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Abstract

The invasion of the Indo-Pacific lionfish (*Pterois volitans* and *P. miles*) to western Atlantic and Caribbean coral reefs has been documented as one of the most rapid and damaging marine finfish invasions to date, demonstrating strong negative impacts on native coral reef fish species. Comparatively little is known, however, about the effects of lionfish on whole reef fish assemblages and whether culling programmes actually make a difference reflected in fish assemblage measures. Data on reef fish assemblage metrics and community composition were collected using a diver-operated stereo-video system, in two locations with differing lionfish abundances in Honduras: Utila, where lionfish culling occurs, and Tela where little control is apparent. Lower lionfish abundance where culling occurred was related to a significant increase in the mean abundance, length, biomass, number of genera and trophic level of reef fish compared with sites where lionfish were not controlled, along with differential effects on specific families and overall changes in assemblage composition. Taxon-specific prey selectivity of lionfish, together with lionfish overall abundance, are suggested to be the key factors affecting reef fish assemblage measures.

Introduction

The invasion of Indo-Pacific lionfish (*Pterois volitans* [Linnaeus] and *P. miles* [Bennett]) to the western Atlantic was first documented off Florida in the 1980s and has now become established along the eastern coast of the USA, the Gulf of Mexico and the Caribbean Sea (Schofield 2009). As one of the most rapid marine finfish invasions in history (Morris et al. 2009), it poses potentially extreme ecological impacts for the coral reef fish assemblages of these areas (Albins & Hixon 2008). The success of the lionfish invasion can be attributed to many factors, such as their ability to consume large quantities of prey (Fishelson 1997; Morris et al. 2009), including a wide range of native fish and invertebrate species (Côté et al. 2013; Morris & Akins 2009; Muñoz et al. 2011), their ability to withstand stresses such as starvation for long periods of time without mortality (Fishelson 1997), and their capacity for frequent reproduction (Morris 2009). Lionfish in the invasive range have a low prevalence of parasites (Morris et al. 2009) and are well defended from predation by venomous fin spines (Halstead et al. 1955), meaning they have a lack of a top-down control mechanism.

These traits, along with others (Morris & Whitfield 2009), make lionfish ideal invaders with densities in some areas reaching as high as 300-650 fish/ha (Green & Côté 2008). This is approximately 5-15 times higher than the densities recorded in their native range (Frazer et al. 2012). Moreover, there is evidence that lionfish in invaded areas are larger, suggesting they have few competitors and fewer constraints on growth (Darling et al. 2011). Their high densities, combined with strong individual effects, result in detrimental impacts on native ecosystems (Parker et al. 1999) by significantly reducing biomass and numbers of native prey species. For example, in the Bahamas, Green et al. (2012a) showed that biomass of both small-bodied prey fish and large-bodied competitors decreased significantly with the introduction of lionfish, while Albins and Hixon's (2008) study on experimental patch reefs highlighted that the introduction of lionfish reduced recruitment of reef fish by up to 80%. These impacts are potentially exacerbated by the ability of lionfish to occupy and feed in important nursery grounds for juvenile reef fish such as mangroves (Barbour et al. 2010; Claydon et al. 2012), and seagrass beds (Biggs & Olden 2011; Claydon et al. 2012).

As a result of the destructive invasion, lionfish removal programs have been established in many countries. Along with promoting the consumption of lionfish to support potential development of fisheries (Morris et al. 2009; Ferguson et al. 2010; Morris et al. 2011), current efforts to control lionfish populations are primarily conducted through culling and manual removal, accomplished during recreational spear and hand-net fishing and lionfish derby-style events (Barbour et al. 2011). Removal efforts have been shown to be effective at reducing the abundance of populations of lionfish in different locations. For example, removal efforts on the island of Bonaire had resulted in a reduction in the abundance of lionfish by a factor of 4.2 compared to the island of Curacao, where there were no removals (De Leon et al. 2011). Another study by Frazer et al. (2012) found that targeted local culls in the Cayman Islands over a 205-day period significantly decreased the abundance of lionfish. Both studies show that persistent culling pressure can be an effective tool for managing lionfish populations, which could potentially minimize the negative impacts of their invasion on the reef system at a local scale (Johnston & Purkis 2015).

Of the body of literature assessing the impact of introduced lionfish on native reef fish assemblages, most only consider specific aspects such as direct reductions in abundance of prey species (Albins & Hixon 2008; Albins 2013), consequences for local predatory species such as the coney grouper (Albins 2013) and nassau grouper

(Raymond et al. 2014), and the destabilisation of mechanisms regulating the local population dynamics of native prey (Ingeman & Webster, 2015). Furthermore, few studies have looked at the effects of lionfish abundance on the composition of the whole reef fish assemblage, the impacts this may entail and the potential consequences of culling programmes on the native reef fish community. It is important that consequences such as these are examined in order to fully understand the extent of the impacts these invaders are having and whether population control aids recovery of native fish assemblages.

It is also important to elucidate differences in lionfish feeding ecology between areas of different densities in order to further assess the impacts of these invaders on reef assemblages. Many of the fish species consumed by the lionfish in the invasive range are among the most abundant forage fishes in coral reef environments that are important diet constituents for economically important species such as snappers and groupers (Morris & Akins 2009). Other prey include important herbivorous species such as damselfish, parrotfish and surgeonfish (Albins & Hixon 2008; Lesser & Slattery 2011), and species such as the bluehead wrasse *Thalassoma bifasciatum*, which is an important facultative cleaner species for many fish (Côté & Maljković 2010). Whilst general patterns in lionfish feeding, in terms of types of prey they consume, are relatively well documented in their native and invasive ranges, more quantitative investigations of lionfish feeding habits, including assessments of prey selectivity, are needed in invaded areas to reveal potential ecological impacts for the whole reef community.

