



Do ecological traits of low abundance and niche overlap promote hybridisation among coral-reef angelfishes?

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Abstract Hybridisation among species of coral-reef fish was previously considered to be rare. However, recent studies have revealed that hybridisation is prevalent in coral-reef fish, highlighting the need to understand the causes of this process. The angelfishes (family Pomacanthidae) have the greatest proportion (~ 30%) of hybridising species to date, with 26 species reported to hybridise. The aim of this study was to examine ecological factors (rarity of parent species and niche overlap) that promote hybridisation in terrestrial environments and to test these factors in the marine environment by examining hybridising angelfishes at Christmas Island (Indian Ocean). Three species of pygmy angelfish (*Centropyge flavissima*, *C. eibli*, and *C. vrolikii*) and their hybrids were investigated to address three objectives: (1) to determine temporal and spatial patterns in abundance and whether these patterns are linked to environmental factors; (2) to test for overlapping patterns in habitat use; and (3) to test for overlapping patterns in diet. Based on 14 yr of surveys, *C. flavissima* was abundant (4.53 individuals per

250 m² ± 0.66), whereas *C. eibli*, *C. vrolikii*, and all hybrid combinations were consistently rare (average abundance < 0.3 per 250 m² ± 0.03). Parent species and their hybrids were more abundant at 20 m depth compared to 5 m. All species and their hybrids had similar patterns of abundance around Christmas Island, with significantly high abundances evident at the most sheltered sites. Parent species and their hybrids also had similar diets that comprised a mix of green, red, and brown algae. The rarity of parent species, their niche overlap, and the harem reproductive strategy likely promote hybridisation in angelfishes at Christmas Island. This study provides empirical evidence that hybridisation in reef fishes conforms to terrestrial-based hypothesis, and thus advances our understanding of the processes underlying hybridisation in coral-reef systems.

Keywords *Centropyge* · Christmas Island · Diet · Reef fish · Habitat overlap · Hybrids

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Introduction

Hybridisation, through the interbreeding between two genetically distinct populations or species (Harrison 1993), can potentially increase or decrease species diversity (Seehausen 2004, 2006). Hybridisation has been documented for at least 10% of animal species and 25% of plant species (Mallet 2007) and is common in terrestrial and freshwater environments (Arnold 1997). Hybrids usually exhibit characteristics from each parent, and although some hybrids are unfit, other hybrids are able to survive to reproduction (Mallet 2007) and can play a vital role in the evolution of plants and animals (Barton 2001; Wissemann 2005). For example, hybrids can colonise unoccupied

ecological niches and increase their fitness (Barton 2001; Karrenberg et al. 2006) or can result in the extinction of one or both of the parent species (Seehausen 2006).

Of all the vertebrate groups, hybridisation is most commonly reported in fish, particularly freshwater fish (Scribner et al. 2000), for which the process has been well studied. Several factors have been proposed to contribute to the high incidence of hybridisation among freshwater fishes, including close relatedness, secondary contact, weak behavioural isolating mechanisms, low abundance of one or both parental species, competition for limited spawning habitat, and shared ecological niches (Scribner et al. 2000). Compared to freshwater systems, few studies have focused on the possible causes of hybridisation in marine systems, most likely due to the process being considered rare (Hubbs 1955; Arnold 1997). However, a recent review of hybridisation in marine fishes found 111 reported hybrids involving 173 species (Montanari et al. 2016) and showed that marine hybrids may be more common than previously thought. Montanari et al. (2016) emphasised the need to evolve from studies focusing on genetic confirmation of hybrids to those that determine the causes of hybridisation. Such information is critical for understanding barriers to reproductive isolation in marine species and determining how to treat hybrids in conservation management programmes (Richards and Hobbs 2015).

In marine ecosystems, few studies have focused on the ecological aspect of fish hybridisation and the possible causes of this process. Similar to terrestrial and freshwater environments, the most important factors driving hybridisation in marine systems appear to be: (1) rarity of parent species; (2) non-assortative mating; and (3) spatial or dietary overlap in parental species (Montanari et al. 2012, 2016; Hobbs and Allen 2014; Richards and Hobbs 2015; Payet et al. 2016), which are also likely to act in combination (Montanari et al. 2012, 2016; Hobbs et al. 2014). The rarity of one or both parental species has been reported in a few families of hybridising marine fishes in both temperate and tropical waters (Montanari et al. 2016). Low densities have also been shown to facilitate the formation of heterospecific pairs and groups (harems) and thereby break down the barriers of assortative mating (Hobbs and Salmond 2008; Hobbs et al. 2013). The lack of conspecific individuals is likely to force a species to interbreed with individuals from another closely related species (Hubbs 1955). In addition, habitat and diet overlap can facilitate hybridisation through increasing social interactions and the chances of encounters between different species of fish (Frisch and van Herwerden 2006; Camp et al. 2016). Despite being considered a vital factor promoting hybridisation, niche overlap has only been examined in a few studies (Randall 1956; Marie et al.

2007; Montanari et al. 2012), highlighting the need for more extensive investigations.

Recent studies in coral-reef fish hybridisation (Hobbs and Allen 2014; Richards and Hobbs 2015) have revealed that the angelfishes (family Pomacanthidae) have the greatest proportion (~ 30%) of hybridising species (Yaakub et al. 2006). The pygmy angelfishes (genus: *Centropyge*), which form the largest genus within the Pomacanthidae (Allen et al. 1998), maintain a harem mating system and exhibit protogynous sex change (Sakai et al. 2003). Hybrids between three species (*C. flavissima*, *C. eibli*, and *C. vrolikii*) in this genus have been observed in heterospecific harems with all harem members interbreeding at Christmas Island (Hobbs and Allen 2014; DiBattista et al. 2016a). The co-occurrence of these hybrids with their parent species in the *Centropyge* genus, therefore, provides us with a natural laboratory to directly examine the ecological factors promoting hybridisation in coral-reef fishes in a natural environment. Here, we aim to determine whether or not rarity of a parent species and niche overlap, in terms of habitat (macro and micro) and diet, are important factors in promoting hybridisation in angelfishes. We examine this at Christmas Island, which together with Cocos (Keeling) Islands is situated on the Indo-Pacific biogeographic border and is considered a global hot spot for hybridisation (Hobbs et al. 2009; Hobbs and Allen 2014).

