SPAWNING AGGREGATIONS OF GROUPERS (SERRANIDAE) IN PALAU

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AUGUST 1999

The Nature Conservancy Marine Research Series Publication No. 1

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Suggested citation: Johannes, R.E., L. Squire, T. Granam, Y. Sadovy, and H. Renguul. 1999.Spawning aggregations of Groupers (Serranidae) in Palau. Marine Conservation Research Series Publ.#1, The Nature Conservancy. 144pp.

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PREFACE

Unencumbered by the usual need for brevity and a tight focus, we take a more expansive approach to our subject in this report than we will when preparing manuscripts on it for submission to journals. We include considerable practical information for fisheries managers and researchers who may wish to extend our work in Palau or elsewhere. We also discuss a range of issues on which our studies impinge, including the cost-effectiveness of fisheries research and management, getting users of coral reef resources involved in studying and managing them, coping with the destructive impacts of the burgeoning live reef food fish trade, reef fish aquaculture, and the large number of other species that may spawn at grouper spawning aggregation sites.

Our study uncovered substantial, interesting and valuable new information on grouper spawning aggregations. But it did not provide all of the knowledge for which we had hoped. The limitations of our study, and the unexpected and sometimes unresolved problems that emerged during the course of it, are as instructive as its successes. By highlighting these difficulties, rather than dwelling only on the successes, we show how they can be used constructively. Collectively, they indicate that it is unlikely that certain methodological problems and data deficiencies will ever be overcome in a cost-effective manner in reef fin-fish stock assessment. We will argue below that, under these circumstances, we must address the urgent need for management now, rather than shelter behind the usual plea for more research support. We believe our conclusions apply not only to the three species of groupers we studied, and not only to Palau, but also to many other species and to the majority of reef fin-fisheries.

ACKNOWLEDGMENTS

Funding for this project was provided by the South Pacific Forum Fisheries Agency, the Pew Foundation, The Nature Conservancy, the Palau Division of Marine Resources and the University of Hong Kong Research Grants Council.

The project was conducted by R.E. Johannes Pty. Ltd., Palau Division of Marine Resources, the Koror State Conservation and Law Enforcement Department, and Ngarchelong State, with the cooperation and assistance of the Palau Conservation Society, the Palau Environmental Quality Protection Board and numerous individuals.

Logistic support was provided by Chuck Cook, Noah Idechong, Theofanes Isamu, Adalbert Eledui, and Gilbert Demei.

The underwater monitoring team at Ngerumekaol included Umang Demei, Grecencio Isaac, Estephan Kintaro, Garry Merep, and Samuel Sanders, with occasional assistance from Randy Alexander, Jimmy Baiei, Ted Brattstrom, Dick Creed, Anna Fahlen, Marty Fujita, Michael Guilbeaux, Mark Heckman, Ile Lucas, Scott Ciment, Drew Bohan, Lisa King, Teruo Remoket, Helena Walkowiak, Jacques Idechong, Totoi Nabeyama and Prescott Umedip.

The tagging team at Ollei included the authors plus Franzier Albert, Jordan Ewatel, Meribal Ewatel, Takami Idep, Shane Melwat, Asap Bukurrou, Hadley Renguul, Flavin Rodas, the late Rodas Rubasch, Lisa King, Sebang, Nimrod Ryungel, Saiky Shiro, Katsushi Skang, Truman Skang, Eric, Kinny Cheroy, Joe Olsudong, Robinson, Maquin, Allen Shiro, Michael Mechaet Temong, Godinez, Abraham, with occasional assistance from Ethan Daniels, Evelyn Oiterong, Elechang Orak, and Denicio Mariur.

Photographic assistance was provided by Michael Pitts, Steve Wolper, Kevin Davidson, Gui Garcia, Bert and Jan Yates and Anna Fahlen. The cover photo was taken by Kevin Davidson.

A big thanks to two Divsion of Marine Resources staff who made special contributions to the project. Asap Bukurrou put a great deal of personal time and effort into making the initial phase of the project a success and did some useful followup work after the project formally ceased. McCarthy Kotaro contributed his time generously and, at considerable cost to himself, let us use his personal boat on a regular basis.

Lisa King devoted many days and nights to assisting us enthusiastically and expertly with our community awareness campaign as well as with data collection and analysis.

The encyclopaedic knowledge of Palauan reef fish spawning aggregations taught to REJ more than 20 years ago by the late Palauan chief and master fisherman, Malsol Ngiraklang of Ngeremlengui (Johannes, 1981) provided the real starting point for this research. Many of today's fishermen of Palau, notably Sanders Kloulechad, also shared their knowledge of grouper spawning aggregations with us and provided historical information.

All or portions of this report were reviewed by Chuck Cook, Noah Idechong, Melita Samoilys, Andrew Richards, Andrew Smith, and Patrick Colin.

SUMMARY

Three species of groupers, *Epinephelus fuscoguttatus, E. polyphekadion* and *Plectropomus areolatus* aggregate to spawn at overlapping locations and during overlapping seasons in Palau, Micronesia. We monitored spawning aggregations of all three species at each of three sites for 2 - 3 years.

The objectives of our research were to:

1. determine the temporal, spatial, structural and behavioral dynamics of spawning aggregations of these groupers
2. determine the best times, and minimum number of times per year that these aggregations can be monitored routinely to provide a useful index of year to year changes in spawning aggregation size
3. recommend appropriate routine spawning aggregation monitoring methodology
4. evaluate the suitability of Palauan government and tradition-based management regulations for groupers, and to determine if they can be improved upon
5. draw on our results to suggest improved monitoring and management measures for groupers and other reef fish species that aggregate to spawn elsewhere in the Pacific islands and beyond

Our results revealed the following:

1. The timing of spawning aggregations of each species varied somewhat between aggregation sites in terms of months of the year and days of the lunar month.
2. The size distributions and sex ratios of *P. areolatus* also varied between aggregation sites. These differences may have been related to differences in fishing pressure and/or recruitment.
3. Some fish were observed to return to a particular spawning aggregation site each lunar month for up to 16 out of 17 spawning aggregation months. No straying from one spawning aggregation to another was observed. Tagging studies also revealed that fish travelled as far as 10 km from their spawning aggregation sites during inter-aggregation periods.

4. Female groupers characteristically outnumber males in spawning aggregations according to the literature. We found, however, that male *P. areolatus* greatly outnumbered females in one spawning aggregation in the most heavily fished area of Palau. The cause of the male-biased sex ratio is uncertain but may have been either poor recruitment or selective fishing pressure on females outside their spawning aggregations or both. This sex ratio may also have been responsible for the abnormal behavior we observed between the sexes and may have prevented spawning. It may also explain the marked lengthening of the spawning aggregation season of this species at this spawning aggregation site in recent years. Our observations indicate that it can be as instructive to monitor sex and size structure in spawning aggregations as it is to monitor aggregation size.
5. The best days of the lunar month and best months of the year were determined for monitoring fish in spawning aggregations of each of the three species in order to provide truly comparative data. The best days of the lunar month for monitoring the sex ratio in *P. areolatus* - the only species where sexes could be differentiated visually by divers - were also determined. Spawning aggregations of all three species can be monitored simultaneously during some months because of their overlap in time and space.
6. *E. polyphekadion* is protogynous (i.e., changes from female to male as it grows). Evidence suggests that *P. areolatus* is also protogynous. Insufficient information was available to determine the sexual pattern of *E. fuscoguttatus*.
7. There is considerable intermonthly and interannual variation in peak spawning aggregation size that is evidently independent of fishing pressure, management measures or recruitment. This background variability makes it difficult to detect changes in aggregation size due to changes in fishing pressure quickly enough to adjust management measures effectively in response.
8. Significant spawning aggregations of *P. areolatus* and *E. polyphekadion* formed outside of Palau’s closed season for grouper fishing.

9. At least 57 other species of reef food fish also spawn in or near the grouper aggregation study sites, including a number of important food fish. Protecting such sites from fishing can therefore relieve fishing pressure on these species as well as on groupers.

We conclude that:

1. In Palau the following actions are desirable:

- lengthening the closed seasons for groupers
- establishing a year-round, no-fishing zone encompassing Ngerumekaol Channel plus a buffer zone
- prohibiting or controlling recreational diving at Ngerumekaol and other important spawning aggregations
- giving the area surrounding and including the reefs of Western Entrance marine reserve status; and
- prohibiting the export of reef food fishes (which includes banning the live reef food fish trade)

2. Management of groupers and other reef fish to achieve optimum yields or stock sizes is not feasible in Palau or elsewhere. Management with the less precise but feasible goal of protecting spawning stock biomass from serious depletion or local extinction, is a practical alternative. Temporal and/or spatial closures designed to reduce fishing on spawning aggregations can be a cost-effective way of implementing such management. Marine resource managers in the tropics should avail themselves of this opportunity more widely - especially in the Indo-Pacific where such management is at least a decade behind the western tropical Atlantic in this regard.

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INTRODUCTION

Many coral reef food fishes aggregate in large numbers at specific locations, seasons and moon phases in order to spawn (e.g., Johannes, 1978a; Thresher, 1984; Sadovy, 1996). These aggregations are prime targets for fishermen, who often take large catches from them. They also present convenient opportunities for monitoring and management - opportunities that have rarely been exploited by fisheries researchers and managers in the Indo-Pacific. Here we describe the results of a three-year project designed to make better use of these opportunities.

Vulnerability of groupers to overfishing

When groupers (as well as snappers) are exploited, they tend to “decline drastically relative to other components of the reef community and the species can even become virtually extinct” (Munro, l987, p. 649; see also Bannerot et al. l987). One of the reasons for this is their tendency to aggregate to spawn at predictable times and locations, making them especially vulnerable to over-exploitation (e.g., Johannes, 1981; Ralston, l987; Sadovy, l997).

Most groupers are protogynous hermaphrodites. That is, individuals typically begin their reproductive life as females but change to males with age. This trait can render them even more susceptible to overfishing (e.g., Bannerot et al. l987; Huntsman and Schaaf, 1994; Vincent and Sadovy, in press, and see below). Stock assessments on a range of grouper species in the western Atlantic indicate that yield per recruit can stay high if individuals recruit into the fishery at a moderately elevated size (age), and/or if fishing mortality remains low. Where these conditions do not occur, yields rapidly decline (e.g., Sadovy 1994; Huntsman et al. 1983).

This vulnerability to overfishing is also reflected in the fact that the only two fully marine species that are candidates for the U.S. Endangered Species List are both groupers. In 1996, 21 species of grouper were proposed for inclusion on the IUCN Red List as vulnerable or endangered - three of them critically endangered (Hudson and Mace, 1996). Groupers constituted more than 15% of the teleost species on the list.

Researchers and fisheries managers in the western Atlantic have a substantial lead over those elsewhere in their employment of management measures focusing on spawning aggregations. Their concern has been prompted by the disappearance of some spawning aggregations of certain species of the genus *Epinephelus* with a history of heavy fishing pressure, as in Puerto Rico (Sadovy, 1993), St. Thomas (Olsen and LaPlace, 1979; Beets and Friedlander, 1992), Florida (Sadovy and Eklund, in press; Coleman et al. 1966), Mexico (Aguilar-Perera and Aguilar-Davila, 1996), Dominican Republic (Colin, 1992), St. Croix and Belize (Carter, 1989), as well as marked declines in spawning aggregation sizes in Bermuda (Bannerot et al. 1987) and elsewhere in the region. Roberts (1997 & pers. comm.) also asserts that overfishing has eliminated groupers of the genus *Mycteroperca* from broad areas of the Caribbean, and Koenig et al. (1996) discuss evidence that fishing over spawning aggregations contributes significantly to population declines of *Mycteroperca microlepis* in the Gulf of Mexico.

Particularly alarming have been declines in landings of the Nassau grouper, *Epinephelus striatus*, in Puerto Rico and the U.S. Virgin Islands. In Puerto Rico this fish was once “a common and very important food fish, reaching a weight of 50 pounds or more” (Everman, 1900). It is now virtually extinct for fisheries purposes (Bohnsack et al. 1986). About one third of the better documented spawning aggregations of this species have disappeared over the last two decades as an apparent result of heavy exploitation. Declines in numbers and/or mean size have been noted in many of the other spawning aggregations (Sadovy, 1997).

Protection of spawning aggregations

Such information, Sadovy (1994, p. 59) states, “provides undeniable testimony of the vulnerability of grouper species to anything but low levels of fishing effort, in the absence of regulation. For almost all stocks assessed, there have been sharp declines in mean size and weight, reduced landings and CPUE, and loss of, or alarming reduction in, spawning aggregations. More stringent management measures, and their enforcement are badly needed.” Under the circumstances, she says, “...at the very least, spawning aggregations should be protected and monitoring programs implemented or refined and management options evaluated.”

A number of researchers has concluded that the protection of spawning aggregations is the most promising of the various management options for groupers (e.g., Sadovy, 1990; 1994; Bohnsack, 1989; Koenig et al. 1996; Beets and

Friedlander, 1998). Speaking of the western tropical Atlantic, Sadovy (l990, p. 371) states, “Given the current law enforcement limitations of the region, this is the measure most likely to succeed.” Because detailed information for management of the grouper *Mycteroperca microlepis* is “time consuming and expensive to obtain,” Koenig et al. (l996, p. 321) recommend “closure of either the spawning areas or (the spawning) season.” Both these statements seem applicable also to many tropical Indo-Pacific grouper fisheries based on our current understanding of grouper biology.

Many central western Atlantic countries have acted to protect grouper spawning aggregations (reviewed by Colin, 1988; Bohnsack, l989; Sadovy, 1996; Domeier and Colin, 1997). Fishing on some spawning aggregations is totally prohibited in Bermuda, the Dominican Republic and Puerto Rico. Bans are in effect during part of the spawning period in St. Thomas, U.S. Virgin Islands. Fishing on spawning aggregations is limited to residents and to hook and line in the Cayman Islands. Fishing on spawning aggregations is prohibited in Mexico (Aguilar-Perera and Aguilar-Davilá, 1996). Grouper spawning aggregations are protected in some marine parks in Belize. However, in the entire Indo-Pacific area we know of only two countries whose governments have moved to protect spawning aggregations - Palau (Johannes, 1991 and see below), and Pohnpei, where commercial sale of groupers during the spawning season, March and April, is prohibited[6].

Although information on grouper stocks in the Pacific is scant, groupers are reported to have been virtually eliminated by overfishing in at least five locations - in four of them fishing over spawning aggregations was specifically implicated.

1. Hooper (l985 and pers. comm.) reports concern among fishermen of Fakaofo Atoll, Tuamotu Islands, over the “almost complete disappearance” from their waters of a grouper species that was once caught in “sufficient quantities to sink a canoe” while in its spawning aggregations.
2. Bell (l980) was unable to locate a single specimen of *Plectropomus leopardus* on the reefs of two populated atolls in French Polynesia during 40 hours of underwater surveying, nor in the fish market at Papeete, nor in collections from fish poison stations. Yet the species used to be common there and was still common around nearby uninhabited islands.

6. Village leaders in a variety of Pacific island countries, have, however, used various measures to protect spawning aggregations in their waters for centuries (Johannes, 1978b).

3. Two species of grouper disappeared from Rarotonga, Cook Islands shortly after the introduction of spearfishing (Sims, l990).
4. A number of spawning aggregations of *Plectropomus laevis* on the outer reefs of the Northern and Cairns sections of the Great Barrier Reef have either disappeared or diminished greatly in the past ten years (Squire, pers. obs.). Certain spawning aggregations of *Plectropomus leopardus* in the mid-shelf reefs in the same area have also disappeared or diminished greatly within the past 5 years (Squire and Samoilys, pers. obs.). Both events are almost certainly the consequence of targeting spawning aggregations with the aid of global positioning systems (GPS) that are now standard equipment on commercial fishing boats on the Great Barrier Reef.
5. At least five grouper spawning aggregations have disappeared since the 1970s in Palau according to Noah Idechong, ex-chief of the Palau Division of Marine Resources, (pers. comm.), presumably due to overfishing. These are at Rebotel (mostly *P. areolatus*, lost in the 1970s), Mesikm near German Channel (mostly *P. areolatus*, lost in the late 1970s), Uchul a Chei on the southern tip of Ngermediu reef (mostly *P. areolatus*, lost in the early 1970s). Mutiar (mostly *E. polyphekadion* and *E. fuscoguttatus*) lost in the 1990s). Also, within the space of three years in the late 1980s, a large grouper spawning aggregation (mostly *P. areolatus* and *E. fuscoguttatus*) was virtually eliminated at Denges by a live grouper export fishing enterprise[7].

These last two examples are manifestations of the rapid expansion of the live reef food fishery, which continues to spread from its center in Southeast Asia. Although the targeting and depletion of grouper spawning aggregations in this connection is poorly documented, it is undoubtedly a fast-growing problem in areas ranging from the Maldives in the central Indian Ocean through south and southeast Asia and Melanesia in the Pacific as far west as the Marshall Islands (Johannes and Riepen, 1995; Bentley, in press, Johannes, unpub.). At least one large live reef food fish supplier in Hong Kong hired expert Chinese fishermen to fly over prospective Solomon Island fishing grounds to pick out likely grouper spawning aggregation sites based on reef topography.

It is not clear exactly what all of the impacts of fishing will be on spawning aggregations. As well as effects on numbers, there could be impacts on reproduction acting through changes in behavior, age structure and/or sex ratio. One goal of our research was to test the idea that monitoring of spawning aggregation offers a

7. This aggregation has reestablished (Idechong and Johannes, unpubl.), perhaps because some of the fish caught there were released from their nearby holding pens when the company that caught them subsequently closed down.

relatively easy way of obtaining information on changes in some or all of the above that will facilitate more informed management. Accordingly, we investigated interannual, seasonal, lunar and daily variations in the size and sex ratio of grouper spawning aggregations, as well as the reproductive behavior of *Epinephelus fuscoguttatus*, *E. polyphekadion* and *Plectropomus areolatus*. These three species dominate the commercial catch of groupers in Palau (Kitalong and Dalzell, 1993). Our studies were carried out at three spawning aggregation sites at which all three species aggregate to spawn, and that were located within fishing grounds subject to different levels of fishing pressure.

Research objectives

The objectives of our research were to:

1. provide baseline data on timing and structure of spawning aggregations of the three species of grouper and the behavior of the fish in them
2. determine the best times of day, lunar month and season, and the minimum number of times per year that these spawning aggregations should be monitored, using underwater visual census (UVC), in order to provide a useful index of the state of the stocks they represent as indicated by aggregation size and structure
3. recommend appropriate routine spawning aggregation monitoring strategies
4. evaluate the suitability of Palauan government and tradition-based protective management regulations for groupers, and determine if they can be improved upon
5 draw on our results to provide suggestions for the monitoring and management of grouper stocks and stocks of other reef fish species that aggregate to spawn elsewhere in the Pacific Islands and beyond

Temporal variation in spawning aggregations

Changes in the size and other characteristics of spawning aggregations will occur not only in response to fishing pressure, fisheries management measures, or other human influences (e.g., habitat degradation), but will also display natural variations. The size of a grouper spawning aggregation can be expected to vary naturally on four time scales (Johannes, 1981; Samoilys and Squire 1994; Colin, 1996; Samoilys, 1977a):

1. during the course of a single day
2. from day to day during the lunar month
3. from lunar month to lunar month, and
4. from year to year.

Understanding such variations is essential in order to design adequate monitoring procedures. As Colin (1996, p. 191) says, "The importance of re-examining fish spawning aggregations at the same season, lunar phase, and daily time as previous observations cannot be overemphasized because . . . slight alterations in the timing of observations could have drastic effects on the data obtained."

For simplicity and cost-effectiveness, routine monitoring should be carried out during the lunar months, on the lunar days and during the hours of the day that facilitate the most reliable year-to-year and site-to-site comparisons of spawning aggregations. One objective of our research was to identify these months, days and time of day. Previous research on these variations in the timing of grouper spawning aggregations reveals the following:

Diurnal variation

Samoilys (1997a) reported that within the space of a single day *Plectropomus leopardus* spawning aggregation sizes varied; numbers were typically lower during the morning than in the afternoon and evening. See also Colin (1992).

Intra-lunar-monthly variation

In no grouper species for which there is relevant published information do all fish arrive at the spawning grounds on the same day. Over a period of several days to more than two weeks the spawning aggregation size increases gradually (e.g., Johannes, l989; Samoilys and Squire, 1994; Sadovy et al. 1994; Samoilys, 1997a). One sex may begin to arrive on the spawning grounds several days before the other [e.g., males first (Johannes, 1989; Samoilys, 1997a], or females first [Olsen and LaPlace, (1978)].

Seasonal variation

The number of groupers that aggregate to spawn at a given site can be expected to vary from lunar month to lunar month during the spawning season (e.g., Colin et al. 1987; Colin, 1992; Samoilys and Squire, 1994; Samoilys, 1997a).

Interannual variation

Spawning aggregation sizes can be expected to vary naturally from year to year in the same calendrical month (e.g., Johannes, 1981; Colin, 1992; Samoilys, 1997a). One likely cause of such variation is natural interannual variation in recruitment. Russ et al. (1996) reported that catches of *Plectropomus leopardus* on some parts of the Great Barrier Reef were strongly dominated for at least four years in a row by the same single year class. In the Bahamas significant interannual variation in settlement has also been reported by Thorrold et al. (1994) for unidentified groupers and by Shenker et al. (1993) for *Epinephelus striatus*.

