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DYNAMICS OF CAMOUFLAGE (*EPINEPHELUS POLYPHEKADION*) AND BROWN MARBLED GROUPER (*EPINEPHELUS FUSCOGUTTATUS*) SPAWNING AGGREGATIONS AT A REMOTE REEF SITE, SEYCHELLES

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ABSTRACT

Aggregations of camouflage, *Epinephelus polyphekadion* (Bleeker, 1849), and brown marbled grouper, *Epinephelus fuscoguttatus* (Forsskål, 1775), were found to overlap spatially and temporally at a reef pass site located in the outer island group of the archipelagic Seychelles. Over three spawning seasons, we investigated the spatial and temporal dynamics of aggregations using a combination of underwater visual census (UVC) surveys and trends in reproductive parameters, notably gonadosomatic index. Interannual variation in aggregation formation was evident for both species, but was more variable in camouflage grouper. Aggregations lasted between 2–3 wks, with peak abundances attained a few days prior to the new moon, soon after which aggregations quickly dispersed. Spawning within aggregations was confirmed using direct (observations of hydrated oocytes) and indirect (gonadosomatic indices, behavior, color changes) signs. Spawning seasons were estimated at between 2 and 3-mo long, occurring between November and February, with interannual variation in onset and termination. Tagged camouflage grouper exhibited site fidelity on scales of weeks to months, and one individual returned to the aggregation the following year. The aggregations are known to fishers and are commercially exploited. Fisher knowledge was found to be a reliable source of information for locating aggregations.

Many reef fish species form aggregations at specific times and places for the purpose of reproduction (Domeier and Colin, 1997). This life history trait is common to the groupers (Serranidae) with a recent review identifying 20 species from this family that are known to form spawning aggregations (Claydon, 2004). Aggregation spawning has been verified for several Indo-Pacific groupers, including the camouflage grouper, *Epinephelus polyphekadion* (Bleeker, 1849), and the brown marbled grouper, *Epinephelus fuscoguttatus* (Forsskål, 1775) (Johannes et al., 1999; Rhodes and Sadovy, 2002a; Pet et al., 2005). Despite the wide distribution of these groupers in the Indo-Pacific (Heemstra and Randall, 1993), verification of spawning aggregations is lacking for the western Indian Ocean (WIO).

Camouflage and brown marbled grouper are highly valued food fishes that are important to small-scale reef fisheries throughout much of the Indo-Pacific. Aspects of their demography and reproductive biology, such as slowness at reaching sexual maturity (Rhodes and Sadovy, 2002b) and protogynous hermaphroditism (Johannes et al., 1999; Rhodes and Sadovy, 2002b; Pears et al., 2006), render these species vulnerable to overexploitation (Sadovy, 1996, 2001). Adding to the vulnerability conferred by these life history traits is the fact that camouflage and brown-marbled grouper are also known to form transient spawning aggregations (Domeier and Colin, 1997; Johannes et al., 1999; Rhodes and Sadovy, 2002a; Pet et al., 2005). Grouper spawning aggregations of the transient type are generally large (Domeier and Colin, 1997), comprising hundreds (Samoilys, 1997) to tens of thousands (Smith, 1972; Olsen and LaPlace, 1979) of individuals, and often involve a considerable migration for participating fish (Colin, 1992; Sadovy et al., 1994; Sadovy, 1996). Moreover, a single spawning aggregation may constitute a significant proportion of the annual reproductive output of participating fish (Shapiro et al., 1993; Samoilys and Squire, 1994). Transient aggregations are highly predictable in time and space and once known to fishers they can be quickly decimated (Colin, 1992; Johannes et al., 1999; Sadovy and Eklund, 1999; Sala et al., 2001; Sadovy and Domeier, 2005).

The Republic of Seychelles is an island nation with an archipelago spread over an exclusive economic zone of 1.37 million km^2 in the WIO. Fish are a major source of protein and fishing is conducted in all areas, including remote atolls. Grouper spawning aggregations are well known to artisanal fishers in Seychelles, particularly those formed by camouflage and brown marbled grouper (Robinson et al., 2004). The outer islands have no indigenous populations, although many have permanent human presence and are managed by parastatal or non-governmental organisations. Reef fish at the outer islands are targeted by inhabitants of the islands or by fishing vessels operating from the inner granitic islands. Prior to this study, little was known regarding the status of grouper aggregations, although several patriarchal fishers reported declines at certain sites. Studies were focused at a site known to fishers which supported a significant commercial aggregation fishery.

The aim of this study was to derive key aggregation parameters for management. The objectives were: (1) to verify fisher reports of grouper spawning aggregations; (2) to investigate the spatial and temporal dynamics of aggregations, and (3) to assess lunar and seasonal periodicity of reproductive activity.

Materials and Methods

Study Site.—The study site (Site A) is located at an atoll situated to the south of the Seychelles archipelago (Fig. 1). Located on the outer reef slope, the site is adjacent to a reef pass and consists of irregular and patchy coral development interspersed with sandy channels and bowls. Depths at the site range from 6 m on reefs bordering the pass, to approximately 15 m at the outer edge of reef development, beyond which a sandy terrace extends on a gentle decline, for around 100 m, to a drop-off. The Admiralty chart for the area notes that currents in the pass reach 2.1 m s^{-1}. Visibility in the pass deteriorates substantially on the ebb tide, from > 30 m to < 5 m. Studies were conducted at the atoll during 7 mo (Dec ’03, Jan ’04, Feb ’04, Dec ’04, Jan ’05, Feb ’05, and Nov ’06) spread over the three seasons of 2003–2004, 2004–2005, and 2006–2007 (Table 1).

Spatial and Temporal Dynamics.—Following nine dives at Site A during a 2-wk period leading up to the new moon (NM) in December 2003, the aggregation site was defined in terms of core and boundary reef areas. The highest densities and abundances, territorial and courtship behavior, and the presence of gravid females were largely confined to two adjacent, oval shaped reef patches, defined as the core area. The core area was located immediately adjacent to the western side of the mouth of the channel. Bordering the core reefs, the boundary reef area was characterised by comparatively high densities, but other indications of spawning behavior were absent. The distribution of aggregating camouflage and brown marbled grouper individuals over core and boundary reefs was similar and the area of these zones was considered to be representative for both species. Estimation of the core (5750 m^2) and boundary (6900 m^2) areas was achieved by placing surface marker buoys around their perimeters and calculating area using a GPS receiver.