Materials and methods

Study site and species

The study was undertaken at Christmas Island (10°30'S, 105°40'E), an oceanic island located approximately 300 km south of Java, Indonesia. The coral reef extends from the shore for about 20–100 m towards the reef drop-off, which usually begins at 15 m depth and then declines steeply to more than 4000 m (Gilligan et al. 2008). The island is exposed to south-easterly trade winds and southerly swells for most of the year (Hobbs et al. 2014; Richards et al. 2016). This study was therefore restricted to the more sheltered north, east, and west coasts of the island. All aspects of the study were undertaken along these coasts (Fig. 1). The study species were *C. flavissima*, *C. eibli*, and *C. vrolikii* and the three hybrids of these species. Although *C. flavissima* in the Indian Ocean has been described as a new species, *C. cocosensis* (Shen et al. 2016), there is debate around this new nomenclature (DiBattista et al. 2016a, 2017). DiBattista et al. (2017) concluded that, to avoid confusion in the existing studies and databases, more taxonomic evidence is needed to accept the new nomenclature. We have, therefore, used the

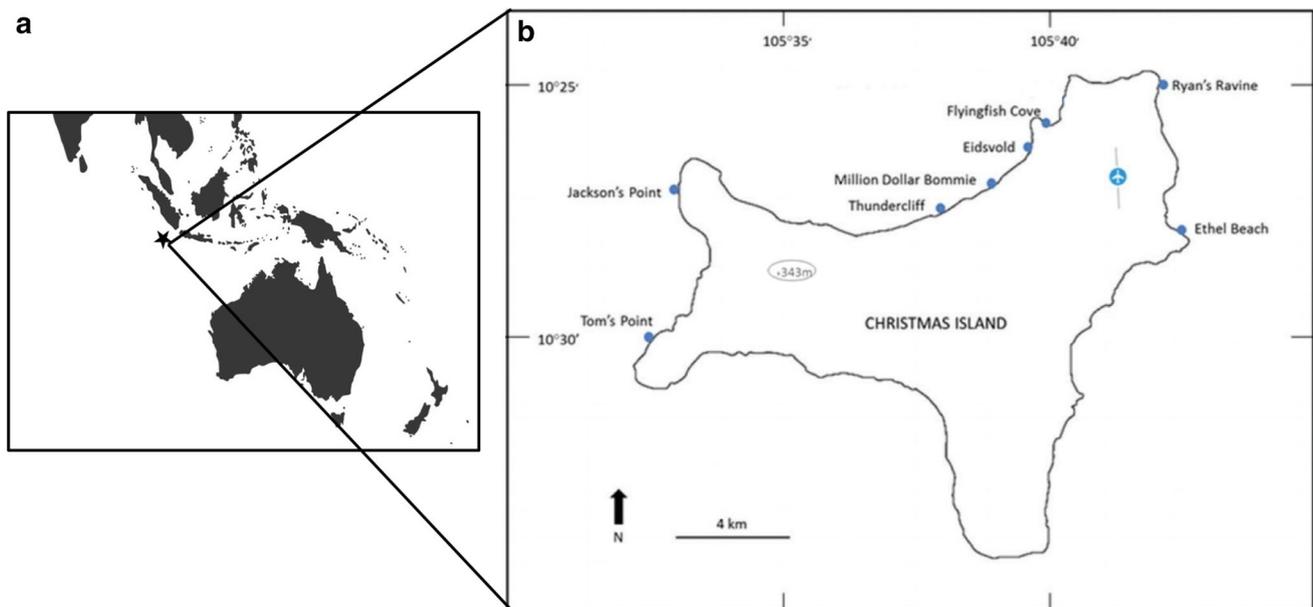


Fig. 1 Map of Christmas Island; dots represent the study sites on the north, west, and east coasts of the island. Due to adverse diving conditions, the south coast could not be surveyed

original species name. Due to the extreme rarity of the two hybrids *C. flavissima* × *C. vrolikii* and *C. eibli* × *C. vrolikii*, analyses were only performed on the three parent species and the most abundant hybrid *C. flavissima* × *C. eibli*.

Hybrids of the three species have been recognised in a range of studies across their geographic range, including Christmas Island (Hobbs et al. 2009; Hobbs and Allen 2014; DiBattista et al. 2016a, 2017). The three parent species have different body colours and patterns, with hybrids displaying a mix of clearly recognisable and consistent colour and patterns (Hobbs and Allen 2014; DiBattista et al. 2012, 2016a, 2017). Furthermore, genetic studies have confirmed that individuals with intermediate colouration are hybrids and not colour morphs of the parent species (Hobbs and Allen 2014; DiBattista et al. 2016a, 2017). One limitation of our study is that some backcrossed individuals have the colouration of a parent species (DiBattista et al. 2016a). Although intermediate-coloured individuals provide reliable identification of first-generation hybrids, they underestimate backcrossed individuals (Yaakub et al. 2006; DiBattista et al. 2015, 2016b). Quantifying this underestimate is challenging due to difficulties in genetically resolving the taxonomy of the parent species (DiBattista et al. 2016a). DiBattista et al. (2016a, 2017) provided examples of intermediate colour variations of hybrids at Christmas Island. Based on these and years of experience of Jean-Paul Hobbs in identifying the parent species and their hybrids, the parent species and the intermediate colouration of the hybrids were visually identified by Jean-Paul Hobbs and Federico Vitelli. At this

stage, we were not able to distinguish between F1, F2, and F3 generations; therefore, all intermediate-coloured individuals were considered as hybrids.

Abundance

To determine whether hybridisation occurs between species with low abundances, the density of each angelfish species (*Centropyge flavissima*, *C. vrolikii*, and *C. eibli*) and their hybrids were estimated by underwater visual censuses on the outer reef slope at eight sites on ten separate occasions over 14 yr (2002–2015). On each occasion, fish abundances were recorded in three replicate 50-m strip transects at 5 and 20 m depths at each site. The approximated width of the transect was 5 metres (~ 2.5 metres on each side of the transect tape).

Niche overlap

Macrohabitat

To determine whether there was overlap in the macrohabitat used by the three angelfish species (and their hybrids) the benthos was recorded at 1-m intervals along the same 50-m transect (point intercept method, 50 points) used to record fish abundance. Benthos was categorised into one of 12 microhabitats based on Hobbs et al. (2010): calcareous coralline algae, turfing algae, branching corals, massive corals, plate corals, corymbose corals, foliaceous corals, encrusting corals, sand, bare rubble, dead hard corals, and soft corals. The wave exposure index and reef

aspect were calculated for each site. The wave exposure index was estimated based on the method of Jennings et al. (1996), by determining the 22.5° sectors (corresponding to traditional compass headings, e.g. E, ENE, NE, NNE, etc.) from which the site was fully exposed to an unobstructed wave fetch of 3 km or more, and summing the product of the mean annual wind speed at each reef complex (km h^{-1}) and duration (proportion) in each of these sectors. Wind data for Christmas Island were obtained from the Bureau of Meteorology website (<http://www.bom.gov.au/wa/forecasts/christmas-island.shtml>). The reef aspect was calculated as the angle of inclination of the reef slope. At each of the two depths (5 and 20 m), the distance of the sampling site from the coastline and reef profile was used to calculate the angle (0° = flat horizontal reef; 90° = vertical reef wall).