Adding a further complication here is the need for “intercalation” - the reconciling of lunar and seasonal timing. To keep such timing in phase over the years requires periodic “correction” of the system by the insertion or “intercalation” of an extra lunar month in the reproductive calendar on the average of about once every three years (Johannes, 1981). This is the same kind of correction that is necessary in connection with the lunisolar calendars devised by various human societies[8].

Intra-regional variation

Johannes (l989) confirmed Solomon Island fishermen’s assertions that a spawning aggregation of *Plectropomus areolatus* begins to form several days later at one particular spawning site in the Marovo area than it does at other nearby spawning sites, although the aggregations dispersed on the same day.

Spatial variation in spawning aggregations

Variation in location of spawning aggregations

Spawning aggregations of a number of grouper species are known to be highly site-specific (e.g., Johannes, 1989; Sadovy et al. 1994; Sadovy, 1997; Colin et al. 1987, and see Thresher, 1984 for a review of the older literature). However, *Epinephelus guttatus* in some parts of the Caribbean aggregate loosely in areas of several square kilometers, but do not always occupy the same sections within such areas every year or every spawning month within a single year (Shapiro et al. 1993; Sadovy et al. 1994). *E. striatus* aggregates at three sites in the Cayman

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8. A given lunar month of a given year is not stricly comparable with any lunar month in the following year because of the lack of synchronicity of lunar and solar cycles. For example, if a new moon occurs on solar day 100 of a given year, new moons will occur on approximately solar days 90 and 119 of the following year and will not occur again on solar day 100 for 19 years. This factor complicates matters for both humans attempting to make year-to-year comparisons of aggregation sizes and, presumably, for the fish themselves, which appear to be attempting to spawn simultaneously on both regular seasonal and regular lunar cycles. For more details see Johannes (1981).

Islands, but not all three sites are necessarily used every year (P. Bush, pers. comm.). Whereas spawning aggregations of *Plectropomus leopardus* were found to be highly predictable spatially at "primary" (major) spawning sites on the Great Barrier Reef, they were less predictable at "secondary" spawning sites (Samoilys, 1997a).

Variation in distribution within spawning aggregations

Samoilys and Squire (1994) established that during the course of a single spawning aggregation of *Plectropomus leopardus*, the spatial distribution of fish changed somewhat over time at aggregation sites. Sadovy et al. (1994). Colin (1992) made similar observations concerning *Epinephelus guttatus* and *E. striatus* respectively.

Components of the study

From May, 1994 through September, 1996 we monitored three spawning aggregations, each composed of the three dominant serranids in Palau's commercial fishery (Kitalong and Dalzell, l994), *Plectropomus areolatus* (**tiau**[9] in Palauan), *Epinephelus polyphekadion* (referred to until recently by most authors as *E. microdon*), (**ksau**), and *E. fuscoguttatus* (**remochel, hludel** or **meteungerel)**[10]. We recorded the temporal trends in aggregation size discussed above. For this purpose 359 monitoring dives were carried out.

We also recorded aspects of the reproductive behavior of the three grouper species (plus more than 50 other species when opportunities permitted), as well as the size structure and the sex ratio of *P. areolatus*.

By means of gonad analyses we obtained information on:

a. reproductive periodicity in spawning aggregations (daily, lunar and seasonal)
b. whether, like many groupers, the species we studied change sex from female to male with increasing size
c. whether all spawners spawn in spawning aggregations
d. whether most fish in spawning aggregations spawn
e. minimum spawning frequency of females within a single spawning month

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9. Although most common fish in Palau have specific Palauan names, most plectropomid species, or coral trout, are commonly lumped under the generic name **tiau.**
10. Palauans often refer to *Epinephelus fuscoguttatus, E. polyphekadion* and other *Epinephelus* species as **temekai.**

Gonad analyses were also used to test the ability of underwater observers to differentiate male and female *P. areolatus*.

By means of tagging of *P. areolatus* and *E. polyphekadion*[11] on two of the spawning aggregation sites we also obtained information on:

a. aggregation fidelity - that is, whether fish moved between different spawning aggregations

b. where groupers went after they left the spawning aggregation site

c. whether individual fish participate in spawning aggregations more than once a year, and

d. how long individual fish stay at a spawning aggregation site

We did not attempt to use tagging data to measure either total population sizes or mortality rates. Past tagging studies of marine fishes have generally failed in this regard (e.g., Hilborn and Walters, 1992).

Recent History of the Palau grouper fishery

Historically, groupers have not been especially esteemed as a food fish among Palauans. Valued more in nearby Guam and the Northern Mariana Islands, groupers have been exported there from Palau for many years. In recent years two other markets assumed increasing importance. Small plectropomids are popular in Palau restaurants, which are proliferating as the number of tourists grow - the latter at an average rate of 16% per year between 1991 and 1996 (Palau Office of Planning and Statistics).

Since l984 a live reef food fish export fishery for groupers has also operated periodically in Palau. Groupers that are kept alive until immediately before cooking, are esteemed by the Chinese in Taiwan, Hong Kong and parts of southeast Asia. This has led to the development of a specialized export fishery for live grouper in Palau. Between 1984 and 1988 at least 54 tonnes of live grouper were exported to Hong Kong (Kitalong and Oiterong, 1991). Exports from l994 through 1996 averaged between 11 and 25 tonnes per year.

The live fishery was operating more or less legally at first; live reef fish tranport vessels came merely to pick up fish caught by local boats and local crews trained by the operators. Frustrated by what they saw as inadequate catches, however, the

11. *Epinephelus fuscoguttatus* were not targeted in the tagging operation due to their large size and strength, and the difficulty of landing them quickly enough to prevent sharks from taking them.

transport vessel operators did more and more fishing on their own (illegal) and in the late 1980s and early 1990s a number of boats were caught and several were confiscated. Legitimate operations more or less ceased in the late l980s, followed by a few years of sporadic poaching, until, in l993, three locally controlled operations began.

The live reef fishery is seen by many Palauan fishermen as contributing to what they say are significant declines in abundance of serranids on certain spawning grounds (e.g., at Denges) and of generally reduced catches per unit effort (e.g Kitalong and Oiterong, 1991; Franny Reklai and Sanders Kloulechad, pers. comm.).

The Marine Protection Act of 1994 prohibits sale or purchase of *Plectropomus areolatus*, *P. leopardus*, *P. laevis*, *Epinephelus polyphekadion* or *E. fuscoguttatus* from April 1 through July 31 each year. This restriction was intended to protect spawning aggregations. The timing of the closure was based on information volunteered by Palauan fishermen on the spawning seasonality of groupers. The law had been widely sought by fishermen for a number of years (Johannes, 1991). In l995 the Act was amended to prohibit any capture of these groupers even for subsistence purposes from April 1 through July 31.

In 1994 traditional leaders of Kayangel and Ngarchelong States also declared a **bul**, or taboo, preventing all fishing at each of eight different sites in their waters during the months of April through July. According to fishermen we interviewed, the locations of these **bul** areas (which include two of our study sites, Ebiil and Western Entrance) were determined on the basis of their observed or assumed importance by fishermen as spawning sites for groupers and other reef food fishes.

A similar four-month **bul** has been enforced sporadically in the state of Ngeremlengui, which shares with Ngatpang State a channel through the western barrier reef. This channel is a spawning aggregation site for several species of groupers. The **bul** was not completely effective in preventing fishing on the spawning aggregations, which were said by Ngeremlengui fishermen to be much smaller than they were 10 years ago.

Since 1976 a national law has prohibited fishing of any kind from April through July in Ngerumekaol Channel, one of our spawning aggregation study sites. Until recently however, as we will discuss further below, enforcement was lax and poaching widely asserted to have been common.

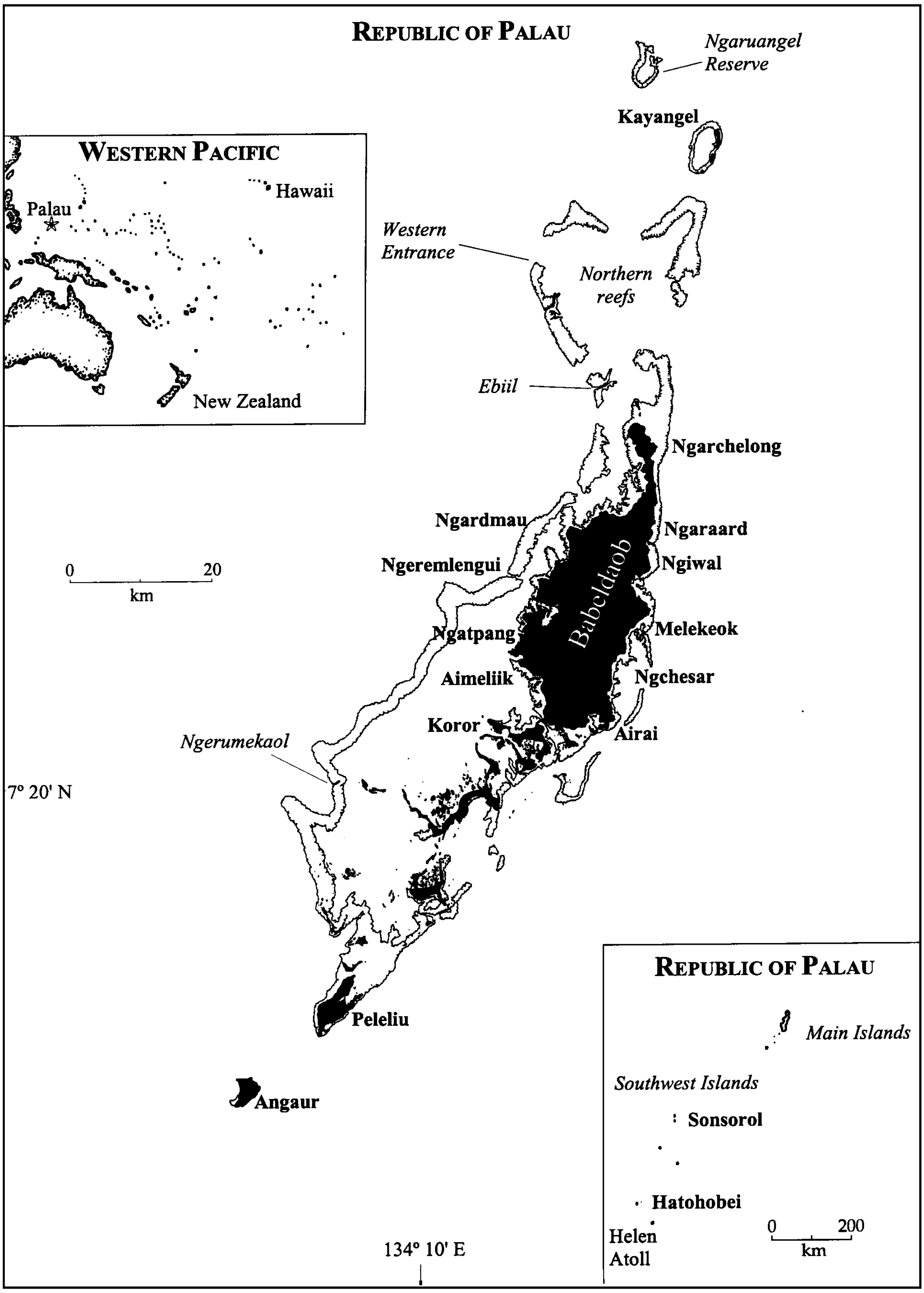
One of the objectives of our research was to evaluate the suitability of these government and tradition-based regulations, and to consider whether they can be improved.

FIELD SITE DESCRIPTIONS

After preliminary reconnaissance of spawning grounds and interviews with fishermen knowledgeable about grouper spawning aggregations, we decided to focus our research on the Ngerumekaol spawning site in Koror State (Fig. l) and, on the Ebiil spawning site in Ngarchelong State (Fig. 1). Reasons for these choices were:

1. During our initial reconnaissance of these sites, in late April 1994, concentrations of the three species of grouper were present in waters shallow enough, and in areas small enough, such that two observers could enumerate all, or a significant fraction of them within less than an hour.
2. Both spawning aggregations had been fished in the past, but were now closed to fishing during the main grouper spawning period.
3. The Ngerumekaol spawning aggregation is only 30 minutes by boat from Koror, Palau's district center, thus providing more convenient access than other Palauan spawning aggregation sites (or most other grouper spawning aggregation sites we know of throughout the tropics).
4. Koror State had requested assistance in formulating a policy for the improved protection of the grouper spawning aggregations at Ngerumekaol.
5. Ngarchelong and Kayangel States had recently requested assistance in formulating a management policy for fishing over grouper spawning aggregations in their shared waters.

Figure 1. Map of Palau showing study sites, Ngerumekaol, Ebiil and Western Entrance.



To obtain permission to study spawning aggregations in tenured waters we held meetings with fishermen of Ngarchelong and Kayangel States and with appropriate authorities in Koror State. Permission was granted in each case[12].

In 1995 we added the Western Entrance spawning aggregation site to our study. It is further from human settlements than the other two sites (Fig. 1). The intention was to provide a spawning aggregation site in an area that was very lightly fished, in contrast with the intermediate fishing pressure in the Ebiil area (coming mainly from nearby villages) and the relatively high fishing pressure in the vicinity of Ngerumekaol. The latter site is within a few miles of Koror, where well over one half of Palau's population resides.

Ngerumekaol

Ngerumekaol is the location of Palau's best known serranid spawning aggregation site, in a dead-end channel situated west of Ulong Island on the southwest side of Palau's main barrier reef in waters of the state of Koror (Fig. 1). It runs ENE for about 1300m from the outer reef edge about two thirds of the way across the reef, where it slopes upward onto a reef flat. It is about 90m wide and averages 7 - 15 m deep (mean depth roughly estimated at 9m).

The steep sides of the outer half of the channel (the portion where groupers were found to aggregate) are covered largely with live coral[13] interspersed with coral rock and occasional small pockets of sand. The bottom is mainly sand with coral and extensive coral rock outcrops. The channel narrows near the mouth, then grades into a less deeply incised coral bottom and wide coral apron sloping gently seaward to the edge of the outer reef drop-off at 10 to 12m. The drop-off plunges almost vertically to a depth of 42m. To the north of the channel mouth between the reef flat and the seaward drop-off is a gradually sea-ward sloping coral and coral rock community. Monitoring was carried out in this area as well as in the channel.

Long-time fishermen in the area agree that the sizes of grouper spawning aggregation at Ngerumekaol have dropped substantially since the 1960s.

Despite the nominal protected status of Ngerumekaol, it did not qualify as an unfished site. As will be discused further below, poaching by both line-fishers and spearfishermen occured.

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12. The rights to fish and to control fishing activities in nearshore waters in Palau have for centuries been owned by the adjacent fishing communities (Johannes, 1981). These rights are legally recognized in Palau's constitution, which states, "Each state shall have exclusive ownership of all living and non-living resources, except highly migratory fish, from the land to twelve (12) nautical miles seaward from the traditional baselines; provided, however, that *traditional fishing rights and practices shall not be impaired."* (Section2, Article 1, TERRITORY).(emphasis added).
13. Coral diversity in the channel has been described as "exceptional", with 90 species counted (Maragos et al.1994).

Ebiil

Ebiil is the site of a channel averaging about 400m wide, leading from the outer reef edge on the northeast side of Palau's main barrier reef and running for about 4 km into the lagoon (Fig. 1). The fishing there is under the traditional control of the people of Ngarchelong State. Until recent years *Plectropomus areolatus*, *Epinephelus fuscoguttatus* and *E. polyphekadion* aggregated on either side of the mouth of this channel. But in recent years, perhaps as a result of fishing pressure, only a few groupers have aggregated on the northern side. The spawning aggregation area on the south side of the channel near its seaward entrance became our second study site.

The spawning aggregation at this site was first fished heavily in about 1988. Until our tagging study began in 1995, line fishing from Ollei had ceased in about 1991 because sharks took too many hooked fish according to fishermen. Some fishermen switched to spearfishing. [Johannes (1981) describes how Palauan spearfishermen protect their speared fish from sharks]. Fishing at night by spearfishermen from outside Ollei also continued and possibly increased (see below).

Spawning aggregations of *E. polyphekadion* extended into areas too deep and too extensive to monitor routinely in a practical period of time. The depth of the portion of the spawning aggregation site we examined varied from 35m at the outermost end to 12m at the innermost. Like the Ngerumekaol site, it was one of moderate coral and rubble coverage interspersed with sand patches.

Western Entrance

Western Entrance, the location of the third spawning aggregation study site, is a 3.3km wide pass between two large reef complexes located on the western side of Palau's main barrier reef close to its northern limit (Fig. 1). The site, which is about 20km northwest of the northern tip of the island of Babledaub, is located at the south side of the entrance. The site is roughly rectangular and measures 45 by 265m. The deepest, northwest portion of the site is about 35m and the shallowest portion, in the east, is 12m deep.

The spawning aggregations at Western Entrance were clearly circumscribed, and our counts encompassed the entire aggregation of each of the three species.

Because of its distance from the nearest fishing village (about 20 km to Ollei) the Western Entrance spawning aggregation was discovered by fishermen only in about 1988 [although its existence was predicted some years earlier by Johannes (l981)]. Fishermen told us that it has been fished much less than the other two study aggregations because of its relative remoteness. Additional disincentives for spearfishermen are its reputation for being frequented by numerous large sharks, including thresher sharks (*Alopias* sp.) and silvertips (*Carcharhinus albimarginatus*), and the belief that the ghost of a Filipino fisherman who died there haunts the area.

METHODS

Survey area and underwater visual census method

Preliminary reconnaissance revealed that the spawning aggregations of the three species overlapped considerably in both space and time at all three spawning aggregation sites. At each of the three study sites, a core area for monitoring was delineated to include the largest proportion of the aggregation that it was possible to count on a single SCUBA tank. The core area of each spawning aggregation was determined by swimming transects across it.

Monitoring all three species simultaneously required making a trade-off between completeness and the time involved. The outer boundaries of some aggregations were ill-defined and somewhat fluid along certain portions of site peripheries. This made total counts of the visible members of some spawning aggregations impractical during the peak spawning aggregation periods because of the long searches that would have had to be made at the edges of these aggregations.

At Western Entrance the entire spawning aggregation of all three species was always contained within the core (monitored) area. But during peak spawning aggregation periods at Ngerumekaol and Ebiil, aggregations expanded beyond core areas. In addition, a portion of the *E. polyphekadion* aggregation at Ebill (possibly more than 50%, see Appendix II) that was was too deep to monitor routinely. Therefore, although we were able to determine day-to-day, seasonal, and interannual *trends* in aggregation size, we were unable to determine absolute numbers of fish in these aggregations.

In the Ngerumekaol area, spawning aggregations of all three grouper species were concentrated in the outer half of the channel and/or seaward of it, especially in and to the immediate north of the channel apron. We monitored numbers of fish both in the channel and in the northern near-mouth area.

All visible fish of the three species were counted. One count was made on the northern side of the channel, another on the southern side. All fish visible in the area extending from the upper edge of the channel to about two meters onto the sandy bottom of the channel from the reef edge were counted. After divers were trained in size estimation (see below) the lengths of individual *P. areolatus* within 5cm were also estimated. Size estimates of the other two species were rarely made because of time constraints. The sex of each *Plectropomus areolatus*, a species for which the sexes were visually distinguishable on the spawning grounds (see below) was also recorded.

At Ngerumekaol, SCUBA was always used near the channel mouth and in the middle of the channel. Snorkel was used during the first year along the channel walls. The team then switched to SCUBA along the deeper of the two channel walls. (See Appendix II for a discussion of the rationale for, and logistics of, making this switch).

Total counts of fish in the Ngerumekaol spawning area were impractical at times of peak spawning aggregations in what was deemed a reasonable amount of time for two divers - that is, one hour or less. Searching for the shifting limits of the aggregation outside the channel each time a count was made would have required too much time for the purpose of routine monitoring. The areas we selected for counts were those in which the fish were consistently most concentrated, and, based on transect surveys, we estimate that our counts accounted for well over half of the fish that were visible on the entire spawning ground during periods of peak aggregation periods.

At Ebiil and Western Entrance, the counts were always performed using SCUBA.

E. polyphekadion attained much higher densities on the spawning grounds than the other two species - so high, in fact, that it was sometimes difficult to count them individually. Under these conditions we found it helpful to count them in units of five or ten.

We refer to the counts we made at the three study sites and discussed below as “research monitoring”, and distinguish it from “routine monitoring”. Research monitoring involved counting of fish during various periods of daylight, during most tidal stages and lunar phases, and throughout the spawning season of the three species over the three years of the study.

One of our objectives was to design a routine monitoring protocol. This entailed deciding upon a practical compromise between comprehensiveness and costs for estimating spawning aggregation sizes and composition, based on the results and experience gained during research monitoring. Routine monitoring is for use during the shortest period of the spawning season that can provide a useful measure of year-to-year and month-to-month changes in maximum spawning aggregation sizes - and, in the case of *P. areolatus*, sex ratios.

Both Western Entrance and Ebiil spawning aggregation sites bottomed at about 35m, with a working depth for monitoring of 20 - 30m. Bottom time on SCUBA was therefore restricted at these sites. This made less time available than at Ngerumekaol for general behavioral observations; most dive time had to be used for counting. At peak aggregation times the counts took 45 - 55 minutes; divers had to work to dive tables and stay as shallow as practical to do the counts.

The monitoring team consisted of a boat handler and a two-person (or occasionally one-person) dive team[14]. One diver was responsible for the primary count, the other, if present, acted as buddy and secondary observer. The buddy position was often taken by divers being trained to become familiar with the site, with the underwater identification of grouper species, and with monitoring procedures.

