Using Environmental DNA (eDNA) to Improve the Accuracy and Efficiency of Managing the Aquatic Invasive Pacific Red Lionfish in the Caribbean

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Introduction

The Pacific red lionfish (*Pterois volitans*) originates from the Indo-Pacific region, but has invaded the Atlantic Ocean, Caribbean Sea, and Gulf of Mexico over the last few decades (Schofield P. J., 2010). Lionfish were most likely introduced into Florida waters due to their popularity in the aquaria pet-trade industry (Semmens, Buhle, Salomon, & Pattengill-Semmens, 2004; Whitfield, et al., 2002). Lionfish are considered to be an invasive species, rather than just an exotic species, due to the ecological effects they have on marine ecosystems (Schofield, Morris, Langston, & Fuller, 2017).

In their native territory, lionfish densities are approximately 26.3 individuals per hectare (Kulbicki, et al., 2012). Lionfish abundance in introduced waters are 3-15 times their natural densities, with 72.7 individuals per hectare in parts of the Atlantic Ocean (Whitfield, et al., 2007) and more than 390 individuals per hectare in the Bahamas (Green & Coˆte, 2009). Lionfish have been able to rapidly invade and establish themselves since their introduction; it is estimated that they have invaded an area of approximately 7.3 million km² of the Western Atlantic Ocean, Caribbean Sea, and the Gulf of Mexico (Côté, Green, & Hixon, 2013). It is anticipated that lionfish will successfully invade the entirety of the Caribbean, and spread south towards South America into Brazil coastal marine waters. (Schofield P. J., 2010). The lionfish's success in these invaded areas is likely due to their high reproduction rates, low predation pressure, ability to migrate large distances in response to competition, and their veracious appetite (Barbour, Allen, Frazer, & Sherman, 2011)**.**

Lionfish reach sexual maturity early in their life cycles, reproducing within their first year of life (Morris J. J., 2009). In optimal conditions, lionfish can produce as many as 40,000 eggs every 4 days throughout the entire year (Morris J. J., 2009). This means a single female lionfish could potentially produce approximately 2 million eggs each year. This remarkably high reproductive capability makes the lionfish very efficient at rapid colonization, but dependent on survivorship. Lionfish maintain high levels of survivorship in their invaded territories much in the same way as other invaders, by looking and behaving unique to avoid would be predators. The natural predators of lionfish in the Indo-Pacific Ocean, such as groupers and sharks, are also found in the invaded areas of the Caribbean and Atlantic Ocean; however, these same species of fish have yet to acclimatize to the invasive lionfish. Lionfish have bright red coloration and venomous dorsal spines that also aid in warding off most potential predators (Halstead, Chitwood, & Modglin, 1955). However, recent reports suggest evidence that predation of lionfish is beginning to occur. For example, lionfish were found in dissected groupers in the Caribbean (Maljković, Van Leeuwen, & Cove, 2008), and researchers have witnessed a spotted moray eel consuming a live lionfish in the field (Pimiento, Monaco, Barbour, & Silliman, 2013). One management strategy for reducing the impact of lionfish is trying to train predators to eat lionfish by feeding sharks and groupers dead lionfish, but the effectiveness of this strategy is still unknown (Albins & Hixon, 2013). Furthermore, even if training top predators to consume lionfish was effective, the natural top predators in reef ecosystems are extremely overfished, limiting the impact they could have on controlling lionfish populations (Mumby, Harborne, & Brumbaugh, 2011; Sadovy, 1995).

Lionfish are piscivores, meaning their diet consists of other fish. Their ability to consume a large variety and amount of fish has led to the reduction of native reef fish populations by around 80-97% (Albins & Hixon, 2008; Benkwitt, 2015). The depletion of reef fish in invaded

areas is increasing as the lionfish diffuse throughout the Southeastern coast of the U.S., the Gulf of Mexico and the Caribbean Sea. However, the biggest effect their consumption has on reef ecosystems is due to the large number of herbivorous reef fish consumed (Morris & Akins, 2009). Herbivorous reef fish help maintain coral reef health by consuming algae which, if left unchecked, would grow over the coral polyps, blocking the sunlight from getting to the symbiotic organism found in the coral, resulting in coral death and further biodiversity loss (Albins & Hixon, 2013; Lesser & Slattery, 2011; Mumby, et al., 2006). Lionfish are also veracious, generalized predators and are indiscriminate when consuming prey (Morris & Akins, 2009). Researchers have observed a single lionfish consuming over 20 reef fish in as little as 30 minutes (Albins & Hixon, 2008). In addition, lionfish can survive for long periods without eating, despite their typical high consumption of prey, allowing them to migrate great distances (Tamburello & Cote, 2014).

