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Estimating the number of lionfish (*Pterois volitans*) that colonized the Atlantic Ocean

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The invasion of the western Atlantic Ocean by the Indo-Pacific red lionfish (*Pterois volitans*) has had devastating consequences for marine ecosystems. Estimating the number of colonizing lionfish can be useful in identifying the introduction pathway and can inform policy decisions aimed at preventing similar invasions. It is well-established that at least ten lionfish were initially introduced. However, that estimate has not faced statistical scrutiny and is based solely on the number of haplotypes in the maternally-inherited mitochondrial control region observed in samples from the western Atlantic Ocean. To rigorously estimate the number of lionfish introduced, we used a forward-time, Wright-Fisher, population genetic model of the mitochondrial control region in concert with a demographic life-history model to simulate the invasion. The colonization of the western North Atlantic and subsequent spread to the Caribbean and Gulf of Mexico were modeled as single introductions of 1-800 females, which should conservatively estimate the most likely number of colonizing lionfish relative to a model with multiple introductions. Assuming a balanced sex ratio and no Allee effects, the simulations indicate that the Atlantic population was most likely founded by 118 (54-514, 95% HPD) lionfish from the Indo-Pacific, the Caribbean by 84 (22-328, 95% HPD) lionfish from the Atlantic, and the Gulf of Mexico by at least 114 (no upper bound on 95% HPD) lionfish from the Caribbean. Considering the additional possibilities that (1) multiple Indo-Pacific populations colonized the Atlantic and (2) Allee effects (e.g. 50% reduction in fecundity) plagued the colonists, the most likely number of lionfish increased to 180 (88-416, 95% HPD). These results, in combination with all other published data, support the hypothesis that lionfish were introduced to the Atlantic via the aquarium trade, rather than shipping. The most parsimonious introduction pathway is a single release of lionfish in one location, but it seems likely that a combination of smaller and larger releases from a variety of aquarium trade stakeholders occurred near Miami, Florida. Efforts to prevent future invasions via the

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aquarium trade should focus on the education of stakeholders and the prohibition of release, with adequate rewards for compliance and penalties for violations.

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ABSTRACT

The invasion of the western Atlantic Ocean by the Indo-Pacific red lionfish (*Pterois volitans*) has had devastating consequences for marine ecosystems. Estimating the number of colonizing lionfish can be useful in identifying the introduction pathway and can inform policy decisions aimed at preventing similar invasions. It is well-established that at least ten lionfish were initially introduced. However, that estimate has not faced statistical scrutiny and is based solely on the number of haplotypes in the maternally-inherited mitochondrial control region observed in samples from the western Atlantic Ocean. To rigorously estimate the number of lionfish introduced, we used a forward-time, Wright-Fisher, population genetic model of the mitochondrial control region in concert with a demographic life-history model to simulate the invasion. The colonization of the western North Atlantic and subsequent spread to the Caribbean and Gulf of Mexico were modeled as single introductions of 1-800 females, which should conservatively estimate the most likely number of colonizing lionfish relative to a model with multiple introductions. Assuming a balanced sex ratio and no Allee effects, the simulations indicate that the Atlantic population was most likely founded by 118 (54-514, 95% HPD) lionfish from the Indo-Pacific, the Caribbean by 84 (22-328, 95% HPD) lionfish from the Atlantic, and the Gulf of Mexico by at least 114 (no upper bound on 95% HPD) lionfish from the Caribbean. Considering the additional possibilities that (1) multiple Indo-Pacific populations colonized the Atlantic and (2) Allee effects (50% reduction in fecundity) plagued the colonists, the most likely number of lionfish increased to 180 (88-416, 95% HPD). These results, in combination with all other published data, support the hypothesis that lionfish were introduced to the Atlantic via the aquarium trade, rather than shipping. The most parsimonious introduction pathway is a single release of lionfish in one location, but it seems likely that a combination of smaller and larger releases from a variety of aquarium trade stakeholders occurred in the vicinity of Miami, Florida. Efforts to prevent future invasions via the aquarium trade should focus on the education of stakeholders and the prohibition of release, with adequate rewards for compliance and penalties for violations.

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INTRODUCTION

The ability to successfully eradicate an alien-invasive species is directly correlated with the population size (Epanchin-Niell & Hastings, 2010) and the amount of time that has passed since the initial introduction and establishment (see Blackburn et al., 2011). If the alien population becomes invasive and spreads after establishing, eradication becomes nearly impossible without vast financial commitments (Epanchin-Niell & Hastings, 2010). Preventative measures and early detection enable the effective control of alien species before exponential population growth exceeds conventional control methods (Jarrad et al., 2011). Given the costs of control, prevention or minimization of future invasions is the most effective approach to avoid the destruction of ecosystems and protect economic interests (CBD, 2002; Pimentel, Zuniga & Morrison, 2005; Simberloff et al., 2013).

Elucidating the likely route of transport for already established and spreading alien species, and determining how many colonists established the initial population can be used to inform preventative management and education efforts (Estoup & Guillemaud, 2010; Hulme, 2015; McGeoch et al., 2016). While the initial mechanism of introduction is rarely conclusively identified (Brockerhoff et al., 2014), understanding the number of invaders and the frequency of introductions (i.e., propagule pressure) can be used to infer likely invasion routes (Hulme, 2015). Propagule pressure is directly correlated with the ability of an introduced species to become established and spread (Yang et al., 2012; Brockerhoff et al., 2014). To determine the propagule pressure and infer likely invasion routes, it is important to have probabilistic estimates of the number of invaders initially present (García-Díaz et al., 2015).

Genetic methodologies are particularly useful in reconstructing the history of introduced species (Estoup & Guillemaud, 2010; Rius et al., 2015; Cristescu, 2015). For example, biological invasions that occur following an initial population bottleneck will typically exhibit founder effects, which can be exploited to estimate the initial number of founders (Azzurro et al., 2006; Golani et al., 2007). Integrated population genetic (Fisher, 1922; Wright, 1931) and demographic models can be used to infer and predict founding population sizes and diversity (Ficetola, Bonin & Miaud, 2008; Hulme et al., 2008; Tran, Hofrichter & Jost, 2012; Benson et al., 2016).

