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ORIGINAL PAPER

# Genotyping confirms significant cannibalism in northern Gulf of Mexico invasive red lionfish, *Pterois volitans*

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**Abstract** DNA barcoding is used in a variety of ecological applications to identify organisms, including partially digested prey items from diet samples. That particular application can enhance the ability to characterize diet and predator–prey dynamics but is problematic when genetic sequences of prey match those of consumer species (i.e., self-DNA). Such a result may indicate cannibalism, but false positives can result from contamination of degraded prey samples with consumer DNA. Here, nuclear-encoded microsatellite markers were used to genotype invasive lionfish, *Pterois volitans*, consumers and their prey (n = 80 pairs) previously barcoded as lionfish. Cannibalism was confirmed when samples exhibited two or more different alleles between lionfish and prey DNA across multiple microsatellite loci. This occurred in 26.2% of all samples and in 42% of samples for which the data were considered conclusive. These estimates should be considered conservative given rigorous assignment criteria and low allelic diversity in invasive lionfish populations. The highest incidence of cannibalism corresponded to larger sized consumers from areas with high lionfish densities, suggesting cannibalism in northern Gulf of Mexico lionfish is size- and density-dependent. Cannibalism has the potential to influence population dynamics of lionfish which lack native western Atlantic predators. These results also have important implications for interpreting DNA barcoding analysis of diet in other predatory species where cannibalism may be underreported.

**Keywords** Lionfish · Cannibalism · DNA barcoding · Microsatellite genotyping · Self-DNA

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## Introduction

A range of genetic techniques have been developed to identify organisms when visual identification is problematic (Symondson 2002; Hebert et al. 2003). One of the more widely used of these molecular approaches is DNA barcoding, a technique based upon a highly conserved 650 base pair region of the mitochondrially-encoded cytochrome *c* oxidase subunit I (COI) gene (Hebert et al. 2005; Ivanova et al. 2007). This molecular marker is species-specific and can be used to identify species with great accuracy if voucher sequences exist in globally available databases (Frézal and Leblois 2008; Ward et al. 2009). Given its utility,

DNA barcoding is employed by ecologists, as well as taxonomists and forensic scientists, to investigate biodiversity, food safety, illegal wildlife trade, and predator–prey interactions (Teletchea et al. 2008; Valentini et al. 2009). This last application is particularly useful in marine fish ecology given that prey items are often too degraded from digestion to identify visually (Sheppard and Harwood 2005; Ward et al. 2005; Dahl et al. 2017).

Confounding factors can arise when applying DNA barcoding to identify prey items due to the sensitivity of PCR amplification, the use of universal primers, and the relative lack of COI diversity within species. When a prey sample is identified as the same species as the consumer (i.e., self-DNA with respect to the consumer), it is not possible with COI sequences alone to distinguish this result as a true indication of cannibalism versus a false positive (Sheppard and Harwood 2005; O'Rorke et al. 2012; Jo et al. 2014). Across a range of marine consumer taxa, authors of diet studies applying DNA barcoding frequently report the amplification of consumer species DNA among prey items (e.g., Sheppard and Harwood 2005; Jo et al. 2014). Some authors have discarded results that indicate prey are the same species as the consumer given potential issues with contamination, ignoring potential cannibalism (Bartley et al. 2015; Moran et al. 2015), while others have reported all detections of consumer DNA as the existence of cannibalism, potentially overestimating the true rate (Braid et al. 2012; Valdez-Moreno et al. 2012; Côté et al. 2013; Arroyave and Stiassny 2014).

In a recent study, results from DNA barcoding of visually unidentifiable invasive red lionfish, *Pterois volitans* (hereafter lionfish), fish prey (n = 696) from the northern Gulf of Mexico (nGOM) indicated thirty-four prey species including potential instances of cannibalism (Dahl et al. 2017). Lionfish have exhibited an extensive invasion across the tropical and subtropical western Atlantic since the late 1980s and first entered the Gulf of Mexico in 2009 (Schofield 2010). As highly effective, generalist predators that consume a wide variety of fishes and invertebrates (Albins and Hixon 2008; Morris and Akins 2009; Muñoz et al. 2011; Dahl and Patterson 2014), lionfish are capable of directly altering community and trophic structure of native reef fishes across a variety of western Atlantic ecosystems (Lesser and Slattery 2011; Albins 2015; Dahl et al. 2016), potentially causing extirpations (Ingeman 2016). The success with which lionfish have invaded the introduced Atlantic range suggest native communities exert little biotic resistance to invasion, resulting in reduced interspecific competition (Albins 2013), few constraints on growth (Darling et al. 2011), and few effective, novel parasites (Sikkel et al. 2014). Furthermore, predation by native predators does not appear to be regulating lionfish populations (Hackerott et al. 2013), which have reached higher densities and body sizes than are observed in their native Indo-Pacific (Darling et al. 2011; Kulbicki et al. 2012; Dahl and Patterson 2014; Pusack et al. 2016). Concurrently, lionfish have experienced ecological release from natural population control mechanisms (e.g., predators, diseases, parasites) within their native range (Albins and Hixon 2013; Sikkel et al. 2014; Tuttle et al. 2017). While recently invaded, the nGOM region has some of the higher lionfish densities in its invasive, western Atlantic range (Dahl et al. 2016), and characterizing diet composition and potential cannibalism are important for understanding the impacts of lionfish on local reef fish communities as well as factors that may substantially limit lionfish populations.

Though 100 lionfish were detected as prey via DNA barcoding, indicating potential cannibalism on juveniles in the northern GOM, lionfish consumers were not barcoded by Dahl et al. (2017) to compare with the prey, and so, contamination of degraded prey tissue with consumer DNA could not be ruled out. Further, given low haplotype diversity documented in western Atlantic lionfish populations, it is unlikely there would be detectable differences in COI sequences between predators and prey, especially in the nGOM (Ricardo et al. 2011; Toledo-Hernández et al. 2014; Johnson et al. 2016). Cannibalism in invasive lionfish populations has seldom been reported from visual inspection of gut contents (Valdez-Moreno et al. 2012; Villaseñor-Derbez and Herrera-Pérez 2014; Dahl et al. 2017), and if confirmed, DNA barcoding might suggest that cannibalism in this species has been underestimated in the invaded range.

