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Selection of a spawning aggregation site by *Chromis hypsilepis* (Pisces: Pomacentridae): habitat structure, transport potential, and food availability

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ABSTRACT: Spawning aggregations form when fishes migrate to a site from their normal feeding grounds and form temporary groupings for breeding. Spawning aggregation sites are spatially rare, and demonstrating differences between a spawning aggregation site and unselected sites nearby is the first step towards understanding the benefits provided by the aggregation site. *Chromis hypsilepis* (Pomacentridae) is a demersally spawning reef fish, one population of which reproduces in a large, single aggregation at a rocky reef off the central coast of New South Wales, Australia. This study compared the habitat structure (rugosity, reef slope, substratum particle size, and abundance of preferred spawning microhabitat) of the spawning aggregation site and several non-spawning sites, and tested the hypotheses that the spawning aggregation site provided greater off-reef larval transport and prey availability for brooding males. Substratum rugosity was significantly greater and the preferred spawning microhabitat was significantly more abundant at the spawning aggregation site. Reef relief and substratum particle size were not significantly different from non-spawning sites. Passively drifting surface current drogues released at the spawning aggregation site were more rapidly transported off the reef, but did not travel further or faster, than drogues released at a non-spawning site over a 12 h period. Biomass of the preferred prey (copepods of 0.441 to 1.49 mm equivalent spherical diameter) was not significantly greater, but was less variable, at the spawning aggregation site.

KEY WORDS: Habitat preference · Larval dispersal · Reef fish reproduction · Spawning aggregation · Spawning site selection · Temperate reef fish

INTRODUCTION

Spawning aggregations occur where fishes form temporary aggregations for breeding after migrating from their normal feeding grounds (Domeier & Colin 1997, Claydon 2004). Spawning aggregations form on a daily, semi-lunar, lunar, or annual cycle and endure for periods of 1 to 2 h (for daily spawners) to 7 d (for species spawning in a single annual spawning event). At least 119 species (18 families) of reef fishes are known to form spawning aggregations, most belonging to the families Serranidae, Lutjanidae, Acanthuridae, Scaridae, and Siganidae (Cornish 2005). Most aggregative species are pelagic spawners (but see Gladstone 1994, 2007). Sites where spawning aggregations occur are spatially rare (in comparison to the total amount of reef habitat) and are often used by many different species over many successive spawning seasons (Domeier & Colin 1997, Claydon 2004). Species that spawn at aggregation sites reach them after migrating considerable distances (in relation to their body length) that range from hundreds of metres to hundreds of kilometres (Bell & Colin 1986, Sadovy et al. 1994). These observations suggest that the act of aggregating, or the aggregation site, may provide benefits to the spawners and/or their propagules that outweigh costs involved in migrating to the aggregation site.

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Aggregation sites may provide a number of benefits to the spawners and/or their offspring. For example, distinctive physical structures at these sites may act as a visual signal to assemble individuals that are widely dispersed outside the spawning season (Moyer & Zaiser 1981). Aggregation sites may provide a reduced risk of predation for spawners because of some unique habitat attributes (Sancho et al. 2000a). The most widely applied hypothesis for the selection of spawning sites by aggregative and non-aggregative species is that currents at the site favour the rapid transport away from the reef of newly fertilized eggs and hatching larvae (the off-reef transport hypothesis). Rapid off-reef transport may be beneficial to eggs and larvae because it: (1) reduces the risk of predation by reef-based planktivorous fishes and invertebrates (Johannes 1978), (2) facilitates widespread dispersal and therefore increases the likelihood of settlers locating another reef (Barlow 1981), or (3) delivers them into eddies that enhance retention within the vicinity of the natal reef until larvae are competent to settle (Lobel & Robinson 1988). Although the off-reef transport hypothesis was developed largely from observation of pelagic spawners, it is also applicable to demersal spawners that deposit their eggs in nests at positions on a reef that may favour the dispersal of hatching larvae (Gladstone 1994).

Observations that suggest off-reef transport may be an important factor in spawning site selection include (1) frequent formations of aggregations in areas of reefs that are well flushed by currents, or on the downcurrent edges of reefs, (2) frequent spawning on a falling tide, and (3) the correlation between slight changes in the position of spawners in an aggregation site and changes in current patterns (Johannes 1978, Samoilys 1997). Surprisingly, there are few tests of the off-reef transport hypothesis (but see Appeldoorn et al. 1994 and Hensley et al. 1994). In one study, dye released at spawning and non-spawning sites differed in some variables (initial speed and depth) but differences were not consistent between reefs of different sizes (Hensley et al. 1994). Thus, there is a clear need for further tests of this hypothesis.

Fishes aggregating for spawning may incur several energetic costs arising from migration to and from the spawning site, deferred feeding opportunities while migrating, territorial defense, courtship, and spawning. For example, the feeding rates of demersal spawners, while engaged in brooding activities, are reduced by 24 to 85% (Robertson et al. 1990, Gladstone 2007). Zooplankton abundance around reefs is spatially and temporally variable and planktivorous fishes will migrate to areas where zooplankton density is greater (Hobson 1991). It is therefore possible that the energetic costs of broodcare to a planktivorous species will be minimised if males select sites with predictably higher prey availability. Thus, spawning aggregations may form where prey availability is high as males collectively attempt to reduce the overall energetic costs of broodcare.

This study was undertaken to test hypotheses that have been proposed to explain the selection of spawning aggregation sites on the basis of differences in habitat structure (e.g. visual cues, reduced predation), off-reef dispersal, and energetic costs of broodcare. The aims of this study were: (1) to compare attributes of habitat structure between a spawning aggregation site and several non-spawning sites, (2) to test the hypothesis that larvae hatching at a spawning aggregation site are more quickly transported from the reef than larvae hatching at non-spawning sites (using drogues as a proxy for newly hatched larvae), and (3) to test the hypothesis that a spawning aggregation site provides a greater biomass of prey for the brooding males than other sites.

MATERIALS AND METHODS

Study species. *Chromis hypsilepis* (Pomacentridae) is a planktivorous damselfish (maximum length 120 mm) occurring on rocky reefs off southeastern Australia, Lord Howe and Norfolk Islands (between Australia and New Zealand), and northern New Zealand (Kuiter 2000). It is most abundant on rocky reefs off New South Wales, Australia, where it feeds from mid-water to just below the surface in schools of hundreds to thousands of individuals (author's pers. obs.). *C. hypsilepis* forms spawning aggregations of between 3500 and 33 000 ind. on a semi-lunar cycle from September to February. Spawning occurs demersally in temporary individual territories established by males at the spawning aggregation site, and is followed by a 4.5 d period of paternal brood care. The feeding rates of males that are brooding developing embryos are reduced by 85% compared with non-spawning periods (Gladstone 2007).