Lionfish are successful predators due to their coloration and cryptic nature. Lionfish have neutral coloration with elongated fin rays that native fish might mistake as seaweed, crinoids, or even tubeworms; effectively camouflaging lionfish from potential prey (Albins & Hixon, 2013). Lionfish have a unique hunting strategy compared to native predators; they blow jets of water towards their prey, causing them to turn and orient themselves in a fashion that allows lionfish to consume prey fish head-first (Albins & Lyons, 2012). Fish in invaded areas are unfamiliar and not adapted to lionfish's hunting strategies, making them easy targets (Albins & Hixon, 2013).

Current conservation and management efforts have been focused at the local level, with small scale attempts to reduce lionfish populations and prevent invasion in specific areas by using spear fishing and netting practices. This technique would only be effective at reducing lionfish density in a small routinely monitored area, but eradication of the lionfish using these techniques is unlikely. Furthermore, current fishery management techniques have also been deemed either ineffective or too costly to implement in the fight to control the invasive lionfish. For example, common tracking techniques used to monitor other marine fish species are impeded by the lionfish's cryptic nature, patchy distribution, migration patterns and rapid colonization due to the high reproduction rate of individual lionfish (McCreedy, Toline, & McDonough, 2012). Similarly, traditional fish surveying techniques of getting into the water (e.g. scuba diving) and looking to determine if lionfish are present has been shown to be a costly, time consuming and inaccurate approach. A 2013 study estimated that conventional visual survey methods underestimate lionfish abundance by approximately 200% (Green, Tamburello, Miller, Akins, & Côté, 2013). The National Park Service has noted that a recommended amount of survey time is 5 minutes for every 50 square meters surveyed for simple habitats; requiring a large amount of time to properly conduct a visual survey of structurally complex reef ecosystems (McCreedy, Toline, & McDonough, 2012). The National Park Service has also evaluated several tools currently available to control lionfish, but they are all either costly, ineffective, damaging to ecosystems, or a combination of those qualities (McCreedy, Toline, & McDonough, 2012). More efficient, cost effective and accurate methods are necessary to combat the devastating effects of this invasive species. With the goal of site-specific management for this invasive species, determining what areas have not been invaded is critical. Therefore, being able to efficiently and accurately track lionfish to reduce their threat to coral reef ecosystems is essential to improving current lionfish management.

Environmental DNA (eDNA) sampling is a tool used for genetic surveillance of aquatic organisms. This tool can be applied to detecting trace amounts of DNA collected from water samples. With this technique, DNA can be detected from epithelial tissue, feces, gametes, mucous and the cells of dead organisms (Newton, 2014) and only requires the collection of surface water samples from the site of interest. The processing of the water sample is standard for molecular biologists. The water sample is first filtered to capture the eDNA, the DNA is then extracted and analyzed using PCR and run out on a gel (or sequenced) to determine its species composition.

Currently, this efficient and accurate technique has been successfully used to track freshwater invasive species such as Asian carp (*Hypophthalmichthys nobilis*) and silver carp (*Hypophalmichthys molitrix*) along the Mississippi River Basin and in the Great Lakes (Jerde, et al., 2013; Jerde, Mahon, Chadderton, & Lodge, 2011), as well as, used to catalog entire marine communities (Thomsen P. F., et al., 2012). Similarly, because the eDNA technique only requires a water sample, it offers a less intensive and harmful method to monitor rare and endangered species, and has been used to successfully monitor endangered biodiversity in freshwater environments (Thomsen P. F., et al., 2011), and is currently being developed to monitor shark communities (Dr. Mariani and Judith Bakker of UK and Professor Glaholt IU, personal communication). Using eDNA to monitor aquatic organisms has been shown to be more time and cost efficient than current methods (Amberg, et al., 2015). These same eDNA methods currently being applied for tracking invasive and endangered species in aquatic ecosystems can be applied to tracking the presence and movement of the invasive lionfish.

