

# Identification of octocoral recruits using microsatellite primers: Relationships between recruitment and adult distribution of *Pseudopterogorgia* spp.

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## Abstract

Octocorals are commonly identified from the structure of their calcium carbonate sclerites. However, small recruits cannot always be identified in this manner. Primers for microsatellite loci developed for the Caribbean branching octocoral *Pseudopterogorgia elisabethae* were used to identify six common *Pseudopterogorgia* spp. on the Little Bahama Bank. The primers consistently generated polymerase chain reaction products of different molecular weights for each species, and a combination of up to three primers enabled identification of recruits whose identity was otherwise indeterminate. Recruits were collected and adult density was surveyed at three depths at Cross Harbour, Great Abaco, Bahamas, during May and November 2005. More recruits were collected during May than November, suggesting that most of the *Pseudopterogorgia* species reproduced between November and May. There were differences in both recruitment and adult density between the three sites among the surface-brooding species. Covariation between adult and recruit abundance in *P. bipinnata* is consistent with the hypothesis that brooding species exhibit limited larval dispersal. Among the known broadcast-spawning species, there was a significant difference in adult distribution between the sites but no significant difference in recruitment between sites, suggesting that larvae of broadcast-spawning species are more widely dispersed.

There is overwhelming evidence of coral reef degradation worldwide, and while there are arguments over the mechanisms responsible for the decrease in global coral reef health, it is difficult to deny that coral reefs are at risk (Pandolfi et al. 2003; Wilkinson 2004). While the focus of research has been on the mortality of colonies, recruitment of corals into disturbed and degraded reefs will determine the extent and rate of recovery of many populations (e.g., Colgan 1987; Connell 1997; Hughes et al. 2000). Thus, information on the recruitment ecology of corals is essential to understand the resilience of coral reefs. Yet data are often unavailable because the identification of recruits to the species level is difficult. While analyses based on genus or family level identifications are useful, species-level analyses are essential to fully characterize population- and community-level dynamics. Thus, development of

molecular markers that differentiate between closely related species that are difficult to identify morphologically can provide an important tool for understanding the dynamics of many reef cnidarians.

The branching octocorals, commonly referred to as gorgonian corals, are a polyphyletic group of families of alcyonacean octocorals that have internal skeletons. They are found throughout the world's oceans and are ecologically important members of Caribbean coral reef communities. They provide spatial heterogeneity on the reef and are the visually dominant organisms on many Caribbean coral reefs. They are also the source of a diverse array of natural products, and one species, *Pseudopterogorgia elisabethae*, is actively harvested in the Bahamas with a reported market value of \$3–\$4 million (Bruckner 2002). Given their ecological importance and potential as sources of important biomedical compounds, the dearth of literature addressing alcyonacean recruitment ecology is both surprising and in need of correction.

Species-level identification of recruits is also important in analyses of reproductive strategies and in assessing the role of reproductive strategy and postsettlement processes in determining the distribution patterns of recruits and adults. Like scleractinian corals, octocorals exhibit two modes of sexual reproduction: broadcast spawning and brooding. Broadcast-spawning species release eggs and sperm into the water column where fertilization and development occur (Brazeau and Lasker 1989). Brooding species retain eggs, which develop either within the maternal polyp (Theodor 1967), within specialized chambers (Benayahu and Loya 1984), or on the colony surface (Benayahu and Loya 1983). The zygotes produced by

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brooding species are later released as fully developed planulae larvae. Brooded larvae of scleractinian corals may be physiologically capable of dispersal over great distances (Richmond 1987), but many brooding species, both scleractinians and octocorals, are competent to settle shortly after release (Brazeau and Lasker 1990; Harii et al. 2002; Gutiérrez-Rodríguez and Lasker 2004c) and appear to have short dispersal distances (Benayahu and Loya 1983; Carlon and Olson 1993; Vermeij 2006). Recruitment patterns and adult distributions of broadcast and brooded scleractinians are also consistent with a pattern of shorter dispersal among brooding species and longer-distance dispersal among broadcast-spawning species (Babcock 1984; Hughes et al. 1999; Nozawa and Harrison 2005). Gutiérrez-Rodríguez et al. (2005) found high levels of genetic structure among populations of *P. elisabethae*, consistent with the expectation of limited larval dispersal in brooding octocorals, and similarly Gutiérrez-Rodríguez and Lasker (2004c) directly observed the settlement of released planulae within meters of the natal colony. However, species-level recruitment rates and the relationship between recruitment and adult distribution patterns are unknown for most octocorals.

If recruits are of local origin (i.e., the population is “closed”), then settlement and recruitment should mirror adult distribution patterns. If recruits originate from other populations (i.e., the population is “open”), then recruitment and adult density will be independent of each other. Thus, we would expect recruitment of the brooding species to correlate with adult distribution patterns and recruitment of the broadcast-spawning species to be independent of adult abundances.

In this study, we present a methodology for making species-level identifications of recruits in the genus *Pseudopterogorgia* and address how life-history strategy affects recruitment and adult distribution among six common gorgonian corals in the genus *Pseudopterogorgia* in the Bahamas. *Pseudopterogorgia* spp. are abundant on Caribbean coral reefs (cf. Lasker and Coffroth 1983; Yoshioka 1996), and like the vast majority of octocorals, all the *Pseudopterogorgia* spp. that have been studied are gonochoric. *Pseudopterogorgia elisabethae* and *Pseudopterogorgia bipinnata* are surface brooders that reproduce in November or December in the Bahamas (Gutiérrez-Rodríguez and Lasker 2004c; Lasker pers. obs.); *Pseudopterogorgia americana* is a broadcast spawner (Yoshioka 1979) that spawns in October or November in Florida (Fitzsimmons-Sosa et al. 2004); data for *Pseudopterogorgia acerosa* suggest that it also broadcast spawns (Yoshioka 1979). The reproductive strategies of *Pseudopterogorgia rigida* and *Pseudopterogorgia* sp., the other two species at the study site, are unknown at this time, but observations of large seemingly mature eggs in *Pseudopterogorgia* sp. in November 2007 (T. Higgs pers. comm.) suggest that it spawns in late November or early December.