Study area. This study was conducted at Terrigal reef, New South Wales, Australia (33° 27′ 00″ S, 151° 26′ 00″ E) (Fig. 1). Terrigal reef fringes a coastal headland for a distance of 2.2 km. Surveys of the entire reef during 2 spawning cycles in 2004 to 2005 and 2005 to 2006 spawning seasons confirmed that *Chromis hypsilepis* at Terrigal reef spawned only in an area of 1250 m^2 in the sea urchin-barrens habitat at the western extremity of the reef at 7 to 10 m depth (hereafter called the spawning aggregation site). The 7 non-spawning sites that were compared to the spawning aggregation site were located along the northern and southern edges of Terrigal reef and were selected

because of the availability of sea urchin-barrens habitat in the same depth range as the spawning aggregation site.

Habitat attributes. Habitat attributes (rugosity, relief, substratum composition, abundance of preferred spawning microhabitat) were compared between the spawning aggregation site and 7 sites on Terrigal reef where spawning did not occur during a spawning cycle in November 2004 (Fig. 1). Rugosity was estimated by the 'chain-and-tape' method (Risk 1972). A 12.5 m length of chain (link size 10 mm) was laid to follow the contours of the substratum by draping it over the upper surfaces of boulders and pushing it into holes and crevices. After the chain was laid in this way, the straight line distance between the beginning and end of the chain was measured (to the nearest cm), and the ratio of chain length to linear distance used as a measure of substratum rugosity. Five replicates were done in each site. The relief of the reef slope was measured by laying a 10 m tape measure down the reef slope from a depth of 7 m, which is the depth at the shallow margin of the spawning aggregation site. The tape measure was pulled tight and positioned so that it followed the reef slope. Depth (to nearest 0.1 m) at the end of the tape measure was recorded and, after adjusting depths by the stages of the tidal cycle, a measure of relief was calculated from the formula (shallow depth – deeper depth)/10. Five measurements, haphazardly located and separated by ≥15 m, were made in each site.

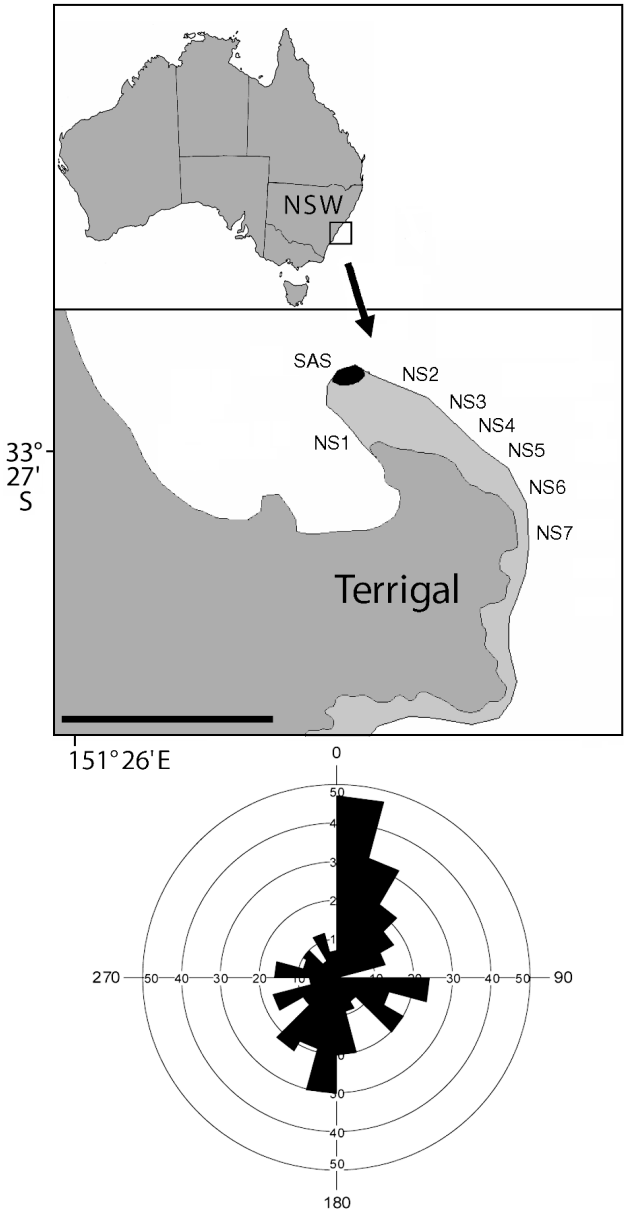

Fig. 1. *Chromis hypsilepis*. Location of study site in Australia, and positions of spawning aggregation site (SAS, black) and non-spawning sites (NS1–NS7). Light grey shaded area: reef (scale bar = 800 m). Wind rose: wind directions at the times eggs hatched in the 2005/2006 spawning season (0 north, 90 east, 180 south, 270 west). Wind direction recorded at Norah Head, 22 km north of study site (source: www.bom.gov.au). NSW = New South Wales

The significance of the differences in mean values of rugosity and relief between the spawning aggregation site and the 7 non-spawning sites were tested by asymmetrical analysis of variance (ANOVA) (Underwood 1992). The asymmetrical ANOVA was performed in 3 steps: (1) a 1-way ANOVA was done with no distinction between the spawning aggregation site and non-spawning sites to obtain a value of sum of squares (SS) for the comparison of all sites; (2) a second 1-way ANOVA was done using only the values for the non-spawning sites; (3) a value for the SS for the spawning aggregation site was obtained by subtracting the SS for the non-spawning sites from the SS for all sites. Degrees of freedom and mean square (MS) values were similarly derived. An *F*-value for the comparison of the spawning aggregation site and non-spawning sites was obtained by dividing MS mating area by MS non-mating area. When the test for non-spawning sites (Step 2) was not significant at $p > 0.25$, the SS for non-spawning sites was pooled with the SS residual and the corresponding MS value was used as the denominator in the test of the spawning aggregation site. The assumption of homogeneity of variances was tested by Cochran's *C*-test and, where necessary data were transformed. ANOVA was done with GMAV software (Institute of Marine Ecology, University of Sydney).

Substratum composition was quantified by laying a 20 m tape measure (n = 3 replicates) and assigning the substratum at each 1 m interval to one of the following 8 categories from a modified Wentworth scale: 8–16 mm (medium gravel); 16–32 mm (coarse gravel); 32–64 mm (very coarse gravel); 64–256 mm (cobble);

256–1000 mm (boulder); 1000–3000 mm (large boulder); >3000 mm (very large boulder); bedrock (Folk 1980). The substratum was assigned to one of the size categories by divers using a length of PVC conduit tubing marked to represent each length category. The complete data set of occurrence of each substratum size category in each replicate transect was analysed (without transformation) as a multivariate data set. Principal Components Analysis (PCA) was used to determine whether the spawning aggregation site could be distinguished from the non-spawning sites on the basis of a particular substratum size. The data for each substratum size category in each replicate transect were its total occurrence. The multivariate set of substratum sizes of each transect was displayed on an ordination of the first 2 principal components. PCA was undertaken with PRIMER5 software (Primer-E). The hypothesis that the multivariate set of substratum sizes of the spawning aggregation site differed significantly from the non-spawning sites was tested with permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001), using the same logic as asymmetrical ANOVA. Each factor in the analysis was tested individually against its appropriate denominator with DISTLM v.5 (Anderson 2004) using 9999 permutations of the appropriate permutable units. Euclidean distance was used as the measure of dissimilarity in the PCA and PERMANOVA analyses.