The invasion of the western Atlantic Ocean by Indo-Pacific lionfish (*Pterois volitans, P. miles*) has been recognized as a global environmental problem (Sutherland et al., 2010; Hixon et al., 2016). In the Atlantic, alien-invasive lionfish consume both economically and ecologically important species (Morris & Akins, 2009; Côté et al., 2013). The net effect of adding lionfish to the ecosystem has been a reduction in recruitment and biomass of native species that fall prey to the lionfish. In some cases severe reductions have been observed (Albins & Hixon, 2008; Green et al., 2012; however, see Hackerott et al., 2017). Lionfish have also been indirectly tied to a shift from coral to algal dominated habitats through the consumption of herbivorous fishes (Albins & Hixon, 2011; Lesser & Slattery, 2011). Given their range expansion and population size, conventional eradication is not a viable option for controlling these effects (Côté, Green & Hixon, 2013) despite the efficacy of local management (Green et al., 2014; Usseglio et al., 2017).

Lionfish were transported from the Indo-Pacific to the Atlantic via the aquarium trade and likely released by aquarists into the wild initiating the introduction (Semmens et al., 2004). The initial sightings of lionfish in the western Atlantic occurred in Florida in the mid-1980s (Courtenay, 1995; Morris & Akins, 2009). While initially slow to spread outside this region (Whitfield et al., 2002), an indication of Allee effects (Taylor & Hastings, 2005; Tobin et al., 2007), by 2007 the invasion was characterized by large populations (Whitfield et al., 2007) and exponentially increasing population size (Green et al., 2012). Between 2007 and 2010, lionfish spread across the Caribbean (Betancur-R. et al., 2011). In 2010 the invasion front entered the Gulf of Mexico, originating from the Caribbean population (Johnson et al., 2016). The stepping-stone pattern of this invasion has resulted in three genetically distinct lionfish populations, one in each of these regions, with each subsequently colonized region exhibiting progressively lower genetic diversity (Johnson et al., 2016).

The absolute minimum number of *P. volitans* individuals that could have colonized the Atlantic has been estimated to be between six and 10 (Betancur-R. et al., 2011). However, this estimate is based upon counts of unique haplotypes from a 674 bp fragment of mtDNA and is not a robust reflection of the number of individual lionfish that were introduced because it is not rooted in a rigorous probabilistic framework. Therefore, this bare-minimum estimate is not useful to inform either a further understanding of the processes leading to the establishment of the invasion or policy aimed at preventing similar future invasions. Further, the lower estimate of six colonists postulates that four haplotypes may have originated through mutation in the Atlantic, but this is unlikely. The behavior of allelic diversity in expanding populations is well established (Nei, Maruyama & Chakraborty, 1975; Maruyama & Fuerst, 1985), and is characterized by an excess of rare alleles. However, this pattern takes time to develop because mutation rates, even in mitochondrial DNA, are relatively slow (see Lynch, 2010). The invasive lionfish population has yet to exhibit the characteristic genetic signature of an expanding population, despite a large documented population size and widespread expansion. Thus, it is exceedingly unlikely that any of the haplotypes observed in the Atlantic are the result of mutations that arose in the Atlantic. Indeed, in the well-documented invasion of Hawai‘i by the bluestriped snapper (*Lutjanus kasmira*), which predates the Atlantic lionfish invasion by roughly

20 years, no evidence of population expansion is evident in the mitochondrial control region either (Gaither et al., 2010).

This current best estimate of the number of *P. volitans* that colonized the Atlantic (at least nine females and one male) supports the hypothesis that releases by home aquarists triggered the invasion. This hypothesis is tenuous, however, because it does not account for the genetic diversity of the source population or the frequencies of haplotypes in the Atlantic. We hypothesized that when considering additional available genetic information, it was highly improbable that only one male and nine female lionfish (with nine unique haplotypes) established the Atlantic population. If many more lionfish colonized the Atlantic, then it could indicate that the scale of releases by home aquarists is larger than previously thought or that home aquarists alone are not sufficient to explain the mechanism by which lionfish were introduced to the Atlantic.

Here we simulate the lionfish (*P. volitans*) invasion and estimate the most likely number of lionfish to colonize the western Atlantic Ocean, Caribbean, and Gulf of Mexico using a demographic-population genetic model. The results of the simulation are used to re-evaluate the mechanism by which lionfish were introduced to the Atlantic and how this affects the management of future invasions.

METHODS

Characterizing Genetic Composition of Wild Populations

A total of 1,294 mitochondrial control region sequences consisting of 30 unique haplotypes were gathered from GenBank and published literature (Table 1; Freshwater et al., 2009; Betancur-R. et al., 2011; Toledo-Hernández et al., 2014; Butterfield et al., 2015; Johnson et al., 2016). The sequences were obtained from *P. volitans* in Indonesia and the western Atlantic Ocean. Johnson et al. (2016) report that lionfish in the western Atlantic exhibit genetic structure among the western North Atlantic, Caribbean, and the Gulf of Mexico, but not within these regions. Consequently, sampling locations within these regions of the western Atlantic were pooled together for this analysis.

Because the Indo-Pacific population that was the source of the Atlantic lionfish invasion has not been sampled, we needed to estimate its genetic makeup. We used the mean number of pairwise differences and the observed number of segregating sites of a population sample of Indonesian lionfish (Freshwater et al., 2009) to estimate the population parameter, θ ($2N_e\mu$, where N_e is the effective population size and μ is the mutation rate) and its standard deviation (s) using ARLEQUIN V 3.5 (Excoffier & Lischer, 2010). The mitochondrial control region from the Indonesian population sample was tested for conformation with the assumptions of the infinite alleles and infinite sites models of nucleotide evolution (Kimura, 1969; Watterson, 1975; Tajima, 1996), which are nearly identical for linked nucleotides like those in the mitochondrial control region (Hartl & Clark, 2006), using the Ewens-Watterson test (Ewens, 1972; Watterson, 1978), Tajima's D (Tajima, 1989), and Fu's F_s (Fu, 1997) based on 10,000 simulations in ARLEQUIN v 3.5 (Excoffier & Lischer, 2010).