Microhabitat structure within harems

To determine microhabitat use and overlap among the three species (and their hybrids), point intercept transects were completed within the home range of each harem. The species composition was recorded for 274 harems at three sites on the sheltered (northern) side of the island. The harems were randomly selected at depths between 5 and 25 m at each site during a 30-min swim. *Centropyge flavissima* was the only species observed in monospecific harems, whereas *C. eibli*, *C. vrolikii*, and the hybrids were only found in mixed harems. Therefore, the surveyed harems were divided into the following categories based on the species composition: (1) pure harems of *C. flavissima*; (2) *C. eibli* + *C. flavissima*; (3) *C. vrolikii* + *C. flavissima*; and (4) harems including hybrids. To quantify microhabitat use, the home range of each harem was determined by observing the behaviour and movements of the individuals for approximately 6 min. Based on previous studies (e.g. Hobbs and Allen 2014; Hobbs personal observations), 5 min of observation is enough to allow the fish to move across their home range and allow the diver to establish the area of the harem. Prior pilot studies using 5-min observations showed that the home range was usually wider than 3 m, and therefore, 3-m transects were considered adequate to represent the spatial extent of each home range to determine microhabitat structure. Once the home range was determined for each harem, a 3-m line intercept transect was placed on the substrate through the centre of the harem, and the amount of each microhabitat within that area was recorded every 10 cm (following Hobbs et al. 2010). Microhabitat was recorded following the same 12 categories used for macrohabitat surveys.

Diet

Since food resources provide another dimension of potential niche overlap that could lead to greater interactions between parent species (Montanari et al. 2012), the diets of *C. flavissima*, *C. vrolikii*, and *C. eibli* and their hybrids were determined by collecting 30 individuals of each species and their hybrids by spearfishing at eight (non-survey) sites along the north coast in September 2015. Since prior observations indicated that these species feed throughout the day (Hobbs personal observations), fishes were collected at anytime during the day. Stomachs were removed from each fish, and the gut contents were later examined in the laboratory by cutting the stomach open and spreading the contents onto a gridded petri dish. Gut contents of each individual were examined under a stereodissecting microscope and quantified by recording the food type present at 100 randomly assigned point intercepts on the grid (Buckle and Booth 2009). All stomachs were found full, and food items inside were not digested, indicating that fish fed throughout the day. Food items were therefore easily identified, apart from some of the smallest fragments of algae (< 1 mm), which were grouped under other unidentified material. Less than 2% of the gut contents across all samples were classified as other unidentified material. Dietary items were identified to the lowest possible taxon.

Data analysis

To examine patterns in the density of angelfishes and hybrids, analyses were only performed on the three parent species and the most abundant hybrid *C. flavissima* × *C. eibli*. The other two hybrids, *C. flavissima* × *C. vrolikii* and *C. eibli* × *C. vrolikii*, were too rare to analyse. Total abundance estimates of the three species of *Centropyge* and the hybrid were compared among years, sites, and depths using PERMANOVA (permutational analysis of variance). PERMDISP analysis in the PERMANOVA+ (Anderson et al. 2008) package in PRIMER 6 (Clarke and Gorley 2006) was run to test for homogeneity of the variances, and based on this, analyses on the fish assemblage and on single species were performed on untransformed data. The only exception was *C. flavissima* which was square root transformed. As the PERMDISP test did not result in homogeneity of data for the factor 'site', the alpha level for this factor was accepted at a lower level ($P < 0.01$) (Underwood 2011). An orthogonal PERMANOVA design was used for the fish assemblage and *C. flavissima*, with year (fixed factor, 11 levels), site (random factor, 8 levels), and depth (fixed factor, 2 levels). The rare species *C. eibli*, *C. vrolikii*, and the hybrid *C. flavissima* × *C. eibli* were absent or virtually absent at 5 m depth. Therefore, for these

species only data from 20 m depth were used and analysis proceeded using only 2 factors (year and site). Post hoc tests in PERMANOVA were run as necessary to investigate statistically significant main effects or interactions between factors.

A distance-based linear model (DistLM) in the PERMANOVA+ (Anderson et al. 2008) package in PRIMER 6 (Clarke and Gorley 2006) was used to determine the influence of habitat characteristics on the distributions of the three angelfish parent species and the *C. flavissima* × *C. eibli* hybrid. This analysis selects the environmental variables that best explain the variation in the density of fish over the entire survey area. Univariate resemblance matrices of the densities of each of the angelfish species and the hybrid were constructed from untransformed data using the zero-adjusted Euclidean coefficient (Clarke et al. 2006). Habitat predictor variable data were not transformed. DistLM was performed using the best selection procedure and the AICc, which reduces bias in linear regression models and improves model selection with small sample sizes (Hurvich et al. 1990) and has been used in similar studies (e.g. Saunders et al. 2014).

Differences in microhabitat composition among types of social groups (harems) (fixed factor harems, 4 levels) and differences among diets between species and hybrids (fixed factor species, 4 levels) were examined using PERMANOVA. For the microhabitat analysis, the majority of the 274 surveyed harems were pure harems of *C. flavissima*, so we randomly subsampled 20 harems per harem type beforehand. PERMDISP analysis indicated that the untransformed microhabitat data had homogeneous variances, whereas square root transformation was required for the percentage dietary data to satisfy the assumption of homogeneity of variances. Non-metric multidimensional scaling (nMDS) plots were used to visually represent the relative similarity of microhabitat composition from each species and the hybrid.

Results

Abundance

Two of the three parent species were rare at Christmas Island. The mean density of *C. flavissima* [4.53 ± 0.2 (mean \pm SE) fish per 250 m^{-2}] was more than 20 times higher than those of *C. eibli* (0.16 ± 0.03), which was the second most abundant species, followed by *C. vrolikii* (0.04 ± 0.008), and the hybrid *C. flavissima* × *C. eibli* (0.04 ± 0.01) over the 14 yr of sampling. There were no significant differences in abundances among years when species were analysed individually or as the whole fish assemblage (Table 1).

