

FINAL REPORT
South Carolina State Wildlife Grant T-54-R-1; F10AF00443
South Carolina Department of Natural Resources
January 1, 2015 – December 31, 2015

NOTE: This project falls under the older grant originally titled, “Monitoring Impacts of Yellow Pine Restoration on Avifauna in the SC Mountains” which was extended in December 2014 to include this new scope of work. The entire grant date range is 2010-2015.

Project Title

Relative abundance (CPUE) and distribution (habitat use) of Lionfish (*Pterois* sp.) in the Southeast region based on reef fish survey (video) observations.

Principal Investigator Dr. Walter J. Bublely
Assistant Marine Scientist
Marine Resources Research Institute
South Carolina Department of Natural Resources
217 Fort Johnson Road
Charleston, SC 29412

Co-Principal Investigator(s) Dr. Joseph C. Ballenger
Assistant Marine Scientist
Marine Resources Research Institute
South Carolina Department of Natural Resources

Dawn Glasgow
Biologist I
Marine Resources Research Institute
South Carolina Department of Natural Resources

Dr. Peter Kingsley-Smith
Associate Marine Scientist
Marine Resources Research Institute
South Carolina Department of Natural Resources

Background

The invasion of the Indo-Pacific Lionfish (*Pterois* sp.) in the western Atlantic Ocean has been well documented, as are the adverse impacts on the local ecosystem and fisheries resources (Morris and Whitfield 2009; Green et al. 2012). Given its deleterious impacts to natural communities, the 2015 South Carolina State Wildlife Action Plan (SWAP) identified lionfish as an invasive species known to threaten South Carolina’s native wildlife and habitat. Various studies have documented the impact of Lionfish on native species, including those identified as priority species under the SWAP (see <http://www.dnr.sc.gov/swap/species2015.html>), either as a predator of juvenile snappers, groupers, flatfishes, Tomtate (*Haemulon aurolineatum*), and Penaeid shrimp (Dahl and Patterson 2014; *SERFS unpublished data*), or as a competitor with species such as Red Snapper (*Lutjanus campechanus*) and Gag Grouper (*Mycteroperca microlepis*) for common prey species or habitat (Whitfield et al. 2007; Wells et al. 2008; Munoz et al. 2011). However, few quantitative estimates of relative abundance or distribution for Lionfish are available for SC or off the Southeast region of the United States in the Atlantic Ocean. These estimates are critical to determine the current or future effects of this invasive species on native organisms, develop management options (including the identification of suitable habitat which could lead to a targeted removal strategy), and to judge the effectiveness of such management.

The South Carolina Department of Natural Resources' (SCDNR) Marine Resources Monitoring, Assessment and Prediction (MARMAP) program has collected information on relative abundance of reef fishes and other species off the Southeast coast of the US for more than four decades. Since 2009 and 2010, respectively, this program has collaborated with SCDNR's Southeast Area Monitoring and Assessment Program-South Atlantic (SEAMAP-SA) and the Southeast Fishery Independent Survey (SEFIS, housed at the NOAA Fisheries Lab in Beaufort, NC) to increase sampling of reef habitat. Currently, these programs are collectively called the South East Reef Fish Survey (SERFS). SERFS is a long-term fishery-independent monitoring program designed to monitor long-term changes in relative abundance, age compositions, length frequencies, and other information regarding reef fish species on live-bottom and/or hard-bottom habitats distributed from waters off the continental shelf and shelf edge between Cape Hatteras, NC, and St. Lucie Inlet, FL (Figure 1) from April through October each year (Figure 2). Currently, there are approximately 3,500 reef habitat stations in the sampling universe, ranging in depth from 9 to 109 m, although the vast majority of stations are generally shallower than 100 m. The chevron trap and camera placements were standardized since 2010 (Figure 3). A full description of the SERFS chevron trap-video survey and gears can be found in Smart et al. (2015).

Based on the catch data from traditional gears (e.g. chevron trap, longlines, and rod and reel) it appeared that the abundance levels of Lionfish were very low off the southeastern United States. In 2010, a subset of deployed traps was outfitted with video cameras, and in 2011 video cameras were included on all chevron traps. Based on early examination of these pictures and videos, Lionfish were regularly visible, indicating that abundance estimates based on traditional sampling gears did not represent the true abundances due to gear selectivity with this species. Glasgow (2010) provided the first Lionfish CPUE estimates from a fisheries-independent survey that focuses on natural live-bottom areas for SC and the southeast region in her MS thesis. This was based on digital cameras affixed to some traps starting in 2006, with all traps equipped with cameras by 2009. They took pictures every five minutes during the approximately 90 minute soak.

By characterizing invasive Indo-Pacific Lionfish (*Pterois sp.*) populations in the western Atlantic off of the southeastern United States, in terms of abundance, distribution, and potential effects on native species, while also identifying potential habitat for this invasive species from un-sampled areas, it will provide data required for management purposes in terms of the past, present, and future effects of this invasive species on native species. To this end, we analyzed the Lionfish information from the SERFS database and 1) constructed a time series (2011-2014) of relative abundance using a nominal and standardized index for waters off South Carolina and the southeast region; 2) related lionfish abundance with habitat characteristics to construct a habitat model; 3) based on observations and the results of the habitat data, investigated temporal and spatial shifts in Lionfish distribution in the South Atlantic Bight, with particular focus to shifts in distribution of the South Carolina coastline; and 4) using multi-variate methods, examined fish assemblages in relation to Lionfish presence.

Objective 1

Construct a time series (2011-2014) of relative abundance using a nominal and standardized index for waters off SC and the Southeast region.

Background:

Here, we report on the development of a nominal and standardized relative abundance index of Lionfish derived from the SERFS video survey during the years 2011-2014. The standardized index accounts for annual sampling distribution shifts with respect to covariates that affect numbers of Lionfish seen on videos. Data presented in this report are based on the combined SERFS database accessed on July 16, 2015.

Methods:

Survey Design

Abundance Data

The relative abundance of Lionfish on videos was estimated using the MeanCount approach (Conn 2011; Purcell et al. 2014; Schobernd et al. 2014). MeanCount was calculated as the mean number of Lionfish over a number of video frames in the video samples (Bacheler 2013). Zero-inflated modeling approaches (see below) require count data instead of continuous data like MeanCount. Therefore, these analyses used a response variable called SumCount that was simply the sum of all individuals seen across all video frames. SumCount and MeanCount are exactly proportional when the same numbers of video frames are used in their calculation (Purcell et al. 2014). Therefore, SumCount values were only used from videos where 41 frames were read (>99% of all samples). For determination of SumCount, only data available from Canon Vixia HFS-200 cameras were used; no data from GoPro Hero cameras were used for determination of SumCount.

Covariates

Sample Level Data

Associated with each deployment of a chevron trap with an attached video is information on the time, spatial location, and sampling depth of the event. Date and time for each deployment are recorded. Time is recorded in Greenwich Mean Time (GMT). For index development, this information is used to calculate a day of year (DOY) metric representing the numeric (to nearest integer) day of the year the deployment occurred. For location, precise latitude and longitude of deployment is recorded for each chevron trap deployment.

Hydrographic Data

Hydrographic data is collected via a conductivity, temperature, and depth instrument (CTD) to complement most gear deployments. A variety of SeaBird® CTDs have been used at various time points within the time series, all of which measured depth, temperature, and salinity. CTD casts are conducted while sets of chevron traps with video cameras are soaking. Bottom temperature (°C), defined as the temperature of the deepest depth recording within 5 m of the bottom, was extracted and considered for inclusion as a covariate in the relative abundance index.

Habitat Data

Trap-level Habitat Data

In addition to relative abundance data for index development, cameras were used to identify and quantify microhabitat features in the immediate vicinity of the deployed trap. Microhabitat characterization was completed individually for each camera available on a given trap using five different metrics: substrate size, substrate relief, substrate density, biota height, and biota density (Table 1). A single characterization of the microhabitat for a given chevron trap was assigned for discrete habitat variables treated as ordered factors (substrate size, substrate relief, and biota height), that being the highest ranked factor level observed using any available video data for that trap. For continuous habitat variables (substrate density and biota density), an average for all cameras with respect to the variable was calculated and assigned to the trap level microhabitat feature.

Unfortunately, there is likely a scale miss-match between the scale of the microhabitat features measured and the scale of habitat important to individual fish. For the discrete habitat metrics substrate size, substrate relief, and biota height, as well as continuous habitat metrics, substrate density and biota density, we used a weighted *k*-nearest neighbors approach to classify individual video observations to a given class or to obtain regression estimates, respectively.

Water Property Data

Finally, cameras were used to quantify three water property metrics at the site of deployment: water clarity, current direction, and current magnitude. Descriptions of discrete categories for each variable are found in Table 1.

Relative Abundance Index

Data and Nominal Relative Abundance Estimation

Data available for use in catch per unit effort (CPUE) estimation for each video (deployment) included a unique collection number, day of year of deployment, latitude, longitude, bottom depth, catch code, SumCount (see above) of Lionfish observed, bottom temperature, water clarity, current direction, current magnitude, substrate size, substrate relief, substrate density, biota height, and biota density. Estimates of nominal CPUE, or relative abundance, are given as the SumCount of Lionfish observed per video. Prior to modeling, all SERFS video data missing covariate data, videos deemed unreadable for any reason (e.g. too dark, camera out of focus, files corrupt), or chevron trap-videos that did not fish properly (e.g. bouncing or dragging due to waves or current, trap mouth was obstructed) were removed from the analysis.

Standardized Relative Abundance Estimation

Background

CPUE was standardized among years using a zero-inflated negative binomial general linear model (ZINB). This error distribution was chosen as ecological data is expected to have far more zeroes in count data than would be expected in other distributions, which can cause bias in the estimated parameters and standard errors or overdispersion (Zuur et al. 2009).

Model Structure

ZINB models can account for effects of different covariates on observed counts. The same or different covariates can be included in the binomial sub-model and catch sub-model. In initial investigations we considered the continuous covariates day of year (DOY), latitude, bottom depth, bottom temperature, substrate density, and biota density. We also considered the discrete covariates water clarity, current direction, current magnitude, substrate density, and biota height. Based on reduction of covariates due to collinearity or limited data in terms of sample or range, final covariates considered for the full model, in addition to the discrete variable year, included the continuous covariates latitude, depth, bottom temperature, day of year, and biota density and the discrete covariates water clarity, current direction, current magnitude, substrate size, substrate relief, and biota height. Continuous covariates were modeled as polynomials in the full ZINB model to allow for non-linear effects of these covariates on Lionfish relative abundance. Preliminary generalized additive models (GAM) were used to investigate the potential polynomial order for each of these covariates. All continuous covariates were centered and scaled prior to inclusion in the model.

Model Selection

Selection of the covariates included in the final model (both zero-inflation and count sub-models) was done based on Bayesian information criterion (BIC; Schwarz 1978).

Results:

Sampling Summary

From 2011 to 2014 the SERFS collected video data from 4,708 chevron trap deployments made throughout the South Atlantic Bight that were appropriate for use in the development of a Lionfish relative abundance index, averaging 1,177 collections per year. Across all years, Lionfish were present in approximately 9.3% of videos, ranging from 6.0 to 14.5% over the time series (Table 2). Descriptive statistics for continuous covariates are provided in Table 3.

Relative Abundance

Nominal SumCount per video generally increased during the survey years, peaking in 2014 at approximately 44% greater than the series average (Table 4 and Figure 4).

The final best fit model using BIC had the following form:

Zero-Inflation Sub-Model

$$\text{SumCount}^* = \text{Year} + \text{Current Magnitude} + \text{Depth}^3 + \text{Depth}^2 + \text{Depth} + \text{Latitude}^2 + \text{Latitude} + \text{Biota Density}^2 + \text{Biota Density}$$

Count Sub-Model

$$\text{SumCount} = \text{Year} + \text{Water Clarity} + \text{Substrate Size} + \text{Depth}^3 + \text{Depth}^2 + \text{Depth} + \text{Latitude} + \text{Bottom Temperature}^3 + \text{Bottom Temperature}^2 + \text{Bottom Temperature} + \text{Day of Year} + \text{Biota Density},$$

where SumCount* represents the Sumcount data transformed to presence/absence data and SumCount represents the observed SumCount data.