While fully developed colonies of these species have distinctive appearances, the recruits are often indistinguishable from each other based on gross morphological characteristics. Species-level taxonomy of octocorals is generally based on the morphology of the sclerites, the

calcium carbonate spicules embedded in the tissue. However, identification of very small recruits from analyses of sclerites appears to be problematic (Jamison and Lasker unpubl. data). Identification procedures based on population genetic techniques can circumvent this difficulty, and techniques such as RAPDs, AFLPs, ISSRs, and RFLPs have been used to distinguish scleractinian and octocoral species (Coffroth and Mulawka 1995; Lasker et al. 1996; Shearer and Coffroth 2006). We present a molecular method that uses the PCR products generated from microsatellite primers to distinguish six common *Pseudopterogorgia* species: *P. elisabethae*, *P. bipinnata*, *P. acerosa*, *P. rigida*, *P. americana*, and *Pseudopterogorgia* sp.

Microsatellites are regions within the genome in which slippage during DNA replication generates tandemly placed repeats of a group of nucleotides. This process can occur multiple times at a particular locus during the evolution of a lineage, producing many alleles and highly polymorphic populations (Queller et al. 1993). Microsatellites are typically used to study the evolution of one particular species. However, in some cases microsatellite primers designed for one species generate polymerase chain reaction (PCR) products in closely related species, and PCR primers developed for one species have been used for microsatellite studies among multiple species (Coughlan et al. 2006; Papakostas et al. 2006). The rapid evolution of microsatellites suggests that interspecific variability should be common, and in this study we demonstrate the utility of using microsatellite primers designed for one species to identify recruits of other species and examine how recruitment and adult distribution are related and how reproductive strategy affects this relationship.

## Material and methods

*Molecular identification*—*Pseudopterogorgia elisabethae* microsatellite primers Pel-19, Pel-32, and Pel-1, which were previously described by Gutiérrez-Rodríguez and Lasker (2004a,b) and Gutiérrez-Rodríguez et al. (2005), were tested on five additional common Caribbean *Pseudopterogorgia* spp. to determine if they would be useful for species-level differentiation. Our understanding of the behavior of the microsatellite loci in *P. elisabethae* is based on surveys of 22 populations in the Bahamas and one in Florida (Gutiérrez-Rodríguez et al. 2005 and unpubl. data). Pel-1 is a “CTGC” repeat; Pel-19 and Pel-32 are both “CA” repeats. Primers for Pel-1 are given in Gutiérrez-Rodríguez and Lasker (2004b). PCR conditions for amplification were [MgCl] = 50  $\mu\text{mol L}^{-1}$ , [dNTPs] = 200  $\mu\text{mol L}^{-1}$ , IRD800 fluorescent M13 labeled primers [forward] = 0.05  $\mu\text{mol L}^{-1}$ , [reverse] = 0.1  $\mu\text{mol L}^{-1}$ ; PCR cycles, 94°C, 2 min; (94°C, 30 s; 60°C, 30 s; 72°C, 45 s)  $\times$  32; 72°C, 5 min. In *P. elisabethae*, Pel-1 has alleles with up to four repeats in PCR products of 131–147 base pairs (bp). The 131- and 135-bp alleles have combined allele frequencies that are never less than 0.93 at the Florida and Bahamas *P. elisabethae* populations that we have surveyed. Details about the primers and reaction conditions for Pel-19 and Pel-32 are described in Gutiérrez-Rodríguez and Lasker (2004a). In *P. elisabethae*, Pel-19 is found with up to

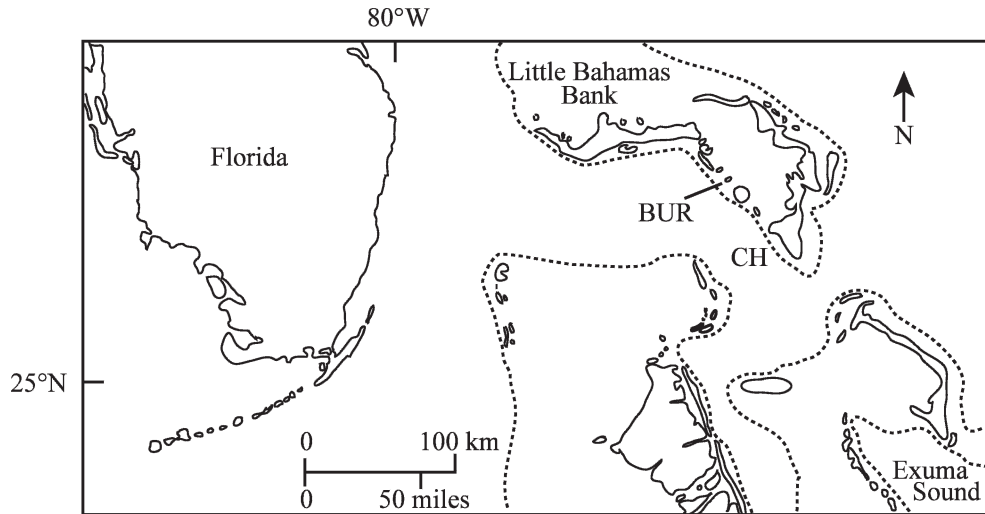


Fig. 1. Collection sites of *Pseudopterogorgia* spp. adults and recruits in the Bahamas. CH = Cross Harbour; BUR = Burrows. The dashed line shows the edge of the shallow water banks.