This study therefore examined two sites in Honduras with differing lionfish removal efforts – high removal efforts around the island of Utila and low removal efforts in the bay of Tela on the mainland – to address the following questions: (1) Is there any difference in the abundance of lionfish between the two areas to indicate successful impact of the culling? (2) If so, do contrasting abundances of lionfish link to differences in the native reef fish assemblages? (3) Can any differences in reef fish assemblage composition be explained by comparative aspects of the feeding ecology of lionfish in the two areas?

Methods

Study sites
This study took place at two sites: around the southern shore of the island of Utila and the bay of Tela, Honduras (Fig. 1), which are located at the southern end of the Mesoamerican Barrier Reef (MABR). Utila is a widely known and popular dive destination with approximately 11 dive shops, of which around 8 actively partake in lionfish culling programs. On the other hand, Tela has only 1 dive shop with a limited, irregular lionfish culling regime. Dive locations in Utila were relatively close to shore with minimum depths of around 2-3m, whilst dive locations in Tela were on the newly discovered offshore reef system of Banco Capiro with a minimum depth of around 10m. In order to test for effects of both 'site' (Utila/Tela) and 'depth' (shallow/deep) on lionfish abundance and different aspects of the reef fish assemblage composition, depth categories were defined as follows: shallow ≤ 10m, and deep ≥ 15m.
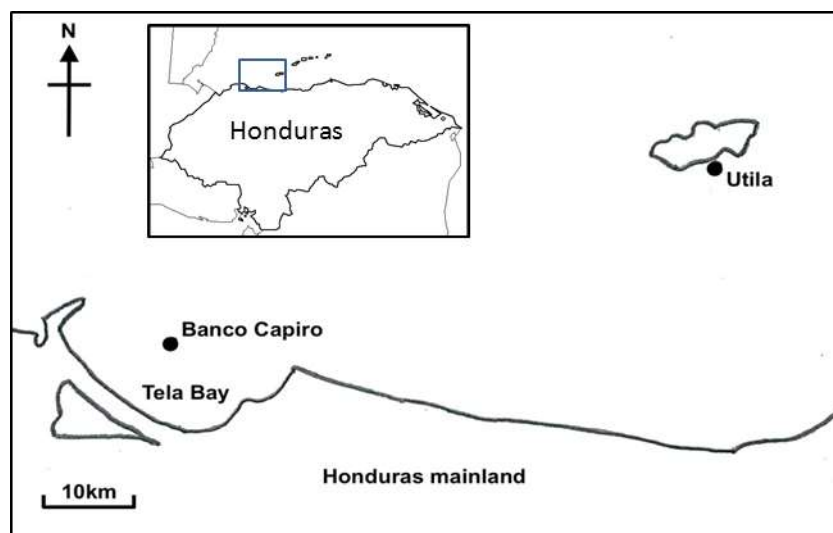
Figure 1: Study sites. The near-shore fringing reefs of Utila where culling efforts are high, and the offshore reef system of Banco Capiro reef system in Tela bay where culling efforts are low

Lionfish abundance surveys and collection

Lionfish abundance was surveyed between June and August 2015 from five locations within both Utila and Tela sites, using lionfish-focused search (LFS) surveys, where divers swim in an S-shaped pattern, as described in Green (2012), 5m either side of a 25m transect (total area = 250m^2). Three surveys at each depth of 5, 10 and 15 m in Utila and 10 and 15m in Tela were completed at all five locations. All crevices and overhangs were examined carefully for lionfish. Upon discovery, lionfish were recorded, speared and collected for further analysis in the lab. The collection of lionfish during abundance surveys was unlikely to affect the outcome of further surveys as lionfish in their invasive range have been shown to exhibit extremely high site fidelity within a 10 m range (Jud & Layman 2012). Transect locations (both within and between sites) were far enough apart (≥10 m) to ensure lionfish were unlikely to move between them.

On 3rd July 2015, a further 229 lionfish were collected during a derby style event in Utila. Each lionfish, once collected, was put on ice and dissected throughout the day.

Two species of lionfish (*Pterois volitans* and *P. miles*) have been recorded in the invasive region (Morris 2009; Coté et al 2013). Morris (2009) estimated 93% of lionfish off the East coast of the US are *P. volitans,* with other regions (e.g. Bahamas) only appearing to consist of this species. Whilst it is likely that most, if not all, lionfish encountered in Honduras are *P.volitans,* the species are extremely difficult to separate visually (Coté et al 2013) so for the purposes of this study "lionfish" refers to *Pterois* spp.

Reef fish assemblage composition

Data on the native reef fish assemblage composition across the two sites were collected via a diver-operated stereo-video system (stereo DOV) technique, similar to that used by Harman et al. (2003) and Watson et al. (2005). This method has shown to significantly improve accuracy and precision when determining fish lengths compared to another widely used survey technique (underwater visual census – UVC; Harvey et al. 2001) and hence was chosen for use in this study. The stereo DOV system used two Canon HFS21 video cameras in underwater housings mounted 0.7 m apart on a base bar and inwardly converged at 8 degrees, with a synchronising diode mounted in front of the cameras.

The stereo DOV transects were completed by two SCUBA divers, one operating the stereo DOV system and the other measuring the distance swam with a transect tape. Divers swam slowly (1 m every 3 s) approximately 30 cm above the substrate. Six replicate, 50 x 5 m transects were completed at each depth category (≤ 10 m, ≥ 15 m) in each of the five locations that were used for lionfish abundance surveys within Utila and Tela. The transect width (5 m) was defined during post-processing of the stereo-imagery.

The videos were reviewed and analysed using SeaGIS software (SeaGIS Pty Ltd., EventMeasure version 3.50) where each fish seen was identified to the lowest taxonomic level possible. If the fish was present on both the left and right cameras the total length (from the tip of the snout to the tip of the caudal fin) was recorded by clicking on the respective areas of the fish. From these data, biomass and trophic level of the fish was calculated using data and equations provided by FishBase (www.fishbase.org, Froese & Pauly 2009). If the fish was present only on one camera, only the taxonomic grouping and trophic level were recorded, as 3D measurements were not possible.