No study to our knowledge has used this technique to track an invasive marine species. We propose to be the first to implement eDNA techniques to track an invasive marine species by determining the leading edge of the Caribbean lionfish invasion. To achieve this goal, we need to first develop accurate methods of tracking lionfish under controlled laboratory and field conditions. These methods were then applied in a field study to determine accuracy and cost effectiveness of identifying areas invaded by lionfish in order to help mitigate the effects they have on reef ecosystems. This will be done by identifying areas that have not been invaded to prevent further invasions because current tracking methods have not proved to be effective enough to stop the dispersal of lionfish. The eDNA technique offers a cost-effective, efficient, and accurate way to detect lionfish presence in an ecosystem that requires minimal time and effort to perform.

Our research adapts current eDNA methods to track and monitor lionfish populations in the Caribbean. eDNA offers a powerful technique to help mitigate future negative ecological impacts from lionfish and other invasive species. This technique provides managers with an affordable, efficient, and practical method to track and manage lionfish and other invasive species. We are aiming to improve the efficiency of monitoring endangered, rare, and invasive species in marine environments.

Methods

The following experiments were designed to detect the presence or absence of lionfish, determine if eDNA concentration correlates with lionfish abundance, measure the duration of time eDNA persists in the environment, and test this method to determine its applicability in the

field. The lionfish used in each of these experiments were collected from various sites off the coast of Bonaire (N 12º09.605' W 068º16.919') by certified divers associated with CIEE Bonaire and ranged in size from 3.4g to 24.1g with mean of 10.8g ± 5.4g.

Lionfish Detection & Abundance Laboratory Experiment

Objective: This experiment was designed to determine if eDNA can detect the presence or absence of lionfish, and if eDNA signal strength correlates with lionfish abundance.

Experimental Setup: The experiment was conducted at the CIEE Research Station in Bonaire, Caribbean Netherlands (N 12º09.619' W 068º16.859') using 12- 10 gallon glass aquariums. All aquariums were cleaned using a 10% bleach solution and rinsed with freshwater to remove any DNA before being filled with unfiltered, homogenized ocean water collected off the beach outside of CIEE Research Station. Each aquarium was randomly assigned one of three lionfish abundance treatments (Low, Medium, and High) or a control. All treatments were replicated three times. All treatments consisted of the same density of lionfish, with only lionfish abundance varying among treatments. The control aquariums were filled with 4 L of homogenized seawater and contained 0 lionfish. Low abundance treatment contained 1 lionfish, and were filled with 4 L of homogenized seawater. Medium abundance treatment contained 2 lionfish and 8 L of homogenized seawater. High Abundance treatment contained 4 lionfish and 16 L of homogenized seawater.

Experimental Conditions: The experiment was conducted outside the CIEE Field Research Station to simulate the natural temperature and light levels found in the Caribbean. Water temperature was recorded every 10 minutes using loggers place inside each experimental tank. An aeration system was set up to maintain D.O. levels above 5ppm (ranging from 5.4 – 5.7 mg/L) to prevent stressful conditions. Other water quality measurements (i.e. total chlorine, free chlorine, total hardness, total alkalinity, and pH) were taken using Hach AquaChek water quality strips at the start and end of each experiment. All tanks were covered with opaque plastic material at all times, except when samples were taken, to prevent cross contamination or extraneous DNA from entering the water.

Sampling: Prior to placing the lionfish in the treatment tanks, water samples (300 mL) were collected (T_0) from the control tank and each of the experimental tanks to determine the amount of background lionfish eDNA in the seawater. A second water sample (300 mL) was taken from all tanks 24 hours after the lionfish had been put in the tanks (T_{24}). All water samples were taken using bleach wiped 300 mL Nalgene High-Density Polyethylene bottles. Quality control samples (i.e. blanks) were taken of the Milli-Q ultra clean rinse water used to clean all filtering equipment between samples to monitor potential environmental contamination. All water samples were filtered within 1 hour after collection.