17 repeats in PCR products that varied from 190 to 218 bp. The 194-, 196-, and 198-bp alleles are the most common alleles (with a frequency of 0.49 or greater) in all but one of the previously surveyed population. The primers for Pel-32 generate PCR products of 135–195 bp in *P. elisabethae*. The 137-bp allele is the most common allele in the Bahamian populations. These allelic patterns among *P. elisabethae* provided the “standard” against which the other species were compared.

The focus of this study was to identify reproducible, genetically based differences between the *Pseudopterogorgia* species that can be visualized using our microsatellite primers. The PCR products produced by the microsatellite primers were interpreted as phenotypes, and we made no assumptions regarding the homology of products in our interpretation of the data. Sequencing of the bands from all the *Pseudopterogorgia* spp. and all the loci would be needed to determine if they are all homologous microsatellite loci. However, determining homology is not necessary for species identification.

Adult samples of each of the six species were collected during May 2005 and November 2005 from two sites near Burrows Cay on the southern edge of the Little Bahama Bank (Burrows North, 26°23'N, 77°46'W, 18-m depth; Burrows South, 26°20'N, 77°45'W, 18-m depth) and three sites at Cross Harbour on the southern coast of Great Abaco Island (Cross Harbour 30 [CH30], 25°57'N, 77°19'W, 10-m depth; Cross Harbour 60 [CH60], 25°56'N, 77°20'W, 17-m depth; Cross Harbour Ridge [CHR], 25°57'N, 77°20'W, 21-m depth) (Fig. 1). A small branch was clipped from each of 21–50 colonies of each species, and a portion of the tissue was preserved for DNA analysis in either 95% ethanol or salt-saturated 20% dimethyl sulfoxide (DMSO) solution. The remaining portion of the sample was dried for morphological sclerite analysis to confirm the species identification (Bayer 1961). DNA was extracted using either the Prep-A-Gene DNA extraction kit (Bio-Rad Laboratories) or the 2× CTAB protocol described in Coffroth et al. (1992). DNA was

amplified with primers for *P. elisabethae* microsatellite loci Pel-1, Pel-19, and Pel-32 (Gutiérrez-Rodríguez and Lasker 2004a,b). PCR amplification was performed in 10- $\mu$ L reactions following the conditions described in Gutiérrez-Rodríguez and Lasker (2004a). PCR products were separated on 7% polyacrylamide denaturing gels and visualized on a LI-COR NEN® Global IR2 DNA Sequencer System using fluorescently labeled primers (Gutiérrez-Rodríguez and Lasker 2004a). PCR product sizes were determined by comparing them to size standards (LI-COR Biotechnology Division, 50–350 bp). The consistency of the product sizes resulting from PCRs using the microsatellite primers was compared within a species and then between the species to determine if the primer could be used to differentiate the six species.

*Recruitment and adult density of Pseudopterogorgia spp.*—Recruits were collected and adult density surveys performed at three Cross Harbour sites. The sites, which varied in depth, were separated by a maximum distance of 2.5 km. Recruitment was measured in 20 randomly chosen, 1-m<sup>2</sup> quadrats that were established and permanently marked in November 2004 at both the CH60 and the CHR sites. The quadrats were randomly placed along each of four 20-m-long transects. The transects were randomly positioned using a randomly selected heading and distance within a 50-m radius of an arbitrarily selected point at each site (Fig. 2). At the CH30 site, the same recruit sampling design was used, but the transects were not permanently marked. *Pseudopterogorgia* spp. recruitment was measured by collecting all individuals less than 5 cm in length from each of the quadrats during May 2005 and November 2005. Recruits were preserved for DNA analysis in either 95% ethanol or salt-saturated DMSO.

The recruits were not distinguishable based on morphological characteristics, and species identification was accomplished using molecular analysis as described previously. The only morphological characteristic used to aid identification was that *P. americana* and *Pseudopterogorgia*

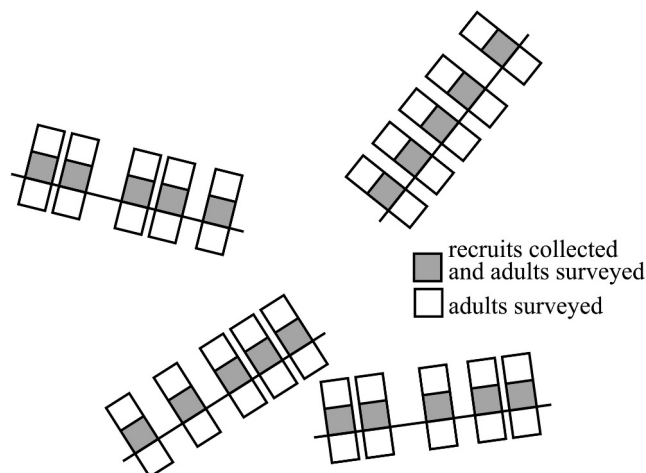


Fig. 2. Diagram of the sampling design at the Cross Harbour sites. Each of the four lines represents a 20-m transect. Each square indicates a 1-m<sup>2</sup> quadrat. The origin and direction of the transects and position of quadrats on the transects were randomly selected at each of the three sites.

sp. recruits produced large amounts of mucus and were slimy to the touch, while the other four species were not slimy. At CHR, recruits from only four randomly selected quadrats (1 from each transect) were identified using molecular analysis because of the large number of recruits (often >125 m<sup>-2</sup>) found in the surveyed quadrats at this site.