External and internal measurements of lionfish
Lionfish were processed at Coral View Research Centre in Utila, and Honduras Shores Plantation in Tela. Measurements were taken as soon as possible after collection (usually within an hour); however when this was not possible, lionfish were kept on ice until taken to the lab. For all collected lionfish (both from surveys and LFS dives), dissection protocol followed that of Green et al. (2012b) for measurements and recordings of: total length, total weight, gape width and gape height. An index of gape cross sectional area (Arnold 1983) was calculated as the area of an ellipse, with major and minor axes measurements equal to gape length and width:

$$Gape\ index = \frac{\pi(gape\ length)(gape\ width)}{4}$$

All stomach contents (including from lionfish caught in the Utila derby) were identified to the lowest taxonomic level possible using identification guides such as Humann & DeLoach (2002) and given a digestion level score as described in Green et al. (2012b). For all prey items at digestion level 1 or 2, the total length and weight of the items were taken. All other prey items were considered too digested to accurately discern body shape (and therefore total length).

Data analysis
To determine any significant differences between 'site', and 'depth' on the abundance of lionfish a two-way Analysis of Variance (ANOVA) was used, where 'site' and 'depth' were fixed factors. Similarly, two-way ANOVAs were also used to detect any significant differences between 'site and 'depth' on the abundance, mean length, total biomass, mean trophic level and number of genera of native fish, as well as the abundance of the most common taxa: wrasse (Labridae) and damselfish (Pomacentridae). Levene's test was used to determine any deviation of homogeneity of variances and data were transformed if significant. If data still showed non-homogeneity of variances after transformation, the two-way ANOVA was carried out with caution as recommended by Underwood (1997).

Between-site and between-depth differences in native reef fish assemblage composition were quantitatively explored using multivariate analysis in the software PRIMER version 6 (Primer-E Ltd., Plymouth Marine Laboratory, Plymouth, UK, www.primer-e.com) (Clarke & Warwick 2001). A Bray–Curtis similarity matrix using fourth-root transformed species abundance values from the stereo DOV data served as input for the analysis.

Multidimensional scaling (MDS) was then used to produce ordinations of the native fish assemblages between both sites and depths. To test the significance of these results, a two-way crossed Analysis of Similarity (ANOSIM) between site and depth was used. Importance of fish species in the contribution to dissimilarity between the two sites was assessed using a Similarity Percentages (SIMPER) analysis.

To determine any significant difference between the total length or weight of lionfish between Utila and Tela a Student's t-test and a Mann-Whitney U-test were used, respectively, due to considerations of non-normality of total weight data.

Ivlev's prey selectivity index (Ivlev 1961) was calculated for families of prey that were present in both stereo DOV data and in the stomachs of lionfish:

$$E = \frac{r - p}{r + p}$$

where r is the proportion of the prey family in the lionfish diet and p is the proportion of the availability of the prey family in the study site. As there were only 4 easily identifiable individual fish found in the stomachs of the lionfish caught in Utila, prey families from the lionfish derby were also used in this analysis. However, caution was taken when interpreting results as the depths the lionfish were collected from (and therefore likely to be feeding at) were unknown. Prey selectivity index values range form -1 to +1, where a value of -1 would suggest the prey category is selected by the predator much less than its abundance in the study area, and more than its abundance in the study area for values of +1. Prey with selectivity values of 0 are considered to be consumed in proportion to their availability. Families of teleost fish that were present in stereo DOV data, but not found in the stomach contents at either site (Lutjanidae, Tetradontidae, Chaetodontidae, Carngidae, and Grammatidae) were removed from the analysis, as it was clear lionfish were avoiding them. Prey families found in lionfish stomachs but not seen in stereo DOV data (Gobiidae, Synodontidae, Holocentridae, and Monacanthidae) were also removed as they represent species that are not easily detectable in the environment, due to size or cryptic nature.

Results

Overall, 156 lionfish were caught throughout the 8-week period, of which 19 were from Utila and 136 were from Tela. The size of lionfish ranged from 16.8 to 38.8cm in Utila and 9.5 to 38.2cm in Tela with mean sizes (±SE) of 25.9 ± 1.5cm and 23.8 ± 0.6cm respectively; however there was no significant difference in either the total length (t_{153}=1.356, p=0.177) or weight of invaded lionfish (U=1265.0, p=0.883) between Utila and Tela.

In total, 72 species of native fish were recorded over the two sites, with 62 of these present in Utila and 56 present in Tela.

Number of lionfish
There was a significant effect of site, but neither an effect of depth nor an interaction on the number of lionfish (Fig 2, Table 1a). The number of lionfish was lower in Utila than in Tela ($F_{1,56}$=9.431, p<0.005).

Impacts on native fish assemblages

Total assemblage metrics
There was a significant effect of site and depth on the total abundance of reef fish (Fig 3a, Table 1b). The number of fish was higher in Utila than in Tela and there was a higher

number in shallower compared to deeper depths. There was also a significant interaction effect of site and depth on the number of fish (Table 1b). In shallow depths there were more fish in Utila than Tela; however in deep water, the number of fish was similar across sites. There was no difference in fish abundance between depths in Tela.
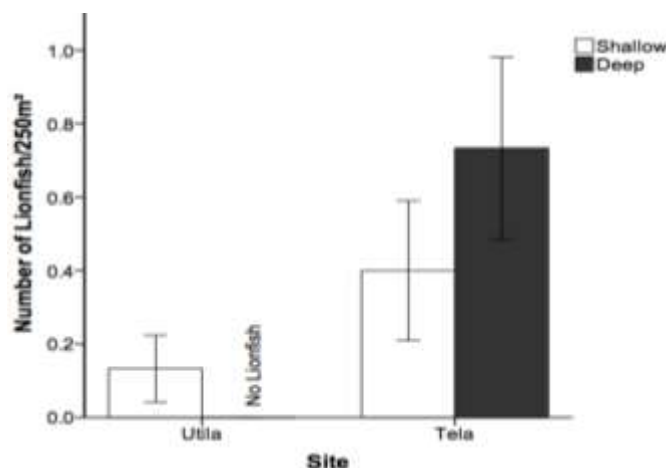

Figure 2: Number of lionfish/250m^2 (mean ± SE) in Utila and Tela in shallow (≤10m) and deep (≥15m) water. An active lionfish culling programme occurs at Utila.

