the T using a cable tie. *Dictyota* spp. and *Lobophora* spp. were chosen because long-term reef monitoring at our study sites shows they are the most common algal genera. The third alga, *Sargassum* spp. was chosen as it was particularly abundant in the year this study was conducted. We selected common reef algae to prevent bias in grazing rates (Littler and Littler, 2007) that could occur if native species exhibit orienting responses towards novel stimuli (Post and von der Emde, 1999). Turf algae and all three macroalgal genera used in our algal assay are consumed by herbivorous fish in the western Atlantic (Burkepile and Hay, 2008, Morrison, 1988). Algae were only identified down to genus level due to the difficulty of identifying algae in-water.

Figure 4. Algal grazing assay deployed on the reef.



Macroalgae were collected on the same dive that the grazing assays were deployed, whilst turf algal tiles were prepared in advance and then collected and maintained in tanks of seawater in the lab for a maximum of 48 hours before being deployed on the reef. All non-turf algae and other benthic organisms were removed from the tiles before they were used (Korzen et al., 2011). Turf algal tiles were created from unglazed terracotta tiles measuring 10 x 10 cm, which were used as settlement tiles on which turf algal communities could develop. A custom-made PVC frame was used to hold the tiles so that they could be deployed on the reef and raised up off the seabed by approximately 15 cm. The frame was surrounded by 1.5 cm diameter mesh to exclude large herbivores, however, small herbivores were still able to enter. The frame was placed at a depth of 12 m at Canyon (Supplementary table 1) and left for a minimum of ten weeks. After this time, algal turf communities had established and covered the entirety of the exposed surface of the tile.

Changes in wet mass of algae were not recorded because algae were not taken to the laboratory between collection and use in algal assays. We chose not to transport algae to the laboratory to be weighed because preliminary trials found that small pieces of algae (particularly *Dictyota* spp.) often broke off during transit between the laboratory and reef, making any recorded change in mass inaccurate. Changes in mass may also be inaccurate if fish graze at the bottom of the fronds, causing biomass to be lost into the water column but not consumed (Shantz et al., 2017).

Video analysis

Every fish that appeared in each video was identified to species level using identification guides (Charteris, 2012, Humann and Deloach, 2014), however, large schools of fish that were seen far away in midwater were not recorded. The abundance of each species that appeared in the video, whether grazing or not, was recorded as MaxN (the maximum number of individuals present at any one time; Ellis and Demartini, 1995) to prevent over-estimation, which would occur if every individual was counted. We initially recorded grazing on naturally occurring algae, the algal tile, and each of the species in the algal assay separately, but we subsequently pooled all grazing because bite rates on the algal tile and algal assay were low (≤1 bite per hour in three of the ten replicates). Grazing was

divided into feeding bouts, where a feeding bout is a collection of bites that occur close together and are separated from other bites by periods of non-feeding activity. Total number of bites, number of bites by damselfish, number of bites by parrotfish and number of feeding bouts were converted to counts per hour to account for differing video lengths between replicates.

We summarised the data to create a single value for each treatment at each replicate in the following categories: (i) number of bites per hour for all fish, damselfish only and parrotfish only, (ii) number of feeding bouts per hour, (iii) number of bites per feeding bout, (iv) total fish abundance, (v) piscivore abundance, (vi) species richness, and (vii) diversity calculated using the Shannon-Weaver index (Shannon and Weaver, 1949). Fish were assigned as piscivores according to the Atlantic and Gulf Rapid Reef Assessment (AGRRA) database (Marks, 2018). The piscivores we observed in our study were: *Caranx ruber*, *Cephalopholis cruentata*, *Epinephelus striatus*, *Mycteroperca interstitialis*, *Haemulon plumierii*, *Lutjanus apodus*, *Lutjanus jocu*, *Lutjanus mahogoni*, *Lutjanus synagris* and *Ocyurus chrysurus*. We also observed one grouper and one snapper that could not be identified to species level, so we recorded these in our data as unknown grouper and unknown snapper.

We suspected that occasional large schools of fish that passed close to the camera may have taken large numbers of bites and thus skewed the results, in a similar way to how the abundance of schools can skew prey abundance (Tuttle, 2017). However, we found that all feeding bouts with more than ten recorded bites came from groups of less than seven individuals, most commonly *Scarus iseri*, so we chose not to remove large fish schools from the analysis.

Statistical analyses

We used mixed models to test the effect of treatment (lionfish-present and lionfish-culled) on a selection of response variables relating to herbivory and fish communities. All models included the main effects of treatment (present and culled) and site (Aldrid's, Butterfingers, Canyon, Kisci's garden and Mushroom Mountain) and the interaction between treatment and site. We included

replicate as a random effect to account for multiple testing, as each replicate was tested as both lionfish-present and lionfish-culled.

Robust linear mixed models (robustlmm) were used for continuous data to account for the presence of outliers (Koller, 2016), which skewed the distributions and led to heteroscedasticity. There are no *p*-values provided in the output of robustlmm, so we calculated *p*-values using Satterthwaite approximated degrees of freedom following Geniole et al. (2019). This approach to calculating *p*-values limits type-I errors, even for small sample sizes (Luke, 2017). We ran a separate robustlmm for each response variable. We also used non-metric multidimensional scaling (nMDS) with Bray-curtis distances to visualise differences in the fish communities observed between the lionfish-present and lionfish-culled treatments. We then ran analysis of similarities (ANOSIM) to test for a significant difference between the treatments.

All analyses were conducted in R version 3.6.2 (R Core Team, 2019). Robustlmms were run using the robustlmm package (Koller, 2016). Shannon-Weaver diversity was calculated using the vegan package (Oksanen et al., 2019), which was also used to run the nMDS and ANOSIM. All plots were produced using the ggplot2 package (Wickham, 2016) and the interaction plots were coloured using the colour-blind friendly viridis colour palette from the viridis package (Garnier, 2018).

Ethics statement

All experimental protocols were approved by the University of Oxford Department of Zoology Animal Welfare and Ethical Review Body. Lionfish culling was carried out in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (Leary et al., 2013). No specific permits were required to collect lionfish. A research permit for this work was obtained from the Instituto de Conservacion Forestal, Honduras (ICF-508-2019).

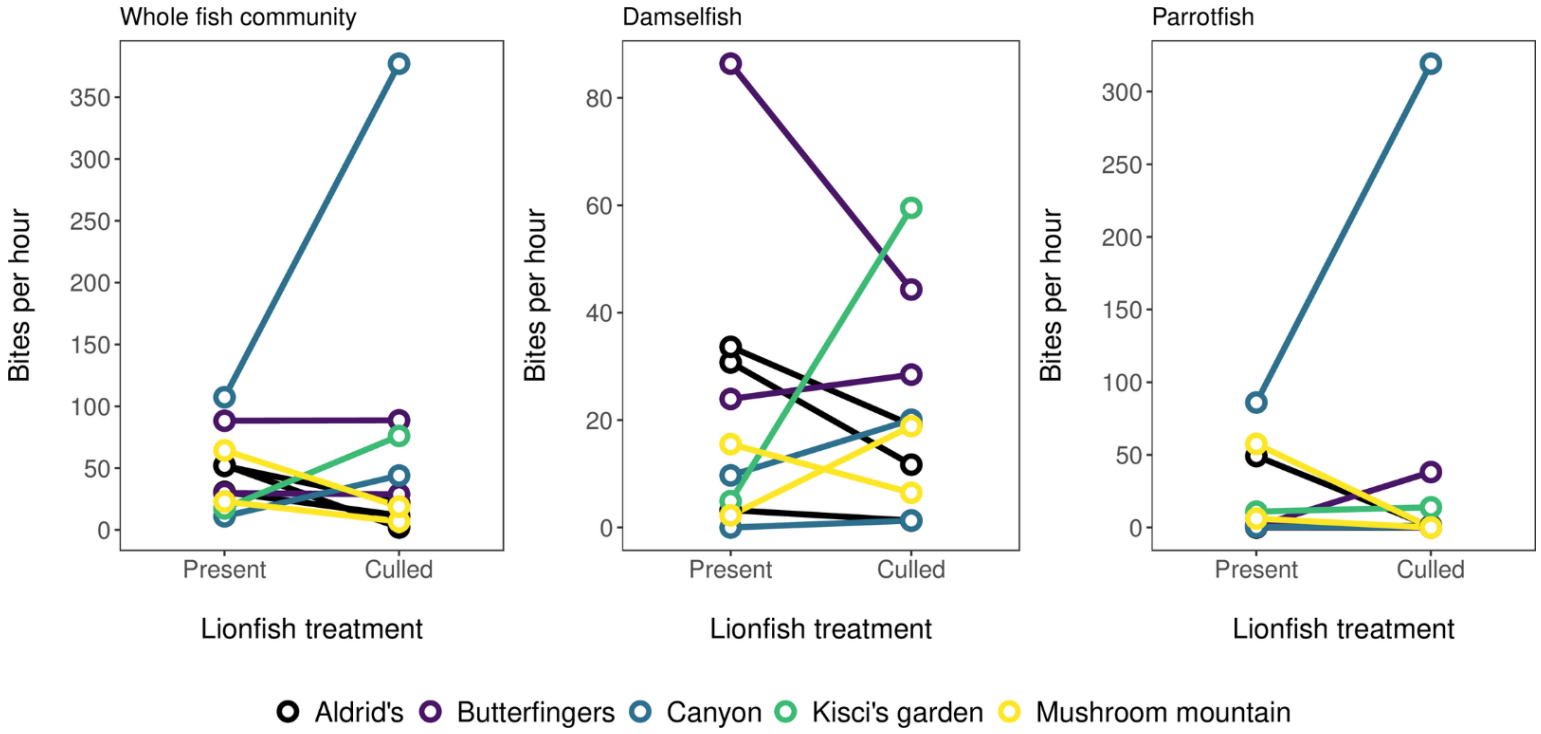
4.4 Results

Impacts of lionfish on herbivory differ by site

There was no main effect of treatment (lionfish-present or lionfish-culled) on bites per hour for the whole fish community (t=2.31, df=5, p=0.069), for damselfish only (t=1.30, df=5, p=0.251) or for parrotfish only (t=0.525, df=5, p=0.622). However, we did find significant interaction effects between treatment and site for Canyon (t=-8.17, df=5, p<0.001) and Kisci's garden (t=-3.21, df=5, p=0.024) in the whole fish community model, Kisci's garden (t=-3.64, df=5, p=0.015) in the damselfish model and Canyon (t=-2.79, df=5, p=0.038) in the parrotfish model. These interactions occurred because the number of bites decreased from present to culled in Aldrid's (the reference level for site) but increased from present to culled for Canyon and Kisci's garden (Figure 5).

We found no main effect of treatment on number of feeding bouts per hour (t=1.51, df=5, p=0.193) or the number of bites per feeding bout (t=1.16, df=5, p=0.300) but we found significant interactions between treatment and site for Kisci's garden (t=-4.24, df=5, p=0.008) in the feeding bouts per hour model and Canyon (t=-2.91, df=5, p=0.033) in the bites per feeding bout model. These interactions occurred because the number of feeding bouts and number of bites per feeding bout decreased from present to culled for Aldrid's (the reference level for site) but increased for Kisci's garden (number of feeding bouts model) and Canyon (bites per feeding bout model).

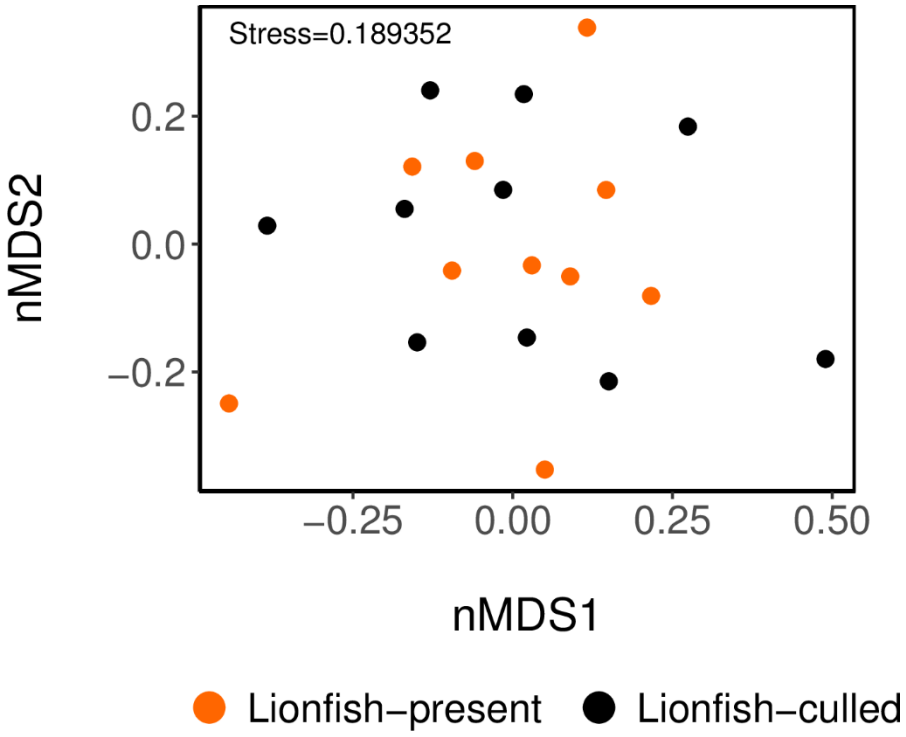
Figure 5. Interaction plots of lionfish treatment (lionfish-present and lionfish-culled) and site (Aldrid's, Butterfingers, Canyon, Kisci's garden and Mushroom mountain) for the total number of bites per hour by the whole fish community, damselfish only and parrotfish only. Lines connect the two treatments conducted at each of the ten replicates. Note the differing y axis scales, indicating that most bites were taken by parrotfish.



No effect of lionfish on observed fish communities

Lionfish treatment had no effect on the total abundance (t=-0.979, df=5, p=0.372), piscivore abundance (t=-0.730, df=10, p=0.482), species richness (t=-1.21, df=10, p=0.256) or diversity (t=-1.34, df=5, p=0.238) of the fish observed in each replicate. Site had no effect on piscivore abundance, species richness or diversity, however, total fish abundance was significantly higher at Butterfingers (t=3.15, df=7.8, p=0.014) and Canyon (t=2.56, df=7.8, p=0.035) compared with Aldrid's. Unlike herbivore grazing, we found no interaction between treatment and site for any of these whole community metrics. The nMDS plot (Figure 6) supports the results of our robustlmms and this was confirmed with an ANOSIM (R=-0.076, p=0.953), indicating no difference between treatments.

Figure 6. nMDS plot of fish communities observed in the lionfish-present (orange) and lionfish-culled (black) treatments. Each replicate has two points: one for lionfish-present and one for lionfish-culled.



4.5 Discussion

Although we found no significant treatment effects across all grazing metrics, we did find significant interactions between treatment and site, indicating different trajectories between sites. These different trajectories cancelled out and resulted in no significant main effect of treatment.

We observed increases in grazing following culling at Butterfingers, Canyon and Kisci's garden, indicating that grazing was lower in the presence of lionfish. This result supports previous research that found significant negative effects of lionfish on herbivorous fish grazing (Eaton et al., 2016, Kindinger and Albins, 2017). Parrotfish have been shown to reduce their bite rate by 50% when a lionfish was present in a laboratory study (Eaton et al., 2016) and an *in situ* study that pooled all herbivorous fishes found a significant reduction in bite rate of small (<10 cm) herbivorous fish in the presence of lionfish on a reef with high lionfish density (Kindinger and Albins, 2017). Herbivorous fish reduce their grazing rates in the presence of native piscivores as a response to increased predation risk (Catano et al., 2016), which suggests that the reductions in grazing in the presence of lionfish occur because native fish respond appropriately towards lionfish. Predator detection, particularly using olfactory cues, can be a learnt behaviour (Kelley and Magurran, 2003), so native fish may have learnt to recognise invasive lionfish as a predator. Alternatively, native fish may display generalised predator avoidance behaviour (Berchtold and Cote, 2020) and thus avoid novel stimuli such as invasive lionfish.

We observed decreases in grazing following culling at Aldrid's and Mushroom Mountain, indicating that herbivory was higher in the presence of lionfish. The lack of a negative response by herbivores to lionfish presence may relate to prey naivety, where prey display ineffective anti-predator behaviour because they have not evolved with the invasive predator (Sih et al., 2010). Naivety of prey fish towards lionfish has been detected in several native fish species including damselfish and parrotfish (Anton et al., 2016, Kindinger, 2015). A study on bicolour damselfish (*Stegastes partitus*) showed they spent less time feeding in the presence of a native predator than in the presence of a lionfish,

indicating prey naivety towards lionfish (Davis, 2018). However, the lack of response may also relate to herbivorous fish size, as fish that are too large to be consumed by lionfish may not display anti-predator behaviour towards them. We did not record fish size in our study, but we suggest that future studies should either use a stereo-video system to enable accurate calculation of fish lengths or record the life stage of each fish to enable an approximate estimation of size.

The ratio of site-attached to roving herbivores observed in each replicate may also have influenced our results. Site-attached herbivores may continue to display anti-predator behaviour even in the absence of lionfish, as demonstrated in a study where herbivorous fish grazing rates were lower on high lionfish density reefs than low lionfish density reefs even in the absence of a lionfish nearby (Kindinger and Albins, 2017). In contrast, roving herbivores may display anti-predator behaviour in relation to predator presence whilst feeding, so they would not display lionfish-induced anti-predator behaviour during our lionfish-culled treatment. Our replicates that exhibited no difference between lionfish-present and lionfish-culled treatments may thus have been dominated by site-attached herbivores that continued to display anti-predator behaviour for the duration of our study. Whereas our replicates that exhibited a reduction in grazing in the presence of lionfish may have been dominated by roving herbivores, which only displayed anti-predator behaviour during the lionfish-present treatment. The foraging behaviour of each species (site-attached or roving) should thus be considered in future studies.

We found no effect of lionfish treatment on the fish communities observed and no effect of lionfish treatment or an interaction between treatment and site for total fish abundance, species richness, diversity or piscivore abundance. The lack of effects suggest that the observed differences in grazing between sites and replicates were not driven by changes in prey or predator communities.

The reduction in grazing in the presence of lionfish at some sites may suggest that native fish recognise lionfish, whilst the lack of effect at other sites may suggest that native fish are naïve towards lionfish. However, we do not expect both scenarios to occur within the same reef system. As

lionfish can travel large distances, with mean daily movements of 265 m and movements of 960 m in just four days (Dahl and Patterson, 2020), we expect lionfish to move freely between our five sites, which are located on a single reef system and separated by a minimum of 120 m and a maximum of 1.25 km. It is therefore unlikely that native fish would have learnt to recognise lionfish on one site but not on another site. Similarly, piscivore abundance did not differ between sites, thus we do not expect generalised predator avoidance behaviour to be greater on one site than another. We therefore assume that the type of response towards lionfish, either recognition of lionfish or generalised anti-predator behaviour, is the same across all sites and any differences observed between sites or replicates are due to differences in perceived predation risk.

Habitat complexity can influence predation risk by providing refuges for prey (Steele, 1999) and reducing predator hunting success (Bartholomew et al., 2000, Scharf et al., 2006). Recent research also suggests that habitat complexity may influence fear acquisition in prey, with prey from low complexity habitat becoming more wary of predators (Crane et al., 2020). Complex habitat can be detrimental if it prevents prey from detecting or escaping from predators, as suggested by a study of grazing behaviour where the presence of a predator decoy reduced herbivore grazing in high rugosity areas but not in low rugosity areas on coral reefs (Catano et al., 2016). We did not record habitat complexity in our study, but we suggest that variation in complexity at the scale of individual replicates may contribute to the observed variation in lionfish effects. Future studies should record habitat complexity of each replicate using habitat assessment scores (HAS; Gratwicke and Speight, 2005), which record several measures of complexity including rugosity and live coral percentage cover.

Predator density also influences predation risk, resulting in stronger anti-predator behaviour on reefs with greater predator biomass (Madin et al., 2010b). Lionfish density has been shown to influence herbivore grazing behaviour, with significantly higher grazing levels on reefs with low lionfish density than on reefs with high lionfish density (Kindinger and Albins, 2017). Although we found no difference in piscivore abundance between sites, we did not measure lionfish density and so this may

have partially driven the observed changes. Future studies should record lionfish densities by conducting multiple belt transect surveys at each site and following the lionfish-focussed search method (Green et al., 2013).

In addition to habitat complexity and predator density, characteristics and behaviour of individual lionfish may also impact perceived predation risk and thus grazing rates. Predator movement can influence predator detection in prey fish (Wisenden and Harter, 2001), thus the activity of the lionfish in our study may have contributed to the differences between replicates. Although generally considered a crepuscular species (Cure et al., 2012), lionfish move and hunt during the day in their invaded range (Benkwitt, 2016). We observed lionfish moving in and out of our quadrats during the lionfish-present treatment, therefore it is possible that lionfish activity and behaviour may have differed between replicates. To account for potential effects of lionfish movement on herbivorous fish grazing behaviour, we suggest that future studies should record lionfish activity as one of four categories: (1) inactive, (2) active- hovering (3) active- swimming and (4) active- hunting, following Green et al. (2011). Lionfish may not display the same activity throughout each 2-3 hour trial, so we suggest recording the dominant activity for each trial.

The movement of lionfish also means that the distance between native fish and lionfish varied during each video and between replicates. Distance between predator and prey significantly affects grazing rates of herbivorous fish, with significantly more bites taken when predators are further away (Catano et al., 2016). Distance effects have also been observed between native fish and lionfish; grazing by small herbivorous fish is significantly higher when lionfish are >3 m away compared with <0.25 m away (Kindinger and Albins, 2017), indicating that distance between native fish and lionfish influences native fish behaviour. Although the distance between lionfish and native fish cannot easily be measured during the trial, we suggest recording the proportion of time that lionfish are within the 2 x 2 m quadrat to indicate whether lionfish are generally close to or far from the herbivorous fish observed in the videos. Difference in the size of lionfish between replicates may also have impacted grazing rates because reef fish display stronger anti-predator behaviour towards larger predators

(Helfman, 1989) and larger predators cause greater reductions in herbivorous fish grazing than smaller predators (Rizzari et al., 2014). Future studies should record an approximate total length of lionfish (to the nearest 5 cm) *in situ* so that lionfish size is recorded even if lionfish are not successfully captured. For lionfish that are captured, an accurate total length (to the nearest mm) should be recorded during lionfish dissection so that analyses can also be run on a sub-set of the data where more accurate lengths of lionfish have been recorded.

Our results suggest that under certain circumstances, lionfish may exert non-consumptive effects on herbivorous fish, as has been observed in other studies (Eaton et al., 2016, Kindinger and Albins, 2017). Non-consumptive effects can simultaneously affect a whole population, rather than a single individual (Rizzari et al., 2014) and can even impact prey in the absence of predators nearby (Kindinger and Albins, 2017) if prey respond to predator density, rather than the proximity of predators. Prey may also change their behaviour in response to a single encounter with a predator; some fish continue to display anti-predator behaviour for more than 24 hours after a short-term exposure to a predator (Rahel and Stein, 1988). The time delay between our lionfish-present and lionfish-culled treatments varied from 24-48 hours and may thus have influenced whether we detected an effect. Future studies could conduct lionfish-culled treatments at varying times after lionfish removal in each replicate to determine when herbivorous fish resume normal behaviour following exposure to lionfish.

Herbivorous fish are important for grazing macroalgae on reefs (Hughes, 1994) and reductions in herbivore abundance lead to phase shifts (Hughes et al., 2007, Lirman, 2001), thus reductions in herbivore grazing caused by lionfish are also likely to contribute to phase shifts. Our results suggest that lionfish may negatively impact grazing in some areas, but these non-consumptive effects are not universal even within a small reef system and likely rely on several other factors.

Changes to implement in future studies

Throughout this discussion I have identified the following variables that were not included in my study but that may influence herbivore grazing behaviour: herbivore size, herbivore site fidelity, habitat complexity, lionfish density, lionfish activity, distance between lionfish and herbivorous fish, lionfish size and time delay between lionfish-present and lionfish-culled treatments.

In addition to expanding the number of variables in the models, I also suggest a change to the experimental method. The current study does not contain a control and thus cannot rule out the possibility of the observed changes in herbivore behaviour being driven by factors other than lionfish removal, such as disturbance by divers or the presence of the remote cameras. These factors may lead to fish changing their behaviour between the lionfish-present and lionfish-culled replicates, which would be detected as an effect of lionfish removal in the current study. I therefore suggest that future studies should use a Before-After-Control-Impact (BACI) design. BACI designs compare data collected before and after an impact at both a control and an impacted site (Green, 1979), with a significant interaction between treatment (before or after) and type of site (impact or control) indicating a significant effect of the impact (Ingeman et al., 2017, Smith, 2014). The impact in our study is lionfish removal, however, in the current study all replicates are impacted sites and we do not have control sites. I therefore suggest that future studies should also collect data from control sites. These control sites will be replicates with lionfish but where lionfish are not culled following the lionfish-present treatment. Divers will still swim around within a 25 m radius of each site as if searching for lionfish to ensure that experimental conditions at the impacted sites are exactly replicated at the control sites, except that lionfish are not removed at the control sites. These control sites will require lionfish to remain in the same location over consecutive days, so that both treatments are conducted with lionfish present.

Future studies should be analysed using mixed effects models, as in the current study, but with a few differences. The model should include all new variables that may influence herbivore behaviour as fixed effects, in addition to treatment (before or after) and dive site. A new variable should also be

included: type of site, which distinguishes between impacted sites (where lionfish are removed) and control sites (where lionfish are not removed). As future studies should use a BACI design, the models must include the interaction between treatment (before or after) and type of site (impact or control), as the significance of this interaction will determine whether lionfish removal had a significant effect. Replicate should be included as a random effect, as in the current study, to control for multiple testing of each replicate (once as lionfish-present and once as lionfish-culled).

4.6 Conclusion

Our results suggest that lionfish may exert non-consumptive effects on herbivorous fish, but these effects are highly variable even within a small reef system and between study sites in close proximity. We provide potential explanations for the variation in effects observed between sites and replicates, in addition to discussion on how to record these variables and how to improve the study design for future research. Our research highlights the importance of testing for the effects of invasive species across multiple sites and not extrapolating the results from a single site or study.

4.7 Covid-19 impact statement

This data chapter was impacted by the Covid-19 pandemic because my final fieldwork season in Honduras was cancelled due to lockdown and travel restrictions. The work I have presented in this chapter was intended to be a pilot study, prior to the full study being conducted in summer 2020. The pilot study suffers from low sample size because a large part of the fieldwork season was spent on adjusting the data collection methods, leaving less time to conduct the chosen data collection methods. After analysing the pilot study data, I planned a series of changes to the data collection methods that would have been implemented during the full study in summer 2020. The changes include: (1) substantially increased sample size to ensure reliability and allow a mixed model with interaction to be used without over-parameterisation, (2) only record grazing on naturally occurring algae as grazing rates were low on the algal assay, (3) use a quadrat to standardise the survey area because survey area in the pilot study was dependent on camera field of view, (4) record the number

of bites per individual, as data from the pilot study recorded number of bites per group, thus it is not known whether all bites were from one individual or distributed across the group, (5) record depth and habitat complexity of each replicate as these factors may influence fish communities and their responses to predators, (6) record lionfish size, as predator size can affect the strength of anti-predator behaviour.

4.8 Acknowledgements

We thank all the Operation Wallacea volunteers and staff who assisted with data collection. This work was financially supported by the Natural Environment Research Council (grant number: NE/L002612/1) and Operation Wallacea.

4.9 Author contributions

Conceptualisation: Christina L. Hunt, Dominic A. Andradi-Brown, Dan A. Exton

Formal analysis: Christina L. Hunt, Dominic A. Andradi-Brown, Dan A. Exton

Investigation: Christina L. Hunt, Joshua Bennett-Williams, Jess Sweeney, Dennis Duffin, Emma Taylor-Clark

Supervision: Dominic A. Andradi-Brown, Dan A. Exton

Visualisation: Christina L. Hunt

Writing – original draft: Christina L. Hunt.

Writing – review & editing: Christina L. Hunt, Dominic A. Andradi-Brown, Joshua Bennett-Williams, Jess Sweeney, Dennis Duffin, Emma Taylor-Clark, Dan A. Exton

4.10 Supplementary information

Supplementary table 1. Co-ordinates and number of replicates conducted at each of our five study sites in Tela Bay, Honduras. Coordinates are given in WGS84.