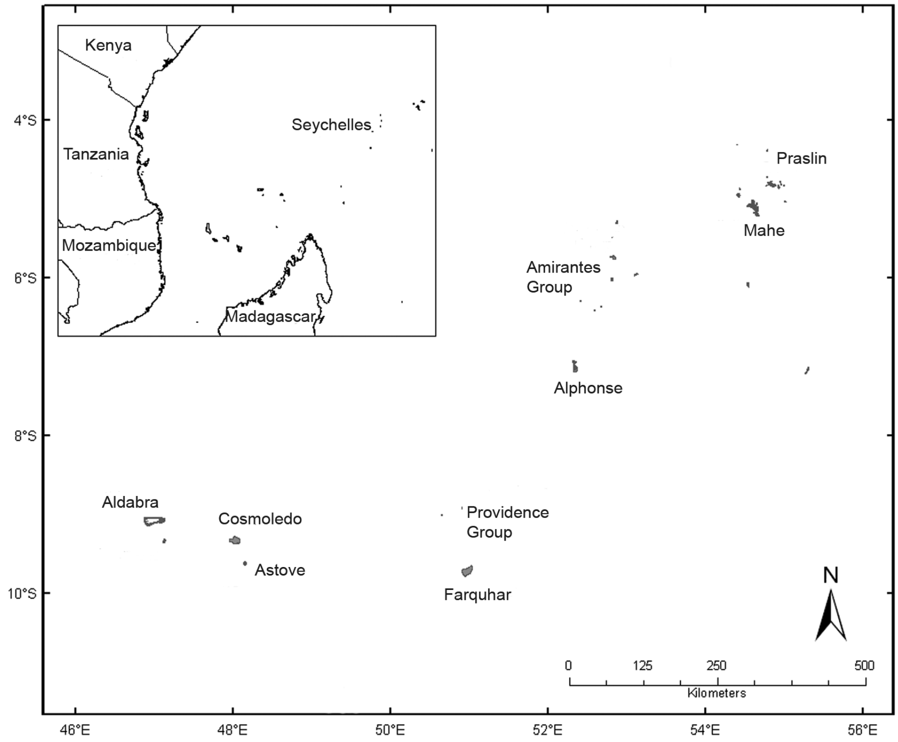

Figure 1. The Seychelles archipelago in the western Indian Ocean (inset). The inner granitic islands are centered on Mahé and Praslin, while other islands constitute the outer island groups. The exact location of the study site is not shown due to sensitivity of the data.

Underwater visual census (UVC) was used to determine spatial and temporal variation in camouflage and brown marbled grouper densities and abundances. Counts for both species were performed over 100 × 4 m permanently demarcated belt transects in the core and boundary areas. We established this systematic method as brown marbled grouper were found to be extremely wary of divers (Johannes et al., 1999). Counts were initiated after four transects were installed (two each in the core and boundary reef areas), 4 d prior the NM in December 2003. A further transect was added (in the core) to the census prior to December 2004, giving five in total. Depths of the transects ranged from 7 to 13 m. Diving was limited to either high or low water slack tide so that census times varied between days, being conducted between 0930 and 1730 across months (Table 1). Diel variability in density was investigated by conducting morning and afternoon counts on 4 d within a week of NM in December 2004 and January 2005, with counts separated by at least 5 hrs.

The census team consisted of a pair of SCUBA divers, the first swimming several meters ahead of the second and estimating brown marbled grouper according to the following abundance classes; 0, 1–10, 11–30, 31–50, 51–100, 101–500, 501–1000, > 1000 (Colin et al., 2003). Use of abundance classes was necessary due to the wariness of brown marbled grouper and its propensity to be "herded" by divers (Johannes et al., 1999). The second diver performed discrete counts of camouflage grouper.

For the purposes of documentation and to facilitate interpretations of fish behavior, video footage was obtained during several dives, notably within periods close to new moon. Sex-specific observations and their interpretation are considered putative, but were employed after initial assessments revealed that behavior and color phases were consistent with sex-determined observations by other authors (Johannes et al., 1999; Rhodes and Sadovy, 2002a).

Table 1. Details of studies at Site A. Sampling days refers to the number of days each month that catches were available for biological sampling. The dates of underwater visual census (UVC) surveys (not bracketed), the number of UVC surveys per day (no d^{-1}) and the total number of underwater visual census (UVC) surveys per month [no mo^{-1}] are given. The UVC times are given as a range based on the earliest and latest survey start time for that month. Note: dusk occurs at between 1830 and 1900 in December and January.

Season	Mo/yr	Study dates	New moon	Sampling days (no mo^{-1})	UVC surveys: (no d^{-1}) [no mo^{-1}]	UVC times
I	Dec ’03	07–31	23	7	19, 21, 23, 26, 29, 31; [6]	0945–1730
	Jan ’04	01–22	21	8	03, 06, 12, 14, 16, 18, 19, 21; [8]	1000–1530
	Feb ’04	13–22	20	6	No surveys	
II	Dec ’04	04–14	12	0	06, 07, 09 (2), 10, 12; [6]	0950–1710
	Jan ’05	06–14	10	7	07 (2), 08 (2), 09 (2), 10, 12, 13; [9]	1010–1720
	Feb ’05	01	08	1	Qualitative survey	
III	Nov ’06	11–13	20	0	12; [1]	1000

Spawning Verification and Periodicity.—To verify spawning at the aggregations and to investigate seasonal and lunar patterns in spawning periodicity, several direct and indirect indicators were employed (Colin et al., 2003), including occurrence of hydrating oocytes, patterns of sexual maturation, and gonadosomatic index (GSI). Subsistence and commercial aggregation catches landed by island staff fishers were sampled in their entirety when small or sub-sampled randomly when daily catches were large, notably in January 2005. Prior to sampling, fishers sometimes removed larger individuals from the catch for salt fish preparation. This meant that aggregation sex ratios could not be derived from catch sampling data and may have led to biases in size data and distributions. Fish were measured to the nearest 1 mm (total length, TL) and weighed to the nearest 50 g. Gonads were removed, weighed to the nearest 5 g and sexed macroscopically. GSI values were calculated for adult females using the formula: Gonadosomatic index (GSI) = gonad weight/gonad-free body weight × 100.