Therefore, to reexamine potential lionfish cannibalism, consumers and prey were genotyped with previously reported nuclear DNA microsatellites. Microsatellites are short sequence repeats that exhibit high levels of allele diversity and when assayed across multiple loci can provide a unique genotype profile for

each individual examined, providing a high resolution way to distinguish between cannibalism and contamination (Chistiakov et al. 2006). While microsatellite genotyping is widely used in fisheries and aquaculture to address questions about the relatedness of individuals, genetic diversity, and population association, it has also been applied in studies of predator–prey interactions (Kvitrud et al. 2005; Sundqvist et al. 2008), including an examination of filial cannibalism (DeWoody et al. 2001). Here, lionfish microsatellites were employed to (1) test whether lionfish DNA in prey samples is unique from that of consumer lionfish, and (2) determine the degree of cannibalism among previously barcoded samples. Results of this study have implications for interpreting self-DNA detections from DNA barcoding analysis of diet, as well as for management of invasive lionfish.

## Methods

### Sample collection

Lionfish were sampled for diet analyses (Dahl and Patterson 2014; Dahl et al. 2017) by scuba divers from April 2013 through March 2014 on nGOM natural and artificial reefs at depths of 24 to 35 m. Individuals were captured by spearing immediately posterior to the spinal column and then placed in a saltwater ice slurry upon surfacing. Each lionfish was weighed to the nearest 0.1 g and measured to the nearest mm total length (TL). Lionfish samples were categorized into small: $< 200$ mm TL, medium: 200–250 mm TL, and large: $> 250$ mm TL size classes (e.g., Fig. 1). White muscle tissue ($\sim 5$ g) was dissected from each lionfish at the time of capture and frozen at $-80$ °C until DNA extraction. Stomachs and all prey contents were removed from each sample lionfish and fixed in 100% (200 proof) molecular grade ethanol in plastic bags (Dahl and Patterson 2014) All applicable institutional and/or national guidelines for the care and use of animals were followed during the course of this study.

### DNA barcoding of unidentified lionfish prey

Previously, DNA barcoding was performed for lionfish prey items that were identified as fish but could not be identified to a taxonomic level lower than family (n = 696) (Dahl et al. 2017). Samples were processed by first removing any external layer of tissue that had been in contact with the lionfish's stomach wall or fluids with sterile scalpels and forceps, and then muscle tissue was excised from each unidentifiable prey item. To prevent cross-contamination between tissue extractions, tools were rinsed with 70% ethanol and flame sterilized. For detailed information about DNA barcoding protocols, see Dahl et al. (2017).

Potential cannibalism was revealed by DNA barcoding in 100 consumers, where *Pterois volitans* (i.e., self-DNA) was the most frequently identified prey taxon (14.4%) among unidentified fish prey examined. These prey sequences matched voucher specimens of red lionfish from the western and southern Caribbean, and Brazil (Accession Numbers: KJ739816, KM488633, and KP641132, respectively) most closely ($\geq$ 99.7% pairwise similarity). From the samples in which potential cannibalism was detected, 80 had sufficient DNA material remaining to undergo microsatellite genotyping.

### Microsatellite analysis

Original DNA extractions of prey tissue (n = 80) identified as lionfish via DNA barcoding (Dahl et al. 2017) were stored frozen ($-20$ °C) and then secondarily subjected to microsatellite genotyping. Genomic DNA of lionfish consumers was extracted from 15 to 25 mg of muscle tissue with DNeasy blood & tissue kits (Qiagen, CA). All DNA extractions were diluted $10\times$ with ultrapure water prior to PCR.

All fish were genotyped at four nuclear microsatellite loci (Table 1). The four microsatellite loci (*PVM12*, *PVM14*, *PVM31*, and *PVM42*) were chosen from a previously published primer note (Schultz et al. 2013) after testing for consistent amplification. All loci were amplified using primers developed by Schultz et al. (2013), with PCR conditions being modified to obtain strong amplification. Microsatellite genotyping was conducted via PCR amplification in 15 µl reactions containing up to 2 ng of DNA template, 1x Colorless GoTaq Flexi PCR Buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs each, 0.75 U GoTaq Flexi DNA polymerase, 0.12 mM forward labeled primer, and 0.3 mM reverse primer (Table 1). The forward primer from each primer pair was labelled with a fluorescent label of either VIC®, FAM™, or NED™ dye (G5 dye set, Applied Biosystems). All

**Fig. 1** Lionfish (*Pterois volitans*) samples collected from the northern Gulf of Mexico south of Pensacola, FL for feeding ecology analyses. Lionfish were weighed to the nearest 0.1 g and measured to the nearest mm total length (TL), then categorized into small: $<$ 200 mm TL, medium: 200–250 mm TL, and large: $>$ 250 mm TL size classes

**Table 1** Microsatellite loci used to determine individual identities of consumers and prey to identify cannibalism events

| Locus | Repeat motif | G5 dye color | Size range (bp) | Primers | $N_a$ | $H_s$ | $N_e$ |
|---|---|---|---|---|---|---|---|
| *PVM*12 | $(ACAG)_{11}$ | VIC | 190–246 | F: TGGTTGGGACTATGCAGACA | 9 (20) | 0.810 | 5.041 |
| | | | | R: CCCACACTCAATACCAGCAC | | | |
| *PVM*14 | $(AGAT)_{12}$ | FAM | 256–302 | F: GGATTCTTTCAGGGCAGGTT | 6 (12) | 0.821 | 5.335 |
| | | | | R: TTGTGACCATGACAGCATCA | | | |
| *PVM*31 | $(ACT)_9$ | NED | 176–221 | F: TTGGTCCTCCATTTCTGAGG | 5 (9) | 0.747 | 3.883 |
| | | | | R: AGCCTCACTGAGTCCACCAT | | | |
| *PVM*42 | $(ATC)_{11}$ | NED | 227–236 | F: GTGTGTCAGACGCTGAAGGA | 4 (3) | 0.519 | 2.064 |
| | | | | R: ACGTACAGCGGGTTAGGATG | | | |

Loci were isolated and primers designed by Schultz et al. (2013). ABI dye set G5 dye colors are indicated for each primer set, where forward primers were dye labeled, reverse primers were unlabeled. The number of alleles ($N_a$) observed for each locus in this study are reported alongside those reported by Schultz et al. (2013) in parentheses. Unbiased gene diversity ($H_s$) and effective number of alleles ($N_e$) are also shown

PCR reactions were performed under the same cycling conditions consisting of initial denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 15 s, annealing at 62 °C for 15 s, 72 °C for 30 s, followed by 10 cycles of 94 °C for 15 s, 53 °C for 15 s, and 72 °C for 30 s, and a final extension 72 °C for 5 min.