Centropyge flavissima was the only species found consistently in shallow (5 m) waters, but densities were generally higher in deeper (20 m) waters (Fig. 2, Table 1). The densities of this species varied across sites (Fig. 2), but there was no consistent pattern with depth or year, as shown through the interactions in the PERMANOVA (Table 1). *Centropyge eibli*, *C. vrolikii*, and the hybrid *C. flavissima* × *C. eibli* were either absent or virtually absent in shallow reef habitats (Fig. 2). In deeper reef habitats (20 m), their densities varied across sites but were consistent across years (Table 1), *C. eibli* was more abundant at Eidsvold and Flying Fish Cove, and *C. vrolikii* reached its maximum densities at Flying Fish Cove. The hybrid *C. flavissima* × *C. eibli* showed the same pattern as the two rare parent species, reaching its maximum densities at Eidsvold and Flying Fish Cove (Fig. 2). The other hybrids *C. flavissima* × *C. vrolikii* [0.006 ± 0.003 (mean \pm SE) fish per 250 m^{-2}] and *C. eibli* × *C. vrolikii* (0.002 ± 0.002) were rare across the 14 yr of surveys.

Macrohabitat use

All parent species and their hybrids overlapped in their use of macrohabitats and achieved their highest abundance at the depth of 20 m (Fig. 2). The linear relationships between environmental variables and fish abundances were generally weak (except for *C. flavissima*) with low R^2 and slope values. Reef aspect was repeatedly identified as a positive explanatory variable for the variation in abundances of all species (Table 2). Variation in *C. flavissima* density was positively related to reef aspect and turf algae, which contributed to 35% and 7% of the total variability, respectively. Branching corals were negatively associated with the density of *C. flavissima*, explaining 26% of the variation (Table 2). Foliose corals were included in the model and were positively correlated with the presence of *C. eibli*, *C. vrolikii*, and the hybrid *C. flavissima* × *C. eibli*. Sand was positively associated with the abundance of *C. eibli* and the hybrid (Table 2). Wave exposure also explained variations among *C. vrolikii* abundance, which was negatively correlated with this variable.

Microhabitat use

The three species overlapped in their use of microhabitats. Of the three species, *C. flavissima* was the only species to form monospecific harems, which contributed 54% of the 274 observed harems. This species was also present in all the remaining harems, which comprised mixed species of either *C. eibli* (18%) or *C. vrolikii* (12%), or the two hybrids *C. flavissima* × *C. eibli* (19%) and *C. flavissima* × *C. vrolikii* (3%).

Table 1 Results of PERMANOVA testing the differences in abundance of the three angelfish parent species and their hybrid between years, sites, and depth

Factor	<i>Fish assemblage</i>				<i>C. flavissima</i>		
	<i>df</i>	MS	Pseudo- <i>F</i>	<i>P</i> (perm)	MS	Pseudo- <i>F</i>	<i>P</i> (perm)
Year (<i>Y</i>)	10	2.18	1.40	0.1288	6.94	1.52	0.1485
Site (<i>S</i>)	7	1.04	9.47	0.0001	12.80	7.15	0.0001
Depth (<i>D</i>)	1	2.24	26.97	0.0014	0.49	26.84	0.0015
<i>Y</i> × <i>S</i>	68	0.27	2.05	0.0001	1.20	2.11	0.0002
<i>Y</i> × <i>D</i>	10	2.45	1.15	0.3229	4.26	1.10	0.3754
<i>S</i> × <i>D</i>	7	0.18	21.11	0.0001	0.80	31.26	0.0001
<i>Y</i> × <i>S</i> × <i>D</i>	68	0.07	2.76	0.0001	0.44	3.34	0.0001
Res	344	0.05			0.33		0.1485
Total	515	0.24					

Factor	<i>df</i>	<i>C. eibli</i>			<i>C. vrolikii</i>			Hybrid		
		MS	Pseudo- <i>F</i>	<i>P</i> (perm)	MS	Pseudo- <i>F</i>	<i>P</i> (perm)	MS	Pseudo- <i>F</i>	<i>P</i> (perm)
Year (<i>Y</i>)	10	0.51	0.50	0.9430	0.06	1.01	0.4492	0.16	1.75	0.0760
Site (<i>S</i>)	7	10.70	2.99	0.0045	0.22	3.61	0.0010	0.20	2.22	0.0310
<i>Y</i> × <i>S</i>	68	1.03	2.01	0.0007	0.06	0.99	0.4800	0.09	1.01	0.4148
Res	172	0.51			0.06			0.09		
Total	257									

The table shows results for the whole fish assemblage (parent species and hybrid together) and for each single species. Three-factor design for the fish assemblage and *C. flavissima* with years (fixed factor, 10 levels), sites (random factor, 8 levels), and depth (fixed factor, 2 levels). Two-factor design with years (fixed factor, 11 levels) and sites (random factor, 8 levels) for the other two species and hybrid. Abundance data were untransformed for all species except for *C. flavissima* which was square root transformed

Significant results (*P* values) are in bold

Harems comprised of any of the parent species or hybrids showed no significant differences in microhabitat use (Table 3, Figs. 3, 4). The main types of substratum present inside both pure and mixed angelfish harems included massive and encrusting corals, and turf algae, which combined covered more than 65% of the substratum in the harem's territory, followed by foliose corals, coral-line algae, and sand (Fig. 3).