eDNA Longevity Laboratory Study

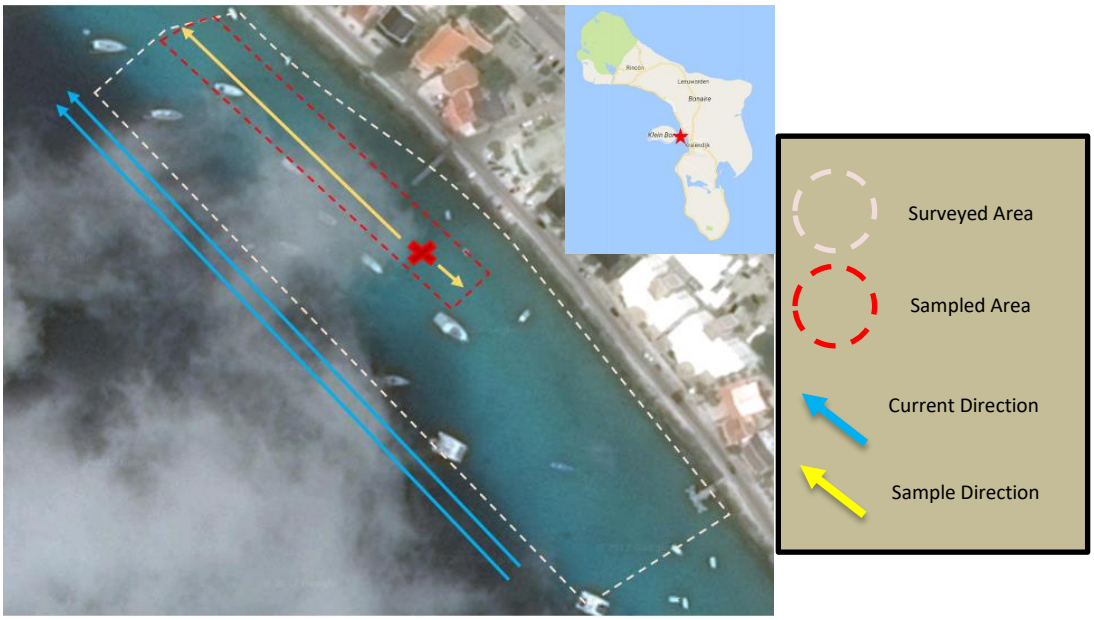
Objective: This experiment was designed to determine how long eDNA persists in the environment.

Setup and Sampling: After the 24 hour water samples were taken for the abundance experiment, the lionfish were removed from the tanks. The water in each replicate of the Low abundance treatment tank was retained for 4 more days to test the duration eDNA persists in the water. Water samples of 300 mL were collected every 24 hours for 4 days using the same sampling procedure as described above. Blanks were also taken during every sampling session to monitor for potential environmental contamination.

Field Validation of eDNA Study

Objective: This experiment was designed to determine how far eDNA can be detected from a single caged lionfish.

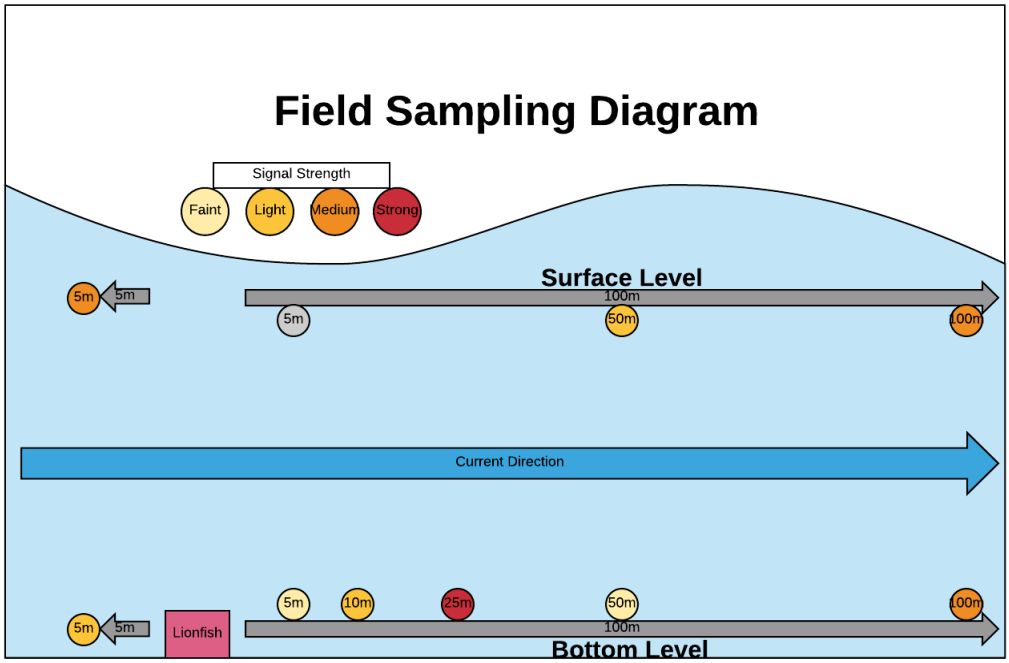
Setup: The field validation study was conducted at the Yellow Submarine dive site on Bonaire (N 12º09'34.8" W 068º16'55.4"). Researchers used a caged (dimensions: 30 cm long x 15 cm diameter with 1 cm^2 hole size) living lionfish (76.5 g) secured 0.5 m off the ocean floor in 2.75m depth of water in an area determined to be free of lionfish by surveying a 100m radius from where the caged lionfish would be placed for 6 hours prior to the experiment (see diagram below).