PCR amplicons were analyzed at the TAMU–CC Core Genomics Lab, electrophoresed on an ABI 3730XL automated capillary sequencer (Applied Biosystems) with the GeneScan™ 600 LIZ™ Size Standard (Applied Biosystems) in each lane. Size fragments were scored using GeneMarker® software and visual verification of all allele sizes was made to ensure correct calls. For a subset of pilot samples, PCR amplicons were electrophoresed on 6% polyacrylamide gels with an ABI Prism 377 sequencer (Applied Biosystems) and the GeneScan™ 400HD ROX™ Size Standard (Applied Biosystems) in each lane following the methods of Renshaw et al. (2013). Size fragments were scored manually, using GENESCAN

v. 3.1.2 (Applied Biosystems) and GENOTYPER v. 2.5 (Perkin Elmer). In all cases, consumer DNA and that of its prey were analyzed on the same machine with the same size standard and the allele calls were made with the same software to ensure comparability of allele calls. Any reactions that failed were repeated up to three times.

To assess the genetic diversity of microsatellite markers employed for this study, the number of alleles, unbiased gene diversity, and effective number of alleles were estimated for each microsatellite locus from consumers with GENODIVE v. 2.0 (Nei 1987; Meirmans and Van Tienderen 2004). Unbiased gene diversity ($H_s$), a corrected expected heterozygosity measure, is simply the probability that two sampled alleles will be different within a population (Nei 1987). The effective number of alleles ($N_e$) is a measure of the number of alleles in the sampled population weighted by their frequencies, thus accounts for alleles that are more common than others (Kimura and Crow 1964).

Visual inspection of matching and non-matching alleles at individual loci allowed for direct microsatellite genotype comparisons between prey and consumer individuals. Prey template DNA had the potential to be of low quality, especially if extensive digestion had occurred within the consumer lionfish's stomach prior to sampling. Therefore, sources of genotyping error (Taberlet et al. 1996; Hoffman and Amos 2005) were considered when developing protocols for determining whether microsatellite data supported cannibalism. For example, allelic dropout, or the failure of one allele of a heterozygous individual to be amplified via PCR, can lead to incorrect genotyping of that individual as a homozygote (Gagneux et al. 1997; Soulsbury et al. 2007), and null alleles, or alleles that do not amplify by PCR, can lead to blank or incorrectly identified genotypes (Shaw et al. 1999; Van Oosterhout et al. 2004). Another source of genotyping error stems from PCR artifacts (i.e., stutters) in which amplification products are generated that can be misinterpreted as true alleles (Taberlet et al. 1996; Goossens et al. 1998; Bradley and Vigilant 2002). Thus, MICRO-CHECKER v. 2.2.3 (Van Oosterhout et al. 2004) was used to screen the data for the presence of null alleles, stuttering, and scoring errors by comparing expected ($H_e$) versus observed ($H_o$) homozygotes for all loci.

We considered cannibalism to have occurred when at least two different alleles were observed between a lionfish and its prey across the four loci tested. When no allele differences were observed and all loci were amplified, we scored the pair as indicative of no cannibalism. We considered two types of results to be inconclusive with respect to cannibalism or the lack thereof. The first occurred when no allele differences were observed between consumer and prey but one or more loci failed to amplify. We also considered a single-allele difference between consumer and prey to be inconclusive, regardless of the number of loci amplified successfully. The proportion of cannibalism observed from genotyping was then calculated using two approaches to obtain minimum and maximum estimates of cannibalism. We calculated the proportion of cannibalism occurring across the total number of samples, regardless of amplification success, as well as among only the sample pairs with conclusive results from genotyping.

## Results

PCR amplification was successful in 76–93% of samples depending on the locus. The observed number of alleles in consumers sampled from the nGOM was lower for all loci except *PVM42* compared to those results previously reported by Schultz et al. (2013), and ranged from 4 to 9 alleles (Table 1). The effective number of alleles was lower than those observed from consumers sampled in the nGOM and ranged from 2.06 to 5.34 alleles. Gene diversity ranged from 0.52 to 0.82 across all loci (Table 1). Results from MICRO-CHECKER showed evidence of null alleles (i.e., general excess of homozygotes) at *PVM14*, but not for other loci (Table 2). There was no evidence of stutter peaks or scoring errors at any locus (Table 2).

Conclusive genotypes were obtained for 50 of the 80 consumer-prey pairs in which DNA barcoding indicated the prey may be a lionfish (Dahl et al. 2017; Table 3). Twenty-one of the paired samples had two or more different alleles between lionfish and prey DNA across the assessed microsatellite loci (Table 3), thus cannibalism was confirmed in 26.3% (21/80) of the total consumers and in 42% (21/50) of consumers for which the data were considered conclusive. Among those samples, there were as many as seven different alleles observed between lionfish consumers and

**Table 2** Results from micro-checker analyses (van Oosterhout et al. 2004) showing the number of expected homozygotes ($H_e$) and observed homozygotes ($H_o$), and presence/absence of stuttering, scoring errors and null alleles

| Locus | $H_e$ | $H_o$ | Evidence of stuttering | Evidence of scoring error | Evidence of null alleles | Significant probability test |
|---|---|---|---|---|---|---|
| *PVM*12 | 11.3 | 18 | None | None | Possible | No |
| *PVM*14 | 11.6 | 28 | None | None | Possible | Yes |
| *PVM*31 | 19.8 | 16 | None | None | None | No |
| *PVM*42 | 36.8 | 34 | None | None | None | No |

Results from probability tests for null alleles are also shown

cannibalized prey (Table 3). Cannibalism was found to occur in lionfish collected from both natural (n = 10) and artificial (n = 11) reef habitats, and across all size classes of lionfish, but was more frequently detected in medium (n = 10) and large size classes (n = 9) (Table 3). Cannibals ranged in length from 138 to 316 mm TL (Table 3). Cannibalism was documented during all four seasons, but more instances occurred in fall (n = 5) and winter (n = 10). When placed in the broader context of all 934 lionfish sampled for the visual diet study by Dahl and Patterson (2014), cannibalism was confirmed in 2.7% of samples that had prey in their stomachs, and in 2.2% of fish overall. The 100 lionfish prey detected via DNA barcoding reported by Dahl et al. (2017) corresponded to 4.87% of all lionfish diet by percent mass (%M); thus, confirmed cannibalism constituted as much as 2.01% diet by mass. Notably, for consumers in which cannibalism was detected via barcoding, cannibalized prey constituted a significant proportion of the diet by mass (mean %M = 71.1%).

Beyond the 50 consumer-prey pairs that were successfully genotyped, results from the remaining pairs of samples (n = 30) were deemed inconclusive. This was because some loci did not amplify in either the prey or consumer and exhibited zero or single-allele differences (n = 23), or because there was only a single-allele difference between the consumer and the prey from complete genotypes (n = 7). Notably, in two such cases of single-allele differences between consumer and prey, the difference was seen at *PVM42*, a locus with only four possible alleles, two of which are rare (Tables 1, 3), which may be indicative of cannibalism but was below our conservative threshold.