Sexual maturation stages of camouflage and brown-marbled grouper were evaluated using a single index adopted from Rhodes and Sadovy (2002a), which combined criteria for both macroscopic and microscopic (histological) approaches. However, for the microscopic evaluation, we included observations of migratory nucleolus stage (MNS) oocytes in the second mature (or “ripe”) stage, as these are indicative of the start of hydration (West, 1990; reviewed in Samoilys and Roelofs, 2000). Microscopic evaluation was also used to validate macroscopic sexing. Sub-samples of gonads for histological preparation were obtained by dissecting a thin section from the anterior portion of one of the lobes. Gonad sub-samples were fixed in 4% neutral buffered formalin, embedded in paraffin, sectioned at 6–8 μm and stained in hematoxylin and eosin.

Site Fidelity.—To investigate site fidelity on lunar and seasonal scales, camouflage grouper were tagged and released at Site A during two main periods; 16–30 December 2003 and 13–21 January 2004. Fish were caught in the core, at depths ranging from 6–10 m, using hook-and-line gear. TL was recorded to the nearest 0.5 cm (all fish lengths were over-estimated due to the curvature of the sling used for tagging fish) and the fish sexed, where possible, from the appearance of the gonopore and by stripping for the presence of milt or eggs. Fish were tagged, using a T-bar anchor tag inserted between the dorsal pterygiophores, and released at the point of capture. Observations of tagged individuals were made through recaptures by fishing and underwater at the aggregation site prior to or after UVC counts, although tag numbers could not be read underwater.

Data Analyses.—Aggregation sizes were calculated from core and boundary reef abundances, based on mean density counts from respective transects and area estimates. For

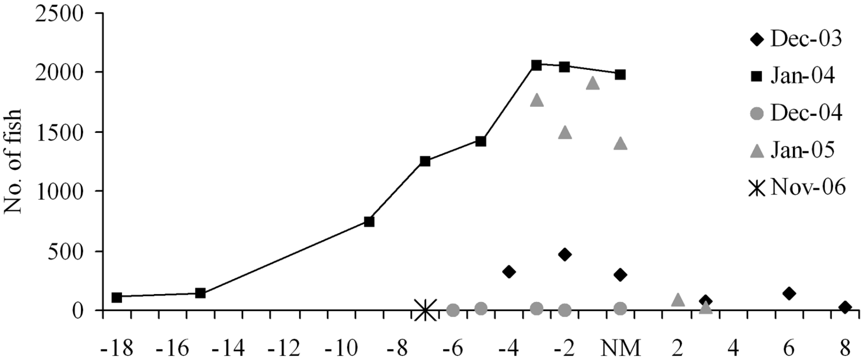

Figure 2. Aggregation (Dec-03, Jan-04, Jan-05) and non-aggregation (Dec-04, Nov-06) abundances of camouflage grouper at Site A on census days relative to the new moon (NM). The line indicates the trend in abundance for the month (Jan-04) with the longest time series of observations.

brown marbled grouper, a conservative approach was adopted and the minima of the abundance classes were used for estimation of overall aggregation abundances. Trends in aggregation densities (using class mid-points for brown marbled grouper) were used in the interpretation of spatial and temporal patterns in behavior. To determine diel variability, densities were compared statistically between morning and afternoon counts using Wilcoxon Signed Ranks Tests as assumptions of parametric tests for paired samples could not be met. The nonparametric Mann-Whitney U-tests were used to determine differences in mean TL between sexes.

Results

Habitat Use and Behavior of Camouflage Grouper.—Aggregation abundances peaked at more than 1900 fish a few days prior to NM in both January 2004 and January 2005 (Fig. 2). More than 300 fish aggregated over a 4 d period prior to the NM in December 2003, while aggregations were not present in December 2004 or November 2006. A qualitative survey made by the lead author during one dive 8 d before the NM in February 2005 indicated that more than 30 camouflage grouper were present in the core.

Camouflage grouper had largely dispersed from the aggregation site a week after the NM of December 2003 and abundances 2–3 wks before the NM of January 2004 were < 5% of the peak abundances for that month (Fig. 2). Subsequently, in January 2004, the month with the longest time-series of observations, densities started to climb on core reefs around 2 wks prior to the NM (Fig. 3). From appearance, size and behavior, it appeared that males started to aggregate at the site prior to females. Males in the core established territories 1–3 m in diameter over coral substrate. Densities on boundary reefs were an order of magnitude lower than in the core but peaked several days prior to NM, coinciding with large groups of females arriving at the aggregation and passing through to the core. Females were recognizable by their greatly distended abdomens (Fig. 4A). Concomitant with peak aggregation densities, dense clusters of females (up to 10 ind.) were observed in the core, largely but not exclusively confined to the pass edge of the shallower patch reef. Patterns in density were similar in January of both years, and in 2005 dropped sharply after the NM (Fig.