Genetic diversity was used to characterize the alien-invasive lionfish populations of the western North Atlantic, Caribbean, and the Gulf of Mexico, so they could be compared with simulated invading populations. Haplotype richness was calculated as the number of unique haplotypes that have been observed in each population. Since sample sizes from each of the invasive lionfish populations were at least 188 and the maximum number of haplotypes was nine, we see little justification for rarefication of the richness values. Haplotype diversity was calculated as the probability of drawing two different haplotypes at random from the population with 95% confidence intervals calculated based on 1,000 bootstraps sampled with replacement (Nei & Tajima, 1981).

Simulating Colonization

The colonizations of the western North Atlantic, Caribbean, and Gulf of Mexico were simulated as single introduction events, which represent the minimum number of introductions required to trigger invasive populations with the observed allelic richness and mitochondrial haplotype diversity. The number of introduced female lionfish was varied from 1 to 800. We assumed that an equal number of males accompanied the females, given the ~1:1 ratio of males to females in wild populations (Fogg et al., 2013; Downey-Wall, 2016), but the model only requires enough males to fertilize the eggs of the females. Females were sampled from either (1) the inferred Indo-Pacific population using an estimate of θ and the Ewens' sampling formula (Ewens, 1972; Crane, 2016) when simulating the invasion of the western North Atlantic or (2) the observed multinomial distribution of sampled haplotype frequencies in the western North Atlantic and Caribbean populations when simulating the invasions in the Caribbean and Gulf of Mexico, respectively. To test for model sensitivity to error in the estimate of θ (greater or lesser genetic diversity or more source populations), simulations assuming that the source population was characterized by $\theta \pm s$ and $\theta \pm 2s$ were also run.

Demographic model

An individual-based model adapted from the stage-based matrix model developed by Morris, Shertzer & Rice (2011) was used to simulate lionfish population growth. The model was initialized with colonizing adult females, which minimizes the number of colonists required to begin an invasion, relative to the introduction of larvae or juveniles. The model proceeded in monthly time-steps and divides the lionfish life history into three primary life stages (larvae, juvenile, and adult). We further divided the juvenile life stage into 11 juvenile stages of one month to appropriately simulate the delay in the reproductive maturity of Atlantic derived lionfish, which would not be accurately simulated by the model of Morris, Shertzer & Rice (2011). Life-stage-specific demographic parameters from lionfish were used, as outlined by Morris Shertzer & Rice (2011; also see for additional information regarding the estimation and sensitivity of these parameters).

Briefly, adult fecundity (194,577 eggs/month/adult) was calculated from the reproductive contribution (number of eggs) per female per spawn (R_A = 35,315, Morris 2009) and the spawning rate of 7.9/month/female. The eggs had a mortality rate of 0.31/day (McGurk, 1987) before hatching after three days (Morris, 2009). The larval stage lasted for the rest of the month (Ahrenholz & Morris, 2010) with a mortality rate of 0.35/day (McGurk, 1987). Ultimately, the proportion of larvae that survived and metamorphosed into juveniles was 0.00003 (G_L). The 11 juvenile stages had a mortality rate of 0.165/day (m_J). The adult life span was controlled by mortality rate, which was 0.052/month (m_A, Lorenzen, 1996).

To simulate the effect of a slower growth rate at the outset of the invasion due to Allee effects caused by low population densities (Stephens, Sutherland & Freckleton, 1999), we adjusted the monthly fecundity to 25%, 50%, 75%, and 100% (relative fecundity, r) of that reported by Morris, Shertzer & Rice (2011). Allee effects can reduce the fecundity to 25-50%, or lower, in natural populations (Roll et al., 1997; Morgan, 1999; Berec, Angulo & Courchamp, 2007).

A series of four discrete, stage-based equations were used to calculate the number of individuals for each haplotype at each subsequent time point ($t + 1$) from the number at time point (t):

$$n_L(t+1) = rR_A n_A(t) \tag{1}$$
$$n_{J,1}(t+1) = G_L n_L(t) + (1 - m_J) n_{J,1}(t) \tag{2}$$
$$n_{J,s+1}(t+1) = (1 - m_J) n_{J,s}(t) \quad 1 < s < 11 \tag{3}$$
$$n_A(t+1) = (1 - m_J) n_{J,11}(t) + (1 - m_A) n_A(t+1) \tag{4}$$

where $n_{[L,J,A]}$ represent the number of individuals at the larval, juvenile (s represents the 10, monthly juvenile stages), and adult stages, respectively (other variables are defined in the description of the demographic model, above). The model was run for 15 years to encompass the time from the initial lionfish sighting to range expansion (Whitfield et al., 2002).

Genetic drift model

The demographic growth model was used to determine the number of larvae and adults in a Wright-Fisher, forward-time model of genetic drift with overlapping generations (Fisher, 1922; Wright, 1931). Each month, haplotypes are drawn from an infinite pool of eggs produced by the adult females to create the new cohort of lionfish larvae. This model assumes a mutation rate of zero due to the short time scale of the invasion, and there is no evidence of mutations driving mtDNA diversity in western Atlantic lionfish populations. Running the model for 15 years was sufficient time for haplotype frequencies to stabilize without mutation due to decreased genetic drift with increasing population size.

Assessing Simulations

Following the simulation, a sample was drawn from the simulated population that was equal in size to the number sampled and reported by researchers in the destination population.