## Discussion

Microsatellite genotyping of lionfish consumers and their prey that had been previously identified as lionfish via DNA barcoding provides definitive evidence of cannibalism in the nGOM. While the invasion is relatively recent in this region, lionfish densities have increased exponentially and those reported from nGOM artificial reefs are among the highest in the western Atlantic (Hackerott et al. 2013; Dahl and Patterson 2014; Dahl et al. 2016). Lionfish are opportunistic feeders, consuming a wide diversity of native, reef-dwelling organisms in this region (Dahl and Patterson 2014; Dahl et al. 2017). Their increasingly high densities appear to be forcing them to switch to other prey besides reef fishes, such as non-reef associated fishes, pelagic fishes, and invertebrates (Dahl and Patterson 2014; Dahl et al. 2017). Cannibalism reported here may also be a response to growing lionfish densities and increasingly limited prey supply (Polis 1981). Notably, cannibalized *P. volitans* in this study were generally small, most weighing under 0.3 g wet mass (Dahl et al. 2017), indicating that juveniles are most commonly cannibalized.

Cannibalism was confirmed in consumers of all size classes, on both natural and artificial reefs, and across all seasons. However, cannibalism frequency of occurrence increased with increasing consumer size and from spring to winter but was observed only slightly more frequently on artificial reef habitats compared to natural reefs. The high frequency of lionfish cannibalism observed in winter coincides with the period when lionfish were at their highest density and their largest mean size during the study period (Dahl and Patterson 2014). The patterns observed here confirm those reported in Dahl et al. (2017) for prey

