1 **Large-scale invasion of western Atlantic mesophotic reefs by lionfish**

2 **potentially undermines culling-based management**

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1 **Abstract**

2 The detrimental effects of invasive lionfishes (*Pterois volitans* and *Pterois miles*) on

3 western Atlantic shallow reefs are well documented, including declines in coral cover

4 and native fish populations, with disproportionate predation on critically endangered

5 reef fish in some locations. Yet despite individuals reaching depths >100 m, the role

6 of mesophotic coral ecosystems (MCEs; reefs 30-150 m) in lionfish ecology has not

7 been addressed. With lionfish control programs in most invaded locations limited to

8 30 m by diving restrictions, understanding the role of MCEs in lionfish distributions

9 remains a critical knowledge gap potentially hindering conservation management.

10 Here we synthesise unpublished and previously published studies of lionfish

11 abundance and body length at paired shallow reef (0-30 m) and MCE sites in 63

12 locations in seven western Atlantic countries and eight sites in three Indo-Pacific

13 countries where lionfish are native. Lionfish were found at similar abundances across

14 the depth gradient from shallow to adjacent MCEs, with no difference between

15 invaded and native sites. Of the five invaded countries where length data were

16 available three had larger lionfish on mesophotic than shallow reefs, one showed no

17 significant difference, while the fifth represented a recently invaded site. This

18 suggests at least some mesophotic populations may represent extensions of natural

19 ontogenetic migrations. Interestingly, despite their shallow focus, in many cases

20 culling programs did not appear to alter abundance between depths. In general, we

21 identify widespread invasive lionfish populations on MCE that could be responsible

22 for maintaining high densities of lionfish recruits despite local shallow-biased control

23 programs. This study highlights the need for management plans to incorporate

24 lionfish populations below the depth limit of recreational diving in order to address all

25 aspects of the local population and maximise the effectiveness of control efforts.

Introduction

Fewer than 16 % of marine environments are now considered to be free of invasive species and the threat to biodiversity they represent (Molnar et al. 2008). The major impacts they cause to natural ecosystem health and functioning, whether as predators, competitors, pathogens or parasites are well documented (Simberloff et al. 2013) and present an enormous dilemma for conservation practitioners. The ability to cross environmental barriers combined with high reproductive output facilitates the rapid spread of many marine invasive species (Côté et al. 2013) while the difficulties associated with removing individuals makes complete eradication almost impossible once invasives become established across a large geographical range (Thresher and Kuris 2004). Instead managers are often forced to limit their efforts to local control, and to focus on mitigating the most severe impacts rather than seeking complete eradication (Williams and Grosholz 2008).

The appearance of lionfish in the western Atlantic has led to one of the fastest marine invasions ever recorded (Schofield 2010). Native to the Indo-Pacific, the lionfishes *Pterois volitans* and *Pterois miles* (Family: Scorpaenidae) were introduced to the western Atlantic in the early 1980s and have since become a widespread conservation concern in the region (Côté et al. 2013). Lionfish have proved to be highly effective predators on invaded shallow coral reefs benefiting from young age of maturity combined with high fecundity, highly generalist feeding strategy, lack of natural predators and parasites, and prey naivety (Côté et al. 2013). Invasive

population densities are consequently five times greater than those of lionfish in their

native range, although the presence of additional co-occurring Indo-Pacific lionfish

species may partially reduce this overall disparity (Darling et al. 2011). Invasive

lionfish have been reported to reduce native fish recruitment by up to 79 % (Albins

and Hixon 2008) and drive declines in overall prey fish biomass of up to 65 % (Green

et al. 2012) on shallow patch reef systems. Their impacts on mesophotic reefs in The

Bahamas have shown their potential to cause declines in coral cover (Lesser and

Slattery 2011), while dietary analysis has suggested disproportionate predation on

critically-endangered reef fish species in some locations (Rocha et al. 2015). Lionfish

density appears limited by food availability (Benkwitt 2013), with invasive

individuals 1.5 times longer and three times heavier on average than those in their

native range (Darling et al. 2011).

Invasive lionfish are now established from North Carolina, USA in the north,

throughout most of the Greater Caribbean and Gulf of Mexico (Schofield 2010), with

an individual collected as far south as Brazil (Ferreira et al. 2015). This wide spatial

distribution makes complete eradication impossible with current methods that focus

on active culling to reduce local population densities. Culling efforts are often

managed/regulated by government and conducted by volunteer recreational SCUBA

divers using Hawaiian sling hand spears or pole spears (Morris et al. 2009). Evidence

suggests that culling successfully reduces lionfish abundance on shallow reefs (Frazer

et al. 2012), and such reductions aid the recovery of native fish communities (Green

et al. 2014). However, continued culling is crucial as lionfish populations quickly

recover after culling ceases (Arias-González et al. 2011).