The ZINB standardized index normalized to the series average indicates that the relative abundance of Lionfish steadily increased throughout the survey period (Figure 4). Plots of annual coefficient of variation (CV), and variance indicate that 10,000 bootstraps were sufficient for these measures to stabilize.

The individual covariate effects varied in magnitude and shape (Figure 5, Figure 6, and Figure). Water clarity and current magnitude exhibited the *a priori* relationship where the predicted relative abundance of Lionfish decreased with poorer visibility and high current magnitudes (Figure). Concerning visibility, it is likely that this effect doesn't truly represent Lionfish abundance, but rather our ability to detect Lionfish on videos as visibility decreases. A similar mechanism likely explains the relationship between Lionfish relative abundance and current magnitude, with Lionfish perhaps sticking closer to the bottom (and hence making them harder to detect) at higher magnitude currents. There is not as obvious of a mechanism for the relationship between the habitat metrics biota density and substrate size on the relative abundance of Lionfish (Figure). The apparent parabolic relationship between Lionfish relative abundance and biota density likely represents a trade-off between Lionfish preferring structurally complex habitats (i.e. high biota density) and our ability to detect Lionfish at high biota densities due to their ability to camouflage in their surroundings. The increase in Lionfish relative abundance as substrate consolidation increases reflects the importance of hard bottom habitat to Lionfish. The most varied predicted relationship between Lionfish relative abundance and covariates occurs when looking at the effects of environmental variables on Lionfish abundance (Figure). The relative effects of the covariates depth and bottom temperature are larger than all other considered covariates, indicating these are strong drivers of Lionfish relative abundance. With regards to depth, Lionfish appear in highest relative abundance at depths of 40-50 m, which is deeper than most inner-shelf reefs of the region and suggest that they are first colonizing deeper mid-shelf and outer-shelf reefs. This distribution pattern may be related to Lionfish strongly preferring higher water temperatures, as indicated by their relative abundance generally increasing exponentially as a function of bottom temperature. The deeper reefs are closer to the influence of the Gulf Stream, likely making them less susceptible to wide swings in annual water temperature. The increase in relative abundance of Lionfish as a function of Day of the Year may be related to either increasing bottom water temperatures throughout the year or due to within year recruitment of Lionfish.

Accomplishments:

We were successful in creating an index of relative abundance of Lionfish during for the period 2011-2014 using the SERFS video index. This index clearly indicated that the relative abundance of Lionfish in the region increased substantially during the last four years. Additional efforts should allow for the inclusion of earlier years, perhaps back to 2008. SERFS video survey data will be available in subsequent years to further investigate how Lionfish relative abundance changes in the future.

Significant deviations:

Unfortunately, we were unable to extend the relative abundance index back to 2008 as originally planned in the project proposal. Several factors contributed to this result, most notably the changes in methodology used to assess the relative abundance of species throughout the time series. During the period 2008-2010, the majority of image data available for analysis was derived from still cameras attached to chevron traps. These still images were collected at a different frequency and amplitude than the frames collected via the video cameras used in the analysis during the period 2010-2011. Further, these still cameras had a different field of view than the Canon video cameras. Further, although some chevron traps were outfitted with GoPro video cameras in 2010, these continued to differ from those used in 2011-2014. Due to these differences, additional work is needed to understand the relationship among data derived from the still cameras, GoPro cameras, and Canon Vixia video cameras so that resultant relative abundance data are directly comparable. Project staff plan to investigate this using a calibration study that should allow for the development of calibration factors to convert relative abundance measures derived from still cameras and GoPro cameras to Canon Vixia relative abundance measures.

Objective 2

Relate lionfish abundance with habitat characteristics to construct a habitat model.

Accomplishments:

For this objective, we wanted to understand what environmental and habitat characteristics were affecting the distribution of Lionfish. A model was formulated to describe the distribution of Lionfish within the study area. Logistic regression analysis was used since the presence or absence of Lionfish was known for each site. That is, generalized linear models with the logit link function were formulated in R (R_Core_Team 2015). A total of 4,234 video samples were used for this analysis. The presence or absence of Lionfish was determined for each of these samples based on MeanCount data provided by the SERFS employees as described above.

The original variables thought to possibly have an effect on the distribution of Lionfish were year, latitude, longitude, salinity, temperature, depth, mean substrate density, mean biota density, biota height, substrate size, substrate relief, current magnitude, and current direction. First, correlation between covariates was examined. Mean substrate density and mean biota density were found to be positively correlated ($R^2=0.45$) so only mean biota density was used in future analyses, as with the index development. Furthermore, temperature was negatively correlated with depth ($R^2=0.21$), and thus only depth was used in formulating the models. Salinity was also excluded due to the very narrow range of salinity values for our samples. Also, it appears that lower salinity values were only found in very low sample depths. Finally, longitude was left out of the analyses due to it being correlated with depth.

Models were created for each possible combination of the variables of interest, which were then selected for based on Akaike's information criterion (AIC). AIC was chosen as the model selection method due to the exploratory nature of the analyses (Aho et al. 2014). It was found that the best model to describe the presence of Lionfish contained the following variables: year, latitude, depth, mean biota density, biota height, substrate size, substrate relief, and current magnitude. The best model had an AIC value significantly lower than the AIC value for the null model. However, the best seven models based on the automated model selection process differed in AIC values by less than 2.5.

Individual variables chosen for model inclusion were removed from the best model individually to determine changes in the AIC values and thus show the most important variables to the model. Removing depth from the model had the largest impact on the AIC value, increasing it by 47.1 (Table 5). Depth, year, substrate relief, mean biota density, and substrate size all changed the AIC value of the model by more than 10 when removed, and hence are most likely important in determining the presence of Lionfish (Table 5). On the other hand, biota height, latitude, and current magnitude had little impact on the AIC value when removed from the model.

The effects of individual variables on the probability of Lionfish occurrence were also examined. It was found that all variables, except current magnitude and current direction, significantly affect Lionfish distribution. In particular, the probability of Lionfish occurrence significantly increases with depth and mean biota density (Fig. 8). This increase in probability with depth becomes more prominent in the later years of the study period (Fig. 9).

Significant deviations:

Logistic regression analysis was chosen instead of occupancy modeling to understand the factors driving Lionfish distribution. This was due to the requirement of repeated sampling for occupancy modeling. That is, there must be temporally and/or spatially repeated sampling of sites within a season (MacKenzie et al. 2003), which was difficult to achieve since we were analyzing data after it had already been collected.

Objective 3

Based on observations and the results of the habitat data, investigate temporal and spatial shifts in Lionfish distribution in the South Atlantic Bight, with particular focus to shifts in distribution of the South Carolina coastline.

Accomplishments:

Logistic regression analysis was used to examine temporal changes in distribution. A total of 4,234 video samples were analyzed, of which 438 were found to have Lionfish present from 2011-2014. When separated by year, the proportion of videos with Lionfish present was 0.0892, 0.0658, 0.0919, and 0.1491 for 2011 -2014, respectively (Table 6). Furthermore, the logistic regression analysis shows that the probability of Lionfish occurrence has been increasing since 2012 (Fig. 10), similar to the nominal and standardized indices.

In addition to Lionfish increasing with time, there has also been a change in distribution during the study period. It is clear from maps that the distribution of Lionfish has expanded from 2011-2014 (Fig. 1). In particular, it appears that more Lionfish have been observed to the north and closer to shore in recent years (Fig. 1). Furthermore, logistic regression analysis showed an increased probability of Lionfish occurrence for higher latitudes in later years (Fig. 11).

Significant deviations:

None.

Objective 4

Using multi-variate methods, examine fish assemblages in relation to Lionfish presence.

Accomplishments:

Because of ecological connectivity in marine ecosystems, changes in one component of a community, such as the introduction of an invasive species (e.g. Lionfish), can have effects on the species assemblage as a whole, due to faster acting direct effects, such as predation or competition or longer term, slower acting, indirect effects through trophic cascades (Mack et al. 2000). Community data contain large amounts of species and multiple collections over a temporal and/or spatial frame; therefore, traditional univariate techniques and parametric modeling is inappropriate in assessing changes (Clarke and Warwick 2001a). Species assemblage should be assessed by examining as large a component of the entire community as possible, including not only presence/absence, but abundance as well. Multi-variate methods provide a means to assess the entire community structure, without having to limit the data, while also incorporating environmental variables in an

attempt to determine causality. The SERFS survey has provided this abundance of data, which in turn can be used to examine the potential ecological impact of the invasive Lionfish.

Previous studies examining ecological effects of Lionfish invasion have relatively small spatial or temporal coverage and have ranged from no effect to causing a drastic change in the community assemblage (Green et al. 2012; Elise et al. 2015). This study incorporates consistent sampling over five years and a large spatial scale from North Carolina to Florida, which will allow for a more regional view into the effects that increased Lionfish abundance and range has had on the community structure in its non-native habitat. We will examine spatial and temporal differences potentially associated with Lionfish presence/absence or abundance using the SERFS video and trap surveys in the Atlantic Ocean along the Southeastern United States.

Materials and Methods:

The SERFS video survey, as described above, was the starting point for this analysis since Lionfish do not recruit well to the chevron trap survey. A drawback with the video dataset, though, is that not all species are counted during a typical video read. In fact, the majority of species enumerated are larger bodied grouper and snapper species, which are not expected to have short-term, direct effects from Lionfish presence. The adult grouper and snapper species are most assuredly not affected directly due to predation by Lionfish and are most likely not competing for prey, as Lionfish tend to prey upon small individuals by comparison in relation to their body size (Morris and Akins 2009). For this reason, the chevron trap survey over this same time period is supplementing the video survey, with the Lionfish on the videos serving as a proxy for abundance as the cameras are affixed to the traps. Because every fish is identified to the lowest taxonomic level, counted, and measured in the trap survey, this will provide a better picture in terms of the community assemblage of those species most likely competing with Lionfish, including those species not counted in the video reads.

Effects taking longer to realize could occur if juveniles of these larger snapper/grouper species are potential prey sources or through indirect trophic cascades—though because of the time scale that the video survey has been going—there is less of a chance of observing that for this study. The trap data do not supplement the video data for the direct impacts of Lionfish in terms of monitoring juveniles of the larger snapper/grouper species, as the juveniles tend to be infrequent in the sampling habitat of the SERFS survey and/or too small to be retained by the chevron trap as they can fit through the mesh. In summary, the expected impacts from Lionfish that have the highest probability of being observed in this study are the direct, short time scale effects brought on by competition and to a lesser extent, predation.

Multivariate analyses were performed using PRIMER-E (Clarke and Warwick 2001b), unless otherwise noted. Because certain continuous variables had to be binned to create discrete depth, latitude, and year bins before continuing analysis, those bins that were either not or very sparsely populated were removed from analysis to minimize variability or control for differences across bins. For this reason, unless otherwise noted; latitude bins at 27° and 35°N latitude were removed from the analysis due to small sample size throughout the survey; The year 2010 was removed due to relatively limited spatial coverage; and the depth bin relating to the highest Lionfish observations for the survey (30-55m) was used to make comparisons while holding the depth factor constant.

The approach taken to examine potential species assemblage changes was three-fold. The first involved examining spatial and temporal change in assemblages and integrating environmental variables to identify potential causes. The second approach was more focused on the Lionfish impact, by examining if there were any differences between sites based on absence/presence of Lionfish, both spatially and temporally. Finally, species were identified which had the highest risk of being affected by both at present levels and if they continued to become more abundant.

Environmental Effects

Assemblage and environmental data from videos were integrated and analyzed together using the BIO-ENV procedure in PRIMER-E. This non-metric procedure determines which subset of environmental variables

best explain the assemblage patterns. One component of this study that makes it so appealing, the large spatial scale and long time series, also introduces obstacles and noise into the analysis, making it more difficult to observe a signal that may be there. The environmental data were square-root transformed and normalized. Correlations between environmental variables—latitude bins, temperature, mean biota density, biota height, substrate size, and substrate relief—were tested.