**Table 3** Microsatellite genotypes of consumers and prey pairs analyzed to evaluate potential cannibalism

| | | | | | MICROSATELLITE LOCI | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *PVM*12 | | | | *PVM*14 | | | | *PVM*31 | | | | *PVM*42 | | | | | | |
| | | | | | *a* | | *b* | | *a* | | *b* | | *a* | | *b* | | *a* | | *b* | | | | |
| Consumer-Prey Pair | Consumer TL (mm) | Size Class | Season | Habitat | Con | Prey | Con | Prey | Con | Prey | Con | Prey | Con | Prey | Con | Prey | Con | Prey | Con | Prey | Allele Differences | Complete | Positive Cannibal ID |
| **1** | 127 | S | S | NR | --- | --- | --- | --- | 280 | 280 | 280 | 280 | 153 | 153 | 180 | 180 | 211 | 211 | 211 | 211 | 0 | No | Inconclusive |
| **2** | 127 | S | S | AR | --- | --- | --- | --- | --- | 268 | --- | 280 | 174 | --- | 183 | --- | 211 | 211 | 214 | 214 | 0 | No | Inconclusive |
| **3** | 157 | S | F | NR | 200 | 200 | 240 | 240 | 280 | 280 | 280 | 280 | 153 | 153 | 180 | 180 | 214 | 214 | 214 | 214 | 0 | Yes | No |
| **4** | 90 | S | W | AR | 188 | 188 | 188 | 188 | 244 | 244 | 252 | 252 | 153 | 153 | 183 | 174 | 211 | --- | 211 | --- | 1 | No | Inconclusive |
| **5** | 107 | S | W | AR | 196 | 196 | 200 | 200 | 248 | 280 | 280 | 280 | 153 | 153 | 183 | 183 | 211 | 211 | 214 | 214 | 1 | Yes | Inconclusive |
| **6** | 130 | S | W | NR | 188 | 188 | 240 | 240 | 260 | 260 | 260 | 260 | 174 | 174 | 183 | 183 | 211 | 211 | 214 | 214 | 0 | Yes | No |
| **7** | 132 | S | W | AR | 188 | 188 | 192 | 192 | 268 | 268 | 268 | 268 | 153 | 153 | 153 | 153 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **8** | **138** | **S** | **W** | **AR** | **192** | **200** | **196** | **200** | **244** | **280** | **244** | **280** | **174** | **153** | **180** | **174** | **214** | **211** | **214** | **214** | **7** | **Yes** | **Yes** |
| **9** | 155 | S | W | AR | --- | --- | --- | --- | --- | 252 | --- | 252 | --- | 159 | --- | 159 | 208 | --- | 211 | --- | NA | No | Inconclusive |
| **10** | 175 | S | W | AR | 188 | 188 | 200 | 200 | 252 | 252 | 268 | 268 | 153 | --- | 174 | --- | 211 | 211 | 214 | 214 | 0 | No | Inconclusive |
| **11** | **190** | **S** | **W** | **NR** | **---** | **196** | **---** | **196** | **---** | **248** | **---** | **280** | **153** | **153** | **180** | **183** | **211** | **211** | **214** | **211** | **2** | **No** | **Yes** |
| **12** | 196 | S | W | AR | 200 | 200 | 200 | 200 | 248 | 248 | 268 | 268 | 174 | 174 | 174 | 174 | 211 | 211 | 214 | 214 | 0 | Yes | No |
| **13** | 216 | M | S | AR | 188 | --- | 200 | --- | 252 | 252 | 268 | 268 | 180 | 180 | 183 | 183 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **14** | 224 | M | S | AR | 200 | 200 | 200 | 200 | 280 | 280 | 280 | 280 | 180 | 180 | 183 | 183 | 205 | 205 | 211 | 211 | 0 | Yes | No |
| **15** | **235** | **M** | **S** | **AR** | **---** | **200** | **---** | **200** | **248** | **244** | **248** | **244** | **180** | **---** | **183** | **---** | **---** | **208** | **---** | **211** | **2** | **No** | **Yes** |
| **16** | **236** | **M** | **S** | **NR** | **---** | **188** | **---** | **200** | **248** | **252** | **260** | **252** | **153** | **---** | **183** | **---** | **211** | **205** | **211** | **211** | **3** | **No** | **Yes** |
| **17** | 243 | M | S | AR | 188 | 188 | 240 | 240 | 244 | 244 | 280 | 280 | 153 | 153 | 180 | 180 | 211 | 208 | 211 | 211 | 1 | Yes | Inconclusive |
| **18** | 244 | M | S | NR | 188 | 188 | 200 | 200 | 248 | 248 | 248 | 248 | 153 | 153 | 174 | 174 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **19** | 247 | M | S | AR | 196 | --- | 196 | --- | 252 | --- | 252 | --- | 180 | 180 | 180 | 180 | 211 | 211 | 214 | 214 | 0 | No | Inconclusive |
| **20** | 204 | M | U | NR | 204 | --- | 216 | --- | 252 | 252 | 280 | 280 | 153 | 153 | 180 | 180 | 211 | 211 | 214 | 214 | 0 | No | Inconclusive |
| **21** | 210 | M | U | NR | --- | 192 | --- | 216 | 244 | 244 | 248 | 248 | 153 | 153 | 174 | 174 | 211 | 211 | 214 | 214 | 0 | No | Inconclusive |
| **22** | **215** | **M** | **U** | **NR** | **200** | **---** | **204** | **---** | **248** | **---** | **248** | **---** | **174** | **174** | **174** | **174** | **211** | **208** | **214** | **211** | **2** | **Yes** | **Yes** |
| **23** | 216 | M | U | NR | 192 | 192 | 200 | 200 | 252 | 252 | 260 | 260 | 174 | 174 | 183 | 183 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **24** | 220 | M | U | NR | 200 | 200 | 216 | 216 | 252 | 252 | 252 | 252 | 153 | --- | 174 | --- | 211 | 211 | 214 | 214 | 0 | No | Inconclusive |
| **25** | **239** | **M** | **U** | **AR** | **188** | **192** | **240** | **192** | **248** | **---** | **248** | **---** | **180** | **---** | **183** | **---** | **211** | **---** | **214** | **---** | **2** | **No** | **Yes** |
| **26** | 240 | M | U | AR | 192 | 192 | 216 | 216 | 248 | 248 | 268 | 268 | 153 | --- | 183 | --- | 208 | 211 | 211 | 211 | 1 | No | Inconclusive |
| **27** | **240** | **M** | **U** | **AR** | **200** | **212** | **200** | **212** | **280** | **244** | **280** | **244** | **153** | **174** | **174** | **180** | **211** | **214** | **214** | **214** | **7** | **Yes** | **Yes** |
| **28** | 248 | M | U | AR | 196 | --- | 196 | --- | 280 | 280 | 280 | 280 | 174 | 174 | 180 | 180 | 214 | 214 | 214 | 214 | 0 | No | Inconclusive |
| **29** | **250** | **M** | **U** | **AR** | **---** | **212** | **---** | **216** | **248** | **268** | **268** | **280** | **174** | **153** | **180** | **180** | **211** | **211** | **214** | **214** | **3** | **No** | **Yes** |
| **30** | 230 | M | F | NR | --- | 184 | --- | 188 | --- | 280 | --- | 280 | 174 | 153 | 174 | 174 | 211 | 211 | 211 | 211 | 1 | No | Inconclusive |
| **31** | 233 | M | F | NR | --- | 196 | --- | 196 | --- | 248 | --- | 252 | 153 | 153 | 183 | 183 | 214 | 214 | 214 | 214 | 0 | No | Inconclusive |
| **32** | 234 | M | F | NR | 196 | 196 | 200 | 200 | 248 | 248 | 280 | 280 | 180 | 180 | 180 | 180 | 211 | --- | 214 | --- | 0 | No | Inconclusive |
| **33** | **244** | **M** | **F** | **NR** | **196** | **196** | **196** | **240** | **248** | **244** | **252** | **268** | **153** | **153** | **174** | **183** | **214** | **211** | **214** | **214** | **5** | **Yes** | **Yes** |
| **34** | 244 | M | F | AR | 216 | 216 | 216 | 216 | 252 | 252 | 252 | 252 | 153 | 153 | 174 | 174 | 214 | 214 | 214 | 214 | 0 | Yes | No |
| **35** | 200 | M | W | NR | 188 | 188 | 200 | 200 | 248 | 248 | 248 | 248 | 153 | 153 | 183 | 183 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **36** | 211 | M | W | NR | 200 | 200 | 216 | 216 | 248 | 248 | 268 | 268 | 153 | 153 | 183 | 183 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **37** | **226** | **M** | **W** | **NR** | **---** | **200** | **---** | **216** | **---** | **248** | **---** | **268** | **174** | **153** | **180** | **183** | **211** | **211** | **211** | **211** | **2** | **No** | **Yes** |
| **38** | 236 | M | W | NR | 200 | 200 | 200 | 200 | 260 | 260 | 280 | 280 | 153 | 153 | 153 | 153 | 211 | 211 | 214 | 214 | 0 | Yes | No |
| **39** | 237 | M | W | AR | 188 | 188 | 200 | 200 | 248 | 248 | 252 | 252 | 174 | 174 | 180 | 180 | 211 | 211 | 214 | 214 | 0 | Yes | No |
| **40** | **237** | **M** | **W** | **AR** | **---** | **212** | **---** | **216** | **---** | **260** | **---** | **268** | **174** | **153** | **186** | **153** | **---** | **---** | **---** | **---** | **2** | **No** | **Yes** |
| **41** | 239 | M | W | NR | 216 | 216 | 244 | 244 | 280 | 280 | 280 | 280 | 153 | 153 | 183 | 183 | 208 | 208 | 211 | 211 | 0 | Yes | No |
| **42** | **240** | **M** | **W** | **AR** | **188** | **188** | **192** | **192** | **280** | **280** | **280** | **280** | **183** | **153** | **183** | **183** | **211** | **211** | **214** | **211** | **2** | **Yes** | **Yes** |
| **43** | 249 | M | W | AR | --- | --- | --- | --- | --- | 252 | --- | 268 | 153 | --- | 174 | --- | --- | 211 | --- | 214 | 0 | No | Inconclusive |
| **44** | 292 | L | S | NR | --- | 192 | --- | 196 | --- | 280 | --- | 280 | 153 | 153 | 180 | 180 | 211 | 211 | 214 | 214 | 0 | No | Inconclusive |
| **45** | 252 | L | U | AR | 188 | --- | 188 | --- | 252 | 252 | 260 | 260 | 174 | 174 | 174 | 174 | 211 | 211 | 211 | 211 | 0 | No | Inconclusive |
| **46** | 254 | L | U | NR | 200 | 200 | 216 | 216 | 252 | 252 | 252 | 252 | 174 | 174 | 183 | 183 | 211 | 211 | 214 | 214 | 0 | Yes | No |