Site	Number of replicates	Longitude (ºW)	Latitude (ºN)
Aldrid's	3	87.49841	15.86496
Butterfingers	2	87.49525	15.86351
Canyon	2	87.50662	15.86414
Kisci's garden	1	87.50017	15.86550
Mushroom mountain	2	87.49730	15.86497

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5 Temporally offset additive mixed models show invasive lionfish drive fish community shifts across the western Atlantic

Christina L. Hunt, Dominic A. Andradi-Brown, Thomas C. Cameron, Craig P. Dahlgren, Judith C. Lang, Owen T. Lewis, Melanie McField, Pamela J. Schofield, Dan A. Exton

5.1 Abstract

Despite continued growth in the rate of species invasions, many studies fail to consider their impacts at multiple time points or locations. Using invasive lionfish as a model species, we investigated their ecological impact over large spatial and temporal scales whilst controlling for a comprehensive set of contextual variables and using temporally offset data. We combined an 18-year dataset comprising 11,000 transects, representing multiple reef types from eight countries, with regional remote sensed and modelled data to control for 14 biophysical, social and management variables known to affect reef fish communities. Lionfish invasion occurred across six years in our dataset, so we centred data on year of lionfish arrival, thus reducing the influence of large-scale point impact events on our results. We found clear shifts in native fish communities between pre-invasion and two or more years post-invasion. When we investigated trends in fish abundance, species richness and diversity, we detected pre-invasion increases in all metrics that continued into the initial years of the invasion, however, we detected simultaneous declines in all metrics at five years post-invasion. Using staggered invasion dates and a comprehensive set of contextual variables, our results suggest that invasive lionfish may begin to negatively impact native fish abundance, species richness and diversity approximately five years post-invasion. Our study highlights the importance of: (i) large spatial and temporal scales for uncovering region-wide trends and (ii) including contextual variables and sites with staggered invasion dates to disentangle the impacts of invasive species from other variables.

5.2 Introduction

As the global rate of species invasions continues to grow (Seebens et al., 2017), it becomes increasingly important to understand their effects. However, a thorough understanding of invasive species effects is often hindered by poor study design. Many studies of invasive species effects conduct a simple comparison of pre- and post-invasion (Parker et al., 1999), with 40% of studies failing to report the length of time since the species invaded (Strayer et al., 2006). Additionally, most studies consider a single site (Stricker et al., 2015), with few studies considering multiple sites that were invaded at different times (Strayer et al., 2006).

To gain a full understanding of the impacts of invasive species, studies must account for changes that may occur over time or space. Throughout the course of an invasion, changes may occur in the invading species (Phillips and Shine, 2005), native species (Cattau et al., 2018) or ecological processes (Yelenik and D'Antonio, 2013), all of which may alter the impacts of the invasive species. Climatic or anthropogenic stressors may also change throughout an invasion and often increase in intensity over time (Halpern et al., 2015), therefore it is crucial that studies of invasive species control for these confounding factors. In addition to changes that occur over time, differences in the invasive species or native community may also occur between habitats (Thiele et al., 2010) or geographical areas (Kollmann and Banuelos, 2004) and the measured effect of invasive species may be influenced by spatial scale of the study (Powell et al., 2011). Differences between geographical areas and spatial scale of the study mean that the results from one region may not be applicable elsewhere in the invaded region, thus highlighting the importance of regional-scale studies.

Two extensively studied invasive species are the marine fish *Pterois volitans* and *Pterois miles* (Schofield, 2009), hereafter collectively referred to as lionfish. The consumptive effects of lionfish have been a major research focus, with numerous studies reporting declines in reef fish abundance (Ballew et al., 2016), species richness (Albins, 2015) and recruitment (Albins and Hixon, 2008). These effects have mainly been investigated in small-scale patch reef experimental studies over

several weeks (Albins, 2013, Albins and Hixon, 2008, Ingeman, 2016), but it has been suggested that patch reefs are highly unrepresentative of most reefs within the western Atlantic (Hackerott et al., 2017). The non-linear effects of lionfish on prey communities (Benkwitt, 2015) indicate that scaling up per-capita effects from small-scale studies will likely lead to over-estimates of lionfish effects at larger spatial scales.

Some observational studies have investigated the effects of lionfish on fish communities across larger reefs (Elise et al., 2015, Hackerott et al., 2017) and on a single native species at a sub-regional scale (Ballew et al., 2016). In contrast to small-scale studies, these larger-scale studies also cover longer temporal scales, ranging from 2-18 years post-invasion (Ballew et al., 2016, Elise et al., 2015). Recent declines in lionfish abundance have been reported from the Bahamas (Benkwitt et al., 2017) and Northern Gulf of Mexico (Harris et al., 2020), therefore it is important to study the effects of lionfish over long temporal scales to account for changing lionfish densities.

In this study we use lionfish as a model species to investigate the ecological effects of an invasive species over large spatial and temporal scales, whilst accounting for a comprehensive set of contextual variables and using temporally offset data. We investigated whether lionfish had a detectable impact on native fish abundance, biomass, and community structure across the invaded western Atlantic region and in doing so provide the first suggestion of region-wide lionfish impacts. We combined the largest western Atlantic coral reef monitoring data set — comprising 18 years of data spanning both pre- and post-invasion in each country — with regional remote-sensed and modelled data to control for 14 potential biophysical, social and management confounding factors. As the lionfish invasion began in the early 2000s but took almost two decades to reach its full range extent (USGS, 2020), there is temporal staggering in lionfish arrival across western Atlantic reefs. We centred our data on year of lionfish arrival, thus reducing the potential influence of large-scale point impact events on our results.

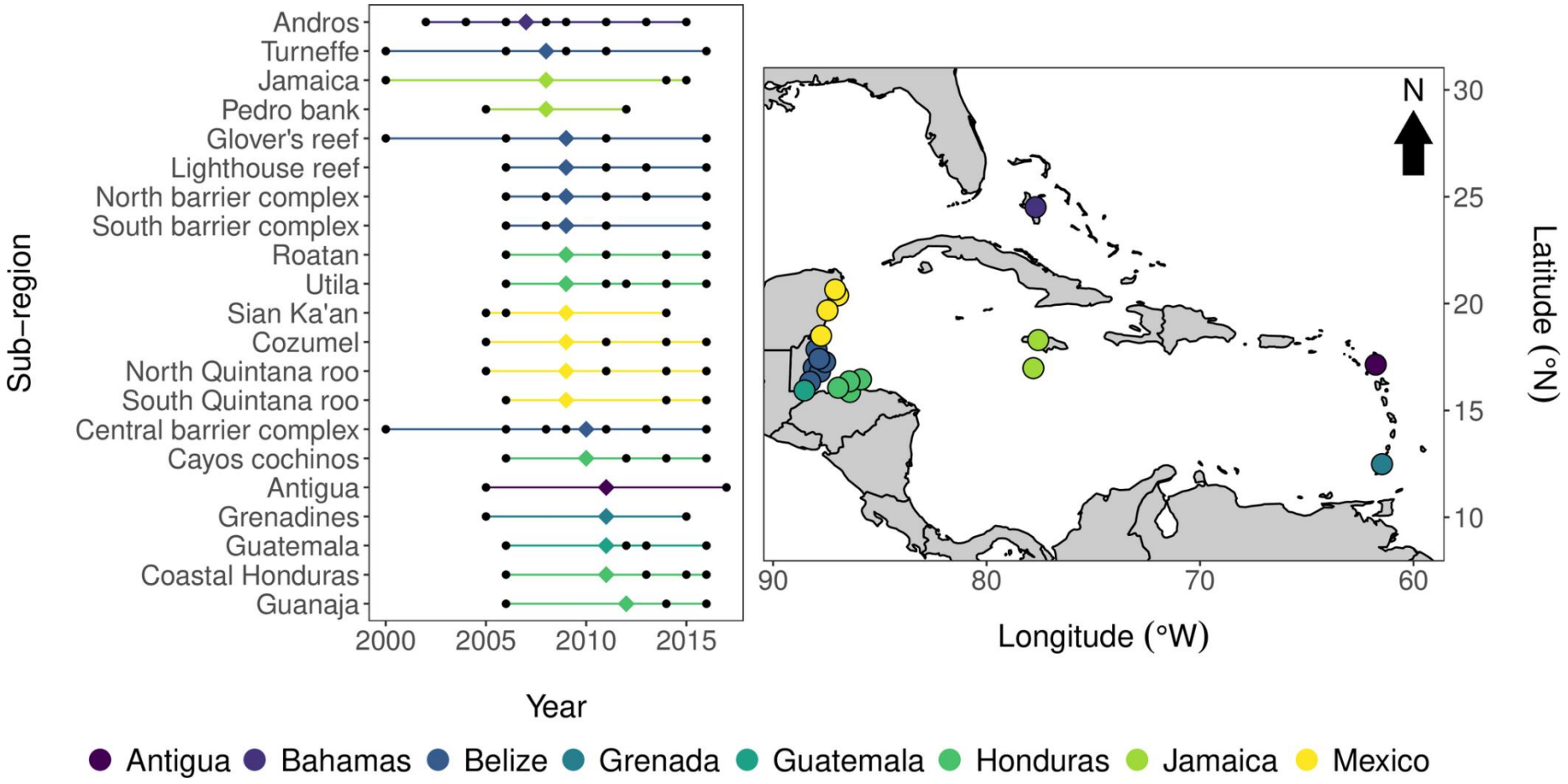
5.3 Methods

Study sites

We used data from the Atlantic and Gulf Rapid Reef Assessment (AGRRA) database (Marks, 2018), which contains belt transect data from coral reefs across the western Atlantic. AGRRA survey protocols have changed over time and preliminary analyses suggested that surveys prior to version three were inconsistent, often recording significantly different values than other survey protocols in the same year, thus we limited our data to versions three, four and five. The AGRRA data is labelled by country, sub-region, site and transect. When multiple transects were conducted at a site in a single day, we averaged these to create a single data point for each specific site and date. We only selected sub-regions with data from at least one pre-invasion and one post-invasion year, with a minimum of two sites per sub-region per year. We removed all sites for which we had incomplete contextual data (following the collection of contextual data as described later).

Our final dataset includes 11,753 transects, 584 sites, 21 sub-regions and eight countries (Figure 1; Supplementary table 1). Our sites covered multiple reef types, including fringing, barrier and patch reefs. The data spans a maximum of ten years pre-invasion and eight years post-invasion. The year of lionfish invasion ranges from 2007 in Andros, Bahamas to 2012 in Guanaja, Honduras. We obtained data on year of lionfish invasion in each sub-region from the USGS Nonindigenous Aquatic Species database (USGS, 2020) except for Guanaja, which was not listed in the USGS dataset. Instead we recorded the year that lionfish were first recorded on an AGRRA transect in Guanaja. Although we did not record the year of invasion for each individual site, we assume that lionfish invaded all sites within a sub-region in the same year because lionfish records indicate fast spread both within and between sub-regions (Schofield, 2010). We only considered lionfish presence/absence in this study, rather than lionfish density, because lionfish are cryptic species that rarely show up in standard fish underwater visual census transects (Green et al., 2013) and thus we do not have good density estimates for all our sites.

Figure 1. Data available for each of the sub-regions (left) and locations of the sub-regions used in our study (right). Coloured diamonds on the left figure indicate year of invasion for each sub-region and black points show the years that we have data for.



Temporal offsetting of data

Point impact events such as bleaching and hurricanes may impact native fish communities and if not accounted for correctly, their impacts could be wrongly interpreted as being driven by lionfish. To reduce the potential influence of these events, we (i) used temporal data offsetting and (ii) controlled for a range of contextual variables. Temporal data offsetting centred our data on year of lionfish arrival in each sub-region. The impact of lionfish was then analysed in relation to years since invasion, with negative values for pre-invasion years, zero for the year of lionfish arrival, and positive values for post-invasion years. As lionfish invasion occurred across six years in our dataset (Figure 1), a single disturbance event that occurred in a single calendar year (e.g widespread coral bleaching) would be spread across six different years since invasion, thus reducing the potential for lionfish impacts to be confounded by the disturbance event.

AGRRA fish data

We obtained data on fish size distributions from the AGRRA database (Marks, 2018). These data were collected using standardised 30 x 2 m wide belt transects. Surveys were conducted at a median depth of 7 m but ranged in depth from 1 – 22 m, so we included depth as a contextual variable in our analysis to account for the differing survey depths. Divers swam at a speed of approximately 6 mins per transect and recorded fish that were within and above the transect boundaries. Only a select group of species were recorded (Supplementary table 2). Lionfish were only recorded in later survey protocols and thus were removed from the dataset. The total length of each fish was assigned to one of the following size categories: 0-5, 6-10, 11-20, 21-30, 31-40 or to the closest 10 cm for any fish larger than 40cm. All size categories were recorded for most fish (Supplementary table 2), however, 0-5 cm parrotfish, wrasse and grunts were only recorded in later survey protocols, so we decided to remove the 0-5 cm category for these three families.

The mean size of lionfish prey is often in the range of 2-4 cm (Cure et al., 2012, Green and Cote, 2014, Munoz et al., 2011) but lionfish can consume 5-10 cm fish from most families (Munoz et al., 2011), with a maximum recorded prey size of 13 cm (Green and Cote, 2014). Fish within both the 0-

5 cm and 6-10 cm categories can therefore be considered as prey-sized fish. However, we only included 6-10 cm fish in our prey-sized fish category because the abundance of 0-5 cm fish can be too variable to obtain meaningful conclusions (Hackerott et al., 2017) and because 0-5 cm parrotfish, wrasse and grunts were not recorded in versions three and four of the survey protocol.

Biomass calculations

Biomass was calculated by first assigning each fish to the mid-point of each size category, such that all fish in the 6-10 cm category were assigned a length of 8 cm. We then applied species specific constants (Froese and Pauly, 2019) to the following equation to calculate biomass:

$$W = a \times (cL)^b$$

Where biomass (W) is measured in grams and length (L) is total length in cm. The a, b and c are species specific constants. The c constant was used to convert between total length and fork length, as some of species constants required length to be measured as fork length. Total length was used for most species, thus the c constant was equal to one. Biomass was calculated for each size class within each species. Fish within the *Kyphosus* genus were not recorded to species level so we calculated average biomass constants from the two *Kyphosus* species that are found in the western Atlantic (*K. incisor* and *K. sectatrix*).

Contextual variables

To enable lionfish effects to be separated from other factors, we controlled for 14 biophysical, social and management variables that are known to influence fish communities. We included ten biophysical, two social and two management variables (Supplementary table 3). The variables we included were: (1) wave exposure, (2) live stony coral percentage cover, (3) macroalgae percentage cover, (4) depth, (5) sea surface temperature, (6) productivity, (7) distance to deep water (>60 m), (8) distance to shore, (9) exposure to hurricanes, (10) sedimentation, (11) human population density, (12) distance to the nearest city, (13) Marine Protected Area (MPA) presence, and (14) scuba diving intensity. As the main management intervention to control lionfish populations is diver-based culling,

which occurs throughout the western Atlantic (Malpica-Cruz et al., 2016), we included scuba diving intensity as a proxy for lionfish culling intensity.

We did not include structural complexity as a variable because this was not measured consistently across all survey years and because another of our variables, stony coral percentage cover, correlates positively with structural complexity (Alvarez-Filip et al., 2011). Although we intended to include reef zone as a variable, this was recorded inconsistently and often confused with reef exposure in the AGRRA surveys, thus we excluded it from our analysis. We used MPA presence in our analysis because more detailed management data, such as the presence of no-take zones, was missing for most of the MPAs that encompassed our study sites.

Three of our variables (scuba diving intensity, exposure to hurricanes and sedimentation) were calculated using the gravity concept (Anderson, 2011), which states that interactions between two places are positively related to their mass and inversely related to the distance between them (Cinner et al., 2018). We calculated gravity as intensity of impact (number of dive centres, hurricane wind speed and sediment output) divided by the squared distance between reef and impact source, following the equation in Cinner et al. (2018). Several of our variables were available in the AGRRA database (stony coral percentage cover, macroalgae percentage cover and depth) or other online databases (wave exposure, sea surface temperature and productivity), however, most were calculated during this study.

Extraction and calculation of contextual variables

Data on live stony coral percentage cover, macroalgae percentage cover and depth were obtained directly from each transect in the AGRRA database (Marks, 2018). Data on wave exposure, sea surface temperature and productivity were obtained for the co-ordinates of each site from online databases (Assis et al., 2018, Tyberghein et al., 2012, Yeager et al., 2017). We compiled all other variables during this study, using spatial data in the form of rasters, points, lines and polygons. All spatial data analysis was conducted in R using the following packages: sp (Bivand et al., 2013,

Pebesma and S., 2005), raster (Hijmans, 2020), rgdal (Bivand et al., 2019), rgeos (Bivand and Colin, 2019), geosphere (Hijmans, 2019), dplyr (Wickham et al., 2020).

Wave exposure

We obtained data on wave energy (kW m^{-1}) for each site from the Marine Socio-Environmental Covariates database (Yeager et al., 2017). The wave energy values in this database were obtained from the NOAA Wavewatch III model (Tolman, 2009). The resolution of the Wavewatch III grid varies between geographical areas, with 91% of our sites located within a ten arc-minute grid and the remaining 9% located within a 30 arc-minute grid or four arc-minute grid.

Sea surface temperature and productivity

We obtained the mean sea surface temperature (^{o}C) and mean surface primary productivity (g $m^{-3}day^{-1}$) for each site from the Bio-ORACLE database (Assis et al., 2018, Tyberghein et al., 2012), which provides data at five arc-minute resolution.

Distance to deep water

We obtained water depths from the ETOPO1 Global Relief Model (Amante and Eakins, 2009), which provides data on land topography and ocean bathymetry at one arc-minute resolution. We converted a raster file of the data into a regular grid of points, with one point centred on each raster cell. The points were then sub-set to include only those with a water depth of at least 60 m. Distance to deep water was calculated as the distance between each site and the nearest depth point.

Distance to shore

We obtained a spatial polygon file of global shorelines from the Global Self-consistent, Hierarchical, High-resolution Geography database (GSHHG; Wessel and Smith, 1996). Distance to shore was calculated as the minimum distance between each site and a shoreline polygon.

Exposure to hurricanes

Data on hurricane paths was obtained from the NOAA National Hurricane Center (NOAA, 2020). We extracted data on the windspeed and position of each hurricane (which was recorded every six hours), from a separate pdf file for each hurricane. Although hurricane track shape files were provided from 2015 onwards, these were not available for earlier years, thus for consistency we obtained all data from the hurricane pdf files.

For each hurricane we used R to create a spatial line object by joining up each consecutive pair of hurricane position co-ordinates. The spatial line represented the hurricane's path. We then calculated the minimum distance between each site and each hurricane path. The closest point on the hurricane path was termed the intersection point. We calculated windspeed at the intersection point for all sites. To remove the influence of distant hurricanes, windspeed was set to zero if the minimum distance between a site and a hurricane path was ≥100 km. We assumed that windspeed changed at a constant rate between consecutive position points, allowing windspeed at the intersection point to be calculated using the proportional distance of the intersection point between the position points either side of it. We used the minimum distance and windspeed values to create a hurricane impact gravity value for each site, following the gravity equation of Cinner et al. (2018). Hurricane impact gravity was calculated using the following equation:

$$Hurricane\ impact\ gravity = \frac{Windspeed\ at\ intersection\ point}{(Distance\ between\ site\ and\ intersection\ point)^2}$$

For each AGRRA survey, we calculated a hurricane impact score for the current year by summing the hurricane gravity values for all hurricanes in the months up to and including the month of the survey. By taking survey month into account, we ensured that hurricanes occurring after the survey were not included in the hurricane impact score. We also calculated a hurricane impact score for the previous year, by summing hurricane gravity values for all hurricanes that occurred in the year prior to the survey. We only used one year prior to the survey because Caribbean hurricanes exert short-term

impacts in the following year, but no long-term impacts (Gardner et al., 2005). To obtain our final hurricane impact score for each AGRRA survey, we summed the current year and previous year hurricane impacts scores.

Sedimentation

We used a dataset of sediment output from >3000 rivers in the western Atlantic (World Resources Institute, 2004). We calculated the distance between each site and each river mouth then excluded any rivers that were >100 km away from each site. Sedimentation gravity for each site-river combination was calculated using the following equation:

$$Sedimentation\ gravity = \frac{Sediment\ output\ at\ river\ mouth}{(Distance\ between\ site\ and\ river\ mouth)^2}$$

Sedimentation gravity for each site was calculating by summing the sedimentation gravity values from all rivers within 100 km.

Human population density

To calculate human population density surrounding each site, we obtained a raster file of human population density at 30 arc-second resolution (CIESIN, 2018) and summed the values of all raster cells within 100 km of each site.

Distance to the nearest city

We used data on the locations of cities, defined as national and provincial capitals, provided as a spatial points file (ESRI, 2018). We then calculated the distance between each site and the nearest city.

MPA presence

To determine whether each site was within an MPA, we used a spatial polygon file of global MPAs (UNEP-WCMC and IUCN, 2020). We then recorded whether each site overlapped a polygon and was thus within an MPA. MPAs were designated in different years, so we only defined a site as being within an MPA if the MPA was designated during or prior to the survey year.

Scuba diving intensity

Locations of dive centres across the western Atlantic were obtained by searching for 'dive centre', 'dive shop' and 'scuba diving' for each sub-region and country (Google maps, 2020). These searches provided a list of dive site co-ordinates that we converted into a spatial points file. We rasterized the dive shop data to create a grid of 1 x 1 km cells, each containing a value for the number of dive centres within the cell. The raster file was then converted back to points to enable distances to be calculated between each site and the centre of each 1 x 1 km cell. The number of dive centres per grid cell and the distance between each site and grid cell were used to calculate diving gravity using the following equation:

$$Diving\ gravity = \frac{Number\ of\ dive\ shops\ in\ grid\ cell}{(Distance\ between\ site\ and\ grid\ cell)^2}$$

Scuba diving intensity was calculated as the sum of diving gravity values for all grid cells within 100 km of each site.

Response variables

We investigated the effects of the lionfish invasion on abundance, biomass, species richness, species diversity (using the Shannon-Weaver diversity index; Shannon and Weaver, 1949) and species presence/absence for the whole fish community. We also investigated the abundance of 6-10 cm Scaridae (parrotfish), Pomacentridae (damselfish) and Haemulidae (grunts), all of which are found in lionfish diets (Ballew et al., 2016, Chappell and Smith, 2016, Dahl and Patterson, 2014, Eddy et al.,

2016, Morris and Akins, 2009). We had intended to include Labridae (wrasse) — another common family in lionfish diet (Eaton et al., 2016, Morris and Akins, 2009) — but we found a large change in abundance of 6-10 cm wrasse between AGRRA survey versions four and five, which we suspect was caused by the change in protocols (with 0-5 cm wrasse being recorded in version five but not four) influencing search effort for wrasse. No change in protocols occurred for parrotfish or damselfish. A change in protocol occurred for grunts, with 0-5 cm individuals being recorded in version five but not four, but this did not affect 6-10 cm grunt abundance and so we included 6-10 cm grunts in our analysis.

Statistical analysis

First, we used non-metric multidimensional scaling (nMDS) to visualise changes in fish community composition throughout the lionfish invasion. Surveys were grouped into five invasion stages: pre-invasion and 0-1, 2-3, 4-5 and 6-7 years post-invasion. Data were then averaged to produce a single data point for each invasion stage within each sub-region. Abundance and biomass data were fourth-root transformed prior to analysis to reduce the influence of highly abundant species (Komyakova et al., 2018). Separate nMDS analyses using Bray-Curtis distances were run for abundance, biomass, species richness, diversity and presence/absence of the whole community and abundance and presence/absence of 6-10 cm fish. Analysis of Similarities (ANOSIM) was used to test for significant differences between invasion stages. As all ANOSIMs were significant, we ran Similarity Percentages (SIMPER) to identify species with the greatest contribution to the Bray-Curtis distance. Additionally, we ran nMDS analyses using years since lionfish invasion as the grouping variable and using invasion status (pre- or post-invasion) as the grouping variable.

Generalised Additive Mixed Models (GAMM) were used to test the effect of years since lionfish invasion and the contextual variables on several fish community metrics. GAMMs were chosen as we expected to see non-linear trends in the data, but we did not have an *a priori* hypothesis about the shape of the trends. All GAMMs were run using restricted maximum likelihood (REML). Model selection was carried out using the double penalty approach, which penalises both the range space and

null space of each smooth term, thus allowing uninformative smooth terms to be completely removed from the model (Marra and Wood, 2011). The double penalty approach only removes smooth terms from the model, so we tested whether to include MPA presence (a factor variable) by running each model with and without MPA presence and selecting the model with the lowest AIC value as the best model (Anderson and Burnham, 2004). The final models are listed in Supplementary table 4.

We ran separate GAMMs for the response variables species richness, species diversity, abundance and biomass of the whole fish community and for abundance of 6-10 cm parrotfish (Scaridae), 6-10 cm damselfish (Pomacentridae) and 6-10 cm grunts (Haemulidae). The Gaussian distribution was used with data that were normally distributed following either natural logarithm or square-root transformation, whilst the Tweedie distribution was used with data that were continuous but zero-inflated. For the Tweedie distributions, the index parameter (a measure of the shape of the distribution) was estimated from the data to ensure the correct Tweedie distribution was used. The default basis size of ten was used for all variables in all GAMMs. The model assumptions of normality, homogeneity, model fit and sufficient basis size were checked using the gam.check() function from the mgcv package (Wood, 2011). We used the Variance Inflation Factor (VIF) to check for collinearity between variables and we found that all values were below three, indicating a lack of collinearity (Zuur et al., 2010). The *p*-values for each GAMM were obtained from Analysis of Variance (ANOVA) tables following Brandell et al. (2020).

The first derivative and simultaneous confidence intervals of the 'years since lionfish invasion' smooth were calculated using the derivatives() function from the gratia package (Simpson, 2020). We identified periods of significant change as time points where the simultaneous confidence interval did not include zero (Simpson, 2018).

We compared the relative importance of each predictor in the GAMMs using percentage deviance explained. For each GAMM we ran the full model, null model and a series of models in which one smooth term or factor was dropped at a time. In the models where a term was removed, all other

smooths were set to their estimates from the full model. Percentage deviance explained for each term was then calculated following Whitlock et al. (2015), whereby the difference between deviance explained by the full model and the model without the term of interest was divided by the deviance explained by the null model. Models varied in the total percentage deviance explained (Supplementary table 4) so to enable a direct comparison between models of the percentage deviance explained by each predictor, we scaled percentage deviance values to sum to 100% for each model.

All analyses were conducted in R version 3.6.2 (R Core Team, 2019). We calculated Shannon-Weaver diversity and ran the nMDS, ANOSIM and SIMPER analyses using the vegan package (Oksanen et al., 2019). GAMMs were run using the mgcv package (Wood, 2011) and plotted using the visreg package (Breheny and Burchett, 2017) combined with the ggplot2 package (Wickham, 2016). All other plots were made with ggplot2. We used the colour-blind friendly viridis colour palette from the viridis package (Garnier, 2018) for all coloured plots.