Diet

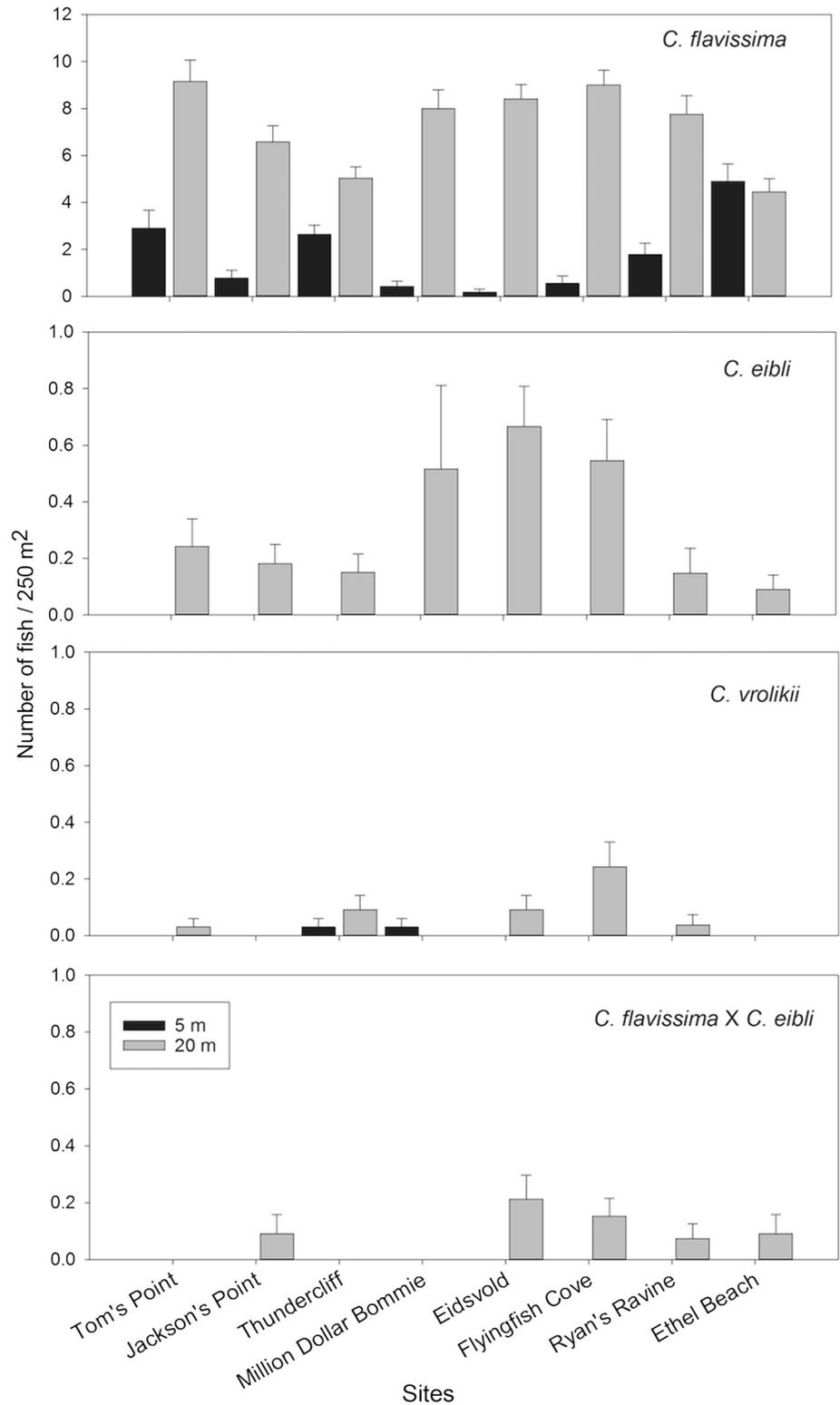
There were no significant differences in diet among parent species or between parent species and hybrids (Table 3, Figs. 5, 6). A variety of red (Rhodophyta), green (Chlorophyta), and brown (Heterokontophyta) algae were ingested by the three parent species and the hybrid *C. flavissima* × *C. eibli*. The most frequently consumed species (frequency of occurrence, %*F*; mean percentage volume, %*V*) were the red alga *Caulacanthus* spp. (%*F* = 100–96.6% and %*V* = 25.2–19.5%), followed by the brown alga *Sphacelaria* sp. (%*F* = 100–90% and %*V* = 24.8–17%), the red alga *Caulerpella* sp. (%*F* = 100–93.3% and %*V* = 14.5–10.7%), the green alga

Bryopsis sp. (%*F* = 96.7–88.6% and %*V* = 9–7.9%), and different species of sponges (%*F* = 97.7–90% and %*V* = 13.8–6.7%) (Table 4, Fig. 5).

Discussion

Two of the three angelfish species known to form hybrids at Christmas Island were rare, thereby supporting the hypothesis that hybridisation occurs where there is a scarcity of conspecific partners. There was strong niche overlap with all three parent species exhibiting similar diets and habitat use (macro and micro). Collectively, niche overlap and a lack of conspecific partners facilitate the mixing of species and the formation of heterospecific harems, whose members then interbreed to produce hybrids. Our results support the hypothesis derived from terrestrial and freshwater systems that rarity of parent species and niche overlap contribute to the breakdown in assortative mating and subsequent hybridisation. However, in order to conclusively determine if these factors promote hybridisation, an experimental comparison to a high-abundance situation would be ideal. This is the first long-

Fig. 2 Mean (+ SE) densities per 250 m² of the three angelfish parent species and the most abundant hybrid across sites at different depths (5 and 20 m). Abundance data were averaged among 14 yr of surveys. Please note differences in scales on the Y-axes



term abundance monitoring study on coral-reef fish hybridisation combined with habitat and dietary analysis and shows that the abundance patterns that lead to hybridisation have been consistent across 14 yr. Thus,

hybridisation is not a rare one-off event, but has been happening over a considerable time period. Indeed, hybrids of these angelfish have been observed at Christmas Island since the first detailed fish surveys were undertaken in 1978

Table 2 Most suitable model/s for explaining variance in angelfish parent species and hybrids abundance

Species	Model	AICc	R ²
<i>C. flavissima</i>	Branching corals ⁻	671.72	0.44243
	Aspect ⁺		
<i>C. eibli</i>	Turf algae ⁺	74.088	0.14704
	Foliose corals ⁺		
<i>C. vrolikii</i>	Sand ⁺	- 321.12	0.07233
	Aspect ⁺		
	Foliose corals ⁺		
Hybrid	Wave exposure ⁻	- 268.83	0.07949
	Aspect ⁺		
	Foliose corals ⁺		
	Sand ⁺		
	Aspect ⁺		

A distance-based linear model (DistLM) approach with a ‘best’ selection procedure (Anderson et al. 2008) and the corrected Akaike information criterion (AICc) was used for model selection. For the model with the lowest AICc value for each species, the environmental variables included in each model, corrected AIC (AICc) value and the R² value are reported. ‘+’ and ‘-’ indicate a positive and a negative relationship, respectively, between the variable and fish abundance

(Hobbs and Allen 2014), and genetic studies of these fishes indicate a long history of hybridisation (DiBattista et al. 2016a).

The three species of angelfishes at Christmas Island displayed a strong disparity in their abundances over the 14 yr of the study, with *C. flavissima* being at least 20 times more abundant than *C. eibli* and *C. vrolikii*. This abundance pattern was consistent at all sites across the 14 yr of study. All three species co-occurred mainly in deeper (20 m) reef habitats, which coincided with the presence of hybrids. It is likely that the lack of conspecific partners facilitates hybridisation among the three species of angelfishes. That is, *C. eibli* and *C. vrolikii* join harems and interbreed with the closely related species *C. flavissima* because they cannot find a conspecific partner. Indeed, we

Table 3 Results of a one-factor analysis of variance on percentages of substratum categories present inside territories of angelfish harems composed by different angelfish species and hybrids (test 1:

	Microhabitat				Diet				
	df	MS	Pseudo-F	P	df	MS	Pseudo-F	P	
Harem	3	1673.0	1.6411	0.0728	Species	3	730.46	1.3953	0.108
Res	115	1019.5			Res	126	523.49		
Total	118				Total	129			