Adult density was surveyed at the CH60 and CHR sites during November 2005 by identifying and measuring the height of each *Pseudopterogorgia* colony  $\geq 5$  cm within each permanent quadrat and its two adjacent 1-m<sup>2</sup> quadrats, that is, those not on the transect and on either side of the permanent quadrats (Fig. 2). A total of 60 m<sup>2</sup> were surveyed at each site. The adult density surveys at the CH30 site were performed in November 2005 using the same design, but because transects at this site were not permanent, the recruits collected during May 2005 were not from the same quadrats as the adult surveys. For analysis purposes, each quadrat sampled for recruits was used as a replicate and each 3-m<sup>2</sup> group used as a replicate in the analysis of adult densities. Distribution data were analyzed in several ways. Density data were heteroscedastic, even after transformation and differences in density between sites were examined with the nonparametric Kruskal-Wallis tests. Hierarchical log-linear analyses (SPSS) were used to test whether the distribution pattern of each species was independent of site (i.e., whether the relative proportion of individuals found at each site was the same for all species). The analysis is identical to a test of independence of a two-way contingency table using a *G*-test. The log-linear analysis was repeated for adults and for recruits. The log-linear analysis was also used to determine whether the relative distribution of recruits and adults was independent of species (i.e., whether the relative proportion of adults and recruits was the same for each species). That analysis was repeated for both the CH60 and CHR sites. Finally, Pearson correlation coefficients between numbers of adults and recruits in the quadrats were calculated to determine

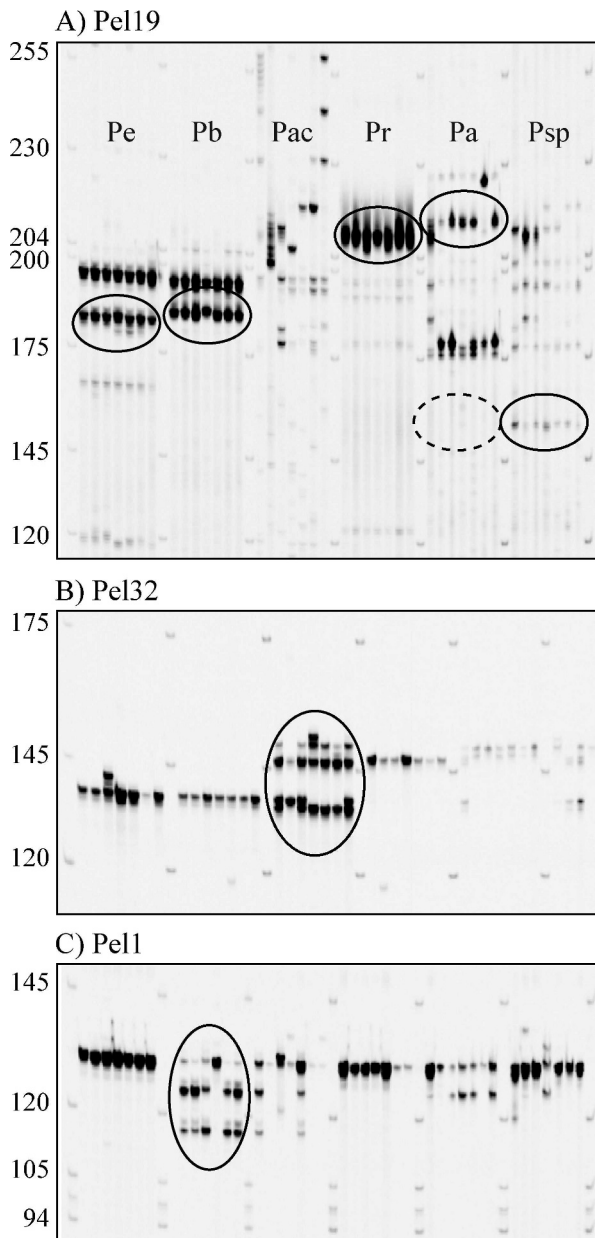


Fig. 3. Image of polyacrylamide gels from a LI-COR NEN® Global IR2 DNA Sequencer System with adult samples from six *Pseudopterogorgia* spp. at microsatellite loci (A) Pel-19, (B) Pel-32, and (C) Pel-1. The same samples were used in the same order for both gels. The diagnostic band(s) for each species are circled. The dotted circle emphasizes the absence of a diagnostic band. Pe = *P. elisabethae*; Pb = *P. bipinnata*; Pac = *P. acerosa*; Pr = *P. rigida*; Pa = *P. americana*; Psp = *Pseudopterogorgia* sp. Size markers are labeled at the left edge of the image.

whether the number of recruits was positively related to the abundance of adults.

## Results

*Molecular identification of the Pseudopterogorgia spp.*—A total of 174 adult samples were used in the development of the identification method. Species identity of the adult

Table 1. Characteristics of PCR products used to distinguish Bahamian *Pseudopterogorgia* spp. The products were generated using microsatellite primers Pel-19, Pel-32, and Pel-1. Bold denotes identifying traits. The use of Pel-32 and Pel-1 is required only for those specimens for which Pel-19 yields ambiguous results.

Primer	PCR product size (bp)	<i>P. elisabethae</i>	<i>P. bipinnata</i>	<i>P. rigida</i>	<i>P. acerosa</i>	<i>P. americana</i>	<i>Pseudopterogorgia</i> sp.
Pel-19	214					+	
	<b>210</b>			+ (smeared)			
	>196	1 or 2 distinct bands	1 or 2 distinct bands				
	<b>188</b>		+				
	<b>184</b> <b>155</b>	+					+ (low intensity, always present)
Other bands	Only very faint/low-intensity products			Numerous bands >175 bp varying in size and intensity			
Notes	In rare cases when 184/188 are both present or both absent, these two species can be distinguished with Pel-1			Pel-32 is needed to differentiate between <i>P. acerosa</i> and <i>P. americana</i>			
Pel-32	<b>147</b>			+	+		
	<b>139/137</b>	+	+		+		
Pel-1	131	+	+	+	+	+	+
	<b>125</b>		+				
		multiple smaller bands					

samples could be discerned based on the products produced using the three microsatellite primers. In many cases only a single primer, Pel-19, was needed to identify a colony (Fig. 3). The diagnostic products generated for each of the species are characterized in Table 1.