There was a significant effect of site, but neither an effect of depth nor an interaction on the mean length of fish (Fig 3b, Table 1c). The length of fish was greater in Utila than in Tela.

There was a significant effect of site and depth on the total fish biomass/250m^2 (Fig 3c, Table 1d). Utila had a higher biomass than Tela and there was higher biomass in shallow water compared to deep. There was a significant interaction effect between site and depth on the total fish biomass/250m^2 (Table 1d). In shallow depths there was a higher total biomass in Utila than Tela; however in deep water, the total biomass was similar across sites. There was no difference in biomass between depths in Tela.

There was a significant effect of site on the number of genera/250m^2 but no effect of depth (Fig 3d, Table 1e). The number of genera was significantly higher in Utila compared to Tela. There was, however, a significant interaction effect between site and depth on the number of genera (Table 1e). In shallow depths there was a higher number of genera in Utila than Tela; however in deep water, number of genera were similar across sites. There was no difference in the number of genera between depths in Tela.

There was a significant effect of site on the mean trophic level but no effect of depth (Fig 3e, Table 1f). The mean trophic level was significantly higher in Utila than in Tela. There was also a significant interaction effect between site and depth on the mean trophic level (Table 1f). In shallow depths there was a higher mean trophic level in Utila than Tela; however in deep water, there was no difference in trophic level between sites. There was also no difference in trophic level between depths in Tela.

Individual fish groups

There was a significant effect of both site and depth on the number of wrasse (Fig 3f, Table 1g). The abundance of wrasse was higher in Utila than in Tela and higher in shallower depths compared to deeper depths. There was a significant interaction effect between site and depth on the number of wrasse (Table 1g). There is no significant difference in the number of wrasse between sites at deeper depths, whereas at shallower depths Utila had higher numbers of wrasse than Tela.

There was a significant effect of both site and depth on the number of damselfish (Fig 3g, Table 1h). The abundance of damselfish was lower in Utila than in Tela and higher in shallower depths compared to deeper depths. There was also a significant interaction effect between site and depth on the number of wrasse (Table 1h). At shallower depths Utila had greater numbers of damselfish compared to Tela, whereas at deeper depths Tela had more damselfish than Utila.
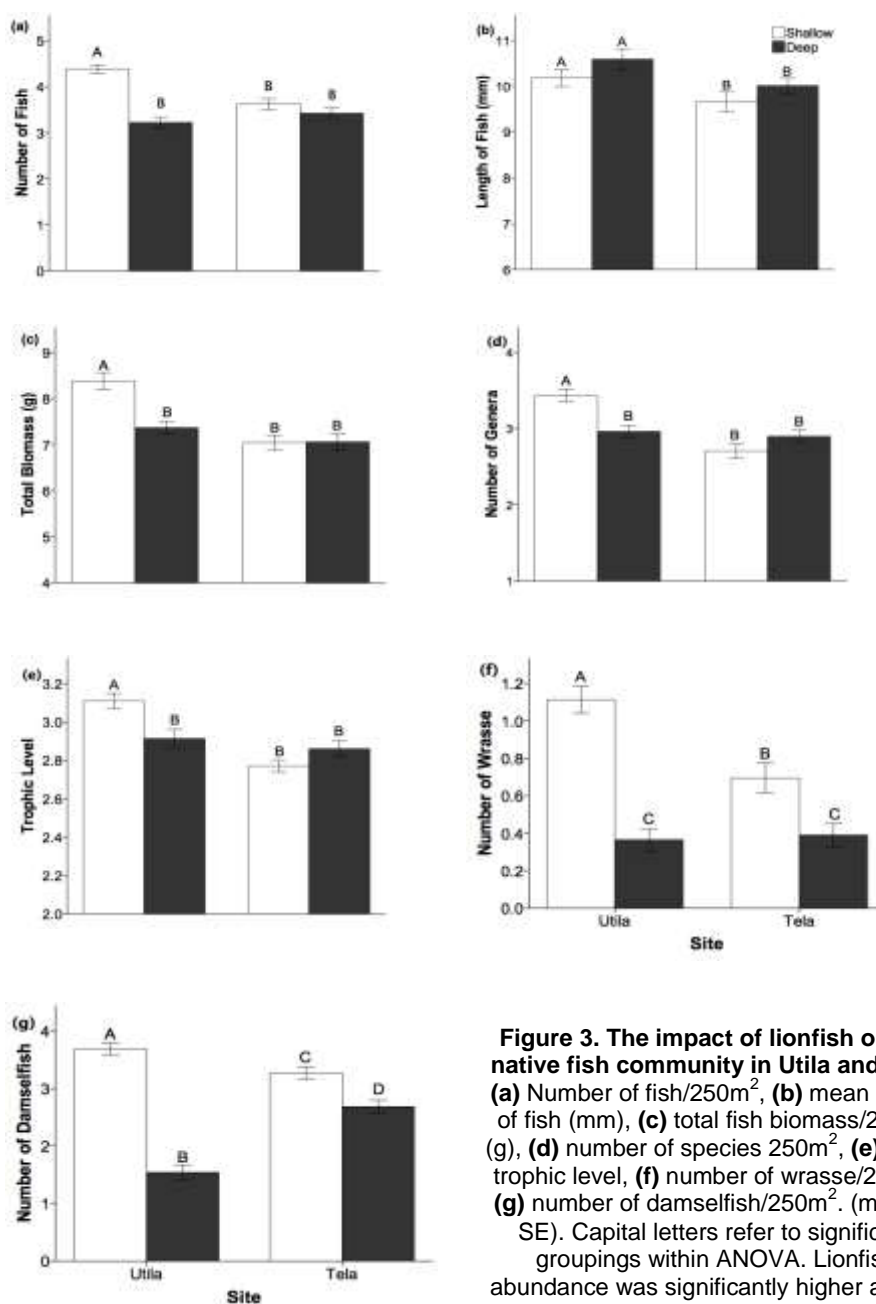