By utilizing multivariate analysis to examine potential species assemblage changes, it allows us to observe signals that cannot be assessed using univariate statistics. An analysis of similarity (ANOSIM) is the multivariate equivalent to the analysis of variance (ANOVA) by allowing significant differences to be detected within a similarity matrix using discrete variables assigned *a priori*. Because latitude can affect species assemblage, data were subset by latitude and individual ANOSIMs were run on both the video and trap survey data to examine the temporal effect on species assemblages region-wide. The concern for excluding 2010 in other analyses was truncated sampling in terms of latitudinal coverage with video compared to the survey range, but because latitudes are separated for analysis, all years were included and results reflect the greatest range between years for each latitude. A similarity percentages analysis (SIMPER) was run to examine the species that make up these observed changes.

Lionfish Presence

Vital data to have with the introduction of any invasive species is the effect that it has on native populations. Examining fish species assemblage change in relationship to Lionfish presence is just that. In this case, the presence of Lionfish is the treatment that was examined, with the samples absent of Lionfish acting as the default control.

Based on the findings of the environmental effects, latitude, year, and depth are three variables which greatly affect species assemblages and surveys spanning multiple years, and larger areas of study tend to incorporate a wider range of all three. For this analysis, the depth was held constant (30-55m) to account for this potential difference, but the latitude and year must be accounted for (2,499 samples). Fortunately, there is a way to examine the potential effect of Lionfish presence, while accounting for latitudinal variability. A two-way crossed ANOSIM can take into account the latitudinal differences in order to isolate the effect of Lionfish on large scale species assemblages. In essence, this 2 way crossed ANOSIM examines the effect of Lionfish presence while blocking the samples into latitude bins of 1°. Both the video and chevron trap data were analyzed by this means. A SIMPER was also run to determine the species most responsible for the differences between sites with and without observed Lionfish.

Species Similarities

Characterizing species which regularly show up in similar videos/catches with Lionfish is important in identifying which ones are at greatest risk due to the effects of this invasive species. Increased interactions due to being in regular proximity with Lionfish, has the potential for increased competition/predation with this invasive species. By utilizing a multivariate clustering analysis, these most vulnerable species can be identified. Both the trap and video survey data were handled similarly. The species list was decreased to the top 20 based on a minimum cut-off of proportion observed/caught at any one location. Lionfish were not included in the top 20 species list for trap catch, so they were added for comparative analysis. All depth and latitude zones were included. The procedures from Clarke and Warwick (2001b) were then followed, including a standardization, creation of a resemblance matrix, and finally, the creation of a species similarity cluster analysis and non-parametric multi-dimensional scaling analysis. This identified those species most closely associated with each other in relation to occurrence.

Results:

Environmental Effects

The combination of variables which best explained the biotic structure capable of explanation for assemblage patterns were latitude bin, biota height, and substrate size ($\rho = 0.171$).

When comparing between years, there are not consistent differences within latitudes and between years for the video or trap data in regards to assemblages (Table 7). Interestingly, the trap data shows a larger contribution to differences on average than the video survey as well as having more differences between years within latitudes.

Lionfish Presence

The ANOSIM results of the video data revealed that there is no difference in assemblages between sites in which Lionfish were present versus those that were not (two-way crossed ANOSIM; $R = 0.01$; $p = 0.267$), with the year and latitudinal effect (two-way crossed ANOSIM; $R = 0.149$; $p = 0.001$) driving this change in assemblages. The SIMPER analysis shows that the majority of species showed an increase in areas in which Lionfish were observed, with Black Sea Bass (*Centropristis striata*) and Red Snapper (*Lutjanus campechanus*) showing declines (Table 8).

The ANOSIM results of the trap data revealed a different pattern than the video data. There is a difference in assemblages between sites in which Lionfish were present versus those that were not (two-way crossed ANOSIM; $R = 0.042$; $p = 0.018$), though not as much as the year and latitudinal effect (two-way crossed ANOSIM; $R = 0.156$; $p = 0.001$), which accounted for more of the variability. The SIMPER analysis, though, does not show a general trend of increasing fish as it did with the video data (Table 9). The two species that showed declining trends with Lionfish presence in the video data, Black Sea Bass and Red Snapper, once again showed that trend with the trap data. There were also multiple species showing this same decline between sites that had Lionfish present and those that did not, such as Bank Sea Bass (*Centropristis ocyurus*), Sand Perch (*Diplectrum formosum*), Tomtate (*Haemulon aurolineatum*), Vermilion Snapper (*Rhomboplites aurorubens*), and Scup (*Stenotomus spp*). There was also a noticeable increase in species, such as Scamp Grouper (*Mycteroperca phenax*), Knobbed Porgy (*Calamus nodosus*), Red Porgy (*Pagrus pagrus*), Gray Triggerfish (*Balistes capriscus*), and White Grunt (*Haemulon plumieri*) when Lionfish were present, a trend also seen in the video data.

Species Similarities

The species similarity analysis of the video data identified Scamp, Hogfish (*Lachnolaimus maximus*), White Grunt, and Gag Grouper (*Mycteroperca microlepis*) as those found most consistently with observations of Lionfish (Figure 12). The species similarity analysis of the chevron trap data identified Scamp, Knobbed Porgy, and Blue Angelfish (*Holacanthus bermudensis*) as those found most consistently with observations of Lionfish (Figure 13).

Discussion

Environmental Effects

BIO-ENV

Latitude was the only variable that was included in every subset of variables chosen by the BIO-ENV. But also latitude may also be a factor of shelf width and/or proximity to shelf edge. The northern and southern latitudes of the sampling range coincide with a narrower shelf where assemblages may be more rapidly altered by disturbance, versus the wider shelf area in the mid latitudes (and middle of the SERFS sampling range), where disturbance effects may be dampened and/or take much longer to affect assemblage patterns. In addition, proximity to the shelf edge includes greater influence from the Gulf Stream, such as recruitment, upwellings, and stable temperatures. Latitudinal correlation with shelf width and proximity to shelf edge are currently being investigated.

Substrate size is based on the percent of *exposed* consolidated sediment. The SERFS survey targets hard bottom areas, and images and videos taken from trap mounted cameras over the years have shown a variety

hard bottom types, including not only areas of this exposed rock/reef of varying relief and biotic cover, but also areas where the biota, in varying heights and densities, is attached to a solid substrate underneath a veneer of sand. Therefore, it is not surprising that biota height (and to some degree, biota density) are accounted for within the subset along with substrate size. These variables potentially provide refuge for smaller “prey” species that attract larger predatory species (including Lionfish), who may also exploit these factors as cover/camouflage, and as would be expected, provides some explanation of the assemblage patterns in both the video data and the trap data.

ANOSIM and SIMPER

The results of the BIO-ENV led to further investigation to determine if there were changes in assemblage patterns, within a subset of each latitude surveyed by SERFS.

It is notable, that significant differences in community composition and abundance were detected at latitudes N28°, and N34° for both the video data and trap catch data, but may not be surprising given the width of the shelf and proximity to the shelf edge as described above. In addition, there were no video samples (and therefore, no Lionfish data) within the depth bin examined for latitude N34° until 2012. Conversely, the lack of significant dissimilarities at all other latitudes for the video data may also be due to the much wider shelf area, and proximity to the Gulf Stream. It must also be noted that the assemblages within the video data reflect only the counts for larger bodied, “priority” species. However, there are significant changes in assemblages at all latitudes (except for latitude 33) in the trap catch data which is mostly likely due to the greater numbers of species accounted for in the trap survey.

The SIMPER analysis revealed that any significant dissimilarities are largely due to a similar set of species at varying contributing percentages such as Red Snapper, Black Sea Bass, Red Porgy, Vermilion Snapper, Gray Triggerfish, Jacks, etc. for the video data set (reflective of the exclusive priority species counts), and species such as Black Sea Bass, Bank Sea Bass, Tomtate, Vermilion Snapper, Red Porgy, Red Snapper, Gray Triggerfish, etc. for the Trap Catch (reflective of trap selectivity). Although it is interesting to see how the native species rank per latitude, it is even more impressive, and particularly notable for this study, that Lionfish, have also contributed to the dissimilarities at varying percentages for each latitude analyzed with one exception at latitude N34° in regard to abundance based on the trap catch. Again, this may be a factor due to lack of samples within that range.

Understanding assemblage patterns of native species is crucial to understanding how they may be influenced by invasive species. Although this study covers a very broad area, there may be signals that Lionfish may be influencing current assemblages where they have had more time to establish their populations, particularly in the southern portion of the SERFS survey. Subsequently, this may also additionally serve as a baseline study for all other latitudes considered.

Lionfish Presence

The differences between the two surveys, which are not independent from each other as the cameras are attached to the traps, has an explanation based on the rationale behind bringing the trap survey into this analysis regardless of this gear not sampling Lionfish well. As mentioned above, the only species counted in the video reads were priority species that typically consisted of larger-bodied and longer-lived species. These species would not be expected to have much competitive interactions with Lionfish as they may prey on species larger than those targeted by Lionfish. Because of this, the time frame for showing effects due to Lionfish presence is expected to be indirect and taking a longer time to manifest. In contrast, the trap survey, while accounting for these larger species, more importantly accounts for the smaller species. The majority of the species that showed a decline in the trap survey were not accounted for in the video reads, as they are smaller, less economically important species, though they may have an important impact ecologically. Except for Red Snapper, most of those species are similar in size to Lionfish and could be expected to compete for resources. They are also faster-growing and shorter-lived fish, meaning that if Lionfish were preying upon juveniles, this effect would most likely show up faster in these species than the longer-lived, slower-growing ones. While Lionfish may have an effect in

the species assemblage sampled by the trap survey, there is also the potential that environmental characteristics could be driving this difference, and Lionfish show a similar preference to those fish which increased in the presence of Lionfish and vice versa to those that declined.

Species Similarities

As with the differences attributed to Lionfish presence, the differences found between the video and trap surveys was expected. Certain species, such as Hogfish, do not recruit well to the chevron trap gear for whatever reason, explaining some of the differences in those identified in the video that were not in the trap catch. Those found in the trap data and not the video can be explained by the limited species that are accounted for during the video read process. Species like Knobby Porgy and Blue Angelfish are not counted in standard video reads.

Scamp shows up in both analyses because it is a priority species that is read in the videos, yet still caught with enough frequency to show up in the trap catch. Surprisingly, White Grunt does not cluster closely with Lionfish in the analysis using the trap data, though it does in the video. This either indicates a discrepancy between the surveys that have also been noticed with other species or the addition of more species in the trap data that cluster closer to Lionfish, thus making it appear White Grunt are further removed.

While the two surveys were utilized to cover a wider range of species in terms of size and ecology, there is still an important component of fish not accounted for here. The smallest fish, which are not priority species counted in the videos and are too small to be captured by the trap, are still not accounted for. The importance of these species in determining change due to Lionfish presence would be valuable as they are potential prey. Finding a way to relate the species assemblages, including these smallest fish, would provide valuable information on the effect of Lionfish predation on their non-native communities. The direct interactions with Lionfish that most of the fish in the videos and caught in traps would be competitive in nature.

Significant deviations:

None.

Estimated Federal Cost: \$15,199.81

Recommendations

Close the grant.

Literature Cited

- Aho, K., D. Derryberry, and T. Peterson. 2014. Model selection for ecologists: the worldviews of AIC and BIC. *Ecology* 95(3):631-636.
- Bacheler, N.M., C.M. Schobernd, Z.H. Schobernd, W.A. Mitchell, D.J. Berrane, G.T. Kellison, and M.J.M. Reichert. 2013. Comparison of trap and underwater video gears for indexing reef fish presence and abundance in the Southeast United States. *Fisheries Research* 143: 81–88.
- Clarke, K. R., and R. M. Warwick. 2001a. A further biodiversity index applicable to species lists: variation in taxonomic distinctness. *Marine ecology progress series* 216:265-278.
- Clarke, K.R, Warwick R.M. 2001b. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E, Plymouth, 172pp.
- Collins, M. R. 1990. A comparison of three fish trap designs. *Fisheries Research* 9(4):325-332.
- Conn, P. B. 2011. AN EVALUATION AND POWER ANALYSIS OF FISHERY INDEPENDENT REEF FISH SAMPLING IN THE GULF OF MEXICO AND U.S. SOUTH ATLANTIC. NOAA Technical Memorandum NMFS-SEFSC 610:i-iv.

