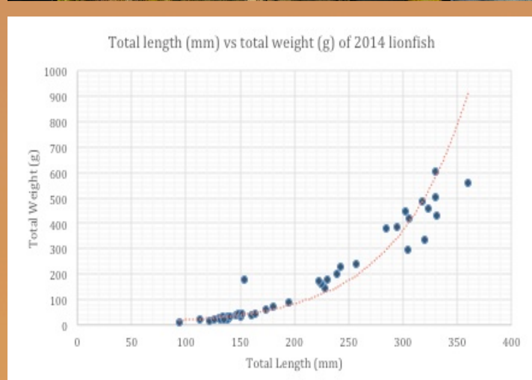
LIONFISH ECOLOGICAL REPORT 2015

Project Report

Lionfish population study of Port Honduras Marine Reserve: 3 year summary report 2011-2014

Results of 2014 landing site surveys & comparison with previous studies

Holah H, Foley J 2015



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A continuation of the lionfish diet and abundance survey in Port Honduras Marine Reserve, Belize

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Abstract

The invasive lionfish species *Pterois volitans* and *Pterois miles* has rapidly colonized many areas of the Western Atlantic and Caribbean in the past 20 years, spreading from Boston, USA to the northern shores of South America. Once established, lionfish pose a serious threat to their new environment, both through direct predation of crustaceans and juvenile fish and through out-competing local predators. Their extremely fast growth and reproduction rates allow them to quickly overwhelm ecosystems, and their efficient hunting techniques and venomous spines mean they have met little resistance from native Caribbean fish.

In Port Honduras Marine Reserve (PHMR), southern Belize the lionfish invasion has been relatively recent and *P. volitans* populations are considered not yet as expansive or as well established as in nearby areas such as Placencia. However lionfish still pose a great threat to the region as their effects both on the local fishing community and the protected conservation area could be severely detrimental. Previous surveys in 2012 and 2013 have characterised the population size and density trends of lionfish within the reserve and provided comparisons with the Placencia population to determine the probable stage of the invasion in PHMR. This report continued the data collection of the two previous years and summarized the findings of all three studies. The population in PHMR has continued to grow and expand into the reserve since at least 2012, a total of 41 lionfish were recorded in 2014, 23 of which were juveniles. The diet of the PHMR population has shown a gradual shift from fish to shrimp dominated over time, with small juveniles showing a far stronger preference for shrimp than mature individuals.

1. Introduction

The colonization of the Western Atlantic, Caribbean Sea and Gulf of Mexico by the invasive lionfish species *Pterois volitans* and *Pterois miles* has occurred rapidly over the course of around 20 years (1992-2014) (Schofield, 2009). Lion fish now occupy almost the entire eastern seaboard of North America, with confirmed sightings as far north as Boston and as far south as the northern coasts of Panama, Colombia and Venezuela (Fig 1).

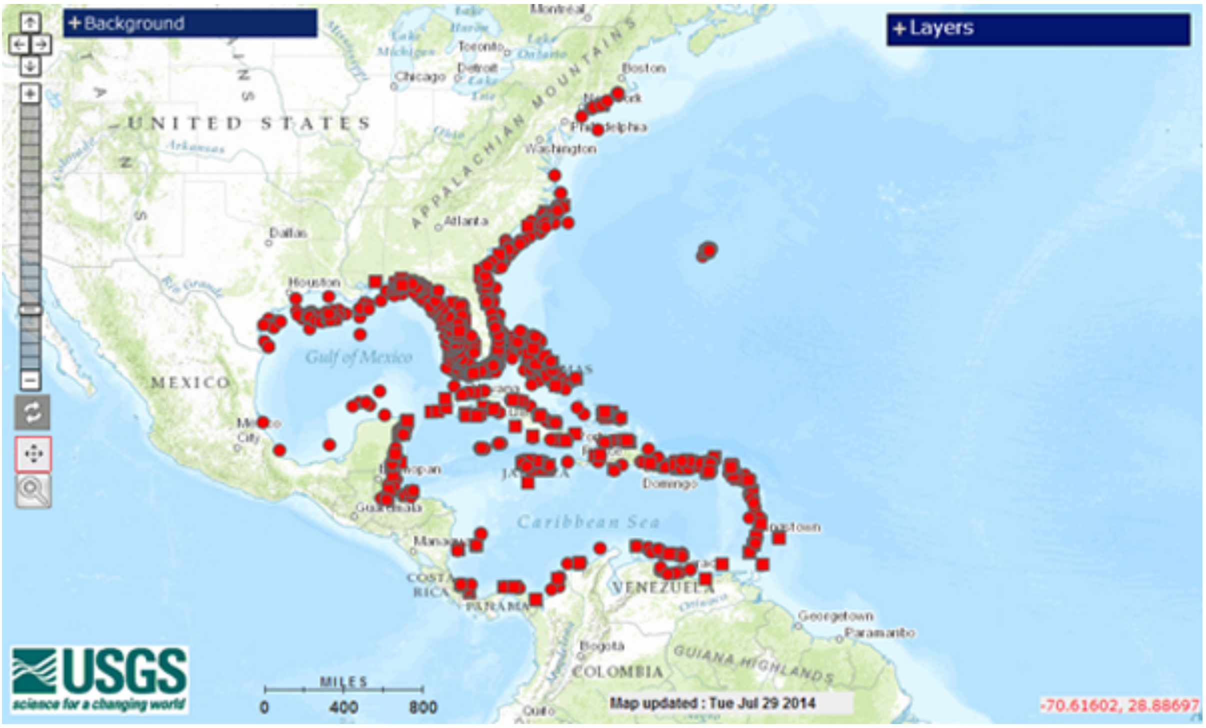

Figure 1: United States Geological Survey map showing the current spread of the lionfish invasion as of 2014 (USGS, 2014)

P. volitans display many characteristics that make them particularly successful invaders; their individual growth and reproductive rates are unusually high, with some mature females capable of spawning every 2-3 days (Gardner, 2015) and possessing an annual fecundity of over two-million eggs per individual (Morris & Whitfield, 2009). These features lead to remarkable growth rates, resulting in invasive lionfish now showing far greater population densities than in their natural Indo-Pacific range, with densities at some sites in the Bahamas reaching over 390 fish per hectare (Green, 2009). They are generalist predators, preying mainly on teleosts and crustaceans (Morris, 2009), and are protected from most potential predators by venom secreted from their 13 dorsal fin spines, 3 anal fin spines and 2 pelvic fin spines (Ruiz-Carus, 2006). The cryptic colourations and hunting behaviours shown by *P. volitans* are unfamiliar to many Atlantic fish, meaning their prey species are often too naïve to these behaviours to evade predation, resulting in lionfish having far higher consumption rates than similarly sized native predators that are outcompeted (Albins, 2013). They are also highly adaptable, capable of overwintering in temperatures as low as 10°C (Kimball, 2004), living at depths varying from shallow reefs to mesophotic depths of 91m (Lesser, 2011) and occupying a range of habitats including coral reef, mangrove, seagrass, sandy beach and in some cases even canal and estuarine habitats (Schofield, 2009; Jud, 2011), suggesting they may be able to tolerate relatively low levels of salinity.

In the study area of the Port Honduras Marine Reserve (PHMR) in southern Belize the lionfish population is currently less established and extensive than those of other sites in the region, such as areas nearby the resort town of Placencia, meaning that it may be possible to predict future population trends to better inform management and environmental control techniques before the invasion reaches a stable level. The presence of lionfish in an area has severe effects on native fish populations both via predation and competition; juvenile populations are particularly at risk as lionfish may reduce net recruitment by up to 79% (Albins, 2008). With the Port Honduras area supporting both a local fishing economy and a marine protected area, the establishment of a lionfish population could have devastating effects on both the local economy and the biodiversity and overall health of the reserve ecosystem.

2. Background/Rationale

Previous surveys conducted in the summer months of 2012 and 2013 confirmed the presence of lionfish at several sites within the reserve and determined local trends in population and individual size distributions (McMahon et al, 2012; Flores et al., 2013). Whilst both years found a lower population density and smaller average individual size than in the comparison population at Placencia the 2013 survey showed a significant increase in lionfish catch per unit effort, and that lionfish were now present in three previously unoccupied sites, though the majority of individuals were still found in the deeper waters on the outer edge of the reserve (Flores et al., 2013).

Between 2012 and 2013 the average individual size of PHMR's lionfish had increased, with the majority now occupying the 20-30cm range, indicating a more mature population. *P. volitans* reaches sexual maturity at 1-2 years old and 10 & 18cm in length respectively for males and females (Morris, 2009), as all of the lionfish found in 2013 were above this size it is likely that there is now a breeding population in the reserve. However, no lionfish of juvenile size (<18cm) were found in the 2013 survey; many Caribbean reef fish species remain in nursery sites such as seagrass beds until they are large enough to move to more dangerous areas (Jud, 2012). It is not currently known how lionfish use different habitats throughout their lifespan, and the Caribbean population show significant behavioural differences to lionfish in their native range, however it is important to establish whether or not recruitment is occurring as the presence of juveniles would indicate that population figures are likely to continue to rise in upcoming years.

In the past two years surveys Port Honduras lionfish have shown a substantial dietary preference of fish over shrimp (92% by number of 2013 stomach contents were identified as fish), particularly when compared with Placencia's population (36% of 2013 stomach contents identified as fish). Whilst these differences may simply be due to localised preference it is possible that the PHMR juvenile fish populations have not yet been sufficiently reduced for lionfish to start targeting shrimp as a primary food source, indicating that it may still be possible to prevent the devastating losses to biodiversity seen in other Caribbean locations with carefully planned management techniques. As complete eradication of invasive lionfish is unlikely to be achieved the population must be managed as effectively as possible, which will require extensive knowledge of population trends and behaviours.

3. Aims and Objectives

Objective 1: To collect data on life history parameters including gender, maturity, total length (mm) and weight (g) of the PHMR lionfish population

Objective 2: To examine trends in abundance and distribution of the PHMR lionfish population over the years 2012-2014

Hypothesis 1: Both total abundance and total catch per unit effort (CPUE) within PHMR will have increased from 2012 to 2014

Hypothesis 2: Abundance and CPUE will have increased at the 12 comparison sites surveyed across all three years

Objective 3: To confirm the presence of a breeding population containing juveniles, and to examine the possible existence of nursery sites within seagrass or mangrove habitats

Objective 4: To investigate possible dietary trends, both over time and between subsets of the population (e.g.: juveniles/adults)

Hypothesis 3: The population will show a shift in dietary preference from fish to shrimp over time

Hypothesis 4: Juveniles will show a preference for shrimp over fish, whilst adult lionfish will prefer fish to shrimp

Objective 5: To observe and record the behaviors shown by the PHMR lionfish

Objective 6: To investigate a possible link between the presence or absence of eyestalks and lionfish maturity

Hypothesis 5: As maturity increases from juvenile to adult prevalence of eyestalks will decrease

Objective 7: To make recommendations for future monitoring and management

4. Methodology

4.1 Pilot Study

During the period of the 15th to 19th of September a five day pilot study was undertaken in which ten sites were surveyed to test the proposed methodology and identify any flaws that required addressing. Potential sites were identified using information from previous studies, however some sites that had not been surveyed before were also included. Sites were selected to include a variety of depths and habitat types, including sea grass, patch reef and coral habitats. Of the ten sites chosen two were sea grass habitats, three fringing reef, one patch reef and four bank reef. The distribution of sites (Fig 2) allowed for good coverage of the deeper areas of the reserve, where lionfish showed the strongest presence in previous years, as well as including some shallower coral and sea grass sites. Two of the sites chosen – Daily Bank and Spanish Bank – were located in the deep waters outside of the reserve and provided insight into population sizes in the surrounding area. In the main study 7 of the 10 sites from the pilot study were surveyed again and 3 new sites – Moho Caye, Middle Snake Caye and Abalone Caye – were selected to replace the sites not being revisited.