Figure 3. The impact of lionfish on the native fish community in Utila and Tela. **(a)** Number of fish/250m^2, **(b)** mean length of fish (mm), **(c)** total fish biomass/250m^2 (g), **(d)** number of species 250m^2, **(e)** mean trophic level, **(f)** number of wrasse/250m^2, **(g)** number of damselfish/250m^2. (mean ± SE). Capital letters refer to significant groupings within ANOVA. Lionfish abundance was significantly higher at Tela

[29]

Table 1: Two-way ANOVA results testing for an effect of site (Utila/Tela), depth (shallow ≤10m, deep ≥15m) and the interaction between the two factors (Site*Depth) on the **(a)** number of lionfish/250m^2, **(b)** number of native reef fish/250m^2, **(c)** mean length of fish (mm), **(d)** total fish biomass/250m^2 (g), **(e)** number of species 250m^2, **(f)** mean trophic level, **(g)** number of wrasse/250m^2, **(h)** number of damselfish/250m^2. NS = no significant difference, * = p<0,05, ** = p<0.005, *** = p<0.001.

(a)	DF	MS	F	p	Sig.
Site	1	3.750	9.431	0.003	**
Depth	1	0.150	0.377	0.542	NS
Site*Depth	1	0.817	2.054	0.157	NS
Error	56	0.398			

(b)	DF	MS	F	p	Sig.
Site	1	2.367	6.266	0.014	*
Depth	1	13.963	36.957	<0.001	***
Site*Depth	1	6.993	18.508	<0.001	***
Error	116	0.378			

(c)	DF	MS	F	p	Sig.
Site	1	6.529	5.091	0.026	*
Depth	1	3.374	2.631	0.108	NS
Site*Depth	1	0.159	0.124	0.726	NS
Error	116	1.282			

(d)	DF	MS	F	p	Sig.
Site	1	20.265	23.629	<0.001	***
Depth	1	7.466	8.706	0.004	**
Site*Depth	1	7.946	9.265	0.003	**
Error	116	0.858			

(e)	DF	MS	F	p	Sig.
Site	1	4.725	21.417	<0.001	***
Depth	1	0.599	2.713	0.102	NS
Site*Depth	1	3.317	15.035	<0.001	***
Error	116	0.221			

(f)	DF	MS	F	p	Sig.
Site	1	1.150	22.734	<0.001	***
Depth	1	0.086	1.700	0.195	NS
Site*Depth	1	0.612	12.104	0.001	**
Error	116	0.051			

(g)	DF	MS	F	p	Sig.
Site	1	1.158	8.101	0.005	**
Depth	1	8.327	58.239	<0.001	***
Site*Depth	1	1.469	10.273	0.002	**
Error	116	0.143			

(h)	DF	MS	F	p	Sig.
Site	1	0.747	10.158	0.002	**
Depth	1	10.595	144.001	<0.001	***
Site*Depth	1	3.458	47.006	<0.001	***
Error	116	0.074			

Multivariate assemblage analysis

MDS ordination of native fish communities shows a clear division between locations (Fig 4a) and a depth division within sites (Fig 4b); almost no overlap was apparent between the two locations. A two-way crossed ANOSIM confirmed the significant differences in assemblage composition between both sites (R=0.578, p<0.001) and depths (R=0.224, p<0.001).

The average dissimilarity between native fish assemblages across Utila and Tela was 73.73%, where 11 species contributed to 50% of the dissimilarity between sites (Table 2, SIMPER analysis). *Chromis cyanea, Stegastes adustus, S. partitus* and *Canthigaster rostrata* together contributed to over 25% of the dissimilarity between sites. *Chromis* and *Canthigaster* were more abundant at Utila, whilst the two *Stegastes* species were more common at Tela.
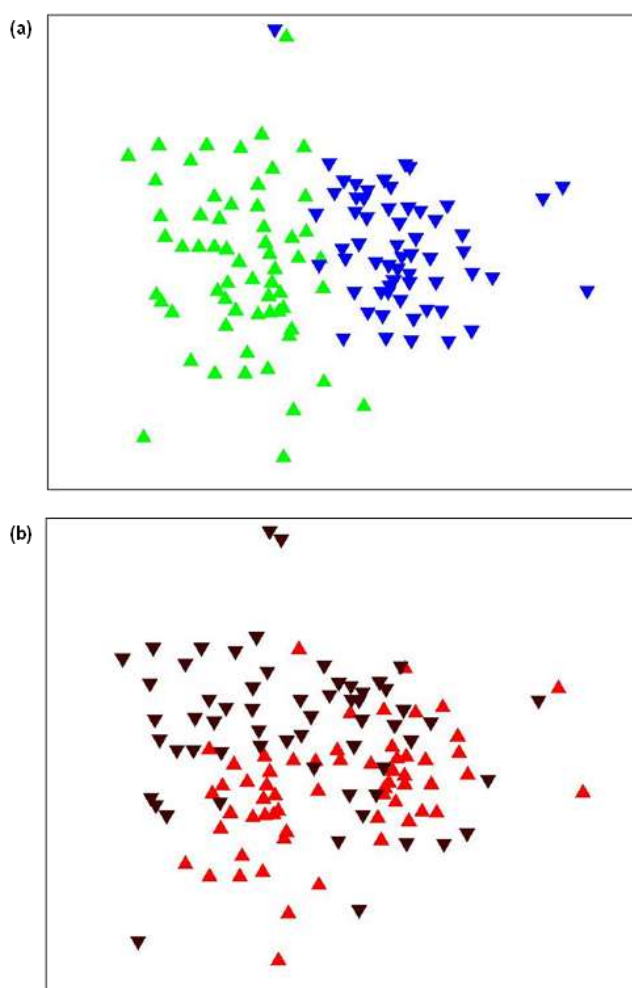