Preliminary observations revealed that 5 microhabitats were utilized for spawning (Fig. 2a). The availability of each microhabitat in the spawning aggregation site was quantified by laying replicate 50 m tape measures (n = 3) along the substratum and recording the microhabitat type present below the tape measure at 1 m intervals. The proportional availability of each microhabitat was the total number of occurrences of each microhabitat expressed as a proportion of the total number of occurrences of all microhabitats. The proportional use of each microhabitat for spawning was determined from the positions of all egg clutches recorded during surveys of 50 × 1 m transects (n = 3) in the spawning aggregation site in November 2004. A total of 120 egg clutches was observed. The preference of *Chromis hypsilepis* for spawning on each microhabitat was tested with the formula $\hat{w}_i = o_i / \pi_i$, where o_i is the proportional use of microhabitat type i, π_i is the proportional availability of microhabitat type i, and $\hat{w}_i$ is the preference score for microhabitat type i (Manly et al. 1993). Ninety-five CIs for $\hat{w}_i$ were used to determine the statistical significance of preference scores. When the upper confidence interval was <1, the microhabitat was significantly avoided. When the confidence interval fell between <1 and >1, the microhabitat was used in proportion to its availability (i.e. no preference or avoidance was exhibited). When the lower confidence interval was >1 the microhabitat was significantly preferred.

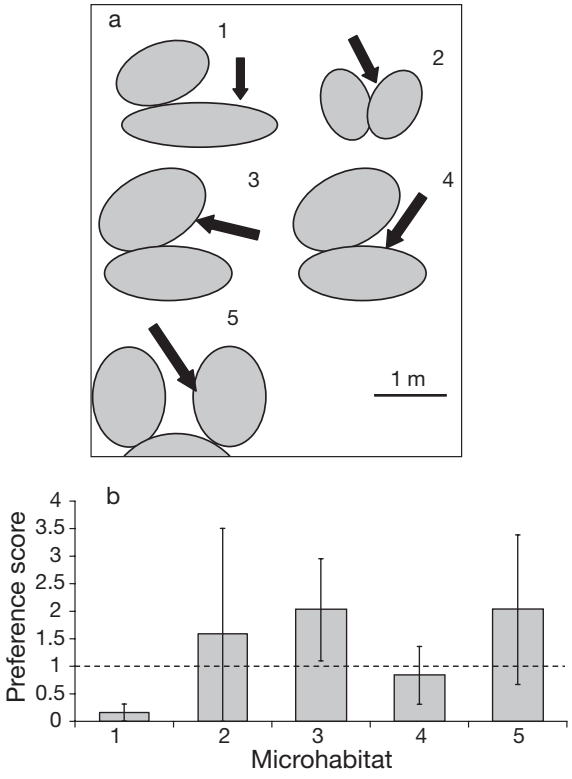

Fig. 2. *Chromis hypsilepis*. (a) Diagrammatic representations of microhabitats used for spawning. Shaded shapes: rocks, arrows: the positions where egg clutches were deposited. (1) Exposed flat rock surface <1 m from the nearest rock; (2) V-shaped crevice between 2 rocks and within 0.3 m of the base of the crevice; (3) underside of an over-hanging boulder; (4) ledge immediately below an over-hanging boulder; (5) vertical wall of a boulder <1 m from the nearest vertical boulder wall. (b) Preference scores (±95% CIs) for spawning on different microhabitats. Preference scores with a lower confidence limit >1 (shown by the dashed line) indicate a significant preference for that microhabitat. The preferred spawning microhabitat is (3)

The abundance of the preferred spawning microhabitat was then determined in the 7 non-spawning sites using the same method that had been employed in the spawning aggregation site. The significance of the difference in mean number of preferred microhabitats between the spawning aggregation site and the 7 non-spawning sites was tested by asymmetrical ANOVA (as described previously).

Off-reef transport. Surface current drogues with portable Global Positioning System (GPS) receivers (hereafter called 'drifters') (Austin & Atkinson 2004) were used to track the movement of surface water. The drifters were constructed of PVC pipe and consisted of 4 rectangular panels (160 × 280 mm, 3 mm thick) per-

pendicular to one another that were attached to a cylindrical watertight housing (100 mm width, 300 mm length) that contained a Garmin eTrex GPS unit. Each drifter was weighted so that only 8 cm of the cylindrical housing protruded above the water's surface. Field experiments with this design has shown that drifters are unaffected by wind and their tracks follow the movement of surface water (Austin & Atkinson 2004).

Two drifters were deployed simultaneously at the spawning aggregation site (SAS; see Fig. 1) and a non-spawning site (NS6; see Fig. 1) in February and March 2006 and allowed to drift for 12 h. The February deployment coincided with a spawning aggregation on days when eggs were hatching (based on underwater observations of the spawning aggregation site). The March deployment was done after spawning had concluded to test whether the spawning aggregation site was consistently different from the non-spawning site (Appeldoorn et al. 1994). Deployment began at 06:30 h because of the practical difficulties of tracking the drifters at night. Although the period of deployment differed from the actual times of hatching (22:00 to 02:00 h, Gladstone 2007), the tide and wind conditions experienced by the drifters were within the range of those experienced by hatching larvae. The most frequent wind directions at the times of hatching were approximately northeast and southeast (Fig. 1). On the first day of deployment, high tides occurred at 03:30 and 16:27 h, and winds varied between southeasterly (33 km h^{-1}) and south-southeasterly (22 km h^{-1}). On the second day of deployment, high tides occurred at 10:46 and 23:14 h, and winds varied between east-southeasterly (26 km h^{-1}) and southeasterly (22 km h^{-1}).

The drifters logged their positions at approximately 1 min intervals. Three variables were quantified: (1) the average speed of each drifter in each 5 min interval over the duration of the deployment, (2) the time spent by each drifter above reef; and (3) the straight line distance between the final position of each drifter and its starting point. The time spent by each drifter above reef is assumed to be a measure of the risk of predation by reef-based planktivorous fishes on newly hatched larvae. The third variable is a measure of the dispersal distance of newly hatched larvae over the time of the deployment. Two-way ANOVA was used to test the significance of differences between locations (spawning versus non-spawning) and times (time 1 versus time 2) in the mean time spent by the drifters over reef and the mean dispersal distance. Paired *t*-tests were used to test the null hypothesis that the average difference in speed of the drifters released at the spawning aggregation site and the non-spawning site was zero.