The microsatellites were developed and PCR conditions optimized for *P. elisabethae*. Thus, it is not surprising that some of the most distinct patterns were found in the products generated from *P. elisabethae* and the closely related *P. bipinnata*. The Pel-19 banding patterns for *P. elisabethae* and *P. bipinnata* consisted of a 196-base-pair (bp) or greater product and a lower-molecular-weight band at either 184 or 188 bp. The higher-molecular-weight band was generated by the microsatellite locus described in Gutiérrez-Rodríguez and Lasker (2004a). The 184- and 188-bp bands were produced by a second priming site with flanking regions that were identical to the Pel-19 locus (Lasker and Hannes unpubl. data). The 4-bp difference between the 184- and the 188-bp band was generated by a (CA)<sub>2</sub> repeat. The diagnostic band for *P. elisabethae* was the 184-bp band, while *P. bipinnata* colonies exhibited the band at 188 bp. In 6% of the *P. elisabethae* and *P. bipinnata* adult samples, either both the 184- and the 188-bp PCR products were missing or both were present. In those cases an additional primer, Pel-1, was used to differentiate the two species. Pel-1 was useful only for samples in which primer Pel-19 did not differentiate *P. elisabethae* and *P. bipinnata*. The Pel-1 primers generated a 131-bp product for both species and a diagnostic 125-bp product for *P. bipinnata*. Multiple bands at molecular weights lower than 125 bp were often present in *P. bipinnata* and never occurred in *P. elisabethae* samples. Other species had bands at 131 bp and lower molecular weights, but Pel-1 was used only when it was determined that the sample was either *P. elisabethae* or *P. bipinnata* based on criteria from Pel-19. Some *P. elisabethae* and *P. bipinnata* did not

generate 125-bp products, but when the results of both Pel-19 and Pel-1 were combined, 99% of the adult *P. elisabethae* and *P. bipinnata* samples could be identified.

*Pseudopterogorgia rigida* generated a very distinctive 210-bp product when amplified with Pel-19. The band appears smeared on the image from the Licor sequencer and would appear as a broad peak in a genotyping electropherogram. *Pseudopterogorgia rigida* also generated numerous smaller and very faint products when amplified with Pel-19. *Pseudopterogorgia rigida* samples generated a band at 147 bp when amplified with the Pel-32 primer and generated a 131-bp band shared by all six species when amplified with the Pel-1 primer.

*Pseudopterogorgia americana*, *Pseudopterogorgia* sp., and *P. acerosa* were the most difficult of the species to differentiate from each other. While the microsatellite primers generated product(s) for each of these species, the products were highly variable in intensity, and there were often numerous products. When amplified with the Pel-19 primer, the distinguishing characteristic of *Pseudopterogorgia* sp. was a band at 155 bp, while *P. americana* typically had a band at 214 bp but never at 155 bp. *Pseudopterogorgia acerosa* did not have a consistent banding pattern using the Pel-19 primer and could be confused with *P. americana*. Amplification with the Pel-32 primers identified a 137- and/or a 139-bp product in *P. acerosa* samples that was missing in *P. americana* samples.

A total of 1,925 *Pseudopterogorgia* recruits were collected from the 60 quadrats at the CH30, CH60, and CHR sites: 1,739 recruits were collected during May 2005, and 186 recruits were collected during November 2005. All of the recruits from CH30 and CH60 were analyzed (21 and 211, respectively). Only 372 of the 1,693 recruits collected at CHR were used in the analysis. The CHR collections were subsampled by analyzing only recruits from a single quadrat from each of the four transects. The stepwise

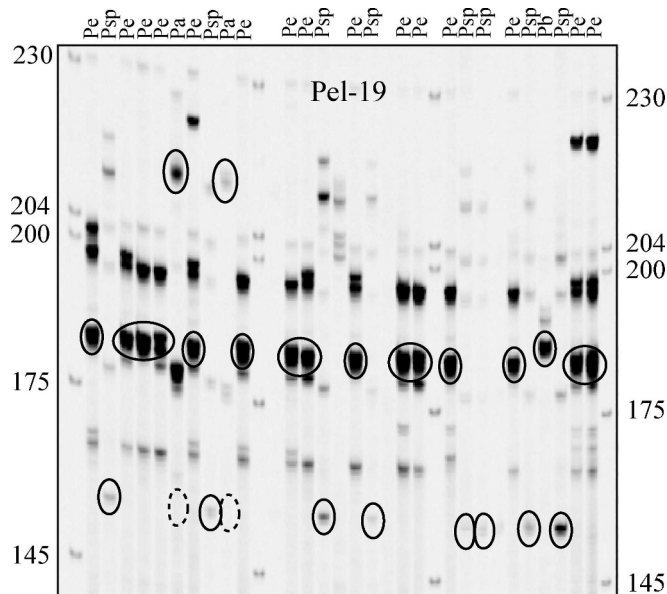


Fig. 4. Image of *Pseudopterogorgia* spp. DNAs extracted from recruits and amplified with microsatellite locus Pel-19. Species designations are listed at the top of the gel and size markers at the edge. The diagnostic band(s) for each species are circled. The dotted circles emphasize the absence of a diagnostic band. Pe = *P. elisabethae*; Pb = *P. bipinnata*; Psp = *Pseudopterogorgia* sp.

molecular analysis of the recruits collected at Cross Harbour generated species identifications for 94% of the 604 recruits that were analyzed (Fig. 4).