Figure 4: Multidimensional scaling ordinations of native fish communities. (a) Between sites: Utila (▲) and Tela (▼), (b) between depths: shallow (≤10m) (▲) and deep (≥15m) (▼)

[31]

Table 2: For each species derived from the stereo-video abundance data, average abundance (Av.Abund) in Utila and Tela, and percentage contribution to dissimilarity (Contrib%) are shown in descending order of contribution until the cumulative percentage contribution to dissimilarity between sites (Cum.%) reaches ≥ 50%.

	Utila	Tela		
Species	**Av.Abund**	**Av.Abund**	**Contrib%**	**Cum.%**
Chromis cyanea	1.60	0.05	8.46	8.46
Stegastes adustus	0.39	1.78	7.84	16.3
Stegastes partitus	0.58	1.35	5.3	21.6
Canthigaster rostrata	1.06	0.5	4.53	26.13
Scarus iseri	0.84	0.39	4.17	30.3
Scarus taeniopterus	0.58	0.64	3.9	34.2
Sparisoma viride	0.79	0.54	3.86	38.06
Acanthurus coeruleus	0.67	0.34	3.67	41.72
Thalassoma bifasciatum	0.9	0.84	3.66	45.38
Chaetodon capistratus	0.43	0.52	3.21	48.59
Microspathodon chrysurus	0.25	0.55	2.83	51.42

Lionfish feeding ecology

Lionfish gape index was not a significant predictor of prey length ($R^2_{adj}=0.199$, $F_{1,35}=1.442$, $p=0.238$). This was also true for within site analysis: Utila ($R^2_{adj}=-0.117$, $F_{1,7}=0.162$, $p=0.699$) and Tela ($R^2_{adj}=0.031$, $F_{1,26}=1.860$, $p=0.184$) (Fig 5).

Teleost fish dominated lionfish stomach contents by number in both Utila (54.3%) and Tela (71.1%). Approximately 10.5% and 20.4% of lionfish stomachs in Utila and Tela respectively were empty. Teleost prey from stomachs consisted of 8 families in Utila and 6 families in Tela.
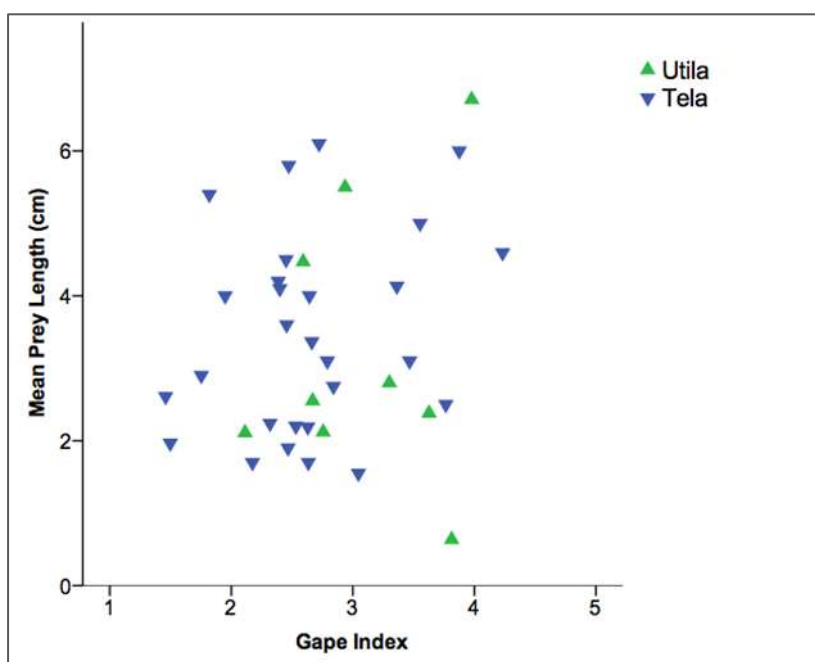

Fig 5. Mean length (cm) of prey items found in the stomach with a digestion score of 2 or less as a function of gape index (π(gape length)(gape width)/4) of lionfish, in Utila (▲) and Tela (▼)

Damselfish (Pomacentridae) were the most abundant potential prey in both Utila and Tela (Table 3). Damselfish were consumed disproportionately less than their abundance in Utila, but relatively in proportion to their abundance in Tela. Scarridae (parrotfish) were also found disproportionately less in lionfish diets than their abundance in Utila, but were not found at all in the stomach contents of lionfish in Tela. Lionfish in both sites selected wrasse (Labridae) in greater numbers than their abundance, slightly more so in Utila than Tela. *Acanthuridae* (surgeonfish, tangs, etc.) were not found in the stomach contents of any lionfish in Tela; however they were preyed upon in proportionally greater numbers than their abundance in Utila. Lionfish in Utila did not have any grunts (Haemulidae) in their stomachs, whereas they were present in lionfish in Tela, but at a disproportionally lower abundance than was available. Pomacanthidae (angelfish) were not found in any stomach contents of lionfish in Tela, but were found in those in Utila at a higher proportion than was found on the reef. Serranidae (bass, groupers etc.) were consumed disproportionately more than their abundance in both Utila and Tela. This family has the highest prey selectivity index value compared to other families in both sites.