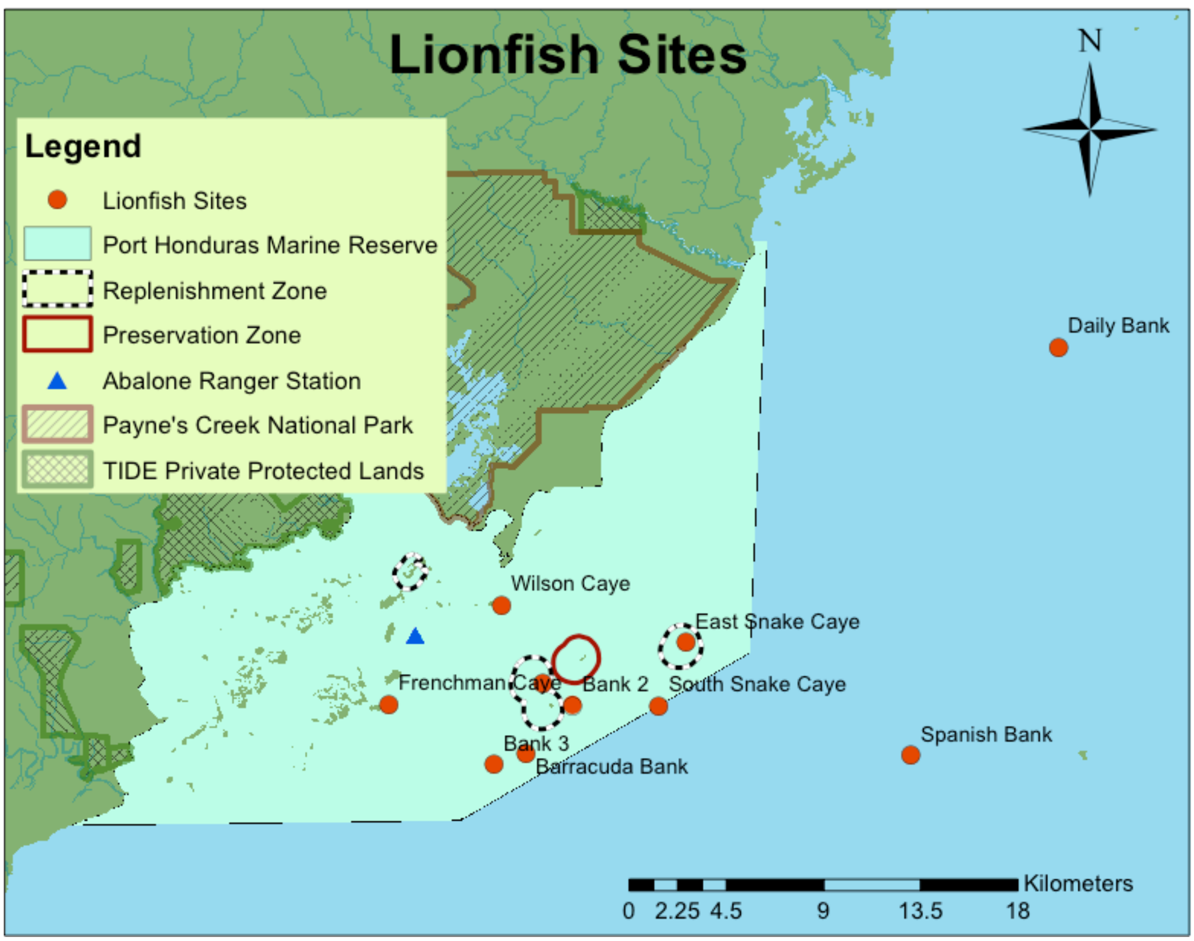

Figure 2: Distribution of dive sites surveyed during the pilot study

4.2 Dive and Observation Methods

At each site a survey dive was conducted using a rover diver method by 1-7 divers (with the majority of sites being surveyed by 2 divers) within a 100m radius of an initial GPS location taken just prior to entering the water. Dive times ranged from 29 to 50 minutes with a mean time of 36 minutes. Where lionfish were sighted their behaviour was observed and recorded, as well as their proximity to other fish and whether they were solitary or found in a group, using a behavioural ethogram (Fig 3) on a waterproof slate. If any individual regurgitated its stomach contents upon spearing this was also recorded due to its effect on later dissection data.

Behaviour	Individual 1	Individual 2	Individual 3	Individual 4
Active (Hunting)				
Active (Swimming)				
Active (Hovering)				
Inactive (Immobile in contact with coral/seafloor				
Alone (no other lionfish present)				
Grouped (other lionfish present)				
Close to other fish (within 1m)				
Isolated (no fish within 1m)				
Regurgitation upon spearing				

Figure 3: Ethogram designed to record lionfish behaviours observed before spearing

To capture lionfish a triple pronged trident spear was used. These spears are currently legal to use on lionfish as they pose a minimal threat to the surrounding environment. The short range of these spears means they are only effective when fired from within 5 inches of the fish, and are not effective at all on larger fish such as groupers. After spearing lionfish were stored in a cooler without ice until dissection.

4.3 Dissection methods

Dissections were carried out on each individual according to the methodology set out by the NOAA (Green et al., 2012). First any excess water was removed by blotting the lionfish with a paper towel so as to avoid inaccuracies when measuring total weight. The individual was then placed on a scale ensuring that no part of the body was touching the table (Fig 4) and total weight (g) was recorded.

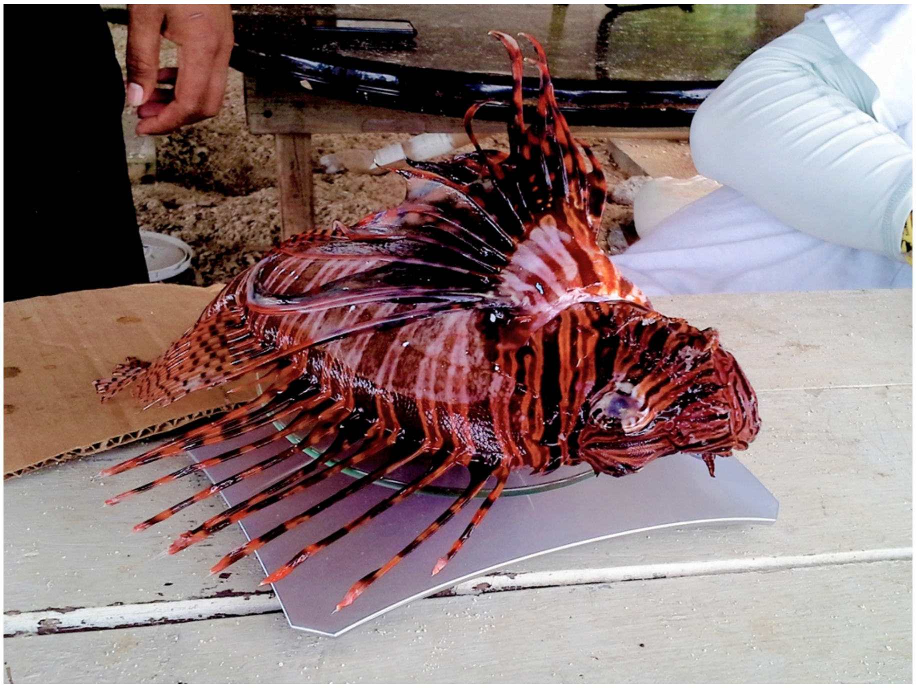

Figure 4: Measuring total individual weight (g) after removing excess water

After weighing, Total Length (mm) – defined as the distance from the tip of the snout to the longest point of the caudal fin – and Standard Length (mm) – the distance from the tip of the snout to the last vertebra – were measured with a meter rule and recorded prior to beginning dissection, whilst calipers were used to determine gape width and height (mm) with the mouth extended to its fullest extent. The number of eyestalks present was also noted at this stage. Once all the external measurements had been made the venomous spines were removed to minimize the risk of being stung during dissection.

To access the gut cavity an incision was made from the urogenital opening to the rear edge of the gill arches, and then upwards towards the dorsal fin before lifting the flank to expose the internal organs. The gonads were identified and used to determine each individual's gender and reproductive stage (Appendix 1 Fig 1). The stomach was removed by severing the esophagus at its termination, and then opened with a cut along the length of the stomach wall. Stomach contents were removed and the weight (g) and length (mm) of each individual item was measured and recorded (Fig 5). In keeping with the two previous studies prey items were described as either 'whole' or 'degraded' and identified to the highest possible level.

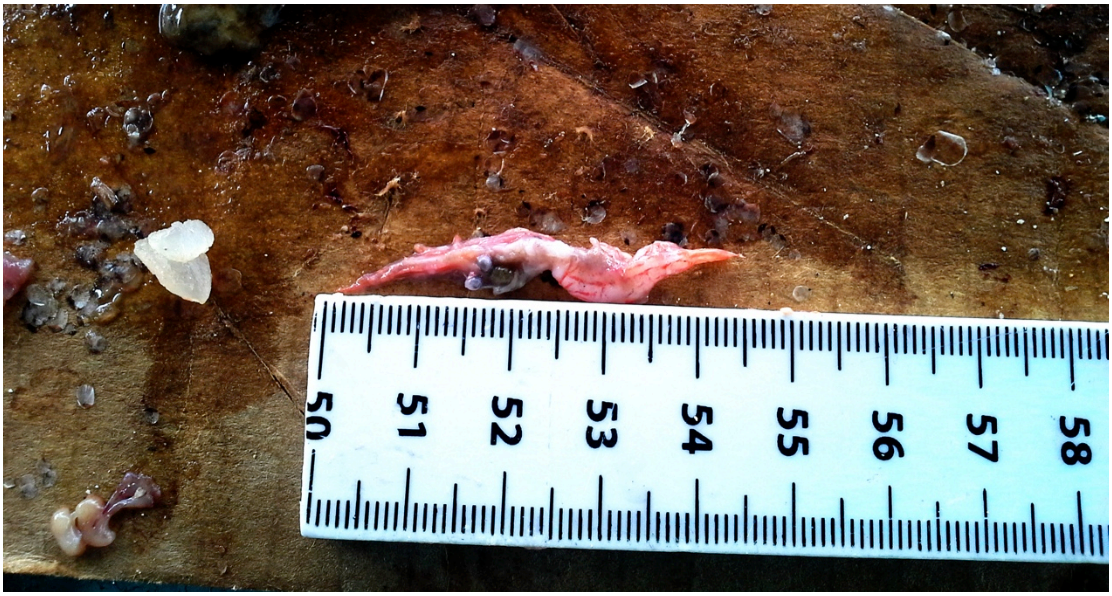

Figure 5: Measuring individual gut content items

In order to determine age the sagittal otoliths were removed from each lionfish and sent for processing. First the head was removed with a vertical cut from the dorsal spine to the gill openings, then the gill filaments were removed to allow easier access to the cranial cavity. Once the cranial cavity was located a cut was made on its rear side through the spine to open the cavity. The free floating otoliths were extracted using tweezers, taking care not to cause any damage. After extraction otoliths were cleaned with deionized water and dried before storage.