5.4 Results

Native fish communities change following lionfish invasion

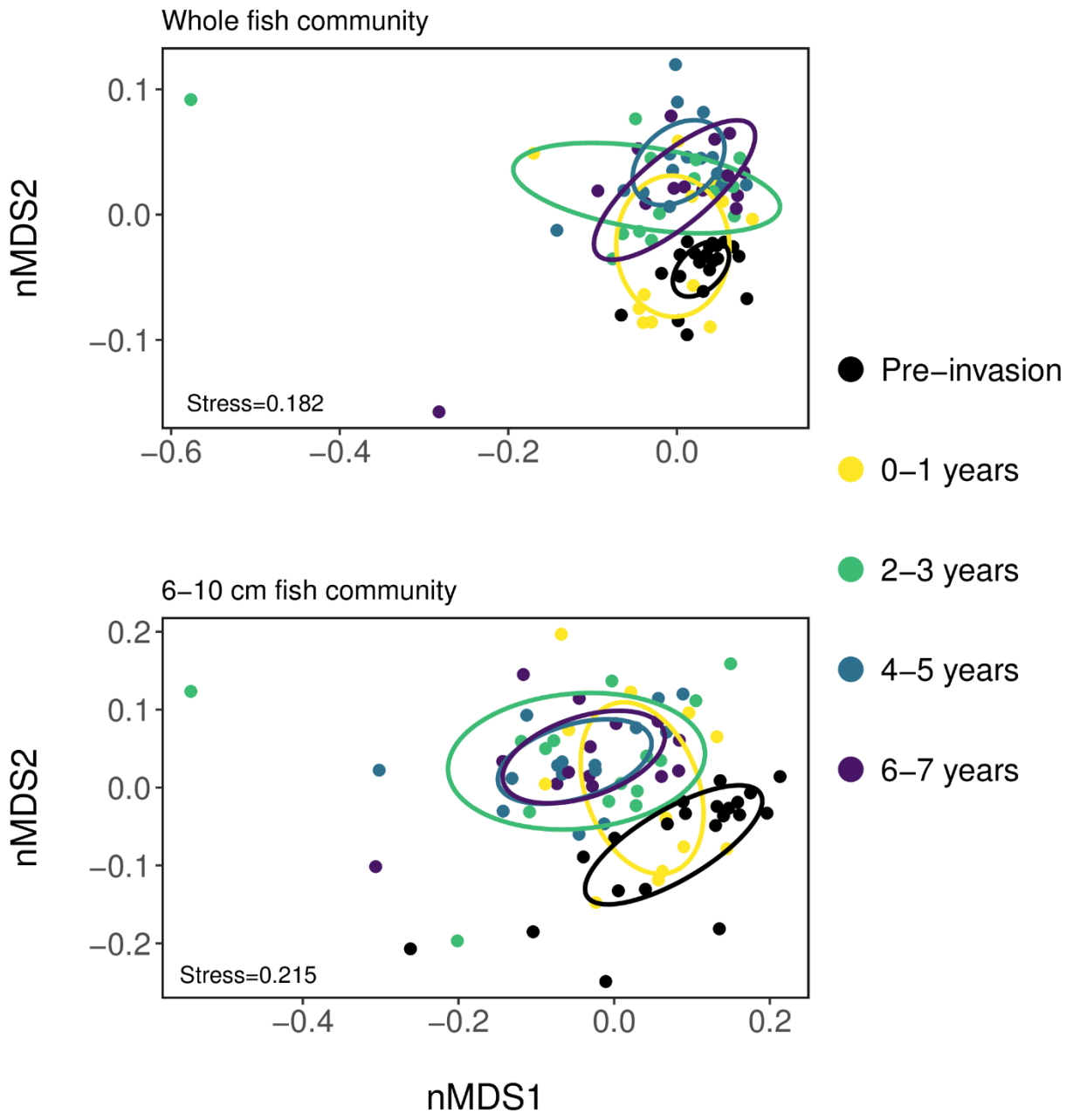
For all variables except biomass, nMDS plots showed a clear community-level shift from pre-invasion to two or more years post-invasion. Significant differences between invasion stages were detected for all response variables (Table 1), however, there was no obvious separation between pre- and post-invasion communities for biomass. For all other variables, nMDS plots showed that communities sampled 0-1 years post-invasion were intermediate between the pre-invasion group and the two or more years post-invasion group. This trend was particularly pronounced in the presence/absence nMDS plots for the whole fish community and the 6-10 cm fish community (Figure 2). Our nMDS plots using years since lionfish invasion and invasion status (pre- or post-invasion) support our suggestion of lionfish driving native fish community shifts, as pre-invasion and post-invasion points form separate groups (Supplementary figures 1 and 2).

SIMPER analyses indicated that parrotfish, wrasse, grunt and damselfish generally contributed the most towards dissimilarity between groups. Species with high contributions to dissimilarity across most SIMPER analyses included *Scarus iseri* (striped parrotfish), *Scarus taeniopterus* (princess parrotfish), *Halichoeres bivittatus* (slippery dick wrasse), *Halichoeres garnoti* (yellowhead wrasse), *Haemulon sciurus* (blue-striped grunt) and *Stegastes planifrons* (three-spot damselfish).

Table 1. ANOSIM statistics and *p*-values for tests of differences between invasion stages (pre-invasion and 0-1, 2-3, 4-5 and 6-7 years post-invasion). Abundance and biomass data were fourth-root transformed prior to analysis.

Response variable	ANOSIM R statistic	*p*-value
Total fish abundance	0.232	0.001
Total fish biomass	0.124	0.001
Whole fish community presence/absence	0.300	0.001
6-10 cm fish abundance	0.180	0.001
6-10 cm fish community presence/absence	0.223	0.001

Figure 2. nMDS plots for presence/absence of the whole fish community and the 6-10 cm fish community. Colours are used to distinguish the invasion stages (pre-invasion and 0-1, 2-3, 4-5 and 6-7 years post-invasion). Each coloured point represents a single sub-region in a single year category. No sub-region has data for all five invasion stages. Ellipses show 95% confidence intervals based on standard deviations.



Negative effects become apparent five years after lionfish invasion

Whole fish community

Years since lionfish invasion was a significant predictor of species richness, diversity, abundance and biomass of the whole fish community (Table 2). All metrics show an increase that began prior to lionfish invasion, with species richness, diversity and abundance showing declines that begin approximately five years after lionfish invasion and continue until the end of the study (Figure 3). The only significant period of change after lionfish invaded was an increase in biomass between one and three years post-invasion (Supplementary figure 3). First derivative plots of our GAMM smoothers show that by the end of our study (eight years after lionfish invasion) the first derivative was negative for species richness, diversity and abundance, indicating a decline in these variables (Supplementary figure 3).

Years since invasion explained a high percentage of the deviance for species richness (30%) and abundance (27%), but a low percentage of the deviance for diversity (6%) and biomass (8%). Stony coral percentage cover explained a high percentage of the deviance in all four models and sea surface temperature explained a high percentage of the deviance in all four models except abundance (Figure 4; Supplementary figure 4). Stony coral percentage cover was similar in importance to years since invasion in the species richness (26%) and abundance (29%) models and was the second most important variable in the diversity (23%) and biomass (24%) models. Sea surface temperature was the most important variable in the diversity (26%) and biomass (42%) models.

Table 2. Significance of the 'years since lionfish invasion' variable in GAMM models.

Response variable	*p*-value
Species richness	<0.001
Diversity	0.019
Total fish abundance	<0.001
Total fish biomass	<0.001
6-10 cm parrotfish abundance	<0.001
6-10 cm damselfish abundance	<0.001
6-10 cm grunt abundance	<0.001

Figure 3. Smoothers and partial residuals for years since lionfish invasion in GAMM models of species richness, diversity, abundance and biomass of the whole fish community. The confidence bands are shaded in grey. The black vertical line indicates the year of lionfish invasion and the dashed, red vertical line indicates five years after lionfish invaded, which is the beginning of the observed declines in most metrics.

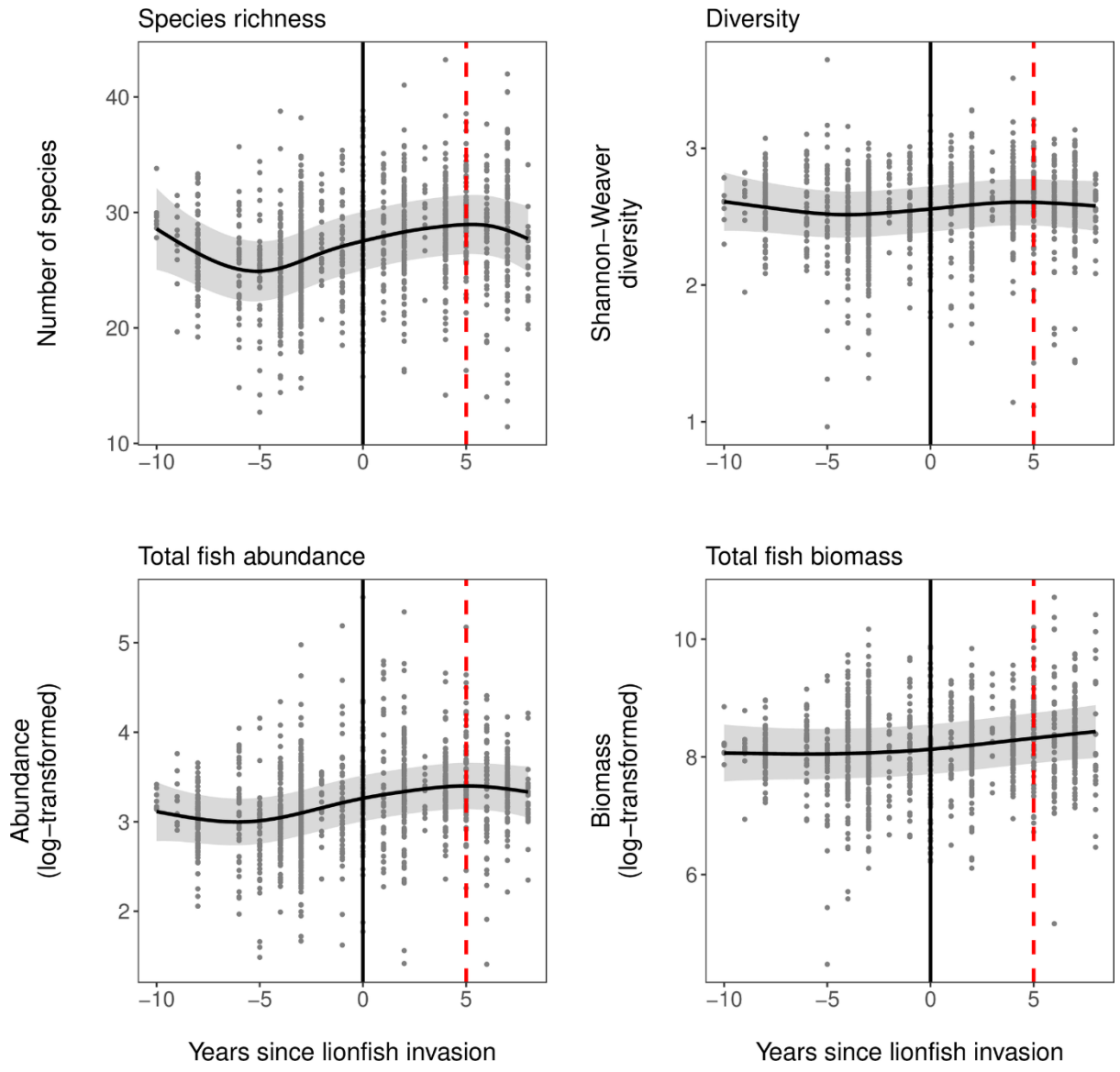
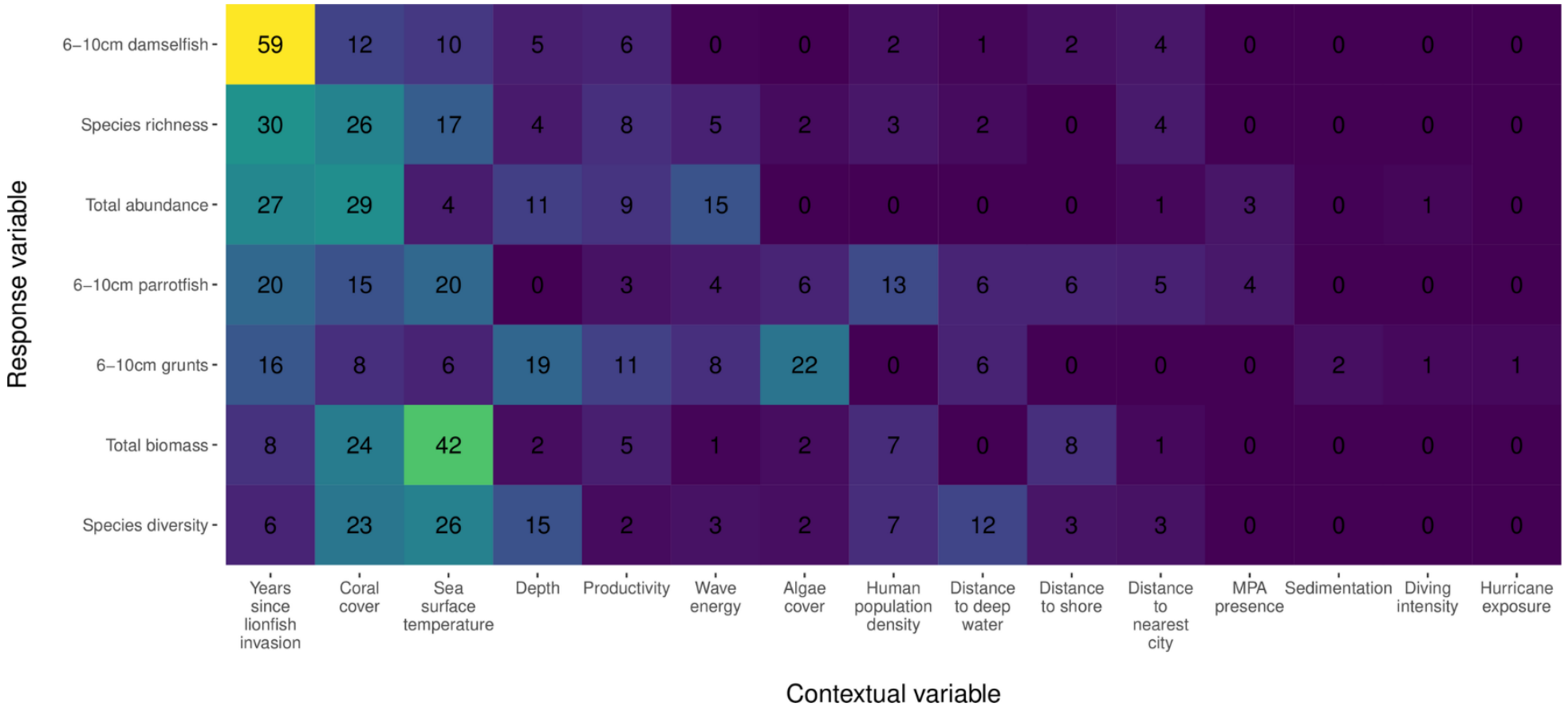


Figure 4. Relative percentage deviance explained by each variable in our GAMMs. Values were scaled so that each model summed to 100%, then values were rounded to the nearest integer. Variables with large contributions to explained deviance are yellow and variables with small contributions to explained deviance are purple. Contextual variables are ordered from highest importance (left) to lowest importance (right) when values were summed across all models. Response variables are ordered from highest contribution (top) to lowest contribution (bottom) of years since lionfish invasion.

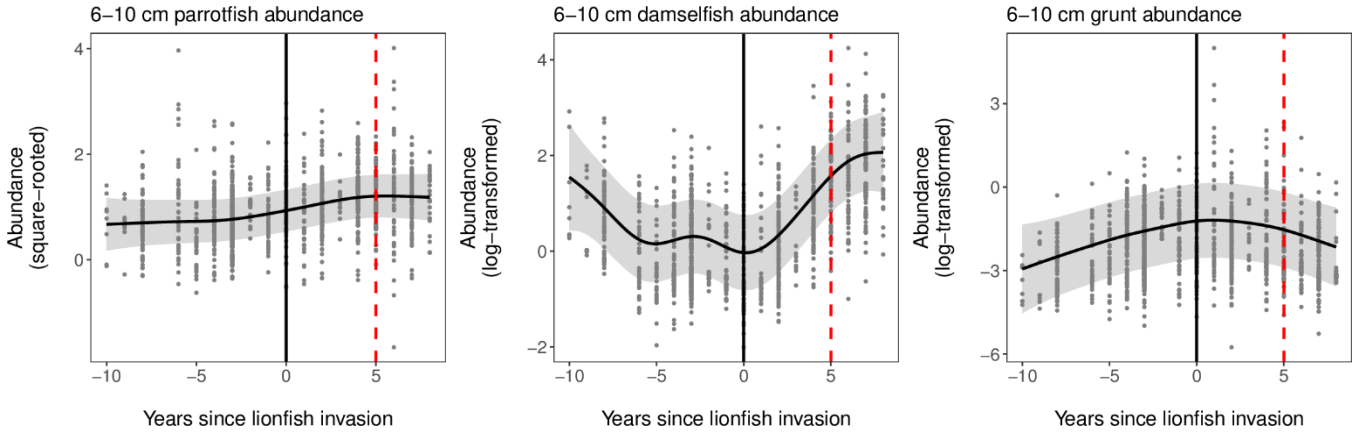


6-10 cm fish community

Years since lionfish invasion was a significant predictor of 6-10 cm parrotfish, damselfish and grunt abundance (Table 2). Parrotfish and damselfish abundance increased at the beginning of the lionfish invasion, but both show changes at approximately five years since lionfish invasion, with parrotfish abundance beginning to decrease and damselfish abundance reaching a plateau (Figure 5). In contrast, grunt abundance increased prior to lionfish invasion and decreased from approximately one year after lionfish invasion until the end of the study (Figure 5). We identified significant increases in abundance from 0-3 years post-invasion for parrotfish and 2-6 years post-invasion for damselfish but no significant change for grunts (Supplementary figure 3). First derivative plots of our GAMM smoothers show that by the end of our study (eight years after lionfish invasion) the first derivative was either negative (indicating a decline) or had reached zero (indicating no change) for all three groups (Supplementary figure 3).

The percentage deviance explained by years since lionfish invasion varied between parrotfish, damselfish and grunts (Figure 4; Supplementary figure 5). Years since lionfish invasion and sea surface temperature were equally important in explaining parrotfish abundance (20% for both), with stony coral percentage cover being the next most important variable (15%). In contrast, years since lionfish invasion was the most important variable in explaining damselfish abundance (59%), with stony coral cover (12%) and sea surface temperature (10%) being the next most important variables. For grunt abundance, the most important variables were macroalgae percentage cover (22%) and depth (19%), with years since lionfish invasion explaining 16%.

Figure 5. Smoothers and partial residuals for years since lionfish invasion in GAMM models of 6-10 cm parrotfish, 6-10 cm damselfish and 6-10 cm grunts. The confidence bands are shaded in grey. The black vertical line indicates the year of lionfish invasion and the dashed, red vertical line indicates five years after lionfish invaded, which is the beginning of the observed declines in most metrics.



5.5 Discussion

We found a clear shift in native fish community structure during the initial stages of the lionfish invasion. Pre-invasion communities were distinct from communities sampled two or more years post-invasion, whilst communities sampled 0-1 years post-invasion were transitional between these two groups. Lionfish only exert negative effects on fish communities when their density is above a reef-specific threshold (Green et al., 2014), thus a transition period would be expected whilst lionfish abundance increases following invasion. Research from Belize found distinct pre-invasion and post-invasion prey fish communities (Hackerott et al., 2017) but no transitional communities as we found in our study. This difference in results may be because we studied multiple sites, whose communities are likely to have changed at different rates (Svensson et al., 2009), whereas Hackerott et al. (2017) only studied a single reef system. Despite the difference in presence or absence of a transition period, both our study and that of Hackerott et al. (2017) support the notion that changes caused by lionfish result in stable post-invasion fish communities.

The families with the largest contribution to the dissimilarity between invasion year groups were parrotfish, wrasse, grunt and damselfish. Within these families, the most influential species that we identified are all found in lionfish diets across the invaded range (Ballew et al., 2016, Chappell and Smith, 2016, Dahl and Patterson, 2014, Eddy et al., 2016, Morris and Akins, 2009). This result indicates that consumptive effects on prey species are more pronounced than non-consumptive effects on non-prey species that can include reductions in feeding (Eaton et al., 2016, Kindinger and Albins, 2017), competition for shelter use (Hunt et al., 2020, Trehern et al., 2020) and reductions in fecundity (Davis, 2018).

Our GAMMs showed a consistent continuation of pre-invasion trends in native fish communities during the initial years of invasion, although these trends were only significant in the biomass, parrotfish abundance and damselfish abundance models. These trends represented increases in all response variables except 6-10cm grunts, which decreased after the year of first invasion. These trends typically began several years prior to lionfish invasion and continued for approximately five years post-invasion, when a downturn was seen in most response variables. These positive trends are unexpected, as most studies indicate a negative effect of lionfish on prey fish abundance and richness (Albins, 2013, Albins, 2015, Ballew et al., 2016, Ellis and Faletti, 2016, Peiffer et al., 2017), with a few studies indicating no effect (Hackerott et al., 2017, Harms-Tuohy et al., 2018). However, native fish can continue to increase in abundance or biomass even when lionfish are present, provided that the number or density of lionfish is below a reef-specific threshold (Benkwitt, 2015, Green et al., 2014). Therefore, the positive trends we observed are likely caused by other factors that acted prior to lionfish invasion, but lionfish populations were too low to counteract these other factors and cause declines in the initial years of the invasion.

We observed a region-wide change in the later stages of the invasion, with most metrics showing declines at five years post-invasion. Although we observed positive trends in fish community and abundance metrics at the beginning of the lionfish invasion, these did not continue throughout the invasion. All GAMM smooth plots except biomass show declines or plateaus at eight years since

lionfish invasion, which is the extent of our dataset. Furthermore, the decrease (or plateau for 6-10 cm damselfish) begins at approximately five years post-invasion for species richness, diversity, total fish abundance, 6-10 cm parrotfish and 6-10 cm damselfish. Although none of the negative trends observed were significant, we observed synchronicity across whole community metrics and two different families, suggesting that a single driving force acts approximately five years after lionfish invade an area. Because lionfish invaded our study regions between 2007 and 2012, five years post-invasion does not equate to the same calendar year across all study sites. Synchronised declines at five years post-invasion are therefore not the result of a single region-wide event such as an extreme climatic or anthropogenic stress. Instead, we suggest that the changes at five years post-invasion may be caused by lionfish reaching a critical density at which they begin to negatively impact native species at a detectable regional scale.

Although many studies show negative effects of lionfish on fish abundance, these studies are often conducted on small-scale patch reefs and over short temporal scales of just a few weeks (Albins, 2013, Albins and Hixon, 2008, Ellis and Faletti, 2016, Ingeman, 2016). Small-scale patch reef studies may be unrepresentative of larger-scale trends due to differences in lionfish movement between patch and continuous reefs (Tamburello and Cote, 2015) and non-linear scaling of lionfish effects (Benkwitt, 2015). Short-term studies may also be unrepresentative of long-term trends if lionfish populations increase or decrease over time (Benkwitt et al., 2017) or if native prey adapt to invasive lionfish through phenotypic plasticity or genetic changes (Berthon, 2015).

Only two studies have investigated the effects of lionfish across whole reef systems and on timescales of two to four years but both found no negative effects of lionfish (Elise et al., 2015, Hackerott et al., 2017). The only study of lionfish effects at a sub-regional scale found a significant reduction in tomtate grunt (*Haemulon aurolineatum*) abundance on reefs that were invaded by lionfish, however, this negative effect was not detected until 7-12 years after lionfish invaded (Ballew et al., 2016). This is consistent with our results, which suggest that lionfish may exert negative effects approximately five years after invasion. The size of prey fish that are studied may also impact whether negative

effects of lionfish are detected. Both our study and that of Hackerott et al. (2017) studied 6-10 cm fish, however, lionfish prey is often in the range of 2-4 cm (Cure et al., 2012, Green and Cote, 2014, Munoz et al., 2011). Although we might have detected greater and potentially significant effects of lionfish if we had studied the 0-5 cm prey size category, our results suggest that lionfish may also exert negative effects on larger prey fish, indicating that lionfish invasion may lead to community-level changes.

The importance of years since lionfish invasion was similar to other variables in most of our models, except the diversity and total fish biomass models, where years since invasion was of low importance. Other important variables include sea surface temperature, stony coral percentage cover, macroalgae percentage cover and depth. For four of the five response variables that showed changes at five years post-invasion (species richness, total fish abundance, 6-10 cm parrotfish abundance and 6-10 cm damselfish abundance), years since lionfish invasion was one of the most important variables in the GAMMs. However, our results do not provide conclusive evidence that lionfish invasion is the driver of the observed changes. All variables fluctuated prior to lionfish invasion, thus it is possible that other contextual variables not included in our analysis may have driven the observed changes. The observed post-invasion declines are of similar or lesser magnitude than the pre-invasion declines, indicating that the effects of lionfish over the course of our study may be less severe than normal stochastic variation or stressors that have occurred in the past.

Interestingly, the importance of management variables including MPA presence and diving gravity were low in all models. We expected MPA presence to have a large effect because there is substantial evidence on the benefits of MPAs for fish communities (Edgar et al., 2014, Halpern and Warner, 2002, Lester et al., 2009). However, for the MPAs we had data for, management methods differed, from complete no-take to completely fished. MPA management significantly affects conservation outcomes (Gill et al., 2017) so not accounting for this may have led to the low importance of MPAs in our models. We also expected diving gravity to have a large effect because it is a proxy for lionfish culling pressure, which reduces lionfish densities (de Leon et al., 2013, Green et al., 2017) and can

allow native fish populations to recover (Green et al., 2014). We suggest that the low importance of diving gravity in our study may relate to the coarse resolution at which it was calculated, rather than a lack of effect of lionfish culling. Lionfish culling intensity and effectiveness can vary between regions and over time in relation to lionfish densities (Malpica-Cruz et al., 2016), the type and number of participants in derby events (Green et al., 2017, Malpica-Cruz et al., 2016), awareness of the lionfish invasion (Morris, 2012), no-take fishing regulations (Morris, 2012) and country-specific management plans (Graham and Fanning, 2017). Our proxy for lionfish culling pressure did not consider differences in culling intensity across space or time and thus we cannot conclude from our study whether lionfish culling has an effect. Future studies should take these factors into account to create a more accurate representation of lionfish culling pressure before any conclusions on the effectiveness of lionfish culling can be made.

5.6 Conclusion

Our results demonstrate the importance of combining a wide geographical and temporal spread of data with contextual variables and sites with staggered invasion dates when studying the regional impacts of invasive species. Using this approach, we provide the first suggestion of lionfish impacts across the entire western Atlantic range. We observed pre-invasion increases in fish community metrics that continued into the initial years of the invasion, however, we observed simultaneous declines in species richness, diversity, total fish abundance and prey fish abundance from five years post-invasion until the end of our study. The staggered invasion dates across the invaded sub-regions in our study, from 2007-2012, significantly reduce the potential influence of region-wide point impact events as drivers of the observed changes. By controlling for, and reducing the influence of, confounding variables, our results suggest that lionfish may exert a region-wide impact at five years post-invasion.

5.7 Acknowledgements

This work was financially supported by the Natural Environment Research Council (Grant number: NE/L002612/1) and Operation Wallacea. We thank Owen Lewis for comments that improved the manuscript.

5.8 Author contributions

Conceptualisation: Christina L. Hunt, Dominic A. Andradi-Brown, Dan A. Exton

Formal analysis: Christina L. Hunt, Dominic A. Andradi-Brown, Thomas C. Cameron, Dan A. Exton

Investigation: Christina L. Hunt

Resources: Craig P. Dahlgren, Judith C. Lang, Melanie McField, Pamela J. Schofield

Supervision: Dominic A. Andradi-Brown, Dan A. Exton

Visualisation: Christina L. Hunt, Dominic A. Andradi-Brown, Dan A. Exton

Writing- original draft preparation: Christina L. Hunt

Writing- review and editing: Christina L. Hunt, Dominic A. Andradi-Brown, Thomas C. Cameron, Craig P. Dahlgren, Judith C. Lang, Owen T. Lewis, Melanie McField, Pamela J. Schofield, Dan A. Exton

5.9 Supplementary information

Supplementary table 1. Countries and sub-regions used in this study. All years of first occurrence were obtained from the USGS Nonindigenous aquatic species database (USGS, 2020) except Guanaja, which was obtained from the AGRRA database (Marks, 2018). The years for which we have data are listed for both pre-invasion and post-invasion. The year that lionfish invaded is denoted zero years since invasion and is listed in the post-invasion years column.