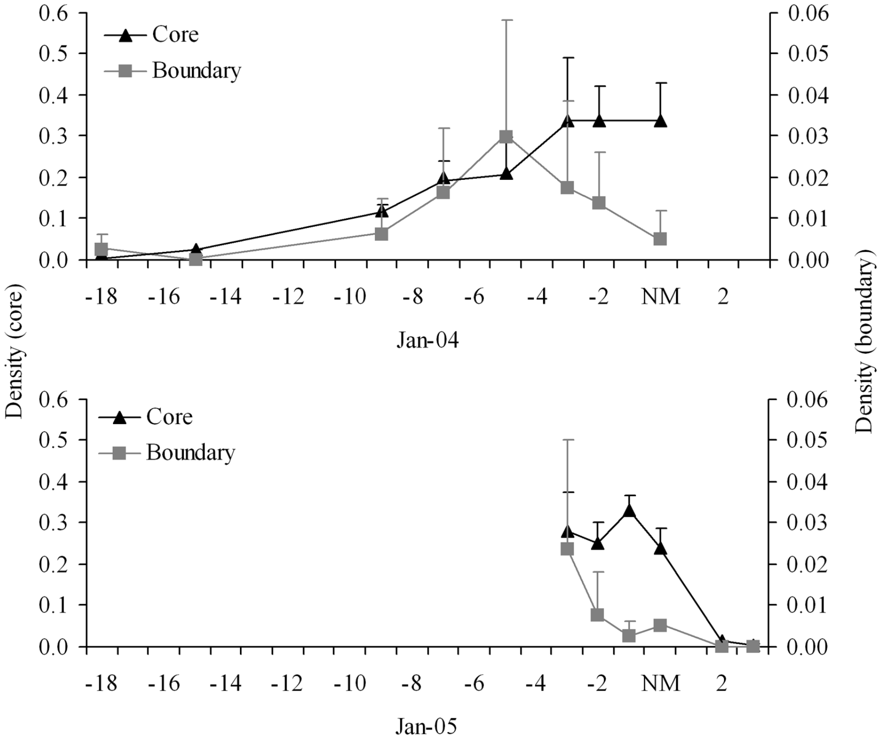

Figure 3. Trends in mean densities (fish m^{-2}) of camouflage grouper in core and boundary reef areas (Site A) on census days relative to the new moon (NM) during the two months of the largest aggregations. Where sample sizes were sufficient, error bars are standard deviations; N = 2 for each area in Jan-04, while in Jan-05, N = 2 and 3 in the boundary and core areas, respectively.

3), indicating that aggregations persist for over 2 wks. No diel variability in mean density was detected (Wilcoxon Signed Rank: $Z = -0.91$, $P > 0.05$).

At high aggregation densities, territorial disputes between males frequently led to aggression, commonly involving two individuals facing each other in a cheek-to-cheek position, with occasional bouts of pushing by one individual at the flanks of the other (Fig. 4B). Other acts of aggression were briefer and included snout-to-snout snapping and sidling. Encounters typically ended in retreat or the intruder was pushed out of the territory. Individuals involved in aggressive encounters usually underwent a color change, termed "ghosts", blanching to display a uniform light brownish coloration with no obvious markings except the black spot on the caudal peduncle and the two black dots above the upper lip. Females displayed no major disruption of the common color pattern. Spawning rushes and gamete release were not observed.

Habitat Use and Behavior of Brown Marbled Grouper.—Aggregations were smaller than those formed by camouflage grouper, peaking at around 1050 individuals on the NM in January 2004 and 1 d before NM in January 2005 (Fig. 5). After a decline to 163 fish 3 d after the NM in December 2003, abundances recovered to around 50% of peak aggregation size by early January 2004. Significant brown marbled grouper aggregations also formed in December, although these varied in abundance among years. More than 50 brown marbled grouper were observed in

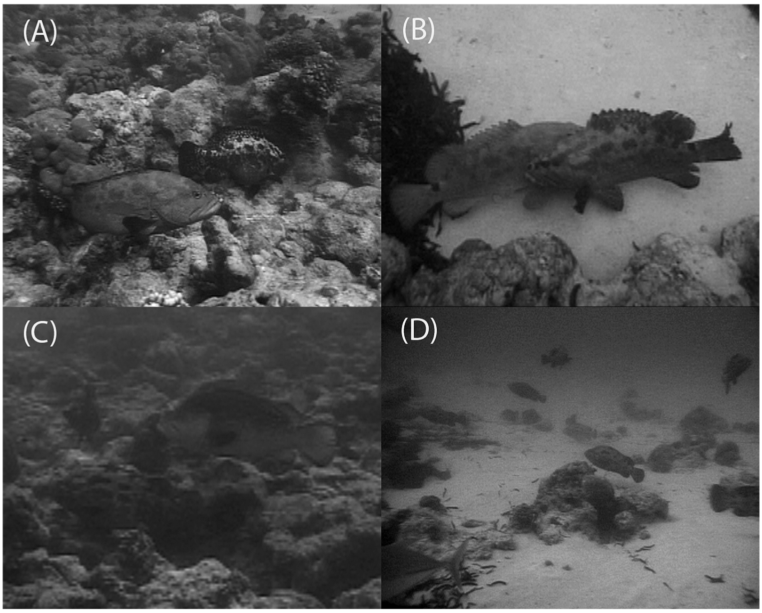

Figure 4. (A) Male and (gravid) female camouflage grouper with male showing "ghost" color phase and the female the normal color phase, (B) male camouflage grouper in cheek-to-check territorial display, (C) brown marbled grouper displaying normal coloration (lower individual) and bicolor phase (upper individual), and (D) brown marbled aggregating over sand at the interface of the pass and core reefs, with individual hanging high in water column.

the core site 8 d before the NM of February 2005. Aggregation formation had not occurred a week prior to NM in November 2006.

During the early stages of aggregation development in January 2004 (Fig. 6), small groups (3–20 ind.) of brown marbled grouper were observed in caves and fissures in the boundary area, but territorial behavior or color changes were not observed. In the core, males established territories several meters in diameter over coral or sandy substrate, and frequently changed in coloration ("bicolor phase") to reveal a uniform pale grey color on the lower abdomen, operculum, cheeks and lips, and a dark grey/

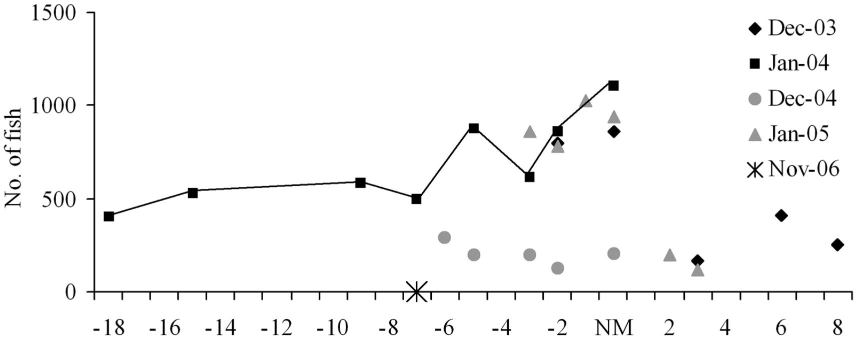

Figure 5. Aggregation (Dec-03, Jan-04, Dec-04, Jan-05) and non-aggregation (Nov-06) abundances of brown marbled grouper at Site A on census days relative to the new moon (NM). The line indicates the trend in abundance for the month (Jan-04) with the longest time series of observations.