**Table 3** continued

| | | | | | MICROSATELLITE LOCI | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *PVM*12 | | | | *PVM*14 | | | | *PVM*31 | | | | *PVM*42 | | | | | | |
| | | | | | *a* | | *b* | | *a* | | *b* | | *a* | | *b* | | *a* | | *b* | | | | |
| Consumer-Prey Pair | Consumer TL (mm) | Size Class | Season | Habitat | Con | Prey | Con | Prey | Con | Prey | Con | Prey | Con | Prey | Con | Prey | Con | Prey | Con | Prey | Allele Differences | Complete | Positive Cannibal ID |
| **47** | 266 | L | U | AR | 200 | --- | 216 | --- | 268 | 268 | 280 | 280 | 153 | 153 | 183 | 183 | 211 | 211 | 211 | 211 | 0 | No | Inconclusive |
| **48** | 274 | L | U | AR | 188 | --- | 192 | --- | 248 | 248 | 268 | 268 | 153 | 153 | 153 | 153 | 211 | 211 | 214 | 214 | 0 | No | Inconclusive |
| **49** | 275 | L | U | AR | 196 | 196 | 196 | 196 | 280 | 280 | 280 | 280 | 153 | 153 | 174 | 174 | 211 | 211 | 214 | 214 | 0 | Yes | No |
| **50** | 296 | L | U | NR | 200 | 200 | 240 | 240 | 248 | 248 | 248 | 248 | --- | --- | --- | --- | 211 | 211 | 214 | 214 | 0 | No | Inconclusive |
| **51** | 355 | L | U | NR | 188 | 188 | 240 | 240 | 252 | 252 | 260 | 260 | 174 | 174 | 174 | 174 | 211 | 211 | 214 | 214 | 0 | Yes | No |
| **52** | 256 | L | F | AR | 200 | 200 | 216 | 216 | 260 | 260 | 280 | 280 | 153 | 153 | 174 | 174 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **53** | 264 | L | F | AR | 216 | 216 | 216 | 216 | 252 | 252 | 260 | 260 | 174 | 174 | 183 | 183 | 211 | 211 | 214 | 214 | 0 | Yes | No |
| **54** | **266** | **L** | **F** | **NR** | **196** | **200** | **200** | **200** | **268** | **252** | **268** | **268** | **174** | **153** | **183** | **153** | **211** | **211** | **211** | **211** | **4** | **Yes** | **Yes** |
| **55** | **266** | **L** | **F** | **NR** | **---** | **192** | **---** | **240** | **---** | **252** | **---** | **280** | **153** | **---** | **174** | **---** | **208** | **211** | **208** | **211** | **2** | **No** | **Yes** |
| **56** | 270 | L | F | AR | 188 | --- | 188 | --- | 244 | 244 | 248 | 248 | 153 | 153 | 180 | 180 | 211 | 211 | 214 | 214 | 0 | No | Inconclusive |
| **57** | 270 | L | F | NR | 196 | 196 | 196 | 196 | 244 | 244 | 280 | 280 | 153 | 153 | 174 | 174 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **58** | 270 | L | F | NR | 196 | 196 | 196 | 196 | 244 | 244 | 280 | 280 | 153 | 153 | 174 | 174 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **59** | 271 | L | F | NR | 200 | 200 | 216 | 216 | 268 | 268 | 268 | 268 | 153 | 153 | 183 | 183 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **60** | 273 | L | F | NR | --- | 212 | --- | 216 | --- | 268 | --- | 280 | 153 | --- | 186 | --- | --- | 211 | --- | 214 | NA | No | Inconclusive |
| **61** | 283 | L | F | AR | 212 | 212 | 216 | 216 | 260 | 260 | 280 | 280 | 174 | 174 | 183 | 183 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **62** | 284 | L | F | NR | --- | --- | --- | --- | 248 | 248 | 252 | 252 | 174 | 174 | 174 | 174 | 211 | 211 | 211 | 211 | 0 | No | Inconclusive |
| **63** | **284** | **L** | **F** | **AR** | **---** | **200** | **---** | **240** | **---** | **248** | **---** | **280** | **174** | **153** | **180** | **174** | **214** | **211** | **214** | **214** | **3** | **No** | **Yes** |
| **64** | 300 | L | F | NR | 196 | 196 | 200 | 200 | 244 | 244 | 268 | 268 | --- | --- | --- | --- | 211 | 211 | 211 | 211 | 0 | No | Inconclusive |
| **65** | 302 | L | F | NR | 196 | 196 | 200 | 200 | 252 | 252 | 268 | 268 | 174 | 174 | 174 | 174 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **66** | **309** | **L** | **F** | **NR** | **188** | **196** | **200** | **200** | **244** | **268** | **252** | **268** | **183** | **174** | **186** | **183** | **211** | **211** | **214** | **211** | **6** | **Yes** | **Yes** |
| **67** | 321 | L | F | NR | 188 | 188 | 204 | 204 | 260 | 260 | 260 | 260 | 153 | 153 | 183 | 153 | 211 | 211 | 214 | 214 | 1 | Yes | Inconclusive |
| **68** | 330 | L | F | NR | 196 | 196 | 240 | 240 | 244 | 244 | 280 | 280 | 174 | 174 | 186 | 186 | 211 | 211 | 214 | 214 | 0 | Yes | No |
| **69** | 257 | L | W | NR | 196 | 196 | 216 | 216 | 248 | 248 | 252 | 252 | 174 | 174 | 183 | 183 | 211 | 211 | 211 | 211 | 0 | Yes | No |
| **70** | 259 | L | W | NR | 216 | 216 | 216 | 216 | 252 | 252 | 252 | 252 | 153 | 153 | 174 | 153 | 205 | --- | 211 | --- | 1 | No | Inconclusive |
| **71** | 265 | L | W | AR | 200 | 200 | 200 | 200 | 260 | 260 | 260 | 260 | 174 | 174 | 174 | 174 | 211 | 211 | 214 | 214 | 0 | Yes | No |
| **72** | **272** | **L** | **W** | **AR** | **200** | **200** | **200** | **200** | **280** | **280** | **280** | **280** | **174** | **153** | **174** | **180** | **211** | **211** | **214** | **214** | **2** | **No** | **Yes** |
| **73** | **276** | **L** | **W** | **NR** | **188** | **196** | **200** | **196** | **---** | **---** | **---** | **---** | **174** | **174** | **183** | **183** | **211** | **---** | **214** | **---** | **2** | **No** | **Yes** |
| **74** | **279** | **L** | **W** | **AR** | **---** | **200** | **---** | **200** | **---** | **248** | **---** | **280** | **180** | **153** | **183** | **183** | **211** | **205** | **211** | **211** | **2** | **No** | **Yes** |
| **75** | 279 | L | W | NR | --- | 200 | --- | 240 | --- | 248 | --- | 260 | 153 | 153 | 183 | 183 | 208 | 208 | 211 | 211 | 0 | No | Inconclusive |
| **76** | **303** | **L** | **W** | **AR** | **---** | **---** | **---** | **---** | **---** | **252** | **---** | **280** | **153** | **153** | **153** | **153** | **205** | **211** | **211** | **214** | **2** | **No** | **Yes** |
| **77** | 304 | L | W | NR | 188 | 188 | 200 | 200 | 268 | 268 | 280 | 280 | 153 | 153 | 183 | 183 | 205 | 205 | 211 | 211 | 0 | Yes | No |
| **78** | **316** | **L** | **W** | **NR** | **---** | **200** | **---** | **240** | **---** | **252** | **---** | **276** | **153** | **153** | **180** | **174** | **211** | **211** | **211** | **214** | **2** | **No** | **Yes** |
| **79** | 335 | L | W | NR | 192 | 192 | 200 | 200 | 280 | 280 | 280 | 280 | 153 | 153 | 180 | 180 | 211 | 211 | 214 | 214 | 0 | Yes | No |
| **80** | 369 | L | W | AR | --- | 192 | --- | 192 | --- | 248 | --- | 248 | 153 | 153 | 174 | 174 | 211 | 211 | 214 | 214 | 0 | No | Inconclusive |