Country	Sub-region	Year of lionfish invasion	Number of sites	Number of transects	Pre-invasion years	Post-invasion years
Antigua	Antigua	2011	16	196	-6	6
Bahamas	Andros	2007	63	2439	-5, -3, -1	0, 1, 2, 4, 6, 8
Belize	Central barrier complex	2010	46	881	-10, -4, -2, -1	1, 3, 6
	Glover's reef	2009	21	426	-9, -3	0, 2, 7
	Lighthouse reef	2009	18	359	-3	0, 2, 4, 7
	Northern barrier complex	2009	16	314	-3, -1	0, 2, 3, 4, 7
	Southern barrier complex	2009	44	603	-3, -1	0, 2, 7
	Turneffe	2008	22	428	-8, -2	1, 3, 8
Grenada	Grenadines (Grenada)	2011	16	229	-6	4
Guatemala	Guatemala	2011	13	206	-5	1, 2, 5
Honduras	Cayos cochinos	2010	14	528	-4	0, 2, 4, 6
	Coastal Honduras	2011	38	728	-5	0, 2, 4, 5
	Guanaja	2012	18	495	-6	0, 2, 4
	Roatan	2009	27	856	-3	0, 2, 5, 7

	Utila	2009	17	486	-3	2, 3, 5, 7
Jamaica	Jamaica	2008	70	777	-8	6, 7
	Pedro bank	2008	36	382	-3	4
Mexico	Sian Ka'an	2009	28	313	-4, -3	5
	Cozumel	2009	16	227	-4, -3	2, 5, 7
	Northern Quintana roo	2009	27	546	-4, -3	2, 5, 7
	Southern Quintana roo	2009	18	334	-3	5, 7

Supplementary table 2. Fish species that were recorded in the AGRRA surveys.

Family	Species recorded	Sizes included in our dataset
Acanthuridae	All species	All
Chaetodontidae	All species	All
Scaridae	All species	> 5 cm
Haemulidae	All species	> 5 cm
Lutjanidae	All species	All
Pomacanthidae	All species	All
Balistidae	*Balistes vetula, B. capriscus, Melichthys niger, Aluterus scriptus, Cantherhines pullus, C. macrocerus*	All
Carangidae	*Caranx ruber, Trachinotus falcatus*	All
Diodontidae	*Diodon holocanthus, Diodon hystrix*	All
Kyphosidae	*Kyphosus spp.*	All

Labridae	*Lachnolaimus maximus, Halichoeres radiatus, Halichoeres bivittatus, Bodianus rufus, Halichoeres garnoti*	> 5 cm
Monacanthidae	*Cantherhines pullus, Aluterus scriptus, Cantherhines macrocerus*	All
Ostraciidae	*Lactophrys bicaudalis*	All
Pomacentridae	*Stegastes planifrons, Microspathodon chrysurus*	All
Serranidae	*Epinephelus* spp. and *Mycteroperca* spp.	All
Sparidae	*Calamus bajonado, Calamus pennatula, Calamus calamus, Calamus penna*	All
Sphyraenidae	*Sphyraena barracuda*	All
Tetraodontidae	*Sphoeroides spengleri*	All

Supplementary table 3. Description of each contextual variable and justification for inclusion in the study.

Variable	Category	Data type	Description	Justification for inclusion	Data source
Wave exposure	Biophysical	Continuous	Mean wave energy (kW m^{-1})	Wave exposure has been shown to influence the density and biomass of fish communities (Floeter et al., 2007, Friedlander et al., 2003)	Wave exposure obtained from Yeager et al. (2017)
Stony coral percentage cover	Biophysical	Continuous	Percentage cover of living scleractinian corals	Hard coral cover is an important predictor of fish biomass on coral reefs (Williams et al., 2015) and reef fish communities have been shown to change as coral cover declines (Emslie et al., 2014)	Coral cover obtained from the AGRRA database (Marks, 2018)
Macroalgae percentage cover	Biophysical	Continuous	Percentage cover of macroalgae	Macroalgae cover is an important driver of reef fish density and biomass in the Caribbean (Valles and Oxenford, 2015)	Macroalgae cover obtained from the AGRRA

					database (Marks, 2018)
Depth	Biophysical	Continuous	Site depth (m)	Fish abundance and community composition changes with depth (Brokovich et al., 2008)	Depth obtained from the AGRRA database (Marks, 2018)
Water temperature	Biophysical	Continuous	Mean sea surface temperature (^{o}C)	Water temperature is an important driver of fish biomass and density in the Caribbean (Valles and Oxenford, 2015)	Sea surface temperature obtained from (Assis et al., 2018, Tyberghein et al., 2012)
Productivity	Biophysical	Continuous	Mean surface primary productivity (g $m^{-3}day^{-1}$)	Chlorophyll-a is used as a proxy for productivity and has a positive	Primary productivity obtained from

				correlation with species richness in the Caribbean (Sandin et al., 2008)	(Assis et al., 2018, Tyberghein et al., 2012)
Distance to deep water	Biophysical	Continuous	Minimum distance between site and water >60 m deep (m)	The boundary between shallow and mesophotic communities occurs at 60 m (Lesser et al., 2019) and in a global study, sites with higher than expected fish biomass (based on environmental and social predictors) were often within 5 km of a deep-water refuge (Cinner et al., 2016).	Water depth obtained from Amante and Eakins (2009)
Distance to shore	Biophysical	Continuous	Minimum distance between site and shoreline (m)	Globally, distance from shore is an important predictor of fish biomass (Gill et al., 2017)	Shoreline positions obtained from

					Wessel and Smith (1996)
Exposure to hurricanes	Biophysical	Continuous	Sum of hurricane impact gravity values for the year of, and one year prior to, each AGRRA survey. Hurricane exposure gravity for each hurricane-AGRRA survey combination was calculated as: $\frac{Windspeed\ at\ closest\ point\ of\ hurricane\ path}{(Distance\ between\ site\ and\ hurricane\ path)^2}$ This gravity measure assumes that distant hurricanes and hurricanes with lower windspeed have less effect on reef communities.	Hurricanes can physically damage reefs (Hughes, 1994). In a global study, sites with lower than expected fish biomass (based on environmental and social predictors) were often those that has been exposed to environmental shocks, such as coral bleaching or cyclones (Cinner et al, 2016)	Hurricane positions and windspeeds obtained from NOAA (2020)
Sedimentation	Biophysical	Continuous	Sum of sedimentation gravity values for all rivers within 100 km of each site. Sedimentation was calculated as: $\frac{Sediment\ output\ at\ river\ mouth}{(Distance\ between\ site\ and\ river\ mouth)^2}$	There is a significant negative correlation between sedimentation and fish density (Bejarano and Appeldoorn, 2013)	River sediment output obtained from World Resources Institute (2004)

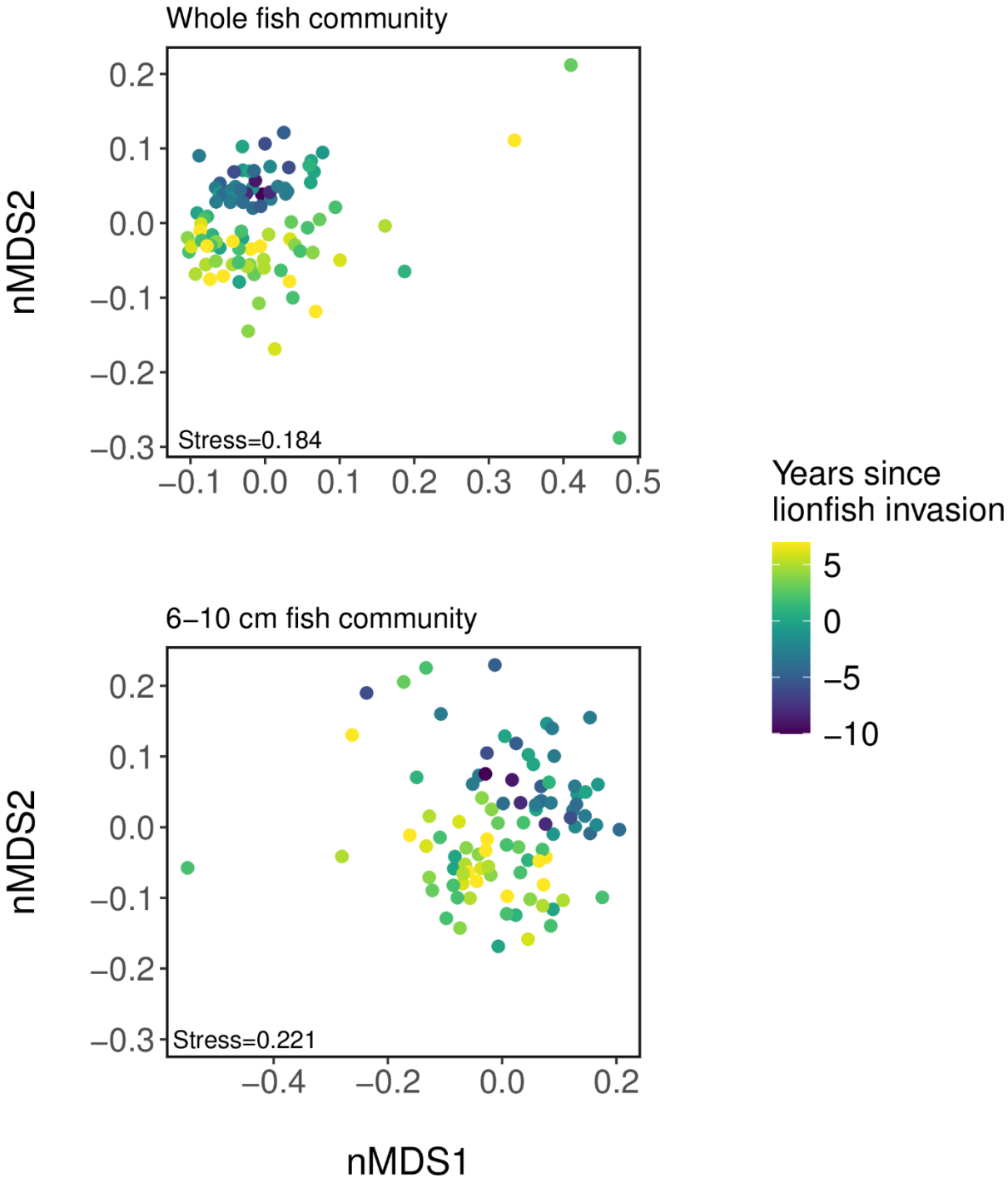
			This gravity measure assumes that distant rivers and rivers with lower sediment output have less effect on reef communities.		
Human population density	Social	Continuous	Number of people within 100 km of the reef	Human population density is an important predictor of fish biomass in central and western Pacific coral reefs (Williams et al., 2015) and across the world (Cinner et al., 2013)	Human population densities obtained from CIESIN (2018)
Distance to the nearest city	Social	Continuous	Distance to the nearest city, defined as the national or provincial capital (km)	Using distance to city as a proxy for distance to market, a global study found that fish biomass remained relatively constant when markets were within 14 km of a reef, but increased exponentially as distance	Location of cities obtained from ESRI (2018)

				to market increased further (Cinner et al., 2013)	
Marine Protected Area (MPA) presence	Management	Categorical	Presence of absence of an MPA during the year of the AGRRA survey (yes/no)	In a global study of MPAs, species biomass, density and richness were all higher inside MPAs than outside (Lester et al., 2009)	MPA locations obtained from UNEP-WCMC and IUCN (2020)
Scuba diving intensity	Management	Continuous	The sum of diving gravity values for all 1 x 1 km grid cells within 100 km of each site. Diving gravity was calculated as: $= \frac{Number\ of\ dive\ shops\ in\ grid\ cell}{(Distance\ between\ site\ and\ grid\ cell)^2}$ This measure of gravity assumes that distant dive centres and areas with a low density of dive centres exert lower lionfish culling pressure	Lionfish culling is common across the region (Malpica-Cruz et al., 2016) and has been shown to reduce lionfish density (de Leon et al., 2013), which can allow prey fish densities to recover (Green et al., 2014, Peiffer et al., 2017).	Location of dive centres obtained from Google maps (2020)

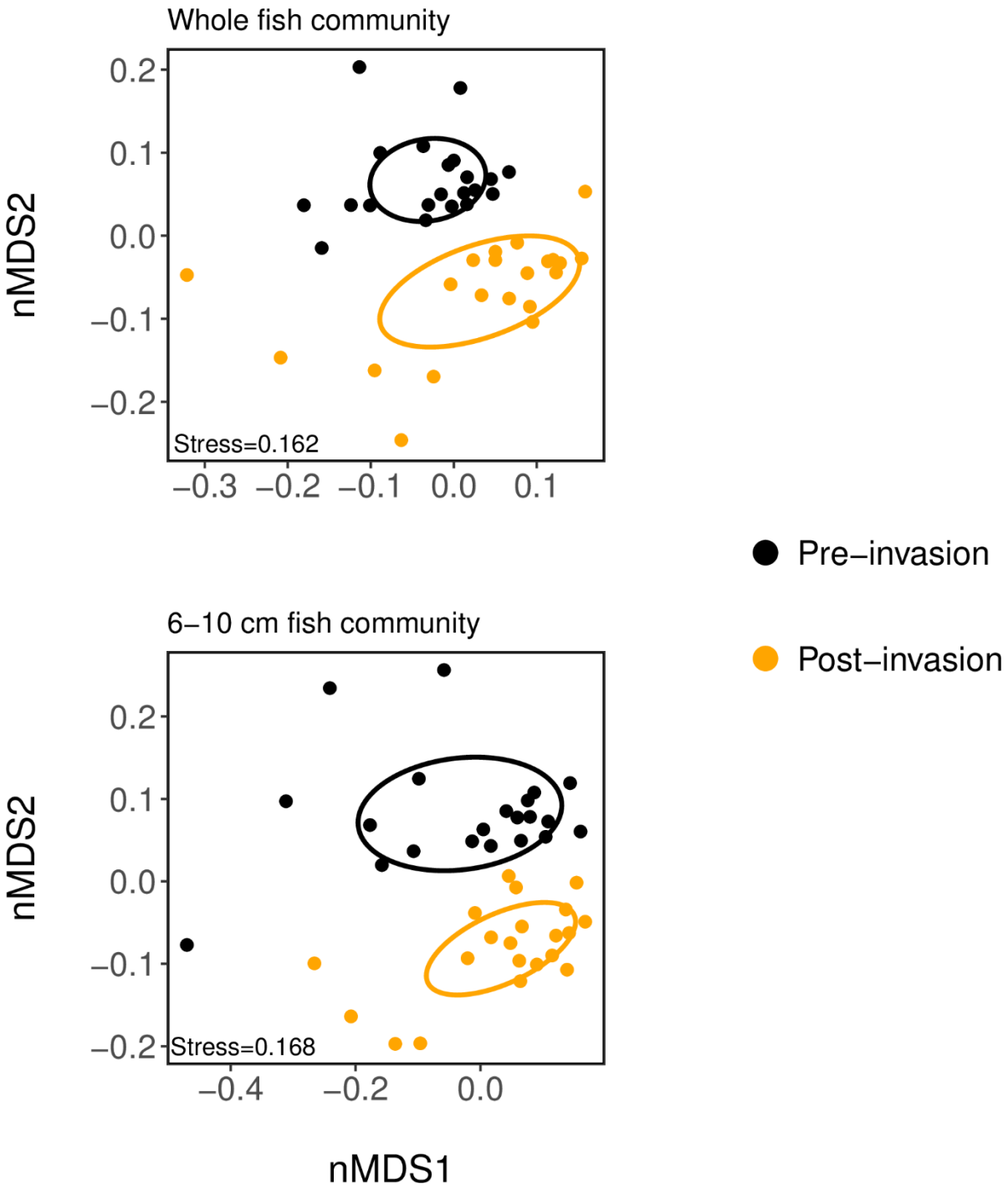
Supplementary table 4. Data transformations and distributions used in the GAMMs along with the percentage deviance explained by each model.

Model	Transformation	Distribution	Percentage deviance explained
Species richness	No transformation	Gaussian	59
Species diversity	No transformation	Gaussian	52
Total fish abundance	Natural logarithm	Gaussian	43
Total fish biomass	Natural logarithm	Gaussian	56
6-10 cm parrotfish abundance	Square-root	Gaussian	64
6-10 cm damselfish abundance	No transformation	Tweedie	67
6-10 cm grunt abundance	No transformation	Tweedie	65

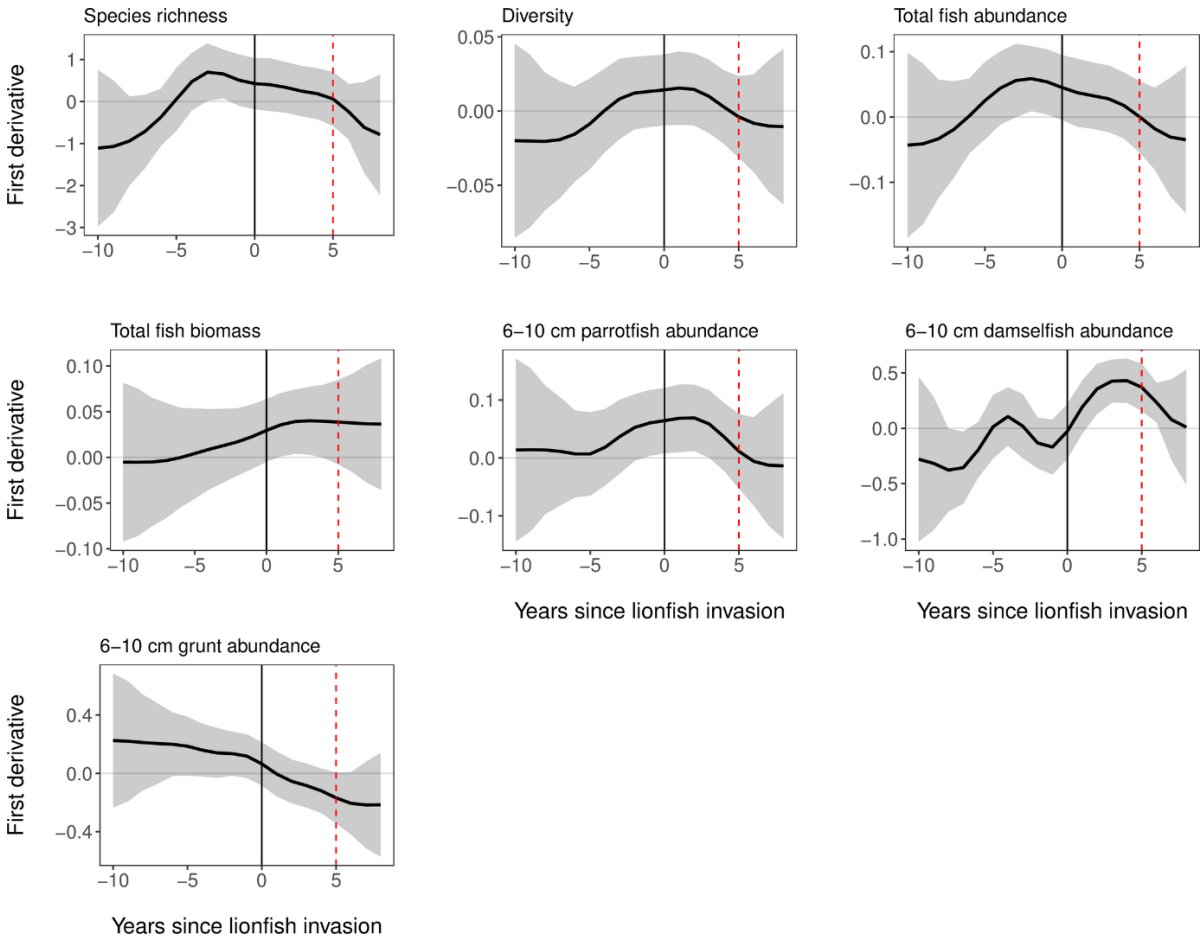
Supplementary figure 1. nMDS plots for presence/absence of the whole fish community and the 6-10 cm fish community. Colours are used to distinguish the number of years since lionfish invasion, with purple/blue representing pre-invasion communities and green/yellow representing post-invasion communities. There were too few data points per year to calculate 95% confidence intervals.



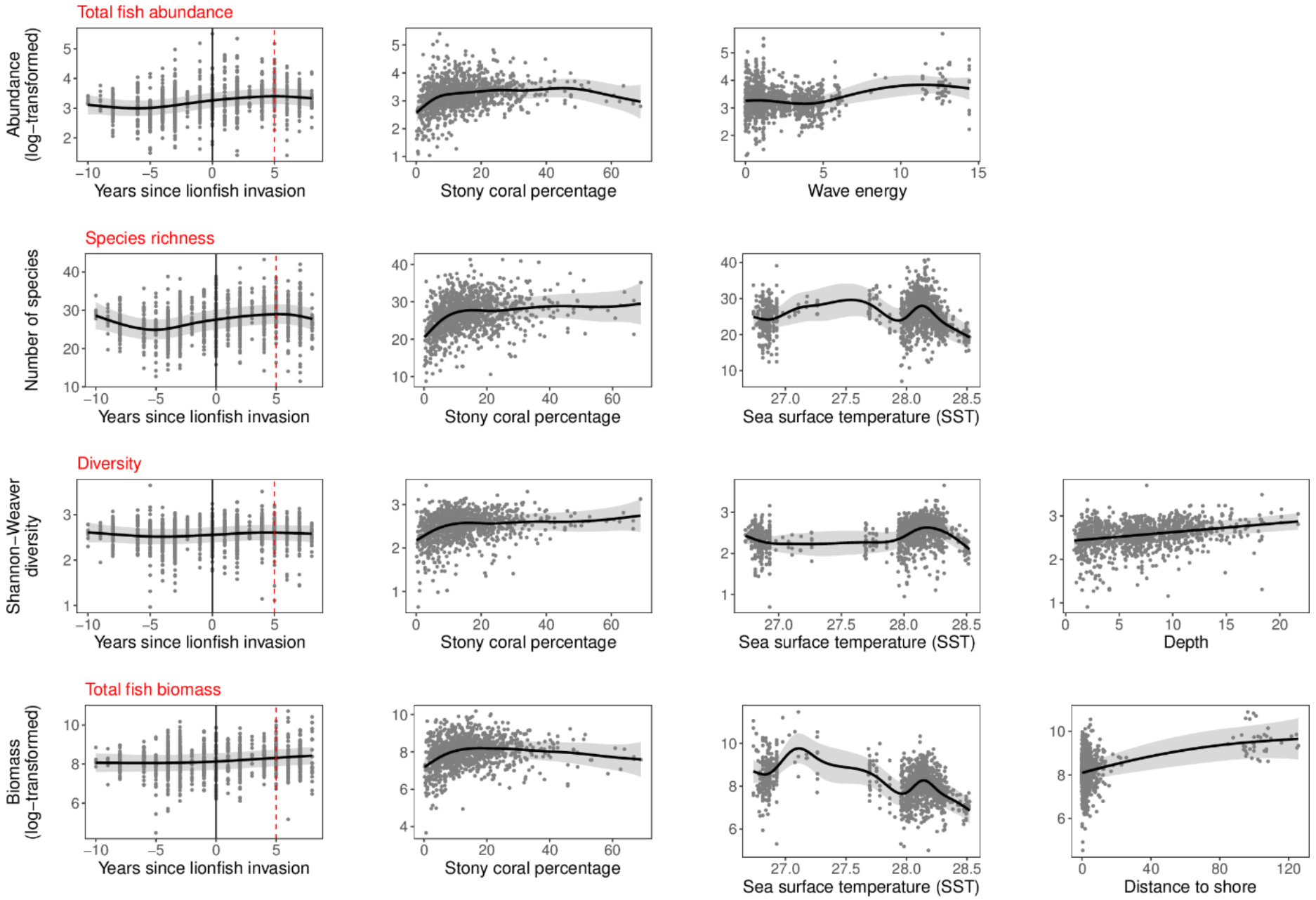
Supplementary figure 2. nMDS plots for presence/absence of the whole fish community and the 6-10 cm fish community. Colours are used to distinguish pre-invasion communities (in black) from post-invasion communities (in orange). Ellipses show 95% confidence intervals based on standard deviations.



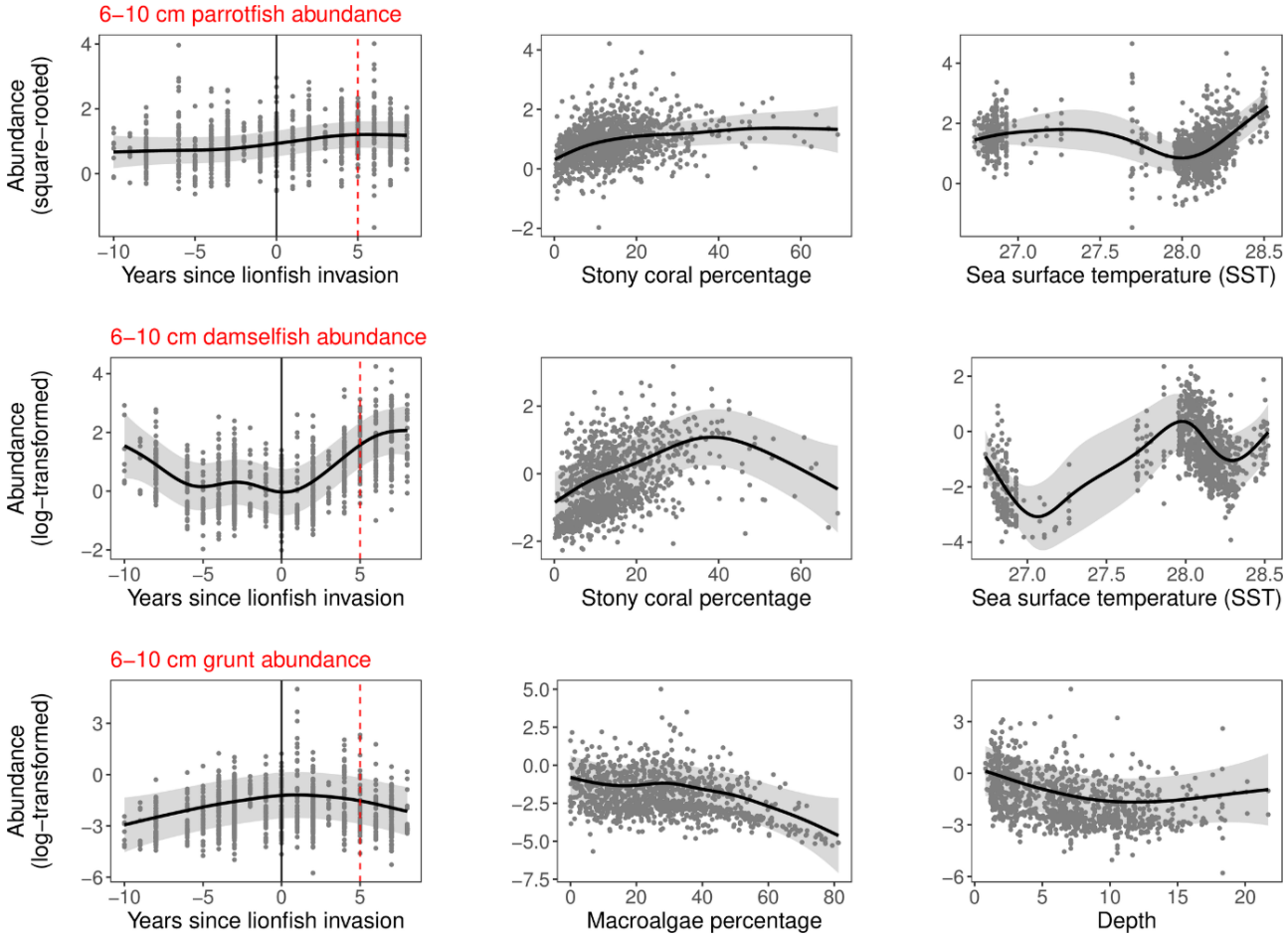
Supplementary figure 3. Plot of the first derivative of the smoother for years since lionfish invasion. The 95% simultaneous confidence interval is shaded in grey. The black vertical line indicates the year of lionfish invasion and the dashed, red vertical line indicates five years after lionfish invaded, which is the beginning of the observed declines. The horizontal grey line indicates where the first derivative is equal to zero. We identified periods of significant change as time points where the simultaneous confidence interval did not include zero (Simpson, 2018).



Supplementary figure 4. Smoothers and partial residuals for GAMM models of abundance, species richness, diversity and biomass of the whole fish community. Years since lionfish invasion is shown for all models, along with the top three most influential variables in each model. Only two additional variables are shown for the abundance and species richness models as years since lionfish invasion was included in the top three variables. The confidence bands are shaded in grey. The black vertical line indicates the year of lionfish invasion and the dashed, red vertical line indicates five years after lionfish invaded, which is the beginning of the observed declines in most metrics.