Food availability. Twenty *Chromis hypsilepis* specimens of both sexes and in the same size range as reproductively active adults (170 to 190 mm total length) were collected in January 2006 for gut content analysis. Specimens were collected during a non-spawning period, because the low feeding rate of brooding males (Gladstone 2007) suggested that sufficient amounts of gut contents would not be obtainable for an analysis of diet. I therefore assumed that the diet of non-spawning individuals was similar to brooding males. Specimens were frozen at –20°C within 2 h of collection. All prey items were removed from the stomach and intestine of each fish and identified to a coarse taxonomic level (e.g. copepod, ostracod, polychaete). The maximum length and width of each intact prey item (n = 291) was measured, and the equivalent spherical diameter (ESD) was calculated (Mustard & Anderson 2005). Prey items were assigned to one of 23 ESD bin sizes that ranged from 0.225–0.256 mm (smallest bin) to 2.304–2.401 mm (largest bin). Ninety-five percent of prey items occurred between bins 0.441–0.484 mm and 1.764–1.849 mm.

The densities of plankton at the spawning aggregation site and at 3 non-spawning sites (NS1, NS3 and NS6 in Fig. 1) were quantified by sampling with plankton nets (23.5 cm diameter net, 100 µm mesh, n = 3 replicates) propelled separately by divers above a 100 m tape measure laid over the substratum. Each diver swam with the net held forwards to minimize the likelihood of the divers' presence causing plankton avoidance behaviour. Replicate samples were separated by approximately 5 m. Sampling occurred 1 m above the substratum (equivalent to a depth of 6 to 7 m), corresponding to the distance above the substratum at which male *Chromis hypsilepis* feed while brooding eggs (Gladstone 2007). To account for the anticipated temporal variation in plankton abundance, sampling occurred at different times on 2 successive days in each of 2 periods (brooding, non-brooding) in January to February 2006. The order in which sites were sampled was randomized on each sampling day, and sampling of the 4 sites on each day was completed within 1.5 h. Samples were immediately fixed in 10% formalin in seawater.

Particle sizes (in ESD) of preserved plankton samples were analyzed with an optical plankton counter (OPC) using the same bin sizes that were used for the analysis of gut contents. The biomass of each bin size was determined by calculating the volume of each bin size (using the geometric mean ESD) and assuming a density of 1000 kg m^{-3} (Suthers et al. 2004). Detritus occurred in all plankton samples in approximately equal amounts and accounted for only ~5% of total sample volume. Total biomass of each sample was the sum of the biomass of particles in each bin size for the bin sizes representing 95% of the gut contents of *Chromis hypsilepis*. The significance of the difference

in total biomass of plankton between the spawning aggregation site and the 3 non-spawning sites was tested by asymmetrical ANOVA.

RESULTS

Habitat attributes

Mean (±SE) substratum rugosity of the spawning aggregation site (1.9 ± 0.07) was significantly greater than the mean of the non-spawning sites (1.5 ± 0.06) (Fig. 3a, Table 1). There was no significant variation in rugosity of the non-spawning sites. The reef slope (Fig. 3b) changed from low relief on the northern edge of Terrigal reef (between NS7 and NS2 in Fig. 1) to a moderate relief at the spawning aggregation site, then

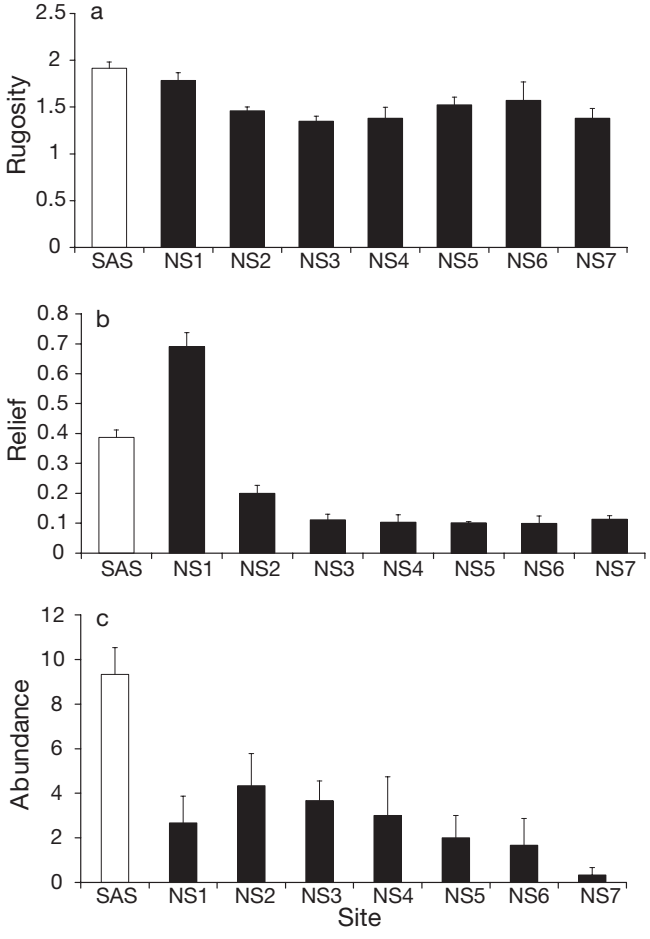

Fig. 3. *Chromis hypsilepis*. Habitat attributes of the spawning aggregation site (SAS) and non-spawning sites (NS1–NS7). Values shown are means + SE for (a) rugosity (n = 5), (b) relief of reef slope (n = 5), and (c) abundance of the preferred spawning microhabitat (the underside of an over-hanging boulder; n = 3)

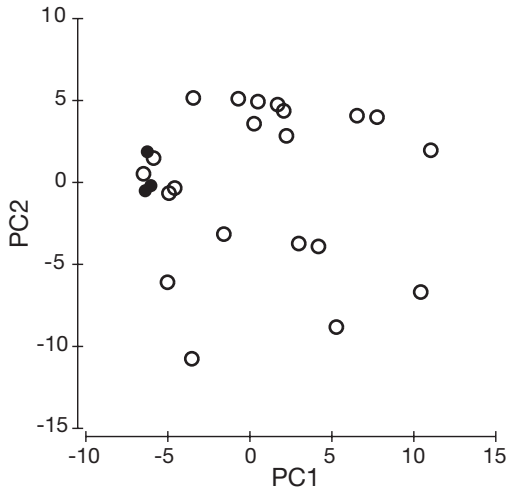

Fig. 4. *Chromis hypsilepis*. Principal component analysis (PCA) biplot showing substratum categories explaining most variation in substratum composition between the spawning aggregation site (●) and the non-spawning sites (○). The PC1 axis represents a gradient of increasing occurrence of very large boulders (>3000 mm) and the PC2 axis represents a gradient of increasing occurrence of large boulders (1000–3000 mm)

to a high relief on the southern, sheltered section of the reef (NS1). The spawning aggregation site was therefore part of a steeper section of the reef at its western extremity that included SAS and NS1. However, the mean relief of the spawning aggregation site (0.4 ± 0.02) was not significantly different from the mean relief of the non-spawning sites (0.2 ± 0.08) (Fig. 3b, Table 1).