It is vital that the ecology and distribution of the invasive species is fully

understood to design the most effective form of control. If invasive species are widely

dispersed habitat generalists, such as lionfish, then they will likely exhibit source-sink

population dynamics, with individuals in certain areas disproportionately contributing

to recruitment (Travis and Park 2004). Depth is recognised as a fish refuge from

harvesting pressure, with major differences in biomass and species composition

detected across even small depth gradients (Tyler et al. 2009; I. Bejarano et al. 2014).

Yet few studies have considered how control programs are affected by depth, despite

several major shallow reef marine invasive species being recorded across wide depth

ranges (Andradi-Brown et al. 2016).

Lionfish are found on mesophotic coral ecosystems (MCEs; reefs from 30 m

to 150 m) in their native range, having been reported at 65 m in the Red Sea

(Brokovich et al. 2008), from trawl surveys at 75 m in New Caledonia (Kulbicki et al.

2012) and remotely operated vehicle (ROV) observations below 80 m in American

Samoa (Wright 2005). Lionfish have also been anecdotally observed on MCEs

throughout much of their invaded range, although comprehensive sampling at

mesophotic depths remains lacking. For example, lionfish have been recorded down

to 55 m in Puerto Rico (I. Bejarano et al. 2014), over 100 m in The Bahamas (Lesser

and Slattery 2011), 112 m in the northwestern Gulf of Mexico (Nuttall et al. 2014),

and 120 m in Honduras (Schofield 2010). Even though many western Atlantic MCE

observations report high abundances, logistical difficulties associated with accessing

mesophotic reefs (Pyle 1998) mean that culling is largely restricted to shallow reefs.

With active management interventions absent from most MCEs it is therefore feasible

that deeper reefs are providing a refuge environment for invasive lionfish.

Many coral reef fish species are known to undergo ontogenetic migrations,

typically from shallow habitats (mangroves, seagrass beds and nearshore reefs) to

deeper reef habitats (Appeldoorn et al. 2003). These ontogenetic movements are

1   believed to be driven by trade-offs between maximising food availability (and thus

2   growth rates), while minimising predation risk (Kimirei et al. 2013). Some fish even

3   demonstrate multiple stages of ontogenetic habitat shifts, incorporating transition

4   habitats during intermediate life stages (Grol et al. 2014). Ontogenetic inter-habitat

5   migrations by lionfish have been previously reported, with juveniles found in

6   mangroves, seagrass beds and shallow sheltered reefs before migrating to reef slopes

7   (10-30 m) (Claydon et al. 2012). It is not clear whether lionfish populations on MCEs

8   represent a continuation of this ontogenetic migration, with lionfish primarily moving

9   from the shallows to MCEs, or a less depth-structured population with lionfish

10 movement in both directions between shallow reefs and MCEs.

11   This study synthesises data from multiple studies throughout the invaded and

12 native range of lionfish, encompassing both shallow and mesophotic depths, in order

13 to explore the role of deeper reef environments in invasive lionfish ecology and to

14 inform future management efforts. We use a meta-analysis approach to compare

15 adjacent shallow-mesophotic paired reefs to identify whether anecdotal large-scale

16 patterns in lionfish distributions are supported by available evidence. Specifically, we

17 address: (i) the relative abundance of lionfish across the depth gradient in both native

18 and invaded ranges; (ii) whether body size varies between shallow and mesophotic

19 reefs; and, (iii) how these patterns with depth are affected by the availability of hard

20 substrata, time since site invasion, and active management via culling.

21

Materials and methods

23 **Study design**

24   Study sites were identified through discussions at the Second International

25 Mesophotic Workshop (Eilat, Israel, October 2014). Mesophotic researchers at the

1　workshop, along with key collaborators, provided lionfishes (*P. volitans* and/or *P.*

2　*miles*) abundance and/or body length data from reef sites shallower than 30 m

3　(shallow) and adjacent deep reefs between 30-150 m (mesophotic). These depth zones

4　follow the widely accepted definition of shallow and mesophotic reefs (Hinderstein et

5　al. 2010), allowing broad patterns between the two to be tested. MCEs are broadly

6　defined as reef communities harbouring zooxanthellate corals, though the dominant

7　reef organisms can also be non-zooxanthellate corals, sponges or algae, that occur at

8　depths from 30 m to >150 m (Hinderstein et al. 2010). Lionfish abundance measures

9　were based on a variety of visual and video-transect techniques, but were consistent

10　within each shallow-mesophotic pairing (summarised in Table 1). Fish length

11　measurements used a combination of underwater visual estimates (broad size

12　categories), post-dive measurement after spearing, and stereo-video in situ

13　measurements (Table 1). Full details of all methods and site locations are included in

14　the Electronic Supplementary Materials.