Map of field sampling area

Sampling: Sampling of the caged lionfish eDNA occurred 6 hours after placing the lionfish in the sampling area, to allow lionfish eDNA to be released into the water. A total of 10- 1 L water samples were taken by hand at surface level and ocean floor level. Samples were collected at surface level and ocean floor to determine if eDNA follows laminar flow or is distributed throughout the water column. Two samples were taken as controls at 5 m upstream from the caged lionfish to test for background lionfish eDNA; one at surface level, the other along the floor of the ocean. Eight samples were taken along a 100 m transect downstream from the lionfish, to determine how far a lionfish could be detected using eDNA sampling. Three of the eight samples were taken at surface level at 5 m, 50 m, and 100 m away from the caged lionfish.

Five of the eight samples were taken along the ocean floor at 5 m, 10 m, 25 m, 50 m, and 100 m away from the caged lionfish (see diagram below).



After collection, the outside of all sample bottles were bleach wiped before being transported into the filtering area to avoid contamination. All samples were processed following the same procedure as the previous experiment and described in more detail below.

Filtering, Extraction & PCR

All samples were filtered within 24 hours after collection, following a standard filtering and extraction procedure. The filtering area and equipment were cleaned with bleach wipes to prevent contamination before processing each sample. Water samples were filtered using 300 mL Pall® filter funnels onto 1.2 µm PCTE filters (Millipore RTTP Isopore Membrane Filter Cat# RTTP04700). Filters were then folded inwards and placed into 2 mL tubes filled with 700 µL of CTAB warmed to 65 ºC (Doyle & Doyle, 1987) and left to incubate at 65 ºC for 10 minutes. The 2 mL tubes were then placed into a 20 °C freezer until DNA extractions were performed.

The eDNA extraction followed a modified chloroform-isoamyl alcohol (hereafter “CI”) DNA extraction and isopropanol precipitation as described in (Renshaw, Olds, Jerde, McVeigh, & Lodge, 2014) and outlined here: [1] the 2 mL microcentrifuge tubes containing the filter and 700 uL of CTAB based hyb solution were incubated in a 65°C water bath for a minimum of 10 minutes; [2] 700 µl of CI (24:1, Amresco) was added to each tube and samples were vortexed for 5 seconds; [3] tubes were centrifuged at 15,000 g for 5 minutes and 500 µl of the aqueous layer was transferred to a fresh set of 1.5 mL microcentrifuge tubes; [4] 500 µl of ice cold isopropyl alcohol and 250 µl of 5M NaCl were added to the 500 µl removed from the aqueous layer and tubes were precipitated at -20 °C overnight; [5] the precipitate was pelleted by centrifugation at

15,000 g for 10 minutes and the liquid was decanted; [6] 150 µl of room temperature 70% ethanol was added to each tube to wash pellets; [7] tubes were centrifuged at 15,000 g for 5 minutes and the liquid was decanted; [8] 150 µl of room temperature 70% ethanol was added to each tube to wash pellets a second time; [9] tubes were centrifuged at 15,000 g for 5 minutes and the liquid was decanted; [10] pellets were dried in a vacufuge at 45 °C for 15 minutes, followed by air drying until no visible liquid remained; and finally, [11] pellets were rehydrated with 100 µl of 1X TE Buffer, Low EDTA (USB). Extracted samples were then processed for PCR using GoTaq polymerase and following standard PCR techniques. The following 12s primers designed specifically for lionfish were used: F- 5' CCA TCT TAA CAT CTT CAG TG 3' and R- 5' CAT ATC AAT ATG ATC TCA GTAC 3' (Freshwater, et al., 2009). As a positive control for our PCR we used DNA extracted from tissue samples taken from our experimental lionfish, as well as, non-lionfish DNA (i.e. Daphnia) as a negative control for our PCR. PCR products were visualized on a 1% agarose gel to determine lionfish eDNA presence and concentration in all samples.