The 3 substratum sizes present in the spawning aggregation site were: large boulders, occurring at 63.3% of all points sampled; boulders, 33.3%; and cobbles, 3.4%. The substratum sizes that were most abundant in the non-spawning sites were: large boulders, 42.7%; very large boulders, 22.6%; and boulders, 18.9%. Bedrock occurred at 11.6% of all points sampled. The least abundant substratum sizes were: cobble, 2.8%; very coarse gravel, 1.2%; coarse gravel, 0%; and medium gravel, 0.2%.

The PCA biplot indicates that the multivariate set of substratum sizes at the spawning aggregation site was not distinctly different from the substratum sizes at the non-spawning sites, shown by the overlap with all replicate transects from NS2 and 1 transect from NS1 (Fig. 4). The PC1 axis represents a gradient of increasing occurrence of very large boulders and the PC2 axis represents a gradient of increasing occurrence of large boulders (Table 2). The spawning aggregation site had a low occurrence of very large boulders and high occurrence of large boulders. The PC1 and PC2 axes together accounted for 76.0% of total variation. The variation in substratum sizes at the spawning aggrega-

Table 1. Asymmetrical ANOVA comparing habitat attributes of the spawning aggregation site (SAS) and non-spawning sites (NS). MS_{denom} = term for the denominator mean square in the calculation of *F*-ratios. The pooled SS for NS and Residual (Res) NS was used as the denominator MS when NS was not significantly different at $p > 0.25$

Source of variation	df	SS	MS	*F*	p	MS_{denom}
Rugosity[a]						
Site	7	1.48	0.21			
SAS	1	0.78	0.78	6.50	0.04	NS
NS	6	0.70	0.12	1.98	0.11	Res NS
Residual	32	1.73	0.05			
Res SAS	4	0.09	0.02			
Res NS	28	1.64	0.06			
Relief[b]						
Site	7	0.29	0.04			
SAS	1	0.03	0.03	0.75	0.42	NS
NS	6	0.26	0.04	70.95	<0.001	Res NS
Residual	32	0.02	0.0006			
Res SAS	4	0.002	0.0003			
Res NS	28	0.02	0.0006			
Abundance of preferred spawning microhabitat[c]						
Site	7	153.63	21.95	5.16	0.003	
SAS	1	121.73	121.73	12.73	0.002	NS + Res NS
NS	6	31.90	5.32	1.25	0.34	Res NS
Residual	16	68.00	4.25			
Res SAS	2	8.67	4.33			
Res NS	14	59.33	4.24			

[a]Untransformed, variances heterogeneous
[b]$\sqrt{x + 1}$ transformed, variances homogeneous
[c]Untransformed, variances homogeneous

tion site was within the range of variation of substratum sizes at the non-spawning sites, as shown by the closeness of the replicate transects on the PCA biplot (Fig. 4). Asymmetrical PERMANOVA confirmed that the multivariate set of substratum sizes at the spawning aggregation site was not significantly different from the non-spawning sites (shown by the non-significant *F*-ratio for SAS versus NS in Table 3).

Microhabitats utilized for spawning were as follows: exposed flat rock surface <1 m from the nearest rock (6% of all egg clutches), a crevice between 2 rocks and within 0.3 m of the base of the crevice (8%), the underside of an over-hanging boulder (42%), a ledge immediately below an over-hanging boulder (18%), and the vertical wall of a boulder that was <1 m from the nearest vertical boulder wall (26%). The relative availabilities of spawning microhabitats in the spawning aggregation site were: exposed flat rock surface <1 m from the nearest rock (occurring at 38% of all points sampled), a crevice between 2 rocks and within 0.3 m of the base of the crevice (5%), the underside of an over-hanging boulder (22%), a ledge immediately below an over-hanging boulder (22%), and the vertical wall of a boulder that was <1m from the nearest vertical boulder wall (13%).

Chromis hypsilepis exhibited a significant preference for spawning on the undersides of over-hanging boulders (Fig. 2b). The fish avoided spawning on exposed flat rock surface <1 m from the nearest rock and showed no preference for crevices, ledges immediately below an over-hanging boulder, or vertical walls of boulders that were <1 m from the nearest vertical boulder wall. The undersides of over-hanging boulders (the significantly preferred spawning microhabitat) were significantly more abundant in the spawning aggregation site (9.3 ± 1.2 per 50 m, SE) than non-spawning sites (2.5 ± 0.5 per 50 m, SE) (Fig. 3c, Table 1).

Off-reef transport

On the first day of deployment, the 2 drifters released at the spawning aggregation site immediately started to drift in a northwesterly direction and passed across the edge of the reef and into open water after 30.5 ± 2.5 min (SE) (Fig. 5a). The 2 drifters released at the non-spawning site moved in a circular path (of approximately 65 m diameter) for 94 to 96 min before drifting in a northwesterly direction over the reef, and eventually drifted across the edge of the reef and into open water 211.5 ± 7.5 min after deployment (Fig. 5a). On the second day of deployment, the 2 drifters released at the spawning aggregation site left the reef after 18.0 ± 2.0 min and drifted in a northeasterly direction for approximately

Table 2. *Chromis hypsilepis.* Summary of principal component axis loadings (only the first 2 PCs are shown). Components with an absolute value of >0.5 (**bold**) are important (Tabachnick & Fidell 2001)

Variable (mm)	PC1	PC2
8–16	0.011	0.008
16–32	0	0
32–64	−0.011	−0.078
64–260	−0.068	−0.122
260–1000	−0.416	−0.434
1000–3000	**−0.526**	**0.599**
>3000	**0.671**	0.419
Bedrock	0.309	**−0.506**
Cumulative % total variation explained	44.2	76.0

Table 3. PERMANOVA for asymmetrical comparison of substratum composition (8 categories, no transformation) between spawning aggregation site (SAS) and 7 non-spawning sites (NS). Measure of dissimilarity used was Euclidean distance. p-values were obtained from 9999 Monte Carlo samples from the asymptotic permutation distribution. MS_{denom} = term used for the denominator mean square in calculation of *F*-ratios

Source of variation	df	SS	MS	*F*	p	MS_{denom}	Permutable units
Site	7	1141.66	163.09				
SAS	1	133.76	133.76	0.79	0.51	NS	8 Site cells
NS	6	1007.90	167.98	5.73	0.0002	Res NS	21 Raw data units
Residual	16	418.00	26.12				
Res SAS	2	8.00	4.00				
Res NS	14	410.00	28.29				

120 min, then drifted back towards the coast and followed it until the end of the deployment (Fig. 5b). The 2 drifters released from the non-spawning site drifted initially in a circular path for 12 to 43 min before each drifted separately in a northwesterly direction over the reef. Both drifters moved southwards over the reef and into the shallow sheltered bay on the southern side of Terrigal reef, where they each circled for periods of 297 and 410 min, respectively. After leaving the bay, each drifter moved northeastwards over the reef and eventually passed across the reef edge. Excluding the time spent circling in the sheltered bay (where the substratum consisted of sand and seagrass), the 2 drifters deployed from the non-spawning site spent an average of 172.5 ± 43.5 min over the rocky reef (Fig. 5b).