Total fish abundance
Abundance (log−transformed)
Years since lionfish invasion
Stony coral percentage
Wave energy
Species richness
Number of species
Years since lionfish invasion
Stony coral percentage
Sea surface temperature (SST)
Diversity
Shannon−Weaver diversity
Years since lionfish invasion
Stony coral percentage
Sea surface temperature (SST)
Depth
Total fish biomass
Biomass (log−transformed)
Years since lionfish invasion
Stony coral percentage
Sea surface temperature (SST)
Distance to shore

Supplementary figure 5. Smoothers and partial residuals for GAMM models of 6-10 cm parrotfish, damselfish and grunt abundance. The top three most influential variables are shown for each model; years since lionfish invasion was in the top three variables for all models. The confidence bands are shaded in grey. The black vertical line indicates the year of lionfish invasion and the dashed, red vertical line indicates five years after lionfish invaded, which is the beginning of the observed declines in most metrics.



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6 Discussion

6.1 Overview

This thesis provides novel insights into lionfish behaviour, non-consumptive effects and regional-level consumptive effects. Within this thesis I provided the first evidence for the driver of lionfish aggregations (chapter two), investigated non-consumptive effects of lionfish on shelter use and activity levels of native invertebrates (chapter three), provided additional evidence for non-consumptive effects of lionfish on herbivorous fish grazing behaviour (chapter four) and finally, using data from across the western Atlantic, provided the first suggestion of region-wide impacts of invasive lionfish (chapter five). Chapter five expands the scope of this thesis beyond invasive lionfish by providing a novel approach for large-scale analysis of invasive species impacts.

6.2 Key findings and implications

6.2.1 Lionfish are attracted to broad-scale complexity

Although large aggregations of lionfish are often observed around natural and artificial structures on both shallow (de Leon et al., 2013, Garcia-Rivas et al., 2017, Jud and Layman, 2012) and mesophotic (Andradi-Brown, 2019, Harter et al., 2017, Sherman et al., 2013) reefs, prior to this thesis, little was known about the drivers of lionfish aggregations. In chapter two I investigated the drivers of lionfish aggregations and found that aggregations were not driven by social attraction, but instead occurred coincidentally when multiple lionfish were attracted to the same areas of reef based on structural complexity. Also in chapter two, I took a novel approach to studying lionfish habitat preference by calculating complexity at a range of spatial scales using 3D models of the reef created with structure-from-motion photogrammetry.

At small spatial scales of 1-5 cm, lionfish were associated with low habitat complexity. This may be because predatory fish hunt less effectively in habitat that is complex at fine scales (Gotceitas and Colgan, 1989). We found the opposite result at broader spatial scales of 30-60 cm and 60-120 cm,

with lionfish attracted to high complexity at these scales. High broad-scale complexity is created by caves, crevices and overhangs, which lionfish are often reported to associate with (Biggs and Olden, 2011, Garcia-Rivas et al., 2017, Whitfield et al., 2014). We suggest that lionfish association with broad-scale complexity is related to hunting success. The low light environments created by caves and overhangs may enhance prey capture success, as lionfish eyes are highly sensitive to dim light (Hasenei et al., 2020) and prey capture rates are higher on overcast than clear days (Cote and Maljkovic, 2010). The vertical surfaces of caves and crevices may also benefit lionfish hunting, as lionfish have been observed herding prey towards vertical and concave surfaces before striking (Cote and Maljkovic, 2010). Substratum height is inversely related to the availability of prey refugia (Bejarano et al., 2015), therefore broad-scale complexity may enhance lionfish hunting success because of a lack of refugia for prey. Lionfish that are actively hunting are mostly found around complex structures (Garcia-Rivas et al., 2018) and a laboratory experiment found that lionfish spent more time hunting around cinder blocks and PVC pipes, which are complex at broad scales, than around algae, which is complex at fine scales (DeRoy et al., 2020), further supporting our theory of broad-scale complexity benefitting lionfish hunting.

My finding that the scale of habitat complexity influences lionfish habitat preference highlights the importance of considering spatial scale when recording complexity. In the laboratory experiment by DeRoy et al. (2020), cinder blocks and PVC pipes were considered low complexity, whilst algae was considered high complexity, indicating that the authors were considering fine-scale complexity. However, when lionfish are reported to use complex habitat, this usually refers to broad-scale complexity such as coral heads (Tilley et al., 2016), caves (Garcia-Rivas et al., 2017) or artificial structures (Dahl and Patterson, 2014). The scale at which complexity is measured is also relevant to the study of other reef organisms, as many species choose shelters that scale with their body size (Bodmer, 2019, Eggleston and Lipcius, 1992, Hixon and Beets, 1993) and thus may only be attracted to complexity at a particular scale. The commonly used chain-and-tape method for measuring rugosity measures fine-scale complexity, with chain link sizes of 1-1.5 cm commonly used (Bayley et al., 2019, Luckhurst and Luckhurst, 1978, Trebilco et al., 2015). Studies that use the chain-and-tape

method therefore lack an understanding of relationships with broad-scale complexity. It is thus important that future studies clearly state the scale of complexity that has been measured.

Knowledge of lionfish habitat preference can be used to improve lionfish removal efficiency by targeting culling to areas of higher broad-scale complexity, informing the design of artificial aggregation devices or improving lionfish traps. Existing lionfish traps already involve some complexity (Gittings et al., 2017, Harris et al., 2020b), but could benefit from incorporating broad-scale crevices and minimising fine-scale refugia. Artificial aggregation devices and traps could extend the depth limits of culling by targeting the large populations of lionfish on mesophotic reefs (Andradi-Brown et al., 2017, Gress et al., 2017), which are believed to include the most reproductively active individuals (Andradi-Brown et al., 2017).

In chapter two I showed that lionfish are attracted to broad-scale complexity, but I have not tested why lionfish display this habitat preference. I hypothesise that broad-scale complexity may benefit lionfish hunting by providing ideal conditions for successful hunting (low light and vertical or concave surfaces) and few refugia for prey. Future studies should aim to test why lionfish display this habitat preference as this may help improve the design of artificial aggregation devices and traps.

6.2.2 Lionfish do not out-compete native invertebrates for shelter

In chapter two I found that lionfish are attracted to broad-scale complexity, which led me to question whether lionfish may compete with native species for shelter. Shelter competition has previously been studied between lionfish and native fish (Raymond et al., 2015, Trehern et al., 2020) and has been suggested between lionfish and spiny lobsters (*Panulirus argus*; Akins et al., 2012, Curtis-Quick et al., 2014). Although other invertebrates also use shelters on the reef (Berger and Butler, 2001, Davis, 2019, Lozano-Alvarez and Briones-Fourzan, 2001, Moran and Reaka, 1988), prior to this thesis no research on shelter competition had been conducted between lionfish and any native invertebrate except for spiny lobster.

In chapter three I studied the potential for shelter competition between invasive lionfish, native spiny lobsters (*Panulirus argus*) and native long-spined sea urchins (*Diadema antillarum;* hereafter referred to as *Diadema*). I found that all three species preferred high complexity habitats, indicating the potential for shelter competition. However, in a laboratory experiment I found that neither spiny lobsters nor *Diadema* reduced their shelter use in the presence of a lionfish, which contrasts with other studies of shelter competition between native and invasive species (Griffiths et al., 2004, Hobbs et al., 2017, Van Kessel et al., 2011). A surprising finding was that lionfish reduced their shelter use in the presence of lobsters and *Diadema*. If the use of shelters benefits lionfish hunting success, as I suggested in chapter two, then a reduction in shelter use may reduce lionfish consumptive effects and could lead to reductions in lionfish body condition and fecundity in the long term. The poor recovery of *Diadema* across much of the Caribbean following the mass mortality event in 1983-1984 (Bodmer et al., 2015) may have benefitted lionfish by freeing up shelters, which may have enhanced the negative effect of lionfish on native fish communities. In chapter three I also found differences in activity levels of lionfish and spiny lobsters. Lionfish became more active in the presence of *Diadema*, whilst spiny lobsters became more active in the presence of lionfish. Both lionfish and another species of lobster (*Homarus americanus*) show increased activity during intraspecific competition (Benkwitt, 2016, Spanier et al., 1998) thus I suggest that the observed changes in activity are related to competition between the species.

Although lionfish have been suggested to cause reductions in commercial spiny lobster catches by out-competing spiny lobsters for shelter (Henderson and Cotê, 2011, Lazarre et al., 2013), my results do not support this idea. My results show it was lionfish, not spiny lobsters, that reduced their shelter use when the two species were together, which suggests that spiny lobsters may exclude lionfish from traps. If the strength of behavioural change in lionfish is related to spiny lobster abundance (which was not tested in my study) then this would explain why lionfish are found in traps with small, but not large, numbers of lobsters (Lazarre et al., 2013).

Prior to this study, I hypothesised that lionfish may hinder restoration efforts of *Diadema* by out-competing them for shelter. Lack of predation refugia is thought to be a barrier to recovery of *Diadema* following the 1983-1984 mass mortality (Bodmer et al., 2015), so the provision of artificial shelters has been used to promote recovery (Bodmer, 2019). Lionfish are attracted to and aggregate around complex structures, including both natural structures (as shown in chapter two) and artificial structures (Dahl and Patterson, 2014) and may thus impact the use of natural and artificial shelters by *Diadema*. However, the results of my study are positive for *Diadema* restoration, as *Diadema* did not change their shelter use or activity in the presence of lionfish.

This chapter provides the first assessment of the non-consumptive effects of invasive lionfish on native invertebrates. However, there are still many unanswered questions. My study only investigated heterospecific pairs, but future studies should consider varying numbers of lionfish and native species competing for shelter, as all three species can be found in groups on the reef (chapter two; Davis, 1971, Herrnkind.W, 1969). Studying varying numbers of lionfish and spiny lobster may help to explain the effects of lionfish on commercial spiny lobster catches. The effects of prior residency should also be investigated by placing one species inside the shelter before adding the competitor, as prior residency influences shelter competition in other aquatic species (Braddock, 1949, Peeke et al., 1998). Different sizes of the study species should be investigated as size can influence the outcome of shelter competition between native and invasive species (Zhang et al., 2019). The lobsters we used were below the legal harvesting size, so future studies should be conducted using legal sized lobsters to gain a better understanding of how lionfish may impact commercial spiny lobster fisheries. Different shelter sizes should also be investigated, as I found that lionfish used larger shelters than lobsters or *Diadema in situ* and many species select shelters that scale with their body size (Bodmer, 2019, Eggleston and Lipcius, 1992, Hixon and Beets, 1993), thus the outcome of competition may differ depending on whether the shelter is a preferred size.

6.2.3 Lionfish impacts on herbivorous fish grazing vary within a reef system

In addition to impacts on individual species (chapter three), lionfish may also impact whole communities through trophic cascades, where consumptive or non-consumptive effects on native species exert knock-on effects on lower trophic levels (Ripple et al., 2016). Herbivorous fish are important for grazing algae and maintaining Caribbean coral reefs in a coral-dominated state (Hughes, 1994), but they can be impacted by both consumptive (Eddy et al., 2016, Mizrahi et al., 2017, Munoz et al., 2011) and non-consumptive (Eaton et al., 2016, Kindinger and Albins, 2017) effects of invasive lionfish. Despite the potential importance of non-consumptive effects on herbivorous fish, to date only two studies have focused on the impacts of lionfish on herbivorous fish grazing (Eaton et al., 2016, Kindinger and Albins, 2017). Only one previous study examined lionfish impacts *in situ*, so my study provides a much-needed comparison.

In chapter four I investigated whether the presence of lionfish impacted grazing behaviour of herbivorous fish or fish community structure. I found that herbivorous fish responses to lionfish were variable even within a small reef system, however, I identified many variables that were not included in the analysis but that may have driven the observed differences between replicates and sites. At some sites I found a decrease in grazing in the presence of lionfish, which supports previous studies (Eaton et al., 2016, Kindinger and Albins, 2017). Herbivorous fish reduce their grazing rates in the presence of native piscivores as a response to increased predation risk (Catano et al., 2016), which suggests that the reductions in grazing in the presence of lionfish occur because native fish display anti-predator behaviour towards lionfish. At some sites I found no effect of lionfish presence on herbivorous fish grazing. The lack of a negative response by herbivores to lionfish presence may relate to prey naivety, where prey display ineffective anti-predator behaviour because they have not evolved with the invasive predator (Sih et al., 2010). Naivety of prey fish towards lionfish has been detected in several native fish species including damselfish and parrotfish (Anton et al., 2016, Kindinger, 2015). However, the lack of response may also relate to herbivorous fish size or site fidelity. Fish that are too large to be consumed by lionfish may not display anti-predator behaviour towards them. Site fidelity may also influence responses as site-attached species may remain wary and

thus not increase their grazing rate even after removal of lionfish, whereas roving herbivores may not respond to prior presence of lionfish and thus display higher feeding rates in the absence of lionfish.

The reduction in grazing at some sites may suggest that native fish recognise lionfish, whilst the lack of effect at other sites may suggest that native fish are naïve towards lionfish. However, I do not expect both scenarios to occur within my study reef system. My study sites were separated by a minimum of 120 m and a maximum of 1.25 km, which is well within the distances travelled by lionfish over several days (Dahl and Patterson, 2020). Additionally, piscivore abundance did not differ between sites, thus the variation in response to lionfish was not caused by differences in generalised predator avoidance behaviour (Berchtold and Cote, 2020). Instead, I suggest that differences between sites occurred due to differences in prey behaviour linked to perceived predation risk between sites. Factors that can influence prey behaviour include habitat complexity (Steele, 1999), predator density (Madin et al., 2010), predator movement (Wisenden and Harter, 2001), distance between predator and prey (Catano et al., 2016) and predator size (Helfman, 1989, Rizzari et al., 2014). The effects of lionfish density and distance between lionfish and prey have already been shown to impact herbivorous fish grazing, with reduced grazing on reefs with high lionfish density and when lionfish are nearby (Kindinger and Albins, 2017). Although I did not include these variables in my study, I suggest they should be included in future studies to improve understanding of the drivers of herbivorous fish grazing behaviour and responses towards lionfish.

My results reiterate the importance of studying the impacts of invasive species across many sites, as responses of herbivorous fish varied between sites even within the same small reef system in my study. Many studies of lionfish impacts are conducted on shallow patch reefs in the Bahamas (Albins, 2015, Albins and Hixon, 2008, Green et al., 2012), but if lionfish impacts vary between reef types and regions as they did between sites in my study then the results of studies from a single site or region may not be applicable across the entire invaded range. In addition to variation amongst sites, I hypothesise that lionfish impacts may also vary over time. Two of the variables I suggested as potential drivers of differences between sites ─ lionfish movement and distance between lionfish and

prey ─ may vary on a minute-by-minute basis, therefore the presence and strength of lionfish impacts may be highly variable.

This chapter provides a basis on which future studies of lionfish non-consumptive effects should be conducted. I have highlighted many variables that can influence herbivorous fish grazing and that should be included in future studies, including habitat complexity, lionfish density, lionfish size and lionfish activity. Many of these variables may influence other non-consumptive effects of lionfish such as changes in shelter use of native species, as studied in chapter three. Additionally, I suggest that future studies should use a BACI design to reduce the influence of confounding variables such as disturbance by divers and the presence of remote cameras. Only a more rigorous experiment with many contextual variables and a BACI design can properly assess the impact of invasive lionfish on herbivorous fish grazing.

6.2.4 Lionfish may reduce fish abundance, richness and diversity at a regional scale

Chapters three and four focused on lionfish impacts in a single study area (Tela Bay, Honduras) and over a short experimental duration, as is common with studies of invasive lionfish impacts (Albins, 2013, Albins and Hixon, 2008, Ingeman, 2016). However, to fully understand the impacts of invasive species it is important to study the entire invaded region. In chapter five I studied the impact of lionfish on native fish abundance, species richness, diversity and community composition across the western Atlantic. In comparison to other studies of invasive species, which often conduct simple comparisons of pre-invasion and post-invasion in a single study area (Strayer et al., 2006), my study spans a maximum of ten years pre-invasion and eight years post-invasion, includes 14 potential confounding variables and includes sites with invasion dates spread over six years. I centred the data on year of lionfish arrival in each location, thus significantly reducing the potential influence of point-impact events as drivers of any observed changes.

I found a shift in fish communities between pre-invasion and two or more years post-invasion. My results support those of Hackerott et al. (2017) regarding fish abundance, indicating that the changes

caused by lionfish result in stable post-invasion fish communities. However, my results contrast those of Hackerott et al. (2017) regarding fish presence/absence, as they found no difference between pre- and post-invasion. This difference may be because I studied all fish, not just prey fish, and may thus have captured trends in non-prey species due to non-consumptive effects of lionfish.

When I investigated trends in native fish abundance, species richness and diversity, I found pre-invasion increases in all metrics that continued in the initial stages of the invasion, however, simultaneous declines in all metrics began approximately five years post-invasion. Although the observed declines were not significant, the synchronicity across metrics suggests a single driving force that acts approximately five years after lionfish invade an area. We suggest that the observed declines at five years post-invasion may be driven by lionfish reaching a critical density at which they begin to negatively impact native species at a detectable regional scale. The observed decreases in fish abundance and species richness support the results of small-scale patch reef studies (Albins, 2013, Albins, 2015, Ellis and Faletti, 2016, Peiffer et al., 2017, Tuttle, 2017) but contrast with the results of two reef-level studies that found no negative effects of lionfish (Elise et al., 2015, Hackerott et al., 2017). These reef-level studies were conducted over short time scales, up to a maximum of four years post-invasion (Hackerott et al., 2017), but my results suggest that longer time scales may be needed to detect negative effects over large spatial scales.

My study is the first to investigate and provide possible evidence for the impacts of invasive lionfish across the western Atlantic. Although small-scale studies often show drastic effects of lionfish in a short period of time (Albins, 2015, Albins and Hixon, 2008, Ingeman, 2016), my results suggest that region-wide impacts may be less drastic and only become apparent several years after lionfish invasion. In comparison to experimental studies, which often attribute any changes in prey communities to consumption by lionfish (Albins, 2013, Albins, 2015, Green et al., 2012), long-term studies such as mine can account for non-consumptive effects, such as reductions in fecundity, that can also affect non-prey species but that may not influence fish populations immediately. The presence of lionfish has been shown to reduce fecundity of bicolour damselfish (*Stegastes partitus*;

Davis, 2018) and may also reduce fecundity of herbivorous fish if a reduction in grazing (as studied in chapter four) leads to a loss of body condition and subsequent reduction in fecundity.

A surprising result from this study was that scuba diving intensity, used as a proxy for lionfish culling pressure, was of low to zero importance in all models. Lionfish culling has been shown to reduce lionfish densities (de Leon et al., 2013, Frazer et al., 2012, Green et al., 2017) and allow native fish to recover (Green et al., 2014), thus I expected to find a large effect of lionfish culling. I suggest that scuba diving intensity was of low importance due to the coarse resolution at which it was calculated, which did not account for differing lionfish culling intensity or effectiveness between regions or over time. Future studies should construct a more detailed lionfish culling variable by considering factors such as general awareness of the lionfish invasion, lionfish density, number and type of participants at lionfish derby events, no-take fishing regulations and country-specific lionfish management plans.

6.3 Wider implications

6.3.1 Native species can negatively impact invasive species

Research on invasive species, including lionfish, often focuses on their impact on native species, however, native species may also detrimentally impact the invasive species in a process termed 'biotic resistance' (Simberloff and Von Holle, 1999). Biotic resistance can constrain the abundance (Levine et al., 2004) and distribution (deRivera et al., 2005) of invasive species but is unlikely to prevent successful establishment (Levine et al., 2004). For lionfish, the only aspect of biotic resistance that had been suggested prior to this thesis was predation by native species, including groupers (Maljkovic et al., 2008, Valdivia et al., 2014) and eels (Munoz, 2017, Ogden et al., 1973). More recently, an ulcerative skin disease caused by an unknown pathogen has been suggested as the driver of lionfish population declines in the Gulf of Mexico (Harris et al., 2020a). In chapter three I provide evidence for potential biotic resistance in the form of exclusion from shelter, as lionfish reduced their shelter use in the presence of native spiny lobsters (*Panulirus argus*) and native long-spined sea urchins (*Diadema antillarum*). If lionfish shelter use enhances hunting success, as I suggested in chapter two,

then a reduction in shelter use may reduce lionfish body condition and lead to reductions in fecundity. However, the lack of recovery of *Diadema* following their mass mortality event (Bodmer et al., 2015), combined with intensive spiny lobster fisheries in the western Atlantic (Cochrane and Chakalall, 2001), may mean that lionfish shelter use is not constrained in some areas, potentially leading to greater consumptive effects of lionfish through enhanced hunting success. Although predation is the most commonly documented aspect of biotic resistance in marine and freshwater environments (Carlsson et al., 2011, deRivera et al., 2005, Ruesink, 2007), shelter use has also been studied (Zhang et al., 2019). My results highlight the importance of investigating biotic resistance, particularly regarding shelter use, as I was able to demonstrate a previously unknown negative impact of native species on invasive lionfish.

6.3.2 Invasive species do not always exert negative effects

Despite most research on invasive species focusing on their negative impacts (Albins and Hixon, 2013, Gherardi et al., 2011, Shine, 2010), invasive species may exert positive impacts on some species or in certain areas of the invaded range (Rodriguez, 2006). In chapter three I showed that lionfish had no effect on the shelter use of spiny lobsters (*Panulirus argus)* or long-spined sea urchins (*Diadema antillarum*) and in chapter four I found that at certain sites, lionfish had no negative effect on herbivorous fish grazing. Also in chapter four, I showed that the impacts of invasive lionfish varied between positive and negative, even within a single small reef system. Although it is acknowledged that the impacts of invasive species may vary between habitats (Thiele et al., 2010), my finding that lionfish impacts may vary even within a small reef system highlights the importance of other biotic and abiotic variables when studying the impacts of invasive species. For example, the outcome of competition between native and invasive crustaceans is dependent on carapace or claw size (Zhang et al., 2019), the anti-predator response of native fish towards invasive lionfish is dependent on lionfish density (Kindinger and Albins, 2017), and predation rates of an invasive fish are influenced by habitat complexity (Alexander et al., 2015). Within chapter four, I suggested several variables that may influence the effect of invasive lionfish on herbivorous fish grazing, including habitat complexity, herbivorous fish size, lionfish activity and lionfish movement. The potentially large collection of

variables that can influence the relationships between native and invasive species mean that the effects of an invasive species from one site or even one time point are not necessarily applicable elsewhere. Research on invasive species should thus focus on repeating research in multiple areas of the invaded range and controlling for a range of contextual variables that may influence native species' responses to the invasive species.

6.3.3 A novel approach for studying the large-scale impacts of invasive species

In chapter five I used lionfish as a model species to investigate the effects of an invasive species over large spatial and temporal scales. I took a novel approach, using temporally offset additive mixed models. I included sites that were invaded in different years, across six years in my dataset, and centred the data on year of lionfish arrival in each location, thus reducing the potential influence of large-scale point impact events, such as climatic or anthropogenic disturbance events. To further disentangle the effects of lionfish from other confounding factors, I included 14 biophysical, social and management variables that are known to influence native fish communities.

My study highlights the importance of studying trends in native species prior to the invasion, as I was able to identify that the positive trends observed in the initial stages of the invasion began prior to the invasion. Some studies of invasive species consider only a single pre-invasion time point (Britton et al., 2013, Hackerott et al., 2017) or even ignore the pre-invasion state altogether (Kreps et al., 2012), both of which may lead to existing trends being incorrectly attributed to the invasive species. Many studies do consider multiple pre-invasion time points, however, they generally consider only a single study site (Doody et al., 2014, Lesser and Slattery, 2011, Zhu et al., 2006). To gain a full understanding of invasive species impacts, studies must be conducted in multiple areas because impacts of invasive species can vary between habitats (Thiele et al., 2010), sites (Doody et al., 2014) and even within a site, as I demonstrated in chapter four.

My results also highlight the importance of studying several post-invasion time points over a long temporal scale. I found increases in all but one variable in the initial stages of the invasion, which

were a continuation of pre-invasion trends, however at five years post-invasion most response variables reached a turning point and began to decrease, albeit non-significantly. Long timescales post-invasion are thus essential in studies of invasive species impacts, as a short timescale in my study would have suggested no effect or even positive effects of the invasive species. Differences in observed effects of invasive species between short-term and long-term studies have also been observed in freshwater (Kelly et al., 2013) and terrestrial (Marchante et al., 2008, Mgobozi et al., 2008) systems.

Although invasive species are often considered as the driver of observed changes, many observational studies do not control for potential confounding factors (Britton et al., 2013, Kreps et al., 2012, Zhu et al., 2006). MacDougall and Turkington (2005) proposed two models of invasive species- the driver model, where invasive species cause changes in native species, and the passenger model, where other variables such as environmental change cause changes in native species. In the passenger model, changes in the native species may be incorrectly attributed to the invasive species. Bauer (2012) introduced the back-seat driver model, where habitat change causes an initial change in native species, but invasive species benefit from this change and cause further changes in native species.

Observational studies often use correlations as evidence of invasive species impacts (Parker et al., 1999), however, these should not be used to infer causation because of potential confounding variables. To provide stronger evidence for invasive species as the cause of observed changes, studies use a Before-After-Control-Impact (BACI) design (Ballew et al., 2016, Matheson et al., 2016, Strubbe and Matthysen, 2009) or include confounding variables in their statistical analysis (Houlahan and Findlay, 2004, Rowe et al., 2019). In a BACI design, data is collected before and after the impact (species invasion) at both a control and an impacted (invaded) site (Green, 1979). A significant interaction between treatment (before or after the impact) and site (control or impacted) indicates a significant effect of the invasive species because the difference between pre-invasion and post-invasion differs between invaded and control sites (Ingeman et al., 2017, Smith, 2014). BACI designs may not be possible if there are no pre-invasion data or control sites, so instead studies can attempt to

control for other variables that may influence the response being measured (Gonzalez-Oreja et al., 2018, Jolly et al., 2015).

My study has demonstrated that it is possible to combine all the features of invasive species studies: long temporal dataset from pre-and post-invasion, wide spatial scale, staggered invasion dates and confounding variables into a comprehensive region-wide study. By combining these features, the impacts of invasive species on native communities can be investigated over an entire region, whilst controlling for potential confounding variables.

6.4 Conclusion

This thesis has provided new insights into invasive lionfish habitat use, behavioural interactions and ecological impacts but is also relevant to invasive species more generally. Lionfish were attracted to broad-scale but not fine-scale complexity, highlighting the importance of reporting what scale of complexity has been measured. Two native invertebrate species did not reduce their shelter use in the presence of lionfish, however, lionfish reduced their shelter use in the presence of the native species, indicating that native species may exert detrimental effects on invasive species. Lionfish impacts varied even within a small reef system, emphasising the potential influence of other variables on invasive species impacts, but also demonstrating that invasive species do not always exert negative effects. By applying a novel analytical approach to understanding the impacts of invasive lionfish on native fish communities across the western Atlantic, I demonstrated a shift in native fish communities within two years of lionfish invasion and provided the first suggestion that lionfish may cause region-wide declines in native fish abundance. My approach, using data centred on year of invasive species arrival, combined with a wide spatial and temporal spread of data and a comprehensive set of contextual variables, can be applied to elucidate the large-scale impacts of other invasive species.

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7 Appendices

7.1 Journal offprint: Aggregating behaviour in invasive Caribbean lionfish is driven by habitat complexity

SCIENTIFIC REPORTS

OPEN

Aggregating behaviour in invasive Caribbean lionfish is driven by habitat complexity

Received: 24 April 2018
Accepted: 30 November 2018
Published online: 28 January 2019

Christina L. Hunt[1,2], George R. Kelly[2,3], Hannah Windmill[2], Jocelyn Curtis-Quick[4], Helen Conlon[2], Max D. V. Bodmer[2,5], Alex D. Rogers[1] & Dan A. Exton[2]

Caribbean lionfish (*Pterois* spp.) are considered the most heavily impacting invasive marine vertebrate ever recorded. However, current management is largely inadequate, relying on opportunistic culling by recreational SCUBA divers. Culling efficiency could be greatly improved by exploiting natural aggregations, but to date this behaviour has only been recorded anecdotally, and the drivers are unknown. We found aggregations to be common *in situ*, but detected no conspecific attraction through visual or olfactory cues in laboratory experiments. Aggregating individuals were on average larger, but showed no further differences in morphology or life history. However, using visual assessments and 3D modelling we show lionfish prefer broad-scale, but avoid fine-scale, habitat complexity. We therefore suggest that lionfish aggregations are coincidental based on individuals' mutual attraction to similar reef structure to maximise hunting efficiency. Using this knowledge, artificial aggregation devices might be developed to concentrate lionfish densities and thus improve culling efficiency.