- Dahl, K., A., and W. F. Patterson. 2014. Habitat-Specific Density and Diet of Rapidly Expanding Invasive Red Lionfish, *Pterois volitans*, Populations in the Northern Gulf of Mexico. *PLoS One* 9(8):1.
- Elise, S, I. Urbina-Barreto, H. Boadas-Gil, M. Galindo-Vivas, and M. Kublicki. 2015. No detectable effect of lionfish (*Pterois volitans* and *P. miles*) invasion on a healthy reef fish assemblage in the Archipelago Los Roques National Park, Venezuela. *Marine Biology* 162(2): 319-330.
- Glasgow, D. M. 2010. Photographic evidence of temporal and spatial variation in hardbottom habitat and associated biota of the southeastern U.S. Atlantic continental shelf. College of Charleston, Charleston, South Carolina.
- Green, S. J., J. L. Akins, A. Maljkovic, and I. M. Cote. 2012. Invasive Lionfish Drive Atlantic Coral Reef Fish Declines. *PLoS One* 7(3).
- Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10(3):689-710.
- MacKenzie, D. I., J. D. Nichols, J. E. Hines, M. G. Knutson, and A. B. Franklin. 2003. Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. *Ecology* 84(8):2200-2207.
- Morris, J. A., Jr., and J. L. Akins. 2009. Feeding ecology of invasive lionfish (*Pterois volitans*) in the Bahamian archipelago. *Environmental Biology of Fishes* 86(3):389-398.
- Morris, J. A., and P. E. Whitfield. 2009. Biology, Ecology, Control and Management of the Invasive Indo-Pacific Lionfish: An Updated Integrated Assessment.
- Munoz, R. C., C. A. Currin, and P. E. Whitfield. 2011. Diet of invasive lionfish on hard bottom reefs of the Southeast USA: insights from stomach contents and stable isotopes. *Marine ecology progress series* 432:181-NIL_0494.
- Purcell, K., M. Bacheler Nathan, and L. Coggins. 2014. Standardized video counts of Southeast U.S. Atlantic Gray Triggerfish (*Balistes capriscus*) from the Southeast Reef Fish Survey. SEDAR41-DW03. http://sedarweb.org/docs/wpapers/SEDAR41_DW03_Purcell_etal_GTFVideoIndex_7.31.2014.pdf. SEDAR, North Charleston, SC. 16pp.
- R_Core_Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Schobernd, Z., H., N. M. Bacheler, and P. B. Conn. 2014. Examining the utility of alternative video monitoring metrics for indexing reef fish abundance. *Canadian Journal of Fisheries and Aquatic Sciences* 71(3):464-471.
- Schwarz, G. 1978. Estimating dimension of a model. *Annals of Statistics* 6(2):461-464.
- Smart, T. I., M. J. M. Reichert, J. C. Ballenger, W. J. Bublely, and D. M. Wyanski. 2015. Overview of sampling gears and standard protocols used by the Southeast Reef Fish Survey and its partners. SEDAR41-RD58. http://sedarweb.org/docs/wsupp/S41_RD58_Smart_etal.2014_SERFSProtocols.pdf.
- Wells, R. J. D., J. H. Cowan, Jr., and B. Fry. 2008. Feeding ecology of red snapper *Lutjanus campechanus* in the northern Gulf of Mexico. *Marine ecology progress series* 361:213-225.
- Whitfield, P. E., J. A. Hare, A. W. David, S. L. Harter, R. C. Munoz, and C. M. Addison. 2007. Abundance estimates of the Indo-Pacific lionfish *Pterois volitans/miles* complex in the Western North Atlantic. *Biological Invasions* 9(2):231.
- Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed Effects Models and Extensions in Ecology with R. Spring Science + Business Media, LLC, New York, NY.

Tables

Table 1: Brief summary of co-variates used to quantify microhabitat and water property data observed by individual video cameras attached to chevron traps. For discrete variables, order from top to bottom indicates lowest to highest rank.

Variable	Distribution	Bins	Description
Microhabitat Variables			
Substrate Size	Discrete, ordered factor	None	No consolidated sediment
		Coarse	≥50% of consolidated sediment <1 m in diameter
		Continuous	≥50% of consolidated sediment >1 m in diameter
Substrate Relief	Discrete, ordered factor	Low	<0.3 m of relief
		Moderate	0.3 – 1 m of relief
		High	>1 m of relief
Substrate Density	Continuous, range 0-100%		Percent of substrate composed of consolidated (hard-bottom) sediment
Biota Height	Discrete, ordered factor	None	No attached biota
		Low	Max height <0.5 m
		High	Max height ≥0.5 m
Biota Density	Continuous, range 0-100%		Percent of substrate covered by biota
Water Property Variables			
Water Clarity	Discrete	Poor	Bottom substrate could not be seen
		Fair	Bottom habitat could be seen, but the horizon was not visible
		Clear	Bottom habitat could be seen, and the horizon could be seen in the distance
Current Direction	Discrete	Towards	Water column flocculants moving towards the camera
		Cross	Water column flocculants moving perpendicular to the camera
		Away	Water column flocculants moving away from the camera
Current Magnitude	Discrete	Weak	Flocculent movement speed relatively slow
		Strong	Flocculent movement speed relatively fast

Table 2: Number of video camera collections on live/hard-bottom areas, proportion of videos positive for Lionfish, and information regarding continuous covariate distribution annually. The microhabitat covariate biota density here refers to reef-level microhabitat estimates as calculated using weighted k -nearest neighbors regression.

Year	n	Prop. Pos.	Depth (m)			Latitude (°N)			Bottom Temperature (°C)			Day of Year			Biota Density (%)		
			Avg	Median	Range	Avg	Median	Range	Avg	Median	Range	Avg	Median	Range	Avg	Median	Range
2011	675	0.0741	40.0	40	14-93	30.70	30.64	27.23-34.54	21.6	21.3	14.8-28.8	209	209	141-301	19.33	16.38	0-76
2012	1222	0.0597	40.2	37	15-106	32.00	32.30	27.23-35.04	22.3	22.8	12.9-27.8	198	200	116-285	15.69	13.40	0-55
2013	1396	0.0795	37.6	34	15-100	31.13	31.15	27.23-35.01	21.8	22.3	12.4-28.1	199	205	116-279	16.86	14.81	0-76
2014	1415	0.1449	39.3	36	15-110	31.96	32.49	27.23-35.01	23.4	23.7	16.1-29.3	194	197	115-296	20.23	17.00	0-70

Table 3: Distribution of discrete covariates with respect to their categorical bins. Microhabitat covariates (e.g., substrate size, substrate relief, and biota height) represent reef-level microhabitat classification based on weighted k -nearest neighbors analysis. Water parameter covariates (e.g., water clarity, current direction, and current magnitude) are defined based on individual video reads.

Variable	Metric	Category 1	Category 2	Category 3
Substrate Size	Description	No Consolidated Sediment	>50% Consolidated <1 m in Diameter	>50% Consolidated >1 m in Diameter
	Number	867	2160	1681
	Proportion	0.1842	0.4588	0.3571
Substrate Relief	Description	<0.3 m of Relief	0.3 – 1 m of Relief	>1 m of Relief
	Number	3603	986	119
	Proportion	0.7653	0.2094	0.02528
Biota Height	Description	No Attached Biota	Max Height <0.5 m	Max Height >0.5 m
	Number	288	2417	2003
	Proportion	0.0612	0.5134	0.4254
Water Clarity	Description	Poor	Fair	Clear
	Number	369	1986	2353
	Proportion	0.784	0.4218	0.4998
Current Direction	Description	Towards	Cross	Away
	Number	163	2434	2111
	Proportion	0.0346	0.5170	0.4484
Current Magnitude	Description	Weak	Strong	
	Number	4267	441	
	Proportion	0.9063	0.0937	

Table 4: Lionfish relative abundance based on the SERFS video survey, 2011-2014. Relative abundance is presented as a nominal (mean SumCount/Year) CPUE and ZINB standardized CPUE. Both indices are normalized to the series mean. Index = relative abundance of Lionfish. Bias = observed bias in bootstrap analysis. CV = coefficient of variation.

Year	Nominal		ZINB Standardized				Confidence Intervals	
	Index	CV	Index	Bias	SE	CV	Lower	Upper
2011	0.7238	0.2125	0.6775	-0.0007	0.1492	0.2203	0.3667	0.9528
2012	0.4988	0.2225	0.8140	-0.0064	0.1688	0.2073	0.4577	1.1140
2013	1.2323	0.2017	1.0736	0.0089	0.1653	0.1540	0.7209	1.3696
2014	1.5451	0.1163	1.4350	-0.0019	0.1756	0.1224	1.0740	1.7658

Table 52: Effects of removing certain variables from the base model on AIC values. Base model is the best model chosen based on AIC values.

Model	AIC	Δ(AIC)
Base	2436.4	0.0
Base – Depth	2483.5	47.1
Base – Year	2464.2	27.8
Base – Substrate Relief	2459.6	23.2
Base – Mean Biota Density	2454.4	18.0
Base – Substrate Size	2447.9	11.5
Base – Biota Height	2438.9	2.5
Base - Latitude	2438.2	1.8
Base – Current Magnitude	2137.7	1.3

Table 6: Sample data for presence of Lionfish per year, as used in the habitat suitability analysis.

Year	Present	Absent	Prop. Positive	Total
2011	53	541	0.0892	594
2012	70	994	0.0658	1064
2013	111	1097	0.0919	1208
2014	204	1164	0.1491	1368
Total	438	3796	0.1034	4234

Table 7. Pairwise tests between years, within latitude bins to identify temporal differences in fish assemblages observed in the SERFS video survey or the trap survey (using Lionfish abundances observed in the video survey), including the contribution to the difference by Lionfish.

Square Root transformation - differences in abundance								
Video Data					Video LF + Trap Catch			
Latitude	Pairwise test - years exhibiting significant differences	R-value	Average Dissimilarity	Lionfish Contribution %	Pairwise test - years exhibiting significant differences	R-value	Average Dissimilarity	Lionfish Contribution %
28	10,14	0.24	84.2	2.19	10,14	0.23	72.4	2.44
29	none				10,14	0.166	83.77	3.34
30	none				11,14	0.119	83.39	2.92
31	none				11,14	0.115	76.44	3.97
32	none				11,14	0.08	77.35	4.86
33	none				none			
34	12,14	0.102	83.4	2.55	12,14	0.132	70.33	0
	<i>No significant differences at latitudes 29-33</i>				<i>No significant differences at latitudes 33</i>			

Table 8. Results from the SIMPER analysis of video data showing the species and their contribution to the assemblage differences in the absence and presence of Lionfish. Only species contributing to the top 90% of dissimilarity are shown here.

Lionfish Absent Lionfish Present

Species	Average Abundance	Average Abundance	Average dissimilarity	Contribution (%)	Cumulative Contribution (%)
Vermilion Snapper	0.98	1.23	13.95	18.75	18.75
Red Porgy	0.87	1.08	11.62	15.62	34.38
Gray Triggerfish	0.5	0.74	7.6	10.22	44.6
Almaco Jack	0.27	0.38	4.78	6.42	51.03
Scamp	0.15	0.39	4.5	6.06	57.08
Greater Amberjack	0.26	0.3	4.36	5.86	62.95
Black Sea Bass	0.33	0.16	3.56	4.78	67.73
Red Snapper	0.37	0.21	3.18	4.27	72.00
white Grunt	0.13	0.29	2.63	3.53	75.54
Hogfish	0.07	0.17	2.45	3.29	78.83
Banded Rudderfish	0.11	0.16	2.28	3.06	81.89
Unidentified Jack (<i>Seriola</i> spp)	0.12	0.13	2.26	3.04	84.93
Gray Snapper	0.11	0.17	1.82	2.45	87.38
Gag	0.08	0.13	1.61	2.16	89.54
Sand Tilefish	0.04	0.11	1.59	2.13	91.67

Table 9 . Results from the SIMPER analysis of trap data showing the species and their contribution to the assemblage differences in the absence and presence of Lionfish. Only species contributing to the top 90% of dissimilarity are shown here.