‘Harem type’ and ‘species’ are fixed factor, 4 levels for harem type (pure harems of *C. flavissima*; *C. eibli* + *C. flavissima*; *C. vrolikii* + *C. flavissima*; and harems including hybrids), and 4 levels for species (*C. flavissima*, *C. eibli*, *C. vrolikii*, and *C. flavissima* x *C. eibli*)

observed many heterospecific harems in the field, and at sunset, all members of the harem interbreed even if it is with another species (Hobbs personal observations). While the lack of conspecific partners appears to facilitate hybridisation in harem-forming species, it also appears to operate in a range of other mating systems exhibited by marine fishes, such as pair spawning and mass spawning (DiBattista et al. 2015; Montanari et al. 2016). A lack of conspecific partners has been observed in the hybridisation of butterflyfish (Hobbs et al. 2013), including at Christmas Island (Montanari et al. 2012), and in other reef fishes such as Pomacentridae, Labridae, and Acanthuridae (Hobbs and Allen 2014). Thus, the lack of conspecific mates leads to the formation of heterospecific harems, which results in the breakdown of assortative mating and subsequent production of hybrids.

Habitat overlap is one of the most important factors promoting hybridisation in freshwater fishes (Scribner et al. 2000), and in this study, all three angelfishes exhibited considerable overlap in resource use. In terms of macro-habitat, all three species were most abundant in deeper waters (20 m) at sheltered sites where they inhabited steep reef walls that were dominated by turfing algae, massive and encrusting corals, rather than occupying the shallow (5 m) reef flat dominated by plates and branching corals. Overlap in habitat use has been reported in some other hybridising reef fishes. In 90% of the hybridising butterflyfishes (Chaetodontidae—the sister family to angelfishes), the parent species overlapped by 50% or more in their depth ranges (Montanari et al. 2012; Hobbs et al. 2013). Similarly, hybridising groupers (Serranidae) and surgeonfishes (Acanthuridae) overlapped in depth range and habitat use (Frisch and van Herwerden 2006; Marie et al. 2007; Hobbs and Allen 2014; Payet et al. 2016; DiBattista et al. 2016b). However, these previous studies generally examined course-scale measures of niche overlap such as depth. To better understand species associations and resource partitioning in reef fishes it is best to study the habitat use with a multi-scale approach (Eagle et al. 2001).

microhabitat) and on percentages of dietary items of the three angelfish parent species and hybrid (test 2: *diet*) at Christmas Island

Fig. 3 Percentage composition of microhabitat types within the territories of angelfish harems at Christmas Island. Pure harems of *C. eibli* and *C. vrolikii* were not found at Christmas Island. Therefore, harems composed by *C. flavissima* only, *C. flavissima* + *C. eibli*, *C. flavissima* + *C. vrolikii*, and hybrids + parent species are compared. Microhabitat surveys were conducted between September 2015 and April 2016

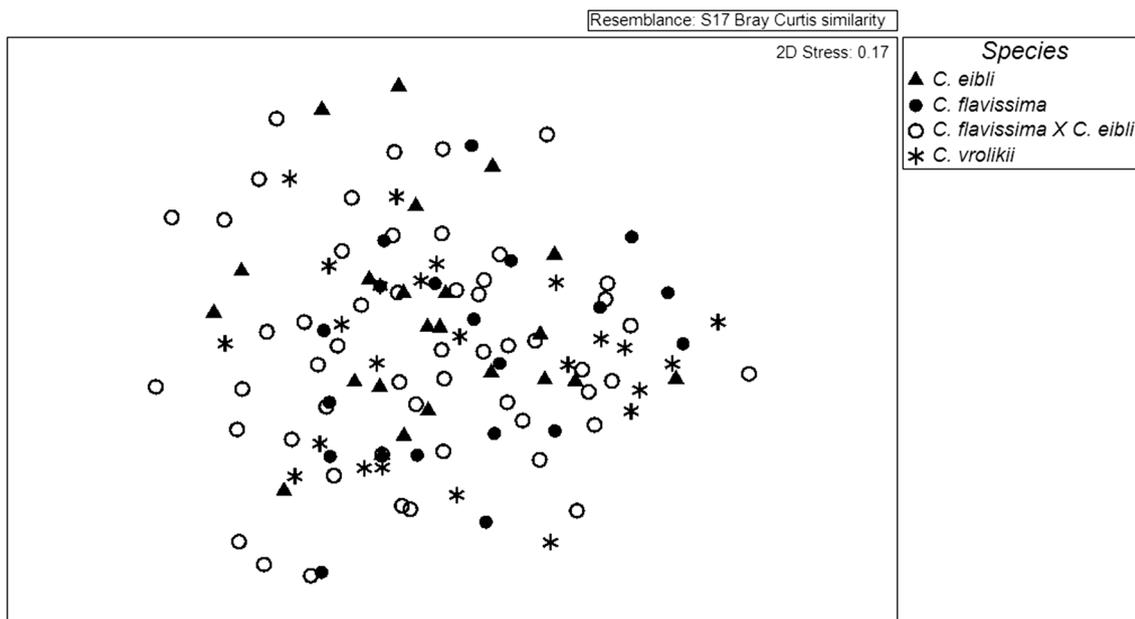
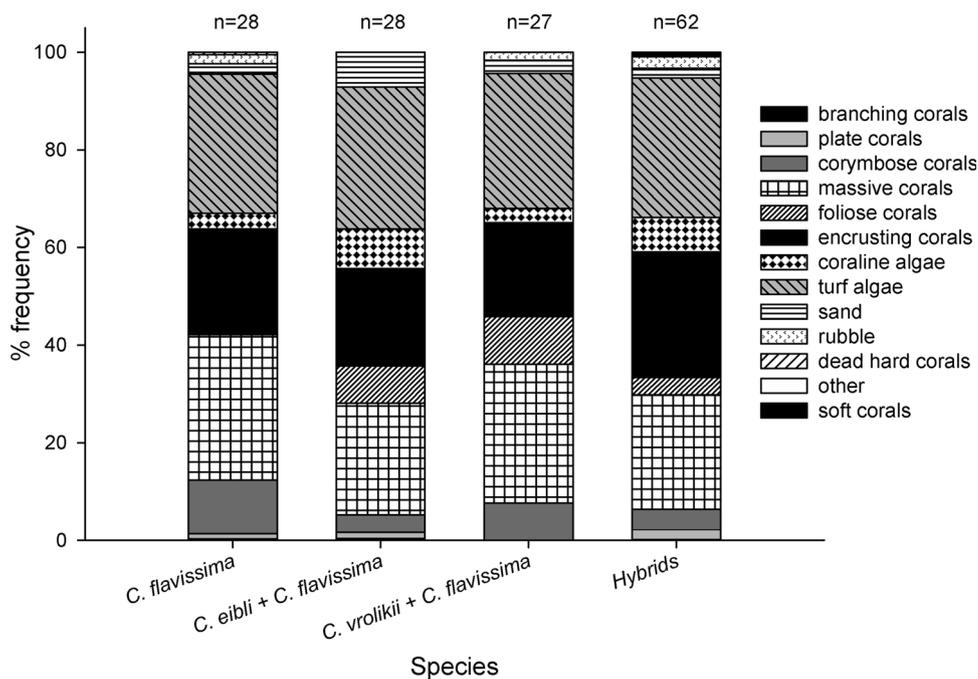