Since their introduction in the early 1980s[1], lionfish (*Pterois volitans* and *P. miles*) have spread throughout the western Atlantic with devastating impacts on the region's coral reefs[2]. Successful eradications of marine invasive species are rare and generally involve species with a small invaded range[3,4]. Complete eradication of lionfish is considered unlikely[5], as they are now well established with a large geographical distribution[6] and have the potential for long distance dispersal[7]. Rather than eradication, management should therefore be focused on population reduction through culling[8].

Lionfish culling is typically performed by recreational SCUBA divers and snorkelers using hand nets and pole spears[8]. This can be opportunistic or as part of organised events involving competing teams of divers and fishermen[9]. On shallow reefs, culling has been successful at reducing lionfish densities[8,10] and allowing native species to recover[11]. However, whilst culling is largely restricted to depths <30 m[12], lionfish are known to have invaded deeper mesophotic and sub-mesophotic habitats to at least 304 m[12,13]. Technical diving provides a partial solution, although cost, expertise and safety concerns will limit the extent of its use[14], and lionfish traps to collect lionfish from deeper water are still being tested[15].

Groups of lionfish have been observed throughout the invaded range[10,16,17] and it has been noted that aggregating individuals often appear larger than solitary individuals[16,17]. By exploiting this natural behaviour and artificially stimulating aggregations, culling or trapping efficiency could be improved. Mass trapping of invasive and pest species has been successful in both aquatic and terrestrial ecosystems[18–20] but requires knowledge of the species' aggregation drivers to inform the design of effective lures to trap them. Although previous work has focused on many aspects of the lionfish invasion[21], nothing is known about the drivers of lionfish aggregations, and quantitative data on their prevalence is lacking.

One possible driver of lionfish aggregations is social attraction, which may be for cooperative hunting (as observed in *P. miles* in its native range[22]), spawning[23], minimising the chance of predation[24] or pooling experiences to solve problems[25]. Social attraction in fish can be initiated by visual[26], olfactory[27], acoustic[26] or a

[1]Department of Zoology, University of Oxford, John Krebs Field Station, Wytham, Oxford, OX2 8QJ, UK. [2]Operation Wallacea, Wallace House, Old Bolingbroke, Spilsby, Lincolnshire, PE23 4EX, UK. [3]Department of Ocean and Earth Science, University of Southampton, National Oceanography Centre, Southampton, SO14 3ZH, UK. [4]School of Earth and Ocean Sciences, University of Cardiff, Main Building, Park Place, Cardiff, CF10 3AT, UK. [5]School of Environment, Earth and Ecosystem Sciences, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK. Rogers A.D and Exton D. A. contributed equally. Correspondence and requests for materials should be addressed to C.L.H. (email: christina.hunt@zoo.ox.ac.uk)

Stimulus	Sample size	Initial preference		Overall preference	
		Proportion of trials where stimulus zone was entered first	*p*-value	Test statistic	*p*-value
Visual	15	0.6	0.6072	NA	0.6072
Olfactory	13	0.62	0.5811	−3.6764	0.00317*
Visual + olfactory	18	0.28	0.09625	−0.35812	0.7247
Artificial model	12	0.67	0.3877	1.2259	0.2458

Table 1. Results of laboratory social attraction experiments. Initial preference *p*-values were calculated using binomial tests with a null hypothesis of 0.5. Overall preference *p*-values were calculated using one-sample *t*-tests (olfactory, visual + olfactory and artificial model cues) and sign tests (visual cue) with a null hypothesis of 0. * indicates $p < 0.05$.

combination of cues[28]. Zebrafish will even aggregate with robotic fish that mimic their shape and movement[29]. Another possible aggregation driver is habitat preference. Fish may seek out specific habitat because it provides a source of prey, good hunting conditions or shelter from predators[30]. Marine habitat complexity can be measured via a range of metrics (e.g[31,32]) and is known to be a strong predictor of fish abundance[33,34]. Although lionfish have been anecdotally reported to be found in more complex habitat[10,16,17], this has not been determined quantitatively.

Here, we provide the first quantitative assessment of lionfish aggregations and their drivers, specifically exploring: (i) morphology and life history traits of aggregating and solitary individuals via *in situ* population surveys and *ex situ* dissections, (ii) social attraction using laboratory binary choice tests focusing on visual and olfactory cues, and (iii) habitat preference and the role of habitat complexity using 3D modelling and visual scores.

Results

Aggregating lionfish are larger and heavier, but otherwise similar to solitary individuals. A total of 283 lionfish were caught and dissected for this study, comprising 178 solitary and 105 aggregating individuals. We recorded 33 aggregations of varying sizes from 2 to 7 individuals, with a mean (±1 SE) of 3 (±0.34) and a median of 2. Lionfish were collected from depths of 3.0–18.4 m, with similar mean depths (±1 SE) for solitary lionfish (10.7 ± 0.28 m) and aggregations (10.6 ± 0.52 m).

Lionfish found in aggregations were significantly larger than solitary individuals ($p < 0.001$) and were also heavier for their size ($p < 0.001$). Mean total lengths (±1 SE) were 22.2 cm (±0.55) for aggregating lionfish and 18.4 cm (±0.54) for solitary lionfish. However, there was no difference in sex ratio ($p > 0.05$), the gonadosomatic indices of males and females ($p > 0.05$ for both) or percentage body fat ($p > 0.05$) between solitary and aggregating individuals.

Lionfish are not attracted to conspecific visual or olfactory cues. The proportion of trials where lionfish entered the stimulus zone first did not differ significantly from chance levels for any of the stimuli tested ($p > 0.05$ for all stimuli; Table 1). Lionfish in the visual, visual + olfactory and artificial model treatments showed no significant preference for either stimulus or control ($p > 0.05$ for each; Table 1). Lionfish in the olfactory treatment showed a significant preference for the control, or avoidance of the stimulus, with a mean (±1 SE) time difference of −229 (±62) seconds.

Lionfish prefer broad-scale, rather than fine-scale, habitat complexity. Habitat assessment scores found that aggregating lionfish inhabited areas with significantly larger refuge sizes ($p < 0.001$), higher percentage live cover ($p < 0.05$) and a greater variety of growth forms ($p < 0.05$) than the areas inhabited by solitary lionfish (Fig. 1). There were no significant differences for rugosity or substratum height ($p > 0.05$ for both).

We also used 3D modelling to test for lionfish preferences towards habitat complexity. As there were no significant differences in the three complexity metrics (linear rugosity, vector dispersion and fractal dimension) between reef areas harbouring solitary lionfish and aggregations, these data sets were pooled to represent complexity with lionfish (lionfish quadrats) and compared with randomly selected areas of reef without lionfish (background quadrats).

Although there was no significant difference in linear rugosity between lionfish quadrats and background quadrats ($p > 0.05$; Table 2), lionfish were found in areas with significantly lower vector dispersion values (mean ± 1 SE; 0.144 ± 0.009 compared with 0.239 ± 0.007), indicating an association with low complexity at fine spatial scales ($p < 0.001$; Table 2). This is supported by assessment of fractal dimension across five spatial scale ranges (Table 2; Fig. 2). Lionfish quadrats were significantly less complex than background quadrats at the finest spatial scale (1–5 cm; $p < 0.001$). Conversely, lionfish quadrats were significantly more complex than background quadrats at the broadest spatial scales (30–60 cm and 60–120 cm; $p < 0.05$ for both). At intermediate spatial scales (5–15 cm and 15–30 cm) there was no significant difference. These results demonstrate an association with low complexity at fine spatial scales but high complexity at broad spatial scales.

Discussion

Prior to this study, lionfish aggregation behaviour had only been observed anecdotally and little was known about its drivers. Through a combination of approaches and both *in situ* and *ex situ* data collection we have made a substantial contribution to this aspect of invasive lionfish research.

We found that aggregating individuals were larger than solitary individuals. Although larger female fish are often more fecund[35], we found no difference in reproductive maturity between solitary and aggregating lionfish.

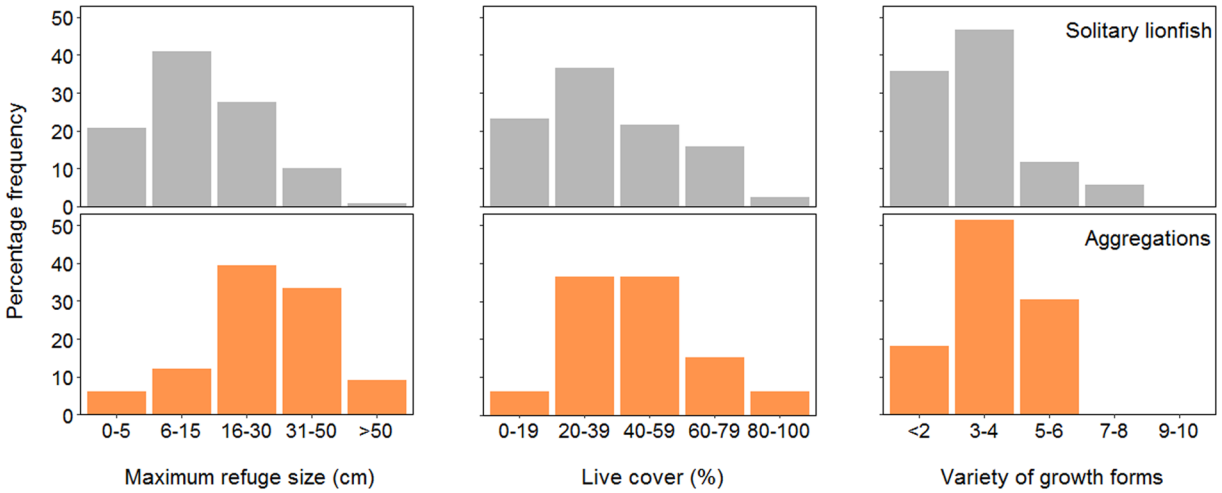


Figure 1. Habitat assessment scores for solitary lionfish and aggregations. The percentage frequency of lionfish in each habitat assessment score category is plotted for solitary lionfish (grey bars; $n = 120$) and aggregations (orange bars; $n = 33$).

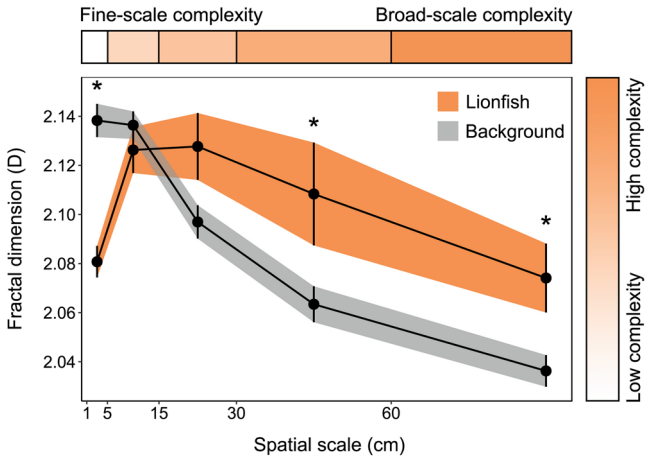


Figure 2. Fractal dimension at varying spatial scales. Fractal dimension values were calculated at five spatial scales: 1–5 cm, 5–15 cm, 15–30 cm, 30–60 cm and 60–120 cm. Means (±1 SE) are plotted at the midpoint of each spatial scale range for both lionfish quadrats (orange; $n = 30$) and background quadrats (grey; $n = 44$). * indicates $p < 0.05$.

Complexity measure		Test statistic	*p*-value
Rugosity		−1.8179	0.07534
Vector dispersion		8.7092	<0.001*
Fractal dimension	1–5 cm	6.3722	<0.001*
	5–15 cm	0.9375	0.3528
	15–30 cm	−1.7142	0.09248
	30–60 cm	−2.2406	0.02899*
	60–120 cm	−2.5757	0.01255*

Table 2. Tests of complexity between lionfish and background quadrats. Complexity was compared between lionfish quadrats (n = 30) and background quadrats (n = 44). Rugosity was tested using a Welch's *t*-test, vector dispersion was tested using a two-sample *t*-test and all fractal dimension spatial scales were tested using ranked Welch's *t*-tests. * indicates $p < 0.05$.

This suggests that the aggregations observed during our study period were not for the purpose of spawning, unlike in other reef fish species[36,37]. Alternatively, aggregating lionfish may be larger because of higher growth rates, which may occur through cooperative hunting (which has been demonstrated to improve hunting success[38]) or because lionfish choose areas that maximise their individual hunting success. Cooperative hunting has been observed in another lionfish species, *Dendrochirus zebra*, in the laboratory[38] and in *P. miles* in its native range[22]. However, in Mexico lionfish were more likely to be seen hunting when solitary than when in aggregations[39]. Although we found no difference in percentage body fat between aggregating and solitary individuals, we did find a difference in the value of Fulton's condition factor, with aggregating individuals being heavier for their size. This difference may result from the benefits of cooperative hunting, or because aggregations form in areas where hunting success is higher.

Many fish that are found in groups exhibit social attraction[40,41] but lionfish showed no preference for any of the cues tested here, both in initial reaction or overall preference. This is not unique; a lack of response to visual cues has been observed in other aggregating fish[27]. The lack of response might have been caused by differing personalities between individuals, resulting in differing motivation to aggregate. Several fish species show personality (e.g[42,43]) and personalities can influence social attraction[43]. Although we did not control for personality differences, the lionfish we caught may have been the boldest individuals[44], thus minimising personality differences between individuals.

Lionfish did show a significant response in the olfactory treatment, however, their preference was for the control. This may have been due to the presence of waste products (e.g. ammonia[45]) in the olfactory cue, which are known to stimulate avoidance behaviour in other fish species[46]. Based on this result we suggest that future studies should use alternative methods of introducing olfactory cues, such as a lionfish behind an opaque, but water permeable, barrier.

Sound has a well-established role in reef fish behaviour[47,48] and lionfish are known to produce sound[49], therefore the use of acoustic cues for social attraction is possible. Acoustic cues were not standardised in our experiment therefore we cannot deduce whether lionfish use acoustic cues for social attraction. However, our findings do suggest that aggregating behaviour in invasive lionfish is not driven by visual or olfactory cues between conspecifics.

Previous studies provide mixed results on the association of lionfish with habitat complexity. Lionfish have been anecdotally reported from more complex habitat[10,16,17] and shown to be associated with areas of greater hard coral cover and overhanging structures[50]. However, other studies have reported no relationship between habitat complexity and lionfish density or biomass[10,51,52]. By using more rigorous methods and quantifying multiple metrics of reef structure, we found a clear link between lionfish, aggregations and habitat complexity.

Firstly, our data show aggregations were associated with larger refuge sizes, higher live cover and a greater variety of growth forms. Preference of lionfish for these habitats may increase food availability; larger refuges can be associated with greater fish abundance[53], increased coral cover may provide preferred habitat for larval settlement of prey species[54] and a greater variety of growth forms can be associated with increased species richness[31].

Lionfish habitat preference varied across different spatial scales. At finer scales (1–5 cm for fractal dimension and 1 cm for vector dispersion) lionfish were associated with lower complexity. Predatory fish may hunt less effectively in habitats that are complex at fine scales[55], therefore lionfish may avoid high complexity at this scale to maximise hunting success. If this is indeed the case, lionfish hunting success may be increased because of reef flattening which is occurring throughout the Caribbean[56]. At broader scales (30–60 cm and 60–120 cm for fractal dimension) lionfish were associated with higher complexity that is often created by large crevices and overhangs. The erosion of broad-scale complexity as part of reef flattening may be less important to lionfish as artificial reefs such as shipwrecks and concrete structures can provide this type of complexity. This may explain why lionfish were found at higher density on artificial, rather than natural, reefs in the Gulf of Mexico[57]. Our study highlights the importance of considering spatial scale in assessments of species associations with habitat complexity. By creating "complexity signatures" using fractal dimension (Fig. 2) a much more detailed picture can be gained of how a species associates with both the quality and quantity of complexity[32].

Caves and overhangs provide low light environments that may increase hunting success, given that lionfish prey capture rates have been shown to be higher on overcast than clear days[58]. The physical structure of caves and overhangs may also benefit lionfish hunting; lionfish have been observed herding prey towards vertical and concave surfaces before striking[58].The inverse relationship between substratum height and availability of prey refugia[59] suggests that these habitats often lack refuges for prey species. However, these habitats may provide shelter for certain prey and mutualist species. For example, overhangs provide shelter for *Gramma loreto*[60], a fish that has been found in lionfish stomachs[61,62] but that is also a potential lionfish cleaner[16].

Based on these findings, we propose that aggregations of invasive lionfish are coincidental and form when multiple lionfish are attracted to the same area of reef, rather than through visual or olfactory attraction to conspecifics. This attraction to broad-scale over fine-scale complexity is likely to be driven by a search for maximised hunting efficiency, characterised by reduced refugia for prey items, leading multiple lionfish to congregate on areas of reef where feeding is easiest.

This knowledge could be used to significantly improve the efficiency of culling efforts. Our results can be used to inform the design of artificial aggregation devices or to improve existing lionfish traps that already involve some complexity[15], but which could benefit from incorporating broad-scale crevices and minimising fine-scale refugia. Artificial aggregation devices and traps could in turn extend the depth limit of culling efforts to exploit the significant populations found on mesophotic reefs[12,13], which are believed to include the most reproductively active individuals[12]. Only through improved understanding of the behaviour and ecology of this widespread invader will managers have the upper hand. By expanding and improving culling efforts using the findings of this study, a long-term solution will be one step closer.

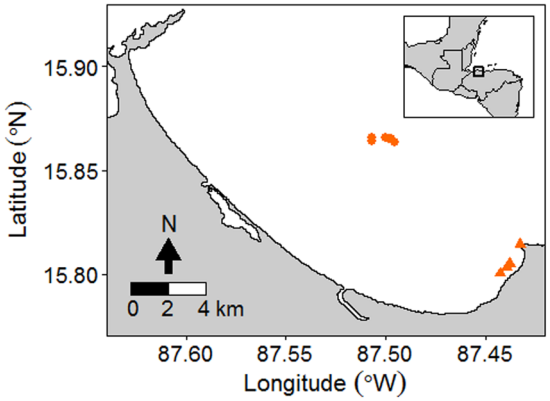


Figure 3. Map of our study sites in Honduras. Population structure data and habitat assessment scores were recorded from all 10 sites. Triangles mark the four sites on the La Ensenada reef system where lionfish were collected live for laboratory social attraction experiments. Circles mark the six sites on the Banco Capiro reef system where lionfish were culled and 3D modelling was carried out. The location of Tela bay, Honduras within central America is shown on the inset map.

Methods

Study sites. All data were collected from reefs in the mainland Tela Bay, Honduras (Fig. 3; Supplementary Table 1) between June and August 2017 using open-circuit SCUBA. Lionfish were culled for dissection at six sites on the offshore reef system of Banco Capiro at depths between 10–18 m (see Bodmer *et al.*[63] for a general site description). Lionfish were collected alive for use in laboratory social attraction experiments from four sites on the shallow nearshore reef system of La Ensenada at depths <10 m. A recent genetic study suggests invasive lionfish in Honduras are likely to all be *P. volitans*[64], and so we assumed this to be the case. However, we did not identify them to species level and so will refer to them simply as lionfish hereafter.

***In situ* population structure surveys and *ex situ* dissections.** Roving diver surveys were conducted, and any lionfish encountered deeper than 10 m were culled using pole spears before being transferred into a ZooKeeper container (ZooKeeper LLC, USA) for transport back to the laboratory. Approximate size, supra-ocular tentacle type and distinguishing features (e.g. unusual patterning) were noted *in situ* to enable later identification of individuals. Depth of capture and, if aggregating, the number of individuals within the aggregation were also recorded. An aggregation was defined here as two or more individual lionfish being found within approximately four body lengths of one another[24].

On return to the laboratory, culled lionfish were refrigerated and dissected within 12 hours. Total length was recorded to the nearest mm from the tip of the snout to the longest point of the tail. Percentage body fat, which was used as a measure of feeding success, was calculated as fat weight (to the nearest 0.1 g) divided by body weight (to the nearest g), then multiplied by 100. Fat weight was obtained by removing all fat deposits from within the gut cavity and weighing these using an electronic balance. Fulton's condition factor, which was also used as a measure of feeding success, was calculated as body weight divided by total length cubed[65,66], with higher values indicating that fish were heavier for their size. Macroscopic analysis of the gonads was used to determine sex[67]. Lionfish reproduce throughout the year[68], which enabled us to measure reproductive maturity during our study. Reproductive maturity of males and females was measured using the gonadosomatic index[68], which was calculated as gonad weight (to the nearest 0.1 g) divided by body weight and then multiplied by 100. Dissection methods followed Green *et al.*[67].

Laboratory visual and olfactory attraction experiments. *Lionfish capture and experimental setup.* A total of 58 lionfish were captured alive using hand nets and transferred to large plastic dry-bags, before being slowly brought to the surface. Lionfish were transported inside the dry-bags to the laboratory by 15-minute boat ride. On arrival at the laboratory lionfish were immediately transferred to the holding tank. Live collections were restricted to <10 m to avoid barotrauma injuries. Lionfish ranged in total length from 4.7–29.5 cm with a mean (±1 SE) of 17.0 (±0.88) cm. Each lionfish was used once to explore one of (i) visual cues ($n = 15$), (ii) olfactory cues ($n = 13$), (iii) visual + olfactory cues ($n = 18$) or (iv) artificial model cues ($n = 12$). Post-trial, some lionfish were used as stimulus fish for up to two trials. Stimulus fish were never re-used as test fish.

The holding and trial tanks measured 245 × 56 × 24 cm (length x width x depth) and were filled with unfiltered natural seawater to a depth of 16 cm. Water in the tanks was maintained at ambient temperature (29 °C). Water changes were conducted at 100% every 24 hours in the holding tank, and 100% between each trial in the trial tank using freshly collected seawater (which was assumed to be constant in aeration and quality). No lionfish were present in the tanks during water changes. Tanks were naturally lit by large windows and thus followed the ambient light:dark cycle. All experiments were conducted during daylight hours between 6:00 am and 6:00 pm. During the trials two artificial lights (10 watt LED lamps) were used above the trial tank to account for shading by the building structure and ensure standardisation of lighting between all trials.

Lionfish were kept in the holding tank for an acclimatisation period of 24 hours during which they were starved to ensure similar hunger levels between individuals[69]. A maximum of six lionfish were maintained in the

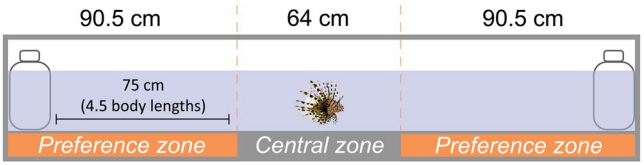


Figure 4. Tank set-up for laboratory social attraction experiments. The tank was divided into two preference zones (stimulus and control) and a central zone. Dotted lines mark boundaries between zones, which were marked by placing tape across the top of the tank. A GoPro Hero5 camera (GoPro Inc., USA) was mounted on the ceiling 1 m above the centre of the tank. Diagram is not drawn to scale. Lionfish photo ©iStock.com/GlobalP.

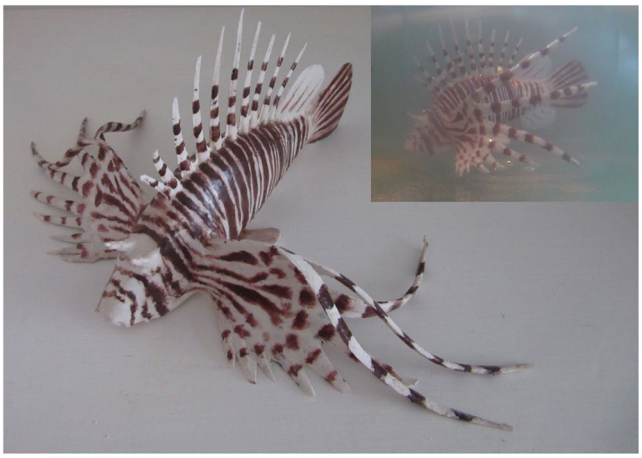


Figure 5. Artificial model lionfish used in the laboratory social attraction experiments. The model was moulded from aquarium grade silicone using a custom-made mould created photogrammetrically using video footage of a live lionfish. The model measures 15 cm total length. Underwater the pectoral fins spread out to give a more realistic appearance (inset photograph).

holding tank at any time. The holding tank was fitted with a Tetra whisper power filter (Spectrum Brands Inc., USA) and Aqua Culture air pump (Walmart Stores Inc., USA) to ensure the water remained clean and aerated.

We used one trial tank that was divided into two preference zones of 90.5 cm (one at either end of the tank) and a central zone of 64 cm, by placing tape across the top of the tank (Fig. 4). The preference zones encompassed a 15.5 cm diameter stimulus container plus approximately 4.5 body lengths distance from the stimulus container, based on the average total length of 17.0 cm for lionfish in this study. This distance encompassed the four body length distance used to define fish aggregations[24] to ensure that, if present, aggregation behaviour was detected. The tank was surrounded by opaque white plastic to prevent interference from external stimuli[70].

Experimental design. Stimuli were added to one end of the trial tank based on the following: (i) live lionfish and water from the trial tank added to a submerged transparent plastic container (visual cue), (ii) water from the holding tank added to a submerged transparent plastic container with 30 evenly spaced 4 mm diameter holes (olfactory cue), (iii) live lionfish and water from the trial tank added to a submerged transparent plastic container with 30 evenly spaced 4 mm diameter holes (visual + olfactory cue), and (iv) 3D printed lionfish model (Fig. 5) and water from the trial tank added to a submerged transparent plastic container (artificial model cue). A control was placed at the opposite end of the tank, comprising water from the trial tank added to a submerged transparent plastic container without holes (visual and artificial model controls) or with 30 evenly spaced 4 mm diameter holes (olfactory and visual + olfactory controls). The end of the tank containing the stimulus/control was randomised between trials and the order of trials was randomised between days.

A randomly chosen lionfish was placed in a 30 cm diameter mesh acclimatisation cylinder in the centre of the tank. After a ten minute acclimatisation period[29] (which allowed the lionfish to acclimatise to the water, stimuli and artificial lighting), the cylinder was lifted and the lionfish was filmed for a trial period of ten minutes[29] using a GoPro Hero5 camera (GoPro Inc., USA) mounted 1 m above the centre of the tank. Subsequently, the videos were analysed and the position of the lionfish (stimulus, control or central zone) recorded every second for the duration of the trial, as well as which preference zone (stimulus or control) was entered first. Position was determined as the zone that the tip of the lionfish's snout was in. Overall preference was calculated as time spent in the stimulus zone minus time spent in the control zone.

Once lionfish had been used in a trial they were humanely culled by cervical transection, followed by pithing to destroy the brain tissue[71]. These lionfish were subsequently dissected as described earlier, except that fat weight was not recorded.

Habitat preference and habitat complexity. Whenever lionfish were encountered on the reef, a 1 × 1 m quadrat was placed over the reef centred on the position of the individual lionfish or the centre of the aggregation. Habitat assessment scores were then performed following Gratwicke and Speight[31], whereby five categories of structure were estimated visually on a five-point scale. Maximum substratum height, refuge size and variety of growth forms were recorded, along with average live cover (percentage cover of sessile organisms such as live corals, macroalgae and sponges) and rugosity scores for each quadrat. Over the course of the study 153 quadrats were assessed. Only one data point for complexity was taken per aggregation.

In addition, 30 large quadrats (2 × 2 m) were placed on reef areas where lionfish were encountered (23 solitary and 7 aggregations). These quadrats were filmed in a lawnmower pattern, approximately 0.5 m above the benthos, using a GoPro Hero3 camera (GoPro Inc., USA). Areas of reef without lionfish present were also sampled to give a background average for the reef system. Transects were laid at randomly chosen directions from each mooring line and quadrats were placed at regular intervals along each transect to give a total of 44 background quadrats. The resulting footage was converted into stills and used to render 3D models in Agisoft Photoscan (Agisoft LLC, Russia), before being analysed for habitat complexity using Rhinoceros 3D (Robert McNeel and Associates, USA), following the method of Young *et al.*[32]. Habitat complexity was measured using three metrics: linear rugosity (2 cm resolution), vector dispersion (1 cm resolution) and fractal dimension. Fractal dimension was measured at five spatial resolutions (1–5 cm, 5–15 cm, 15–30 cm, 30–60 cm and 60–120 cm) to explore patterns between lionfish habitat preference and habitat complexity at varying spatial scales.