The drifters released at the spawning aggregation site spent significantly less time over the reef than the drifters released from the non-spawning site (Fig. 6a, Table 4). There was no significant difference in the total dispersal distance of the drifters released from the spawning aggregation site (780.0 ± 55.0 m) and the non-spawning site (997.5 ± 47.5 m) (Fig. 6b, Table 4).

The average difference in speed between the 2 sets of drifters was not significantly different from zero (t = 0.55, p = 0.58) on the first day of deployment. The average speeds of the drifters released from the spawning aggregation site (0.54 ± 0.04 km h^{-1}) and the non-spawning site (0.37 ± 0.02 km h^{-1}) differed significantly from zero (t = –4.06, p < 0.001) on the second day of deployment. The baseline speed of the 2 sets of drifters was similar, but the drifters released at the spawning aggregation site had periods of much higher speed.

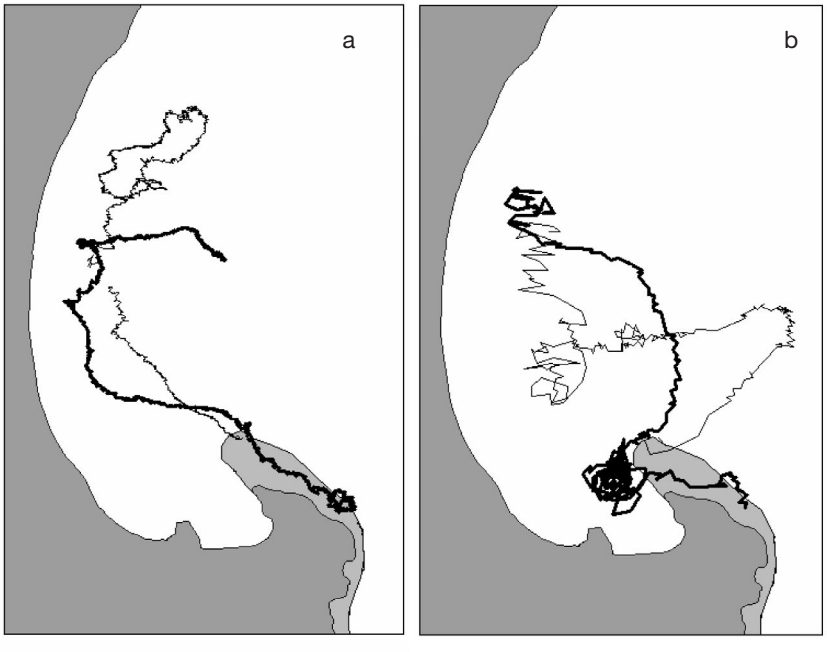

Fig. 5. *Chromis hypsilepis*. Tracks of drifters deployed on (a) 23 February 2006 and (b) 3 March 2006 from spawning aggregation site (light track) and non-spawning site (heavy track). Drifters were deployed for 12 h. Two drifters were deployed simultaneously from each site on each day, but only tracks of single drifters are shown, because deployed drifters remained close together throughout the deployment

Food availability

The dietary items collected from *Chromis hypsilepis* (percentages given below as: abundance of all dietary items, total biomass of identifiable dietary items, and presence in fish stomachs, respectively) included copepods (95.6, 88.1, 90%), fish eggs (1.9, 9.3, 20%), mysids (1.3, 1.2, 10%), amphipods (0.6, 0.6, 10%), polychaetes (0.3, 0.7, 20%) and ostracods (0.3, 0.1, 10%). Only 10 fish contained intact and identifiable prey items. The remaining fish contained

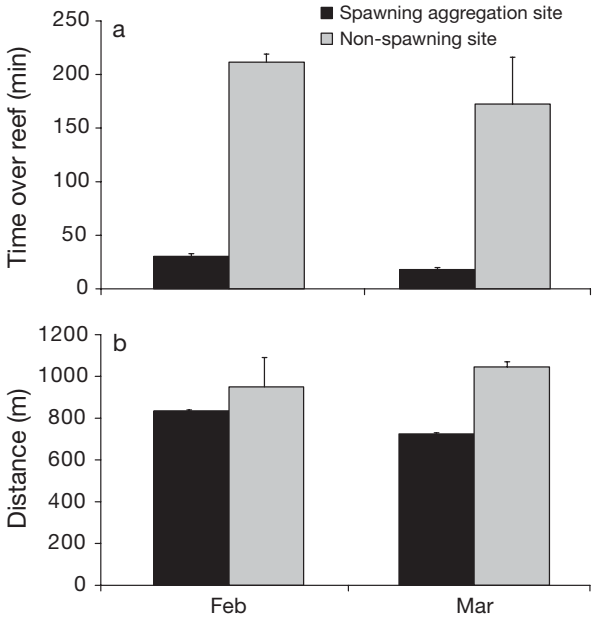

Fig. 6. *Chromis hypsilepis*. (a) Time spent by drifters over reef following their simultaneous deployment from spawning aggregation site and a non-spawning site for 12 h in Feb and Mar 2006, and (b) distance of drifters from their release position. Data are mean + SE (n = 2)

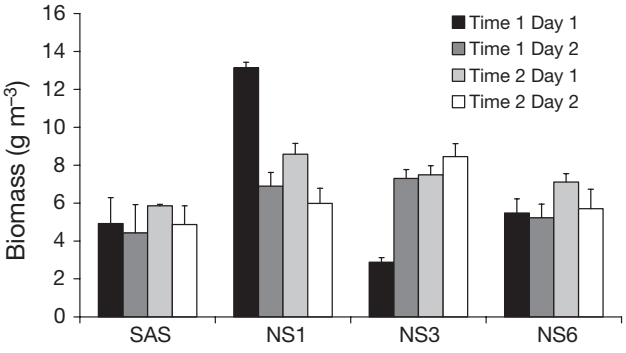

Fig. 7. *Chromis hypsilepis*. Mean biomass (+SE, n = 3) of plankton of equivalent spherical diameter 0.441 to 1.849 mm at the spawning aggregation site (SAS) and 3 non-spawning sites (NS1, NS3, NS6; see Fig. 1) on each of 2 days (Day 1, Day 2) within each of 2 times

only completely digested material (2 fish), completely digested material mixed with fragments of copepods (5 fish), polychaetes (1 fish), or unidentifiable crustaceans (2 fish).