15　　　For inclusion, data from each site were required to include the following

16　information for both shallow and mesophotic depths: (i) mean abundance and/or body

17　length of lionfish; (ii) standard deviation/standard error of mean; (iii) number of

18　replicates; (iv) depths for all replicates; (v) survey year; and, (vi) survey

19　methodology. Where possible, we also recorded the following: (i) year of lionfish site

20　invasion (non-native sites); (ii) shallow and mesophotic percentage hard-substrata

21　cover; and, (iii) presence/absence of regular lionfish culling. Hard substrata included

22　all benthic habitat types excluding mud, sand and rubble following Gratwicke and

23　Speight (2005). Presence/absence of regular lionfish culling was based on information

24　available from the data providers and, because of a general lack of intensity data,

1 culling was treated as a categorical variable defined as regular local spearing of

2 lionfish by divers.

3   In total, data incorporated lionfish abundance from 63 sites in seven western

4 Atlantic (invasive) countries and eight sites from three Indo-Pacific/Red Sea (native)

5 countries, with lionfish length data from 39 sites in six invasive countries and seven

6 sites in three native countries (Figure 1, Table 1). Shallow data ranged 5-30 m, while

7 mesophotic data ranged 30-110 m. While the majority of data are previously

8 unpublished, published data were also extracted from (i) broader fish community

9 studies with no direct comparison of lionfish populations across depth gradients,

10 specifically for The Bahamas (Lesser and Slattery 2011), Israel (Brokovich et al.

11 2008) and Puerto Rico (I. Bejarano et al. 2014), and (ii) a study on lionfish culling

12 effectiveness for Bonaire and some Curaçao sites (de León et al. 2013). When a site

13 had multiple survey depths within a single zone, data were combined and mean depth

14 (weighted by replication) used. Western Atlantic studies were totals of both invasive

15 lionfishes (*P. volitans* and *P. miles*), while Indo-Pacific studies were for *P. volitans*

16 (Micronesia, Philippines) and *P. miles* (Israel) separately, as these species do not co-

17 occur in these locations. While *P. volitans* and *P. miles* are distinct species, they seem

18 to be ecologically and morphologically analogous (Freshwater et al. 2009).

19 **Data analysis**

20   To compare sites surveyed using different methods, summary data from each

21 site were converted into a standardised effect size, Hedges' *d* (Hedges and Olkin

22 1985; Koricheva et al. 2013). Hedges' *d* (Equation 1) is based on the standardised

23 mean difference (SMD) between two groups (here depth zones), where $\overline{X_s}$ and $\overline{X_m}$

24 represent mean lionfish abundance or body length (respectively) at a single shallow-

25 mesophotic pairing. Hedges' *d* values can be positive or negative indicating the

1   direction and magnitude of effect, with d=0 representing no difference. Negative

2   effect sizes indicate greater abundances or larger lionfish at mesophotic depths, while

3   a positive effect size indicates the reverse. Pooled standard deviation (s_{pooled}) was

4   calculated from the standard deviation of means for shallow and mesophotic depths

5   (Equation 2), where s_s and s_m represent standard deviation, and n_s and n_m represent

6   number of replicates within a site for shallow and mesophotic reefs respectively. J

7   (Equation 1 and 3) is a correction for small sample sizes, allowing Hedges' d to be

8   reliable with small numbers of replicates.