5. Results

5.1 Objective 1 – Life History Parameters

A total of 55 lionfish were observed and 41 caught in 2014. For lionfish that were observed but not caught behavioral data and estimated size (to the nearest 10cm) were recorded, but no physical measurements could be taken. The recorded total lengths varied from 94 to 360mm, with a mean of 210mm. The minimum and maximum recorded weights were 12g and 603g respectively, with a mean weight of 185g. Figure 6 shows the total length and weight of each lionfish caught in 2014. See Appendix 2 Table 1 for all length and weight values.

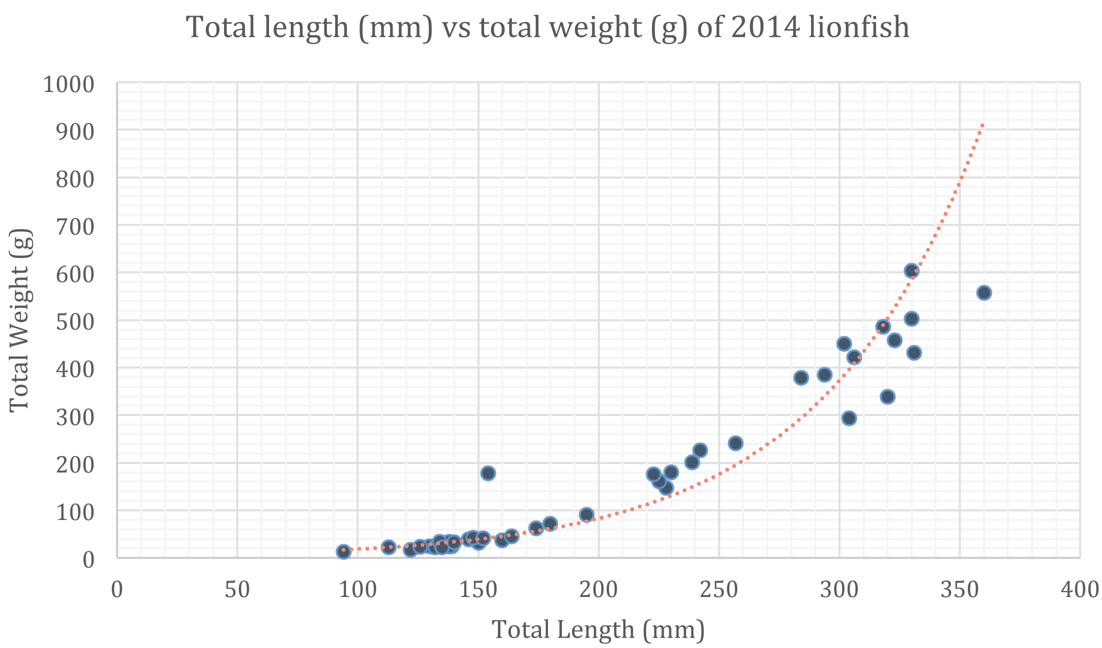

Figure 6: Total lengths (mm) and total weights (g) of the 2014 PHMR lionfish population

Of the 41 individuals caught in 2014 17 were male and 7 female, 17 juveniles were too immature for their gender to be accurately determined by dissection alone (Figure 7).

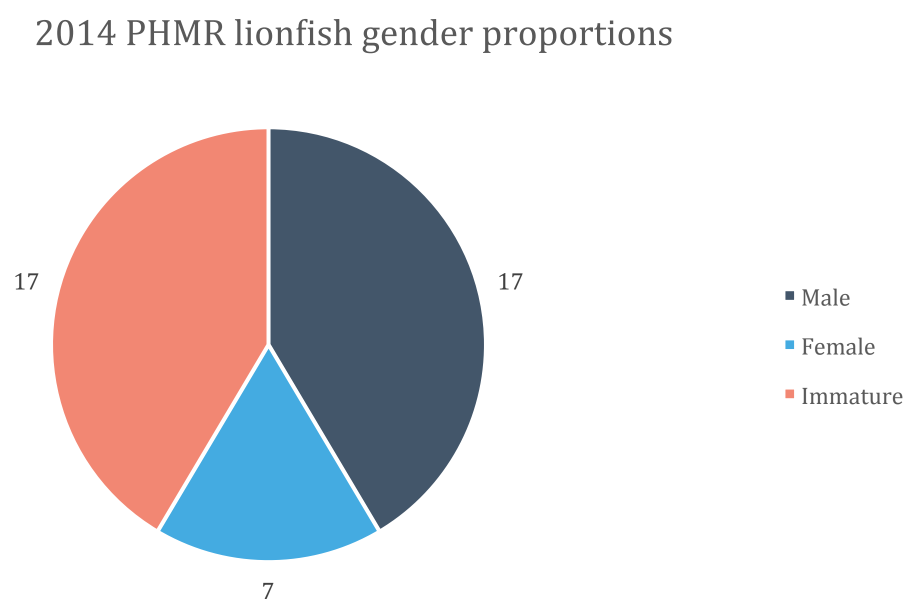

Figure 7: Gender proportions of the 2014 PHMR lionfish population

Figure 8 shows the levels of maturity of the 2014 population; although both juvenile and spawning capable individuals were recorded, no actively spawning females (females carrying eggs) were found.

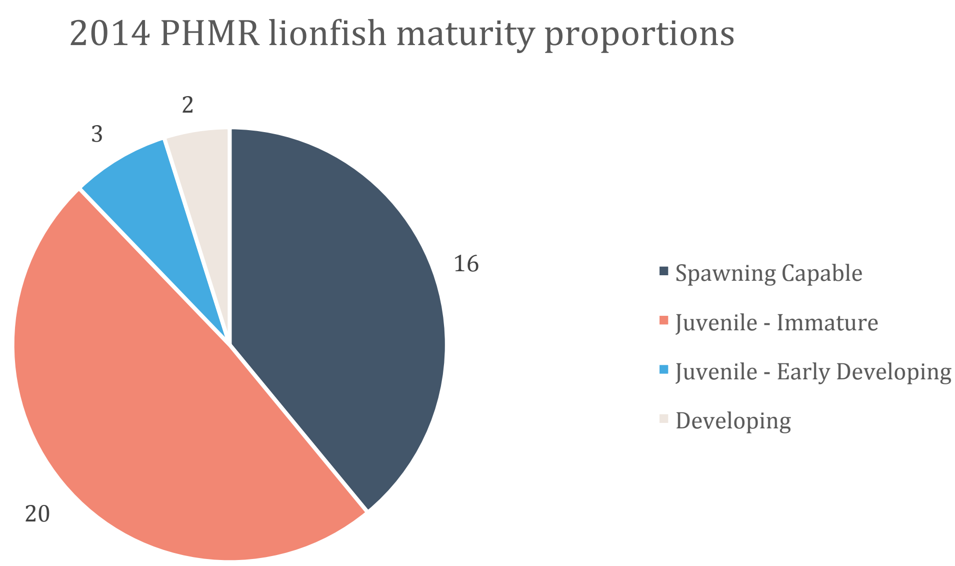

Figure 8: Maturity proportions of the 2014 PHMR lionfish population

5.2 Objective 2 – Abundance and Distribution

Lionfish were found to be present at 10 of the 14 sites surveyed in 2014. The sites with the greatest number of lionfish were Middle Snake Caye (9 caught, 12 observed) and Bank 3 (8 caught, 9 observed). No lionfish were found at East Snake Caye, Bank 2, Frenchman Caye or Wilson. Figure 9 shows the number of lionfish caught and observed at each of the 2014 survey sites.

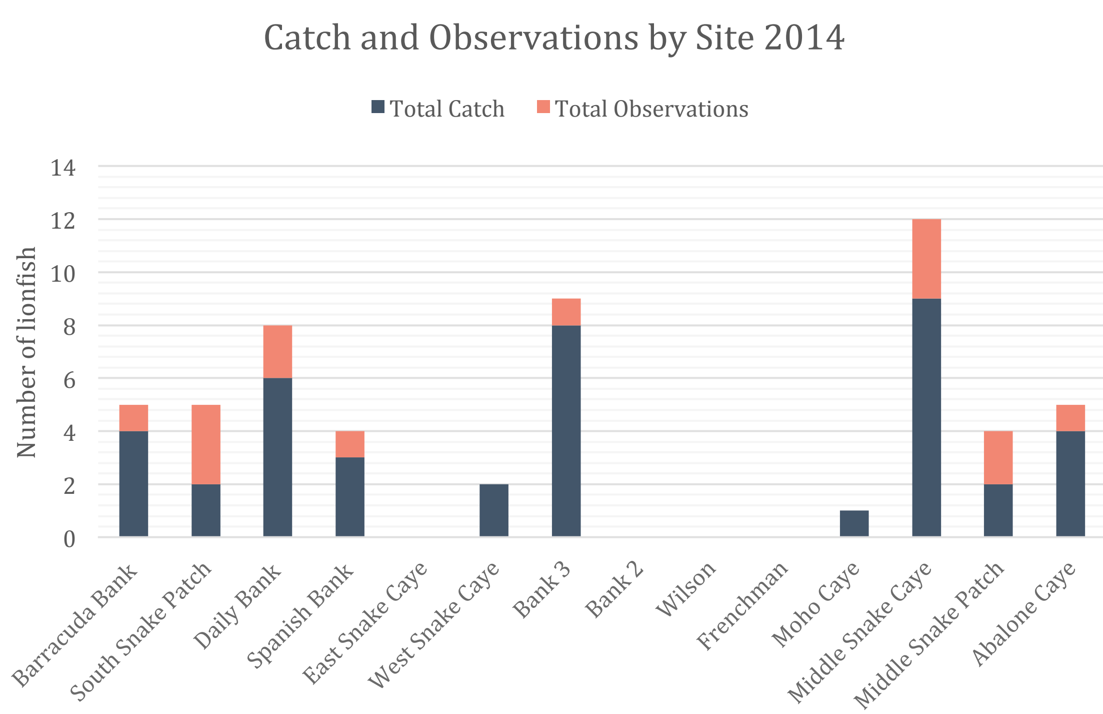

Figure 9: Total lionfish catch and observations at each of the 2014 survey sites

Catch per unit effort (CPUE) was calculated for each site by dividing total catch by total man hours of search time (total man hours = total search time X no. of divers). Figure 10 shows the CPUE and OPUE (observations per unit effort: total observations/total man hours of search time) for each of the survey sites. Middle Snake Caye again shows the highest prevalence of lionfish, however here Abalone Caye shows the second highest CPUE and OPUE – higher than several sites which showed a larger total catch.