Tagging

Tagging was conducted on spawning aggregations from March through August 1995 at Ebiil and Western Entrance during the ten day periods prior to the new moon (i.e., during peak aggregation periods). This maximized the number of tagged fish per unit effort. The tagging operation typically consisted of four vessels (usually 21 - 23ft Yamaha boats with 70 - 100hp outboard motors) with two fishermen and one tagger/recorder in each, fishing conventionally with hook and line according to local custom.

When the catch was coming in too fast for the tagger/recorder to handle alone, one fisherman assisted on a rotating basis. Once caught, a fish was held in a tub of seawater to maintain it in good condition, measured, then sexed if possible by stripping, i.e., pressing the abdomen to expel some eggs or milt. Excess gas was released from the swim bladder using a hypodermic needle. [Research on other species indicates that this treatment, if done properly, does not decrease long term survival measurably (e.g., Bruesewitz et al. 1993; Keniry et al. 1996)].

The fish was then tagged. Ten to 12cm Hallprint[15] dart tags were used. The tag was inserted into a sharpened hollow tube, which was then inserted into the muscle below a point half way between the last dorsal spine and the posterior edge of the soft dorsal. The tag was inserted at an acute forward-pointing angle slanting inward toward a point above the vertebral column so that the barb would catch and lock onto one of the vertebral spines or one of the pterygiophores, i.e., the spines extending below the soft dorsal. When the tube was withdrawn the tag remained, anchored by the barb.

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14. Wildly unreasonable scientific diving safety regulations have created bureaucratic and economic nightmares for marine researchers in places such as the U.S., U.K. and Australia and discouraged many underwater research projects altogether. If we had been forced to abide by such regulations it would have increased the cost of our research by several tens of thousands of dollars and amplified logistic problems substantially while achieving little obvious increase in safety.
15. 27 Jacobsen Crescent, Holden Hill, South Australia, 5089

Each tag had a unique number stamped at either end, so that if the number at the exposed end of the tag became obscured by fouling organisms or by other fish biting the tag, the duplicate number, buried in the fish near the dart end of the tag, would still be visible when the tag was removed. Different colored tags (white, yellow, blue or green) were used for different tagging runs.

During line fishing over the spawning aggregations for the tagging program, grey reef sharks *Carcharhinus amblyrhynchos* and white-tip reef sharks *Triaenodon obesus* often attacked hooked fish. At least 47 fish were lost to sharks during tagging operations, 1.7% of the fish hooked.

When sharks became a problem[16], line fishermen tended to pull in hooked fish faster than otherwise. The rapid change in pressure experienced by such fish would result in the eyes, gut and swim bladder protruding from the fish. This was the main reason for death of landed fish. Gill-hooking was the second most common cause of injury to fish.

Training

An important facet of our project was the training of Palauans in routine monitoring of spawning aggregations and in tagging.

A three-day training course in size estimation of groupers was conducted for Koror State Rangers and Division of Marine Resources staff using the procedures described in the Manual for Assessing Fish Stocks on Pacific Coral Reefs (M. Samoilys, ed., 1997).

Cutout wooden silhouettes of groupers ranging in length from 15 to l00cm in 1cm intervals were used. Students had to assign each of these model fish, strung out at random on six lines in the water, to appropriate 5cm size categories. Divers were considered to have passed the course when they could repeatedly assign 75% of the silhouettes presented to them underwater to the correct size category. Some students achieved an accuracy of over 90%.

Twenty-four Ollei fishermen participated in the tagging project and were taught how to tag fish and record appropriate data. They were also shown the correct procedure for releasing excess gas from the swim bladder - i.e., inserting a hypodermic needle into the body cavity below the pectoral fin, rather than through the anus or mouth. (Insertion at either of the latter two locations results in the penetration of the gut wall and the introduction of infection into the body cavity).

16. Sharks never posed serious problems to divers, however.

Gonad analysis

Two hundred thirty eight gonads (ovaries and testes) of the three grouper species were examined histologically, and more than sixty others were examined macroscopically in circumstances where the fish could not be dissected (fish market, tagging operations). The fish from which they were obtained came from Ebiil and Western Channel spawning aggregation sites, miscellaneous fishing grounds and the Koror fish markets. Sampling of gonads from fish at the Ngerumekaol aggregation site could not be carried out since the area is off limits to all fishing during the peak spawning season.

Fish were measured for length in millimeters. The gonads were removed and fixed in 10% formalin. They were then preserved in 70% alcohol, embedded in paraffin, sectioned at 6 - 8 microns and stained in haematoxylin and eosin.

Additional groupers were measured at fish markets and sexed by stripping (i.e., using pressure on the abdomen to extract eggs or sperm in ripe fish). One of us (HR) developed a means for sexing dead fish whose near - mature gonads could not be extruded simply by applying pressure. He inserted a finger in the vent, then broke up the gonad internally, making it easy thereafter to extrude a sample.

Stages of sexual maturation were assigned on the basis of criteria described by Sadovy et al. (1994). Reproductively active females contained late stage vitellogenic (yolk - bearing) oocytes (eggs) (called stage 4) and hydrated oocytes (ripe eggs that have taken up water shortly before being spawned) (stage 5).

Ovaries of fish that had recently spawned could be distinguished by the presence of post - ovulatory follicles (collapsed egg envelopes). These remain visible in gonad sections for a day or so after eggs are released, and thus can be used as evidence of recent egg release.

Reproductively active males contain sperm within the seminiferous tubules and sperm ducts. At the height of the reproductive season, the testis is essentially a bag of sperm, with none of the earlier stages of spermatogenesis evident.

Sexual patterns were determined on the basis of the size distributions of males, females and specimens with gonads transitional between male and female phase according to the criteria of Sadovy and Shapiro (1987). Sex was also determined on the spawning grounds during tagging operations, when practical. Sex determination of aggregating groupers by stripping or cannulation was not always possible because some individuals of both sexes were fully ripe yet would not yield gametes. Fish caught early on the day of peak aggregation size (which was also usually the day of peak spawning activity, see below) were found to be harder to strip in the morning than in the afternoon closer to their probable spawning time. Overall, 44% of the fish examined were able to be sexed in this manner.

Cannulation, which involves sucking a portion of eggs or sperm from the fish through a small tube, proved unpalatable to Palauan team members, so it was seldom used on the fishing grounds.

Developing community support for the study

During introductory meetings with the Ollei community, team members became aware that distrust had been generated by the activities of past researchers. Villagers said that previous researchers had collected valuable information from them and from the surrounding environment without the community being notified of the resulting findings or conclusions. Villagers were left feeling cheated. They were thus initially skeptical concerning the value to them of our project.

To reassure them, as well as to enlist their considerable involvement in the project, we set up a community awareness program. Fortunately, locally respected Division of Marine Resources personnel were active team members. As well as carrying out their roles in the research, they also interpreted, liaised, assisted with logistics, advised on cultural etiquette and generally helped smooth the way for the rest of the team. As a result, an excellent rapport with the Ollei community developed, along with the feeling that fishermen and researchers were genuinely on the same team, working for a better understanding of local grouper spawning aggregations and better management of grouper fisheries.

We gave presentations about grouper spawning aggregations and marine conservation at Palauan schools, targeting 6 - 8 graders, and conducted a coloring contest. The children colored a picture of a tagged grouper swimming on a reef. Winners received a Grouper Aggregation Study T-shirt at a special awards assembly. This approach had two advantages. First, teachers became familiar with the project and turned into project-supporters within the community. Secondly, students learned about the study and talked about it with their parents; many of their fathers were fishermen. The overall effect was that the community saw a high time-investment by researchers in other local activities beside the actual study, and realized that the researchers really did care about the community and its future.

We also adopted an open house policy and encouraged fishermen to stop by our accommodation and talk. Evenings became a social time to become better acquainted and share ideas. Children would also drop in to ask for additional coloring materials or to ask questions.

We also called special evening meetings to update the community about the project, sometimes providing transportation to the meetings. The team acted as hosts and refreshments were provided.

Story boards are boards with stories depicting Palauan legends carved into them. We developed “paper story boards” using stick figures and plain language to tell the story about why the team was in Palau and what they hoped to accomplish. The paper storyboards were used to help explain the project and were later hung on the walls of elementary school libraries. The feature of grouper biology that most fascinated our audiences was the fact that groupers change sex.

Boat owners who assisted the tagging project were paid U.S.$20.00 per day and had fuel supplied. Fishermen were paid U.S.$15.00 a day with a bonus of U.S.$1.00 for each fish tagged. T-shirts with a special grouper logo were given to fishermen who caught tagged fish. All tagged fish caught during the tagging periods were returned to the water except for a few that arrived at the surface in poor condition (e.g., gill-hooked or bitten by sharks). Fishermen were informed that all tags would be placed in a bin and that the owners of three tags selected at the end of the project would win one of three prizes consisting of vouchers for U.S.$100, U.S.$75 and U.S.$25 worth of fishing equipment. (One outstandingly persistent fisherman won two of the three cash prizes and 28 T-shirts!).

At the end of the project we had a final party/meeting with the fishermen at Ollei, at which we presented them with their prizes, summarized our results and promised to send them a specially written summary and suggestions for management once we had completed the analysis of our results.

We also provided information regularly to appropriate government agencies. In addition, regular appointments were made with relevant state governors and interested non-governmental organizations, to keep them updated on the project and the channels of communication open.

As a consequence of these activities, we suspect our project became one of the most widely understood and supported research projects that has ever been carried out in Palau. (One government official proved to be an emphatic and troublesome exception).

RESULTS

Temporal variations in spawning aggregations

Diurnal variation

At Ngerumekaol we made four successive counts of each between 11:00 and 17:00 on two different occasions. We found no significant variation in numbers of any of the three study species during different times of day. We think it inevitable, however, that a more thorough study would have revealed significant changes in numbers during the day - a subject we deal with in Appendix I.

Intra-lunar monthly variation

Peak counts of all three species occurred between one and seven days before the new moon. [In fact, in all but one case (*E. fuscoguttatus* at Ngerumekaol) they occurred between 2 and six days before the new moon (Table l)]. The calculations involved in describing these and other statistics that relate to lunar periodicity of spawning aggregations of the three species at each of three sites over three years were lengthy. The details are discussed in Appendix I. They include calculating the best lunar days on which to monitor in order to get comparable data from one month or year to the next.

Table 1. Lunar days on which spawning aggregation counts peaked during the study

Location	Days Before New Moon
Ngerumekaol	
Plectropomus areolatus	**2,3,4**
Epinephelus polyphekadion	**4,6**
Epinephelus fuscoguttatus	**1,2,4,7**
Ebiil	
Plectropomus areolatus	**3,4,5**
Epinephelus polyphekadion	**3,4,5,6**
Epinephelus fuscoguttatus	**4,5,6**
Western Entrance	
Plectropomus areolatus	**4,5,6**
Epinephelus polyphekadion	**4,5,6**
Epinephelus fuscoguttatus	**4,5,6**

In those months when two or more species aggregated at a given site, the days on which counts of each species typically began to increase, peaked, and on which the fish disappeared from the site (or were reduced to just a few fish) all occurred within two days of each other (Fig. 2).

Figure 2. Typical gradual build-up, plateau and rapid decline of counts of groupers in spawning aggregations.

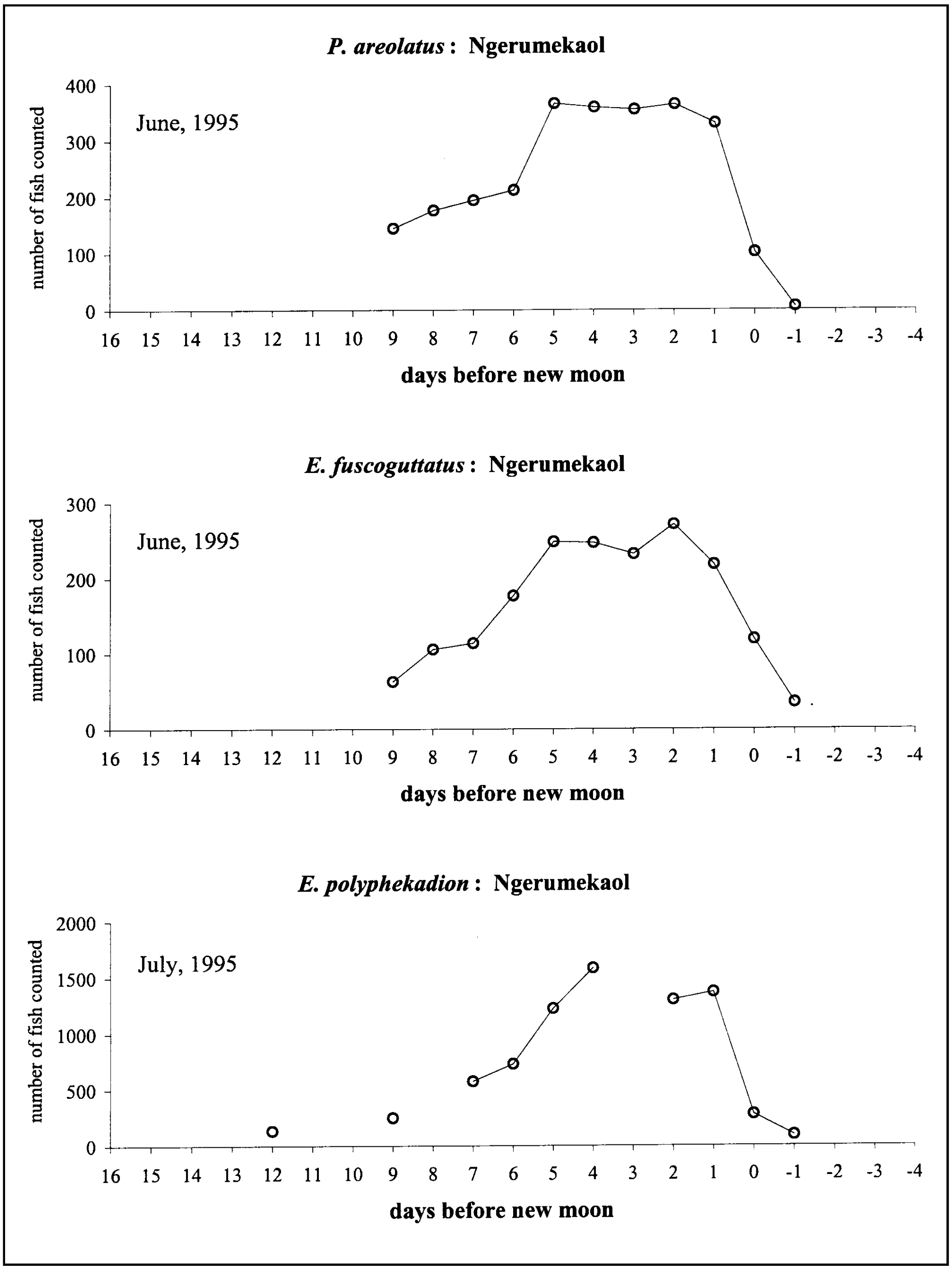
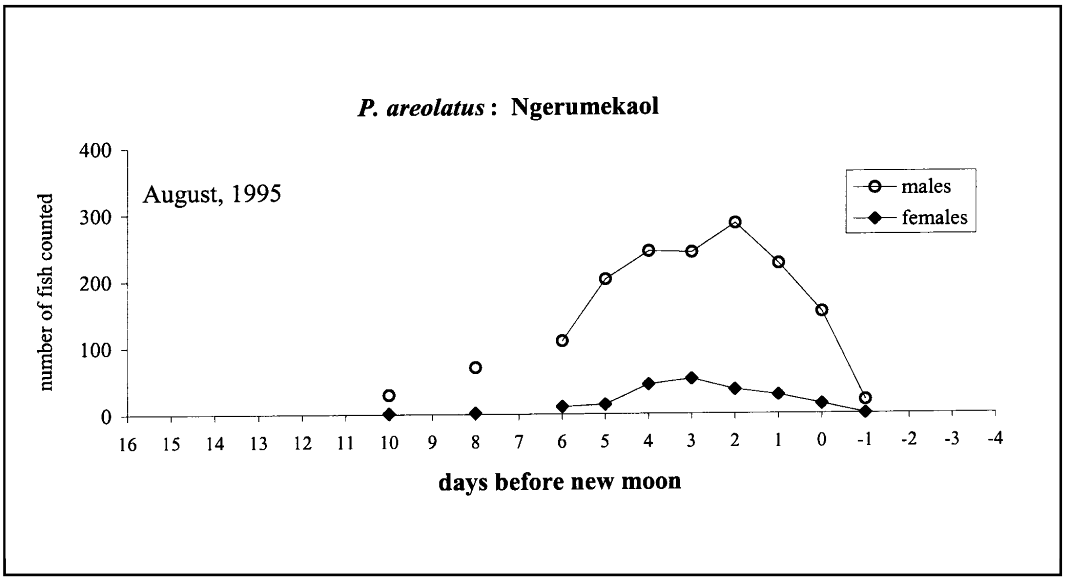


Figure 3. Typical difference in timing of arrival at and departure from a spawning aggregation of male and female *Plectropomus areolatus.*



Each symbol represents the highest count made during each lunar month. A missing symbol indicates that no counts were made during that month.

Male *P. areolatus* typically began arriving earlier than females at the aggregation sites. (How we were able to differentiate between male and female *P. areolatus* while they were in their spawning aggregations is described in a later section of this report). For example, in August 1995, male *P. areolatus* began to increase in numbers at the Ngerumekaol site about 10 days before the new moon (Fig. 3). Females began to appear at the site 4 days later. Johannes (1989) observed similarly that male *P. areolatus* began to aggregate at a spawning site in Marovo Lagoon, Solomon Islands five days before females.

Three aspects of the lunar monthly timing were examined to see if there were significant differences by sex:

1. arrival day (the mean day of the lunar month on which 50% of the maximum numbers of fish were first counted at the monitoring site)
2. peak day (the mean day of the lunar month on which the month's peak number of fish was present)
3. departure day (the mean day of the lunar month on which the fish count had decreased to 50% or less of the month's peak)

For each of these three aspects, Table 2 shows the mean day of the month (in days before new moon, or "DBNM") for each sex at each of the three sites. Comparisons were made using paired t-tests.

Table 2. Sexual differences in arrival, peak and departure times, in days before new moon, of P*lectropomus. areolatus* for three spawning aggregations.

	Ngerumekaol			Ebiil			Western Entrance		
	n	Male	Female	n	Male	Female	n	Male	Female
arrival	7	7.0*	4.8	12	6.7*	5.5	11	6.3*	5.3
peak	7	3.4	3.6	12	3.9	4.7	11	5.1	5.0
depart	7	-0.1*	1.1	11	1.6*	2.5	11	2.6	3.0

* indicates a significant difference between sexes at the 95% confidence level.

At all three sites males arrived significantly earlier and at two sites they left significantly later than females. The difference in arrival times was about two days at Ngerumekaol and one day at both Ebiil and Western Entrance. The males were found to depart significantly later than the females at Ngerumekaol and Ebiil (1 day), but not at Western Entrance. At none of the three sites was the mean day of peak counts found to differ significantly between the sexes.

Histological analysis of gonads of *P. areolatus* (n=86 fish) indicated that peak reproductive activity occurred from 2 to 5 days before the new moon in each of the four months of 1995, May through July, for which samples were available. Spent individuals were observed in Koror fish markets from one day before to two days after the new moon. Peak spawning activity thus occurred with pronounced lunar periodicity and concurrently with peak aggregation counts in each of the four months during which gonads were sampled.

The rapid decrease to near-zero counts of *P. areolatus* typically observed near new moon at each of the sites occurred as follows: at Ngerumekaol, it occurred on the day of, or day after new moon; at Ebiil, it occurred from two days before, to the day of new moon; and at Western Entrance it occurred one to three days before new moon.

The mean day on which all three species reached peak counts (and, in the case of *P. areolatus*, peak f:m ratios) was between 3 and 6 days before the new moon (Table 3).

Table 3. Mean day before new moon of peak count of three grouper species and of peak f:m ratio of *Plectropomus areolatus*. (standard deviations in parentheses)

	Ngerumekaol	Ebiil	Western Entrance
P. areolatus	3.1 (0.7)	3.9 (1.07)	5.1 (0.8)
peak f:m ratio	3.2 (1.2)	5.6 (1.43)	5.4 (0.8)
E. fuscoguttatus	2.9 (2.0)	5.3 (0.82)	4.5 (0.9)
E. polyphekadion	4.5 (1.0)	5.7 (0.96)	5.3 (0.7)

These mean peak days were compared statistically between species at each site and between sites for each species. Correlation analysis was used to determine whether the peak days were related among species and sites. For example, if the timing of the peak day for one species was later than usual in a given month, was the timing of the other two species also later than usual? If the timing of all three species at a given site was later than usual, was the timing at the other sites also later than usual? Answers to these questions can help reveal whether or not these species are cueing to environmental variables other than lunar and solar rhythms and/or to each other.

At Ngerumekaol, there was no significant difference in the timing of peak aggregation counts of *P. areolatus* and *E. fuscoguttatus*. The mean peak day for *E. polyphekadion*, however, was about two days earlier than those for both *P. areolatus* ($p = 0.04$) and *E. fuscoguttatus,* although the latter difference was not statistically significant ($p = 0.08$).

At Ebiil, the mean peak day for *P. areolatus* aggregation counts was also later than for both other species, but only the difference with respect to *E. fuscoguttatus* was significant ($p = 0.03$). The mean peak days for aggregations of the two epinephelids were almost identical.

At Western Entrance the mean day of peak counts for each species was not significantly different from that of the other two species.