Table 3: Mean prey abundance (250 m^{-2}) and prey selectivity (E) for prey families seen both in stereo DOV data and stomach contents data in Utila and Tela. Prey selectivity for stomach contents of lionfish caught during the derby in Utila were included in the Utila analysis.

Prey Family	Abundace Utila	Abundace Tela	E (Utila inc. Derby)	E (Tela)
Pomacentridae	3.79	4.70	-0.303	0.131
Scarridae	2.93	2.15	-0.486	-1.000
Labridae	1.71	1.60	0.406	0.349
Acanthuridae	0.90	0.57	0.305	-1.000
Haemulidae	0.40	0.62	-1.000	-0.417
Pomacanthidae	0.17	0.21	0.330	-1.000
Serranidae	0.23	0.09	0.630	0.478

Discussion

The difference in abundance of lionfish seen between Utila and Tela clearly demonstrates the efficacy of continued removal efforts on Utila, resulting in a significantly smaller lionfish population than in Tela. This result coincides with other studies (DeLeon et al. 2011; Frazer et al. 2012), which show that persistent culling pressure, such as that seen in Utila, can be effective in reducing lionfish abundance at a local scale. Even though removal efforts are unlikely to fully eradicate the invasive lionfish (Arias-González et al. 2011; Barbour et al. 2011; Green et al. 2014), it is undoubtedly possible for populations to be effectively managed, thereby potentially reducing their impacts on the reef system.

Seeing that no statistically significant difference of lionfish abundance between depths was found, it can be speculated that any differences in fish communities found between depths are likely to be due to natural differences in the composition of reef fish assemblages, as opposed to differing impacts of lionfish. It is interesting to note, however, that despite no significant interaction effect of depth and site on the number of lionfish found, no lionfish were found in deeper waters in Utila, whereas in Tela there were more lionfish in deeper waters than in shallower waters. Regardless of these differences in lionfish abundance, many of the assessments of fish assemblage metrics

were similar between deeper waters in both sites. This could be due to one of two reasons: (1) lionfish in deeper waters at Tela are having non-significant impacts on the reef fish assemblages, or (2) actual lionfish abundance in Utila were higher than observed due to wariness of lionfish of divers. Both explanations are plausible, however there is little research on how lionfish impacts change with depth. Therefore, more research should be undertaken in order to determine whether behaviour or feeding ecology of lionfish change with depth. A previous study has shown that in areas of high culling pressure, lionfish become more wary of divers, and hence reside into deeper crevices for protection (Côté et al. 2014). Observations of the reef structure on Utila revealed greater structural complexity, with deeper crevices and overhangs than the reef system in Tela Bay. Although divers took great care in searching for lionfish within these refuges, there is the possibility that some individuals were missed.

Crucially, the results of the current study show a strong likelihood that the high abundance of lionfish in Tela is linked to negative impacts on the native reef fish assemblage in the area. The significantly lower abundance of reef fish seen in Tela compared to Utila is consistent with what would be expected in an area with high densities of lionfish. Albins and Hixon (2008) showed that high lionfish densities significantly reduced recruitment of native reef fish as a result of predation. Reduction in recruitment, together with predation of adult reef fish by lionfish has most likely led to the lower abundance of reef fish in Tela.

Analysis of the metrics of reef fish assemblages in Utila and Tela additionally highlighted the lower mean total length of fish in Tela compared to Utila. It is possible that this is also attributed to the higher numbers of lionfish in the area. Greater densities of lionfish in Tela could have resulted in a reduction in the mean total length of reef fish for a number of reasons, including the possibility that lionfish in Tela are (1) actively selecting larger prey, (2) choosing to predate on juveniles of larger-bodied reef fish, or (3) reducing the abundance and size of large native mesopredators through competition. As lionfish are gape-limited predators (Côté et al. 2013) it would only be possible for larger individuals with bigger gape sizes to actively select larger prey-fish. Examination of the relationship between gape index and prey length showed no such selectivity at either site (lionfish with larger gape index values were shown to be consuming both larger and smaller fish). This suggests it is unlikely that prey size selectivity has led to the reduced mean length of native fish.

Inspection of stomach contents and prey selectivity indices indicated predation on juveniles of large-bodied reef fish was also unlikely to be the reason for the reduced mean length of native fish. Despite lionfish in Tela selecting to consume fish in the families Serranidae and Labridae (which contain species that can grow to be over 25cm [Green et al. 2012a]) disproportionately more than their abundance on the reef, none of the identifiable species within these families (*Halichoeres maculipinna, H. pictus, Liopropoma rubre, L. carmabi* and *Serranus tortugarum*) were those able to grow to notably large sizes.

It seems the most likely explanation, in this case, is that lionfish are reducing the abundance and size of large native mesopredators through competition, resulting in a lower mean length of reef fish. Lionfish are likely to monopolize the important food resource of native predators through predation of reef fish either at the recruitment or adult stage. This competition is likely to lead to a reduction in the size, or even abundance, of large-bodied piscivores due to a limited food resource restricting growth (Henderson 2005) or carrying capacity (Begon et al. 1990) of a population. Green et al. (2012a) demonstrated this is a plausible explanation as they showed that with an

increase in lionfish abundance, the biomass of large-bodied competitor species declined by 44%.

Coupling reduced abundance and length of native fish seems to have ultimately resulted in a reduced overall biomass of fish in Tela. These findings are consistent with models predicting the impacts of invasive piscivorous fish (Pine et al. 2007; Arias-González et al. 2011) in that they are forecast to suppress native fish assemblage biomass through both competition and predatory interactions. It is likely that densities of lionfish in Utila have been reduced to below the predicted threshold densities needed to protect the native fish community biomass from predation-induced declines (Green et al. 2014).