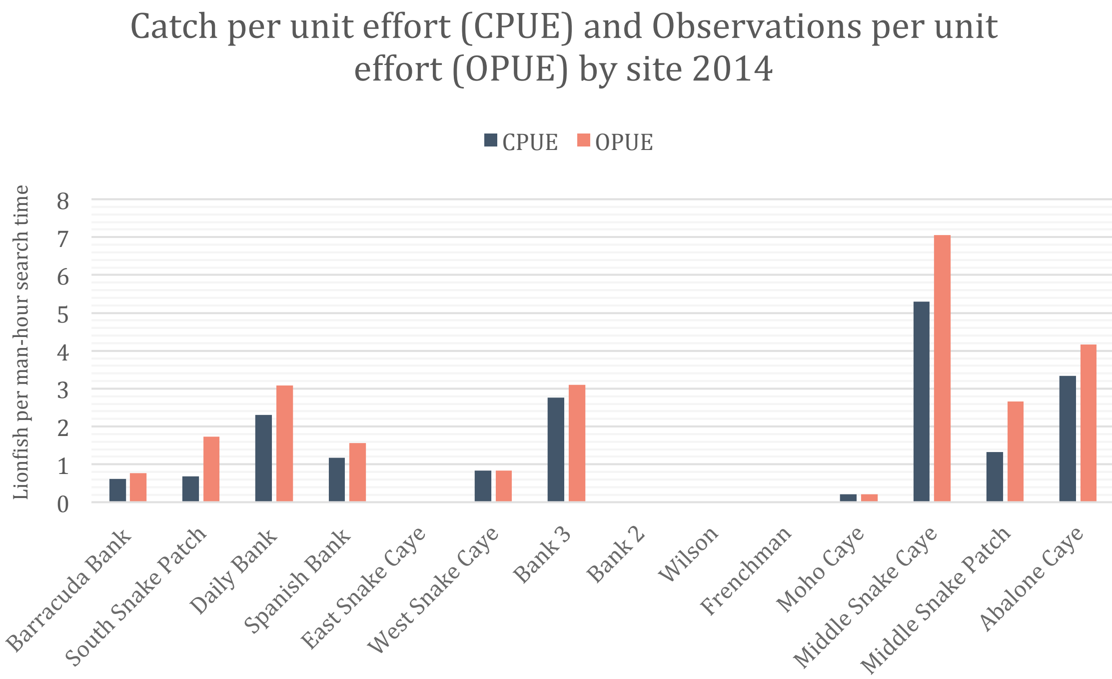

Figure 10: Catch per unit effort and observations per unit effort at each of the 2014 survey sites

The total number of lionfish caught in 2014 was 41, and the total number of lionfish observed was 55, showing a higher total abundance than the figures for either 2012 or 2013 (Figure 11). The total CPUE and OPUE for 2014 were also greater than that of the two previous years (Figure 12), however as different sites were surveyed each year this cannot be used as a direct comparison.

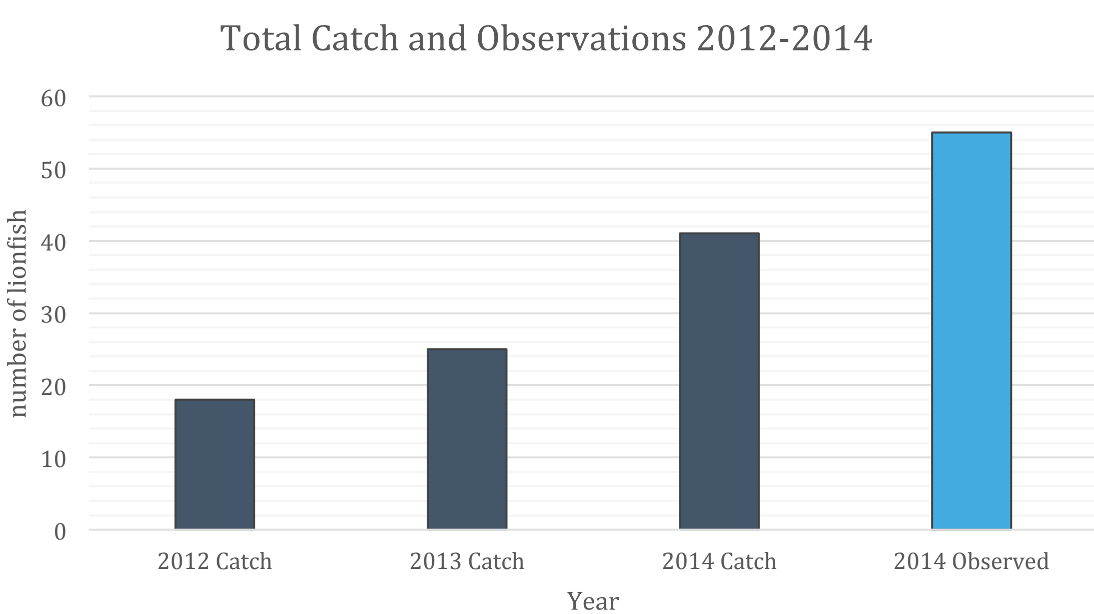

Figure 11: Total number of lionfish caught and observed in PHMR for the years 2012-2014

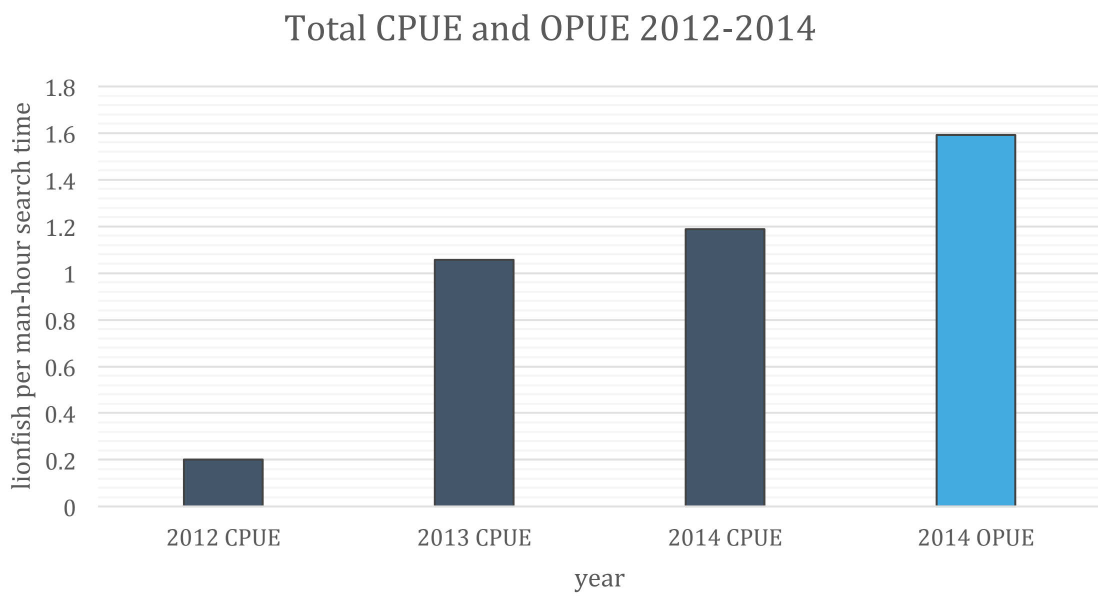

Figure 12: Total catch per unit effort (CPUE) and observations per unit effort (OPUE) of the 2012-2014 PHMR lionfish surveys

To create a fairer comparison 12 sites were selected that had been surveyed every year from 2012 to 2014. Figure 13 shows the catch and observation rates for these sites across the years. In 2014 lionfish were only absent from one site where they had previously been recorded – East Snake Caye. At 7 of the 12 sites lionfish were found in greater numbers in 2014 than in 2013, at 4 of the 12 sites they were present in equal numbers, and only one site showed a decrease in population from 2013 to 2014. However, at 3 sites the number of lionfish caught in 2014 was lower than in 2012. In 2014 lionfish were recorded for the first time at Abalone Caye.

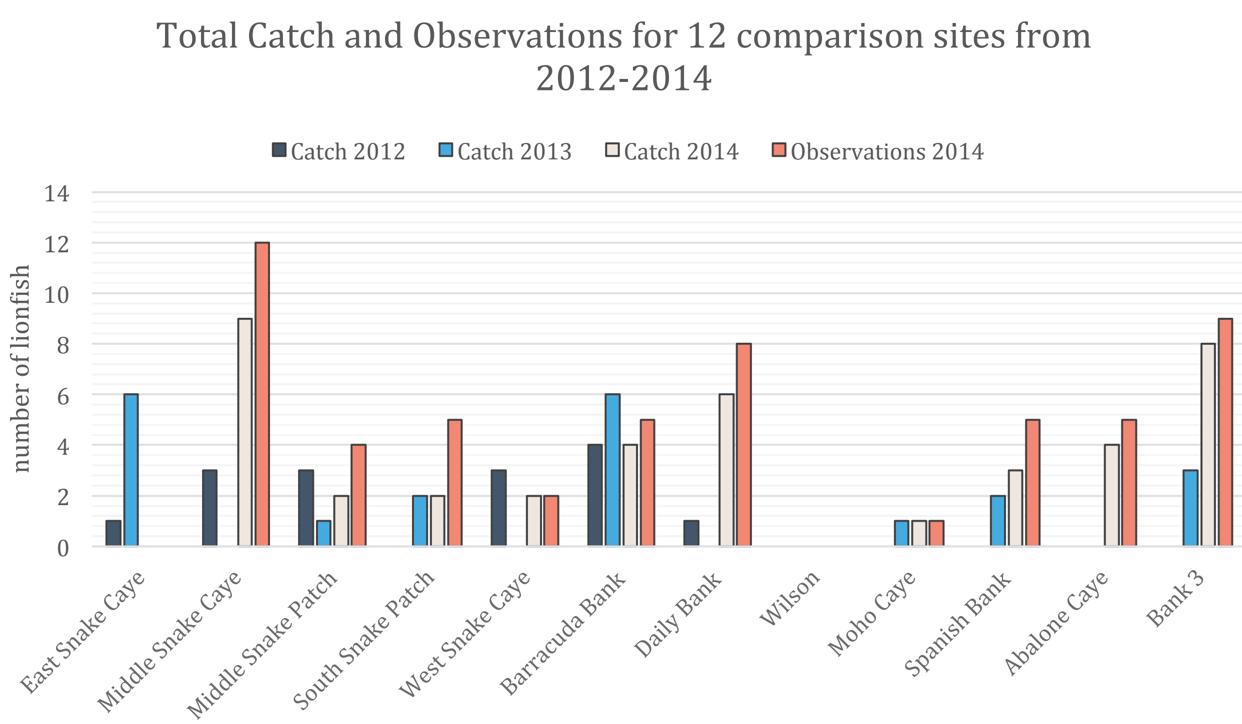

Figure 13: Total lionfish catch and observations recorded at 12 comparison sites surveyed in all three years from 2012-2014

Catch per unit effort values were also calculated for the 12 sites for 2013 & 2014, and observations per unit effort values calculated for 2014 (Figure 14). Here 6 out of 12 sites showed an increase in lionfish abundance from 2013 to 2014, 4 out of 12 sites showed a decrease in abundance, and at two sites CPUE was equal in both years. Unfortunately it was not possible to calculate CPUE values for the sites in 2012.