Statistical analyses. All tests were two-tailed with an a-priori significance level of 0.05 and were conducted in R version 3.4.2 (R Core Team, Austria). Prior to analysis, datasets were checked for normality using a Shapiro-Wilk test, and groups were checked for homogeneity of variance using Fisher's *F* test. When the assumption of normality was violated, transformation was attempted. When data transformation did not normalise the data, a non-parametric test was conducted on the original data.

For the social attraction experiments, initial preference was tested using binomial tests and overall preference was tested using one-sample *t*-tests or sign tests. For tests between two groups (lionfish quadrats and background quadrats or solitary lionfish and aggregations), two-sample *t*-tests were used for continuous, normally distributed data, whilst two-proportions *Z*-tests were used for proportions. For continuous data that violated the normality assumptions, and for ordinal data, Mann-Whitney *U*-tests were conducted. Data that violated both normality and homogeneity of variance assumptions were analysed using a ranked Welch's *t*-test[72,73].

Ethics statement. Lionfish culling was carried out in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals[71]. All experimental protocols were approved by the University of Oxford Animal Welfare and Ethical Review Body. No permits were required to collect lionfish, however, a research permit to allow this work to be conducted in Honduras was obtained from the Instituto de Conservacion Forestal Honduras (permit number: DE-MP-081-2017).

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Acknowledgements

We thank all the Operation Wallacea volunteers and staff who assisted with data collection. We also thank Steve Dey and colleagues at ThinkSee3D Ltd. (UK) for producing the 3D printed lionfish model. This work was financially supported by the Natural Environment Research Council (Grant number: NE/L002612/1) and Operation Wallacea.

Author Contributions

C.L.H., D.A.E. and A.D.R. designed the population structure and habitat complexity aspect of the study, the data for which were collected by C.L.H., G.R.K. and H.W. C.L.H., J.C.Q., H.C., D.A.E. and A.D.R. designed the social attraction experiments, which were performed by C.L.H. M.D.V.B. provided fieldwork support. C.L.H. analysed the data and wrote the manuscript. C.L.H. and D.A.E. produced the figures. All authors reviewed drafts of the manuscript.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-018-37459-w.

Competing Interests: The authors declare no competing interests.

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7.2 Journal offprint: Shelter use interactions of invasive lionfish with commercially and ecologically important native invertebrates on Caribbean coral reefs

PLOS ONE

RESEARCH ARTICLE

Shelter use interactions of invasive lionfish with commercially and ecologically important native invertebrates on Caribbean coral reefs

Christina L. Hunt[1,2]*, **Dominic A. Andradi-Brown**[3], **Callum J. Hudson**[1,2], **Joshua Bennett-Williams**[2], **Frankie Noades**[2,4], **Jocelyn Curtis-Quick**[5], **Owen T. Lewis**[1], **Dan A. Exton**[2]

1 Department of Zoology, University of Oxford, Oxford, United Kingdom, **2** Operation Wallacea, Spilsby, Lincolnshire, United Kingdom, **3** Ocean Conservation, World Wildlife Fund, Washington, D.C, United States of America, **4** School of Environment and Life Sciences, University of Salford, Salford, United Kingdom, **5** Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, Illinois, United States of America

* christina.hunt@zoo.ox.ac.uk

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Citation: Hunt CL, Andradi-Brown DA, Hudson CJ, Bennett-Williams J, Noades F, Curtis-Quick J, et al. (2020) Shelter use interactions of invasive lionfish with commercially and ecologically important native invertebrates on Caribbean coral reefs. PLoS ONE 15(8): e0236200. https://doi.org/10.1371/journal.pone.0236200

Editor: John A. B. Claydon, Institute of Marine Research, NORWAY

Received: April 27, 2020

Accepted: June 30, 2020

Published: August 26, 2020

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: CLH was funded by a NERC PhD studentship (NE/L002612/1, www.nerc.ukri.org) and a Small Research Grant from the Fisheries Society of the British Isles (www.fsbi.org.uk). This work was also financially supported by Operation Wallacea (www.opwall.com). Apart from Operation Wallacea, who supported data collection in the

Abstract

Indo-Pacific lionfish have become invasive throughout the western Atlantic. Their predatory effects have been the focus of much research and are suggested to cause declines in native fish abundance and diversity across the invaded range. However, little is known about their non-consumptive effects, or their effects on invertebrates. Lionfish use shelters on the reef, thus there is potential for competition with other shelter-dwelling organisms. We demonstrate similar habitat associations between invasive lionfish, native spiny lobsters (*Panulirus argus*) and native long-spined sea urchins (*Diadema antillarum*), indicating the potential for competition. We then used a laboratory experiment to compare activity and shelter use of each species when alone and when lionfish were paired with each native species. Spiny lobsters increased their activity but did not change their shelter use in the presence of a lionfish, whilst long-spined sea urchins changed neither their activity nor shelter use. However, lionfish reduced their shelter use in the presence of spiny lobsters and long-spined sea urchins. This study highlights the importance not only of testing for the non-consumptive effects of invasive species, but also exploring whether native species exert non-consumptive effects on the invasive.

Introduction

Invasive predatory species are well known to cause consumptive effects on native species [1–3]. Yet, invasive species may also exert non-consumptive effects, such as competition for food [4] and space [5]. Competition for space occurs in terrestrial [6], freshwater [7] and marine environments [8] and in both plants [9] and sessile and mobile animals [6, 8]. Prior residency may confer an advantage in preventing displacement by a competitor species [9], whereas other factors such as body size may be more important determinants of which species gains access to the shelter [10].

field, the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Coral reef ecosystems are in decline around the globe as a result of stressors including climate change [11, 12], unsustainable fishing practises [13], pollution [14] and invasive species [15]. Invasive lionfish have colonised both the western Atlantic [16] and, more recently, the Mediterranean [17]. Lionfish are habitat generalists found on shallow and mesophotic reefs [18, 19], the deep sea [20], mangroves [21], and seagrass beds [22]. Two species of lionfish have invaded the western Atlantic: *Pterois volitans* and *Pterois miles* [23], hereafter collectively referred to as lionfish. The consumptive effects of lionfish on native fish are well known [24], with numerous studies reporting declines in reef fish abundance [25], species richness [26] and recruitment [1]. However, fish are only one component of lionfish diets. Shrimps are also an important prey item [27], particularly in the diet of smaller lionfish [28]. Despite the importance of shrimps in the diet, the consumptive effects of lionfish on shrimps and other crustaceans have been investigated to a lesser extent [29, 30].

In addition to consumptive effects, lionfish are subject to and can exert non-consumptive effects, for example 'fear effects', whereby prey change their behaviour to reduce the risk of predation in the presence of a predator [31]. In the western Atlantic, some (but not all) native prey species recognise invasive lionfish as predators [32, 33]. For example, some herbivorous fish show reduced grazing [34, 35] and cleaner shrimp stay closer to their anemones [36] in the presence of lionfish. In addition to exerting non-consumptive effects, lionfish can also be subjected to these effects by humans; lionfish hide deeper in the reef and are more wary of humans as a consequence of lionfish culling programs [37, 38]. Lionfish also exert non-consumptive effects through 'transmitter' species, for example lionfish predation on fairy basslet releases blackcap basslet from competition and allows them to occupy prime feeding locations on the reef [39]. Another type of non-consumptive impact is competition. Previous studies have identified competition for prey between invasive lionfish and native grouper [40], potentially affecting native grouper populations. Studies using artificial reefs have demonstrated that limited shelter availability can constrain the abundance of organisms [41], therefore if lionfish occupy shelters on the reef they may exclude native species. This has been suggested by previous studies, indicating that native grouper will avoid a shelter that is occupied by a lionfish [42].

Studies investigating shelter use interactions between lionfish and native invertebrates are lacking. Lionfish show preference for broad-scale complexity such as caves and overhangs [43], which they use as shelter [38], but they avoid fine-scale complexity such as intricate branching corals [43]. Spiny lobsters (*Panulirus argus;* hereafter referred to as lobsters) and long-spined sea urchins (*Diadema antillarum;* hereafter referred to as *Diadema*) are both native invertebrates that use reef shelters. Neither species has been the focus of research on lionfish impacts because spiny lobsters are rarely consumed by lionfish [44] and there are no reports of *Diadema* being consumed by lionfish. Lobster is an economically important species exploited for food across much of its native range [45], whilst *Diadema* is an ecologically important species for its keystone role in macroalgal grazing [46]. Both lobsters and *Diadema* use shelters on the reef to avoid predation [47, 48] and along with lionfish, all three species spend more time using shelter during the day than during the night [49–51]. The availability of reef shelters decreases with increasing shelter size [52], suggesting that shelters at a suitable scale for these three species may be limiting. Research indicating that spiny lobsters and *Diadema* will recruit to artificial shelters that are placed on natural reefs [53, 54] further strengthens our hypothesis that suitable shelters are often limited on coral reefs.

If lionfish show similar shelter preferences as native lobsters and *Diadema*, as seen in grouper-lionfish interactions [55, 56], they may compete for the limited shelters that are available. This could lead to lionfish displacing lobsters and *Diadema* from shelters, thus exposing them to greater predation risk. Similar shelter preferences between lionfish, spiny lobsters and

Diadema have been suggested [57], and shelter use interactions between lionfish and lobsters are believed to occur. Lionfish are highly abundant by-catch species in lobster traps [58] and the presence of lionfish is associated with lower lobster abundance in both traps [59] and condos (non-enclosed shelter traps; [60, 61]). Competition for shelter between invasive lionfish and native species could therefore have significant impacts on lobster and *Diadema* populations, which may affect economic benefits from the lobster fishery and ecological functions provided by *Diadema*.

In this study we investigate whether invasive lionfish compete with native lobsters and *Diadema* for shelter on Caribbean coral reefs. We first tested whether lionfish, lobster and *Diadema* occupy similar habitats on the reef, by conducting *in situ* assessments of habitat complexity. We then tested whether the presence of lionfish influences the behaviour of lobsters and *Diadema*, using a controlled laboratory experiment with limited shelter availability. Our study clearly identifies the overlap in shelter preferences and illustrates the behavioural responses caused by interactions between native invertebrate species and invasive lionfish, highlighting the importance of non-consumptive impacts of invasive species on the ecology of native species.

Methods

Study species and area

Lionfish (assumed to be *Pterois volitans* based on [62]), lobsters (*Panulirus argus*) and *Diadema* (*Diadema antillarum*) were studied in the shallow (3–12 m) near-shore reef system of La Ensenada in Tela Bay, Honduras, at the southern end of the Mesoamerican Reef (S1 Table) from June-August 2019. La Ensenada has a mean coral cover of 8.5% and mean macroalgal cover of 21.4% [54].

In situ habitat associations

Habitat associations of lionfish (n = 35), lobsters (n = 28) and *Diadema* (n = 22) were recorded during roving SCUBA dives at three sites on the La Ensenada reef system (S1 Table). SCUBA dives (n = 43) took place between 8:00 am and 2:00 pm across June, July and August. All three species are generally inactive and inside shelter during the day [49, 51, 63], so we assume that the habitat associations observed represent daytime shelters. Habitat was assessed within a 1 x 1 m quadrat that was placed with the centre directly over the individual of interest. Habitat associations were only recorded from individuals that were removed from the reef, thus preventing pseudoreplication, which could have occurred if the same individual was sampled on multiple occasions. We aimed to select animals with body sizes of approximately 16 cm to match the size of the experimental shelter, since many marine species choose shelters that scale with their body size [47, 54]. However, it was not always possible to find animals of this size. Mean sizes (± standard error) of the specimens used for our habitat association study are 21.5 (± 0.5) cm total length for lionfish, 5.5 (± 0.1) cm carapace length for lobsters and 4.8 (± 0.1) cm test diameter for *Diadema*. Details on the conversion from carapace length or test diameter to overall body size are provided in the experimental design section of the methods. These habitat associations were compared to randomly sampled background areas of reef (n = 36), obtained by laying three 50 m transects in random directions from the mooring line at approximately 5 m depth at each of the three collection sites (S1 Table). A 1 x 1 m quadrat was placed every 12.5 m along the transects with the measurement as the centre point, omitting the quadrat at 0 m to remove bias in the placement of the start of the transect tape.

In all quadrats placed, Habitat Assessment Scores (HAS) for six complexity categories were recorded on a scale from 1–5, with larger values indicating higher complexity, following [64].

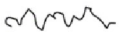
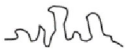
HAS variable	Habitat Assessment Score (HAS)				
	1	2	3	4	5
Rugosity					
Variety of growth forms	<2	3-4	5-6	7-8	9-10
Height (cm)	0-9	10-19	20-39	40-79	≥80
Maximum refuge size (cm)	0-5	6-15	16-30	31-50	>50
Live Cover (%)	0-19	20-39	40-59	60-79	80-100
Hard substratum (%)	0-19	20-39	40-59	60-79	80-100

Fig 1. Habitat Assessment Score (HAS) table. This is a modified version of the HAS table [64] that indicates the criteria for each HAS value. Higher HAS values represent greater complexity.

https://doi.org/10.1371/journal.pone.0236200.g001

Maximum refuge size and substratum height were measured, variety of growth forms was counted (this applies to all sessile organisms and includes categories such as encrusting, branching, filamentous, and massive; see [64] for a full list of growth forms) and average live cover (percentage cover of sessile organisms such as live corals, macroalgae and sponges), average hard substratum (percentage cover that was not sand, silt or rubble) and rugosity (the topography of the reef) were estimated ([64]; Fig 1; S2 Table). When aggregations of lionfish or lobsters were encountered, only one quadrat was recorded at the centre of the aggregation to prevent pseudoreplication. No aggregations of *Diadema* were observed.

Specimen collection

Specimen collections were conducted by recreational SCUBA divers during roving dives in depths of 4.2–12.1 m. The individuals used in our laboratory experiment are a subset of those individuals that were collected during the habitat association aspect of our study. Not all collected individuals were used because the cameras failed during some of the laboratory trials. Lionfish (n = 35) were collected from the reef using hand-nets, whilst lobsters (n = 28) were caught by hand. Following capture, lionfish and lobsters were transferred to separate 57-litre drybags filled with seawater. *Diadema* (n = 22) were collected and transported in a large bucket of seawater. All specimens were slowly brought to the surface and then transported to the laboratory by 15-minute boat ride within the drybag or bucket they were collected in.

Laboratory behaviour experiment

Aquarium setup. Three tanks measuring 100 x 55 x 55 cm (length x width x depth) were used for acclimatisation and two tanks measuring 245 × 56 × 24 cm were used to run the trials. We used the largest tanks available to minimise the impact of confinement on interspecific competition [65]. Trial tanks were the same as used those in a lionfish behaviour experiment [43], which are similar or larger than tanks used in published experiments on spiny lobsters [66] and *Diadema* [67]. Tanks were filled with unfiltered natural seawater to a depth of 17 cm. The air conditioning in the laboratory was controlled to ensure that water temperature in the tanks

remained at 29˚C, matching the ambient sea temperature at the collection sites. Tanks were illuminated from above by sunlight from large windows and thus followed the natural light-dark cycle. Tanks were surrounded by black plastic on four sides to prevent visual disturbance from neighbouring tanks. All acclimatisation and trial tanks contained a shelter that measured 18 x 16 x 15 cm internally (width x height x depth) and was constructed from six concrete bricks (Fig 2). The water level in the tanks extended 0.5 cm above the ceiling of the shelter to ensure that the internal space of the shelter was completely submerged. An internal shelter diameter of approximately 15 cm was chosen to match the previously reported preference of invasive lionfish in Honduras; solitary lionfish most often use 6–15 cm diameter shelters whilst aggregating lionfish most often use 16–30 cm diameter shelters [43]. We aimed to collect animals that matched the internal shelter dimensions to limit shelter sharing and thus create a situation of limited shelter availability.

The shelter was placed at one end of the tank, with the opening facing towards the centre of the tank. Gaps on either side of the shelter were blocked off using extra concrete bricks to prevent animals from sheltering between the edge of the tank and the shelter. Each acclimatisation tank was fitted with a Tetra whisper air pump (Spectrum Brands Inc., Virginia, USA) to maintain oxygenation of the water, however, air pumps were not used in the trial tanks because the much greater surface area would have allowed sufficient oxygen transfer and we did not want the air pumps to interfere with natural diffusion of chemical cues. At the end of every



Fig 2. Lobster inside the shelter in the trial tank.

https://doi.org/10.1371/journal.pone.0236200.g002

acclimatisation period and every trial, water quality (pH, nitrate, nitrite and ammonium) was tested using a saltwater master test kit and all values were found to be within acceptable water quality guidelines (API Fishcare, Pennsylvania, USA).

Specimen acclimatisation. On arrival at the laboratory, each species was placed into a separate acclimatisation tank, with a maximum of two conspecific individuals per tank. Animals were collected between 8:00am and 2:00pm and tested the following morning, resulting in acclimatisation times of 16–24 hours. This was deemed to be enough time because at the beginning of each trial, all individuals were displaying normal day-time behaviour of resting inside shelter [51, 68, 69]. Although shorter acclimatisation times have been used for studies on *Diadema* [67], we chose to standardise the acclimatisation time across species. Animals were not fed during the acclimatisation period to standardise hunger levels between individuals [70, 71].

Experimental design. Single individuals of each species were tested alone (lionfish alone $n = 12$, lobster alone $n = 12$, *Diadema* alone $n = 12$) to determine their activity and shelter use in the absence of other, potentially competing, species. Lobsters and *Diadema* were also tested in the presence of a lionfish (lionfish-lobster together $n = 12$, lionfish-*Diadema* together $n = 7$) to determine whether behaviour differed between these 'together' trials and the 'alone' trials. Each animal was only used once. The individuals used in our laboratory experiment are a subset of those used in the habitat association study, thus we now report the range of sizes used for our laboratory experiment. Lionfish ranged in total length (TL) from 14.6–26.6 cm, with a mean (± standard error) of 21.2 (± 0.6) cm. Lionfish size at sexual maturity varies between the sexes and between locations but on average is 10 cm for males and 17.5 cm for females [72], indicating that the majority of our lionfish were likely to be adults. Lobsters ranged in carapace length (CL) from 4.1–7.1 cm, with a mean of 5.4 (± 0.1) cm, and thus were classed as small juveniles [47]. The minimum carapace size at which lobsters can legally be fished is 7.8 cm [61], thus the lobsters in our experiments were below the legal size. Using a CL to TL conversion for *Panulirus argus* [73], we calculated that the lobsters ranged from 10–18 cm in TL. We only used lobsters with hard exoskeletons, indicative of intermoult condition, because crustaceans can be more vulnerable to predation when they have recently moulted [74], potentially influencing their sheltering behaviour in a way that would confuse interpretation of the results. *Diadema* ranged in test diameter from 4.0–5.6 cm, with a mean of 4.9 (± 0.1) cm, and were classified as adults because they had no banded spines [75]. We only used adults of the more common black phenotype because behaviour is known to vary between phenotypes [67]. Using a test diameter to spine length conversion for *Diadema* spp. [76], we calculated that the *Diadema* individuals used in our experiment ranged from 20–28 cm in total diameter.

To begin the trial, lionfish and/or lobsters were placed at the opposite end of the tank to the shelter, but *Diadema*, always began the trial inside the shelter due to a lack of vision. The artificial shelter would not have provided natural olfactory cues for *Diadema*, thus if they had been placed at the opposite end of the tank, they may not have found the shelter. As we were using two identical trial tanks, we randomised which tank was used for each trial.

Trials were filmed using a Bushnell Trophy Cam HD Aggressor (Bushnell Corporation, Missouri, USA) positioned 1 m from the end of the tank containing the shelter and angled down at 45 degrees. This positioning of the camera allowed us to see into the back of the shelter and 17 cm in front of the shelter. The camera filmed using infrared (850 nm wavelength) during both day and night because preliminary trials found that videos were often underexposed when filmed in daylight without the use of flash. Lionfish, lobsters and *Diadema* show low sensitivity to wavelengths above 600 nm [77–79] so the light emitted by these cameras should have been non-detectable. Trials ran for 24 hours and video recordings were made for 60 seconds every hour.

Following the trials, we telson-clipped the lobsters to ensure that individuals were not re-used in subsequent experiments. *Diadema* were not marked because current tagging methods

are invasive and can impact survival [80]. Instead, *Diadema* were released by the mooring line at the collection site and subsequent individuals were collected a minimum of 20 m away. Average movement each night by *Diadema* is less than 5 m [81] and individuals often return to the same crevice repeatedly [82], thus we assume that 20 m was sufficient to prevent individuals being recaptured. All lobsters and *Diadema* were released back onto the reef within two hours of the trial ending. Immediately after the trial, lionfish were humanely culled using a two-step method for finfish euthanasia, whereby cervical transection was followed by pithing to destroy the brain tissue [83]. All acclimatisation and trial tanks were emptied, cleaned, and refilled with new seawater between trials.

For each 60 second video, the time spent: (i) active vs resting (activity), and (ii) inside vs outside the shelter (shelter use) were recorded. Individuals were recorded as inside the shelter if at least 25% of their body length was inside and were recorded as active if they were moving across the tank. Lionfish often moved their fins and lobsters moved their legs whilst stationary, but we categorised these behaviours as inactive because the individual remained in one location.

For our analyses, we calculated the time spent in each behaviour over the course of the day, but we removed the first hour of each trial to ensure that all animals had acclimatised to the trial tank for a minimum of one hour. Analysed trial data thus spanned 23 hours–comprised of 23 videos of 60 seconds. We calculated activity as the proportion of time spent active out of the time spent in view of the camera, since when animals were out of view we did not know whether they were active or inactive. We calculated shelter use as the proportion of time spent inside shelter out of the total trial time, since there was only one shelter in the tank so even when not in view of the camera we knew that animals were not inside the shelter (S3 Table). In addition to our formal analyses, we recorded the individual that entered the shelter first and the individual that spent the longest length of time inside the shelter (over the 23-hour trial). In the lionfish-*Diadema* together trials, the *Diadema* always started the trial inside the shelter, as previously explained.

Statistical analysis

All tests were two-tailed with an *a priori* significance level of 0.05 and were conducted in R version 3.6.2 [84]. All graphs were plotted using the ggplot2 package [85] with the colour-blind friendly viridis colour palette from the viridis package [86].

To visualise *in situ* habitat associations, we performed non-metric multidimensional scaling (NMDS) with a Bray-Curtis dissimilarity matrix using the vegan package in R [87], and plotted vectors of the HAS categories to aid interpretation of the visual output. We then conducted an analysis of similarities (ANOSIM) test to determine whether complexity differed between quadrats (background, lionfish, lobster and *Diadema*).

To examine *in situ* habitat associations for specific HAS categories, we conducted Kruskal-Wallis tests, which were chosen because the HAS data were ordinal, rather than continuous. We conducted one Kruskal-Wallis test for each of the six HAS categories: rugosity, variety of growth forms, substratum height, maximum refuge size, percentage live cover and percentage hard substratum. Each Kruskal-Wallis test compared HAS between background, lionfish, lobster and *Diadema* quadrats. When the results were significant- indicating a difference in HAS value between quadrat types- we ran post-hoc Dunn tests [88] to compare all treatment combinations: background-lionfish, background-lobster, background-*Diadema*, lionfish-lobster, lionfish-*Diadema* and lobster-*Diadema*. Our post-hoc Dunn tests were run using the Benjamini-Hochberg method to correct for multiple testing (by controlling the type I error rate) whilst retaining greater power than the Bonferroni method [89].

Our data for activity and shelter use were proportions. The denominator of the fractions was large (up to 1380), thus allowing the proportions to take a wide range of values. For this reason our data can be described as non-count-based proportions and thus were analysed using beta regression [90], which can account for the heteroskedasticity and skewness that are common with proportion data [91]. Beta regression does not work with values of exactly 0 or 1, so the response variable must be transformed prior to analysis [90, 91]. We transformed our response variables using equation one (modified from [91]) to scale our response between 0.05 and 0.95. We used the default logit link function for the beta regression analyses because the choice of link function will only affect model fit when one or more of the predictors is continuous [90].

Equation 1. This equation, modified from [91], converts the response variable y, which is a proportion that can range from 0–1, to a new response variable y' that can range from 0.05–0.95.

$$y\prime = \frac{(y \times 9) + 0.5}{10}$$

Model selection followed the two-step process detailed in [92], whereby we first selected the best model from our candidate models assuming fixed precision. We then created a new series of candidate models that incorporated variable precision, which can account for differences in variance between treatments [90] and thus improve model fit. The best model was deemed to be the one with the lowest AICc value [93]. Most of our models differed from the next best model by more than two AICc units and thus our 'best' models have substantial support relative to the next best model [93]. However, two of our models (lobster activity and *Diadema* shelter use) differed from the next best model by less than two AICc units, with the next best model containing one extra parameter. These extra parameters can be considered uninformative [94], thus we selected the simpler models, which had the lowest AICc. We used AICc rather than AIC because of our small sample sizes [93].

Beta regression analyses (hereafter referred to as BR) were conducted using the betareg package in R [91] and AICc values were calculated using the AICcmodavg package in R [95]. Trial type (lionfish alone, lobster alone, *Diadema* alone, lionfish-lobster together, lionfish-*Diadema* together) was always included in the mean models but we also tested whether to include trial tank (left, right) in the mean and precision models. We tested the difference in behaviour (activity and shelter use) between trial types in the 23-hour dataset. Trial tank was never included in the best fitting mean or precision models and thus will not be discussed further.

Ethics

Lionfish culling was carried out in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals [83]. All experimental protocols were approved by the University of Oxford Department of Zoology Animal Welfare and Ethical Review Body. No specific permits were required to collect lionfish, lobsters, or urchins. A research permit for this work was obtained from the Instituto de Conservacion Forestal, Honduras (ICF-508-2019).

Results

Lionfish, lobsters and *Diadema* have similar habitat preferences

There was a significant difference in habitat complexity between the species quadrats and the background quadrats (ANOSIM, R = 0.440, $p = 0.001$; Fig 3), with all three species generally found in more complex areas of the reef when compared with average reef complexity (Dunn

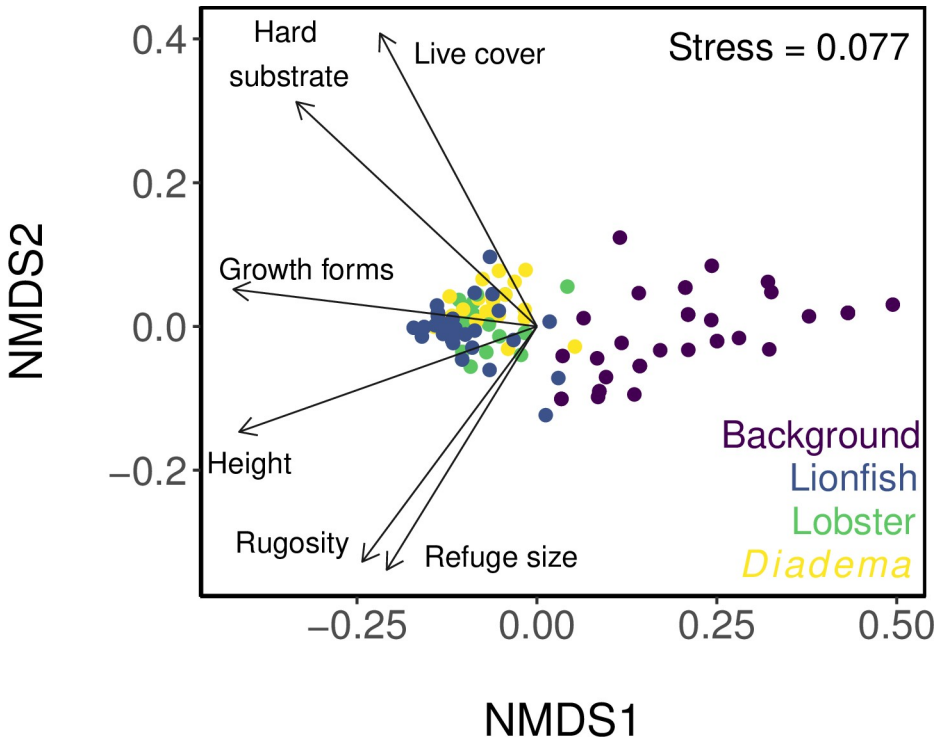


Fig 3. Non-metric multidimensional scaling ordination of reef quadrat complexity. Each point represents a 1 x 1 m quadrat that is either a randomly sampled background quadrat ($n = 36$), or contains a lionfish ($n = 35$), lobster ($n = 28$) or *Diadema* ($n = 22$). The plot was constructed using values of the six HAS categories: rugosity, variety of growth forms, substratum height, maximum refuge size, percentage live cover and percentage hard substratum. Quadrats with more similar complexity are closer together on the plot. Vectors indicate the direction of the gradient for each HAS category.

https://doi.org/10.1371/journal.pone.0236200.g003

tests, $p<0.05$ for all; Table 1; Fig 4). All three species were found in areas with significantly higher values for rugosity, variety of growth forms, percentage live cover and percentage hard substrate (Dunn tests, $p<0.05$ for all; Table 1), but none of the species differed significantly from one another (Dunn tests, $p>0.05$ for all; Table 1). For height, values for all three species were significantly higher than the background, whilst both lionfish and lobsters were found in significantly more complex habitat than *Diadema* (Dunn tests, $p<0.05$ for all; Table 1).