Size categories: S = ≤ 200 mm, M = 200–250 mm, and L = ≥ 250 mm total length; season categories: S = spring, U = summer, F = fall, W = winter; and, habitat types: natural reef = NR, artificial reef = AR. Consumer genotypes are compared against their respective prey genotypes at each allele (e.g. *a* and *b*). A lack of data is indicated by dashes (–). Number of allele differences between consumers and prey are identified, as well as whether a complete genotype was obtained (i.e., all loci amplified for consumer and prey). Cannibalism inference was based on a priori criteria and was "inconclusive" if amplification failures occurred or only one allele difference was observed between genotypes. Consumer-prey pairs in bold indicate cannibalism confirmed by genotyping, where at least two alleles differed between a lionfish and its prey

identified as lionfish via DNA barcoding, and align with studies that consider cannibalism to be an asymmetric interaction, where larger individuals consume smaller individuals (Polis 1981; Pereira et al. 2017).

Estimates of cannibalism from this study should be considered conservative due to cautionary assignment criteria. Our cannibalism assignment criterion of at least two allele differences between consumer and prey genotypes provides unequivocal evidence of cannibalism for consumer-prey pairs that met the criterion, yet one allele difference may also indicate cannibalism. If the instances of single-allele difference between consumer and prey samples were considered to be sufficient evidence of cannibalism, then our upper estimate of confirmed cannibalism would increase from 42 to 49% of barcoding samples that indicated self-DNA was present in prey.

While we established objective, conservative criteria for detecting cannibalism a priori, low allelic diversity in western Atlantic lionfish populations (Johnson et al. 2016), and the greater difficulty in amplifying nuclear microsatellites versus mitochondrial barcodes from partially digested prey samples (Broquet et al. 2007; Oliveira and Duarte 2013) may have precluded cannibalism detection for some of the consumer-prey pairs. Western Atlantic lionfish populations have a well-described genetic founder effect, and genetic diversity is especially low in GOM populations (Johnson et al. 2016). Estimated allele diversity in this study was generally lower as compared to populations originally sampled from North Carolina (Schultz et al. 2013), and the effective number of alleles was fairly reduced relative to the observed number of alleles due to skewed allele frequencies. Thus, while we employed high-diversity microsatellite loci, unique consumer and prey lionfish may not have been genetically distinct at the loci used in this study. Finally, while nuclear microsatellites are relatively stable in degraded DNA and were able to be amplified from most of the digested prey samples in this study, differential digestion likely affected amplification success (Schneider et al. 2004). In degraded or low concentration samples, PCR amplification success is reduced for nuclear DNA microsatellites compared to mitochondrial DNA markers because hundreds more copies of mitochondria are present in a given cell (Broquet et al. 2007; Oliveira and Duarte 2013). Thus, degraded DNA quality may have prevented the detection of more cannibalism events in this study and may partially explain the higher detection of self-DNA via barcoding.

The frequency of cannibalism reported here for the nGOM is high when compared to observations in other regions of the western Atlantic, where cannibalism has been reported infrequently (Valdez-Moreno et al. 2012; Côté et al. 2013; Villaseñor-Derbez and Herrera-Pérez 2014). Authors of DNA barcoding studies in the Bahamas and the Mexican Caribbean reported fewer than twenty instances of self-DNA, which were inferred to indicate cannibalism (Valdez-Moreno et al. 2012; Côté et al. 2013), notwithstanding the inability of barcoding alone to confirm. Rarer yet are reports of cannibalism observed in visual diet studies (Valdez-Moreno et al. 2012; Villaseñor-Derbez and Herrera-Pérez 2014; Dahl et al. 2017). Interestingly, there has been no lionfish DNA observed in DNA barcoding diet studies in some other regions of the western Atlantic, such as Belize in the western Caribbean and the Flower Garden Banks in the western GOM (J.D. Hogan, unpubl. data).

## Lionfish cannibalism: causes and consequences

Cannibalism is commonly observed in size-structured predator populations (Claessen et al. 2004; Rudolf 2008) and is particularly evident in fishes, where it has been recorded in more than 36 teleost families, including Scorpaenidae (Polis 1981; Smith and Reay 1991; Morte et al. 2001). In most fishes that exhibit cannibalism in nature, conspecific prey provide occasional diet supplementation, but the behavior can also be influenced by exogenous factors (Pereira et al. 2017). For example, cannibalism is often observed to be an inverse function of the availability of alternate prey, increasing when other prey are either absent or unavailable (Polis 1981; Juanes 2003). Cannibalism may also result from high conspecific density in combination with low prey diversity or abundance (Pereira et al. 2017). The potential benefits of cannibalism are largely governed by density-dependent processes, including increased survival and growth (Babbitt and Meshaka 2000) or reduced competition (Persson et al. 2000).

In some cases, cannibalism can be triggered or exacerbated by unnatural conditions, such as biological invasion (Polis 1981). Lionfish in their invaded range reach densities far greater than those seen in

their native range, stemming from a high reproductive output coupled with a lack of effective predators, competitors, and parasites (Green and Côté 2009; Darling et al. 2011; Kulbicki et al. 2012; Albins 2013; Hackerott et al. 2013). This is especially true on artificial reef habitats in the nGOM, where lionfish mean density had already reached more than 30 fish 100 $m^{-2}$ by spring 2014 (Dahl and Patterson 2014; Dahl et al. 2016). Individuals of many species maintain fixed spaces or territories in which they are intolerant of conspecifics, and high densities increase the frequency of conspecifics violating this intraspecific space (Polis 1981; Bailey et al. 2001). Ultimately, unnaturally high densities stemming from predation release in invasive lionfish populations may promote cannibalism on juveniles.