Species	Lionfish Absent	Lionfish Present	Average dissimilarity	Contribution (%)	Cumulative Contribution (%)
	Average Abundance	Average Abundance			
Black Sea Bass	2.99	0.98	18.03	22.64	22.64
Tomtate	1.59	1.53	14.12	17.73	40.37
Red Porgy	0.76	0.98	10.65	13.38	53.75
Gray Triggerfish	0.55	0.85	7.56	9.49	63.24
Vermilion Snapper	0.49	0.45	5.24	6.57	69.82
Bank Sea Bass	0.49	0.11	3.53	4.44	74.25
Scup (<i>Stenotomus</i> spp)	0.53	0.06	3.42	4.30	78.55
White Grunt	0.26	0.38	2.73	3.42	81.97
Knobbed Porgy	0.03	0.13	1.81	2.27	84.25
Red Snapper	0.21	0.1	1.69	2.13	86.37
Sand Perch	0.19	0.04	1.59	2.00	88.37
Scamp	0.04	0.13	1.52	1.91	90.29

Figures

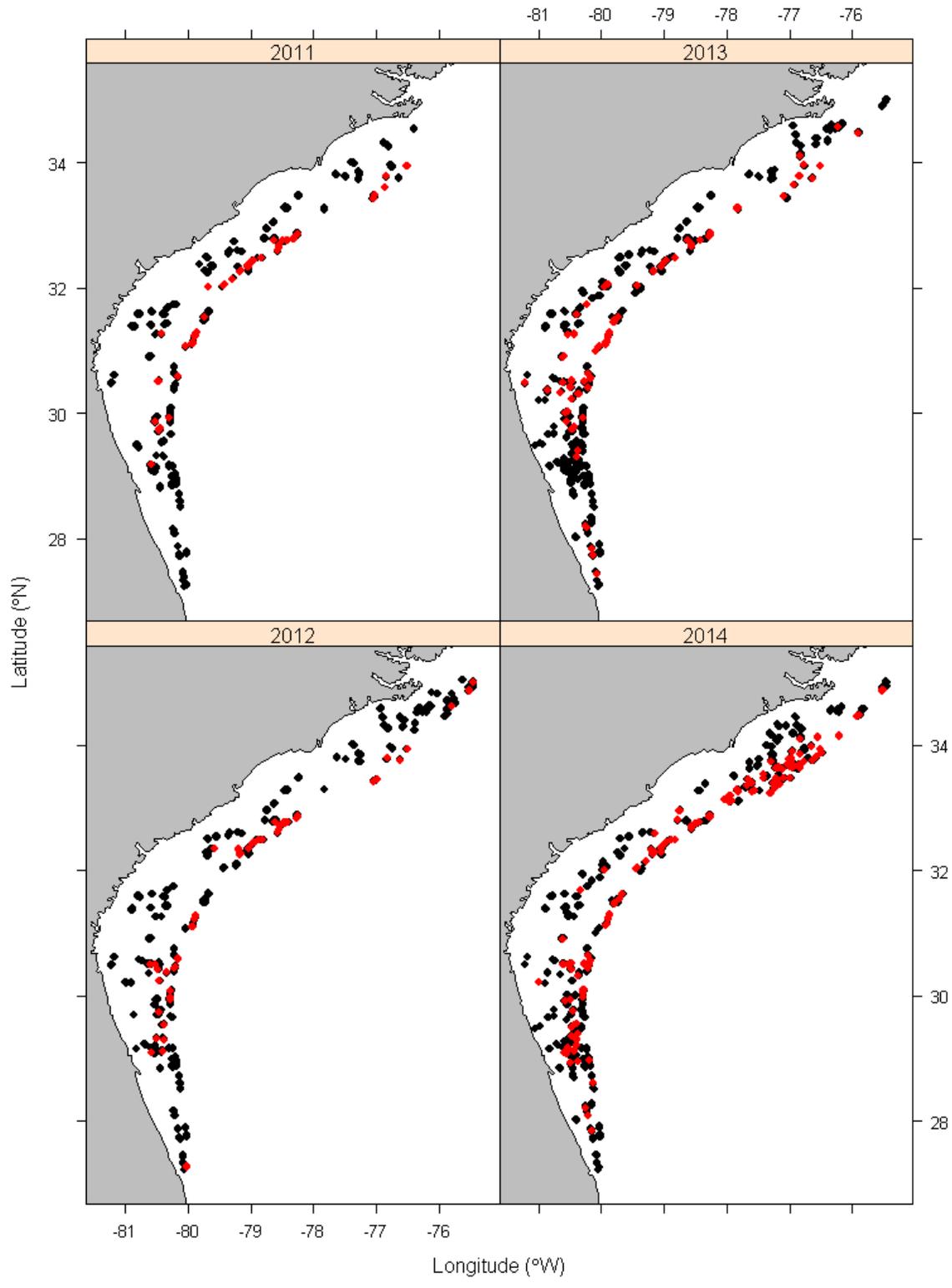


Figure 1: Annual sampling distribution of the SERFS chevron trap-video survey from 2011-2014. Black dots represent video samples absent for Lionfish. Red dots represent video samples where Lionfish were observed.

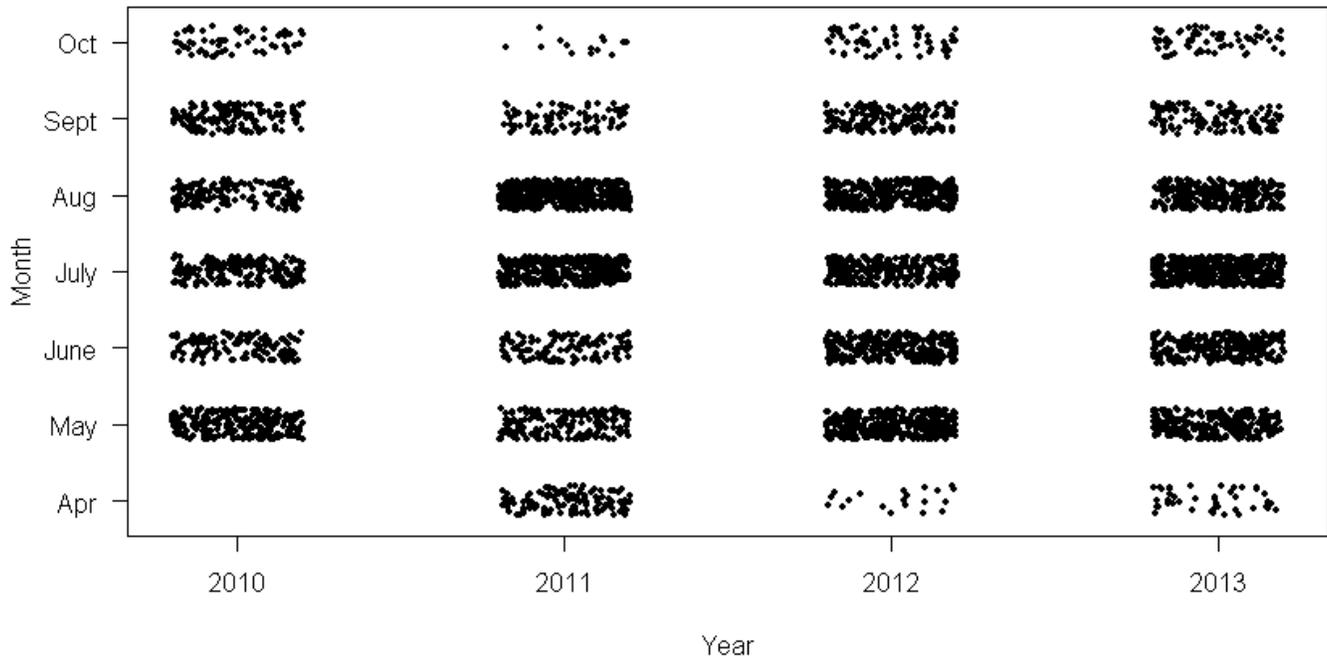


Figure 2: Annual distribution of sampling effort by month and year. Individual data points are jittered to create a cloud to give a sense of the total sample size by month and year combination.

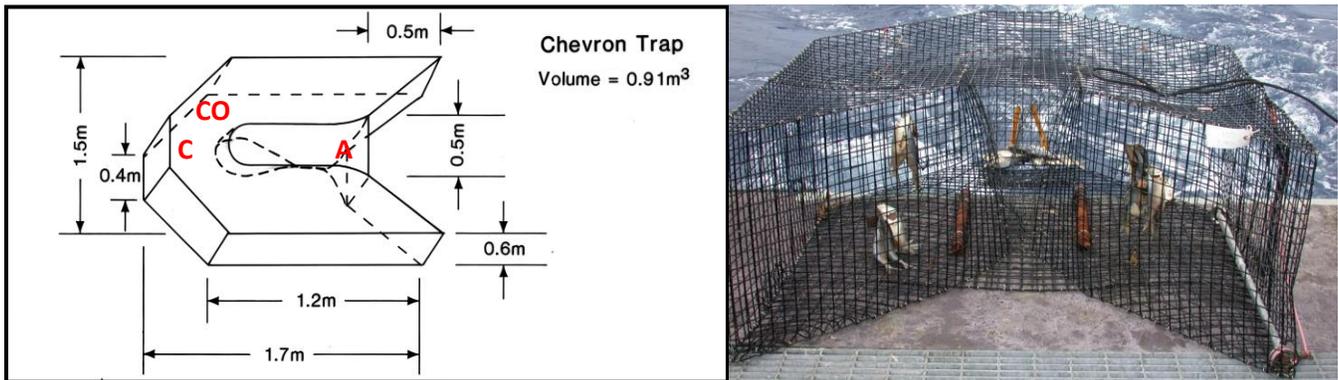


Figure 3: Chevron traps used by SERFS for monitoring reef fish. Left – Diagram with dimensions of chevron trap and video camera placement. A refers to current placement of Canon^{*1} cameras in all partner programs or 2010 placement of GoPro^{*1} cameras, C refers to current placement of GoPro^{*1} cameras on SEFIS vessels, and CO refers to current placement of GoPro^{*1} cameras or former placement of still cameras on MARMAP/SEAMAP-SA vessels. Right – chevron trap ready for deployment baited with clupeids. Iron sashes attached to the bottom weigh the trap down and help maintain the proper orientation of the trap on the bottom. Bottom - Diagram of video location on SERFS chevron traps (based on Collins (9)).