9

10   $$d = \frac{\overline{X_s} - \overline{X_m}}{s_{pooled}} J \qquad\qquad\text{Equation 1}$$

11

12   $$s_{pooled} = \sqrt{\frac{(n_s-1)(s_s)^2 + (n_m-1)(s_m)^2}{n_s + n_m - 2}} \qquad\qquad\text{Equation 2}$$

13

14   $$J = 1 - \frac{3}{4(n_s + n_m - 2) - 1} \qquad\qquad\text{Equation 3}$$

15

16   The variance of each Hedges' d estimate was calculated using Equation 4.

17   $$v_d = \frac{n_s + n_m}{n_s n_m} + \frac{d^2}{2(n_s + n_m)} \qquad\qquad\text{Equation 4}$$

18

19   When fitting models using data obtained using different methods, between-site

20   heterogeneity in effect sizes must be considered (Koricheva et al. 2013). Under

21   standardised methods, heterogeneity between replicates tends to be from sampling

22   variance alone. To account for sampling variation within and between sites, we fitted

23   random-effects models based on Hedges' d estimates and variance. Random-effects

24   models were fitted to calculate summary effects for sites with and without lionfish

culling for each country, and overall for all sites within the invasive and native

ranges. Mixed-effects models were then used to test the influence of potential effect

modifiers on effect sizes and direction. Mixed and random-effects models were fitted

using the DerSimonian-Laird estimator (Koricheva et al. 2013), with sites weighted

by the inverse of their effect size variance for calculations of summary effects. To

identify within-study heterogeneity I^2 and H^2 statistics were calculated. I^2 quantifies

the percentage of heterogeneity that can be explained by between-study variance. By

calculating heterogeneity as a percentage, it is possible to directly compare I^2 values

between meta-analyses with different numbers of studies and effect metrics (Higgins

and Thompson 2002). H^2 is another related measure of heterogeneity with $H^2=1$

indicating homogeneity of treatment effects and larger values providing increasing

evidence of heterogeneity (Higgins and Thompson 2002). For testing the amount of

heterogeneity accounted for when fitting mixed-effects models, a *pseudo-R^2* value

was calculated following Raudenbush (2008). All analyses were conducted in the

statistical program R (R Core Team 2013) with effect sizes calculated using the

'escalc' function and models fitted and heterogeneity estimated with the 'rma.uni'

function in the metafor package (Viechtbauer 2010).

Results

Variation in lionfish abundance with depth

We found no overall difference in the abundance of lionfish between shallow

and mesophotic depths at invaded sites without culling (Figure 2a, SMD=-0.06, 95%

CI= -0.20, 0.32), or those with culling (Figure 2b, SMD=-0.20, 95% CI=-0.43, 0.03).

No individual invaded country exhibited differences in abundance between depth

1 ranges at sites without culling (Figure 2a). Differences were found in Bermuda where

2 culling takes place (Figure 2b), with abundance higher at mesophotic depths (SMD=-

3 1.16, 95% CI=-1.72, -0.61).

4   Native range sites exhibited similar abundance patterns to the western Atlantic

5 (Figure 3a; SMD=-0.14, 95% CI=-0.46, 0.17). When tested in a fixed-effects model,

6 there was no difference between the overall summary effect for Indo-Pacific sites and

7 either non-culled western Atlantic sites (z=-1.32, p=0.19) or culled western Atlantic

8 sites (z=0.38, p=0.70). Invasive lionfish populations therefore appear to exhibit a

9 similar depth-distribution pattern to those in their native range in terms of abundance.

10   Despite no overall difference in lionfish abundance between shallow and

11 mesophotic reefs, the magnitude and direction of each site's observed effect size

12 (SMD) was significantly correlated with both time since invasion and availability of

13 hard substrata (Table 2). There was a negative relationship between SMD and time

14 since invasion (t=-2.07, p=0.038), meaning surveys conducted soon after initial

15 invasion found a greater abundance of lionfish on shallow reefs compared to those

16 conducted at increased time intervals after invasion. Time since first lionfish

17 observation at our study sites ranged from 2-14 years. Shallow hard-substrata cover

18 also negatively correlated with effect size (t=-2.14, p=0.032), indicating that at sites

19 with greater shallow hard-substrata availability a greater abundance of lionfish was

20 found at mesophotic depths. Mesophotic hard-substrata cover was positively

21 correlated with effect size (t=2.76, p=0.006), suggesting that at sites with lower

22 mesophotic hard-substrata availability, a greater abundance of lionfish was found at

23 mesophotic depths. The presence of culling (Table 2) had no impact on the mean

24 difference in abundance between shallow and mesophotic sites. The addition of these

25 effect modifiers to the model accounts for 27.79% of the between-study variation

1   observed, with I^2 reduced from 40.79% (all sites random-effect model with no effect

2   modifiers) to 21.50%.

3

4   **Variation in lionfish body size with depth**

5   We found no overall difference in mean lionfish body length between shallow

6   and mesophotic invaded sites for all sites without culling combined (Figure 4a).