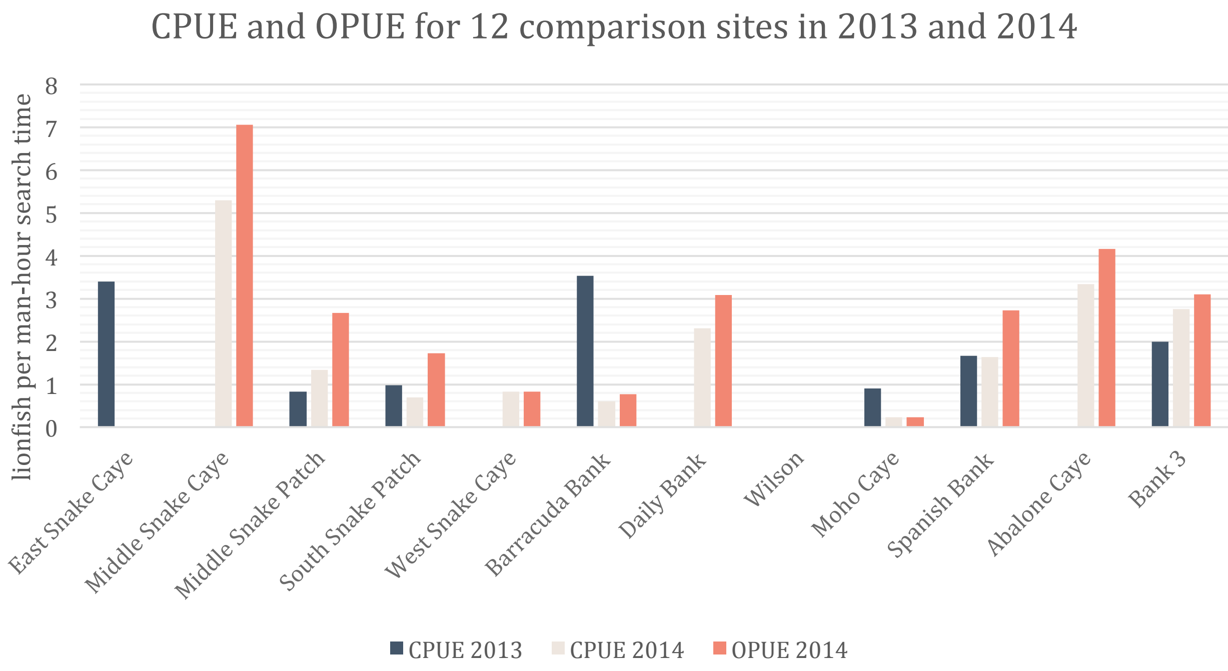

Figure 12: Catch per unit effort (CPUE) and observation per unit effort (OPUE) for 12 sites in 2013 and 2014

5.3: Objective 3 – Confirm the presence of juveniles

Figure 15 shows the total lengths (cm) of lionfish caught in PHMR from 2012-2014. In 2014 a total of 21 individuals of length of 10-20cm were recorded, more than in either 2012 or 2013. Only one individual of length 0-10cm has so far been found. Dissections conducted on the 2014 population found 23 juveniles at varying stages of development (see Figure 20) as well as spawning capable adults of both genders.

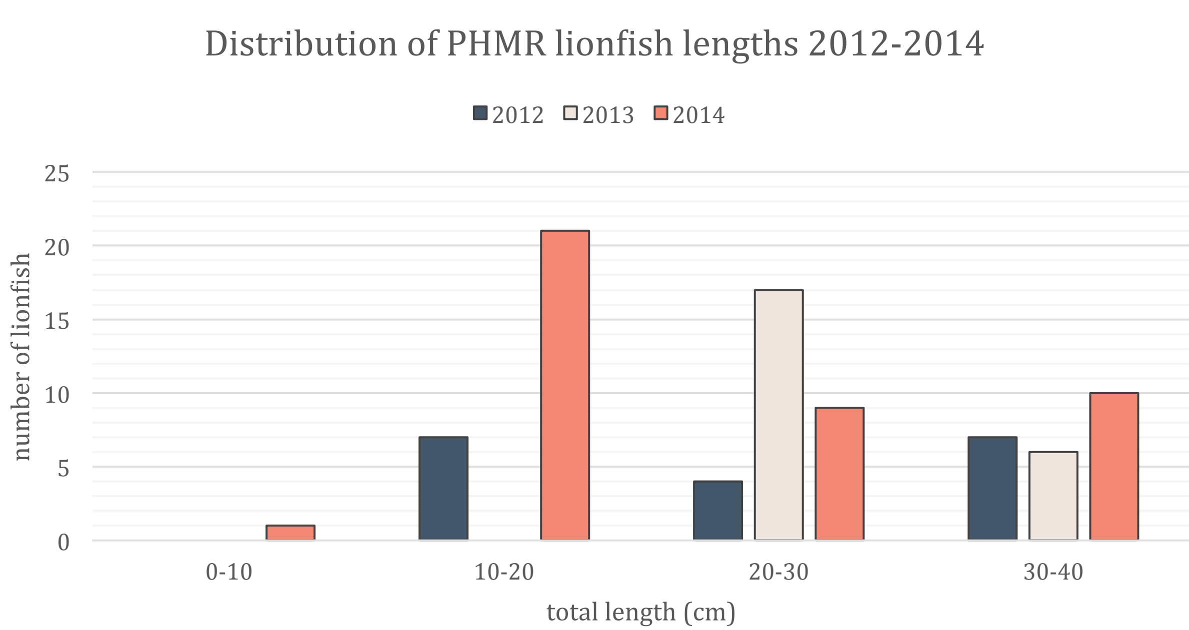

Figure 13: Distribution of total lengths (cm) of PHMR lionfish 2012-2014

5.4: Objective 4 – Investigate possible dietary trends

Figures 16-18 show the percentage by number of total gut content of PHMR lionfish made up by different organisms (e.g. shrimp, fish) for the years 2012-2014 respectively (see Appendix 2 Table 2 for data). In all three years fish accounted for the majority of gut content, making up 64% of the total in 2014. The percentage of shrimp increased by a factor of four from 2013 to 2014, reaching its highest level so far at 32%. Since 2012 no other crustaceans have been identified in the stomach contents of any of the PHMR lionfish surveyed.

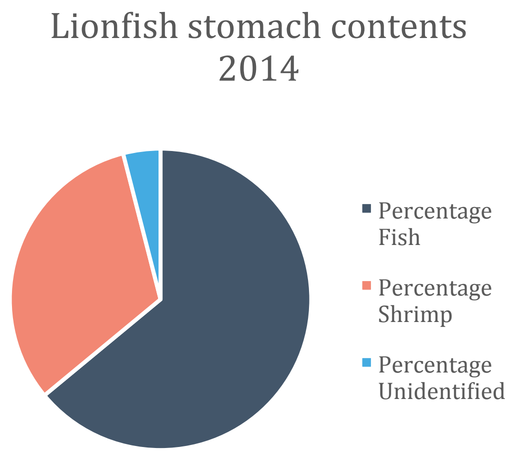

Figure16: Percentage of stomach contents made up by different organisms for PHMR lionfish 2014

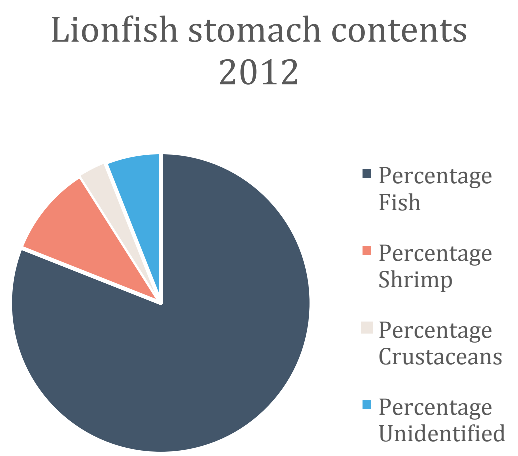

Figure 17: Percentage of stomach contents made up by different organisms for PHMR lionfish 2012

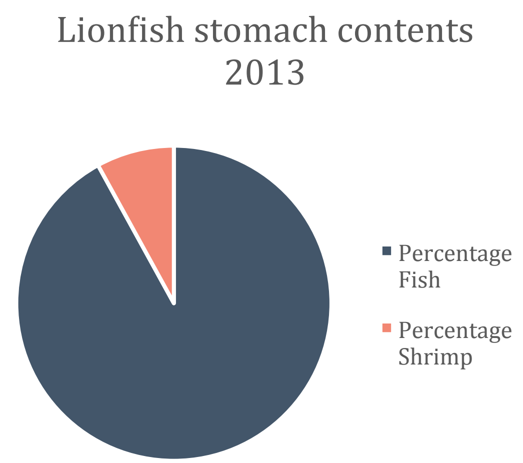

Figure 18: Percentage of stomach contents made up by different organisms for PHMR lionfish 2013

The relationship between maturity and diet is explored in Figure 19 shown below. Shrimp were found to constitute the majority of stomach contents in 12 juveniles from 2012-2014, and were present in 21 juveniles, but only made up the majority in 2 adults, and were only present in the stomachs of 6 mature fish. This meant that juveniles accounted for 78% of instances where shrimp were present, and 86% of instances where shrimp were the preferred food. Conversely, fish were found to be present in 15 juveniles and 38 mature fish, and to make up the majority of stomach contents in 11 juveniles and 35 adults. Mature individuals accounted for over 70% of cases where fish were present, and around 75% where fish were the majority. The number of individuals that showed an equal preference for fish and shrimp was evenly divided at 50% juvenile and 50% mature.

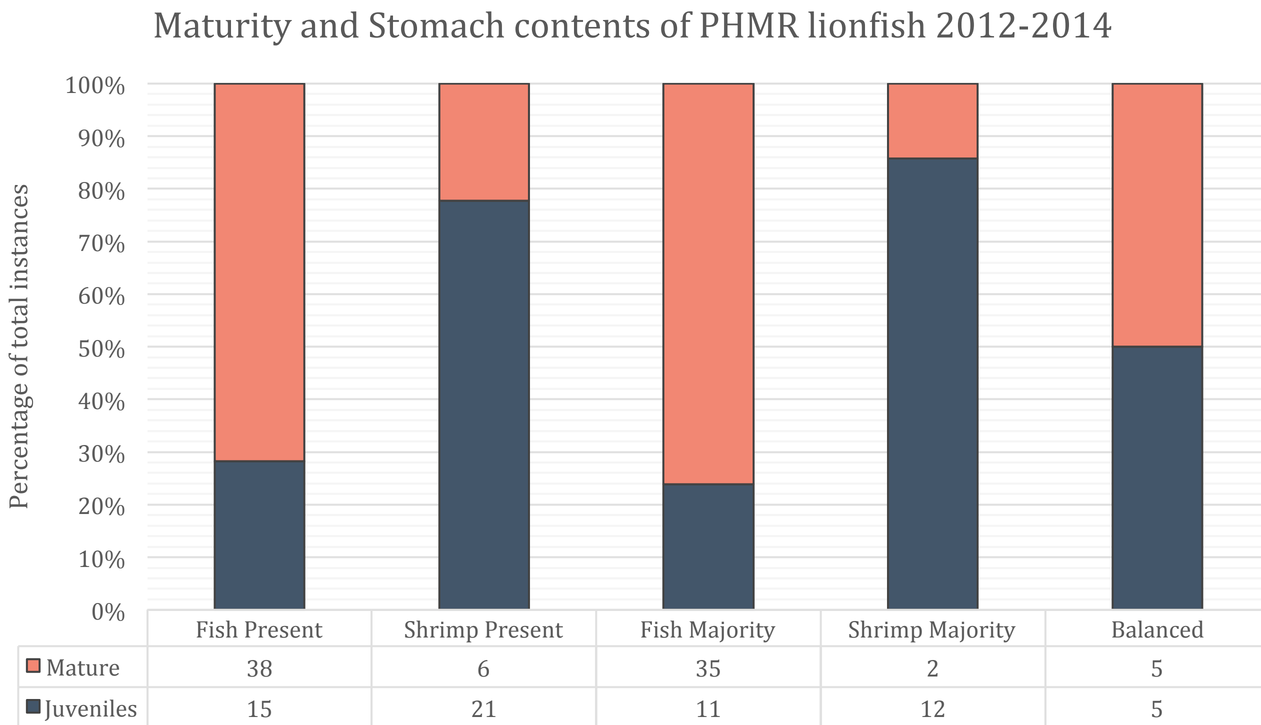

Figure 19: The proportion of adult's vs juveniles showing different dietary preferences for fish and shrimp from 2012-2014

5.5: Objective 5 – Record the behaviors of PHMR lionfish

In 2014 observed lionfish behaviors were divided into two categories: 'active' (with subcategories of 'hunting' 'swimming' and 'hovering') and 'inactive'. Figure 20 shows the percentage of total lionfish observed displaying each behavior type. The most common behavior type demonstrated was 'hovering', shown by 40% of individuals, at 8% 'swimming' was the least common behavior observed.