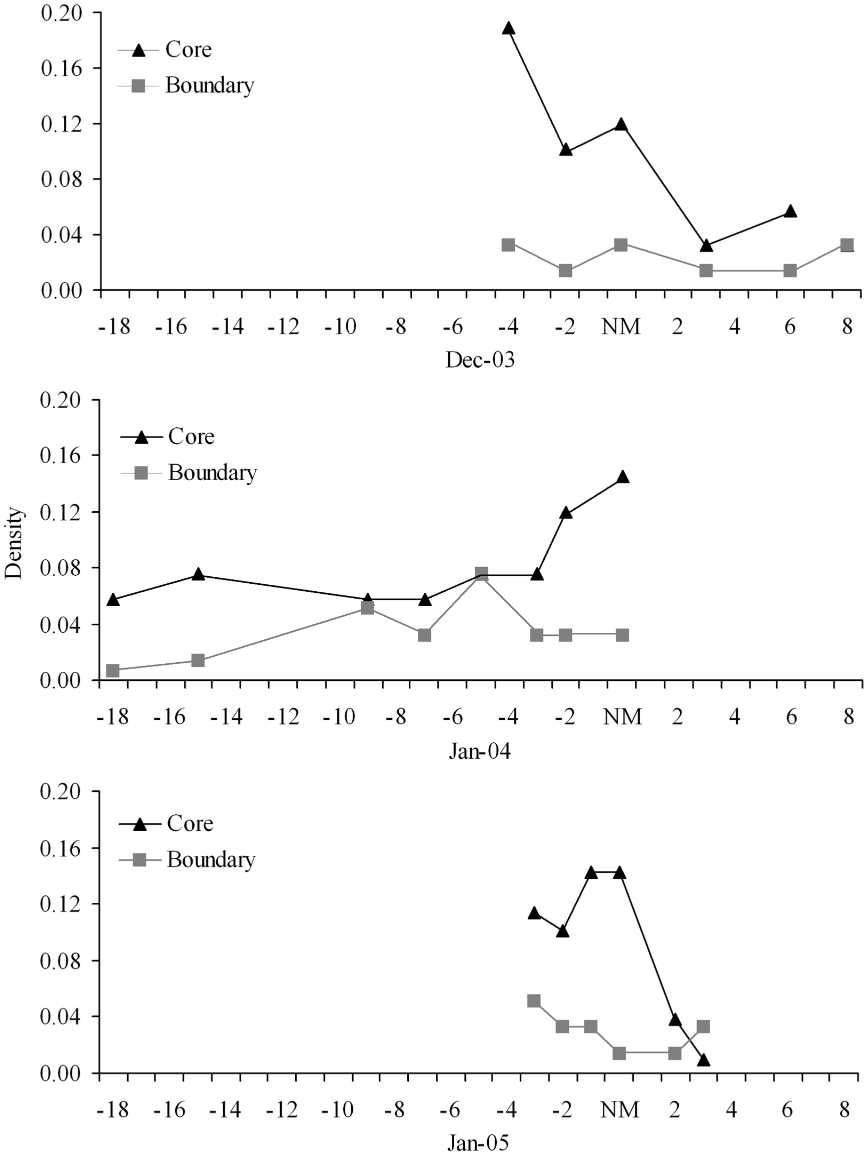

Figure 6. Trends in mean densities (fish m^{-2}) of brown marbled grouper in core and boundary reef areas (Site A) on census days relative to the new moon (NM) during the 3 mo of the largest aggregations. N = 2 for each area in Dec-03 and Jan-04, while in Jan-05, N = 2 and 3 in the boundary and core areas, respectively.

brown color dorsally on the body and head (Fig. 4C). Encroachment by another individual usually resulted in aggressive encounters, typically chasing but sometimes progressing to snapping and biting. Until 5 d before NM, densities in the core and on boundary reefs were not greatly different. Within 5 d of the NM, densities in the two areas diverged as many gravid females and additional males arrived in the core. Core densities exceeded those on boundary reefs by up to a factor of 5 as the aggregation peaked (Fig. 6), concomitant with intense territorial and courtship behavior in the core. Males interacted with females by rubbing alongside them abdomen-to-abdomen, and sometimes appeared to push at the abdominal area of females. Spawning rushes and gamete release were not observed. A week or so prior to NM, groups of between 2 and 10 fish were observed to "hang" high in the water column in the core, approximately 2–4 m above the substratum (Fig. 4D). Divers approaching too closely caused the groups to disperse and swim down to the substrate, but otherwise the groups appeared to be fairly stable, both temporally and spatially. No diel variability in mean density was detected (Wilcoxon Signed Rank: Z = –0.31, P > 0.05).

A high degree of inter-specific spatial overlap was observed in the core with the highest densities of both species occurring along the shallow (< 8 m) reef edge bordering the pass. In that area, camouflage and brown marbled grouper male territories frequently overlapped. High brown marbled grouper densities were also observed in deeper areas (> 10 m) of the core and on sandy patches close to the reefs, habitats which were more sparsely occupied by camouflage grouper. Despite the close association, especially at high densities, inter-specific acts of aggression were not observed although intra-specific behaviors were sometimes disrupted by the other species. For example, check-to-check posturing between camouflage grouper males was sometimes disrupted by brown marbled grouper males chasing each other through the territory. In the boundary reef areas, territory formation and inter-specific interactions were not observed.

SAMPLING DETAILS.—A total of 256 camouflage grouper, including 1 immature individual, and 79 adult brown marbled grouper were sampled from catches during 4 mo (samples were not obtained during the December 2004 and November 2006 expeditions) (Table 2). Samples taken in all months were from catches made directly at the aggregation site (core and boundary), with the exception of February 2004 when 18 of the fish sampled were taken from catches made along the northern outer reef slope, more than 1 km from the boundary reefs, while 1 fish sampled was caught at the aggregation site. In February 2005, three female brown marbled grouper were caught in the pass adjacent to the boundary reef.