7   However, at the country level, those sites without culling in The Bahamas, Curaçao

8   and Honduras showed larger lionfish at mesophotic depths than shallow depths

9   (Figure 4a). Bonaire data showed no difference (SMD=-0.32, 95% CI= -1.24, 0.61),

10   while Puerto Rico had larger lionfish in the shallows (SMD=0.90, 95% CI=-0.36,

11   1.43). At sites where culling takes place, there was no difference in mean body length

12   between depth ranges (Figure 4b, SMD=0.18, 95% CI=-0.21, 0.57). Comparisons

13   within their native-range found no difference in body length between shallow and

14   mesophotic depths (Figure 3b, SMD=-0.37, 95% CI=-0.75, 0.01). This native range

15   summary effect was not different to non-culled invaded sites (z=-0.45, p=0.65), but

16   was different to invaded sites with culling (z=-2.19, p=0.028).

17   The differences in mean lionfish body length between invaded depth ranges

18   were affected by the time since first lionfish observation and the presence/absence of

19   culling (Table 3). Time since invasion had a negative relationship with effect size (t=-

20   3.42, p=0.001), indicating more initial similarity in fish lengths between depths, but

21   progressively larger fish on mesophotic and/or smaller fish on shallow reefs with

22   time. Presence or absence of culling was also highlighted as an important modifier

23   affecting length distributions (t=3.05, p=0.002), with different overall summary

24   effects for invaded sites with and without culling (z=-2.03, p=0.04). Neither shallow

25   nor mesophotic hard-substrata cover had an effect on lionfish body lengths. Including

these factors in the model accounted for 83.39% of the between-study heterogeneity, with I^2 in a random-effects model for all sites without effect modifiers reduced from 44.75% to 10.57%.

Lionfish length distributions have generally been found to be unimodal, however, cohort effects can occasionally be observed, particularly as new invasions occur (Trégarot et al. 2015). To incorporate lionfish length data from all sites, mean length had to be used. To test for potential bimodality, we tested the variance of lionfish lengths against depth for all shallow and mesophotic western Atlantic sites using Spearman's rank correlation coefficient. Our results do not suggest any consistent difference in unimodal or bimodal length distributions in either depth zone, as we found no relationship between length variance and depth for non-culled (*rho*=<0.01, *p*=0.99, *n*=92) or culled (*rho*=-0.191, *p*=0.13, *n*=64) western Atlantic sites.

Discussion

Invasive lionfish have widely colonised mesophotic reefs throughout the western Atlantic and at the time of surveying exist at densities similar to those on nearby shallow reefs, which have to date received the vast majority of research and management attention. Importantly, we also show that individual lionfish in some locations are larger on mesophotic reefs than their shallow counterparts. These findings raise important questions about the role of MCEs in lionfish life history, such as whether MCEs are acting as depth refuges for invasive populations, and so providing a local source of recruits despite removal efforts. Our results also indicate that without direct human intervention via culling the relative abundance and length

1   distributions between shallow and mesophotic depths appear similar for invaded and

2   native sites, suggesting a similar role for MCEs between the two regions.

3

4   **Mesophotic reefs as a potential refuge for invasive lionfish**

5   Across the western Atlantic region, we found no difference in lionfish

6   abundances between shallow and mesophotic depths, highlighting an urgent need to

7   integrate deeper reefs into lionfish management plans, which currently focus almost

8   exclusively on removal from shallow environments. Supported by previous shallow-

9   reef research (e.g. Claydon et al. 2012), combined with our results of (i) no difference

10   in lionfish abundance between depth zones in most locations, (ii) larger lionfish on

11   MCEs in three locations, and (iii) increasing proportional abundance and mean length

12   of lionfish found on MCEs with greater time since invasion, we propose that lionfish

13   population resilience benefits from substantial mature mesophotic lionfish

14   populations (Figure 5). Specifically, lionfish invade new sites at shallow depths via

15   larval settlement from floating egg bundles, with juveniles and then adults

16   subsequently migrating to greater depths, which happen to be below the range of

17   lionfish control programs.

18   At first glance, this presents a major challenge to lionfish managers, as culling

19   programs are often restricted to shallower depths through a reliance on volunteer

20   recreational divers to achieve sufficient effort and by the financial and logistical

21   complications of technical diving (Pyle 1998). Lionfish culling is most effective if

22   adults of all age classes are targeted (Arias-González et al. 2011), with shallow

23   culling resulting in reduced average lengths and abundance of shallow individuals

24   (Frazer et al. 2012), while work on other species has shown that fishing at restricted

25   depth zones can cause fine-scale length-distribution changes (Lindfield et al. 2014).