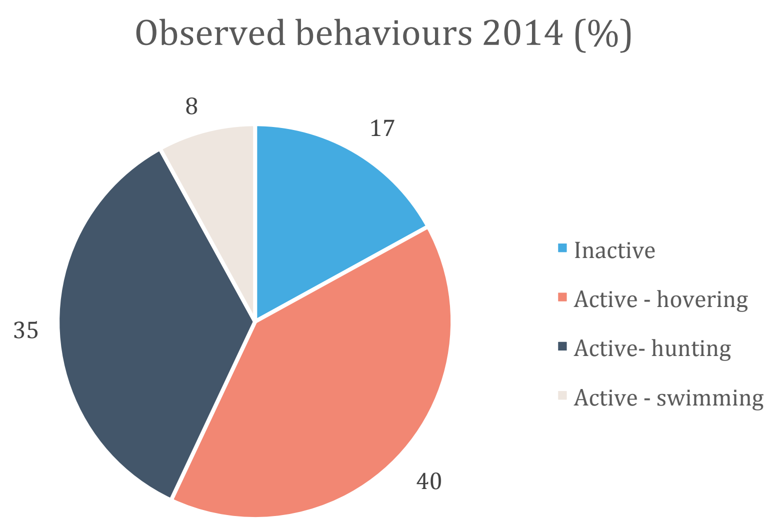

Figure 20: The percentage of total lionfish observed in 2014 displaying each of the recorded categories of behaviour

Figure 21 shows the number of lionfish found in groups and alone across all 3 years. In 2014 23 lionfish were found alone and 32 in groups; the largest group observed consisted of 4 individuals.

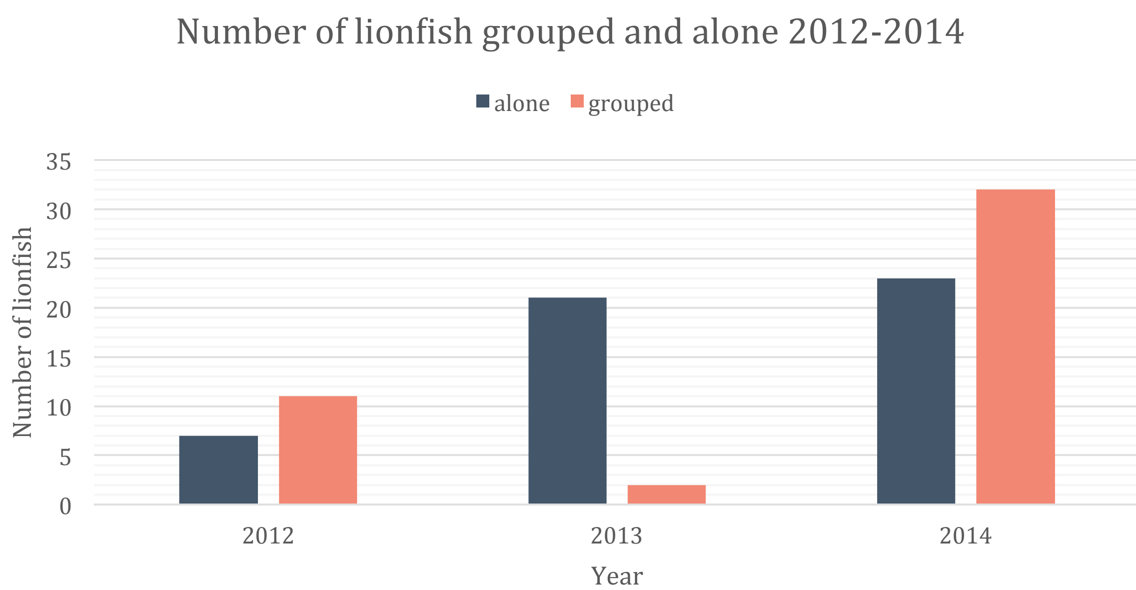

Figure 21: The number of lionfish observed in groups or alone in PHMR for the years 2012-2014

5.6: Objective 6 – Investigate the link between eyestalks and maturity

The presence or absence of eyestalks was recorded for each lionfish caught in 2014 and plotted against their maturity (Figure 22). Out of a total 23 juveniles 22 had 2 eyestalks and 1 had none. 13 of 18 mature individuals had no eyestalks, 2 had 1 eyestalk and 3 had 2 eyestalks. Due to a lack of dissection data it was not possible to compare maturity with number of eyestalks for 2012 and 2013.

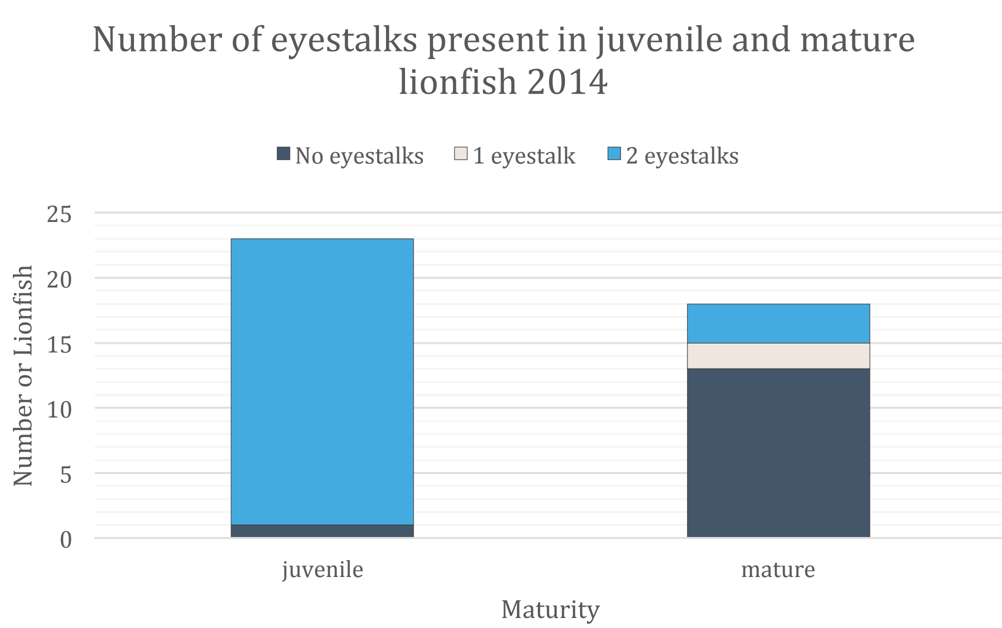

Figure 22: The number of eyestalks shown by mature and juvenile lionfish caught in 2014

6. Discussion

6.1 Objective 1

When the size data for all three years is compared the trend from 2012-2013 was an increase in both mean length and mean weight, however in 2014 the mean length and mean weight are both smaller than in either previous year (Table 1). This is most likely due to the much larger prevalence of juveniles in 2014. Table 1 also shows the ranges of length and weight in each year. The range of individual lengths was larger in 2014 than 2012 & 2013, again likely due to the presence of very small juveniles, however weight showed a much smaller range than in previous years with a maximum value of 603g, around 200g smaller than that of 2013.

Table 1: Measures of length (cm) and weight (g) values for PHMR lionfish from 2012-2014

Year	Measure	Min	Max	Range	Mean
2012	Length	14.2	37.5	23.3	24.64
	Weight	12	653	641	246.93
2013	Length	21.0	39.0	18.0	28.17
	Weight	57	800	743	341.52
2014	Length	9.4	36.0	26.6	21.00
	Weight	12	603	591	184.78

The curve of length vs weight plotted in Figure 6 shows that in smaller fish both values increase proportionally, but with larger fish there is a much wider variety in weight at any given length. This variability can be attributed to differences in stomach contents and size of fatty tissue deposits around the internal organs; in mature females the presence or absence of eggs would also lead to variation in weight for a given length. When considering cross-year trends in lionfish size/growth length is therefore the more reliable measure to use as weight is too easily effected by dietary or breeding habits.

Gender data was not recorded for 2012-2013 and therefore no ratios could be calculated, however in 2014 the ratio of male: female lionfish caught was roughly 2.4:1 with no actively spawning females found. This differs significantly from the expected ratio of approximately 1:1 shown by similar populations in the Caribbean Sea and the Gulf of Mexico (Fogg et al, 2013; Edwards, 2014; Morris, 2011).

6.2 Objective 2

Out of the 14 sites surveyed in 2014, 4 showed no lionfish activity. 3 of these sites were shallow, predominantly seagrass habitats that had been included only to test the hypothesis that juvenile lionfish may utilize seagrass as nursery sites, and as such were unlikely to contain lionfish. However, 1 of the 4 sites, East Snake Caye, had been found to contain lionfish in both previous years – in fact showing the highest abundance of any site in 2013. The apparent absence of lionfish from this site in 2014 is most probably an anomaly caused by the very low relative search effort devoted to this site (only 34 minutes with 1 diver), and not representative of any significant change in its population.

The 3 sites with the highest abundance within the reserve in 2014 were Middle Snake Caye, Bank 3 and Barracuda Bank; this is in keeping with trends identified in the previous two years that the majority of the PHMR lionfish population is still confined to the deeper waters around the edges of the reserve (Flores, 2013; McMahon, 2012). Two of these sites even showed higher abundance (both in catch and cpue) than Daily Bank and Spanish Bank, the deep water control sites outside of the reserve (see Figure 9). However the presence of lionfish in much shallower sites closer to shore such as Moho Caye and Abalone Caye (which showed the highest CPUE of any site other than Middle Snake Caye) suggests that the PHMR population may be beginning to spread further into the reserve. The lionfish caught in 2014 showed a slight preference for bank reef habitats over other habitat types, with 21 individuals found at bank reef sites, 16 at fringing reef sites and only 4 on patch reefs (Figure 23).