Male camouflage grouper outnumbered females in two of the monthly samples, while females dominated samples in January 2005. Male and female brown marbled grouper were roughly equal in two of the sampled months, and female dominated in other months (Table 2). Female camouflage grouper ranged in size from 44.4 to 67.6 cm TL (mean TL ± 1 SD: 52.1 cm ± 4.1, n = 95), males from 46.0 to 68.4 cm TL (57.2 ± 3.4; n = 160). Female brown marbled grouper ranged in size from 59.3 to 92.9 cm TL (70.6 ± 6.9; n = 51), males from 71.9 to 92.7 cm TL (81.9 ± 4.7; n = 28). Despite considerable overlap in size ranges, males were significantly larger than females

Table 2. Numbers of female (F), male (M), and immature (I) camouflage and brown marbled grouper sampled from catches made at Site A. *Females caught in the reef pass approximately 200 m from Site A.

	Camouflage grouper				Brown marbled grouper			
Month	F	M	I	Total	F	M	I	Total
Dec '03	7	48	1	56	15	16	0	31
Jan '04	24	61	0	85	10	11	0	21
Feb '04	7	6	0	13	6	0	0	6
Jan '05	57	45	0	102	17	1	0	18
Feb '05	0	0	0	0	3*	0	0	3
Total	95	160	1	256	51	28	0	79

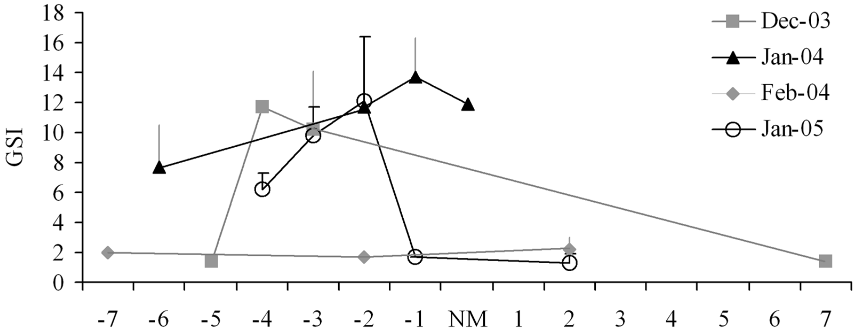

Figure 7. Female camouflage grouper GSI relative to the new moon (NM) for four sampling months. Where sample sizes were sufficient, error bars are standard deviations. Samples in February 2004 were obtained from catches made outside the aggregation site.

for camouflage (Mann-Whitney: Z = −8.92, $P < 0.05$) and brown marbled grouper (Mann-Whitney: Z = −6.11, $P < 0.05$). Sizes and size distributions may have been biased by the removal of some larger individuals prior to sampling.

Males of both species appeared in samples taken from aggregation catches earlier than females, providing evidence for an earlier arrival of males at the site. In January 2004, the month with the longest time-series of sampling, camouflage grouper samples taken 13 and 8 d (n = 13 and 17, respectively) before NM were entirely male, whereas females first appeared in samples (n = 3) 6 d before NM. From catches made over the 2 d immediately prior to NM in January 2004, females (n = 20) dominated over males (n = 5) in the sample. In the same month, male brown marbled grouper were first sampled (n = 5) 8 d before NM, with females appearing slightly later in samples (n = 3) at 6 d before NM. Thereafter, sample sizes were roughly equal for males and females.

Histological Assessment and GSI.—Of the 255 adult camouflage grouper sampled, 85 (33.3%) were examined using histological techniques. Histology confirmed that macroscopic criteria for sexing of camouflage grouper were reliable, with 96% identified correctly. Those sexed incorrectly were spent/re-maturing individuals. Trends in female camouflage grouper GSI indicated that spawning occurred prior to NM (Fig. 7). Based on the comparatively large female sample sizes achieved in January 2004 (n = 24) and January 2005 (n = 57), when significant aggregations had formed, GSI peaked 1 d and 2 d before NM, respectively. Thereafter, sample sizes were small, but females of low GSI were caught on site 2 d after NM in January 2005; the presence of MNS oocytes in one of those females may indicate that protracted spawning occurs in some individuals. Spawning probably occurred in the small camouflage grouper aggregation of December 2003, when a few females of high GSI, with ovaries dominated by late yolk globule stage oocytes, were sampled 3 d (n = 2) and 4 d (n = 1) prior to NM. Mean GSI was low in February 2004 and ovaries examined microscopically were pre-vitellogenic, implying that the spawning season terminated after the NM in January 2004 (Fig. 7).

In total, 53 brown marbled grouper samples were examined histologically, constituting 67% of all samples. Sexing of brown marbled grouper using macroscopic criteria was found to be 100% accurate. Peaks in female GSI were observed over a

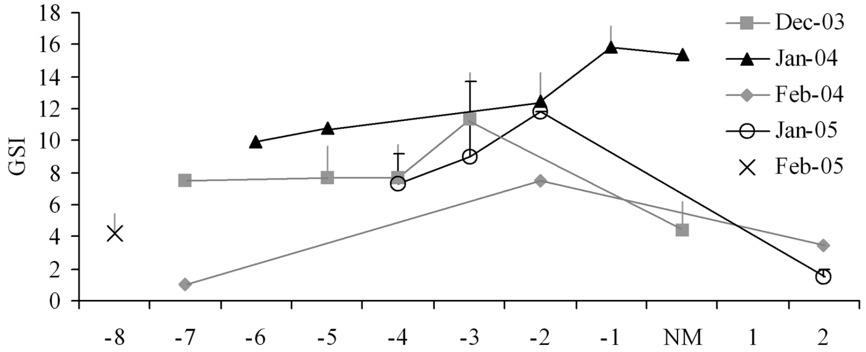

Figure 8. Female brown marbled grouper GSI relative to the new moon (NM) for five sampling months. Error bars are standard deviations. With the exception of a single female (the highest GSI), samples in February 2004 were obtained from catches made outside the aggregation site.

period of 3 d prior to NM (Fig. 8), while the few samples obtained after NM were characterized by post-spawning ovaries. Hydrated and MNS oocytes were observed on the day of the NM in December 2003 and January 2004. The remaining ovaries sampled at the aggregation site were dominated by yolk globule stage oocytes. In February 2004, ovaries from females caught outside the aggregation site were pre-vitellogenic. However, a single ovary sampled from the aggregation site in that month was characterized by high GSI (7.5) and was dominated by yolk globule stage oocytes, suggesting that the spawning season may extend into February for some if not all individuals. Further evidence for spawning in February was provided by the three females sampled 8 d before the NM of that month in 2005, which were dominated by yolk globule oocytes, suggesting reproductive activity in that month (Fig. 8).