1   Previous research into lionfish depth distributions at invaded locations absent

2   of culling has proved inconclusive. Claydon et al. (2012) reported greater sightings of

3   lionfish, per unit effort, at 10-30 m than at 0-5 m in the Turks and Caicos Islands, and

4   Lee et al. (2012) found greater abundances of lionfish with larger body lengths on

5   surveys deeper than, compared to shallower than, 13 m in Jamaica. In contrast, in

6   their native ranges, McTee et al. (2014) found lionfish abundance to be highest at

7   shallow depths in the Red Sea, as did Kulbicki et al. (2012) in their review of Indo-

8   Pacific literature. Our data suggest these differing patterns are likely partially driven

9   by varying availability of hard substrata and linked factors (e.g. structural

10   complexity), which have been shown to be important in lionfish aggregations (S.

11   Bejarano et al. 2014). Although lionfish abundance is significantly higher in the

12   western Atlantic than in their native range (Darling et al. 2011; Kulbicki et al. 2012),

13   we identified similar patterns in relative abundance and mean body length

14   distributions across the depth gradient at native sites as we found at invaded sites

15   without culling. This suggests lionfish are using MCEs in their invaded range in

16   similar ways as their native counterparts and, thus native-range studies can support

17   and inform our understanding of the western Atlantic invasion.

18   Lionfish populations in their native and invaded geographical range limits are

19   likely to face different structuring processes affecting their abundance and

20   distribution. In Bermuda, it has been noted that lionfish are rarely observed in shallow

21   reef habitats, but are much more common on deep fore-reef slopes (Smith et al. 2013).

22   While all sites in our study from Bermuda were culled, it is possible that, at higher

23   latitudes, physical factors such as temperature and wave energy may be the most

24   significant factors driving lionfish-abundance distributions across depth gradients. For

25   example, work by Whitfield et al. (2014) in North Carolina, USA, at the northern

16

1   limit of the established populations, suggests that lionfish abundance across the

2   shallow-mesophotic depth gradient correlates strongly with minimum winter water

3   temperatures. In Bermuda, inshore shallow habitats experience lower winter water

4   temperatures than deeper sites further offshore (Coates et al. 2013). These low winter

5   inshore temperatures are below the temperature threshold at which experimental lab

6   studies have suggested lionfish cease feeding (Kimball 2004). This potentially drives

7   the larger abundances observed on MCEs compared to shallow habitats. We suggest

8   that further studies on lionfish ecology in Bermuda should focus on the interaction

9   between culling-based management and seasonal water-temperature variation in order

10   to better understand whether temperature has a more significant role at the invasion

11   range limit.

12

13   **Lionfish body size and depth**

14   The idea of a deep refuge for invasive lionfish was previously modelled by

15   Arias-González et al. (2011), who found that if larger lionfish exist below depths

16   accessible to culling programs, it will be harder to reduce and control the overall

17   population. There are several possible explanations for larger lionfish being found

18   below culling depths. When spawning, lionfish produce buoyant egg bundles that drift

19   on the ocean's surface for several days before breaking down (Morris et al. 2009),

20   leaving the embryos/larvae free floating with a pelagic larval duration of between 20-

21   35 days (Ahrenholz and Morris 2010). Settlement is then believed to primarily occur

22   in mangroves, seagrass beds, and shallow reef environments (<5 m) (Claydon et al.

23   2012). These shallow ecosystems are used by juvenile lionfish, before individuals

24   migrate to intermediate depth reefs (10-30 m) as they mature (Claydon et al. 2012)

25   (see Figure 5), and this migration could extend into MCEs. Alternatively, the presence

1   of larger lionfish on deeper reefs could be caused by culling biases at shallow depths,

2   disproportionately reducing the number of larger lionfish and so giving the appearing

3   of increasing body size with depth. This could be driven by higher detection rates for

4   larger lionfish than smaller lionfish in culling programs (Green et al. 2013)

5   While we did not find consistent patterns in lionfish body size with depth,

6   additional factors such as time since invasion are likely to explain this discrepancy. In

7   three invaded countries we found larger lionfish at mesophotic depths (The Bahamas,

8   Curaçao, Honduras), in Bonaire, we found no difference in lionfish body lengths with

9   depth, while in Puerto Rico, we found larger lionfish in the shallows. Puerto Rican

10   studies were conducted two years after lionfish invasion, the shortest time interval