For *P. areolatus* the mean day of peak aggregation count was not significantly different from the mean day on which the f:m ratio reached its peak at Ngerumekaol and Western Entrance. At Ebiil, the mean peak day for *P. areolatus* f:m ratio occurred almost two days earlier than when total counts peaked (p = 0.005).

To compare the size of a spawning aggregation from month to month, it is first necessary to choose a descriptor of "size". We chose the peak counts in a given month for the purpose[17]. The timing of the peak spawning aggregation counts for the three species differed from site to site.

The Ngerumekaol spawning aggregations tended to peak later in the lunar month than the aggregations at the two northern sites (Table 3). One environmental factor that could be related to these geographical differences is differences in tidal cycles. The tidal wave crosses northern Palau first[18]. However, the difference in timing of spawning aggregations between Ngerumekaol and the two northern sites is opposite to what might be expected. The day that a given tidal phase occurs at a particular time of day (e.g., high tide at sunset) can be expected to occur about one day later at the northern sites than at Ngerumekaol. Yet peak aggregation counts occurred a day earlier at the northern sites.

For *P. areolatus*, the peak counts occurred, on average, first at Western Entrance, then at Ebiil, then at Ngerumekaol (Table 3). The difference in timing between Western Entrance and Ngerumekaol was significant (p = 0.0008), between Western Entrance and Ebiil significant (p = 0.01), and between Ebiil and Ngerumekaol not significant (p = 0.09).

The day of peak f:m ratios of *P. areolatus* (Table 3) was not significantly different between Ebiil and Western Entrance (p = 0.7). The day of peak sex ratio at Ngerumekaol was significantly later than at both Ebiil and Western Entrance (p = 0.0007 and p = 0.007 respectively).

For *E. fuscoguttatus*, the days of peak spawning aggregation size (Table 3) at the two northern sites were not significantly different (but there were only three common months to compare). The peak day at Ngerumekaol was significantly earlier than at both Ebiil (t = 6.64; p = 0.001) and Western Entrance (t = 6.43; p = 0.02).

For *E. polyphekadion*, there were no significant differences in timing of peaks among the three sites, but only 2 - 4 common months were available to compare (Table 3).

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17. For some purposes peak f:m ratios or other indices might be preferable.
18. The tidal lag was not investigated during this study, but the tidal predictions of the U.S. National Oceanographic and Space Administration indicate that a given tidal phase will occur obout one hour earlier in the areas of Babeldaob (north of Koror) than near Koror.

Duration

We define the duration of an aggregation as the number of days from the first day that 50% of more of the month's peak occurs to the first day that the aggregation size has decreased to 50% or less of that peak. The mean durations for each species at each site are shown in Table 4.

Table 4. Mean duration of spawning aggregations

	Ebiil	Ngerumekaol	Western Entrance
P. areolatus	4.5	6.9	3.6
P. areolatus males	4.8	7.2	5.5
P. areolatus females	3.1	3.7	3.5
E. polyphekadion	4.0	5.0	4.0
E. fuscoguttatus	5.0	5.7	3.3

The differences in durations between sites and sexes were significant as follows:
P. areolatus:: Ebiil vs Ngerumekaol (p = 0.003)
E. fuscoguttatus : Ebiil vs Western Entrance (p = 0.04)
P. areolatus males vs females: Ngerumekaol and Ebiil (p = 0.05)

The mean duration of *P. areolatus* spawning aggregations was several days greater at Ngerumekaol on the average than at Ebiil. The mean duration of spawning aggregations of *E. fuscoguttatus* was significantly greater at Ebiil than at Western Entrance.

The duration of the male component of *P. areolatus* aggregations was significantly greater than that of the female component at Ngerumekaol and Ebiil. This may also have been true at Western Entrance, but, for logistic reasons, there were only two months during which sex ratios were determined here, thus preventing a meaningful test of significant difference.

Seasonal variation

How large a gathering of spawning fish constitutes a "significant" spawning aggregation must be arbitrarily defined. For present purposes we define a significant aggregation as one which, at its peak, is at least 25% of the largest aggregation of that species at that site during that spawning season. Table 5 summarizes the months during which significant aggregations occurred.

Table 5. Months of significant spawning aggregations of groupers

Species	Year	Ngerumekaol	Ebiil	Western Entrance
P. areolatus	1994[1]	May, June, July(?)[1]	na	na
	1995[2]	Feb-March*-Sept	May, July, Aug*	July*, Aug
	1996[3]	Jan.-Feb*-Aug	May-Aug*	May*,June
E. polyphekadion	1994[4]	June*	na	na
	1995[2]	June*, July	July*	July*, Aug
	1996[3]	June*	July*	June, Aug*
E. fuscoguttatus	1994[4]	May*-June	na	na
	1995[2]	May June*-Aug	July-Aug*	May, June*,Aug
	1996[3]	May-June*	June*, July	June,July,*Sept

* denotes months of highest annual spawning aggregation size

[1]Monitored monthly May through December at Ngerumekaol only; monitoring started too late in the year to determine the peak spawning month for *P. areolatus,* nor, with certainty, what the significant spawning months were.

[2]Monitored monthly throughout the year at Ngerumekaol and Ebiil; monitored from January through July at Western Entrance

[3]Monitored monthly January through August

[4]Monitored monthly May through July at Ngerumekaol

Plectropomus areolatus

At Western Entrance *P. areolatus* formed significant spawning aggregations from May through August in l995 and 1996. (This site and Ebiil were not monitored in l994). In 1996 this species also formed significant spawning aggregations from May through August at Ebiil. But in 1995 at Ebiil significant spawning aggregations formed from April through November.

In 1994 *P. areolatus* ceased to form significant spawning aggregations at Ngermekaol in either September or October (no monitoring could be carried out in September) (Table 5). Starting in January 1995, however, significant spawning aggregations of this species at Ngerumekaol formed every month for the last 21 months of our study. This had not been anticipated by fishermen nor ever observed by them in the past.

Peak spawning aggregations of *P. areolatus* were in February or March at Ngerumekaol, in May at Western Entrance and in August at Ebiil (Table 5).

Epinephelus polyphekadion

Significant spawning aggregations of *E. polyphekadion* formed only between June and August (Fig. 5, Table 5). But in one or two of these months (depending on the year) aggregation sizes reached a pronounced peak.
At Ngerumekaol by far the largest spawning aggregation of this species occurred in June and/or July, whereas at Western Entrance and Ebiil (Fig. 6) the largest spawning aggregations occurred for one or two months between late June and August.

At Ngerumekaol, *E. polyphekadion* formed significant spawning aggregations during June and July in 1995 (Fig. 5, Table 5). This was not inconsistent with the single month of aggregation observed in 1994 (early July), in that the two lunar months in 1995 bracketed (on the solar calendar) the single lunar month in 1994. [Johannes (1981) discusses the complications introduced by the need of fish to reconcile their lunar and solar reproductive “calendars”.]

The season’s last significant spawning aggregation of *E. polyphekadion* occurred as late as early August (1995, 1996) at both northern sites, thus extending one month beyond the closed season.

Peak spawning aggregations of *E. polyphekadion* occurred in June at Ngerumekaol, and in July or August at the northern sites.

Epinephelus fuscoguttatus

Depending on the year, either three or four significant spawning aggregations of *E. fuscoguttatus* formed between May and August at all three aggregation sites (Fig. 6, Table 5). In l995 a significant spawning aggregation also formed at Ebiil in September. Significant spawning aggregations of this species therefore occurred as late as two months after the fishing season closed.

At Ngerumekaol, spawning aggregations of *E. fuscoguttatus* were observed in May through August in 1995-96, thus extending a month beyond the seasonal closure.

Peak spawning aggregations of *E. fuscoguttatus* occurred in May or June at Ngerumekaol, June or August at Ebiil and June or July at Western Entrance.

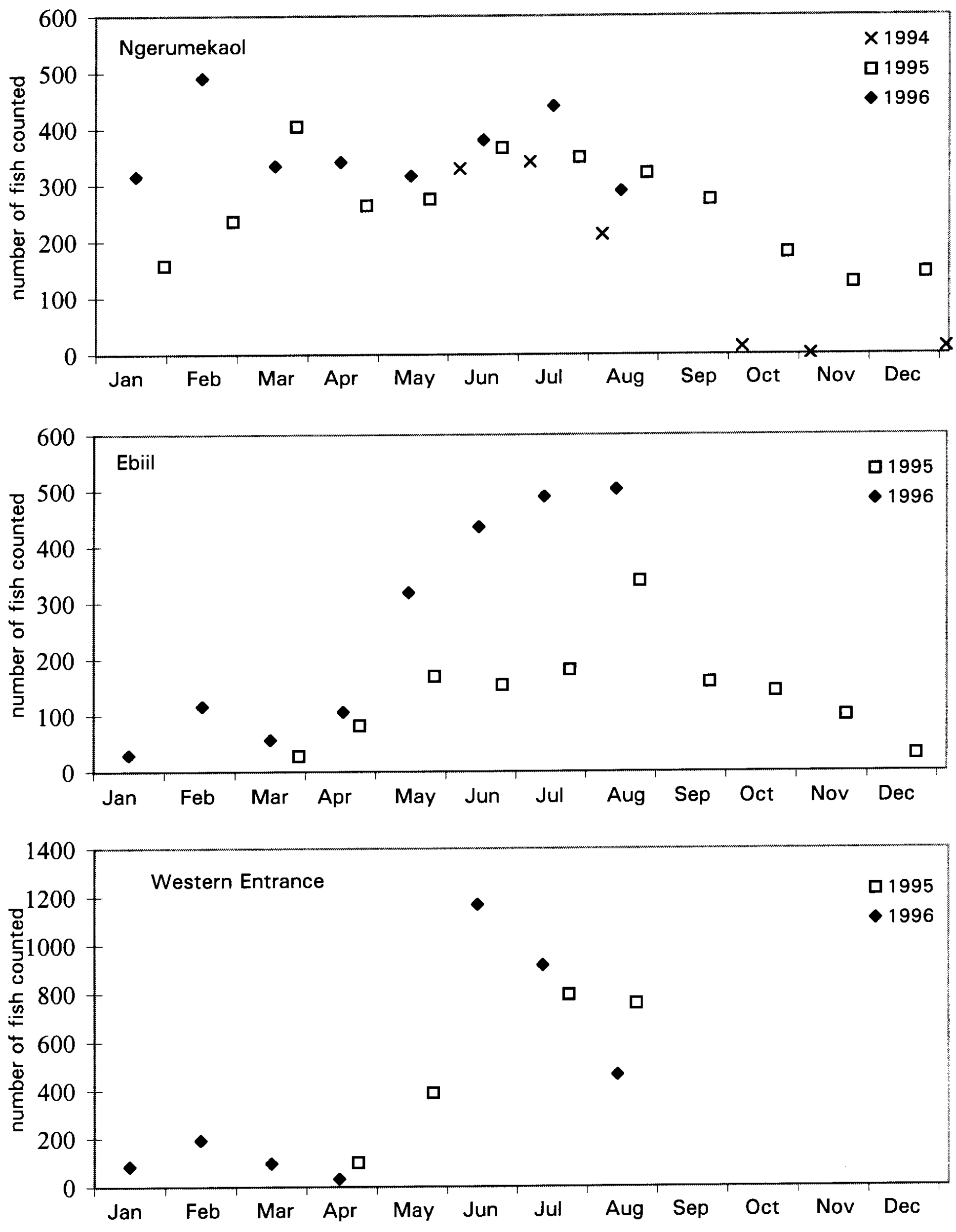
Interannual variation

Starting in June 1995, the area of Ngerumekaol in which fish were counted was expanded in order to cover what was found to be an important area for *E. fuscoguttatus* during peak aggregation periods in peak aggregation months. To make those early fish counts comparable with the later, expanded counts, the early counts were adjusted with coefficients that related the two counts. The fish counts presented throughout this report for the months May 1994 through May 1995 at Ngerumekaol (e.g., in Figs. 4, 5, 6, and Table 6) are therefore not the actual raw counts, but the adjusted counts. The regression functions used to make the adjustments are presented in Appendix II.

The maximum annual spawning aggregation count for all three species varied substantially from year to year (Figs. 4, 5, 6, and Table 6). The maximum annual spawning aggregation count for *E. polyphekadion* in 1996 was more than double that of 1995 at both Western Entrance and Ebiil. The maximum spawning aggregation count for *E. fuscoguttatus* was 61% larger in 1996 than in 1995 at Ebiil. The 1996 maximum annual spawning aggregation count for *E. polyphekadion* at Ngerumekaol was nearly 50% greater than that for l995. Maximum spawning aggregation count of *P. areolatus* was almost 50% greater in 1996 than 1995 at Western Entrance and Ebiil.

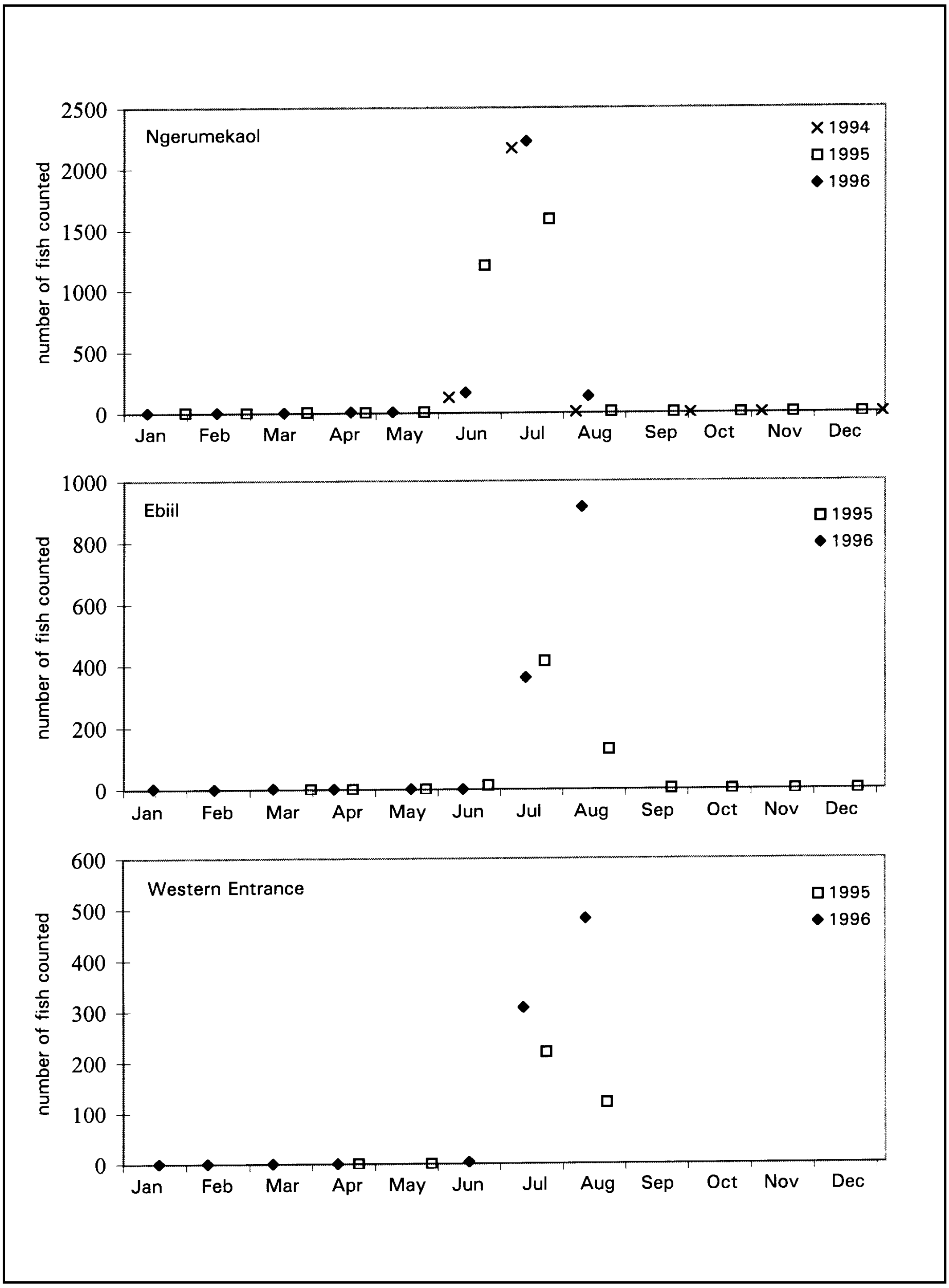
Maximum annual counts of female *P. areolatus* displayed less variability than maximum annual total counts of males and females combined (Table 6), the biggest difference being a 37% increase in maximum annual counts of female at Ngerumekaol between 1995 and 1996.

Figure 4. Peak monthly counts of *Plectropomus areolatus*.



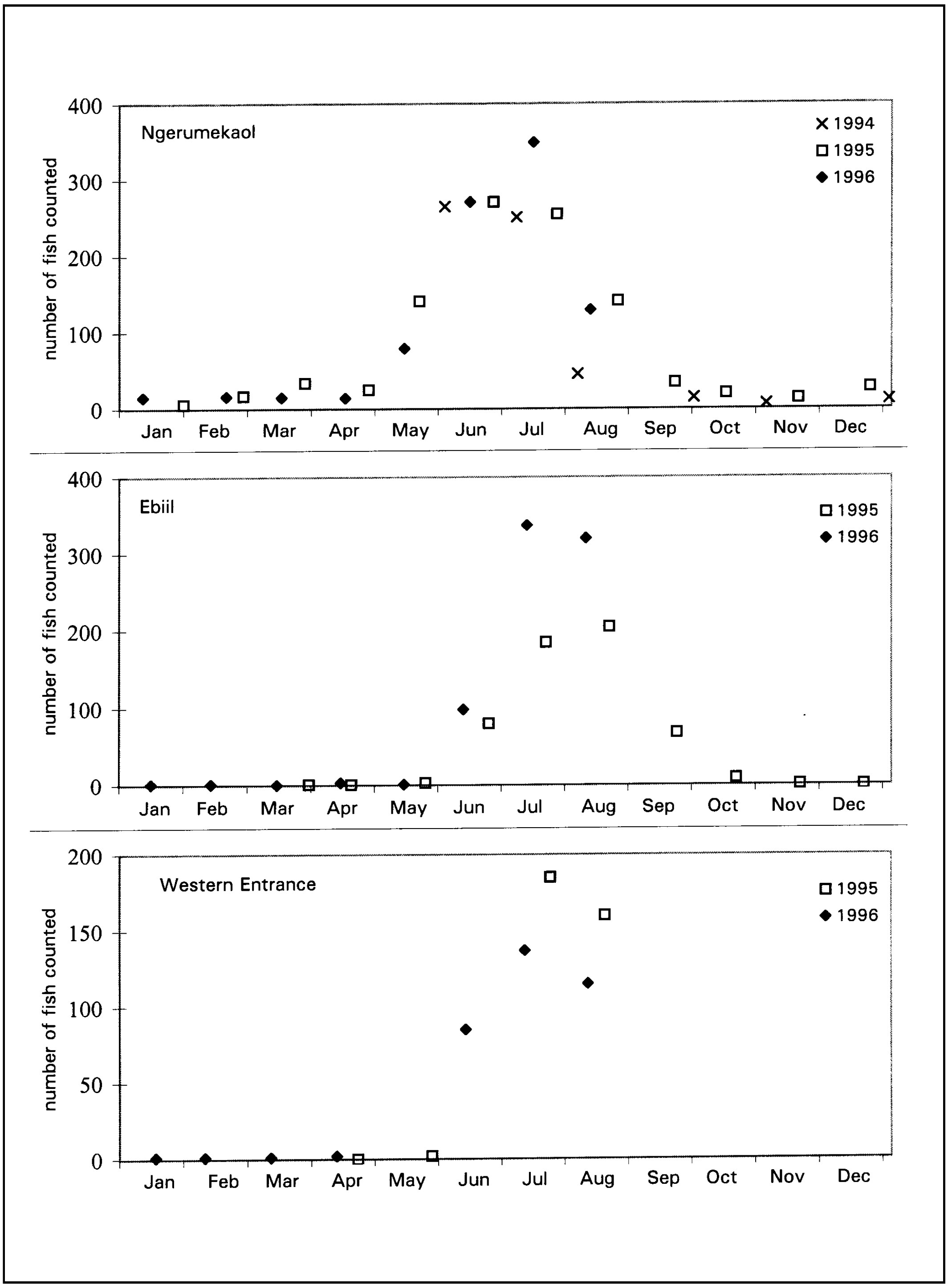
footnote: Each symbol represents the highest count made during each lunar month. A missing symbol indicates that no counts were made during that month.

Figure 5. Peak monthly counts of *Epinephelus polyphekadion*.



footnote: Each symbol represents the highest count made during each lunar month. A missing symbol indicates that no counts were made during that month.

Figure 6. Peak monthly counts of *Epinephelus fuscoguttatus.*



footnote: Each symbol represents the highest count made during each lunar month. A missing symbol indicates that no counts were made during that month.

Table 6. Maximum annual spawning aggregation counts for the three grouper species and maximum f:m ratios of *P*lectropomus areolatus.

	1994			**1995**			**1996**		
	Nger.	Ebiil	W.E	Nger.	Ebiil	W.E	Nger.	Ebiil	W.E
E.f.	265	-	-	271	205	185	349	337	137
E.p.	2,168	-	-	1,587	415	220	2,225	915	482
P.a.	341*	-	-	405	339	795	409	502	1,167
f:m	0.06*	-	-	0.23	0.48	2.47	0.27	0.13	1.78

E.f = *E. fuscoguttatus;* E.p. = *E. polyphekadion;* P.a. = *P. areolatus;* f:m = f:m ratio of *P. polyphekadion* in largest annual aggregations.