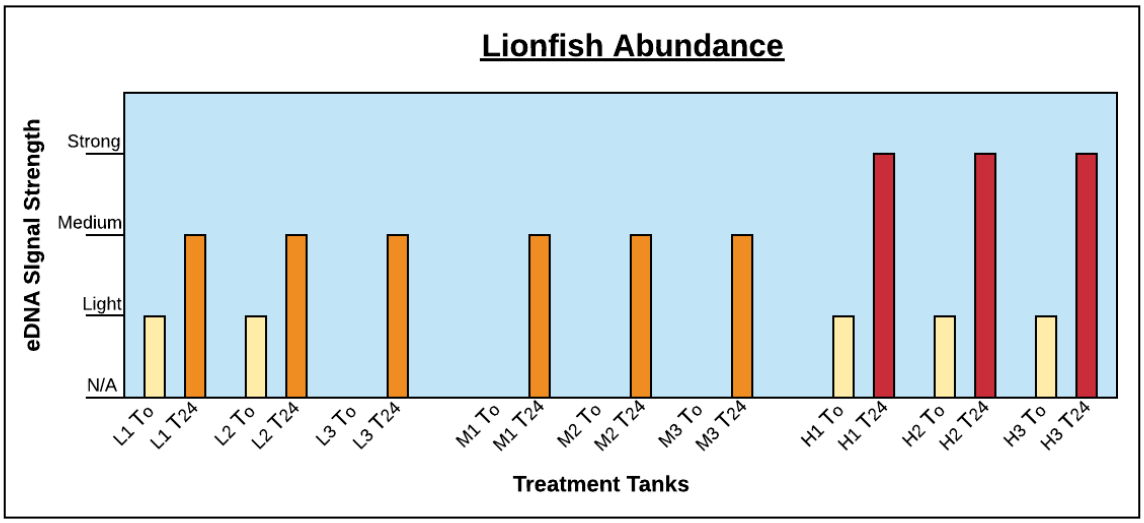
Results

Lionfish Detection & Abundance

Low signals of lionfish eDNA were detected in the unfiltered sea water used in control tanks within the first 24 hours of this experiment, but tested negative after 24 hours. In two of the Low Treatment tanks, water samples taken immediately after lionfish were placed in the tanks (T_o) tested positive for lionfish eDNA for low concentrations of lionfish eDNA, with the third tank testing negative. After 24 hours all three tanks tested positive for lionfish eDNA with medium signal strength. In Medium Treatment tanks, samples taken at T_o tested negative for lionfish eDNA. After 24 hours, the Medium Treatment tanks tested positive in all three tanks for medium concentrations of lionfish eDNA. In High treatment tanks, water samples taken at T_o tested positive in all three tanks for light concentrations of lionfish eDNA. After 24 hours, all three tanks tested positive for high concentrations of lionfish eDNA. In each treatment the strength of the signal of lionfish eDNA present increased from T_o to T_{24}. Furthermore, the signal strength at T_{24} increased with lionfish abundance (e.g. low abundance means medium to low signal strength, and high abundance will have a stronger signal strength).

Treatment	Time ID	eDNA Signal Detectable	Strength of eDNA Signal
Control	T_o	Yes	Light
Control	T_{24}	No	N/A
L1	T_o	Yes	Light
L1	T_{24}	Yes	Medium
L2	T_o	Yes	Light
L2	T_{24}	Yes	Medium
L3	T_o	No	N/A
L3	T_{24}	Yes	Medium
M1	T_o	No	N/A
M1	T_{24}	Yes	Medium
M2	T_o	No	N/A

M2	T_{24}	Yes	Medium
M3	T_o	No	N/A
M3	T_{24}	Yes	Medium
H1	T_o	Yes	Light
H1	T_{24}	Yes	Strong
H2	T_o	Yes	Light
H2	T_{24}	Yes	Strong
H3	T_o	Yes	Light
H3	T_{24}	Yes	Strong