In the nGOM region, little is known about lionfish larval and early juvenile stages, particularly where lionfish settle following their planktonic larval stage. It has been hypothesized that lionfish may settle preferentially in shallow water nursery habitats, such as seagrasses and mangroves, and shift habitat preferences with ontogeny to deeper reefs (Barbour et al. 2010; Biggs and Olden 2011). Perhaps one reason for the lack of cannibalism documented in other regions is that juvenile and adult life stages naturally occupy different habitats. For example, juveniles may move from mangrove habitats to reef habitats as they mature (Claydon et al. 2012). Such ontogenetic habitat shifts may be an adaptive trait to reduce adult antagonism towards and cannibalism on juveniles (Claydon et al. 2012). In the nGOM, juvenile lionfish occupy the same offshore reef habitats as adults sampled in this study because reefs are typically distant (> 15 km) from estuaries supporting seagrasses, and mangroves are not currently established in these regions (Stevens et al. 2006). Furthermore, inshore water temperatures may limit lionfish distributions in the nGOM, as they can drop below lionfish critical thermal minima (10 °C) in winter, while offshore waters remain warmer (Kimball et al. 2004). Therefore, even if lionfish were to settle preferentially in shallow water nursery habitats, they may not survive low winter temperatures. This may result in a lack of separation between juvenile and adult habitat in the nGOM, leading to higher encounter rates of densely settled adults and juveniles, leading to higher rates of cannibalism.

Cannibalism has the potential to influence population dynamics of lionfish through density-dependent regulation of population size (Ricker 1954; Polis 1981; Claessen et al. 2004). To date, there is little evidence for predation on invasive lionfish by native reef fishes in the western Atlantic, whether due to predator naiveté or deterrence from venomous spines (Hackerott et al. 2013; Diller et al. 2014).This apparent lack of biotic resistance to lionfish from native communities has led to unchecked populations of lionfish in the western Atlantic (Albins and Hixon 2013). However, the degree of cannibalism reported herein may provide regulation for lionfish populations that appear to be plateauing in the nGOM (Dahl et al. 2016). Across the 934 fish sampled for diet analyses and from which cannibalism was detected via DNA barcoding, cannibalism was confirmed in 2.2% of fish via genotyping, and cannibalized lionfish constituted high proportions of the diet when consumed (Dahl et al. 2017). While this is a conservative estimate for reasons stated above, it is notable that even when cannibalism accounts for a small proportion of a species' diet, it may still be a significant source of mortality for the species in question (Polis 1981; Pereira et al. 2017). This may be especially true for invasive lionfish, a species that has escaped natural population control mechanisms (Sikkel et al. 2014; Tuttle et al. 2017). Scant information exists on the frequency of cannibalism reported in wild fish populations with which to compare the case of invasive lionfish (Polis 1981; Pereira et al. 2017). While no information exists on other Scorpaeniform fishes, for flounders, the frequency of juvenile cannibalism is also reported to be relatively low (i.e., frequently $< 5\%$, rarely $< 25\%$) (Tanaka et al. 1989; Pereira et al. 2017). Evidence suggests that cannibalism is a major mortality factor in the regulation of many populations (Polis 1981), and, in some cases, cannibalism appears more common in species residing outside of natural geographic ranges (Gomiero and Braga 2004; Fugi et al. 2008; Pereira et al. 2017). Therefore, the incidence of cannibalism observed in lionfish may not be insignificant, although it remains unknown to what extent cannibalism may regulate invasive lionfish populations. What is known is that nGOM lionfish densities approximately doubled between 2014 and present (Dahl et al. 2016), so the rate of cannibalism may increase further and play an increasing role in population regulation in the region.

Recent evidence indicating lionfish population declines in The Bahamas, another region of high lionfish densities, may indicate cannibalism as a potential density-dependent feedback on populations (Benkwitt et al. 2017).

## DNA barcoding and predator–prey interactions

DNA barcoding is being increasingly used to investigate predator–prey interactions. One of the frequently cited reasons to apply DNA barcoding in diet studies is to resolve bias in diet characterization by extracting high-resolution, species-specific information. Potential issues with employing DNA barcoding to identify trophic interactions, such as secondary predation (i.e., prey within a predator, then eaten by a second predator) (Harwood et al. 2001; Sheppard and Harwood 2005) and scavenging (Symondson 2002), have been reported in the literature, but the issue of how to treat self-DNA results has been largely unexplored to date.

While it is a commonly held perception that cannibalism is widespread in fishes, relatively few reports exist that describe cannibalism in nature (Smith and Reay 1991; Pereira et al. 2017). This may be due in part to diet study methodologies, such as DNA barcoding, that lack the ability to discern it. This study demonstrates how using DNA barcoding to characterize predator–prey interactions (i.e., diet) may be biased toward ignoring or overreporting potential cannibalism. The amplification and identification of DNA barcodes among prey items that match the consumer (i.e., self-DNA) is a frequent occurrence, but results are handled differently among researchers (Sheppard and Harwood 2005). A false-positive for cannibalism can occur when prey samples are handled with non-sterile techniques; however, even with rigorous sterilization procedures in place, trace amounts of consumer DNA may amplify preferentially over prey DNA if the quality of prey DNA is poor due to digestion (Gonzalez et al. 2012; Dahl et al. 2017). Blocking primers that are used in many DNA barcoding diet studies to prevent the amplification of consumer DNA during polymerase chain reaction (PCR) amplification (e.g., Sousa et al. 2016) may be useful for consumer species known not to exhibit cannibalism (Vestheim and Jarman 2008; De Barba et al. 2014). However, inhibiting amplification of consumer DNA would result in lost information in cases where actual cannibalism is occurring.

Future research opportunities exist to examine how cannibalism may influence invasive species population dynamics, and native community structure. Cannibalism confirmed here for nGOM lionfish via microsatellite genotyping is a step towards better documentation and understanding of cannibalism in wild fish populations. We know of only one other study where microsatellite genotyping was applied to address questions about cannibalism in wild fishes (DeWoody et al. 2001). The results here suggest the approach has wide applicability and that detections of self-DNA from consumers under study should be investigated more closely, which should be more straightforward for species such as lionfish for which polymorphic microsatellite markers and primers have already been developed. Ultimately, a greater understanding of cannibalism in invasive fishes would serve to improve our understanding of their population dynamics, sources of mortality, and potential mitigation.

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