*Not comparable with numbers counted in 1995 and 1996, since the study did not start until May 1994 and larger aggregations of this species may have formed or higher f:m ratios may have occurred earlier in the year.

Distribution of groupers outside spawning aggregation sites

At Ngerumekaol in June 1994, during a spawning aggregation period for *P. areo - latus* and *E. fuscoguttatus*, six transects radiating out from various locations at the aggregation site were swum to determine the numbers of groupers outside the site (Table 7).

Table 7. Numbers of groupers counted outside Ngerumekaol spawning aggregation site.

Starting Point	Direction	Distance(km)	*P. areolatus*	*E. fuscoguttatus*
Dropoff	north	6.5	2	3
Dropoff	south	4.6	0	0
Mid-channel	north	3.7	0	0
Mid-channel	south	1.8	0	0
Inner end of channel	northwest	2.8	1	0
Inner end of channel	noutheast	1.8	0	0

No *E. polyphekadion* were seen during these swims. The six groupers that were seen were all located near the aggregation site, and the three *E. fuscoguttatus* were actively swimming toward the aggregation site.

Twenty four standard 50x5m strip transects were also swum in various randomly selected locations around the Ngerumekaol spawning aggregation site. None of any of the three species of groupers was seen.

Gonad analysis

Histological analyses (n = 208 fish) confirmed that spawning of all three species takes place in Ebiil and Western Entrance[19] spawning aggregations on, or shortly before, the new moon around the time of peak aggregation sizes.

Some individual females of all three species spawned more than once during a single aggregation period, but did not necessarily spawn every month during their spawning seasons. For example, some mature-size female *P. polyphekadion* and *P. areolatus* with inactive gonads were occasionally taken in spawning aggregations.

Gonad analyses provided no evidence that spawning occurs outside the spawning aggregation periods or sites. No female *E. polyphekadion* (out of 17 females) or *E. fuscoguttatus* (out of 2 females) taken outside of spawning aggregations during the 1995 spawning season were ripe (i.e., containing yolky eggs). Only one of 13 female *P. areolatus* taken from outside spawning aggregation sites during the 1995 and 1996 spawning seasons was ripe.

In contrast, most (i.e., 88%) of the mature size females taken from spawning aggregations were ripe. Fourteen of 20 female *P. areolatus*, 33 of 34 female *E. polyphekadion* and both *E. fuscoguttatus* taken from spawning aggregation sites during the spawning seasons of 1995 and 1996 were ripe.

These data suggest that few fish, if any, of the first two species spawn at locations other than spawning aggregation sites. Data are inadequate to draw even tentative conclusions in this connection for *E. fuscoguttatus*.

Not all fish taken in spawning aggregations are reproductively active, however. Twelve fish out of a total of 109 (i.e., about 10%) sampled from aggregation sites in 1995 and 1996 were of a mature size but were not reproductively active; one male *E. polyphekadion*, one male *P. areolatus* and 8 female *P. areolatus*. One immature female of each of *P. areolatus* and *E. polyphekadion* was also taken from aggregations.

P. areolatus appear to disperse soon after spawning, as indicated by the rapid subsequent decline in numbers at our spawning aggregation study sites and their disappearance from fish markets.

E. polyphekadion, in contrast, were still caught in significant numbers on the northern spawning aggregation sites for several days after gonad analyses

19. We were unable to take specimens from the Ngerumekaol aggregation for gonadal analyses because of its reserve status. This was unfortunate, since, as we discuss later in this report, we were concerned that conditions at that site may discourage spawning, but were unable to confirm it.

suggested that spawning activity had ceased, and specimens were found in significant numbers in fish markets for up to four days after the new moon.

Plectropomus areolatus

Based on histology, individual females ready to spawn [i.e., with ovaries full of late-stage vitellogenic (ripe) eggs] were sampled in May through August l995 at Ebiil and Western Entrance and, in one instance, in May 1995, 100m south of the Ngerumekaol site.

In spawning aggregations from Ebiil and Western Entrance, individual females were clearly able to spawn on at least two different days during the course of monthly aggregation periods. But not all females spawned in each aggregating month. The presence of ovaries which contained both ripe oocytes ready for release and post-ovulatory follicles, which remain in the ovaries for a day or so after spawning has occurred, showed that individual females were capable of spawning more than once in a monthly aggregation.

Not all females spawn in each spawning aggregation in which they participate, however; in July 1995 one small, unripe female [fork length (FL) = 345 mm] and a total of seven mature-sized females sampled at the Western Entrance spawning aggregation site, for example, were not ready to spawn.

In August, the last month for spawning aggregations for this species in 1995 and 1996, males and females examined were all ripe at both Ebiil and Western Entrance (n = 11, 1995; n = 6, 1996). In 1995, all individuals were reproductively active by around August 22, when counts were highest (new moon was 26 Aug.). In 1996, all individuals examined at Western Entrance were reproductively active on August 10 (new moon was 14 Aug.).

In September (n = 3) and December (n = 1), 1995, (i.e., non-aggregation months) none of the fish sampled was reproductively active.

In late April (21st and 27th), 1995, two adult females collected near the Ebiil spawning aggregation site (but not on it) before the new moon (new moon was Aug. 30) showed signs of degenerating early mature ovaries suggesting that some oocytes had started to mature but subsequently underwent atresia. This phenomenon has been reported in a number of other fish species, including the grouper *Epinephelus aeneus* (Hassin et al. 1997).

Epinephelus polyphekadion

Females spawned over several days during the course of monthly spawning aggregations in July[20] and August 1995 and August 1996 as determined by the presence of post-ovulatory follicles together with large numbers of ripe eggs in fish at Ebiil and Western Channel. In June 1996, two adult females taken from Western Entrance were reproductively inactive.

Histological analyses confirmed that individual females can spawn more than once during the course of a single aggregation month, but that small numbers of adult individuals may not spawn in every monthly aggregation in which they participate. Females in August 1996 spawned more than once during the period 2 - 4 days prior to the new moon as judged by post-ovulatory follices which were particularly evident in ovaries (n = 10 females) from August 10, through August 13, 1996. By August 14 (new moon), spawning had ceased.

Epinephelus fuscoguttatus

Only 7 specimens were available for histological gonad analyses, with 20 more available for macroscopic analysis. In August 1996 spawning probably occurred on or before the night of August 14 since many females obtained from the market on August 15 were spent and all 12 females examined in the market on August 14 (new moon) had hydrated eggs (which indicates spawning is likely to occur within several hours). Females can evidently spawn more than once during an aggregation period, as is the case with the other two species. On August 18, 1995, the presence of post-ovulatory follicles and fully ripe oocytes in a female indicated that it had just spawned and was ready to spawn again (new moon was Aug. 26). Two adult fish taken from non-aggregation sites, one each in April and May, were not reproductively active.

Sexual patterns

Protogyny, sexual maturation and size distribution of sexes

Plectropomus areolatus

Gonad development data for *P. areolatus* were available for 7 different spawning months. Although females were smaller than males on average, there was considerable size overlap. Mature females ranged in fork length between 347

20. Fish taken illegally by fishermen.

and 510mm, mature males between 390 and 557mm. Immature fish sampled from spawning aggregations ranged from 300 to 345mm. These data indicate that sexual maturation was attained at about 346 mm.

It is important to stress, however, that these size ranges are based largely on measurements of fish sampled at the markets. They came from more than one (and sometimes unidentified) spawning aggregation. As will be shown below, size distributions varied significantly between study aggregations.

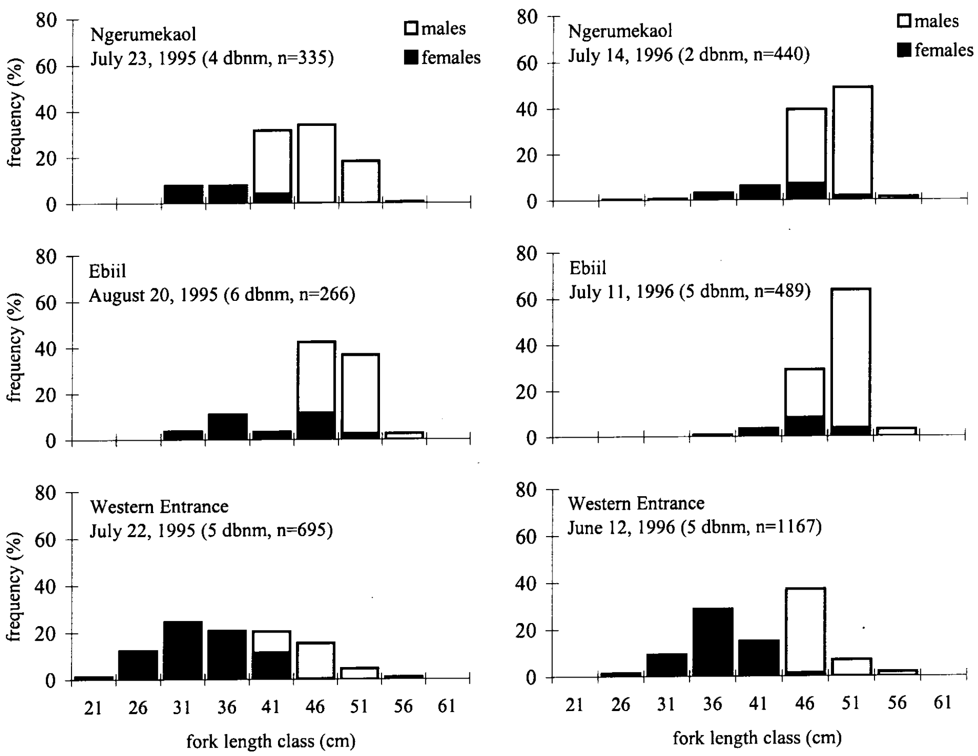
It was not possible to confirm the sexual pattern of this species because of the absence of transitional specimens in our sample of gonads (n = 45). The greater mean size of males than females and lack of any small males support the probability, however, that it is protogynous. Moreover, in 1996 an individual of 510mm FL was collected whose gonads appeared to be in the early stages of sex change. [Two other members of this genus, *P. maculatus* and *P. leopardus*, have been shown by Ferreira (1993, 1995) to be protogynous].

Visual estimates of size distribution in spawning aggregations

The average size of female *P. areolatus* at the lightly fished Western Entrance spawning aggregation was much smaller than in the more heavily fished Ebiil and Ngerumekaol aggregations (Fig. 7). Since the size estimates at Western End and Ebiil were done by the same team member, we considered it appropriate to compare these differences statistically and found them to be significant (p = 0.0008).

In 1996 there was no recruitment of the smallest size category of females into Ebiil and Western Entrance spawning aggregations and negligible recruitment at Ngerumekaol. This is indicated by the absence of the smaller size class when compared to previous years (Fig. 7), and it suggests that larval settlement of this species 2 - 3 years earlier may have been very low.

Figure 7. Size structure of *Plectropomus areolatus* by site and year, as estimated by UVC.



footnotes: Determined on or near the day of the montly peak and during one of the year's peak month.

The size class "21", for example, encompasses fish of lengths between 21 and 26cm.

"dbnm" = days before new moon

Ngerumekaol was monitored by a different observer than were Ebiil and Western Entrance.

Epinephelus polyphekadion

Data on gonadal development in *E. polyphekadion* were available for 10 different spawning aggregation periods. Although females averaged smaller than males, there was considerable size overlap. Mature females were between 301 and 470mm fork length, mature males between 340 and 500mm and immature fish between 239 and 286mm, indicating that sexual maturation occured between about 290 and 300mm fork length.

Strong evidence that *E. polyphekadion* is protogynous is provided by five fish. Two individuals tagged and sexed as females were found to be males when recaptured a year later (total lengths at recapture 430 and 450 mm), and 3 individuals of sizes intermediate between typical males and females (370 and 410 mm), contained both male and female tissue in their gonads. The different size-frequency distributions of males and females (see also Tamaru et al. 1996), and lack of any small males, i.e., <340mm, is additional evidence that this species is protogynous.

Epinephelus fuscoguttatus

Only limited data on reproduction were collected for *E. fuscoguttatus*, from the Koror market. (These fish were not only relatively rarely hooked on the spawning grounds, but also, because of their large size, often managed to retreat into the reef, breaking the line, or fought so long that sharks had ample time to grab them). Twenty fish were sexed and measured. Mature males ranged in size from 698 - 870mm total length, mature females from 420 - 850mm.

Sex-associated color differences

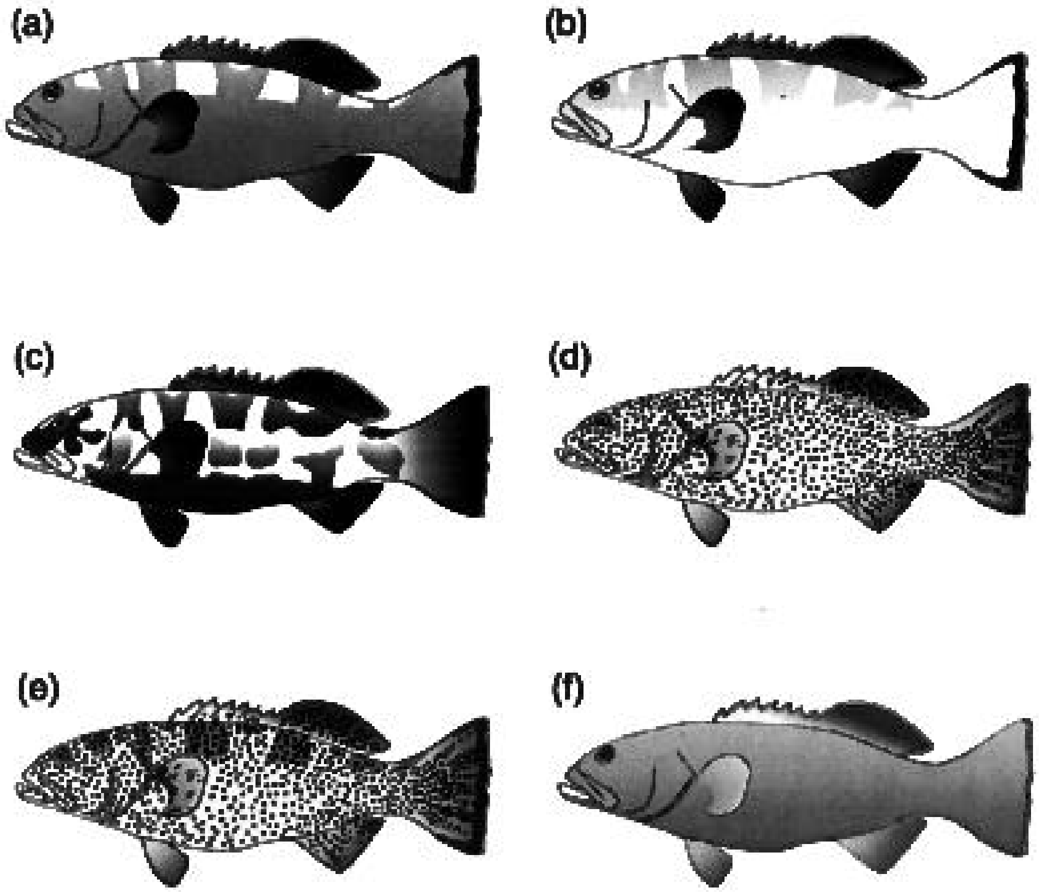
Color differences are described here on the basis of both field observations and video footage taken in the spawning aggregations.

Plectropomus areolatus

Male and female *Plectropomus areolatus* were almost always readily distinguishable in spawning aggregations on the basis of coloration, as well as behavior. When a fish of questionable sex was occasionally seen, the observer watched until color changes or behavior indicated its sex. When spearing *P. areolatus* for gonad samples one of the authors (LS) recorded the sex of 20 fish he targeted, based on the criteria described below. Gonad analyses of these specimens showed 100% agreement with his visual determinations. (A similar check was made on visual estimates of lengths of the speared fish. These estimates were correct to within 1cm 75% of the time and to within 5cm 95% of the time).

Color phases in *P. areolatus* in spawning aggregations are shown in Fig. 8.

Figure. 8. Color phases of *Plectropomus areolatus* in spawning aggregations. See text for explanation.



footnotes:

a. Barred phase: This phase was seen in both males and females. The fins are dark with the dorsal and caudal fins exhibiting a thin white margin. The ventral area is dark, almost black at times. The dorsal area has several distinctly interrupted vertical bars. This color phase is most pronounced when the fish is displaying aggression towards a conspecific. Blue body spots can be seen when close to the fish.

b. Bicolor phase: Males often show this color phase when courting, moving around or fleeing from other, attacking males. There is a distinct black margin to the posterior edge of the caudal fin. The dorsal, anal and paired fins are all dark. On the paler body portions of the underlying small spots are often clearly visible. Males become particularly pale when quivering to (i.e., courting) a female or when fleeing from an aggressor.

c. Camouflage phase: This phase was seen in both sexes when a fish was at rest and not obviously engaged in intense interspecific interactions. The fins are dark with a pale white margin to the dorsal and caudal fins and the body is heavily blotched. Disjunct vertical bands are visible on the dorsal part of the body and on the face. Body spots are visible on lighter areas.

d. Yellow/brown phase: Only females exhibit this color phase. The body color is pale olive/brown and the body is clearly covered with small spots. The fins are a uniform yellow/green. This is the most typical color phase seen in females in spawning aggregations.

Dark phases (females only):

e. The yellow/green hue darkens the body of the female, which also sometimes exhibits some darker dorsal bars and a darker margin to the tail. Instead of being barred, the dorsal half of the body may simply appear darker than the ventral half. This phase cannot be confused with the male bicolor phase since the body pattern is much less distinctly dark and light and the margins of the fins less intensely dark. It is usually seen when a female moves away from an approaching male or moves up into the water column.

f. When presumed females[21] are moving about in schools in the water column and through the spawning aggregation, they can be extremely dark in color. Some details of this color phase are not entirely clear because the fish were moving quickly and were not seen at close range. Females were seen to adopt this coloration when moving in schools into or away from the aggregation (see below). Figure 8f is an approximation of this color phase.

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21. Based on their small size and behaviors.

At close quarters light blue spots can often be seen in the lighter areas of the body of females in all but the black color phase. These spots are much duller and less prominent in males.

Epinephelus polyphekadion and *E. fuscoguttatus*

Male and female *E. polyphekadion* and *E. fuscoguttatus* could often be distinguished on the spawning grounds, but not with enough consistency to enable us to estimate sex ratios reliably. During the afternoon for a few days prior to new moon some females could be distinguished because their hydrated eggs made them "toady" - our description of female groupers whose distended bellies gave them a toad-like appearance.

Males of all three species[22] would "turn off" their male coloration and rise several meters in the water column when they moved about the spawning aggregation area. This may have been to adopt "neutral" colors so as not to invite aggression from other males while passing across their territories. Once these fish moved back to the bottom they immediately reassumed male spawning coloration.

Color changes occurred almost instantaneously when a fish of either sex and of any of the three species was hooked. Landed fish of none of the three species could therefore be sexed based on their coloration.

Sex ratios

Roughly five to ten times as many female *P. areolatus* as males were counted in the most lightly fished area (Western Entrance) as in the spawning aggregations in the more heavily fished areas Ngerumekaol and Ebiil sites (Fig. 9). This pattern was consistent for all months. Only at Western Entrance did the f:m ratio exceed l [and only there during the larger aggregations (Fig. 9)].

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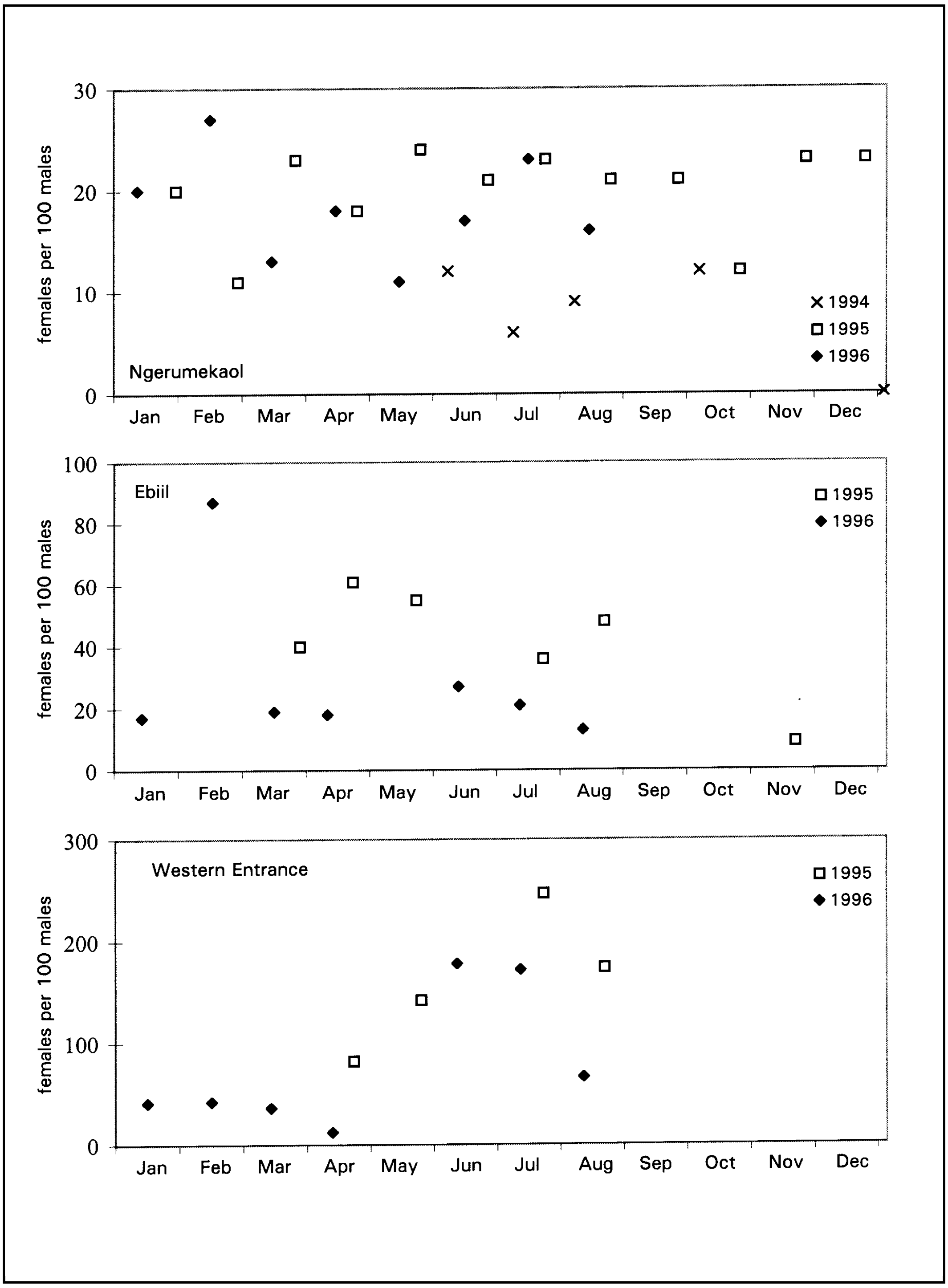
22. Identification of male *Epinephelus polyphekadion* and *E. fuscoguttatus* in spawning aggregations was not always possible, but individuals displaying overtly aggressive interactions and territoriality were assumed to be male.