**Recruitment and adult density of the *Pseudopterogorgia* spp.**—Two features dominate the data sets: the markedly greater abundance of established colonies at CHR and the massive recruitment of *Pseudopterogorgia* sp. at CHR. The distribution of adults was not uniform across the sites and species. Adult densities were significantly different between the sites for all six species (Table 2a). *Pseudopterogorgia elisabethae*, *P. bipinnata*, *P. acerosa*, *P. americana*, and *Pseudopterogorgia* sp. were at their greatest densities at CHR and at their lowest densities at CH30 (Fig. 5). Among those species there was an order-of-magnitude difference in adult colony abundances between CH30 and CHR with an intermediate density at CH60. *Pseudopterogorgia rigida* was the only species that did not follow the pattern of having peak abundance at CHR and lowest abundance at CH30. *Pseudopterogorgia rigida* was most abundant at CH30, present in lower abundance at CHR, and absent from the quadrats at CH60. (The species was observed at CH60 outside the quadrats.) Log-linear analyses of the distribution patterns were limited to *P. americana*, *P. elisabethae*, and *Pseudopterogorgia* sp., which were present across all three sites in sufficient numbers for the analysis. The log-linear analysis indicates that the pattern varied between *P. bipinnata*, *P. elisabethae* and *Pseudopterogorgia* sp. (Table 2b). Although the rank abundances of the species at the three sites were the same, the proportional representation at the three sites differed.

In the recruitment data, Kruskal–Wallis tests identified site-to-site differences in recruitment only among *P.*

Table 2. Comparisons of adult and recruit distributions of *Pseudopterogorgia* spp. at three depths at Cross Harbour, Great Abaco, Bahamas (LR, likelihood ratio).

	Stage	
	Adults	Recruits
Kruskal–Wallis tests		
a. Differences between sites		
<i>P. acerosa</i>	$p=0.005$	$p=0.705$
<i>P. americana</i>	$p<0.001$	$p=0.912$
<i>P. bipinnata</i>	$p<0.001$	$p<0.001$
<i>P. elisabethae</i>	$p<0.001$	$p<0.001$
<i>P. rigida</i>	$p<0.001$	$p=0.350$
<i>Pseudopterogorgia</i> sp.	$p<0.001$	$p<0.001$
Log-linear analyses		
b. Site-by-species interaction		
( <i>P. bipinnata</i> , <i>P. elisabethae</i> , <i>Pseudopterogorgia</i> sp.)		
Adults	LR=236.03, df=4, $p<0.001$	
Recruits	LR=83.9, df=4, $p<0.001$	
c. Stage-by-species interaction		
( <i>P. americana</i> , <i>P. elisabethae</i> , <i>Pseudopterogorgia</i> sp.)		
CH60	LR=161.40, df=2, $p<0.001$	
CHR	LR=173.24, df=3, $p<0.001$	
	(also includes <i>P. bipinnata</i> )	
d. Stage-by-site interaction		
(CH60 and CHR)		
<i>P. americana</i>	LR=12.70, df=1, $p<0.001$	
<i>P. bipinnata</i>	LR=0.506, df=1, $p=0.447$	
<i>P. elisabethae</i>	LR=7.07, df=1, $p=0.008$	
<i>Pseudopterogorgia</i> sp.	LR=84.66, df=1, $p<0.001$	

*bipinnata*, *P. elisabethae*, and *Pseudopterogorgia* sp. (Table 2a). Among those species, log-linear analyses indicated that the distribution pattern across the sites differed for the three species (Table 2b). The difference in the distribution of recruits across the sites was even more striking than the differences among the adults and varied by two orders of magnitude. As is apparent in Fig. 5, that pattern was driven almost entirely by a massive recruitment of *Pseudopterogorgia* sp.

*Pseudopterogorgia* sp. recruits were present at all three Cross Harbour sites, but the recruits were the most abundant at CHR and CH60 and least abundant at CH30. *Pseudopterogorgia* sp. recruits were especially abundant at CHR with an average density of 70 recruits  $m^{-2}$  and a range of 15–252 recruits  $m^{-2}$  across the four quadrats for which all recruits were identified. *Pseudopterogorgia elisabethae* were the second most common recruits at Cross Harbour followed in abundance by *P. bipinnata*, and like *Pseudopterogorgia* sp., both species had their greatest recruit density at CHR with decreasing density at CH60 and CH30.

Recruitment of *P. acerosa*, *P. americana*, and *P. rigida* were similar across sites. *Pseudopterogorgia rigida* recruitment was almost nil at all the sites.

Initial inspection of Fig. 5 suggests that recruitment patterns had limited resemblance to the adult distribution patterns regardless of site. Log-linear analysis of the abundance of adults and recruits at CH60 and CHR led

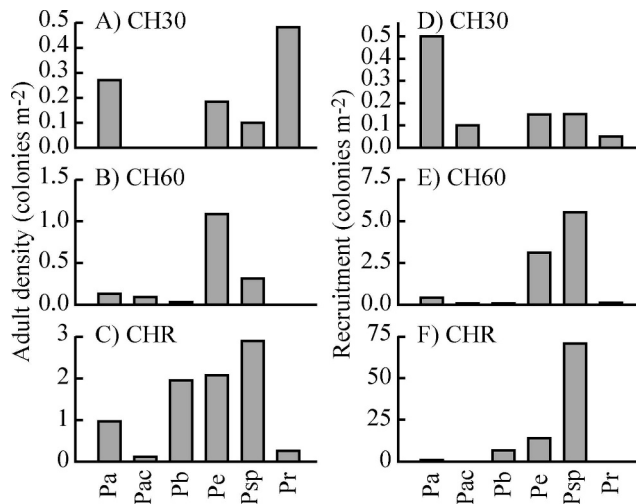


Fig. 5. Adult (A–C) and recruit (D–F) densities of *Pseudopterogorgia americana* (Pa), *P. acerosa* (Pac), *P. bipinnata* (Pb), *P. elisabethae* (Pe), *Pseudopterogorgia* sp. (Psp), and *P. rigida* (Pr) at three locations at Cross Harbour, Great Abaco, Bahamas. Pa and Pac are broadcast spawners, Pb and Pe are surface brooders, and the reproductive patterns of Pr and Psp are unknown. Note the changes in scales between the three sites.

to the rejection of the hypothesis that adult and recruit distributions were similar among species (Table 2c).