For example, 459 lionfish have been sampled from the western North Atlantic (Freshwater et al., 2009; Betancur-R. et al., 2011; Butterfield et al., 2015; Johnson et al., 2016), therefore 459 were sampled from the simulated western North Atlantic population. The haplotype richness and diversity of the simulated invasive populations were calculated for each simulation in the same manner as was calculated for the observed population. The conditional joint probability of finding the observed haplotype richness and haplotype diversity within the 95% confidence interval given the number of female colonists was calculated based on 10,000 simulations. We calculated the 50% and 95% highest probability density interval (HPD) of the initial number of females using the CODA package (Plummer et al., 2006). This process was repeated for each source and destination population.

To test the sensitivity of results to variation in the estimate of θ for the Indo-Pacific source population and the fecundity of the alien population, we performed a MANOVA with both θ and fecundity as potentially interacting independent variables with the point estimate, lower, and upper 95% HPD estimates of the number of colonizing females as dependent variables.

All above analyses were performed in R v 3.3.1 using code written by the authors, with figures made using the package GGPLOT2 (Supplement B, Wickham, 2009; R Core Team, 2015).

RESULTS

Genetic Diversity of Wild Populations

Based on the mitochondrial control region haplotypes present in Indonesia, θ was estimated to be 7.64 (±2.73 SD). The population in Indonesia was generally consistent with the assumptions of the infinite sites model (Tajima's $D = -1.161$, $p = 0.111$; Fu's $F = -8.008$, p =

0.004; Ewen-Watterson test F = 0.077, p = 0.0807). The invasive lionfish populations in the western North Atlantic, Caribbean and Gulf of Mexico exhibited haplotype richnesses of 9, 4, and 3 respectively with associated haplotype diversity estimated as 0.67, 0.47, and 0.55 respectively (Table 1).

Indo-Pacific to Western North Atlantic Simulation

Assuming the θ of the source population is the same as the Indonesian population and no Allee effects in founders, the most likely number of *P. volitans* females to have colonized the western North Atlantic was 59 (27 – 257 95% HPD, Fig. 1C), which can be doubled to 118 (54-514, 95% HPD) females and males if we assume a 1:1 sex ratio. Varying the diversity of the source population (θ) and the strength of Allee effects (fecundity) in the colonizing population had a significant interactive effect on the estimates of colonizing lionfish (MANOVA $F_{(3,14)} \cong 6.37$; Pillai = 0.58; $p = 0.006$; Figs. 1, 2 & 3). Increasing the diversity of the source population by increasing θ resulted in smaller estimated colonizing population sizes (Figs. 1 & 2), which reach a minimum value at or before $\theta+2s$ except when fecundity is 25% of the expected value. Varying the diversity of the source population with θ had little effect on the lower limit of the 95% HPD of the colonizing population size, but had a large effect on the upper estimate (MANOVA $F_{(3,14)} \cong 26.4$; Pillai = 0.85; $p < 0.0001$; Figs. 1 & 2). Similarly, as reproductive output increased, the size of the colonizing population decreased (MANOVA $F_{(3,14)} \cong 14.2$; Pillai = 0.75; $p < 0.0002$; Figs. 1 & 3). Including Allee effects (50% reduction in fecundity) and the additional possibility that multiple source populations in the Indo-Pacific colonized the Atlantic increased the estimate of introduced lionfish to ~180 (88-416, 95% HPD) females and males (Fig. 1E).

Simulations of Caribbean and Gulf of Mexico Colonizations

The most likely number of *P. volitans* females to have colonized the Caribbean from the western North Atlantic is 42 (11-164 95% HPD, Fig. 4A; 84 females and males). The most likely number of females to colonize the Gulf of Mexico from the Caribbean was 158 (57 - >800 95% HPD, Fig. 4B; 316 females and males) but was only able to be estimated when the simulation included the maximum fecundity. The lack of an upper bound on the number of Gulf colonists, which is partially due to only simulating up to 800 colonizing females, is consistent with the low diversity of the Caribbean population and its rapid colonization and expansion throughout the Gulf of Mexico. Notably, the simulation results are congruent with previous research showing strong genetic differentiation between the western North Atlantic and Caribbean, and relatively weak genetic differentiation between the Caribbean and Gulf of Mexico (Johnson et al., 2016).

DISCUSSION

The simulations of the lionfish invasion performed here suggest that many more than 10 lionfish colonized the Atlantic Ocean. Assuming no Allee effects and a 1:1 ratio of females to males, a rigorous, conservative, minimum estimate of the number of colonists is at least 54 lionfish (lower bound of 95% HPD). As the estimated number of female colonists increases, it becomes increasingly improbable for only one male to have colonized the Atlantic given the ~1:1 sex ratio observed in wild populations ($2*10^{-7}$ for 27 female colonists based on the binomial distribution). Therefore, the most likely number of colonizing males is the same as the number of colonizing females, and all further discussion of the number of colonists will double the estimates of females to include both sexes. It is noteworthy that the lower bound of the estimated number of colonists was relatively robust to greater-than-expected genetic diversity in the source population (greater θ equals greater genetic diversity), and that this estimate assumes no reduction in fecundity or other Allee effects, which increase the estimated number of colonists. Thus, it is unlikely that fewer than 54 lionfish established the Atlantic population. It is likely, however, that the number of colonizing lionfish was greater than 54. Five hundred and fourteen colonists are the upper bound of 95% HPD interval, and the most likely number of colonists was 118, assuming a single source population and no Allee effects (Fig. 1C).

Making parameters in the model more realistic improves the estimates of the number of colonizing lionfish. Slower initial population growth rates due to Allee effects are suggested by the lag time between the detection of lionfish and their spread (Schofield, 2009; Morris & Akins, 2009). The number of simulated colonists increased with slower population growth rates caused by Allee effects. It is also possible that several source populations colonized the Atlantic, which would increase the effective θ of the source population. Increasing θ decreases the estimated number of lionfish. If we assume a 50% reduction in fecundity and multiple source populations, or a greater amount of genetic diversity in the source population, 180 (88-416 95% HPD) colonists are predicted (Figs. 1E & 2B). Stronger Allee effects result in a much greater number of colonists, but greater genetic diversity will have little effect.