Figure 9. Peak monthly f:m ratios of *Plectropomus areolatus* by site and year.



footnote: Each symbol represents the highest count made during each lunar month. A missing symbol indicates that no counts were made during that month.

At Ebiil and Western Entrance in 1996 there were no females in what had been the smallest size category in 1995; that is, there was no recruitment of females to these spawning aggregations in 1996. In addition, there was a negligible number of recruits to the Ngerumekaol spawning aggregation (Fig. 7).

Behavior

Spawning and pre-spawning behavior

Despite one attempt at dawn and more than ten attempts around dusk[23] to observe grouper spawning at Ngerumekaol, we saw none of the three species under study spawn, although all three frequently exhibited unmistakable pre-spawning behavior.

No dedicated efforts were made to observe spawning of groupers at Ebiil or Western Entrance because of time constraints on diving due to the greater depth of the aggregation sites there than at Ngerumekaol. At Ebiil in 1996, however, one of our research assistants, Ms. Helena Walkowiak, observed two *P. areolatus* swim rapidly almost straight upward off the reef, coming together at the top of their trajectory before returning quickly to the bottom. No cloud of eggs and sperms was seen, but the observation was made at dusk in failing light. *Plectropomus leopardus* is known to spawn at dusk, while displaying similar behavior (Samoilys and Squire, l994).

Plectropomus areolatus

At Ngerumekaol and Ebiil spawning aggregation sites male *P. areolatus* outnumbered females (Fig. 9). This contrasts with sex ratios reported for other protogynous groupers, including *P. areolatus* in Solomon Islands (Johannes. 1989), and with its congener *P. leopardus* on the Great Barrier Reef (Samoilys and Squire, 1994) where females outnumbered males in spawning aggregations.

Courting behavior at Ngerumekaol and Ebiil seemed to reflect this “shortage” of females. Females were often “harassed” (nudged, chased, collided with) by several males simultaneously and often fled from them. This behavior was noticeably more intense at Ngerumekaol and, in 1996, at Ebiil, where males greatly outnumbered females, than at Western Entrance where f:m ratios were higher. Occasionally, when this behavior was noted near the edge of the Ngerumekaol aggregation, we saw some harassed females depart from the aggregation site in apparent response. The intensity and duration of male:male interactions also

23. Dusk spawning is common in groupers (Sadovy, 1996). We did not attempt to witness spawning at night; lights needed for visibility during night dives disturb those species of groupers that remain awake at night (P. Colin, per. comm.).

seemed greater at Ngerumekaol and at Ebiil in 1996 than at Western Entrance. It seemed to observers that the associated color changes were also greater.

In a Solomon Islands spawning aggregation, where female *P. areolatus* outnumbered males, Johannes (l989) observed that the males were typically attended by several females, and he saw no harassment of females by males.

A number of male *P. areolatus* at the aggregation sites that could be identified by distinctive markings (scarred face, notched caudal fin, misshapen face etc.) returned almost every month for up to more than a year. At Ngerumekaol, where spawning aggregations formed every month for the last 21 months of our study, one male *P. areolatus* was seen at the spawning aggregation site every month for 12 months. Another was seen every month but one for 17 months (Table 8). Several identifiable *P. areolatus* males were seen at the other sites almost every month for four to eight months.

The estimated mean length of stay of these fish at a spawning aggregation site was 5.2 days. It should be stressed that this is a minimum estimate because the distinctive marking would not always have been seen by divers if it was on one side of the body (e.g., SF, Table 8 = scar-face). In addition these fish might also have been on the site for a day or more before the monthly counts began or after they ended.

Unfortunately we could make no similar observations on females because none were observed to have distinguishing marks. This is probably because females fight much less than males, and are less likely to be targeted by spear-fishermen because of their smaller average size.

Table 8. Minimum numbers of days per lunar month and months per year that identifiable individual male *Plectropomus areolatus* were seen in spawning aggregations (January 1995 - August 1996). ***(n = Ngerumekaol, e = Ebiil)***

Month	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J
Individual Fish ID Code																			
MN(n)								11	5	3	4	12	11	7	-	1	1	8	
NS(n)								4	4	2	3	5	8	2	3	1	3	5	9
SF(n)		3	2	5	8	1	8	4	4	1	1	3	8	12	6	-	5	1	5
UL*(n)	2	2	4	8	6	9	7	5											
UN‡(n)				4	9	9	9	7	7	5	1								
6T1(e)										8	3	7	-	7	5	7			

*This fish was badly wounded, either by a shark attack or a spear. The wound was first observed on August 22. The fish appeared very much the worse for wear by August 25, the last day on which it was seen.

‡This fish became increasingly difficult to identify with time as its identifying wound healed.

But clearly, at least some males aggregated at the site during all, or almost all, spawning months. Usually, but not invariably, the above fish were seen repeatedly at the same spot. The areas of the territories defended by two males were measured and found to be 14 and 17 m^2.

Male *P. areolatus* tended to sit in the middle of their territories from early morning until mid-afternoon. Later in the day, the fish were more active, and territorial males would chase off intruding males. In general, females moved around the spawning aggregation sites much more than males.

All-female schools

During aggregation periods several all-female schools of 15-50 *P. areolatus* were seen swimming higher above the substrate than individual females were ever seen to swim. Similar schools were seen swimming along drop-off walls at depths of up to 35m. The schools were swimming towards, within, or away from spawning aggregations. The fish all displayed the dark color phase shown in Fig. 8f. They were invariably swimming with the current except when within spawning aggregations.

Three such schools, each containing 30 - 50 females, were seen on their apparent approaches to Ebiil and Western Entrance spawning aggregation site, on dates ranging from 3 (Western Entrance and Ebiil) to 7 (Western Entrance) days before new moon. Two similar groups were seen leaving the Ebiil spawning aggregation site 3 days before the new moon, i.e., about the time spawning aggregations typically start to break up.

In addition, one all-female school was seen displaying distinctive behavior at the Western Entrance spawning aggregation site on 23 April 1995 at about 11:30am. The school consisted of about 45 females swimming about 5m off the bottom at the speed of a moderately swimming diver. As with all other such schools, the members were a uniformly dark color. The schools were spherical to egg-shaped. They progressed horizontally by "rolling" in such a way that the lead fish were constantly being replaced by other members of the school moving up and over them from behind. During a 10-15 minute period the school traversed the spawning aggregation site three times. When it reached the limit of the aggregation it would reverse and traverse the aggregation in the opposite direction.

At the school's approach, male fish would leave their territories and break quickly into the school, pushing sideways vigorously with their bodies against females, apparently trying to separate them from the school. In the area where the aggregation was densest, as many as 40 males engaged simultaneously in this behavior. In areas of lower aggregation density as few as 3 or 4 males participated.

In apparent response to these efforts, individual females would leave the school and move to the bottom, where they quickly changed from their uniform dark color and adopted the typical coloration of aggregating females — the yellow/brown phase described above. By the time three traverses of the aggregation had been seen (there may have been more traverses before the diver spotted the school) the school had dispersed; all the females had separated and moved toward the bottom.

On two other occasions, 4 and 7 days before the new moon, schools of 20 - 40 females were similarly seen to enter the Ebiil spawning aggregation and elicit the same behavior from males. These schools were dispersed by males before they could fully traverse the aggregation site once.

On two occasions when a school of females was seen leaving the aggregation site, the reverse color transformation occurred to that observed in females joining the school. That is, the typical coloration of aggregating females was quickly replaced by the uniform dark color of the other departing fish in the school.

In one instance we observed a group of females milling at the edge of the spawning aggregation site, then departing down-current at a speed such that they were soon lost sight of by a diver swimming vigorously to try follow them, although underwater visibility exceeded 30m.

Palauan fishermen similarly report seeing schools of small (thus presumably female) *P. areolatus* swimming toward spawning aggregation sites. During non-spawning periods we saw no mobile schools of *P. areolatus* nor have such schools been reported to us at such times by Palauan divers. We also never saw larger fish (i.e., males) travelling in schools.

Line fishermen from Ollei seem to have inadvertently discovered the migration path of the female schools heading toward the Ebiil spawning ground. They reported bursts of good fishing several days before the new moon at the location where we saw most of these schools passing.

Epinephelus polyphekadion

At Ebiil, Palauan taggers preferred to fish over one specific location at the site, and all of the *E. polyphekadion* tagged in 1995 were caught in the same location within the spawning aggregation site. In 1996 most of the tagged fish (tagged in 1995) that were observed in the spawning aggregation were located at this same spot suggesting that individuals of this species are also consistent in the areas they occupy in spawning aggregations.

E. polyphekadion examined in 1996 at four Koror fish markets in August at the end of their spawning aggregation period were noteworthy for their thinness, probably as a result of energy expenditure associated with spawning. The same condition was not evident in the other two species.

E. polyphekadion, presumably males, often engaged in what appeared to be ritual fighting, opening their mouths and pushing gently, mouth-to-mouth against one another. The fish would often allow divers to approach within two meters or less without retreating or terminating this behavior.

Passfield (l996) describes *E. polyphekadion* in spawning aggregations in the Cook Islands, stating (p.20) that "locals who have observed the fish say that they are all lined up on the bottom, pointing out through the passage." Such behavior was not seen in the Palau spawning aggregations of this species.

Epinephelus fuscoguttatus

Large *E. fuscoguttatus* (presumably males) were aggressive toward other large fish of this species. When becoming aggressive their underside flushed whitish. Presumed male *E. fuscoguttatus* could be seen pushing gently against one another, mouth-to-mouth but, unlike *E. polyphekadion*, were easily disturbed at such times by monitors/divers.

Unwariness of spawners

Both *E. polyphekadion* and *P. areolatus* in spawning aggregations exhibited a phenomenon well-known to Palauan fishermen and which Johannes (l981) termed "spawning stupor" - although, in retrospect, the term "stupor" best describes only its more extreme manifestations, which are characteristic of only certain species.[24]

While not behaving as in a stupor, aggregating *P. areolatus* species were, nevertheless, noticeably less wary of approaching divers than at other times.

They did not usually retreat into holes in the reef when approached (as they

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24. We cannot agree, however, with Domeier and Colin's (1997) categorical assertion that reef fishes in spawning aggregations "are keenly aware of their surroundings and courtship or spawning behavior is easily broken off by a diver approaching to (sic) closely or even the exhalation of bubbles from SCUBA gear." With a dip net, one of us (REJ) was able to scoop up large mullet *Crenimugil crenilabus,* as many as five at a time, from the spawning aggregation described by Helfrich and Allen (1975) at Enewetak, and watched as sharks effortlessly consumed fish in this aggregation. When not in spawning aggregations these mullet are extremely wary. Similarly, Robertson reported that when a shark attacked a spawning group of *Acanthurus nigrofuscus,* its biting "rapidly and repeatedly at them" within the "mass of fish ... did not appear to noticeably disrupt the spawning pulse after the attack commenced," (Robertson, 1983, p. 198). REJ has swum with SCUBA into the center of a densely packed spawning aggregation of thousands of cubera snapper *Lutjanus cyanopterus* in Belize without causing the fish to avoid him by more that a few inches nor to cease spawning. Other examples could be given of reef fish so apparently preoccupied with spawning that predatory threats, which would stimulate immediate escape behavior at other times, elicit little or no reaction. Colin, himself, notes that outside of spawning periods *Epinephelus itajara* are "much less easily approached"(Colin, 1993,p.146).

typically do when not in spawning aggregations in waters such as Palau's where spearfishing is common and fish have learned to treat divers with caution). They simply moved away when a diver approached to within about 6 - 8 meters, often moving back to their original location when the diver retreated. Zabala et al. (l997a) observed a similar reduction in wariness in *Epinephelus marginatus*

As noted earlier, *E. polyphekadion* showed no overt concern when divers approached to within 2m or less. Pairs of fish, presumably males, often even persisted with their distinctive open-mouth-to-open-mouth pushing behavior.

E. fuscoguttatus quickly sought shelter within the reef from approaching divers when they were still as much as 15m away, displaying no less wariness than at other times. (For this reason *E. fuscoguttatus* is much harder to spear in spawning aggregations, at least during the day, than the other two species, despite its larger size).

Within-site species distribution and interaction

Although the spatial distribution of all three grouper species overlapped at the spawning aggregation sites, they did not coincide. Areas of highest concentrations of *E. fuscoguttatus* were characterized by large *Porites* coral with relatively large holes and crevices to which the fish readily withdrew when disturbed.

P. areolatus, a smaller species, concentrated in shallower areas on the average, with smaller plate corals, holes and crevices to which they eventually withdrew if sufficiently disturbed. *E. polyphekadion* were often concentrated in areas with little coral cover and seldom sought shelter unless deliberately disturbed at close range by divers. Where *P. areolatus* and *E. polyphekadion* overlapped, the former was invariably dominant, often chasing the latter. There was little behavioral interaction between *E. fuscoguttatus* and the other two species.

Predation

We saw very little predation by or on the three species. We saw a 85 - 90cm size class *E. fuscoguttatus* eat a 50 - 55cm size class *E. polyphekadion*, and *P. areolatus* occasionally attacking schools of small caesionids.

We saw groups of gray reef sharks *Carcharhinus amblyrhynchos* prey in unison on spawning aggregations consisting of thousands of unicornfish *Naso literatus,* at Ebiil and Ngerumekaol. In one instance three grey reef sharks circled the aggregation, herding it into a ball. Then the largest shark, a female, charged into the school biting but not swallowing a number of the unicornifish. Then the two smaller male fish entered the school and, along with the female, attacked and ate the fish that had been injured and disabled by the female.

Tagging results

Several unforseen problems reduced the value of our tagging program, although it nevertheless produced a variety of useful findings. One problem was that fishing activity in the waters of the adjacent Ngaraard State was low, thereby reducing the chances of getting tagged fish from these waters. Nevertheless, we did get a few tag recoveries from there.

Second, the trochus season and a variety of traditional customs (funerals etc.) reduced Ollei fishermen's fishing time for the first few months after our tagging program began.

Third, significant numbers of tagged fish were caught by fishermen from outside the Ollei community. Since they did not want to admit to fishing on Ollei's fishing grounds, they declined to turn in their tags, despite the reward offered. Out of a total of 124 tag recoveries we located 16 tagged but unreported fish in Koror markets and 2 more were recovered in a restaurant.

Fourth, after our tagging study had begun, the Palauan law that prohibits commercial fishing for groupers during their main spawning season was amended to include any fishing whatever, including that for research purposes. This severely disadvantaged our tagging program. It meant not only that we were unable to carry out the planned second year of tagging, but also that we were unable to fish over spawning aggregations from April through July 1996 to recover fish tagged in 1995.

Fortunately, a significant spawning aggregation of *E. polyphekadion* formed after the season opened in August 1996 and we were able to obtain 89 tag returns from it. In addition, underwater site monitoring during the closed season provided observations on tagged fish. Individual fish could not be identified however; even the colors of the tags were indistinguishable by divers because of algal growth on them.

Tagged individuals that were subsequently caught by fishermen were invariably in comparable visual condition to untagged fish, as was their behavior in spawning aggregations. Tagged male *P. areolatus*, for example, were often seen defending territories. Two tagged females were seen distended and ripe. Tagging thus had no apparent significant effect on these fish. When caught at the end of the spawning season aggregating *E. polyphekadion* were noticeably much thinner than they were at other times, but tagged individuals were not visibly thinner than untagged ones.

After about one month all recaptured tags had a heavy growth of algae. After a year almost all tags showed signs of being bitten by other fish, possibly mistaking them for parasites. We used tags of four colors - white, yellow, blue or green. Green was the color that gave us the highest tag returns, possibly because they were less conspicuous to parasite-eating fish.

In August l998, three years after the tagging was done, and after our research program had ceased, two members of the Division of Marine Resources discovered about 50 tagged *E. polyphekadion* at the Koror market, and were able to retrieve 26 of them. All had been captured at Ebiil and all had readable tags.

Palauan fishermen sell their fish whole and so were reluctant to cut open a fish to remove a tag. Consequently they would grab the end of the tag and pull. Often the tag broke, leaving half of it in the fish. This was not a problem in cases where we could read the numbers on the outer half. But often this half was chewed up or bitten off. Very few fishermen used the phone number on the tag in order to report it, and some thought the phone number was the tag number, recording it and throwing away the tag. Therefore, if we were to repeat this exercise we would not put the tag reward phone number on it. (In any event, word of mouth and our tag reward posters turned out to be a more effective way of advertising). Rather, we would print the tag number several times along the entire length of the shaft.

Twelve tags from *E. polyphekadion* - 11 tagged at Ebiil and one at Western Entrance - were returned from fish caught outside these spawning aggregation sites. All were recaptured at reefs that were isolated from the tagging site, implying that the fish were willing to travel not only substantial distances, but also from one reef complex to another in order to join a spawning aggregation. The returns were caught at locations ranging from five to ten km from the tagging sites. One of the fish had traveled four miles in two weeks. These distances were probably traversed over water no deeper than about 30m. *Epinephelus striatus* has been reported to travel distances of up to 110 km (Colin, 1992) and 240km (Carter et al. l994) from spawning aggregation sites in the Caribbean. One red hind, *Epinephelus guttatus*, was reported by Sadovy et al. (l994) to have crossed water at least 194m deep to get to its spawning aggregation site.

At the Ebiil spawning aggregation site, the tagging team recaptured 173 *E. polyphekadion* and four *P. areolatus* that had been tagged there earlier. Some had been tagged the same month as they were recaptured; others had been tagged as much as 14 months earlier.

Fifty *E. polyphekadion* were recaptured at the tag site within l - 5 days of tagging, indicating that individual fish do not simply come to a site, spawn and leave. Fifteen of the 50 recaptures were obtained 4 - 5 days after tagging.

Fifty *E. polyphekadion* were captured in the Ebiil spawning aggregation a month after being tagged there (June-July or July-August), and three fish were caught two months after tagging (June-August). This provides additional evidence that individuals join aggregations in more than one lunar month per spawning season.

In addition, during single dives while conducting UVC at Western Entrance, we saw as many as six individual *P. areolatus* that had been tagged in previous months. At Ebiil, as many as nine individual *P. areolatus* that had been tagged during previous months were seen.

We saw few tagged *E. polyphekadion* at Ebiil, presumably because the tagging team preferred to fish over a deeper portion of the aggregation which the divers did not monitor. Tags were recovered, however, during line fishing at this deeper site. This provides additional evidence that fish not only return to the same aggregation but also to the same general location within the aggregation site.

Tagging provided no evidence of movement of fish from one aggregation to another.

The correlations between CPUE and UVC counts for *E. polyphekadion*[25] were high (Table 9), indicating that CPUE for this species could be a useful index of spawning aggregation size. The statistics for the predictive regression function, UVC = $a + b$(CPUE) are shown in Table 9.

25. Catches of the other two species were inadequate for useful statistical analysis.

Table 9. Correlations between CPUE and UVC counts for *Epinephelus polyphekadion* and *Plectropomus areolatus.*

	E. Polyphekadion		***P. areolatus***	
	Ebiil	Western Entrance	Ebiil*	Western Entrance
F	131.0	26.1 (p<<0.0001)	4.9 (p=0.0003)	0.6
Adjusted R^2	0.74	0.68	0.11	0.08
n	46	13	32	13
a	-6 (p=0.46)	5 (p=0.81)	55 (p<0.0001)	411 (p=0.03)
b	29 (p<<0.0001)	18 (p=0.0003)	22 (p = 0.03)	-60 (p=0.81)

n= number of days that were fished when UVCs were also done.

* Data from July and August were omitted from this analysis, since fishing during those months shifted away from the core of the *P. areolatus* aggregation to where *E. polyphekadion* were more abundant.