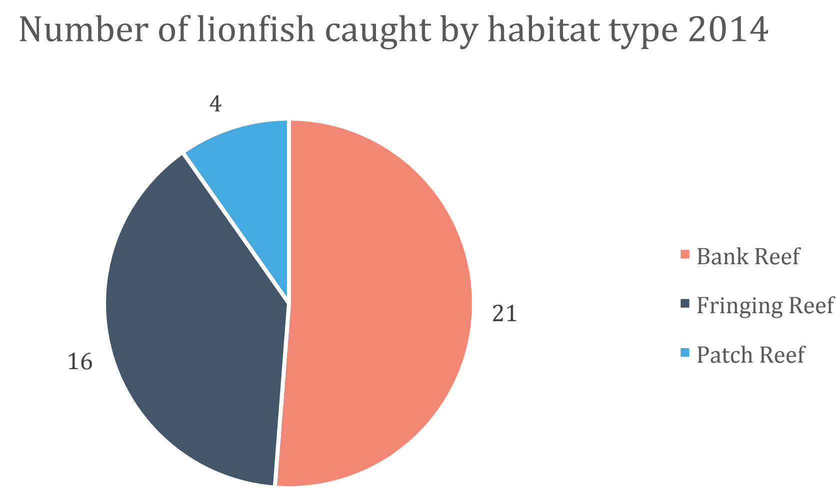

Figure 23: The number of lionfish caught in bank reef, fringing reef and patch reef habitats in 2014

Of all the sites surveyed in 2014, both the highest total catch and highest CPUE were recorded at Middle Snake Caye, within the preservation zone of the reserve. Lionfish were also caught within the no-take zone at West and South Snake Caye, and were present in both 2012 and 2013 within the no-take zone at East Snake Caye. This means that lionfish are now present within 3 of the reserve's 4 Replenishment Zones (RZs) (the fourth RZ Wild Cane Caye has not been surveyed in any year). Given the variety of destructive effects lionfish have on native fish populations (Albins, 2008) the presence of lionfish within these areas could have serious implications for the future success of PHMR's preservation and replenishment zones.

Hypothesis 1: Both total abundance and total catch per unit effort (CPUE) within PHMR will have increased from 2012 to 2014

In cross year comparisons total catch has increased every year since 2012 (Figure 11). From 2012-2013 the total catch increased 39%, and from 2013 to 2014 total catch increased 64%. In 2014 a value for the total number of lionfish observed was also included, regardless of whether or not they were caught, in order to give a clearer picture of the true abundance and to provide a larger set of behavioural data. However, increased catch alone cannot be taken as a definitive sign of an increase in lionfish population. CPUE gives a more reliable impression of changes in abundance. Catch per unit effort has also increased every year since 2012, but with a much smaller change between 2013 and 2014 unless the total observations per unit effort for 2014 are also taken into account (Figure 12). The fact that both total catch and CPUE have continued to increase every year suggests that the PHMR lionfish population is still growing and has not yet levelled off, meaning factors such as food supply or competition have not yet become limiting.

Hypothesis 2: Abundance and CPUE will have increased at the 12 comparison sites surveyed across all three years

Total catch and catch per unit effort for the 12 comparison sites used have both increased over time (Table 2) but CPUE is not radically different between 2013 and 2014.

Table 2: Total catch and Catch per unit effort (CPUE) for the 12 comparison sites from 2012-2014

	Total Catch	Total CPUE
2012	15	NA
2013	21	1.3
2014	41	1.4

The individual comparison sites showed a variety of different changes in CPUE (Figure 24). It is difficult to make accurate comparisons between years as current sampling is sporadic and only occurs over a short time each year, so can only give snapshots of the true population. In their native habitat in the Indo-Pacific, lionfish are crepuscular feeders, most active at sunrise and dusk (Green, 2011), and although there is debate as to whether this behaviour remains the norm in the Caribbean (Cote, 2010; Cure, 2012) time of day could have a significant effect on the number of lionfish found during sampling. Visual surveys have also been shown to underestimate lionfish biomass within an area by around 200% (Green, 2013), perhaps even higher in poor visibility or with untrained observers, meaning it is very difficult to gain an accurate idea of lionfish abundance with only one or two dives per site by a small number of researchers.

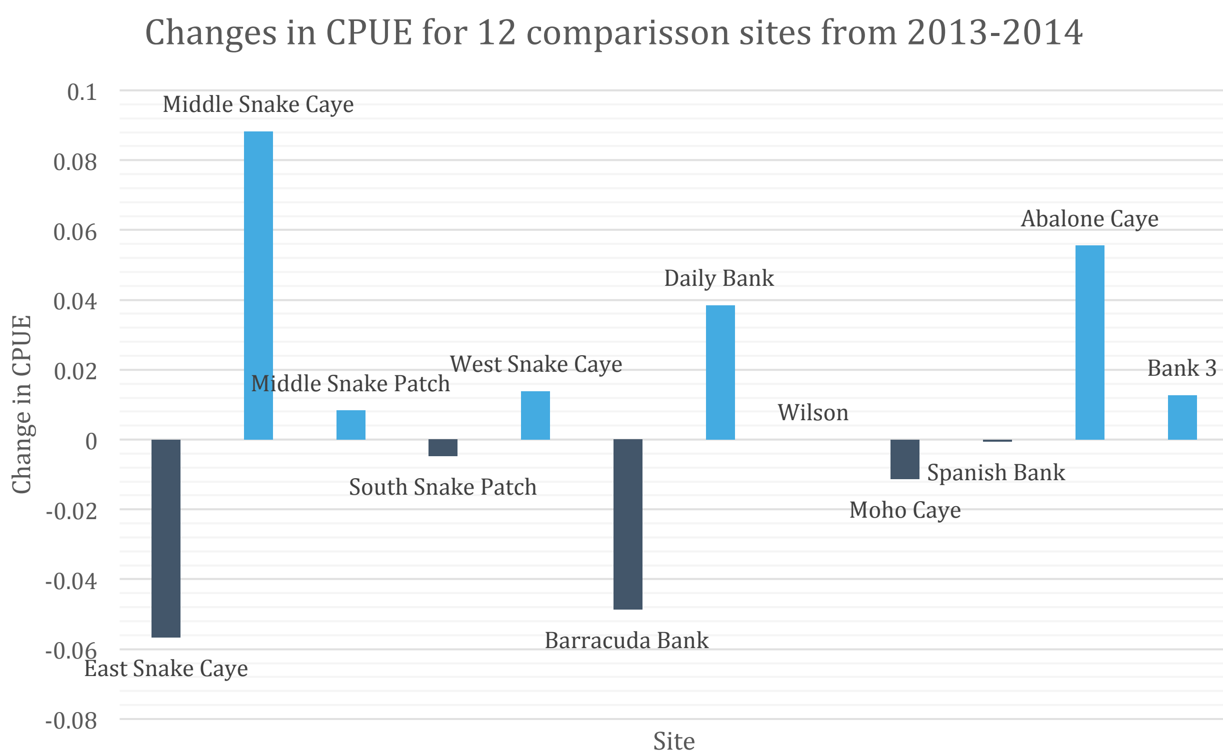

Figure 24:Changes in Catch per unit effort (CPUE) recorded at 12 comparisson sites from 2013-2014

6.3 Objective 3

A total of 23 juveniles were found in 2014. At all 10 sites where lionfish were found juveniles were present. No juveniles were found in the seagrass sites surveyed, suggesting that these are not being utilized as nursery sites. In fact juveniles showed a similar pattern of distribution to mature lionfish, with the highest numbers being found in deeper bank reef habitats, followed by fringing reefs and finally patch reefs (Figure 25). The number of juveniles caught was higher than in either of the previous years, suggesting that the PHMR population is continuing to breed and expand.

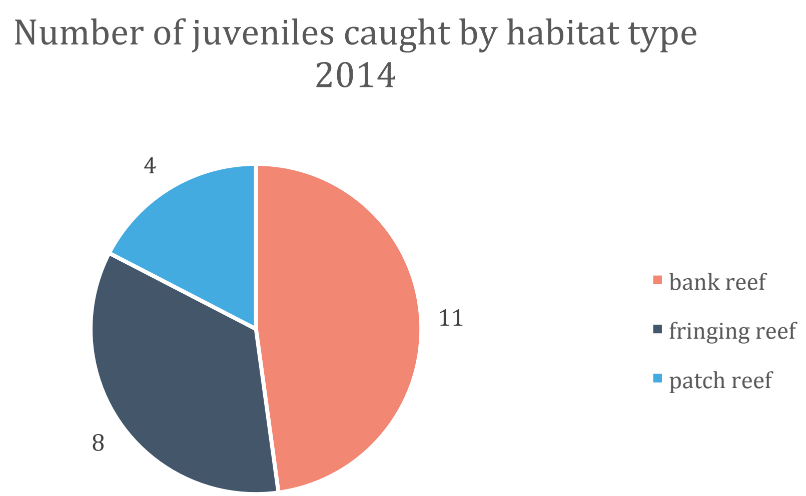

Figure 25: The number of juvenile lionfish caught at bank reef, fringing reef and patch reef habitats in 2014

6.4 Objective 4

Hypothesis 3: The population will show a shift in dietary preference from fish to shrimp over time

In 2014 fish still made up the majority of prey items found, although there was a significant increase in the proportion of shrimp, which had risen 32% from 2013. However from 2012-2013 there was an overall decrease in the amount of shrimp found in lionfish gut content, which does not fit the expected trend of a gradual shift from a fish-based diet to a shrimp-based diet over time, suggesting that other factors rather than just time contributed to dietary preference.

Hypothesis 4: Juveniles will show a preference for shrimp over fish, whilst adult lionfish will prefer fish to shrimp

Maturity was found to play a major role in determining diet. Shrimp were found in the gut content of significantly more juvenile than mature lionfish, with a χ^2 value of 12.77 ($p<0.0005$, 1d.f, one-tailed). Conversely, fish were found in the gut of significantly more mature individuals, with a χ^2 value of 4.37 ($p<0.025$, 1d.f, one-tailed). Shrimp also accounted for the majority of prey items in significantly more juvenile than mature individuals ($\chi^2 = 8.18$, $p<0.01$, 2d.f, one tailed). See Appendix 3 Tables 1-3 for data.

Gape size in particular seems to determine whether or not fish are included in a lionfish's diet. The first recordings of gape size for the PHMR population were made in 2014 and found that the mean gape width of lionfish with shrimp-dominated stomach contents was 11.5mm, compared to a mean of 25.4mm in individuals with a majority gut content of fish. Statistical analysis using Student's t-test showed the difference of means to be significant ($t=3.714$, $p<0.005$, 28d.f, one-tailed) (Appendix 3 Table 4).

The idea that diet changes predominantly with size and maturity rather than only with reduction in available prey fish over the course of an invasion would better fit the changing trends in data shown from 2012-2014 as 2013 showed both the smallest overall percentage of shrimp in gut contents and the fewest small juvenile individuals caught. Other literature has reported similar findings (Morris, 2009) suggesting that size/maturity is one of the main determining factors in lionfish diet.

6.5 Objective 5

It is difficult to make accurate observations about behavioural trends in the PHMR lionfish population as the same behavioural classifications were not used in all 3 years. One change that can be observed from the data is a marked increase in tendency to congregate in groups since 2013, with only 42% of individuals found alone in 2014, compared to 98% in 2013. However the percentage of solitary individuals in 2012 was also 42%. It is likely that grouping behaviour is linked to immaturity as 77% of lionfish found in groups in 2014 were juveniles (Figure 26). Only 4 mature individuals were found in groups and in no case was more than one mature lionfish found in the same group. It is impossible to be certain what proportion of grouped individuals in previous years were juvenile or mature due to a lack of dissection data, however as 2013 shows both the smallest percentage of grouped individuals (8%) and the lowest number of potentially juvenile lionfish it is likely that the trend was sustained throughout all 3 years.