As the lionfish population expanded, the invasion spread beyond the western North Atlantic, into the Caribbean and later the Gulf of Mexico (Johnson et al., 2016). Distinct genetic structure developed among these regions, roughly matching patterns of genetic structure in other species with a similar range (Taylor & Hellberg, 2006). As we have demonstrated here, each expansion of lionfish across a semipermeable biogeographic barrier can be simulated as a new colonization event, and it is likely that ~84 lionfish established the Caribbean population (Fig. 4A).

Due to the small founding population size of the Caribbean, there is an opportunity for genetic drift to cause shifts in genetic composition that perpetuate as the population grows, with a minimal influx of migrants from the founding population (Excoffier & Ray, 2008). This process, termed gene surfing (Hallatschek et al., 2007; Hallatschek & Nelson, 2008), has been observed in bacterial colonies experiencing a range expansion in laboratory settings and predicts that rapidly expanding populations would be characterized by genetic discontinuities, as observed in Atlantic-Caribbean lionfish. First reported by Johnson et al. (2016) for lionfish, to our knowledge, this is the first documentation of gene surfing in a wild population. Given the relatively small number of colonists likely to have spread from the western North Atlantic into the Caribbean (84, Fig. 4A), the most parsimonious explanation for this expansion and shift in genetic composition is simply a range expansion coupled with the phenomena of gene surfing, rather than a secondary introduction as posited by Butterfield et al. (2015; see Johnson et al., 2016).

The ‘tsunami’ of lionfish that rapidly propagated from the Caribbean was unlikely to have experienced an Allee effect or to promote much additional gene surfing. Indeed, we were unable to precisely estimate the number of colonists initiating the range expansion from the Caribbean into the Gulf of Mexico (Fig. 4B), but it is likely that there were at least 114 colonists (Fig. 4B) to produce the observed pattern of genetic diversity. This lack of convergence suggests that while there is a genetic break between the Caribbean and Gulf of Mexico, it is a relatively permeable barrier, which is supported by conventional population genetic studies and larval tracking work (Johnson et al., 2016; Kitchens et al., 2017). We propose that the vast array of oil platforms in the Gulf of Mexico likely provided ample habitat and aided in the rapid establishment and spread of lionfish throughout the Gulf of Mexico (*sensu* Sheehy & Vik, 2010).

It is also important to note that our estimates of the number of initial colonists introduced to each region increases with each additional step. An increasing number of founders in each region aligns with observations of rapid establishment and spread of lionfish as they become introduced to the Caribbean and Gulf of Mexico.

Model Assumptions

All models require making assumptions, and the most critical assumptions made in this effort are that (1) the lionfish that colonized the Atlantic Ocean originated from a single population in the Indo-Pacific, (2) the demographic model is reasonably parameterized, (3) a single colonization event led to the establishment of the populations, and (4) adult lionfish colonized. By manipulating θ, however, we showed that increasing diversity in the source population (which would be the result of multiple populations colonizing the Atlantic) had a relatively small effect on the lower estimate of the number of colonists. Additionally, it may be a reasonable assumption that the lionfish came from few source populations. Indonesia and the Philippines are the primary suppliers of coral reef fishes for the aquarium trade (Wood, 2001; Rhyne et al., 2017; https://www.aquariumtradedata.org/), and these countries are in the center of the range of *Pterois volitans*. With respect to the demographic parameters, the model we employed was designed and parameterized for established lionfish populations, but it is likely that Allee effects would result in slower population growth rates. Consequently, we varied fecundity and found that Allee effects would require even more introductions to explain the observed pattern of genetic diversity in the Atlantic. As pointed out previously and detailed in the next section, modeling a single colonization results in the minimum number of lionfish required to explain the observed pattern of genetic diversity. Finally, assuming that adult lionfish were released minimizes the number of colonists required to establish a population because adults are less likely to die before reproducing than larvae or juveniles. Adult females could even be holding fertilized eggs upon release.

Propagule Pressure and Invasions

Propagule pressure, the number and frequency of introductions (Lockwood, Cassey & Blackburn, 2005), is highly correlated with the establishment of alien invasive populations, and it is likely that the Atlantic was subject to substantial propagule pressure from lionfish. In the context of propagule pressure, there are generally two patterns of introduction prior to establishment: (1) sporadic, infrequent introductions of large numbers of individuals, or (2) frequent introductions of small numbers of individuals (see Simberloff, 2009 for both supporting examples and exceptions). Previous invasions that have been triggered by infrequent introduction of many individuals often involve the intentional release of a species to serve some purpose, after which it becomes a nuisance species (e.g. cane toads throughout the Caribbean and Pacific: Easteal, 1981; various fishes in Hawai'i: Randall, 1987; Gaither et al., 2010). Unintentional large-scale releases are also known to have occurred as a result of escapes from both (1) aquaculture and farming (Naylor, Williams & Strong, 2001; Zapiola et al., 2008; Ramírez et al., 2015) and (2) international shipping in ballast water (Lavoie, Smith & Ruiz, 1999; Drake & Lodge, 2004).

The alternate pathway to the establishment of an alien species, where small introductions occur with relatively high frequency have led to the successful establishment of several alien species (Eurasian house sparrows in North America, European red deer in New Zealand; biocontrol insects in Canada: in Simberloff, 2009). In marine systems this often is a result of the international aquarium trade and many individuals releasing pets into the environment (Padilla & Williams, 2004; Duggan, Rixon & MacIsaac, 2006; Holmberg et al., 2015; Rhyne et al., 2017). These releases may occur when people, seeking a seemingly more humane option than euthanasia, release unwanted pets into the environment (Courtenay & Taylor, 1986; Duggan, Rixon & MacIsaac, 2006).