This analysis shows, for example, that for *E. polyphekadion* at Ebiil, the underwater fish count could best be predicted as being about 29 times CPUE, in fish/fisher-hour minus 6.

For *P. areolatus* the correlations between CPUE and UVC were significant only at Ebiil and only weakly so. This was probably in part because far fewer of this species were caught than *E. polyphekadion*. This may be in part a result of the fact that there was a big difference in catch rates for this species between local fishermen and some researchers. Whereas everyone could catch *E. polyphekadion* readily, certain researchers (REJ, for example) showed a striking lack of ability to catch *P. areolatus* when Palauan fishermen in the same boat were catching them.

Poaching

Despite the nominal protected status of Ngerumekaol, as mentioned earlier it did not qualify as an unfished site. Poaching was said to involve both line-fishing and spearfishing, mainly at night when it is difficult to detect. Night line-fishing would probably remove the two *Epinephelus* species selectively; *P. areolatus* moves into crevices and rests throughout the night (Johannes, 1989) when it is said by Palauan fishermen not to bite. The other two species remain active during the night and are caught by line fishermen. We saw several *E. fuscoguttatus* and *P. areolatus*, and one *P. laevis* at the aggregation site that bore what appeared to be fresh spear wounds. Occasionally we also saw fresh fishing line snagged on corals.

Since the law is not clear on the boundaries of the restricted area, referring to it simply as "Ngerumekaol", some fishermen believe that fishing can be carried out legally over the portion of the aggregation that is found outside the channel in and near the apron area (described above under "Site Descriptions"). Furthermore, fishermen told us that it is easy to get away with line ishing in the channel during the day. When a suspicious boat (i.e. one which might be on fisheries patrol) is seen approaching, one merely lifts the anchor and drifts or motors a few tens of meters away from the channel before the other boat gets close enough to determine where, precisely, fishing has been taking place.

Largely in response to the results of our study, the Koror State government now has legislation that prohibits fishing at and around Ngerumekaol year-round.

A commonly heard comment from fishermen at the dock at Ollei was, "there goes another boat to steal our fish." This was sometimes directed at passing boats from another village in Ngerchelong State that were heading to the grouper spawning aggregation sites, ignoring the **bul** prohibiting fishing over these aggregations and the national law against fishing for groupers from April through July.

In addition, some relatively well-to-do Palauans, most living in Koror, have high-powered boats for which most of those of Ollei fishermen are no match. They sometimes fish on Ngerchelong State's spawning aggregations. One Palauan businessman is known to have employed Filipino fishermen using hookah gear at night to spear groupers at Ebiil. In two successive nights in

1991, for example, his crew was reliably reported to have removed 550kg of *P. areolatus*.

Ebiil fishermen report that line fishing catch rates of this species have dropped markedly since this time, while catch rates of *E. polyphekadion* have also declined, but much less noticeably. (As mentioned earlier, *E. fuscoguttatus* are less often targeted than the other two species by line fishermen because of their large size, or by spearfishermen because of their wariness).

We were forced to remove our marker buoys from the Ollei aggregation site and rely on sunken positional markers to mark the site because poachers were using the buoys as location markers for their night raids on the aggregation.

Such poaching reduces the incentive among Ollei fishermen to observe conservation regulations, since the fish they leave behind can be targeted by outsiders. It also reduced the value of our counts on the spawning aggregations because unknown numbers of fish were periodically removed.

We saw no such evidence at the remoter Western Entrance site. We suspect however, that some poaching occurred there as well. Nevertheless, as discussed above, this site is the farthest from any human settlement, less widely known among Palauan fishermen and poaching there was probably relatively light.

Enforcement of government conservation laws has generally been poor in Palau in past years. This has been due in part to the loss of traditional authority in the villages (Johannes, 1991; Shuster, 1994) and failure of the new national political elite to take seriously their responsibility to regulate the exploitation of the country's natural resources. Enforcement picked up somewhat beginning in the mid-1990s, especially in Koror State.

Other species spawning at spawning aggregation sites

It was not one of our original objectives to record spawning of species other than the three groupers. But the opportunity presented itself frequently, so we recorded our observations. The following behaviors are characteristic of many reef fish prior to spawning. Using them helped us anticipate and thus observe the spawning of 57 species at the three study sites (Table 10). (Spawning of several of them has not been recorded previously). These characteristics are:

1. Aggregating in larger numbers than seen at other times

2. Frequent male-to-male aggression

3. Displaying coloration and markings not seen at other times. In the case of the three aggregating groupers this pertained to the males of all three species, as well as female *P. areolatus* (see above)

4. When egg hydration occurs (typically a few hours before spawning in many species) females' bellies become highly swollen (see above) and the fish become sluggish in their movements

5. Courting of females by males. In the three studied grouper species, for example, a major element in this behavior is a pronounced side to side quivering by the male through the length of his body while facing, or beside, a female. In many species, including these three, males chase females and/or nudge their vent area

6. Other behavior seldom or never seen at other times. It should be noted here that the ability to anticipate and thus to witness spawning is often enhanced considerably by experience. The actual spawning act may take only one or two seconds and is thus easily missed if the observer is not anticipating it and thus not looking in the right place at the right moment. To anticipate it often necessitates being able to recognize unusual behavior, which often precedes it. This, in turn, requires familiarity with the species' more usual behavior, i.e., behavior characteristic of non-reproductive periods. It is the unusual behavior that may first catch the diver's eye. Example: male *P. areolatus* patrol and defend territories; individual males (as well as females with hydrated eggs) often slowly rise and swim several meters above the bottom.

Table 10. Species of reef fish observed spawning at Ngerumekaol

SPECIES	PALAUAN NAME
*Acanthurus nigricauda**	**mesekuuklbad**
*Acanthurus xanthopterus**	**mesekuuk**
Anyperodon leucogrammicus	**choloteachl**
Balistoides viridescens†	**dukl**
Cetoscarus bicolor	**mellemau** (male)
Centropyge bispinosus	**ngemngumk**
*Chaetodon auriga**	**chelebesoi**
*Chaetodon ephippium**	**chelebesoi**
Chaetodon kleinii	**chelebesoi**
Chaetodon lineolatus	**chelebesoi**
Chaetodon semeion	**chelebesoi**
*Cheilinus undulatus**	**maml**
Cirrhilabrus cyanopleura	
Coris aygula	**uluch**
*Ctenochaetus striatus**	**masch**
Decapterus sp	**agie?**
Epibulus insidiator	**ngerengerodl**
Epinephelus malabaricus	**temekai**
Gracila albomarginata	**bekeurasengerruk, mardelucheb**
Halichoeres hortulanus	**sisir**
*Heniochus chrysostomus**	**kaming**
Heniochus singularis	**kaming**
Heniochus monoceros	**kaming**
Heniochus varius	**kaming**
Hipposcarus longiceps	**ngyaoch**
*Lutjanus bohar**	**kedesau**
*Lutjanus gibbus**	**keremlal**
*Lutjanus kasmira**	**dodes**
*Mulloides vanicolensis**	**emisech**

*Naso brevirostris**	**sechou, demrechl**
*Naso hexacanthus**	**borch**
*Naso literatus**	**cherangle**
*Naso unicornus**	**um**
*Naso vlamingii**	**daraboksos, melangesakl**
Oxymonacanthus longirostris	**moichall**
Parupeneus barberinus	**bang**
Parupeneus multifasciatus	**bang**
Plectropomus oligacanthus	**basolokil**?
Pomacanthus sextriatus	**ngemngumk**
*Priacanthus hamrur**	**dechil a deil, dechil ebakl**
Pseudobalistes flavimarginatus†	**dukl**
Pygoplites diacanthus	**ngemngumk**
Scarus bleekeri	**mellemau, besachelutengl**
Scarus chameleon	**mellemau**
Scarus dimidiatus	**mellemau**
Scarus frenatus	**mellemau**
Scarus frontalis	**otord**
Scarus microrhinos	**mellemau**
Scarus niger	
Scarus oviceps	**mellemau, uloitoachl, butiliang** (male)
Scarus schlegeli	
Scarus sordidus	**mellemau, derbetelloi, butiliang** (male)
Siganus argenteus	**beduut**
Siganus puellus	**reked**
*Siganus vulpinus**	**reked**
Thelonota ananus	
Zebrasoma scopas	**masch**

*Species that occurred in noticeably larger groups when spawning than they were seen in at other times.

†Spawning not seen, but the eggs, laid in the characteristic nests excavated by these triggerfishes, were often seen.

Species that appeared to exhibit pre-spawning behaviour but that were not observed spawning include *Plectropomus laevis* (**katuu 'l tiau, mokas**), *Chaetodon melanotus* (**chelebesoi)**, *C. unimaculatus* (**chelebesoi**), *C. trifascialis* (**chelebesoi**), *Centropyge bicolor, Pomacanthus sextriatus*, *Zanclus cornutus* (**karamaramadu**), *Siganus corallinus* (**reked**), *S. punctatissimus, S. puellus* (**reked**), *Scarus altipinnis*, *Pomacanthus xanthometapon*, *Zebrasoma veliferum*, *Hemigymnis melapterus* (**klseib**), *Cantherhines dumerilii,* and *Macolor macu - laris* (**kelalk**).

A more detailed description of these observations is being prepared for publication by LS.

DISCUSSION AND CONCLUSIONS

The value of monitoring more than one spawning aggregation site became apparent as our study progressed. Had we studied only one site we would not have discovered, for example, that grouper spawning aggregations only 20 or so kilometers apart may differ significantly in their lunar and seasonal timing, size distributions and sex ratios.

Best times for monitoring

In order to facilitate comparisons of aggregation sizes in different months and years, we decided to focus on peak spawning aggregation periods. (See Appendix I for further discussion of this choice). We calculated the best lunar days (Figs 10,11,12) and the best months (Figs 4,5,6) during which to obtain peak monthly and annual spawning aggregation counts for all three species at all three aggregation study sites. It is important to stress that these calculations cannot be extrapolated to other grouper spawning aggregations. Timing differs too much from spawning aggregation to spawning aggregation even within Palau's main archipelago (where all our study sites were located) which measures only about 120km long by 30km maximum width. Each other spawning aggregation would have to be monitored for a number of days each spawning period for several years to obtain this information. This subject is discussed further in Appendix I.

Diurnal variation

We found no significant daytime variation in spawning aggregation counts, but the effort we put into attempts to measure it was limited. And, for reasons discussed in Appendix I, such changes would be inevitable. We chose to do our routine monitoring in the late afternoon, a period when, presumably, most of the daytime changes in numbers had occurred. Samoilys (1997a) reported that the highest numbers of *P. leopardus* on the Great Barrier Reef were present in spawning aggregations in the late afternoon. Further justification for choosing this strategy is given in Appendix I.

Lunar variation

There was considerable overlap in the lunar days on which peak aggregation counts of all three species occurred. Peak counts of all three almost always occurred between the 2nd and the 6th day before new moon, depending on species and location. Calculations and data pertaining to this subject are presented in Appendix I.

Seasonal variation

Plectropomus areolatus

Significant spawning aggregations of *P. areolatus* (defined as those spawning aggregations that are greater than 25% of the peak annual aggregation size) formed at Ngerumekaol in 23 of the 26 months during which observations were made (Fig. 5). The only months in which they did not form during our study were from September through December of l994.

This conflicts with Palauan fishermen's widespread and long-held assertion that the main spawning aggregation season for this species at Ngerumekaol is from April through July, with peak numbers typically seen in May and June (e.g., Johannes, 1981). Yet this spawning aggregation is the best known in Palau, is only 30 minutes by boat from the district center, and has been visited by countless Palauan fishermen over many generations. Moreover, Palauans take justifiable pride in their marine lore, much of which has been independently verified by various marine researchers (e.g., Hasse et al. l977; Johannes, 1981). The likelihood that they are fundamentally mistaken about the past seasonal timing of this spawning aggregation is thus highly unlikely. The year-round monthly occurrence of significant spawning aggregations of *P. areolatus* we observed at Ngerumekaol in 1996/97 is thus, we believe, a very recent phenomenon, about which we will have more to say below.

The national and Ngerchelong State closures of the grouper fishery run from April 1 to July 31. An examination of Table 1 shows the extent to which they protected the groupers at the three sites during our study.

Significant spawning aggregations of *P. areolatus* occurred at Ngerumekaol throughout the year in 1995 and 1996, thus occurring during all 8 months of the year that are outside the months of the fishing ban. Moreover, even the *peak* annual aggregations occurred outside the closure period. In l995 they occurred in March; in 1996 in February.

Significant spawning aggregations of *P. areolatus* occurred as late as November in 1995 at Ebiil. Thus the existing closure did not protect aggregations there for the last four months of the 1995 spawning season. (We were unable to monitor during these months in 1996).

Significant spawning aggregations of *P. areolatus* occurred at Western Entrance as late as August, 1995 and 1996, one month beyond the existing closure period.

The existing closures did not protect *P. areolatus* spawning aggregations at Ngerumekaol for 8 months per year in l995-96, nor from August into November at Ebiil in 1995. Nor did they protect the last spawning aggregations of the year of *E. polyphekadion* and the last one or two spawning aggregations of the year of *E. fuscoguttatus* at the two northern sites.

Interannual variation in peak aggregation count

As a reliable reference for interannual comparisons of spawning aggregation sizes, the peak annual spawning aggregation count is not without problems. Data on peak annual counts of *E. polyphekadion* at Ngerumekaol provide an example. This species formed significant spawning aggregations during only one month in each of 1994 and 1996, and the aggregation count in each of those two was similar - about 2,200 fish. In 1995, however, there were significant aggregations during two months - late June and late July. But the peak counts for those two aggregations were only one half to two thirds the peak count of the single aggregations seen in July of 1994 and 1996.

The fact that many tagged or otherwise marked *E. polyphekadion* and *P. areolatus* appeared at the same aggregation site more than one month per year means that many of the fish we counted during the second and, (at least in the case of *P. areo - latus*) subsequent aggregations during the spawning season were the same fish we had counted previously. Thus the total of all peaks for the year does not seem to be a reliable reference for internannual comparisons of spawning aggregation sizes. (It might be a useful index of annual reproductive output, but we do not have data that enables us to examine that question).

There was considerable interannual variation in peak spawning aggregation counts for all three species. Large interannual variation in settlement of grouper larvae is well documented, as discussed above. Are the years of high peak spawning aggregation sizes due to a large number of small females joining the aggregations because of high larval settlement in the area two or three years earlier?

A comparison of the size distribution of *P. areolatus* at all three spawning aggregation sites reveals that this cannot be the explanation. If it were, there should be far more of the smallest-sized female *P. areolatus* in l996 than in 1995. But, in fact, the opposite is true; there was virtually no recruitment of young females into Ebiil and Western Entrance 1996 spawning aggregations and negligible recruitment into the Ngerumekaol aggregation (Fig. 7).

The percentage of adults that spawn might vary with their nutritional state, so that in years when food supplies are poorer, fewer aggregate to spawn. We know of no field data for grouper relevant to this hypothesis. In Saudi Arabia, however, *E. polyphekadion* at first spawned in tanks only in May, but subsequently, when fed an improved diet, spawned in March, April, May and August (Al-Thobaity and James, 1996). Failure of mature females to initiate or maintain egg development when food intake was low has also been reported for several other demersal fishes, e.g., haddock (Hislop et al. (1978), flounder (Burton and Idler, l987) and plaice (Horwood et al. l989).

Large fish outnumbered small in the *P. areolatus* spawning aggregations at Ngerumekaol and Ebiil. If these size structures reflect those of the larger adult populations of which these aggregations are a part, they suggest that the population is unstable and will decline; there are not enough small fish to replace the larger fish as they die. Whether this instability is natural and whether the populations can recover from such skewed size structures are questions that cannot be answered without considerable additional research.

We have insufficient information on size distribution of *E. polyphekadion* and *E. fuscoguttatus*, to accept or dismiss the possibility that variable recruitment underlies the interannual variability in their aggregation sizes.

This high, and apparently natural, interannual variability in peak spawning aggregation size, whatever its source, greatly increases the difficulty of detecting any underlying relationship between spawning aggregation size and changes in fishing pressure (including changes due to implementation or modification of management).

So, what is the magnitude and duration of change in maximum spawning aggregation size that can be accepted as representing a real change in total population of which the aggregation is a part? And what is the magnitude and duration of change in maximum aggregation size that can be accepted as representing an unacceptable decrease in maximum aggregation size requiring immediate management action? Unfortunately these important questions cannot be answered without an unknown number of additional years of monitoring.

We analyzed our data to see if the sex ratio, size distribution or peak female counts in spawning aggregations could have provided a somewhat better index of the state of a spawning aggregation than absolute numbers, but the variability in these measures raised similar difficulties.

Site-based variation in duration

The total annual time spent by *P. areolatus* at an aggregation site was greater where the f:m ratio was lower. At Western Entrance, where females were relatively plentiful, relatively little time was spent at the site per aggregation period and fewer aggregations formed each year compared with Ngerumekaol and Ebill, where the f:m ratios were much lower (Fig. 4, Table 4).

P. areolatus may have been responding to the shortage of females at Ngerumekaol and Ebiil by spending more days per month and more months per year at these sites because the low f:m ratios, along with the possible associated behavioral disruptions discussed above, made spawning difficult or impossible.

Sexual pattern

Gonad analysis, tagging data and sex-related size differences indicate that *E. polyphekadion* is protogynous; that is, smaller mature individuals tend to be females and larger ones males (see also Brusle-Sicard et al. 1992). The sexual pattern of *P. areolatus* is also likely to be protogynous (see also Johannes, 1989) although samples of this species for histological analyses were limited (n = 48). Data on *E. fuscoguttatus* were too few to determine sexual pattern.

In a number of small reef species, some related to groupers, sex change is induced by changes in behavior, sex ratio or size relative to conspecifics or other social group members (e.g., Warner, 1984; Shapiro and Ross, l983). For example, the removal of a male from social group consisting of one-male, and several females often results in the largest female changing sex. In species like groupers, many species of which live widely dispersed and only aggregate for limited periods each year to spawn, information on sex ratios and other population parameters relevant to the 'decision' to change sex, is probably only available to the fish when they are aggregating (Shapiro et al. l993).

The alternative possibility, that sex change occurs at a set size, is not supported by our data since the male-female overlap is broad (Fig. 7).

If a spawning aggregation is severely disturbed by fishing pressure, then aggregating fish may not receive the cues that would otherwise initiate sex change, possibly resulting in sex ratios that lie outside the normal range for the species (Vincent and Sadovy, 1998).

Female *P. areolatus* attained significantly larger sizes in aggregations at Ngerumekaol and Ebiil than at the lightly fished Western Entrance (Fig. 7). Based on current theory of the adaptive significance of protogyny (Ghiselin, 1969; Warner, 1975; Vincent and Sadovy, 1998) the male-biased sex ratios at Ebiil and Ngerumekaol would not be expected to stimulate sex change, since a female would gain no selective advantage by becoming male. Our observations suggest that in a population already containing an excess of males, females receive no social cues to change sex; they thus attain larger sizes.

Changing sex ratio of *Plectropomus areolatus* spawning aggregations

P. areolatus males began to arrive on the spawning grounds several days before females (Table 2 and Fig. 3). Johannes (1989) reported the same trend for *P. areolatus* in Solomon Island[26]. F:m ratios must thus change greatly during the course of an aggregation. The most useful time to judge sex ratio under the cirumstances is when the f:m ratio is highest, which according to our gonad analyses, coincides with when *P. areolatus* spawns and, according to our UVC counts, occurs also at about the time of maximum aggregation size.

The highest f:m ratio recorded for *P. areolatus* at Ngerumekaol during peak aggregation periods was 1:5. This contrasts strongly with the moderate to strong predominance of females that has been reported for most other protogynous grouper species[27] (Table 11).

Larger groupers tend to live in deeper water, and sexual segregation has often been reported during inter-spawning periods (e.g., Shapiro, 1987; McPherson et al. 1988; Williams and Russ, 1994; Coleman et al. l996; Shapiro et al. 1993). Truly comparative observations on sex ratios must therefore entail sampling on the spawning grounds. Moreover, sampling must be done near or at the aggregation peak because, as this and other research shows, one sex may begin to arrive on the spawning grounds several days earlier than the other.

It remains to be seen whether even this strategy is adequate for obtaining an unambiguous determination of sex ratio, since it is possible that some of the early-arrivers of one sex may leave the aggregation before some of the later-arrivers of the other sex. Although our observations provided no evidence that this was true for *P. areolatus*, Zeller(1998) has shown that in one grouper species, *P. leopardus*, individual fish do not always remain in the aggregation until it disperses; females may leave and rejoin a spawning aggregation several times during the course of a single aggregation event. For *P. leopardus*, then, it is not clear when, if ever, grouper population sex ratios can be determined accurately.

Females are reported to outnumber males in groupers of 23 other species confirmed or suspected of being protogynous (Table 11). It should be stressed that

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26. Males are known to begin to arrive on their spawning grounds before females in certain other species including Pacific pollock (Maeda, 1986), orange roughy (Pankhurst, 1988) and Pacific hake (Larkins et al. 1967). Olsen and LaPlace (1078) reported, however, that female *Epinephelus striatus* begin arriving on spawning grounds before males

27. A notable exception is the Nassau grouper *Epinephelus striatus*, which has been recorded as having a 1:1 sex ratio on the spawning grounds (Olsen and LaPlace, 1978). However, this species is gonochoristic, not protogynous like most other grouper species for which sexual pattern has been determined (Sadovy and Colin, 1995).

not all fish reported in these previous studies were taken from spawning grounds. Moreover, those that were taken from spawning aggregations were not necessarily sampled on the optimal days for measuring sex ratios; those days had not been determined.