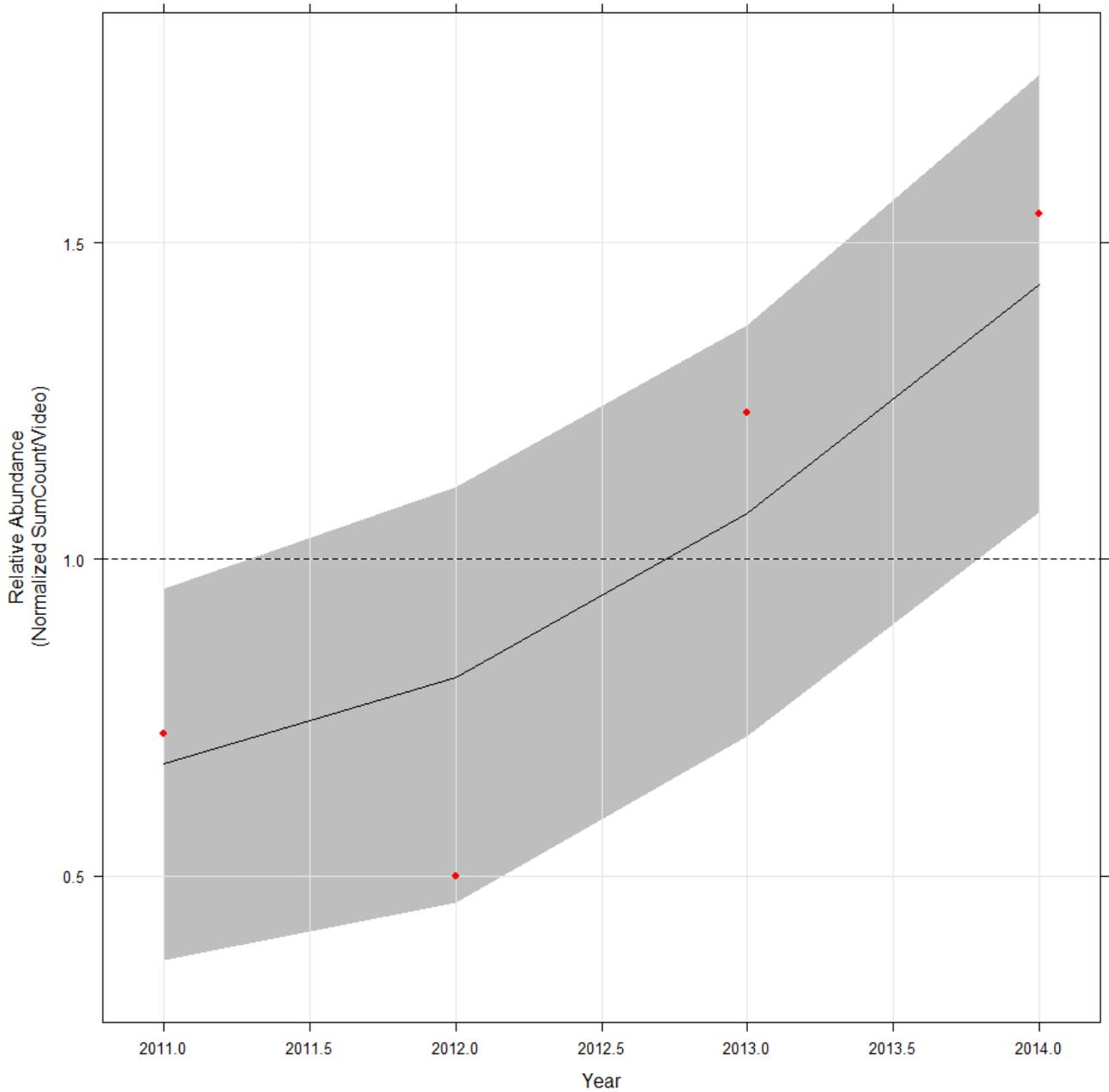


Figure 4: Lionfish index of relative abundance based on the SERFS video survey during the years 2011-2014. The ZINB standardized catch (solid black line) is normalized to the average relative abundance, as estimated by the model, during the period 2011-2014. Red dots represent normalized nominal annual relative abundance. Gray shaded region represents the 95% confidence interval of annual relative abundance based on 10,000 bootstraps.

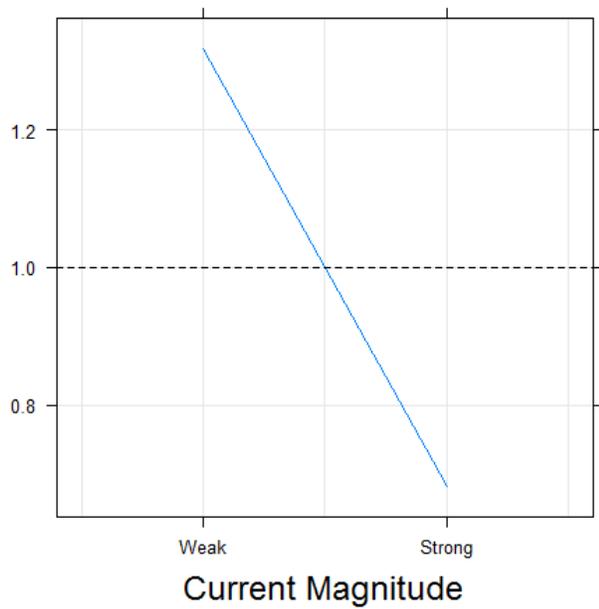
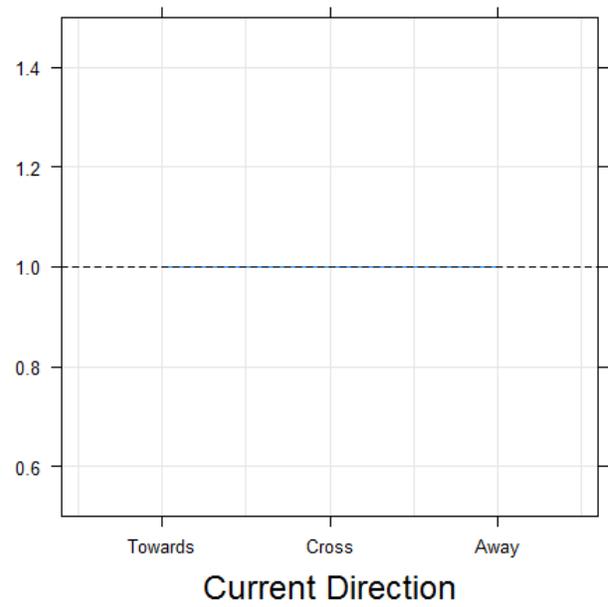
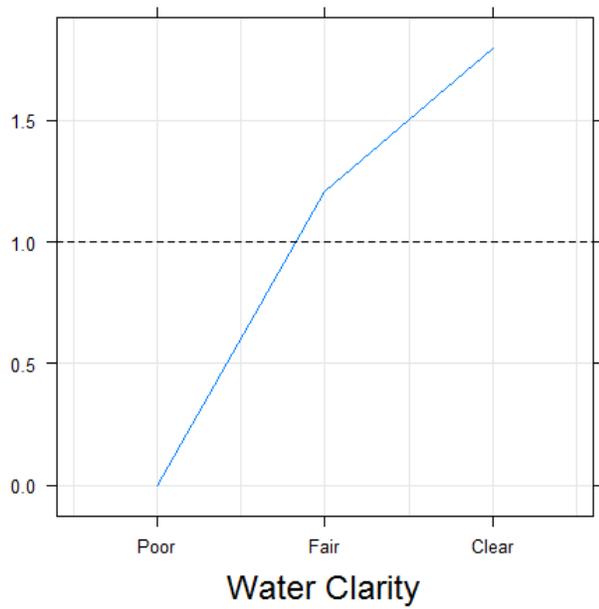


Figure 5: Predicted effect of water clarity, current direction, and current magnitude on the relative abundance of Lionfish.

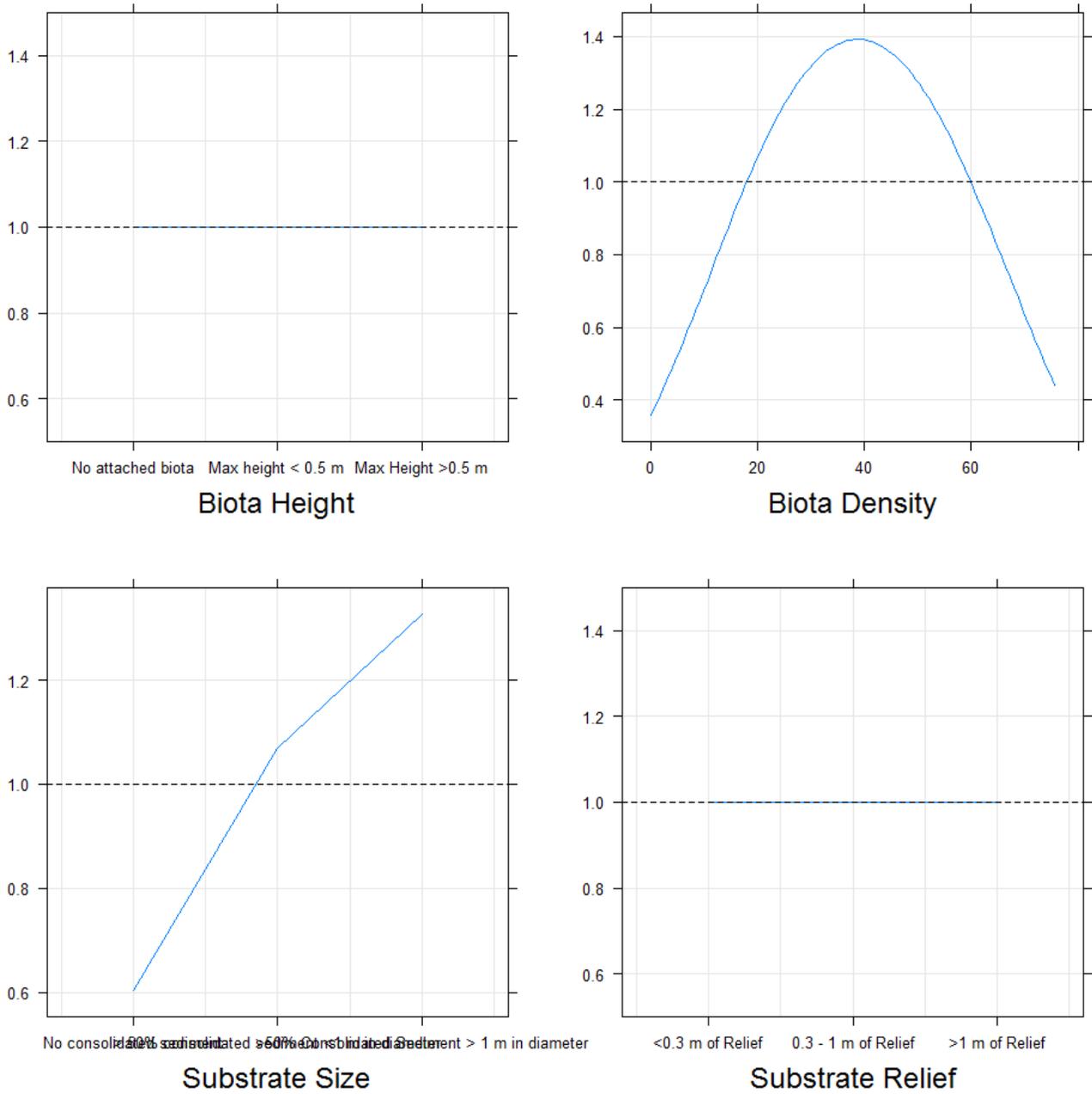


Figure 6: Predicted effect of the habitat covariates on the relative abundance of Lionfish.