Site Fidelity.—In total, 141 camouflage grouper were tagged at the aggregation site during season I (December 2003: n = 36; January 2004: n = 105), of which 50 were males, 17 were females, and 74 were unidentified. During season I, eight individuals were recaptured at the aggregation site by fishers, with time at liberty ranging from 2 to 22 d (Table 3). Two male camouflage grouper tagged 1 d after the NM in December 2003 were recaptured < 8 d before the NM in January 2004, indicating a degree of site fidelity between consecutive aggregation months. Fishing on the aggregation site during season II yielded no recaptures. However, divers observed a single tagged individual in the core during January 2005, the fish having spent at least 352 d at liberty.

Other Spawning Aggregation Observations.—Aggregations of between 50–100 marbled coral grouper, *Plectropomus punctatus* (Quoy and Gaimard, 1824), were also observed at Site A in the boundary reef area, adjacent to the western core reef. These aggregations were not surveyed quantitatively but formed consistently around the NM in December and January of seasons I and II. Evidence of spawning was limited to macroscopic observation of hydrated oocytes in female marbled coral grouper caught at the aggregation 3 d after the NM in December 2003.

Towards the end of our surveys in January 2005, a patriarchal fisher led us to a site (Site B) in the far west of the lagoon where, a few days earlier, he had fished approximately 400 kg of "ripe" camouflage and brown marbled grouper. A qualitative survey of the area by SCUBA divers indicated that aggregations (c. 50 camouflage grouper; <

Table 3. Dates of recapture and dive observations of tagged camouflage grouper (male or sex not determined, ND) with corresponding days spent at liberty.

Sex	Date tagged	Date recaptured	Dive observations	Days at liberty
Male	24/12/03	15/01/04		22
ND	30/12/03	20/01/04		21
Male	24/12/03	13/01/04		20
ND	16/12/03	24/12/03		8
ND	16/12/03	24/12/03		8
ND	15/01/04	20/01/04		5
ND	15/01/04	20/01/04		5
ND	13/01/04	15/01/04		2
ND	D 03 or J 04		08/01/05	> 352

15 brown marbled grouper; c. 100 marbled coral grouper) had formed over shallow (< 10 m) patch reefs just inside of the reef crest and situated a few km from a narrow and shallow reef pass. Based on behavioral observations and fish appearance, including gravid females, it is likely that aggregations had formed for the purpose of spawning. Subsequent interviews with fishers revealed that these aggregations are spatially stable and occur at the same time as those at Site A.

Discussion

A suite of direct and indirect measures (Colin et al., 2003) verified fishers' observations of spawning aggregations of camouflage and brown marbled grouper at Site A (Robinson et al., 2004). The spawning rushes common to many aggregating groupers were not observed, but spawning often occurs after dusk (Domeier and Colin, 1997; Rhodes and Sadovy, 2002a) and the latest in-water observations were made around 30 min prior to this period. Verification based on observations of hydrating oocytes and/or MNS oocytes was limited to a few samples, which is not surprising as hydration tends to begin late in the day and occurs within a few hours of spawning (Rhodes and Sadovy, 2002a). In terms of indirect evidence, behaviors, and appearances of both species were highly consistent with other confirmed reports (Johannes et al., 1999; Rhodes and Sadovy, 2002a; Pet et al., 2005) and trends in GSI were also typical of groupers aggregating for spawning (Beets and Friedlander, 1998; Rhodes and Sadovy, 2002a; Sadovy et al., 1994).

The two groupers formed transient-type spawning aggregations (Domeier and Colin, 1997) with patterns in density, abundance, behavior, GSI, and reproductive maturation strongly associated with the NM; the time of spawning probably varying interannually within a few days leading up to and including NM. There was no evidence of spawning during different lunar periods such as the full moon (FM). However, variation in lunar periodicity may be more common between sites and on regional scales (Shapiro, 1987; Sadovy, 1996). While camouflage and brown marbled grouper spawn on the NM in Palau (Johannes et al., 1999), spawning occurs on the FM in Pohnpei, Micronesia (Rhodes and Sadovy, 2002a), and Komodo, Indonesia (Pet et al., 2005).

In the absence of an annual sequence of monthly ovarian samples, a range of indicators were employed to derive information on spawning season, including histological assessment, observations of fish densities and behavior at the aggregation site, and fisher knowledge (Robinson et al., 2004). The findings confirmed camouflage grouper aggregation formation over two consecutive months in the first two seasons studied (December 2003–January 2004, and January 2005–February 2005). Thus, until *in-situ* observations can be extended temporarily, it is proposed that the camouflage grouper spawning season is 2 mo long and varies on interannual scales between December and February depending on the timing of the NM. This estimate is consistent with the 2–3 mo spawning seasons reported for this species in other regions of the Indo-Pacific (Johannes et al., 1999; Rhodes and Sadovy, 2002a) and for the red hind, *Epinephelus guttatus* (Linnaeus, 1758), in the Caribbean (Nemeth, 2005; Nemeth et al., 2007).

Camouflage grouper aggregations were large in the month of January and similar in size to the largest aggregations surveyed by Johannes et al. (1999) in Palau, while aggregations in the surrounding months tended to be small and their formation variable between years. Interannual variation in aggregation formation and intra-annual variation in aggregation size have been described for several species and sites (Colin, 1992; Samoilys, 1997; Johannes et al., 1999; Rhodes and Sadovy, 2002a). A complex relationship exists between the size of Nassau grouper, *Epinephelus striatus* (Bloch, 1792), aggregations and the timing and yearly number of FM periods (Starr et al., 2007). This dynamic has implications for aggregation monitoring and management.

Brown marbled grouper appear to be reproductively active over a longer period compared to camouflage grouper, with aggregations forming over three consecutive months (December–February, inclusive) in 2004/2005. Although an aggregation had not formed 7 d prior to NM in November 2006, fishers have reported aggregations in November (Robinson et al., 2004), and UVC surveys of the boundary reef area 2-wks before the NM in early December 2002 found relatively high densities of brown marbled grouper (6 fish 400 m^{-2}) compared to non-aggregation counts at the atoll (R. Pears, James Cook University, pers. comm.). Given that brown marbled grouper aggregations may develop 3-wks in advance of the NM, the presence of aggregations in November may relate to monthly timing of the NM in December and will vary interannually. While the available data indicate that the spawning season is possibly 3-mo long, falling between late November and February, there is a need to evaluate other months as this species forms spawning aggregations over longer periods elsewhere (Johannes et al., 1999; Pet et al., 2005).