Most Likely Introduction Vector

While many species can survive in ballast water and be transported by shipping traffic (Medcof, 1975; Carlton, 1985), the introduction of lionfish to the Atlantic via ballast water is less likely than through the aquarium trade (Whitfield et al., 2002), and the results presented here reinforce that. In an analysis of international shipping patterns (ballast), ornamental marine fish imports, and established populations of alien fishes, Semmens et al. (2004) conclude that southeast Florida reefs (the epicenter of the Atlantic lionfish invasion) host an unusually high number of alien species due to aquarium releases. Indeed, lionfish introduced through the aquarium trade are hardy adults that are likely to survive (Wabnitz, 2003). In contrast, fragile larvae have a high probability of mortality upon entering bilge tanks (Gollasch et al., 2000), during transport (Gollasch et al., 2000; Ghabooli et al., 2016) and after release (Padilla & Williams, 2004). Lionfish egg masses float for ~36-72 hours prior to hatching (Fishelson, 1975, Morris, 2009) and are unlikely to enter bilge tanks while floating. Larvae that survive bilge entry will travel for approximately 26 days between the Indo-Pacific region and Miami, given an average speed of 24 knots (Notteboom & Cariou, 2009) and two days to traverse the Panama Canal. Larvae begin feeding four days post hatch, can survive six days of starvation (Thresher, 1984), and thus, must feed in the bilge for at least 16 days on depleted plankton populations to survive the voyage (see Gollasch et al., 2000; Ghabooli et al., 2016). The population of lionfish in the bilge is likely to experience an extreme bottleneck and reduced genetic diversity (Ghabooli et al., 2016). If the population does not go extinct during transport, the larvae must accrue enough resources to metamorphose 20-35 days after fertilization (Ahrenholz & Morris, 2010) and survive for another 11 months before reaching reproductive maturity in the Atlantic (Thresher, 1984). Finally, they must find a mate in order to propagate the species in their new environment.

If ballast water was the primary vector for the lionfish invasion, then we expect that there would have been several successful introductions in other western Atlantic ports between New York and Brazil. It is clear, however, that the lionfish invasion began in one location (near Miami, Florida) in the mid-1980s, and all genetic evidence indicates that there were not successful introductions in other Atlantic locations (Johnson et al., 2016). Further, given the results of the simulations presented here, the offspring of at least 27 mothers, and likely many more, would have to be loaded into ballast tanks, survive the voyage and develop into reproductively mature adults that successfully reproduce. Adult lionfish have been found at densities up to 26.3 ha^{-1} in the Indo-Pacific region (Kulbicki et al., 2012); thus, many ships would have transported lionfish larvae to Florida, exclusively, to produce the pattern of genetic diversity observed in the Atlantic. The ballast hypothesis does not hold water.

We conclude, as others have previously, that the aquarium trade was the most likely vector for the lionfish invasion. In light of the ~180 colonizing lionfish estimated here, it seems most parsimonious to conclude that a combination of small and larger releases caused the lionfish invasion. If the colonization involved several introductions of a small number of fish by home aquarists, then many more lionfish would have to be released to generate the observed pattern of genetic diversity in the Atlantic Ocean. Lionfish are voracious predators and are prime candidates to be a nuisance by consuming other fish in a tank; thus, it is not implausible that many aquarists would discard lionfish from their aquaria in the 1970s and 1980s when the home saltwater aquarium trade skyrocketed (Andrews, 1990; Chapman et al., 1997). These lionfish, however, would need to be released in the same locations (such as ports, harbors or piers) or locate each other and aggregate after introduction. Consequently, its plausible or even likely that wholesalers, aquarium shops, and aquarists were releasing lionfish into the Atlantic, leading to the establishment of the population.

Preventing Future Invasions

Propagule pressure is an important predictor of invasion success (Lockwood, Cassey & Blackburn, 2005), and despite the destructive consequences of the lionfish invasion, there is still a healthy market serviced by the port of Miami. Between 2008 and 2011 approximately 7,000 ± 3,000 (SD) *Pterois* spp. yr^{-1} were imported into the port of Miami, the site of the earliest reports of lionfish in the Atlantic (Schofield, 2009). Approximately 70% were visually-identified as *P. volitans* (Rhyne et al., 2017; http://www.aquariumtradedata.org), meaning that 30% were other lionfish species that can become established if introduced. There are several other species of lionfish in the Indo-Pacific region and one other species, *Pterois miles*, has also established a population in the western North Atlantic (Hamner, Freshwater & Whitfield, 2007). Hybridization of other *Pterois* spp. with the existing invasive lionfish population is also possible (see Wilcox, 2014), providing a potential adaptive subsidy to the invasive population. A small fraction of the lionfish or other species being imported into Miami and other locations in the Atlantic Ocean on a yearly basis could trigger another invasion.

Many invasions have been triggered by very small numbers of individuals (Simberloff, 2009); therefore, management and enforcement should act to safeguard against a similar invasion in the future by eliminating propagule pressure (Caffrey et al., 2014). One focus should be on containment of live alien species to minimize the risk of an accidental large-scale release of potential invaders. A second focus should be on educating wholesalers, retailers, home aquarists, and the public about the dangers to the ecosystem and economy of releasing unwanted pets into the environment. Release into the wild is potentially viewed as a favorable alternative to euthanasia for an unwanted pet, therefore, this education effort could be coupled with some form of buy-back program where unwanted pets can be traded back to a store or government agency as a humane and ecosystem-friendly option (Courtenay & Taylor, 1986; Courtenay & Stauffer, 1990; Duggan, Rixon & MacIsaac, 2006). A third focus should be on implementing penalties for violating regulations and incentives for following them to increase compliance (Furlong, 1991; Keane et al., 2008; Floerl, Inglis & Diettrich, 2016). Game theory dictates that cheating is a viable strategy, unless a combination of the (1) risk of getting caught and (2) the cost associated with getting caught is sufficiently great (Smith, 1982). If regulatory entities and aquarium trade stakeholders take the threat of biological invasions seriously, it should be possible to avoid another devastating invasion like the lionfish in the Atlantic.

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Figure 1(on next page)

Figure 1: Colonization of western North Atlantic from the Indo-Pacific.