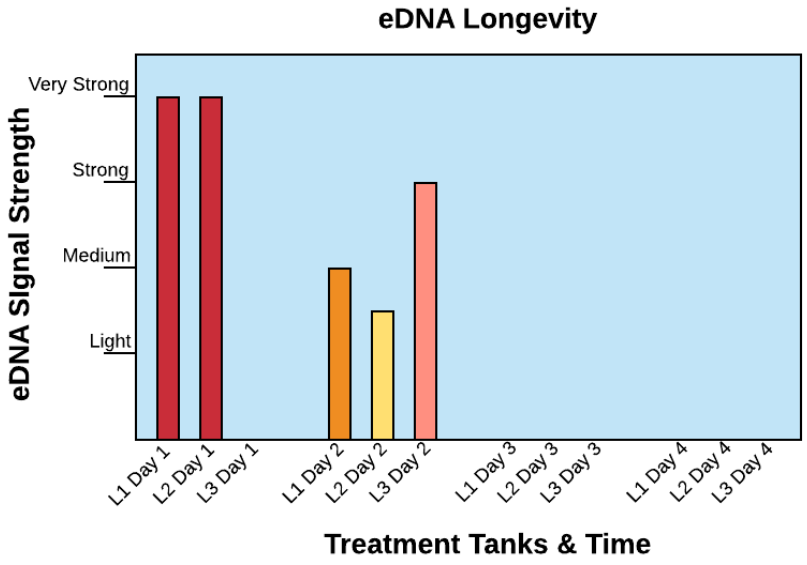


eDNA Longevity

On Day 1 of this experiment, after the lionfish were removed from the Low abundance replicate aquariums, two of the treatment tanks tested positive for lionfish eDNA with a very strong signal, while the third tank tested negative for any lionfish eDNA signal. Twenty-four hours after lionfish were removed all three samples still showed the presence of lionfish eDNA, with signals ranging in strength from light to strong. However, after 48 hours lionfish eDNA was no longer detectable in any of the treatment tanks. Furthermore, all tanks tested negative for lionfish eDNA for the remainder of the 4 day experiment.

Treatment	Time ID	eDNA Signal Detectable	Strength of eDNA Signal
L1	Day 1	Yes	Very Strong
L2	Day 1	Yes	Very Strong
L3	Day 1	No	N/A
L1	Day 2	Yes	Medium
L2	Day 2	Yes	Med/Light
L3	Day 2	Yes	Strong

L1	Day 3	No	N/A
L2	Day 3	No	N/A
L3	Day 3	No	N/A
L1	Day 4	No	N/A
L2	Day 4	No	N/A
L3	Day 4	No	N/A



Field Study

The two-control samples taken 5m upstream from the tethered lionfish to detect background lionfish eDNA both tested positive for lionfish eDNA with light and medium signal strengths. This positive signal indicates the presence of significant background lionfish eDNA at our experimental sight. From there, all samples taken downstream, except for one at the surface level, tested positive for lionfish eDNA with varying signal strengths. Surface level samples and bottom level samples were consistent in their ability to detect lionfish eDNA.

Sample ID	Distance from Lionfish	eDNA Signal Detectable	Strength of eDNA Signal
Upstream- Bottom	5m	Yes	Light
Upstream- Surface	5m	Yes	Medium
Downstream- Surface	5m	No	N/A
Downstream- Surface	50m	Yes	Light
Downstream- Surface	100m	Yes	Medium
Downstream- Bottom	5m	?	Faint
Downstream- Bottom	10m	Yes	Light
Downstream- Bottom	25m	Yes	Strong
Downstream- Bottom	50m	?	Faint
Downstream- Bottom	100m	Yes	Medium

Discussion

Results from all three experiment components indicate the strengths of the eDNA methodology, while also highlighting areas that still need additional research. The lionfish abundance study indicated that the strength of lionfish eDNA signal correlates with number of lionfish present under highly controlled conditions. In this experiment, we controlled for the distance between the sample and the target organism and the level of eDNA degradation by controlling the time at which the eDNA was present in the environment. In the field, both time and proximity to the target organisms are unknown and can each effect the eDNA signal strength, rather than abundance or density. Therefore, while our results support the use of eDNA concentration (i.e. signal strength) to infer abundance (Jerde, Mahon, Chadderton, & Lodge, 2011; Thomsen P. F., et al., 2011), we strongly caution against the use of eDNA as a means to quantify target organisms until more field studies can validate our laboratory experiments. Alternatively, determination of the exact number of individuals could be done using genomic tools such as sequencing and bioinformatics to identify unique genetic differences associated with individuals within water samples collected, to accurately assess the number of individuals and, therefore, estimate population size and density.