The relative distribution of adults and recruits could not be compared at CH30 because of the low number of colonies found at the site. In looking at ranked abundance, *Pseudopterogorgia* sp., *P. bipinnata*, and *P. elisabethae* recruitment across the three sites matched that of the adults. The relative distribution of adult and recruit *P. bipinnata* did not differ between CH60 and CHR (Table 2d). However, the relative proportion of adults to recruits in *P. elisabethae* and *Pseudopterogorgia* sp. differed between sites (Table 2d). *Pseudopterogorgia americana* recruits were equally abundant at all three sites, and given the different adult abundances at the three sites, the proportion of recruits differed between sites (Table 2d). A general lack of correspondence was observed between adult density and recruit density at the sites for *P. acerosa* and *P. rigida*. However, there were too few recruits of either species to accurately characterize the recruitment patterns.

On a finer scale, numbers of recruits found in the 1-m<sup>2</sup> quadrats at CH60 and CHR was correlated with the number of adults in those quadrats for *P. bipinnata* ( $r = 0.45$ ,  $p < 0.05$ ,  $df = 22$ ), *P. elisabethae* ( $r = 0.61$ ,  $p < 0.01$ ,  $df = 22$ ), and *Pseudopterogorgia* sp. ( $r = 0.59$ ,  $p < 0.01$ ,  $df = 22$ ). CH30 was not included in this analysis because the same quadrats were not sampled for recruits and for adult density. The significant correlations are driven entirely by the greater density of both adults and recruits at the Cross Harbour Ridge site, and neither correlation was significant if those four data points are excluded from the analysis. Thus, significance in those tests is confounded by the presence of different ranges of adult density at different sites. A full interpretation of the correlation will have to await comparisons that incorporate a larger number and array of sites. No significant correlations were found

between adult density and recruitment among *P. acerosa* or *P. americana*. *Pseudopterogorgia rigida* recruitment was not tested, as none of the quadrats contained adult colonies and recruits were found in only a single quadrat.

## Discussion

*Molecular identification of the Pseudopterogorgia spp.*—As illustrated in Fig. 3, the microsatellite primers worked very well for some species (i.e., *P. elisabethae*, *P. bipinnata*, and *P. rigida*) and less well for other species (i.e., *P. acerosa*). This may reflect differences in optimal PCR conditions for different species; however, it is more likely indicative that some of the bands from different species are homologous, while others bands are not. For example, sequencing analyses show that Pel-19 is homologous in *P. elisabethae* and *P. bipinnata*, but the multiple bands created by Pel-19 primers in *P. americana* and *P. acerosa* are probably not homologous to the bands in *P. elisabethae* and *P. bipinnata*. While the presence of homologous products among all the species would enable additional analyses, species identification simply requires the presence of reproducible patterns.

Most (94%) of the *Pseudopterogorgia* spp. recruit samples were successfully identified using microsatellite primers Pel-19, Pel-32, and Pel-1. Thirty-six of the 604 samples (6%) could not be identified using this method. Of the samples that could not be identified, three were either *P. elisabethae* or *P. bipinnata* but could not be differentiated using the combination of Pel-19 and Pel-1, and two other samples produced bands that did not fit any of the six *Pseudopterogorgia* spp. molecular patterns. These two samples may not be *Pseudopterogorgia* spp. or may represent a rare *Pseudopterogorgia* sp. that is not commonly found at the Cross Harbour study site. Thirty-one samples were not identified because one or more of the microsatellite primers could not be amplified. A few of the very small recruit samples provided only a small quantity of DNA, making PCR impossible for all three microsatellite primers. However, in most of the 31 cases, the microsatellite primers were unable to amplify a product, and at least three PCRs were performed to confirm this negative result. Cases in which only a single locus failed to amplify probably reflect evolution in flanking regions that prevents the primer from annealing to the template DNA, creating a “null” allele. When consistently occurring in all members of the species, the absence of product for these loci could also be used to differentiate species. For instance, Papakostas et al. (2006) inferred species among rotifers based on whether a microsatellite locus was present (i.e., could be amplified) in samples. However, positive evidence such as used in this study provides greater and more certain resolution of species.

*Pseudopterogorgia acerosa* and *P. americana* were the most difficult species to differentiate using the molecular method. These two species could be sometimes differentiated morphologically because *P. americana* colonies, unlike *P. acerosa*, produced large quantities of mucus that made the colony markedly slimy. However, mucus production could not be used to differentiate small recruits of the two

species because it was often difficult to detect mucus on the smallest recruits. *Pseudopterogorgia* sp. also produced mucus that made it slimy, but the amount of mucus produced was less than *P. americana*. It has generally been assumed by most researchers that all slimy *Pseudopterogorgia* spp. colonies are *P. americana*; however, sclerites of adult *Pseudopterogorgia* sp. were distinctly different from those of *P. americana* (sensu Bayer 1961), and *Pseudopterogorgia* sp. was molecularly and morphologically distinct from the other *Pseudopterogorgia* spp.