The conditional joint probability of simulations resulting in the observed haplotype richness and diversity is plotted against the number of colonizing females. Each panel represents a different estimated value of θ in the Indo-Pacific population, ranging from (A) θ – 2*s* to (E) θ + 2*s*, where θ was estimated from Indonesian *P. volitans* and *s* is the standard deviation. Colors indicate the relative fecundity of individual female lionfish in the model. Solid and dashed vertical bars above the plots represent the 50% and 95% HPD intervals, respectively. The square point along the HPD line indicates the point estimate of the most likely number of females to have generated the observed pattern of genetic diversity. Note that for θ – 2*s* and 25% relative fecundity (panel A), the most likely number of female colonists is much greater than 800 and the HPD intervals cannot be calculated.

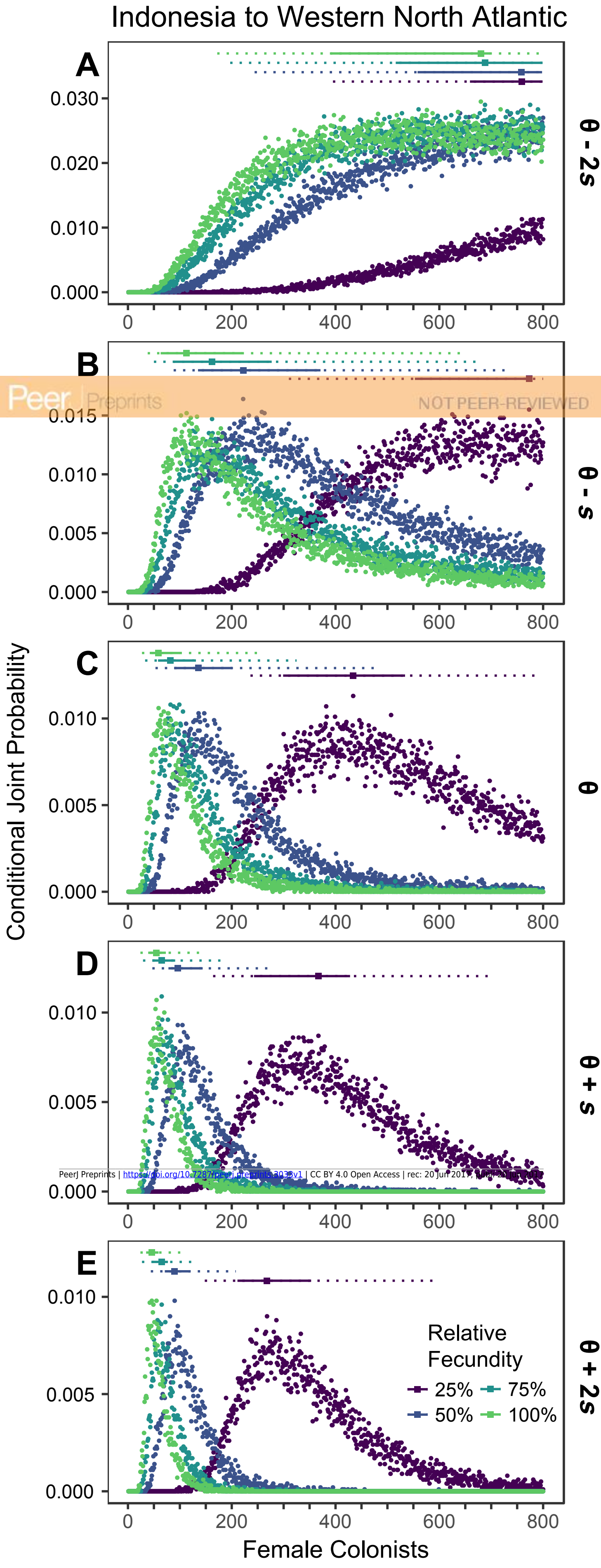
Indonesia to Western North Atlantic
A
B
C
D
E
θ - 2s
θ - s
θ
θ + s
θ + 2s
0.030
0.020
0.010
0.015
0.005
0.000
0
200
400
600
800
Conditional Joint Probability
Female Colonists
Relative Fecundity
25%
50%
75%
100%

Figure 2(on next page)

Figure 2: Effect of θ on the number of western North Atlantic female colonists.

The most likely number of Atlantic female colonists is plotted against the θ of the Indo-Pacific source population, where θ was estimated from Indonesian *P. volitans* and *s* is the standard deviation of the estimate. Solid and dashed vertical bars represent the 50% and 95% HPD intervals, respectively. Colors indicate the relative fecundity of individual female lionfish in the model. Note that for panel A, the most likely number of female colonists at θ – 2*s* is not shown because it is much greater than 800.

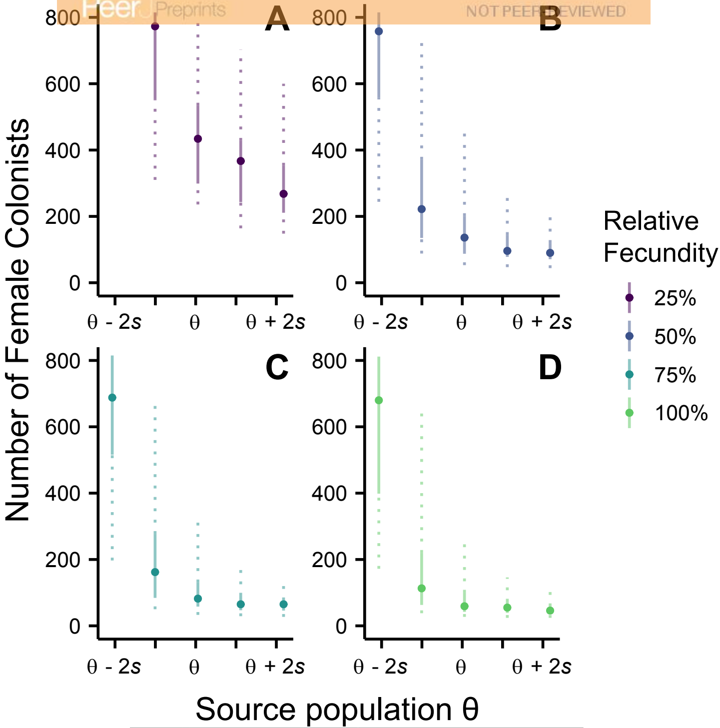
A
B
C
D
800
600
400
200
0
Number of Female Colonists
θ - 2s
θ
θ + 2s
Source population θ
Relative Fecundity
25%
50%
75%
100%

Figure 3(on next page)

Impact of Allee effects on the number of western North Atlantic female colonists.