The biomass of plankton at the study sites ranged from 2886 ± 234.7 mg m^{-3} (NS3 on Day 1) to 13146.6 ± 280.7 mg m^{-3} (NS1 on Day 1) (Fig. 7). The biomass of plankton at the non-spawning sites varied significantly between days within each sampling period (the significant D(T) × NS interaction in Table 5). The significant interaction occurred because plankton biomass varied significantly between non-spawning sites on Day 1 of Time 1 and Day 2 of Time 2, but not on other days. The biomass of plankton at the spawning aggregation site did not vary between days in each time or between the 2 times of sampling. The biomass of plankton at the spawning aggregation site (5019.6 ± 299.9 mg m^{-3}) did not significantly differ from the biomass of plankton at the non-spawning sites (7022.5 ± 716.8 mg m^{-3}) (Table 5).

DISCUSSION

Habitat variation

Habitat variation is an important determinant of the spatial patterns in distribution, abundance and diversity of reef fishes (Caselle & Warner 1996, Friedlander & Parrish 1998) and of variation in reproductive success (Kroon et al. 2000). It is likely that the habitat requirements of fish for reproduction may be one of the factors underlying the selection of spawning aggregation sites. Reproduction by *Chromis hypsilepis* involves a major transition from the pelagic habitat (used for feeding between spawning cycles and during the non-reproductive times of the year) to a benthic habitat for spawning and brood care. The habitat

Table 4. Two-way ANOVA of the effects of Time (Day 1 vs. Day 2) and Site (spawning aggregation site vs. non-spawning site) on the time spent by drifters over reef and their maximum dispersal distance after 12 h

Source of variation	df	Time over reef[a]			Dispersal distance[b]		
		MS	*F*	p	MS	*F*	p
Time	1	0.29	6.76	0.06	112.50	0.01	0.95
Site	1	8.71	201.23	0.0001[c]	94612.50	4.50	0.28
Time × Site	1	0.04	1.00	0.37	21012.50	2.07	0.22
Residual	4	0.04			10137.50		

[a] ln-transformed, variances homogeneous
[b] Untransformed, variances heterogeneous
[c] With Time × Site eliminated, because p > 0.25, and Residual MS used as the MS denominator

Table 5. Asymmetrical analysis of variance of the biomass of plankton in the spawning aggregation site (SAS) and non-spawning sites (NS) (untransformed data, Cochran's $C = 0.22$, $p > 0.05$). MS_{denom} is the denominator MS used to calculate the *F*-ratio. Plankton sampling was done over 2 Times (T; 6 d apart), on 2 Days (D) within each Time, at 4 Sites (1 SAS and 3 NS), and 3 replicate samples were collected

Source of variation	df	SS	MS	*F*	p	MS_{denom}
T	1	2668344.89	2668344.89			
D(T)	2	8541928.72	4270964.36			
Site = S	3	86594429.27	28864809.76			
SAS vs. NS	1	36103133.14	36103133.14	1.43	0.35	NS
NS	2	50491296.13	25245648.07	1.02	0.49	T × NS
T × S	3	49504815.06	16501605.02	1.04	0.44	D(T) × S
T × SAS vs. NS	1	185797.95	185797.95	0.05	0.83	D(T) × SAS vs. NS + Res SAS[a]
T × NS	2	49319017.11	24659508.55	1.03	0.43	D(T) × NS
D(T) × S	6	95519897.34	15919982.89	8.57	<0.001	Res
D(T) × SAS vs. NS	2	48801.98	24400.99	0.006	0.99	Res SAS vs. NS
D(T) × NS	4	95471095.36	23867773.84	19.70	<0.001	Res NS
Residual = Res	32	59424808.32	1857025.26			
Res SAS	8	30353047.79	3794130.97			
Res NS	24	29071760.53	1211323.36			
Total	47	302254223.6				

[a]Tested against pooled D(T) × SAS vs. NS + Res SAS, because D(T) × SAS vs. NS was eliminated (non-significant at $p > 0.25$)

structure of the spawning aggregation site used by *C. hypsilepis* was distinctive in its rugosity and in the availability of the preferred spawning micro-habitat. Rugosity of the reef substratum at the spawning aggregation site was significantly greater than non-spawning sites. Rugosity is a measure of the topographical complexity of the substratum and increases in rugosity, when quantified as in this study, are associated with increased biomass, abundance and species richness of other reef fish assemblages (Friedlander & Parrish 1998).

The greater rugosity of the spawning aggregation site used by *Chromis hypsilepis* may have 2 adaptive explanations. The first hypothesis is that greater rugosity reduces the risks of predation on spawning adults, brooding males or developing embryos. The potential mechanisms for this benefit are that greater rugosity reduces the visibility of *C. hypsilepis* to potential predators, provides more escape sites, or restricts the access of predators. There is sufficient evidence from other studies to indicate that this is a plausible hypothesis. For example, reproduction is often associated with an increased risk of predation (Sih 1994). Spawning fishes have a greater susceptibility to predation because their attention to courtship and spawning activities can reduce their awareness of potential predators (Candolin & Voigt 1998, Sancho 2000). Increased habitat rugosity reduces the risk of predation on fishes (Hixon & Beets 1993), and other studies have shown a relationship between increased rugosity and spawning site selection (Sabo & Orth 1994, Sancho et al. 2000a).

Chromis hypsilepis preferred a specific microhabitat (under ledges) for spawning, and this micro-habitat was more abundant at the spawning aggregation site than at non-spawning sites (although it was not the most abundant microhabitat at the spawning aggregation site). The preferred microhabitat was also available at other sites on Terrigal reef; however, its availability may be too low to allow the formation of spawning aggregations. This is the first demonstration that a physical resource preferred for reproduction is more available at a reef fish spawning aggregation site than at other reef sites. Determining the functional basis for the preferred spawning microhabitat was not one of the aims of this study; however, possible reasons for this preference could relate to the survival of developing embryos. For example, the position of clutches on the undersides of ledges may reduce the amount of sinking particulate matter that accumulates on the developing embryos. There is a need for experimental studies to test for any variation in the mortality of egg clutches laid on the different microhabitats used at the spawning aggregation site.

Rugosity and the abundance of the preferred spawning microhabitat were the only habitat features that differed significantly between the spawning aggregation site and the non-spawning site. However, these 2 attributes may not be independent measures of habitat structure. Rugosity was measured in this study by laying a chain over the substratum so that it followed all surfaces. When several boulders were laying over one another forming numerous ledges, crevices, and flat surfaces, this technique required that the tape mea-

sure be laid across the top of a boulder, around its edge, and across the upper and lower surfaces of the ledge formed by one boulder overlaying another. The preferred spawning microhabitat may be more abundant at the spawning aggregation site because of this site's higher rugosity.