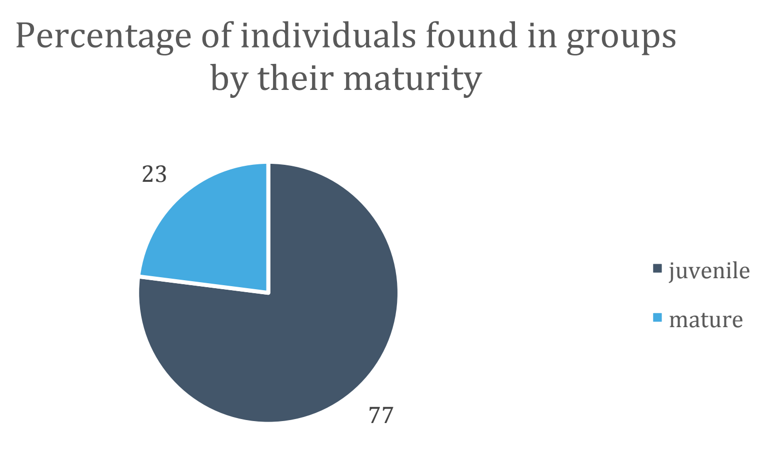

Figure 26: The percentage of total individuals found in groups in 2014 made up by mature and juvenile lionfish

6.6 Objective 6

Hypothesis 5: As maturity increases from juvenile to adult prevalence of eyestalks will decrease

From the data recorded in PHMR there seems to be a negative correlation between maturity and the number of eyestalks. In 2014 96% of juvenile lionfish possessed two eyestalks, compared to only 17% of mature individuals. The presence of eyestalks in juvenile lionfish was found to be significantly higher than in mature adults, with a χ^2 value of 20.78 ($p<0.0005$, 1d.f, one-tailed) (Appendix 3 Table 5). A similar relationship has been found in other literature (Morris, 2008).

7. Recommendations

7.1 Recommendations for monitoring

One of the main drawbacks of this study was a lack of comparable data across the 3 years, making it difficult to confidently identify cross-year trends. It is important that future research is consistent in its methodology and in the measurements taken in order to produce a database of similar data, allowing more accurate comparisons to be made. Monitoring should also take place on a regular basis throughout the year to provide a larger dataset and more reliable representation of annual trends.

Ideally the researchers carrying out this monitoring should be able to conduct dissections including gonadal analysis to determine gender and maturity, and otolith extraction. Specific lionfish targeted searches have been found to be the most accurate survey method in lionfish monitoring (Green, 2013) and these should be carried out by at least 2 divers in order to reduce the risk of missing individuals. This method would also allow 1 diver to record the behavioural data whilst another is spearing the fish, and would also mean more individuals can be caught when found in a group.

As lionfish are crepuscular feeders the most productive times to survey would be at dawn and dusk, when the fish are most active. Repeated surveying should take place at a specific set of comparison sites, preferably including sites that have shown consistently high CPUE values over all 3 years, as well as the reserve's no-take and preservation zones.

The most crucial data to continue gathering is maturity and stomach contents, as changes in the proportion of juveniles within the population and shifts in dietary preference from fish to shrimp can help to track the progression of the invasion. It is also important to record all lionfish observed, regardless of whether they are caught; ideally observational data should be recorded for all lionfish seen in the reserve, even outside of targeted searches.

7.2 Recommendations for management

Culling is still the only currently recognized method of successfully controlling lionfish invasions (Côté, 2014a), although it cannot eliminate them completely, and requires the regular removal of a substantial proportion of the population (27% monthly – Morris, 2011) in order to be effective long term. There is also some evidence to suggest that lionfish become increasingly wary and harder to catch after repeated culls (Côté, 2014b). If culling were to be implemented in PHMR it should be regular, targeted and frequent, ideally taking place on a monthly basis. The effects of culling are more substantial when juveniles are targeted as well as mature individuals (Morris, 2011) and, as with monitoring, efforts should focus on highly inhabited sites and on the more heavily protected no-take and preservation zones.

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This report was written by Hannah Holah, Bangor University Gwynedd, with help from James Foley, TIDE, as field supervisor

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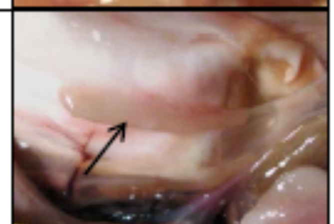
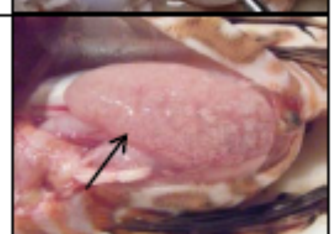
Appendix 1: Dissection guidelines

Fish length (TL)	Appearance	Gender	Stage description	Stage	Image
<180 mm	Gonads are oval masses, cream-pink in colour, with ratio of length:width less than 2.	Female	Additional information required to determine reproductive stage.	Immature (virgin) or Early Developing	See Immature or Early Developing
	Gonads elongated with ratio of length: width greater than 2.	Histology required to distinguish between male and female.	Clear, threadlike structures 1-3 mm in diameter and 5-10 mm in length. Immature ovaries are largely indistinguishable from immature testes.	Immature (virgin)	
>180 mm	Gonads elongated with ratio of length:width greater than 2.	Male	Testes appear threadlike; 1-3 mm in diameter and 5-10 mm in length.	Immature (virgin)	
			Testes appear as cream-colored with well-defined edges. Testes are typically not larger than 10 mm in diameter and 20 mm in length in the largest of specimens.	Spawning capable	
	Gonads are oval masses, cream-pink in colour, with ratio of length:width less than 2.	Female	Ovary is cream colored and round with no edges. Width may vary from 5 - 15 mm. Eggs not visible macroscopically. Ovary is more firm than during the Developing stage.	Early developing	
			Ovary cream colored with some pinkish portions. Eggs visible as small white spheres. Size may vary from a width of 15 mm to 30+ mm. No gelatinous mucus visible around periphery.	Developing	
			Ovary large with clear gelatinous mucus containing visible eggs peripheral to central stroma. Size may vary from a width of 15 mm to 30+ mm.	Spawning capable	
			Large number of clear eggs encompassed in gelatinous mucus visible along periphery of the ovary. Note: ovary wall is removed in picture.	Actively spawning	

Appendix 1 Figure 1: Gonadal staging of lionfish (Green, 2012)

Appendix 2: Results tables

Appendix 2 Table 1: Length (cm) and weight (g) values for all lionfish caugh in PHMR from 2012-2014

Year	Site Name	Individual no.	Total Length (cm)	Total weight (g)	Estimated size (cm)
2012	West/South Snake Caye	1	16	-	-
2012	Middle Snake Caye	2	14.2	-	-
2012	Stuart Caye	3	14.9	12	-
2012	unknown	4	35.5	199	-
2012	Daily Bank	5	30	262	-
2012	East Snake Caye	6	36.5	638	-
2012	South Snake Caye	7	17	50	-
2012	Barracuda Bank	8	32.5	479	-
2012	Barracuda Bank	9	28	309	-
2012	Barracuda Bank	10	20.5	96	-
2012	Barracuda Bank	11	16.5	45	-
2012	Middle Snake Patch	12	14.5	35	-
2012	Middle Snake Patch	13	17.5	65	-
2012	Middle Snake Patch	14	32	-	-
2012	West Snake Caye	15	31.5	420	-
2012	West Snake Caye	16	29	371	-
2012	Middle Snake Caye	17	37.5	653	-
2012	Middle Snake Caye	18	20	70	-
2013	Middle Snake Patch	1	28.5	227	-
2013	South Snake Caye	2	33.5	544	-
2013	South Snake Patch	3	30	454	-
2013	South Snake Patch	4	27.5	272	-
2013	Moho Caye	5	32.5	499	-
2013	Copper Bank	6	28	227	-
2013	Copper Bank	7	27	227	-
2013	Spanish Bank	8	21.5	57	-
2013	Spanish Bank	9	25.5	198	-
2013	East Snake Caye	10	39	800	-
2013	East Snake Caye	11	34	600	-
2013	East Snake Caye	12	29	350	-
2013	East Snake Caye	13	24	200	-
2013	East Snake Caye	14	26	300	-
2013	East Snake Caye	15	21	200	-
2013	Barracuda Bank	16	33	650	-
2013	Barracuda Bank	17	27	350	-
2013	Barracuda Bank	18	29	350	-
2013	Barracuda Bank	19	26	250	-
2013	Barracuda Bank	20	25	200	-
2013	Bank Three	21	28	350	-
2013	Bank Three	22	28	300	-
2013	Bank Three	23	25	250	-
2014	Barracuda Bank	1	33	502	20-30
2014	Barracuda Bank	2	19.5	90	10-20
2014	Barracuda Bank	3	22.8	147	20-30
2014	Barracuda Bank	4	17.4	63	10-20
2014	Daily Bank	5	36	558	30-40
2014	Daily Bank	6	33.1	431	30-40
2014	Spanish Bank	7	30.4	294	20-30
2014	Barracuda Bank	8	-	-	10-20

2014	Moho Caye	9	23	180	10-20
2014	Daily Bank	10	18	72	10-20
2014	Daily Bank	11	13	25	10-20
2014	Daily Bank	12	33	603	30-40
2014	Daily Bank	13	-	-	10-20
2014	Daily Bank	14	-	-	20-30
2014	Daily Bank	15	32	338	20-30
2014	Spanish Bank	16	-		10-20
2014	Spanish Bank	17	9.4	12	0-10
2014	Spanish Bank	18	23.9	201	10-20
2014	Bank Three	19	22.5	161	20-30
2014	Bank Three	20	16	37	10-20
2014	Bank Three	21	14.6	39	10-20
2014	Bank Three	22	12.2	26	10-20
2014	Bank Three	23	29.4	385	30-40
2014	Bank Three	24	-	-	10-20
2014	Bank Three	25	15	31	10-20
2014	Bank Three	26	13.2	22	10-20
2014	Bank Three	27	32.3	458	30-40
2014	Middle Snake Caye	28	13.8	34	10-20
2014	Middle Snake Caye	29	-	-	10-20
2014	Middle Snake Caye	30	31.8	486	30-40
2014	Middle Snake Caye	31	15.4	178	10-20
2014	Middle Snake Caye	32	-	-	40-50
2014	Middle Snake Caye	33	-	-	10-20
2014	Middle Snake Caye	34	28.4	379	20-30
2014	Middle Snake Caye	35	25.7	241	20-30
2014	Middle Snake Caye	36	13.9	25	10-20
2014	Middle Snake Caye	37	12.6	23	10-20
2014	Middle Snake Caye	38	30.2	450	30-40
2014	Middle Snake Caye	39	13.4	34	10-20
2014	Middle Snake Patch	40	-	-	10-20
2014	Middle Snake Patch	41	30.6	421	30-40
2014	Middle Snake Patch	42	-	-	10-20
2014	Middle Snake Patch	43	11.3	22	0-10
2014	South Snake Patch	44	24.2	226	20-30
2014	South Snake Patch	45	13.8	24	10-20
2014	South Snake Patch	46	-	-	10-20
2014	South Snake Patch	47	-	-	10-20
2014	South Snake Patch	48	-	-	10-20
2014	West Snake Caye	49	22.3	175	20-30
2014	West Snake Caye	50	13.5	22	10-20
2014	Abalone Caye	51	14.8	42	10-20
2014	Abalone Caye	52	16.4	45	10-20
2014	Abalone Caye	53	-	-	20-30
2014	Abalone Caye	54	15.2	41	10-20
2014	Abalone Caye	55	14	33	10-20