The most likely number of Atlantic female colonists is plotted against the relative fecundity of female lionfish. The source population depicted has the same θ as estimated from a population of Indonesian lionfish. Solid and dashed vertical bars represent the 50% and 95% HPD intervals, respectively. Colors indicate the relative fecundity of individual female lionfish in the model.

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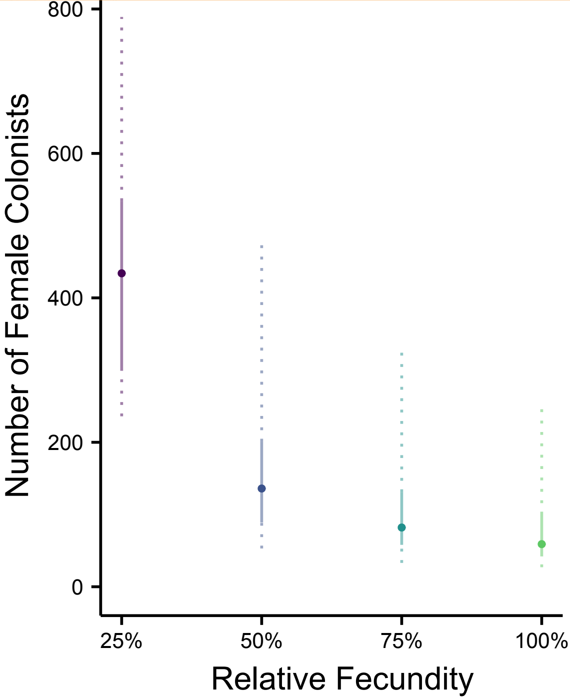

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Figure 4(on next page)

Range expansions from the western North Atlantic to the Caribbean to the Gulf of Mexico

The conditional joint probability of simulations resulting in the observed haplotype richness and diversity is plotted against the number of colonizing females (A) from the western North Atlantic into the Caribbean and (B) from the Caribbean into the Gulf of Mexico. Each point represents 10,000 simulations. Colors indicate the relative fecundity of individual female lionfish in the model. Solid and dashed vertical bars above the plots represent the 50% and 95% HPD intervals, respectively. The square point along the HPD line indicates the point estimate of the most likely number of females to have generated the observed pattern of genetic diversity.

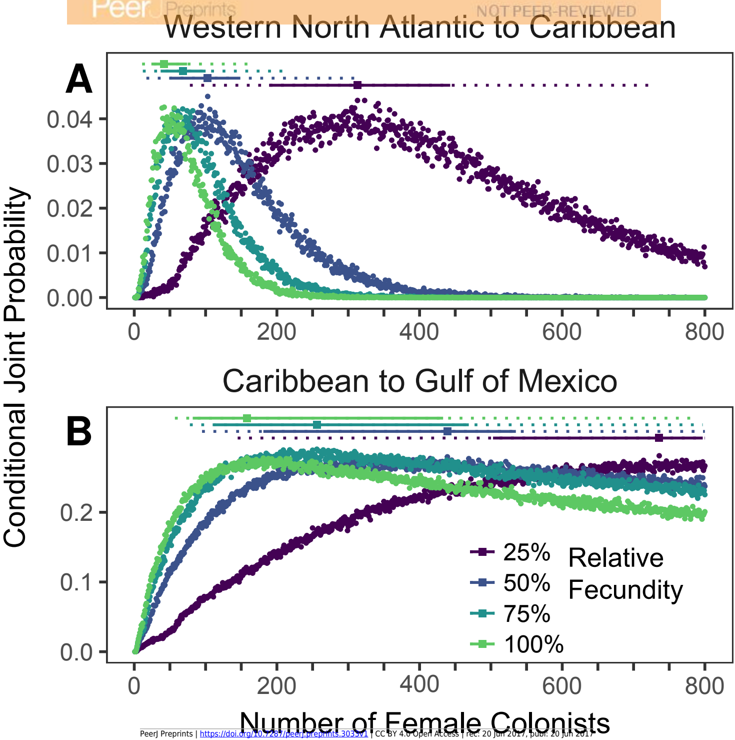
Western North Atlantic to Caribbean
A
0.04
0.03
0.02
0.01
0.00
0
200
400
600
800
Caribbean to Gulf of Mexico
B
0.2
0.1
0.0
0
200
400
600
800
Conditional Joint Probability
Number of Female Colonists
25%
50%
75%
100%
Relative Fecundity

Table 1(on next page)

Source material and population summaries

Pterois volitans mitochondrial d-loop haplotype data utilized in the present study.

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Region	Number of individuals	Haplotype Richness	Haplotype Diversity (95% CI)	References	Accession Numbers
Indonesia	36	21	0.97 (0.95 - 1.00)	1	FJ516418-FJ516438
North Atlantic	459	9	0.67 (0.64 – 0.70)	1, 2, 4	FJ516409-FJ516417
Caribbean	601	4	0.47 (0.43 – 0.50)	2-4	FJ516409-FJ516412
Gulf of Mexico	188	3	0.55 (0.49 – 0.59)	5	FJ516409, FJ516410, FJ516412

(1-Freshwater et al., 2009; 2-Betancur-R. et al., 2011; 3-Toledo-Hernández et al., 2014; 4-Butterfield et al., 2015; 5-Johnson et al., 2016)