11   after invasion of any included studies, so it is possible these studies do not represent

12   the natural length distribution with depth for an established lionfish population. We

13   found a negative correlation between time since invasion and the SMD in lionfish

14   body length, suggesting this short time since invasion explains why we did not find

15   larger lionfish at mesophotic depths in Puerto Rico. Individual country results must be

16   interpreted with caution before generalisation, as some countries are only represented

17   by several sites within the analysis (see Table 1). However, these broad country-level

18   results provide support to the hypothesis that invasive lionfish on MCEs are an

19   extension of the established mangrove, seagrass and shallow reef (>5 m) to

20   intermediate depth reef (10-30 m) ontogenetic migrations (Claydon et al. 2012).

21   However, research studying movement patterns through tagging, stable isotope or

22   otolith age-structure analysis in lionfish across the depth gradient are needed to

23   confirm these ontogenetic movements. These studies should be conducted to allow

24   comparisons between areas with regular culling and those without to establish the

25   influence on culling at finer scales than has been possible in this analysis.

1

Culling changes lionfish movement and behaviour

3   Our data suggest shallow culling (<30 m) leads to increased similarity in

lionfish body lengths across the depth gradient, or even to an increase in larger

individuals at shallow depths compared to MCEs. As we treated culling as a

categorical variable, many subtle impacts of differing culling effort may be hidden.

Future work would benefit from detailed culling records (e.g. frequency, intensity,

time of day, experience of cullers) to better understand its impacts. A likely response

to shallow culling would be changes in movement patterns of lionfish. It has

previously been hypothesised that ontogenetic fish migrations may be reduced when

competition between individuals in shallow habitats is limited (Appeldoorn et al.

2003), meaning those lionfish that avoid culling may mature at shallower depths. Yet

this hypothesis is based on consistent mortality rates, whereas applying culling

increases shallow lionfish mortality. However, we found similar lionfish abundance

effects when comparing shallow and mesophotic reefs regardless of the presence of

culling. This could suggest that culling on shallow reefs did not cause a significant

decline in shallow lionfish abundance, but there is strong evidence to suggest this is

not the case (Frazer et al. 2012). There are several biological explanations possible,

for example: (i) individuals may be re-colonising shallow reefs from adjacent deeper

reefs; (ii) an established mesophotic lionfish population may have been absent before

shallow culling was effectively implemented; or alternatively (3) this was simply

caused by low power in our analysis. Previous studies of lionfish movement have

focused within the shallows and, while the majority were found to have high site

fidelity, there are multiple records of individuals moving more than 1km (Côté et al.

2013; Akins et al. 2014). This would suggest that the relatively small distance

between adjacent shallow and mesophotic reefs is within their natural movement

range. However, movement from deeper areas without culling into shallow areas

under continued culling runs counter to expected fish behaviour, as we would expect

individuals to avoid areas with increased mortality risk (Werner and Gilliam 1984;

Kimirei et al. 2013), although a large reduction in competition could outweigh the

potential mortality risk. Further research is required to address this, focusing on

specific movement studies of individual fish in culled areas across the depth gradient.

Lionfish culling reduces the number of lionfish in culled areas (Frazer et al.

2012), but also affects lionfish behaviour, making lionfish more cautious during

daylight hours (Côté et al. 2014). Therefore, this combination of active removal

combined with increasingly cryptic behaviour suggests we should observe greater

relative lionfish abundances at deeper depths at culled sites than sites without culling.

However, we do not see an effect of culling on lionfish relative abundance

distribution across the depth gradient. When lionfish engage in increased cryptic

behaviour, smaller lionfish become more easily hidden than larger individuals leading

to greater detection rates for larger lionfish on lower structural complexity sites

(Green et al. 2013). This process could explain our observations that higher

abundance was correlated with areas of lower hard substrata, and that following

culling we found similar mean body sizes at shallow and mesophotic depths, yet in

the absence of culling we saw three countries with larger lionfish at depth.

While organised culling has been the focus of most lionfish management

efforts, there is increasing interest in developing fisheries for lionfish and promoting

market demand for invasive lionfish consumption (Bogdanoff et al. 2014). Switching

focus from a government-led invasive species control effort to a market-based

fisheries approach could bring substantial increases in lionfish removal (Bogdanoff et

al. 2014). For example, in The Bahamas at the Cape Eleuthera Institute, a small

marine research centre, their 2011-2015 invasive lionfish-culling program resulted in

the collection of 55 kg of lionfish, whereas a locally developed lionfish fishery

collected 680 kg of lionfish in 2015 alone (J Curtis-Quick personal communication).