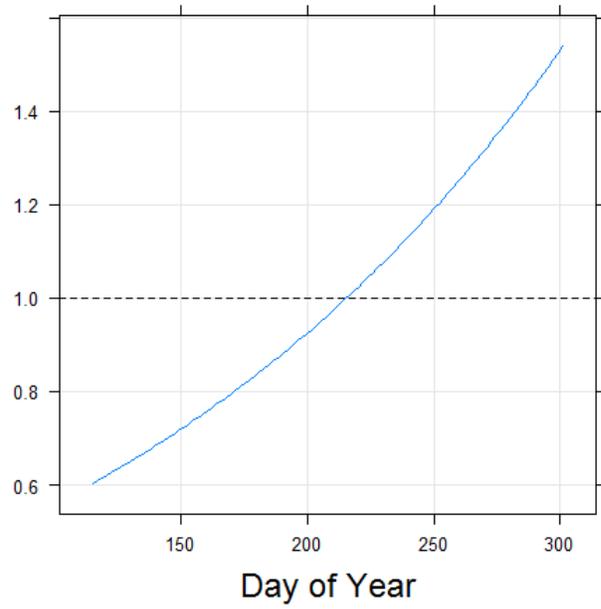
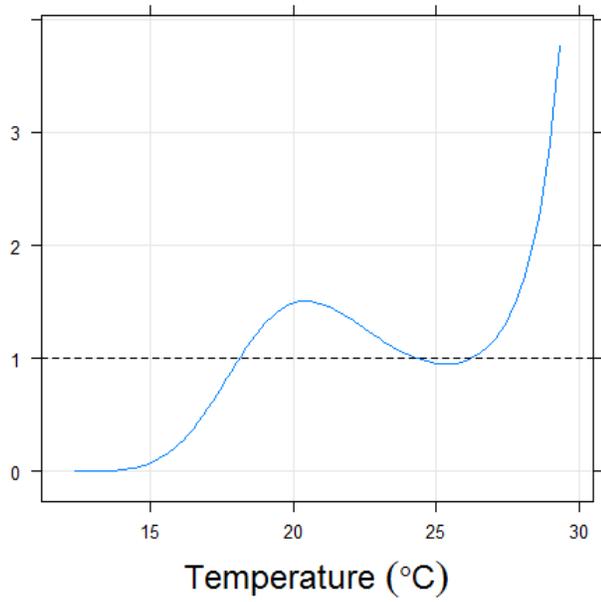
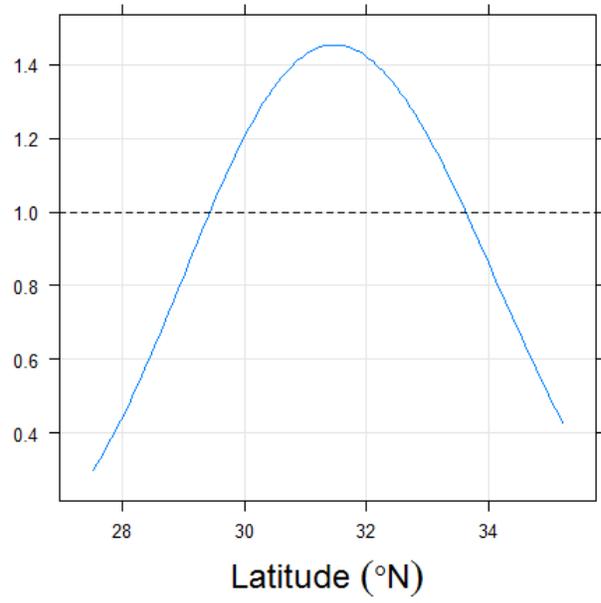
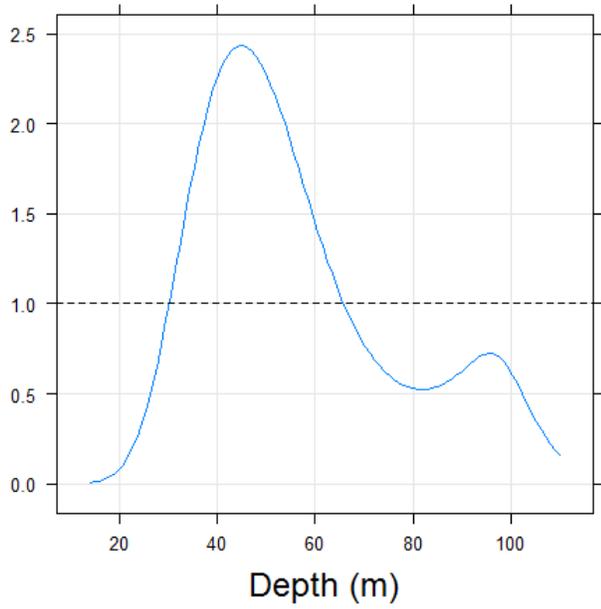


Figure 7: Predicted effect of environmental variables on the relative abundance of Lionfish.

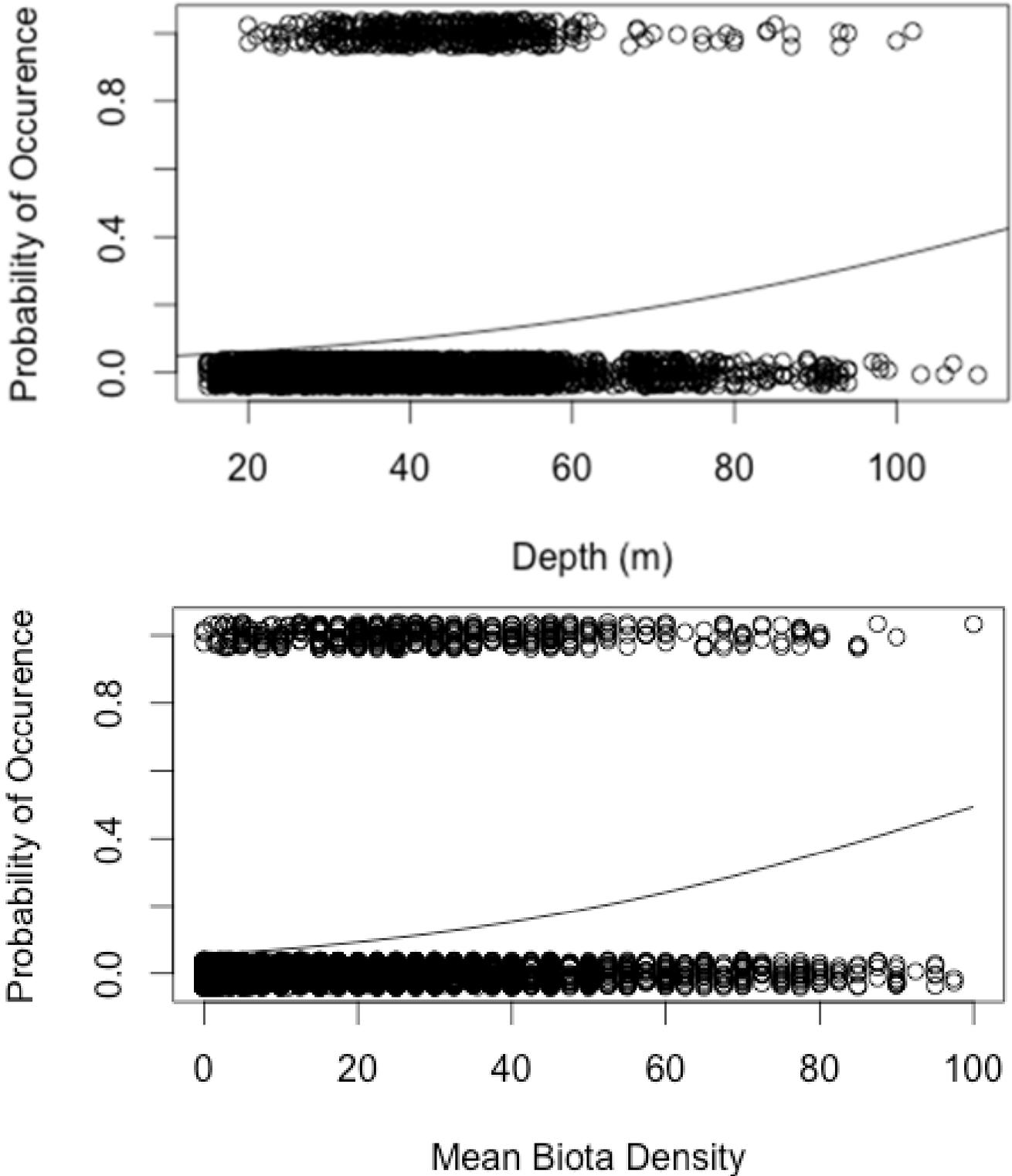


Figure 8: Probability of Lionfish occurrence as a function of depth (top) and mean biota density (bottom).

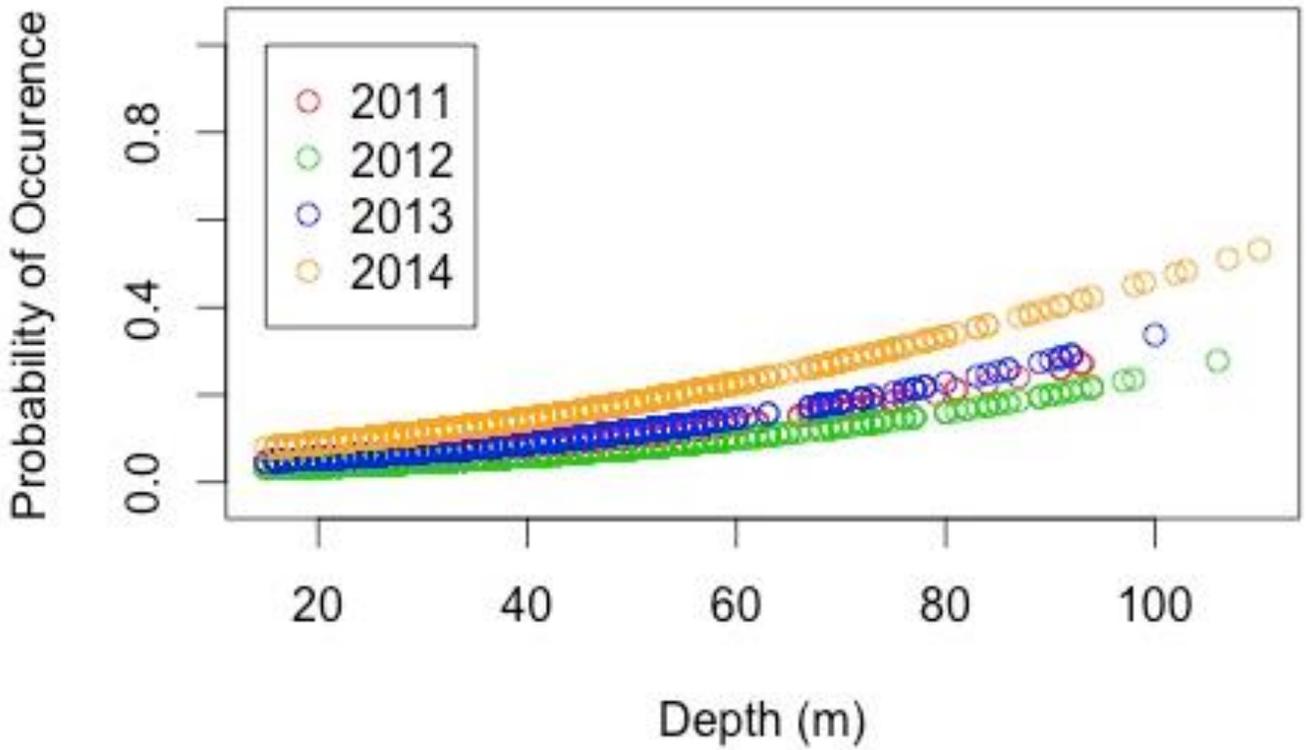


Figure 9: Probability of Lionfish occurrence as a function of depth and year.

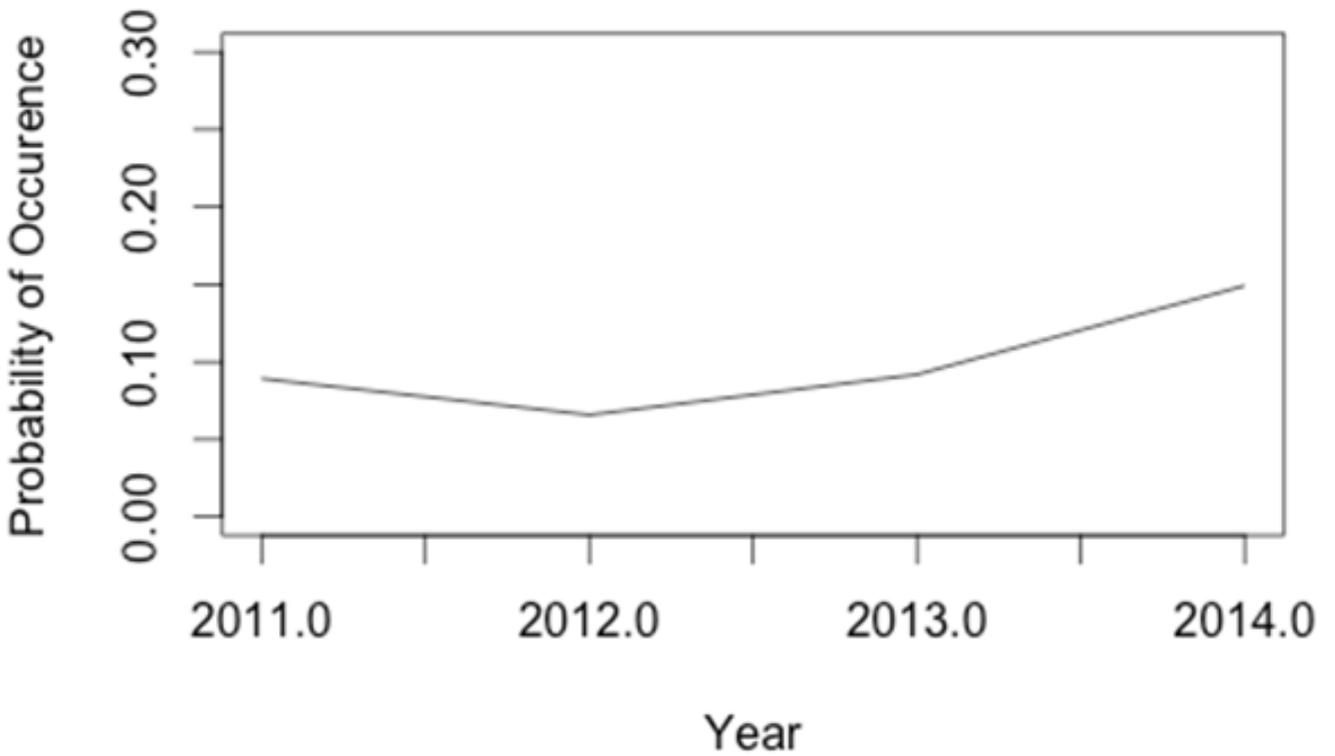


Figure 10: Probability of Lionfish occurrence as a function of year.