eDNA proved to be an effective methodology in determining presence of lionfish under controlled laboratory conditions and in the field. While, due to high background levels of lionfish eDNA in the water, our field experiment was unable to determine the distance a lionfish's eDNA could be detected. We were able to show conclusively that lionfish eDNA is detectable even in the absence of visualization of lionfish. The sensitivity of this technique makes it highly valuable for monitoring species presence and movement in areas of interest (i.e. present or not, invasion/establishment progress, etc.). However, the high sensitivity also makes this method tricky to monitor sub-populations in specific areas, because it is likely that it will also pick up eDNA from separate distant populations up-current. Currently, this technique would be most useful in determining whether lionfish have invaded new areas in the Caribbean, but may be too sensitive to determine habitation in areas on an already invaded small island ecosystem such as Bonaire. More research is needed to fully determine the full potential and limitations of eDNA.

The field component also demonstrated that surface samples were consistent with bottom samples in detecting lionfish eDNA. The ability to take surface samples significantly reduces the time necessary to determine lionfish presence. The ability to take surface samples to detect lionfish using eDNA methodology eliminates the time, effort, and costs associated with divers or snorkelers conducting visual surveys for several hours or days, and enables a boat and a person to collect monitoring samples to be run in the lab. This allows scientist and managers to collect more samples and subsequently increase the frequency of sampling and area sampled to improve monitoring of the lionfish or other invasive species.

Our eDNA longevity experiment clearly showed that eDNA lasts ~48 hours under the light duration and temperatures found on Bonaire. The length of time eDNA persists in the environment is an important variable in determining the maximum distance a positive eDNA signal could detect. Managers in the Caribbean can utilize this information to estimate the maximum distance lionfish might be away from the sampling sight, creating an area of interest based on current speed and direction. The conditions are common among areas found at this

latitude and do not vary much seasonally due to its close proximity to the equator. A 48 hour longevity period not only gives confirmation that lionfish have been in that area in the last 2 days, but it also guarantees that lionfish have been absent in an area for the last 2 days if no eDNA is detected. Lastly, the sensitivity of eDNA detection of lionfish in the field is more accurate than sightings data since lionfish like to hide during the day, making them hard to see. Missing even one adult female lionfish, due to its extreme reproduction capabilities, can make the difference in preventing a new area from being invaded. Thus, eDNA techniques can save environmental managers a substantial amount of time and money, and increase accuracy in monitoring the invasive lionfish.

Conventional methods evaluated by the National Park Service are all either costly, ineffective, damaging to ecosystems, or a combination of those qualities (McCreedy, Toline, & McDonough, 2012). The proposed eDNA methodology saves money by reducing sampling effort, and is itself an inexpensive process. Processing samples for this experiment costs approximately $0.05 per sample, or approximately $2.45 in total. Comparing this cost to conventional methods, which require several surveyors and countless hours to conduct the survey, it is clear how big of a game changer this methodology could be in advancing marine conservation biology.

eDNA has the potential to be a highly valuable environmental management tool for other aquatic species of interest. Most notably in the monitoring of rare or endangered species in marine and freshwater environments. Researchers have already implemented eDNA to monitor endangered freshwater biodiversity (Thomsen P. F., et al., 2011), freshwater invasions (Jerde, Mahon, Chadderton, & Lodge, 2011), and rare species (Thomsen P. F., et al., 2011). However, the marine scientist and managers have been slow to implement eDNA as a tool in their research and conservation efforts. A classic example of where eDNA could be highly effective is in the shark conservation efforts around the globe. Historic methods of collecting site specific community composition data on shark communities is difficult, inaccurate, time consuming, and expensive. Because of this, data on many species of sharks is insufficient. Currently, researchers are attempting to incorporate eDNA into shark community assessment projects. For example, our group is currently working with the National Park Managers on Bonaire to determine the species of sharks around the island as part of a Caribbean wide conservation project. Our group is incorporating eDNA tools into their research to minimize the cost and maximize the efficiency and quality of the shark conservation efforts on Bonaire (STINAPA of Bonaire and Professor Stephen Glaholt of IU, personal communication).

eDNA already has the potential to collect detailed community level data associated with all aquatic ecosystems, however, with additional research eDNA could also be developed into a population level monitoring tool. To develop it as a population estimate tool, detailed molecular and bioinformatics work will need to be conducted. eDNA holds the potential to provide information on population size and community composition at a level of efficiency and accuracy previously unknown to ecologist, making eDNA a real game-changer in conservation biology.

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