Larger lionfish on MCEs could potentially be a target for future fisheries, especially

as fishers have previously reported them from MCEs in lobster traps and by hook and

line (Bogdanoff et al. 2014). While hook and line is not considered appropriate for

widespread lionfish collection (Bogdanoff et al. 2014), there has been much interest

in developing a lionfish trap that can be deployed while minimizing bycatch (Pitt and

Trott 2015). Traps can remove substantial numbers of MCE lionfish – for example,

over 1,200 lionfish were removed from 40-80 m depth around Bermuda as bycatch in

commercial lobster traps between September 2013-March 2014 (Pitt and Trott 2015).

However, in many locations, lionfish trapping has not been achieved effectively at a

large scale (Morris et al. 2011) and further research and development of traps is

required before trapping can be widely adopted. When developing lionfish fisheries as

a conservation management strategy it is important to concentrate on keeping lionfish

populations low, as there is a risk that established fisheries could become focused on

managing for continued lionfish harvests. With these current technological limits

most current MCE lionfish removal is carried out by technical divers using hand

spears. With recent reduced costs, and increased interest from the recreational dive

community in technical diving (Mitchell and Doolette 2013), it is becoming possible

to incorporate deeper culling depths into lionfish management programs. For

example, in 2016 the Bay Islands Conservation Association (the organisation with

responsibility for lionfish management on Utila, Honduras) began to include a

technical diving category in their annual lionfish derby to encourage technical divers

21

1   to become involved in lionfish removal efforts and specifically target MCEs (M

2   Arteaga personal communication).

3   Our results raise critical questions about the role of MCEs as a refuge for

4   lionfish from culling and whether shallow culling program impacts are limited to

5   shallow reefs or able to affect deeper populations. With increased interest from

6   technical divers combined with technological development in lionfish traps, it is

7   becoming possible to incorporate deeper culling depths into lionfish management

8   programs. In addition, if mesophotic lionfish populations are dependent on lionfish

9   recruitment in shallow marine habitats for new individuals, then infrequent deep reef

10 culling combined with intense regular shallow culling could be sufficient to reduce

11 the overall population and maximise the chances of complete localised eradication

12 where culling intensity is sufficient.

13

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36   **Figure Legends**

37   Figure 1. Western Atlantic lionfish survey locations included in this study, and
38   surveyed between 2009-2014. Multiple paired shallow-mesophotic sites were
39   located within the marked region for each country (see Electronic Supplementary

1   Materials for individual sites).
2

3   Figure 2. Summary random-effect models for lionfish abundance at western Atlantic
4   sites with countries presented individually and grouped, showing a) sites without
5   active lionfish culling and, b) sites with active lionfish culling. Diamonds centre
6   represent the mean summary effect, and the width the 95% confidence interval
7   (CI). Diamonds intersecting the dashed vertical line indicate the summary effect
8   is not significantly different to zero, while n= number of study sites (each site
9   contains multiple replicate surveys – see Electronic Supplementary Materials for
10   individual site information), I^2= residual heterogeneity/unaccounted variability,
11   H^2= unaccounted variability/sampling variability, SMD= Standardized Mean
12   Difference, and NA=values not able to be calculated due to small sample sizes.
13   Positive effect sizes (SMD) indicate greater lionfish abundance on shallow reefs
14   than mesophotic reefs, while negative effect sizes indicate the reverse.

15

16   Figure 3. Summary random-effect models for a) lionfish abundance and b) mean
17   lionfish body length at Indo-Pacific reef sites, with countries presented
18   individually and grouped. Positive effect sizes (SMD) indicate greater abundance
19   or larger mean body length of lionfish on shallow reefs than mesophotic reefs,
20   while negative effect sizes indicate the reverse. See Figure 2 legend for full
21   description.

22

23   Figure 4. Summary random-effect models for lionfish body length at Caribbean sites
24   with countries presented individually and grouped, showing a) sites without
25   active lionfish culling and b) sites with active lionfish culling. Positive effect
26   sizes (SMD) indicate larger mean lionfish body length on shallow reefs than
27   mesophotic reefs, while negative effect sizes indicate the reverse. See Figure 2
28   legend for full description
29

30   Figure 5. Schematic diagram of the proposed lionfish distribution across the shallow-
31   mesophotic depth gradient. Lionfish initially invade new sites at shallow depths,
32   before moving to deeper reef areas beyond the range of lionfish control measures
33   (culling).

34