The reduced number of genera in Tela is also in agreement with what would be expected from greater numbers of lionfish in an area. Albins (2013) showed that, on small patch reefs in the Bahamas, a single lionfish could reduce native prey richness by almost 5 species in only 8 weeks. They indicated that one of the ultimate effects of the lionfish invasion is likely to be substantial reductions in the number of native fish species on local reefs. This result could be due to lionfish consuming rare prey species disproportionately more than their abundances on the reef (Almany & Webster 2004), thereby causing local reductions in local prey species richness and potentially number of genera over longer periods of time. It is clear from the prey selectivity indices in the present study that lionfish can have high preferences for particular families of reef fish (especially Labridae and Serranidae). It is also possible, therefore that they could be selective at lower taxonomic levels (genus or species), which would likely lead to the results seen here; however, further analysis would be required in order to confirm this theory.

As mesopredators, lionfish have a central ecological role: they are potential prey to larger native predators, competitors to other native mesopredators, and predators of smaller native fish and invertebrates. For this reason, they have the capacity to cause both direct and indirect impacts at a variety of levels. It has previously been suggested (Albins & Hixon 2008; Morris & Akins 2009) lionfish predation on important herbivorous fish, such as parrotfish and damselfish, may lead to an overall reduction in herbivory on a reef system, hence increasing the potential for a shift to an algal dominated system (Mumby et al. 2006). Evidence of this has been seen on mesophotic reefs in the Bahamas (Lesser & Slattery 2011). In the present study, however, it was found that mean trophic level values were lower in Tela than those in Utila, suggesting that despite having greater numbers of lionfish (and therefore potentially greater predation of herbivores), the reef in Tela may be less likely to undergo a phase shift. It is well known that the specific impacts of an invasive predator on prey community composition are highly context dependent (Hixon 1986). In this case, it seems as though the invasive lionfish have had the opposite impact to that which has been observed (Lesser & Slattery 2011) and predicted (Albins & Hixon 2008; Morris & Akins 2009) elsewhere. As mentioned earlier, lionfish are potentially reducing the abundance of other mesopredator species through competition for food resources. This coupled with the fact that lionfish are selecting to consume higher trophic level families such as Serranidae and Labridae and actively avoiding lower trophic level families such as Scarridae and Acanthuridae in Tela suggests that this observation, although contradictory, is likely to be due to the higher lionfish abundance in Tela compared to Utila.

As well as the assessment of these assemblage metrics, differences in two of the most common taxa found both on the reef and in the stomachs of lionfish were also examined between sites. It was shown that fish in the family Labridae (wrasse) were found in lower abundances in Tela than in Utila. Wrasse were preyed upon disproportionately more

than their abundances on the reef in Tela, suggesting that lionfish are likely to be part of the reason for this difference in abundance. Considering that some fish within this family are important facultative cleaner species (e.g. *Thalassoma bifasciatum* and *Bodianus rufus*), this impact has the potential to lead to further, indirect impacts on species that rely on them. Further study is suggested in order to depict the potential repercussions of this selectivity on both cleaners and clients.

In contrast to this, fish in the family Pomacentridae (damselfish) were found in higher abundances in Tela than in Utila. Many damselfish are herbivorous, so this result supports the previous result of Tela having a lower mean fish trophic level value than Utila. Prey selectivity index values for this family, however, contradict these results to some degree, as lionfish in Tela were found to be feeding on damselfish in proportions slightly higher than their abundance on the reef, whereas in Utila, they were consumed in lower proportions. This suggests that the different abundances of damselfish cannot only be explained by different lionfish abundances. It is possible that the impacts lionfish have on the native predators of the reef may indirectly affect other members of the native reef fish community. This is likely to be the case in this situation as the evidence in this study suggests lionfish in Tela are having a heavy influence on larger bodied competitor species, which include other mesopredators.

Multivariate analysis of between site differences in native reef fish assemblage composition demonstrated that the assemblages in Utila and Tela are substantially dissimilar from one another, with almost no overlap between the assemblages of the two sites. Given other results, these differences are highly likely to be influenced by differences in selective lionfish abundance, and thus predation, between the two areas, the lionfish having differential impacts on different species within the community (Parker et al. 1999) as opposed to simply decreasing the abundance of all members of the community equally. This is supported by the fact that lionfish in Tela are actively selecting some families of fish, whilst completely avoiding others. Indeed, similar prey selectivity results have been found for lionfish in Utila; however, the particularly low densities of invaders in this area are unlikely to show the same severity of impact as seen in Tela.

In spite of all of these results, there is also the possibility that differences in the reef fish assemblages of the two sites could simply be a result of different geographical location. However, given the relative close proximity of the two reef systems and particularly that the outcomes of this study correspond to what would be expected with high lionfish densities, it is highly likely that any differences seen are, at least in part, due to the different abundances of lionfish between sites that has been created by the culling effort in Utila.

Conclusions

From this study, it can be concluded that, (1) lionfish removal efforts in Utila have been successful enough to significantly reduce the abundance of invaders in the area, (2) the consequently greater abundances of lionfish in Tela can be linked to a number of impacts on the reef fish assemblage of the site, including a reduction in the number, length, biomass, number of genera and mean trophic level of reef fish, along with differential effects on specific families and an overall change in assemblage composition and (3) differences in abundance and taxon-specific prey selectivity of lionfish are likely to be the underlying factors determining the potential effects these invaders have on the native fish assemblages, whereas prey size selectivity and feeding rate of lionfish are unlikely to be significant predictors of impact.

These results have important implications since they show the potential for high densities of invasive lionfish to have extensive impacts on different aspects of the native reef fish assemblages and, particularly, that culling of lionfish can make a difference in terms of rehabilitating coral reef communities impacted by this highly successful invader.

Acknowledgements

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