Fig. 4 Non-metric multidimensional scaling (nMDS) plot based on a Bray-Curtis similarity matrix representing the substratum composition inside the territories for harems of the three angelfish parent

species and their hybrids. Microhabitat surveys were conducted between September 2015 and April 2016. The symbol overlap highlights the similarity of the substratum composition

Thus, in this study we also examined microhabitat characteristics of the harem territories, albeit on one sampling occasion, and found strong overlap in microhabitat use of the parent species. By using a multi-scale approach this study demonstrates that hybridising reef fishes overlap in macro- and microhabitat use, thereby providing greater empirical support for the hypothesis that habitat overlap promotes hybridisation.

The three angelfish species also showed strong dietary overlap by feeding on the same species of algae during the sampling occasion in which this was examined. Their diets mainly comprised a mix of green, red, and brown algae, plus some animal material such as sponges and hydroids, which were consumed in similar proportions across species. Overlapping diets showed that the three parent species and hybrids shared the same food resources. Species that

Fig. 5 Percentage of dietary items found in the stomachs of the three parent species of angelfish and the most abundant hybrid. Fish for dietary analysis were collected between September 2015 and April 2016

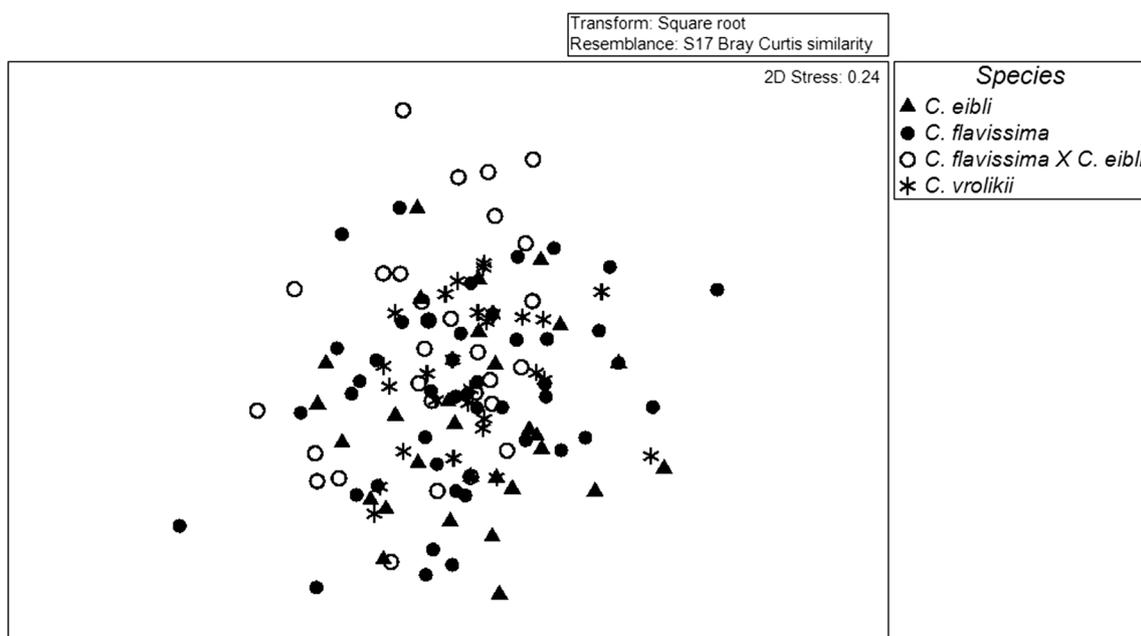
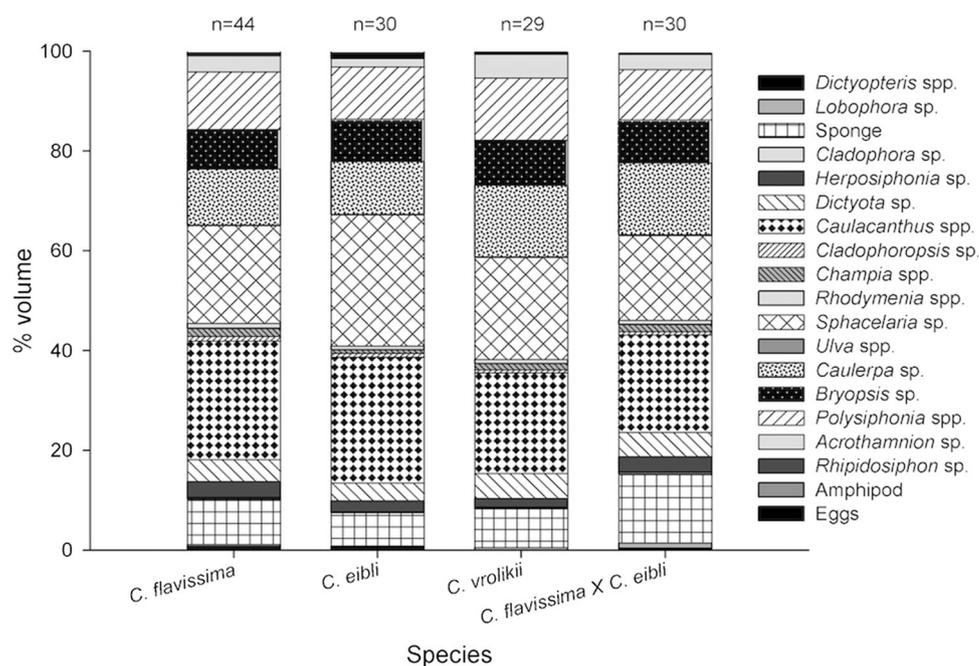


Fig. 6 Non-metric multidimensional scaling (nMDS) plot based on a Bray–Curtis similarity matrix representing the dietary composition of the three angelfish parent species and the most abundant hybrid. Data

were square root transformed. Fish for dietary analysis were collected in September 2015. The symbol overlap highlights the similarity of the substratum composition

feed on the same food resources are likely to have greater chances of encounters and interactions, which could lead to the formation of mixed species harems and, thus, hybridisation (Montanari et al. 2012). In a previous dietary study, *C. eibli* and *C. flavissima* were found to have similar diets; however, *C. vrolikii* had a different diet (Hobbs et al. 2010). The difference between studies could reflect temporal and spatial differences in the collection of *C. vrolikii*,

and therefore their diets. Strong dietary overlap of hybrids and parent angelfish species is therefore similar to hybridising butterflyfishes, based on the only other study to compare diets of hybridising reef fishes (Montanari et al. 2012).