This molecular identification method as well as the specific loci used in this study should be applicable to other studies of *Pseudopterogorgia* spp. However, its application to other areas will require verification that the PCR products found in the Bahamian populations are present and differ between the species. The high levels of polymorphism present in many microsatellite loci are highly advantageous in population analyses but may also lead to geographic differences in some of the PCR products used to differentiate species. While the technique has been extremely useful in our analyses of recruits from the Bahamas, its application to other sites will require analysis of known colonies from those sites. The use of microsatellite loci for species identifications should also be applicable to other genera. The use of highly variable markers such as microsatellites should be particularly useful for anthozoans, which exhibit limited variation in molecules commonly used for "bar coding" such as COI (Shearer et al. 2002). Microsatellites have not generally been used for species identification. Unfortunately, the development of microsatellite loci is time consuming and expensive, and this approach will be most efficient among taxa for which microsatellites have already been described from population genetic analyses. The application of markers to differentiate species within a single genus also requires identification of the sample to genus before the molecular species identification can be undertaken. Fortunately, genus-level identification of adult gorgonian colonies is straightforward, and most recruits are readily identified to genus, even when they are only a few centimeters in height.

*Recruitment and adult density of Pseudopterogorgia spp.*—The number of recruits collected during May 2005 was greater than November 2005 at all three sites, reflecting the species' reproductive biology. *Pseudopterogorgia elisabethae* and *P. bipinnata* are known to reproduce during November and December in the Bahamas (Gutiérrez-Rodríguez and Lasker 2004c). *Pseudopterogorgia americana* spawns in October or November in Florida (Fitzsimmons-Sosa et al. 2004) and appears to have large, mature eggs from September to April in Puerto Rico (Yoshioka 1979). *Pseudopterogorgia acerosa* has been observed spawning in the Bahamas in October (Lasker pers. obs.) and has mature gonads from August to October in Puerto Rico (Yoshioka 1979). A lesser number of *P. elisabethae*, *P. bipinnata*, and *P. americana* recruits were also collected during November 2005. Those colonies probably settled in the fall or winter of 2004 and were missed during the May 2005 collections. The time and mode of reproduction are unknown for *P. rigida*

and *Pseudopterogorgia* sp. However, female *Pseudopterogorgia* sp. colonies contained large seemingly mature eggs in November 2007. That suggests spawning in December or January, which is consistent with the greater number of *Pseudopterogorgia* sp. recruits that were collected during May than November 2005. The size of the recruits collected in May (1–3 cm) as well as observations of single polyp recruits in January 2007 (Lasker pers. obs.) are also consistent with December or early January spawning. Only a few *P. rigida* and *P. acerosa* recruits were collected during this study, and we were unable to make any inferences about reproductive timing in these species.

No significant difference was observed in *P. americana* recruitment between the three sites despite highly significant differences in adult density between the sites. This is consistent with expectations for distribution in broadcast spawners. The eggs of broadcast-spawning species that have been observed are positively buoyant, and development and settlement can take days (Brazeau and Lasker 1989; Lasker and Kim 1996). The detailed behavior of *P. americana* eggs and larvae is not known, but if the eggs are positively buoyant when released, then the *P. americana* recruits settling at the study site probably came from other populations, and, as observed, recruitment would be independent of the local density of adults. The differences in adult abundance that were observed were presumably the result of site-specific differences in postsettlement mortality. Both *P. bipinnata* and *P. elisabethae* surface brood, and recruitment and adult density differed significantly between the sites for both species. Both species had peak recruit abundance at CHR with successively fewer recruits at CH60 and CH30, and the relative abundance of *P. bipinnata* recruits across the three sites mirrored adult abundance. This is consistent with the expectation that planulae from surface-brooding species may not disperse as widely as those of broadcast-spawning species. *Pseudopterogorgia* sp. recruitment and adult density also differed between the sites and also exhibited the pattern of greatest abundance of both adults and recruits at CHR. Either brooding or some combination of larval selectivity and early mortality could generate the same pattern regardless of the reproductive system.

One of the fundamental questions in marine ecology of the past 25 years has concerned the role of larval supply in determining the abundance of species over time and space (i.e., Connell 1985; Gaines and Roughgarden 1985). Among these *Pseudopterogorgia* spp., there appear to be two patterns of recruitment driven in large part by differences in their reproductive biology. Broadcast-spawning species, such as *P. americana*, release eggs that develop and disperse in the water column. Its larvae are widely dispersed, and they recruited uniformly across the three sites that we surveyed. Adult abundance is then determined by site-specific differences in postrecruitment growth and survival. In contrast, species such as *P. bipinnata* and *P. elisabethae* surface brood, their larvae do not travel far, and recruitment patterns reinforce the adult distributions. Thus, while the rarity of *P. elisabethae* and absence of *P. bipinnata* at CH30 may also reflect habitat suitability, the magnitude of the differences between the sites is amplified

by the effects of local recruitment. Whether there is also significant annual variation in recruitment of these species cannot be determined from a single year. However, the *Pseudopterogorgia* sp. recruitment event observed in May 2005 was far greater than that qualitatively observed in May 2004, June 2006, or June 2007 (Lasker pers. obs.), and the presence of many large *P. rigida* colonies and few smaller colonies at CH30 may be indicative of temporally variable recruitment as well.

A correspondence between larval behavior and dispersal has been observed for a number of benthic invertebrates. Looking at scales of meters and hundreds of meters, a variety of studies have related short dispersal distance to patchy distributions among a number of tunicates and corals (Olson 1985; Carlon and Olson 1993; Harii and Kayanne 2003). On scales of kilometers and greater, Hughes et al. (1999) found that brooding scleractinian corals exhibited greater spatial variability in recruitment than broadcast-spawning species, a relationship that they attributed to the more local dispersal of the larvae from brooding species. Similarly, studies of other marine invertebrates have shown how spatial patterns of recruitment can largely determine adult distribution patterns (Ryland 1959; Grosberg 1982). In this study we have seen similar patterns among a group of congeners with similar size eggs that often co-occur on the same reefs and in many cases are found within meters of each other. Studies that include more sites and a greater range of densities are needed, but among these phylogenetically and ecologically similar species, reproductive strategy appears to be well correlated with differences in their distribution patterns.

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