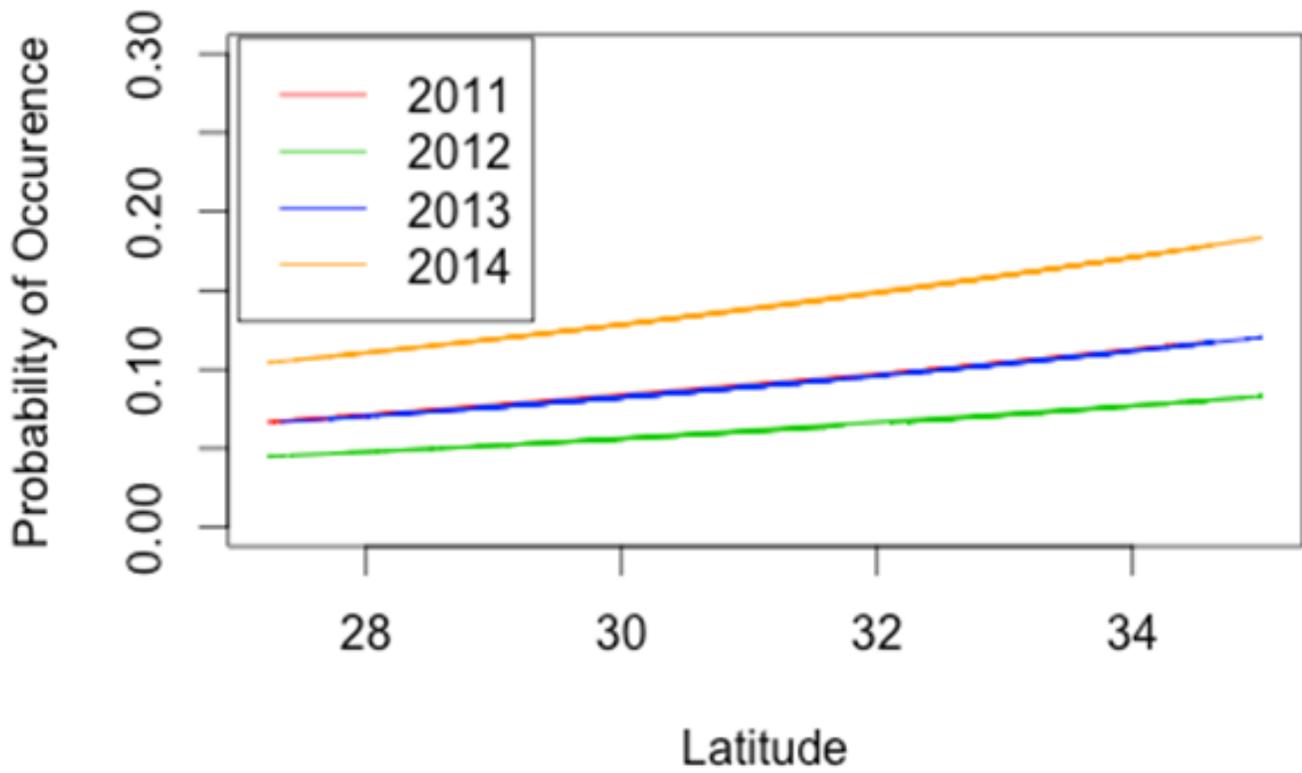


Figure 11: The probability of Lionfish occurrence as a function of latitude and year.

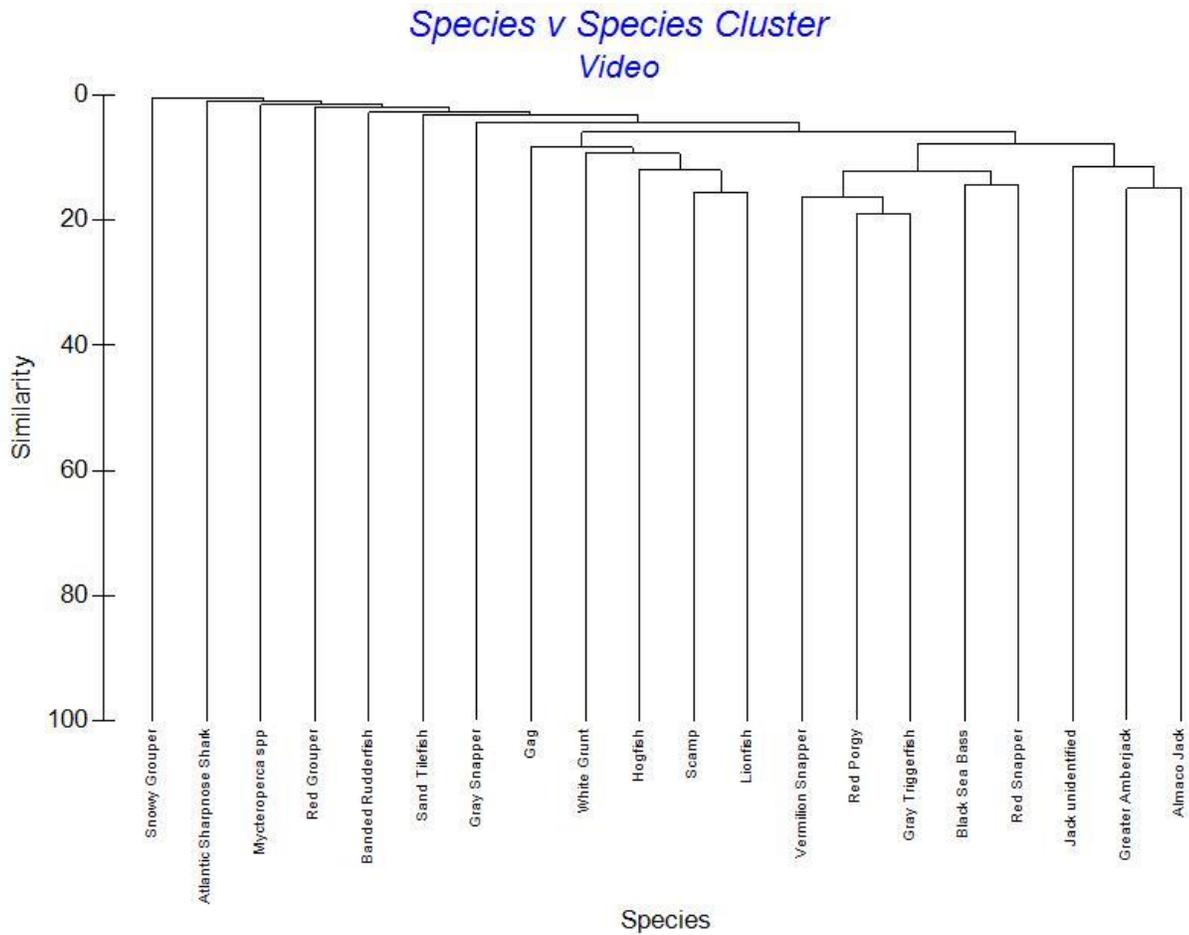


Figure 12. Cluster analysis of top 20 important species from the SERFS video survey selected by a cut-off minimum proportion from any sample. The branches indicate similarities based on the presence and abundance of species pairs over all samples.

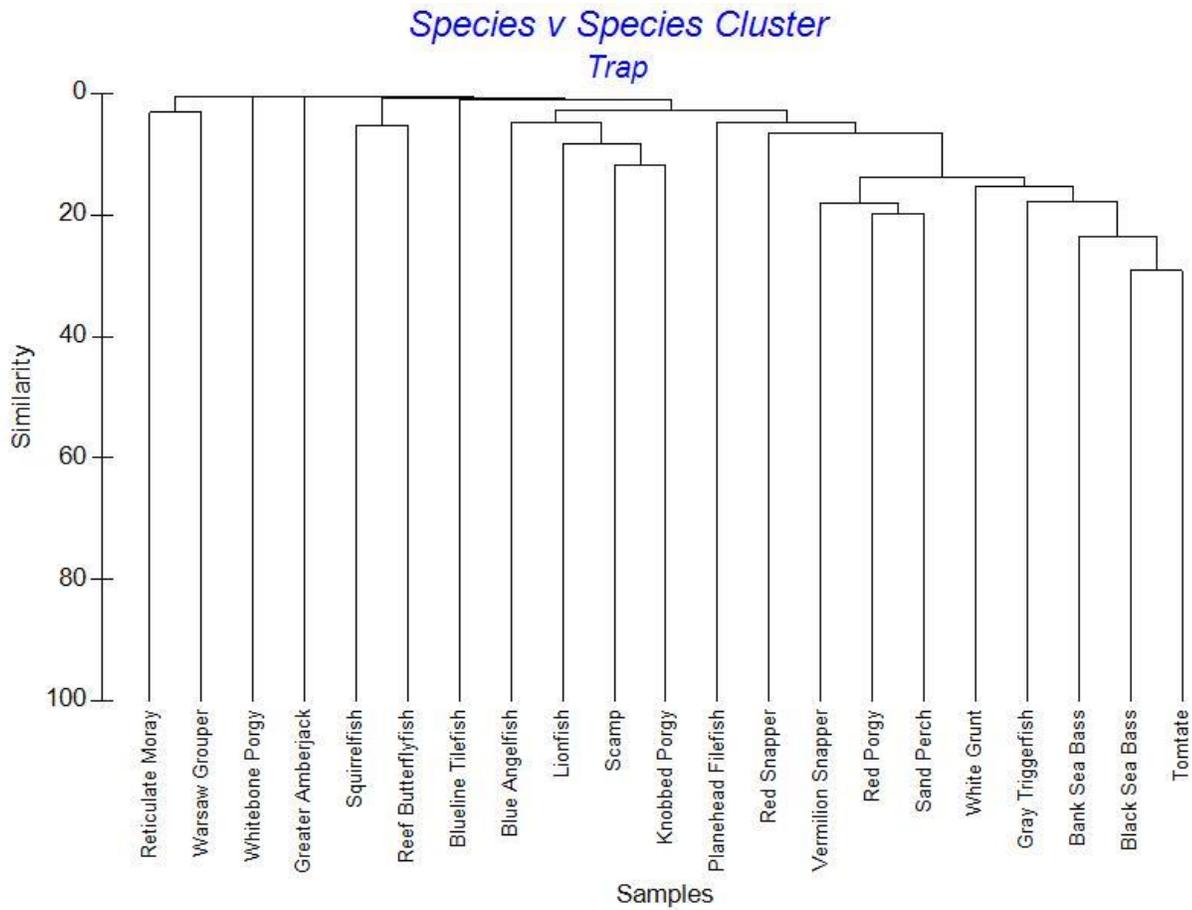


Figure 13. Cluster analysis of top 20 important species from the SERFS trap survey selected by a cut-off minimum proportion from any sample. Lionfish abundances from the video were substituted in this analysis due to poor recruitment of Lionfish to chevron traps. The branches indicate similarities based on the presence and abundance of species pairs over all samples.