By combining information from dietary analyses as well as macro- and microhabitat surveys, this study shows strong overlap in resource of the hybridising angelfishes

Table 4 Frequency of occurrence (%F, percentage of fish that had a particular food item in their stomach) and mean percentage volume (%V) of dietary items in the stomachs of *Centropyge flavissima*, *C. eibli*, *C. vrolikii*, and the hybrid

Species	<i>C. flavissima</i>		<i>C. eibli</i>		<i>C. vrolikii</i>		<i>C. flavissima</i> × <i>C. eibli</i>	
	<i>N</i> = 44		<i>N</i> = 30		<i>N</i> = 29		<i>N</i> = 30	
	S.R. = 52–110 mm		S.R. = 68–100 mm		S.R. = 51–105 mm		S.R. = 43–108 mm	
	%F	%V	%F	%V	%F	%V	%F	%V
Rhodophyta								
<i>Acrothamnion</i> sp.	59.1	3.2	46.7	1.7	72.4	4.8	63.3	3.1
<i>Caulacanthus</i> spp.	100	22.6	100	25.2	96.6	20.2	96.7	19.5
<i>Champia</i> spp.	40.9	1.6	43.3	0.7	51.7	1.2	50	1.5
<i>Herposiphonia</i> sp.	79.5	3.2	53.3	2.3	55.2	1.7	76.7	3.1
<i>Polysiphonia</i> spp.	97.7	11.6	96.7	10.5	96.6	12.5	90	10
<i>Rhodymenia</i> spp.	45.5	1.0	30	0.8	41.4	0.9	33.3	0.8
Chlorophyta								
<i>Bryopsis</i> sp.	88.6	7.9	90	8.5	96.6	9	96.7	8.6
<i>Caulerpa</i> sp.	93.2	11.3	100	10.7	96.6	14.4	93.3	14.5
<i>Cladophora</i> sp.	11.4	0.3	6.7	0.1	17.2	0.4	13.3	0.4
<i>Cladophoropsis</i> sp.	38.6	0.9	36.7	0.8	31	0.6	26.7	0.7
<i>Rhipidosiphon</i> sp.	2.3	0.3	3.3	0.1	0	0	0	0
<i>Ulva</i> spp.	2.3	0.05	0	0.0	3.4	0.04	6.7	0.07
Heterokontophyta								
<i>Dictyopteris</i> spp.	20.5	0.8	23.3	0.6	10.3	0.1	23.3	0.4
<i>Dictyota</i> sp.	84.1	4.4	73.3	3.5	75.9	5	83.3	4.9
<i>Lobophora</i> sp.	15.9	0.4	16.7	0.3	24.1	0.5	30	1
<i>Sphacelaria</i> sp.	100	19.6	96.7	24.8	96.6	20.5	90	17
Animal material								
Amphipod	11.4	0.1	13.3	0.1	6.9	0.07	3.3	0.03
Eggs	15.9	0.6	10	1.2	3.4	0.3	10	0.2
Sponge	97.7	9.1	90	6.7	96.6	7.7	96.7	13.8
Unidentified	11.4	0.7	10	0.4	0	0	10	0.2
Other								
Unidentified material	2.3	0.35	10	1	0	0.09	3.3	0.2

Data are pooled from eight sites; the number of individual per species (*N*) and body size ranges (S.R., TL) are under the names

across multiple niche axes, which would increase the interactions among species and the likelihood that an individual of a rare species (*C. eibli* and *C. vrolikii*) will encounter, and form a harem with, an individual of another species. This is also supported by the fact that multiple species of angelfishes from the genus *Centropyge*, that coexist in overlapping territories, show no signs of competition (Hobbs et al. 2010). Interestingly, the hybrids also showed strong niche overlap with the three parent species, and across the 14 yr of surveys, the abundance of angelfish hybrids was consistently low. Thus, there would be a low probability of one hybrid encountering (and mating) with another hybrid. The hybrids are, therefore, likely to encounter, and form harems, with the parent species. Indeed, hybrids were always observed in harems with the parent species. This would give the hybrids the opportunity

to breed back with the parent species facilitating the transfer of DNA between different species (introgression). This would support previous genetic studies that found strong gene flow among these three angelfish species (DiBattista et al. 2012, 2016a). Given this overlap in habitat and diet between these three species of angelfishes, and their ability to overcome the reproductive barriers, it is interesting how these three species still exist and maintain their particular traits. This can probably be explained by the fact that all three species contain allopatric populations outside of this hybrid zone (i.e. populations where the other two species are not present). It is only in a narrow edge of their geographic range where they overlap and hybridise, and maintenance of the parent species may relate to recruitment of juveniles from the other areas.

This angelfish study system lends itself to further investigation into the process and outcomes of hybridisation in reef fishes. While previous studies in the marine environment have measured the fitness of hybrids in terms of their life span, growth rates, and reproductive output/success (Payet et al. 2016; Montanari et al. 2017), such studies are rare. Growing larger and/or faster for protogynous species influences the transition to males and therefore determines its social rank within a harem and whether it reproduces as a male or female. Thus, determining which species (or hybrid) is rank one (i.e. the male) in the harem will determine their reproductive potential. At this stage, no data are available on the fitness on these study species and hybrids; thus, further investigation is needed to understand possible consequences of hybridisation.

The advancement of molecular techniques has rapidly increased the number of coral-reef fishes implicated in hybridisation. While genetic studies are useful for confirming hybrids and investigating the consequences of hybridisation, determining the causes of hybridisation requires ecological and behavioural studies (Montanari et al. 2016). In this study of angelfishes, field surveys (14 yr for abundances) were used to examine a range of ecological factors, including rarity of parent species, habitat and dietary overlap, and showed how these factors are consistently important in facilitating the formation of mixed species harems that lead to interbreeding. The multifaceted approach used in this study highlights the value in combining field surveys, behavioural observations, and laboratory analyses in identifying factors that contribute to the formation of hybrids. Furthermore, these kinds of studies can help determine the barriers to reproductive isolation that are crucial to understanding speciation in reef fishes.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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