Table 1. Dunn tests results.

		Rugosity		Variety of growth forms		Height		Maximum refuge size		% Live cover		% Hard substrate	
		Z-value	*p*-value	Z-value	*p*-value	Z-value	*p*-value	Z-value	*p*-value	Z-value	*p*-value	Z-value	*p*-value
Comparisons with the background	Background-lionfish	-5.52	<0.001	-6.44	<0.001	-8.17	<0.001	-7.02	<0.001	-6.42	<0.001	-8.34	<0.001
	Background-lobster	-4.28	<0.001	-7.06	<0.001	-6.31	<0.001	-3.94	<0.001	-5.69	<0.001	-7.51	<0.001
	Background-*Diadema*	-3.71	<0.001	-6.17	<0.001	-3.03	0.004	-0.937	0.349	-6.07	<0.001	-7.11	<0.001
Comparisons between species	Lionfish-lobster	0.912	0.434	-0.987	0.486	1.38	0.168	2.66	0.012	0.353	0.724	0.347	1.00
	Lionfish-*Diadema*	1.12	0.393	-0.518	0.725	4.11	<0.001	5.19	<0.001	-0.438	0.794	0.205	1.00
	Lobster-*Diadema*	0.260	0.795	0.383	0.702	2.70	0.008	2.59	0.011	-0.732	0.696	-0.113	0.910

Z-values and Benjamini-Hochberg adjusted *p*-values of post-hoc Dunn tests are displayed for all Habitat Assessment Score (HAS) categories. Highlighted cells represent significant results ($p<0.05$). Z-values are rounded to three significant figures and *p*-values are rounded to three decimal places (except for $p<0.001$).

https://doi.org/10.1371/journal.pone.0236200.t001

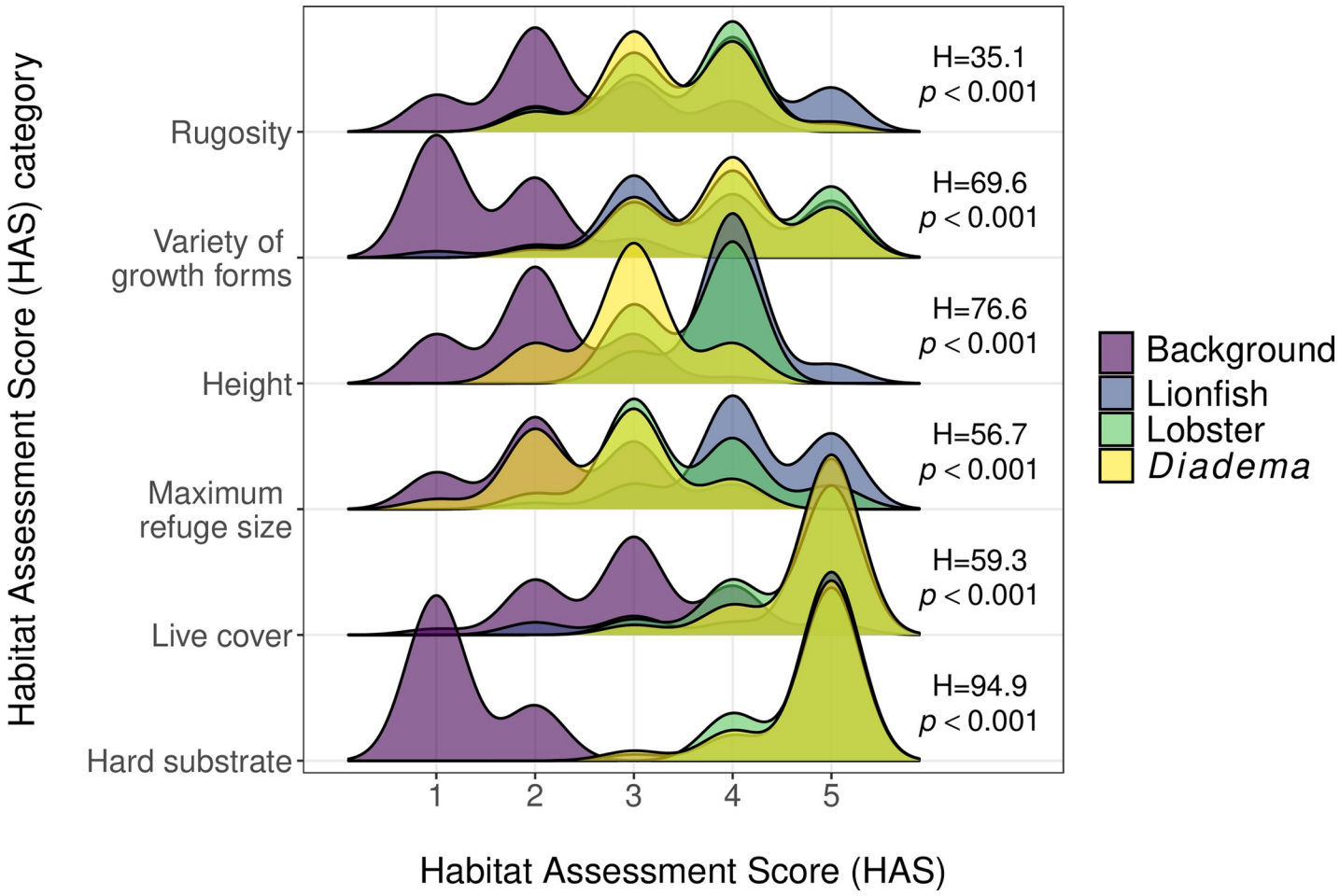


Fig 4. Habitat Assessment Score (HAS) joyplot. HAS are plotted for background (n = 36), lionfish (n = 35), lobster (n = 28) and *Diadema* (n = 22) quadrats. Larger HAS represents greater complexity. HAS are recorded on an ordinal scale from 1–5, thus it is the peaks in this joyplot that highlight the overlap, or lack of overlap, between the four groups. H-values and p-values represent the test statistic and significance of each Kruskal-Wallis test.

https://doi.org/10.1371/journal.pone.0236200.g004

Maximum refuge size showed the greatest difference between species. Lionfish were found in areas with greater maximum refuge sizes than lobsters or *Diadema*, and greater than the background (Dunn tests, $p<0.05$ for all; Table 1). Lobsters were found in significantly more complex habitat than *Diadema* or the background (Dunn tests, $p<0.05$ for both; Table 1). *Diadema* did not differ significantly from the background (Dunn test, Z = -0.937, p = 0.349). The median refuge size HAS were: background (6–15 cm), *Diadema* (16–30 cm), lobsters (16–30 cm) and lionfish (31–50 cm). Despite these differences between species, there was still considerable overlap in HAS for maximum refuge size between species (Fig 4).

Lionfish exert non-consumptive effects, but so do native species

Lobsters were 1.8 times more active (BR, Z = 2.43, p = 0.015; Fig 5), but did not change their shelter use (BR, Z = -0.119, p = 0.906), in the presence of a lionfish during the 23-hour trial. The presence of lionfish had no effect on activity (BR, Z = 1.77, p = 0.077; Fig 5) or shelter use (BR, Z = 0.302, p = 0.763) of *Diadema*. Lionfish and lobsters shared the shelter in one out of twelve trials (8% trials), whilst lionfish and *Diadema* shared the shelter in two out of seven trials (29% of trials). In ten of the twelve lionfish-lobster together trials (83% trials), the species

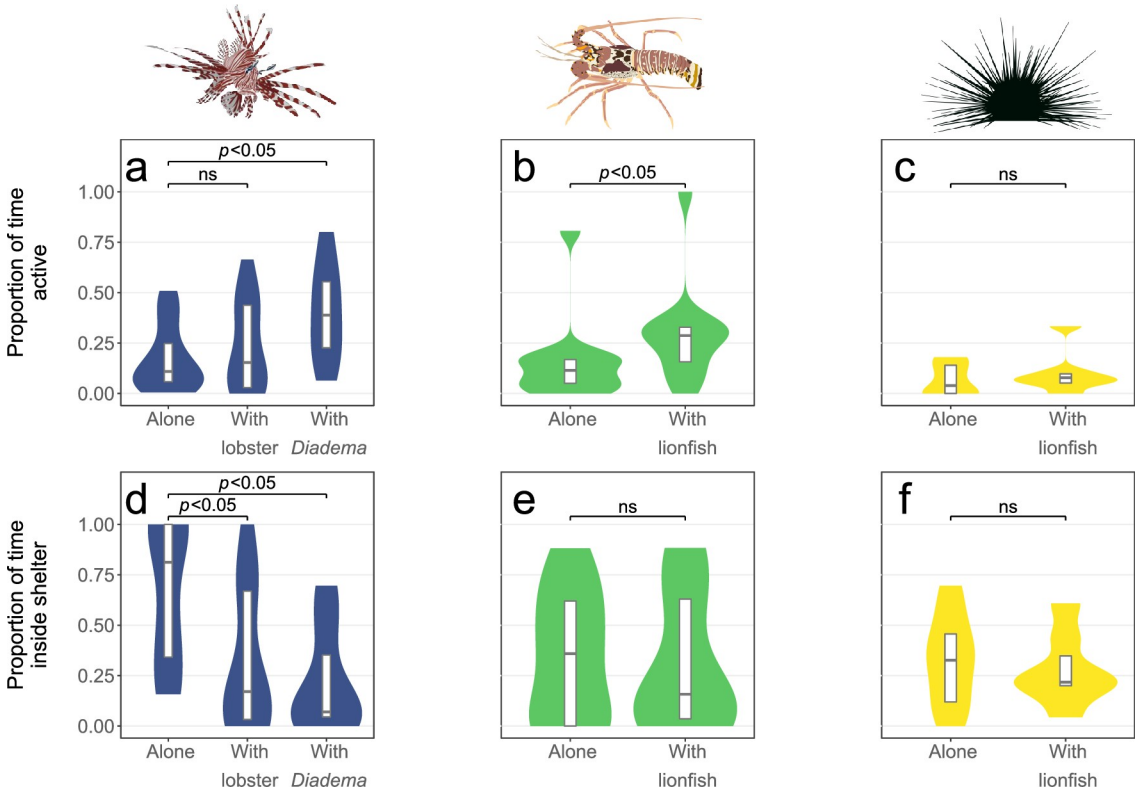


Fig 5. Activity and shelter use of lionfish, lobsters and *Diadema*. The proportion of time that lionfish, lobsters and *Diadema* spent active (a,b,c) and inside shelter (d,e,f) in the 'alone' and 'together' treatments during the 23-hour trial. Each plot represents how the behaviour of the focal species (indicated by the creature image) varies between the 'alone' and 'together' trials. For the lionfish graphs (a and d), the 'alone' bar represents the behaviour of lionfish when alone, the 'with lobster' bar represents the behaviour of lionfish in the presence of a lobster, and the 'with *Diadema*' bar represents the behaviour of lionfish in the presence of a *Diadema*. For the lobster (b and e) and *Diadema* (c and f) graphs, the 'alone' bar represents their behaviour when alone, whilst the 'with lionfish' bar represents their behaviour in the presence of a lionfish. Violin plots represent the spread of the data and are scaled so that the violins on each plot have the same area. Each violin plot is overlaid with a boxplot showing the median and inter-quartile range. All treatments have $n = 12$, except the 'lionfish-*Diadema* together' treatment, for which $n = 7$.

https://doi.org/10.1371/journal.pone.0236200.g005

that entered the shelter first was also the species that spent the most time inside the shelter (Table 2). However, in the lionfish-*Diadema* together trials, lionfish and *Diadema* appeared equally likely to occupy the shelter (dominating the shelter in $n = 3$ and $n = 4$ trials respectively), even though *Diadema* always began the trial inside the shelter.

Lionfish were 1.9 times more active in the presence of *Diadema* than when alone (BR, $Z = 2.39$, $p = 0.017$; Fig 5) during the 23-hour trial. In contrast, the presence of lobsters had no effect on lionfish activity (BR, $Z = 0.487$, $p = 0.626$).Lionfish used shelter 1.9 times more when

Table 2. Shelter occupancy data from lionfish-lobster together trials.

Species that entered the shelter first	Species that spent the most time inside the shelter	
	Lionfish	Lobster
Lionfish	6	1
Lobster	1	4

Trials ($n = 12$) are categorised based on which species entered the shelter first and which species spent the most time inside the shelter.

https://doi.org/10.1371/journal.pone.0236200.t002

alone than when in the presence of lobsters (BR, Z = -2.75, $p = 0.006$)and 2.2 times more when alone than when in the presence of *Diadema* (BR, Z = -2.85, $p = 0.004$).

Discussion

We found invasive lionfish have similar *in situ* habitat preferences as native lobsters and *Diadema* (Fig 4), thus creating the potential for competition when shelter availability is limited. Our experimental trials then indicated that lionfish presence led to increased activity in lobsters but had no effect on *Diadema* (Fig 5). We found the presence of lionfish did not affect shelter use by lobsters or *Diadema*. Lionfish, however, had reduced shelter use when in the presence of lobsters or *Diadema*.

Lionfish, lobsters and *Diadema* preferred high complexity habitats

All three species preferred habitat that was more complex than the background average. For lobsters and *Diadema*, higher complexity habitat provides protection from predation [47, 48]. The preference of lionfish for complex habitat applies across the invaded western Atlantic range (e.g [57, 96, 97]). In areas with frequent culling, lionfish use complex habitat to escape culling by humans [38]. However, lionfish have few natural predators in the invaded range [98], so in areas where they are not culled by divers, habitat preference may instead be related to hunting efficiency [43]. For four of the HAS metrics (rugosity, variety of growth forms, percentage live cover and percentage hard substrate) all three species showed similar habitat preference. This suggests lionfish, lobsters and *Diadema* are likely to compete for reef shelters in the Caribbean. Reef flattening, a reduction in hard coral cover and subsequent decline in architectural complexity, is occurring across the region [99], reducing the availability of shelters and so leading to increased shelter competition.

We found significant differences in maximum refuge size between species. A previous study from Honduras found that lionfish preferred shelter sizes of 6–15 cm and 16–30 cm [43]. However, we found that lionfish preferred shelter sizes of 31–50 cm. This disparity may reflect differences between the availability of shelters on different reefs, with lionfish habitat associations reflecting their preferred habitat based local habitat availability. Spiny lobsters use shelters that scale to their body size when alone [47] but they will use larger shelters when aggregating with conspecifics [100]. Most of the lobsters we found were solitary, which may explain their preference for smaller shelters of the size 16–30 cm. When suitably sized shelters are unavailable, spiny lobsters will use any crevice that is large enough to protect their abdomen [50]. Although we attempted to collect similar sized individuals, the lionfish we collected were larger on average than the lobsters, which may explain the observed difference in preferred shelter size. We used lobsters that were below the minimum legal size, which suggests that the shelter size preference of larger, legal-sized, lobsters may overlap more with that of lionfish. A study on *Diadema* in Utila, Honduras, found that they preferred refuges of the size 5–15 cm [54], which is smaller than the median refuge size of 16–30 cm found in our study. *Diadema* select shelters that scale with their test size to avoid predation [54], therefore this difference in shelter size preference may be related to the high proportion of juvenile urchins present at the sites surveyed by Bodmer (54),whereas we only included adults in our study. By converting lobster carapace size and *Diadema* test diameter to total body size, we found that the *Diadema* were larger in overall size than the lobsters and lionfish. Although *Diadema* were larger, it is likely that they chose smaller shelters than lionfish or lobsters because they choose shelters that scale with their test diameter [54], rather than with their total body size. This indicates that *Diadema* may only be subject to shelter competition from small lionfish.

The relationship between lionfish and native invertebrates

Lobsters became more active in the presence of lionfish. This increase in activity may be related to aggression, as spiny lobsters display several aggressive behaviours [101]. American lobsters (*Homarus americanus*) spend more time active in the presence of other lobsters than when alone and this activity is related to fighting and defending a shelter [102], so the increased activity in our study may be related to shelter defence. Increased activity can reduce the scope for growth in other crustaceans [103], and so could result in reduced growth rates of lobsters. In the absence of shelter, spiny lobsters will remain motionless in the presence of a predator [104], suggesting that increased activity may attract predators and increase predation risk. Lobsters sense their environment using olfactory [66], visual [105] and tactile [106] cues and can detect predator olfactory cues from at least 2 m away [107]. Our experiment cannot distinguish which cues might have been used by the lobsters to detect lionfish, but if they used olfactory cues then the results we observed may be widespread on reefs with invasive lionfish populations, even when lionfish are out of visual range of the lobster. We did not conduct trials with two lobsters, or trials where a lobster was paired with another species, thus we cannot conclusively show that the increased activity of lobsters is driven by the presence of lionfish. Even if the observed response is not specific to lionfish presence, the increase in activity is still cause for concern because as lionfish populations increase, the frequency and duration of lionfish-lobster encounters is likely to increase.

Despite the increase in activity, there was no change in shelter use in the presence of a lionfish, suggesting that the lobsters may be dominant and able to displace lionfish from the shelter, or prevent displacement by lionfish once they are inside the shelter. The lack of change in shelter use suggests that the increased activity observed in the presence of a lionfish is unlikely to be related to submissive behaviour and movement away from the shelter. Lionfish and lobsters shared the shelter in one of our trials, suggesting that even on reefs with limited, and already occupied, shelters, lobsters may still be able to access the shelters. However, we found that prior occupancy influenced shelter use, as in other fish and crustaceans [108, 109]. We found that the first species to enter the shelter was generally the species that spent the most time in the shelter during the trial. This suggests that if shelters are not large enough for lionfish and lobsters to share, then prior occupancy of a lionfish may prevent shelter use by lobsters.

Lionfish had no effect on activity or shelter use of *Diadema*. Several fish families and known predators of *Diadema* [75] and predator cues trigger increased movement [110], so the lack of activity change suggests that lionfish were not considered to be a predator. Alternatively, the lack of a change in activity or shelter use may be because the experimental shelter was considered high quality, as *Diadema* are less likely to flee from a high-quality crevice [82]. In the absence of predators, *Diadema* will avoid shelters containing conspecific odours, which is thought to be a method of avoiding shelter competition [110]. Lionfish and *Diadema* shared the shelter in two of our seven trials, suggesting that lionfish are not considered a shelter competitor and thus the presence of a lionfish inside the shelter does not impact *Diadema* shelter use. However, we found that prior occupancy had no effect on which species used the shelter the most, suggesting that in some cases lionfish may be able to exclude *Diadema* from their shelters.

Impacts of lobsters and *Diadema* on lionfish

Lionfish did not change their activity in the presence of lobsters but they became more active in the presence of *Diadema*. Lionfish increase their activity in response to intraspecific competition [49], thus we suggest that lionfish may become more active in the presence of *Diadema* because they perceive them as a shelter competitor. Lionfish may not have increased their

activity in response to lobsters because the lobsters may have been perceived as a dominant competitor, as is suggested by other studies on shelter use interactions between fish and crustaceans [111, 112]. The differential response of lionfish to lobsters and *Diadema* indicates that lionfish are not simply responding to over-crowding (there are two individuals in the 'together' trials but only a single individual in the 'alone trials). Instead, lionfish behavioural responses are determined by the identity of the competitor, as is observed in other fish species [113].

Increased activity in lionfish may lead to less energy being available for other functions such as growth. In areas where lionfish are culled by divers, increased activity of lionfish may make them more conspicuous and easier to capture, thus aiding culling efforts. But an increase in lionfish activity may not always be beneficial for the native community. Increased activity may increase the likelihood of interactions with native species, potentially leading to other non-consumptive effects, such as reduced grazing by herbivorous fish [34, 35].

In contrast to other invasive species, where shelter use of the invasive is unchanged in the presence of native species [42, 111], we found that lionfish reduced their shelter use in the presence of lobsters or *Diadema*. Our findings lend support to anecdotal evidence that reefs with high urchin densities support few lionfish [57], potentially because urchins occupy all available shelters. In our lionfish-lobster together trials we found an effect of prior residency, where lionfish were less likely to dominate a shelter if a lobster occupied the shelter first. However, the shelter we used in our laboratory experiment (16 cm) was smaller than the preferred shelter size of the lionfish used in this study (31–50 cm), thus the motivation of the lionfish to use the shelter may have been less that it would be had the shelter been larger.Nevertheless, reduction in shelter use by lionfish may mean that if shelter is limiting, it is the natives that gain access to the shelters. Reduced shelter use may be detrimental to lionfish health and survival, meaning that lionfish may be less successful invaders on reefs with limited shelter. Feeding success may be reduced if use of shelters enhances feeding success, as suggested by [43], thus leading to reduced consumptive effects of lionfish on reef communities. Larger and heavier female lionfish produce more eggs [114], so any subsequent loss in body condition may reduce fecundity and thus reduce the population growth rate. Reduced shelter use of lionfish may therefore be beneficial to reef communities.

Management implications

Studies on the commercial spiny lobster fishery have shown that lionfish are often caught as by-catch in traps [58] and the presence of lionfish is associated with reduced lobster abundance in traps [59] and condos (non-enclosed shelter traps; [60, 61]). These results have led to the suggestion that lionfish may deter or out-compete lobsters [59]. Competition between lionfish and lobster may be particularly strong around lobster condos because these are often placed in areas of otherwise low habitat complexity [60]. In our experiments we found that lionfish, rather than lobster, reduced their shelter use when the two species were together, suggesting that lobsters may instead out-compete lionfish. We hypothesise that lobsters exclude lionfish from traps and condos and that the strength of the behavioural change in lionfish may be related to lobster abundance in the trap. This would explain why lionfish are found in traps with small, but not large, numbers of lobsters. The lobsters used in our experiments were below the legal harvesting limit of 7.8 cm carapace length [61] and so our results may not be applicable to larger lobsters. However, if larger lobsters are stronger shelter competitors, as in other lobster species [115], then the effects on lionfish shelter use that we observed may be even stronger when lionfish compete with larger lobsters.

Although our results suggest that lionfish do not affect lobster shelter use, and therefore may not reduce lobster catches, this does not mean that lionfish have no effect on spiny lobster

fisheries. The presence of lionfish in lobster condos increases handling time by fishers as they work more cautiously around lionfish to avoid envenomation and this may lead to reduced earnings as fishers are unable to visit as many condos each day [61]. However, these reduced earnings could be partially compensated by increasing lionfish fisheries and selling the lionfish to restaurants or developing value-added lionfish products–such as jewellery [61, 116].

A lack of structural complexity and the subsequent lack of predation refugia has been suggested as a barrier to recovery of *Diadema* following the 1983–1984 mass mortality [117]. One restoration effort has thus been to provide artificial shelters [54]. Lionfish often use and aggregate around artificial structures [118, 119] and could potentially hinder restoration efforts if they affect *Diadema* shelter use. We initially hypothesised that lionfish would enter the shelter and exclude the *Diadema*, but our results showed that lionfish had no effect on *Diadema* activity or shelter use. This lack of response by *Diadema* suggests that lionfish may not interfere with restoration efforts and that the presence of lionfish on a reef may not a barrier to recovery of *Diadema*.

Lobster traps may prove useful for lionfish management, as studies have shown catches of almost 3000 lionfish in a single lobster fishing season [58]. Trapping will complement current management methods because culling by recreational divers is restricted to 30 m [37], but traps can be deployed much deeper to target the larger and more mature lionfish that are not currently culled [37]. Our results have shown reduced shelter use of lionfish in the presence of lobsters, suggesting that lionfish catches would be maximised in the absence of lobsters. To develop lionfish-specific traps, current lobster traps need to be modified to increase lionfish catch but reduce lobster catch. Some work has been done in this area [120], but lionfish-specific traps are still being tested [121] and are not yet commercially available. Current diver-based culling is insufficient to satisfy demand for international export of lionfish [116], but the development of a lionfish trap fishery could provide a low cost removal method that can be applied to depths inaccessible to divers [18].

Conclusion

Our results have demonstrated the importance of testing for non-consumptive effects of invasive species. We found that lionfish are not detrimental to all native species, as there was no effect of lionfish on *Diadema* activity or shelter use. Invasive species research often focuses on the impacts on the invaded community or on trophic interactions exerted by native species on invasive species, however, we have shown the importance of investigating non-consumptive effects of the native species on the invasive. These effects may prove detrimental to the invader or may lead to additional negative effects on native species. By studying the interactions between lionfish and two different invertebrates, we have demonstrated that non-consumptive effects of native species on invasive species may be widespread. Our research suggests that we should look beyond the consumptive effects of invasive predators and consider how native species may induce changes in invasive species behaviour.

Supporting information

S1 Table. Dive site co-ordinates. Co-ordinates of the mooring lines at each of our three study sites in Tela Bay, Honduras. Lionfish ($n = 35$), lobsters ($n = 28$) and *Diadema* ($n = 22$) were collected from all three sites for use in the laboratory experiments. We recorded Habitat Assessment Scores (HAS) for 12 background quadrats at each site and for each individual that was collected. A total of 43 SCUBA dives took place between 8:00 am and 2:00 pm across June, July and August 2019. Coordinates are given in WGS84.
(CSV)

S2 Table. Habitat Assessment Score (HAS) data. Six HAS values were recorded for each quadrat containing lionfish (n = 35), lobsters (n = 28) and *Diadema* (n = 22) and for randomly sampled background quadrats (n = 36). HAS values range from 1 (low complexity) to 5 (high complexity). Date, site and animal size are also provided.
(CSV)

S3 Table. Raw data and summary values of activity and shelter use across each 23-hour trial. 23 observations of 60 seconds were recorded during each 23-hour trial, thus each trial comprises 1380 seconds of observation. The time that each animal spent active in view of the camera, inactive in view of the camera and inside shelter is shown in seconds (columns G-O). Time spent active was divided by total time in view of the camera (time active + time inactive) to give proportion of time active (columns Q, S and U). Time spent active or inactive was only recorded when the individual was in view of the camera, thus time active + time inactive is not necessarily equal to total trial time (1380 seconds).Time spent inside shelter was divided by total trial time (1380 seconds) to give proportion of time inside shelter (columns W,Y and AA). These proportions were than transformed using equation one to remove any values of 0 or 1 (these transformed proportions are labelled proportion_no01; columns R, T, V, X, Z and AB). Total length of lionfish, lobster carapace length and *Diadema* test diameter are also provided (columns D, E and F).
(CSV)

Acknowledgments

We thank all the Operation Wallacea volunteers and staff who assisted with the data collection, and special thanks goes to Antal Borcsok and the Tela Marine Research Centre. We also thank Olivia Farman for illustrating the lionfish, lobster and *Diadema* in Fig 5.

Author Contributions

Conceptualization: Christina L. Hunt, Dominic A. Andradi-Brown, Jocelyn Curtis-Quick, Dan A. Exton.

Formal analysis: Christina L. Hunt, Dominic A. Andradi-Brown, Dan A. Exton.

Investigation: Christina L. Hunt, Callum J. Hudson, Joshua Bennett-Williams, Frankie Noades.

Supervision: Dominic A. Andradi-Brown, Dan A. Exton.

Visualization: Christina L. Hunt, Dominic A. Andradi-Brown, Callum J. Hudson, Owen T. Lewis, Dan A. Exton.

Writing – original draft: Christina L. Hunt.

Writing – review & editing: Christina L. Hunt, Dominic A. Andradi-Brown, Callum J. Hudson, Joshua Bennett-Williams, Frankie Noades, Jocelyn Curtis-Quick, Owen T. Lewis, Dan A. Exton.

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