The findings indicate that spawning is restricted to the core area. The boundary reefs appear to serve as a refuge area, particularly for brown marbled grouper during the early stages of aggregation development. Although differences between core and boundary area densities were greater for camouflage grouper, densities on boundary reefs close to spawning were more than three-fold greater than non-aggregation periods and contributed significantly to overall aggregation abundance (5%–10%), outlining the importance of a stratified survey methodology. In terms of management, the boundary reef area should be considered integral to the aggregations. Moreover, some groupers are known to follow specific migration routes to and from aggregations. Migrating groupers are vulnerable to exploitation and migration routes and timing should be considered in the management planning process (Fulton et al., 1999; Johannes et al., 1999; Starr et al., 2007). The reef pass, bordering the core and boundary reefs, may constitute part of the migration route, as evidenced by the capture of three reproductively active brown marbled in that location in February 2005.

Further studies should be directed at determining the main migration routes to the site.

The sharing of aggregation sites by camouflage and brown marbled grouper appears to be a pattern that is widespread across their similar geographic ranges (Sadovy, 2005). Whereas the squaretail grouper, *Plectropomus areolatus* (Rüppell, 1830), often completes a trio of groupers known to aggregate at shared sites, this species was replaced by the marbled coral grouper at our study location. The marbled coral grouper aggregations appear to be of similar dimensions and size to those reported for the common coral trout, *Plectropomus leopardus* (Lacépède, 1802) on the Great Barrier Reef (Samoilys, 1997).

A lower than expected rate of recaptures (or sightings) was obtained, notably in the second season. This could have arisen from unsuitability of the T-bar anchor tags, which may have been torn out during aggressive encounters between males or removed by cleaner wrasses. Both these problems have been identified by other authors using similar tags (Johannes et al., 1999). In spite of this, the tagging results suggest that camouflage grouper exhibit a high level of fidelity to the study site, which appears to be typical for aggregating groupers (Luckhurst, 1998; Zeller, 1998; Johannes et al., 1999; Pet et al., 2005; Starr et al., 2007). However, other findings suggest that patterns in site fidelity differ between sexes and species. Behavioral observations support arrival of male camouflage and brown marbled grouper at the aggregation site before that of females, which was further evidenced by the sex composition of samples taken from aggregation catches. Trends in density and abundance reveal that most camouflage groupers disperse from the site following spawning in the first month of the season. By contrast, large numbers of brown marbled grouper appear to remain on site between spawning months, although tagging studies are needed to determine if the same individuals aggregate and spawn across consecutive months in a season. Male red hind are also known to remain on site between spawning months (Nemeth, 2005).

In view of the existence of other spawning aggregation sites at the atoll, determining patterns of site fidelity and identifying catchment areas and migration routes for camouflage, brown marbled and marbled coral grouper will require further study and would benefit from the application of acoustic telemetry (Zeller, 1998). The use of multiple aggregation sites within a reef system has been described for other species (Shapiro et al., 1993; Sadovy et al., 1994; Samoilys, 1997) and understanding the relationships between multiple spawning sites and reefs of (non-aggregation) residence may be critical to effective management.

This study provides the first in situ verification of grouper spawning aggregations in the WIO and underpins the value of local ecological knowledge for locating and understanding the dynamics of these phenomena in the region (Johannes, 1981; Colin et al., 2003). The findings were largely consistent with fisher knowledge in terms of species identification, aggregation location, and months of formation; although knowledge on lunar periodicity was not always clear (Table 4), presumably as aggregations persist for more than 2 wks. The most knowledgeable fishers tended to be those who have been based at the atoll. Fishers from the inner granitic islands have targeted distant grounds sporadically over the last decade, due to increasing operational costs and stagnant local market prices for reef fish, and are not known to have targeted the aggregations at Site A recently. However, the development of tourism in the outer islands and over-exploitation of stocks closer to centers of population may place greater fishing pressure on outer island grouper stocks, warranting proactive management of spawning aggregations.

Even if spawning aggregation sites are closed to fishing, sexual pattern will be an important consideration for management if non-aggregation demersal line fisheries for groupers are further developed in the outer islands. At high fishing pressure, protogynous hermaphrodites are more vulnerable than gonochoristic species, as populations may become sperm limited through the differential loss of larger males (Koenig et al., 1996; Sadovy, 1996). The sexual pattern has not been confirmed for camouflage and brown marbled grouper but protogynous modes are likely (Rhodes and Sadovy, 2002b; Pears et al., 2006). Bi-modality in size distributions was demonstrated here, but this can arise under conditions not related to sexual pattern.

Meetings with fishers and island managers to discuss spawning aggregation fishery issues have resulted in considerable support for management, with fishers recognizing that commercial aggregation fishing is unsustainable. At Site A, an estimated 920 camouflage grouper were caught by island staff over 4 d in January 2005, constituting 48% of the peak aggregation size (1900 ind.). Over the past decade, the island managers and fishers based at the atoll have refrained from targeting aggregations in some years, or much smaller catches relative to January 2005 have been taken to allow for recovery. This informal management has probably contributed to their persistence. Nevertheless, it is recognized that formal and more scientific management of aggregations is required given the risks of heavy exploitation (Pet et al., 2005; Sadovy and Domeier, 2005). Closed seasons and fishery reserves have been proposed to stakeholders as management options.

Seychelles has one of the oldest networks of marine protected areas (MPAs) in the region (Francis et al., 2002), but fisher support for many MPAs is lacking with poaching commonplace amongst adjacent communities who have lost fishing grounds (Wood, 2004). Few MPAs were implicitly designed to incorporate fisheries management objectives, with most sites chosen for aesthetic value, coral reef conservation, or to protect endangered species. Although the establishment of some MPAs inadvertently protected spawning aggregations, the challenge now is to align spawning aggregation protection with the participatory or co-management of reserves that serve to benefit fisheries.

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