Spawning aggregation sites may not exhibit noticeable differences in habitat structure from surrounding reef areas (Colin & Clavijo 1988, Colin 1996). This is partly expected because most studies have concerned pelagic spawning fishes for which there may not be a requirement for specific substratum features for spawning. Other possible reasons for this discrepancy could be a lack of clarity and consistency in descriptions of habitat attributes, interspecific differences in the underlying reasons for the formation of spawning aggregations, or geographic variation within a species in the habitat attributes associated with spawning aggregations. Another explanation is that evaluations of the habitat structure of spawning aggregation sites have been largely qualitative descriptions and have not involved a quantitative comparison with habitat attributes in non-spawning sites. Much work has been done to determine the habitat attributes underlying spawning site selection in freshwater fishes by quantitatively comparing spawning and non-spawning sites (Baxter & Hauer 2000, Labonne et al. 2003) and some marine fishes (Kroon et al. 2000, Sancho et al. 2000b). There is a great potential for applying the same approach to test hypotheses about the importance of habitat structure in the selection of spawning aggregation sites by reef fishes.

The results of the present study are interpreted as indicating selection of a spawning aggregation site by *Chromis hypsilepis* that provides greater early survival of propagules (via rapid off-reef transport) and a reduced predation risk for spawning adults (owing to greater rugosity in the spawning site). An alternative explanation for spawning site selection is traditionality, whereby young individuals copy the choices of older individuals because of this behaviour's selective advantage in eliminating the potentially costly need for individuals to search for and select spawning sites (Warner 1987, 1988). Traditionality in spawning site selection arises when there is an excess of suitable spawning sites (Warner 1988). However, the absence of spawning at other sites on Terrigal Reef and the demonstrated difference between the spawning aggregation site and other sites (in rugosity, availability of preferred spawning microhabitat, off-reef transport) indicates that potentially suitable spawning sites are scarce. In this scenario, traditionality in spawning site selection could be tested by the experimental provision of alternative suitable spawning sites.

Off-reef transport

The passive drifters are useful proxies for tracking the drift of newly hatched larvae of *Chromis hypsilepis*. Hatching experiments have shown that pomacentrid larvae swim upwards towards the surface immediately after hatching and are then largely passive for the next 12 h (Fisher et al. 2000). The drifters released at the spawning aggregation site spent considerably less time over reef than the drifters released simultaneously at a non-spawning site. The depth of reef over which the drifters floated varied between 3 and 12 m. It is therefore possible that larvae hatching at the spawning aggregation site would be exposed to a reduced risk of predation from reef-based planktivorous predators. Therefore, the overall reproductive success of adults spawning at the aggregation site should be greater than spawning at other sites on the same reef. It therefore appears that the benefits for propagules derived from hatching at the spawning aggregation site may occur in the short period of time immediately following hatching.

Working at a shorter temporal scale (109 min) than the present study (12 h), Hensley et al. (1994) found no consistent reduction in time spent over the reef by dye parcels released at the spawning sites of *Thalassoma bifasciatum* compared with non-spawning sites. This was contrary to the hypothesis that spawning sites were selected to hasten the movement of fertilized eggs off the reef. However, Hensley et al. (1994) found that mean depth after 10 min was consistently greater for dye parcels released from spawning sites at the scale of individual reefs and an entire reef complex. It therefore appears that, similar to the results of the present study, the greatest benefits of spawning site selection may occur within a short time of fertilization (for pelagic spawners) or hatching (for demersal spawners).

Spawning at the aggregation site did not provide any advantage in terms of greater offshore dispersal. The drifters released at the spawning aggregation site and the non-spawning sites were in approximately the same position after 12 h. Therefore, the results of this study do not support the hypothesis that spawning at a particular site occurs because it increases the dispersal distance of larvae (Barlow 1981). Although the study lasted only 12 h, the mixing of the drifters suggests that it is unlikely that any differences between the spawning aggregation site and non-spawning site would be manifested had the study had continued longer. This finding reflects the results of another study that compared the trajectories of water masses from spawning and non-spawning sites and found convergence of the trajectories after 16 h (Appeldoorn et al. 1994). The latter authors similarly concluded that spawning at

known spawning sites did not lead to a greater likelihood of fertilized eggs being transported more rapidly to offshore waters.

The spawning aggregation site is located at the western extremity of Terrigal reef and it may seem an unsurprising result that the drifters released there spent less time over the reef. However, the entire reef is relatively narrow (Fig. 1) and all positions on the reef in the same depth range as the spawning aggregation site are relatively close to the reef edge. The interesting result is that the drifters released at the non-spawning site did not immediately drift away from the reef but instead drifted along the reef, after being trapped for a period of time within a local eddy. Small-scale movements of surface waters around reefs are affected by wind strength and direction and tidal phase (Shapiro et al. 1988), however the difference between the spawning aggregation site and the non-spawning site was consistent despite differences in tide phase, wind direction and wind strength.

The different trajectories of the drifters released at the spawning aggregation site and the non-spawning site may be due to small-scale differences in water movements. The drifters released at the spawning aggregation site spent on average 24 min over reef, compared to the drifters released at the non-spawning site that spent an average of 192 min over reef. On both occasions the drifters released at the non-spawning site spent up to 96 min in a local eddy at the release site. After moving out of the eddy, the drifters moved in a northwesterly direction over the reef. Although the non-spawning site was similar to the spawning aggregation site in depth and habitat, it differed in some other features that may have influenced local water movements, viz. the slope of the non-spawning site was less than the spawning aggregation site, the width of the reef was approximately 100 m compared with 25 m at the spawning aggregation site, and the water depth at the edge of the reef where it met sand was 22 m compared with 10 m at the spawning aggregation site. Reef topography is an important determinant of small-scale surface currents (Wolanski & Jones 1980).

Food availability

The hypothesis that *Chromis hypsilepis* selected the spawning aggregation site because it provided greater food availability for brooding males was rejected. The biomass of the planktonic prey of *C. hypsilepis* did not differ between the spawning aggregation site and the non-spawning site. However, the spawning aggregation site, unlike the non-spawning sites, did not experience significant short-term (i.e. between days) temporal variations in prey availability. There was no short-term variation in prey availability during a period when males were engaged in brooding at the aggregation site (Time 1) and also during a non-brooding period. The hypothesis that the spawning aggregation site provides a more reliable supply of prey than other sites on the reef will require further testing in combination with measurements of current velocities.

The opportunities for males to feed are limited by their broodcare duties and this is reflected in their significantly reduced feeding rate (Gladstone 2007). In addition, the concentration of several thousand males engaged in broodcare at the same time at the aggregation site may lead to intra-specific reductions in prey. Males engaged in broodcare may have been at the aggregation site (and feeding at a significantly reduced rate) for up to 8 d (Gladstone 2007). Under these conditions, a significant reduction in prey availability over successive days is likely to substantially increase the energetic costs to males and reduce their reproductive success. This could occur by males compensating for reduced prey availability by filial cannibalism (Rohwer 1978) or reducing the number of occasions they spawn in the reproductive season to allow for recuperation.

CONCLUSION

This study has shown that a species of temperate reef fish selected a spawning aggregation site with greater rugosity, availability of the preferred spawning microhabitat, greater rate of off-reef transport, and more reliable prey availability than other sites on the same reef not used for spawning. The selective advantage of these features may be the improved fitness of spawning adults and their propagules. Further research is currently underway to test these results over a much larger spatial scale.

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