We know of three examples of male-biased sex ratios in groupers in addition to those described above. Munro and Thompson (1983) reported male-biased sex ratios in *Mycteroperca microlepis*. Adams (1996) reported that f:msex ratios in *Plectropomus leopardus* on the Great Barrier Reef varied from 6.2 - 0.32 in different localities. Neither of these species was sampled on the spawning grounds. Tamaru et al. (l996) reported an f:m ratio of l:4 for *E, polyphekadion* (they called it *E. microdon*) taken from spawning aggregations in Palau. They noted that these fish were not fully mature, suggesting that they were caught some time before spawning occurred. If the males of this species arrive on the spawning grounds earlier than the females (as is the case for *P. areolatus*) this would explain this sex ratio.

Table 11. Sex ratios of protogynous groupers

Species		f:m Ratio	Reference
Plectropomus areolatus			
Ngerumekaol	**1994**	0.06	This study
	1995	0.23	
	1996	0.27	
Ebiil	**1995**	0.48	This study
	1996	0.13	
Western Entrance	**1995**	2.5	This study
	1996	1.8	
Plectropomus areolatus			
Solomon Islands		>1	Johannes (1989)
Plectropomus maculatus			
Great Barrier Reef, Australia		2.6	Ferreira (1993)
Plectropomus leopardus			
New Caledonia		5.6	Loubens (1980)
Great Barrier Reef		6.2 - 0.32	Adams (1996)
- mean of 18 non-plectropomid groupers		4.1	reviewed by Shapiro (1987)
Epinephelus guttatus		2.6 - 6.6	Sadovy et al. (1992), Shapiro et al. (1993), Shapiro et al. (1994)
Puerto Rico			
Cephalopholis miniata		4:1	Sphigel & Fishelson (1991)
Red Sea			
Cephalopholis argus		3:1	Sphigel & Fishelson (1991)
Red Sea			

The low f:m sex ratios seen at Ngerumekaol and Ebiil, which are more heavily fished than Western Entrance, could be because, according to Palauan fishermen, females remain in shallow water and are easy targets for line fishermen and spearfishermen during interspawning periods. Larger fish (which are mostly males) are rarely caught except on the spawning grounds. Fishermen say they disappear after spawning, presumably to deeper water where they are generally out of range of fishermen[28].

The selective removal of females by fishermen, coupled with increasing fishing pressure during recent years, could thus explain the high male-to-female ratios we observed. However, where other grouper fisheries similarly target the shallower-living and more accessible female fish during interspawning periods, females nevertheless still predominate on the spawning grounds (e.g., Shapiro et al. 1993; Coleman et al. 1996). Moreover, although we have no relevant data, fishing pressure in the Ebiil area during interspawning periods, it is likely to be at least an order of magnitude lower than it is in the vicinity of Koror, and thus much less likely to contribute significantly to an altered sex ratio. (Koror, near the Ngerumekaol spawning aggregation site, has a population of around 12,300, whereas Ollei, near the Ebiil spawning aggregation site, has a population of about 100 - although a higher percentage of them are fishermen).`

Moreover, if the selection of females by fishermen were the cause of the low f:m ratios we observed, this could be construed as implying that the bulk of the fishing for *E. areolatus* occurs outside the spawning aggregations and therefore that the protection of these aggregations would not be effective in protecting the stock of which such an aggregation is a part. The pronounced peaks in landings of groupers in Palau during the spawning season (Kitalong and Oiterong, 1991) argue against this.

We have noted above, however, that Ollei fishermen had discovered the migration path of the schools of females heading toward the Ollei spawning aggregation site. We found out about this too late to examine the subject in any detail at Ollei - nor to determine if something similar happens near Ngeruemekaol. But this fishing strategy could explain how sexual selection during aggregation periods (but not on the spawning grounds as such) could be a significant factor in reducing the f:m ratios in spawning aggregations.

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28. A number of species of protogynous grouper segregate by sex during interspawning periods, with females tending to be in shallower water (Shapiro, 1987; McPherson et al. 1988; Williams and Russ, 1994; Coleman et al. l996).

Another factor that might have been responsible for, or have contributed to, the low female: male ratio is a low rate of settlement of incoming pelagic juveniles a year or two before our study. There was little or no recruitment of the smallest size category of *P. areolatus* to the spawning aggregations at any of our three study sites in 1996 (Fig. 8).

The peak f:m ratios during peak annual spawning aggregations of *P. areolatus* at Ebiil dropped from 0.48 to 0.13 between 1995 and 1996, while it did not change significantly at Western Entrance only 20 km away. This could also have been due to higher fishing pressure on juveniles, and/or a low settlement in the vicinity of Ebiil a year or two earlier.

For other reef fishes, more than an order of magnitude differences in settlement rates within distances of a few tens of kilometers has been reported by Doherty (l987) and Doherty and Fowler (l994). As the latter authors point out (p. 935), "Wind, topography, and oceanic boundary currents interact with spawnings and larval biology to control cross-shelf larval transport and introduce chaos into recruitment. For a variety of reasons, some sites will be chronically starved of replenishment, while most sites will experience at least occasional years of recruitment failure." ('Recruitment' in this quotation is synonymous with what we refer to in this report as 'settlement'). Thorrold et al. (l994) reported that at 4 sampling stations within a 5km radius in the Bahamas, settlement of larval serranids showed spatial coherence one year but not the next.

Grouper settlement rates can be highly variable interannually in regions with an abundance of nearby recruitment sources, as reported for *Plectropomus leopardus* on the Great Barrier Reef by Ferreira and Russ (l995) and for *Epinephelus striatus* in the Bahamas by Thorrold et al. (1994). Settlement of pelagic juveniles on isolated oceanic reefs such as Palau's might be expected to vary even more from year to year because of greater reliance on a single (i.e., local) source of larvae (see also Medley et al. 1993; Roberts, 1997). Stocks in such self-recruiting areas may thus be especially vulnerable to overfishing superimposed on periodic, naturally occurring low recruitment.

Clearly, more research is needed to verify the cause(s) of the varying sex ratios in our study aggregations.

Egg output of *Plectropomus areolatus*

The reproductive output of a spawning aggregation is a function of both the number and size distribution of females within it that can successfully reproduce. We examined the effects on potential egg output of the differences in female size distributions and of sex ratios between the three aggregation sites studied. Three aggregation days were selected, one from each aggregation site, and each representing the highest f:m ratio recorded for 1996. UVC provided information for these dates on sex ratios and counts of males and females in each of five size classes.

Numbers in spawning aggregations were standardized to 1000 and egg output per 1000 fish was calculated for each aggregation. This allowed us to compare the likely effects of female sizes and sex ratios on egg output in the three different aggregation sites, but eliminates any effects due to differencs in aggregation sizes between sites.

In estimating egg output, a fecundity curve for the congener, *Plectropomus leopardus*, was used (Goeden, 1978). This species is very similar in shape to *P. areolatus* and we assume that the fecundity curve for the former should provide a close approximation to the fecundity curve for the latter.

We make three additional assumptions in this analysis. We assume that sex ratio has no secondary effect on reproductive output, i.e., that egg output per female is not affected by sex ratio. (In fact, we believe that this may not be true, as we discuss elsewhere in this report). We also assume that the fecundity curve is useful for comparative analyses across aggregation sites, even if it does not reflect absolute fecundity. (For the many problems in assessing annual fecundity in batch spawners, such as *P. areolatus*, see Sadovy, 1996). Finally we assume that the selection of days in which the highest f:m ratios were noted are also the days in which egg output is likely to be high and most comparable across aggregation sites.

The three spawning aggregations differ in their sex ratios (see Table 12) and in their female size distributions, with the smallest females, on average, along with the highest f:m ratio found at the least fished aggregation site, Western Entrance.

Table 12. Calculated egg output per 1000 *Plectropomus areolatus* in each spawning aggregation site, based on sex ratios and size distributions.

Aggregation site (sex ratio and date)	Egg output
Western Entrance, f:m = 1.32, 12 June 1996 (mean female size = 39cm)	202,000,000
Ebiil, f:m = 0.2, 11 July 1996 (mean female size = 48cm)	148,000,000
Ngerumekaol, f:m = 0.25, 14 July 1996 (mean female size = 45cm)	142,000,000

The calculations in Table 12 reveal the importance of size distributions and sex ratios in determining potential egg output; the higher mean female size and f:m ratio at Western Entrance result in 25% higher estimated egg output per fish compared with Ebiil and Ngerumekaol. The difference in egg output between Western Entrance and the more heavily fished spawning aggregations would be even greater if a heavy male bias suppresses spawning as we think it may (as we discuss elsewhere in this report).

Behavior

Typical catch rates for shallow reef fishers average about 2 kg/hr (Dalzell, l996, Table 7.3). During peak spawning aggregation periods at Ebiil fishermen caught 8-11 *E. polyphekadion* per line-hour. Since the average weight of these fish was l.03 kg, this converts to 8-11 kg/line hr. This very high CPUE, plus the tendency of this species, as noted earlier, not to flee when approached by divers (e.g., spearfishers), indicates that it is especially vulnerable to overharvesting while in its spawning aggregations.

Differing behaviorof *Plectropomus areolatus* in different spawning aggregations

At Ngerumekaol and Ebiil, f:m ratios were very low (Table 6). Here throughout the study female *P. areolatus* were chased much more by males than was the case at Western Entrance where f:m ratios were much higher (Table 6).

Johannes (1989 and unpubl.) also observed that in a Solomon Island spawning aggregation of *P. areolatus* where females outnumbered males, three to ten females were often seen in a loose association with one to three males. Where there were two or three males in loose association, only one of them was active in courting, and females tended to gather in groups around the males. Harassment of females by males similar to that reported here, was never seen in the Solomon Island spawning aggregation.

Another difference in the Solomon Island spawning aggregations was that females were invariably observed to be in the dark color phase (Johannes, 1989), whereas in Palau they were generally in the pale color phase (Fig. 9) except when travelling in all-female schools.

Differing behavior of two plectropomid species in spawning aggregations

One of us (LS) having observed behavior in spawning aggregations of both *P. areolatus* in Palau and *P. leopardus* on the Great Barrier Reef, each for over 200 hours of dive time, made the following comparisons between the two species.

Table 13. Comparison of spawning aggregation behavior in *Plectropomus areolatus* in Palau and *P. leopardus* on the Great Barrier Reef. (numbers of pluses are proportional to the relative frequency of the activity)**.**

Behavior	*P. areolatus*	*P. leopardus*
Males quivering to females	+	+++
Males aggressive toward females	++	+
Males defending territory	+++	+
Females moving about site	+++	+

In Palau, female *P. areolatus* often moved around spawning aggregation sites more quickly than the relatively sluggish female *P. leopardus* in spawning aggregations on the Great Barrier Reef described by Samoilys and Squire (l994). Male *P. areolatus* were much more aggressive towards other males than *P. leopardus* and spent less time displaying (quivering) to females. These differences may be species-specific, or may reflect the much higher f:m ratios among the *P. leopardus* aggregations that were studied by these authors.

Gonad analyses of Ebiil fish indicate that female *P. areolatus* stay on site for at least several days, during which they spawn fractionally, i.e., release their eggs in several discrete batches. We saw no females leave the aggregations during the peak spawning days, except for a few that fled aggressive males. This is in contrast to the results of tagging studies carried out on the congeneric *P. leopardus*, which revealed that fish, especially females, temporarily left spawning aggregations, sometimes traveling as much as 17km within one to several days before returning (Zeller, 1998; see also Samoilys, 1997a).

Turnover of fish in spawning aggregations

Shapiro (1987) reviewed literature indicating that a variety of species of Caribbean grouper spawn more than once per spawning period. Shapiro et al. (1993, p. 409) suggested that grouper "individuals may remain within the spawning aggregation for much of its duration. Thus, we doubt that frequent replacement of post-spawning individuals by new arrivals substantially increases the number of individuals spawning in the aggregation each year." Zeller, 1998) has shown, however, that some *P. leopardus* move to and from spawning aggregation sites several times during a spawning period.

Analysis of gonads of females of all three species in the Palau spawning aggregations indicated that they spawned more than once per month, and suggested that at least some of them remained on the spawning grounds for at least 2 days. In addition, over a 19 month period, 7 individually identifiable male *P. areolatus* participated in spawning aggregations for minimum average[29] of l0 days per aggregation. Two remained at spawning sites for a minimum of up to 12 days (Table 8).

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29. We use the term "minimum average" here because of the possibility that these fish were present on some days when we did not monitor the aggregation, or did not see them because their distinctive markings were on the side away from the observer.

At what time of day do these groupers spawn?

We know from gonad analysis that spawning of all three species takes place at Ebiil and Western Entrance aggregations on, or shortly before the new moon around the time of peak aggregation sizes. (It was illegal to take fish from Ngerumekaol for gonad analyses in order to make this determination). Why were we unable to observe spawning at Ngerumekaol?

Most species of grouper for which spawning has been described spawn during a period of roughly l/2 hour near dusk around new or full moons (Colin, 1992; Samoilys and Squire, 1994). But observations during ten twilight periods on the part of several of our team with long experience in field observation of spawning reef fish failed to reveal any spawning at Ngerumekaol, although, as described above, pre-spawning activity in all three species was very common for several days before the new moon.

Spawning may have occurred at night after we could no longer see well enough to continue our observations. Some evidence for this was the spontaneous spawning of a pair of *E. fuscoguttatus* in a laboratory tank at the Palau Mariculture Demonstration Center on the night of June 8, 1994, the night before the new moon. At dusk the female was very fat, showing clear signs of egg hydration that indicates the imminence of spawning. We saw no sign of prespawning or spawning activity that evening although we watched until visibility was no longer adequate - about 30 minutes after sunset. Thirty minutes before dawn the next morning the fish were quiescent but the female was noticeably thinner. Large quantities of fertilised eggs were present at and near the surface of the water. The state of development of the eggs, upon inspection, suggested that they had been spawned sometime after 3am (B. Maidraisau, pers. comm.).

Lim et al. (1990) reported that *E. fuscoguttatus* held near Singapore in cages spawned naturally around midnight at, or just after, high tide. Similarly, Al-Thobaity and James(1996) reported that E. *polyphekadion* held in tanks in Saudi Arabia normally spawned at night or early in the morning and that *E. fuscoguttatus* spawned in these tanks in the early morning. One should not draw firm conclusions about spawning behavior in the field based on spawning behavior in captivity, however.

Our inability to witness spawning of *P. areolatus* at Ngerumekaol cannot be explained this way, however, since at dusk this species hides in crevices and appears to remain inactive throughout the night. It seems very unlikely, therefore, that it spawns at night. Solomon Island fishermen described in detail to Johannes (1989) their seeing what appeared to be pair-spawning in *P. areolatus* at both around sunset and sunrise. And Samoilys and Squire (l994) describe its congener *P. leopardus* as a dusk spawner.

One possible explanation for our failure to see spawning of *P. areolatus* at Ngerumekaol is, as mentioned above, that it did not occur. The abnormally low f:m ratios at Ngerumekaol and Ebiil were the apparent cause of a difference in the courtship behavior of this species there compared with Western Entrance. Females at Ngerumekaol and Ebiil were frequently harassed by groups of males. Perhaps, in consequence, spawning was reduced (at Ebiil, where only one possible spawning event was witnessed and gonad analyses confirmed that some spawning occured) or even eliminated (at Ngerumekaol where no spawning was observed despite many attempts to witness it).

If our hypothesis is correct, it could explain why spawning aggregations lasted longer at Ngerumekaol and Ebill than at Western Entrance, and why aggregations continued to form at the latter sites after the normal spawning season; that is, unsuccessful spawners “kept trying”.

We were unable to analyze the gonads of fish from Ngerumekaol for indications of spawning failure because the aggregation was off limits to fishing. But, if our hypothesis is correct, then a trend towards a low f:m ratio in a spawning aggregation is a warning sign. For, as this ratio declines, spawning may decline and cease well before an aggregation is fished to extinction or even before the stock is recognized as being in jeopardy.

There seem to be no published observations of such a phenomenon among fishes. However, there is a description in the mammalian literature of reduction of reproductive output as a result of mobbing of female seals by supernumerary males (e.g., Hiruki et al. 1993).

Trippel and Harvey (1990) were the first to point out the possible association of spawning failure with an imbalanced sex ratio in fish, although in the case they described the problem was too few males, not too many. Coleman et al. (1996, p. 140) discussed evidence that, because of a very high f:m ratio in the grouper

Mycteroperca phenax on its spawning grounds, some females did not spawn and their eggs underwent atresia. These authors pointed out (p. 140) that, as a consequence of unbalanced sex ratios, “reproductive failures can occur before the stocks are recognized to be in jeopardy.”

How do groupers locate their spawning aggregation sites?

Because of their oceanic larval stage, grouper larvae are not likely to recruit with high fidelity to the specific aggregations from which they originated[30]. It therefore seems unlikely that groupers are genetically imprinted for locating their spawning sites. A more likely possibility is that this ability is learned via social transmission, i.e., that of young fish following older, experienced fish. Warner (1988; 1990) provided experimental evidence that the young reef wrasse, *Thalassoma bifasciatum*, learns how to locate the traditional local spawning site for this species from experienced adults. Immature Atlantic cod are known to join adults to make “dummy runs” to spawning grounds (e.g., Woodhead, 1959; Rose, 1993), and diurnal migration behavior of reef fish (grunts) from feeding to resting areas has been shown to be socially transmitted (Helfman and Shultz, 1984). As we have shown, female *P. areolatus* travel to their spawning aggregation sites in schools. In addition, a few immature groupers were found in our study aggregations. Possibly these had joined the female schools or followed males to the aggregations.

Monitoring changes in stock sizes

A spawning aggregation is part of an adult population that may include fish that aggregate elsewhere (although, in the case of the groupers studied here, this seems unlikely for reasons discussed below) or that do not aggregate when other indidivuals are doing so. It would be useful to know the size, structure, range, and degree of isolation of the whole adult population and how these parameters are changing. To obtain this information, however, would be extremely expensive and time-consuming, if not impossible. We began this research with the assumption that year to year changes in the size and structure of a spawning aggregation - which are relatively easily measured - are an index of changes in the larger adult population of which it is a component - which are not easily measured.

30. Earlier we discussed why it is likely that grouper larvae are self-recruiting to Palau as a whole, however.

Operationally, it is more practical to make the aggregation the focus of management, rather than the larger population of which it is part, since we know much less about the latter. This is a precautionary approach. Unless and until evidence to the contrary emerges, it is prudent to assume that sustained major interannual decreases in the size of an aggregating population reflect a roughly proportional decrease in the size of the larger population of which it is part[31]. As discussed earlier, the decline and disappearance of grouper spawning aggregations has been associated with fisheries collapses in a variety of western Atlantic and tropical Pacific areas .

The monthly spawning aggregations of *P. areolatus* that now occur at Ngerumekaol could lead the casual observer or fisherman to conclude that more fish are available there, in the yearly aggregate, than in the past when it is widely agreed by fishermen that significant spawning aggregations occurred only about four times per year instead of the situation in 1995-96 where they occurred throughout the year. Since our tagging data establish that some individual fish joined every monthly spawning aggregation for more than a year, it can be seen that such an assumption is unjustified.

Some sources of variation in counts

To track changes in the size and structure of a given spawning aggregation from year to year, it is necessary to first quantify and account for the natural periodic variation in numbers and structure on seasonal (solar), monthly (lunar), and diurnal (solar) scales.

In order to do so, we must have some grasp of the variation in counts due to other influences, such as environmental conditions. Each count can then be adjusted to minimize the effects of these sources, leaving numbers that are more comparable and representative of the size of the aggregation.

Measurable environmental variables that affected our fish counts include the following:

- visibility[32]
- current direction
- current speed

How we estimated the effects of these factors is discussed in Appendix II.

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31. Over time a stock might come to rely more on isolated pair spawning and less on aggregation spawning because of selection due to heavy fishing pressure on aggregations (e.g., Colin, 1982). In such cases long term interannual changes in the aggregation size would not strictly reflect changes in the larger population. It remains to be demonstrated, however, whether such changes in grouper spawning pattern occur.

32. The "visibility" factor measured here was considered to incorporate all the environmental factors affecting the ability to see fish (e.g. cloud cover, turbidity, position of the sun). These factors were not examined separately (See Appendix II).

Recreational divers were sometimes present at Ngerumekaol. Sometimes they disturbed the groupers, especially *E. fuscoguttatus*, which would flee into holes in the reef on their approach. We avoided counting when other divers were there.

All-female schools

Schools of female *P. areolatus* swimming to, from or within spawning aggregations seem to be the only example of single-sex schooling behavior we know of among groupers of this genus. Johannes (l989) also observed a school of ten presumed females of this species in the vicinity of a spawning aggregation in the Solomon Islands. Similarly, groups of *Epinephelus striatus* (sex not determined) numbering up to 500 individuals have similarly been observed in the Caribbean moving parallel to the coast or along the shelf edge prior to spawning, probably in transit to spawning aggregation sites (Colin, 1992; Carter et al. 1994; Aguilar-Perera and Aguilar-Dávila, 1996).

Possibly the entry into spawning aggregations of female *P. areolatus* in schools functions to prevent the mobbing of individual females by large numbers of males. But this does not explain the fact that females also travel in schools for periods before reaching and after leaving the aggregation. Perhaps female schooling is an anti-predation measure, since the fish are much further from shelter during these migrations than they generally are as individuals during interspawning periods, and, being smaller than males, are more vulnerable to predation.

As discussed above, the selective targetting by line fishermen of female schools heading to or from spawning aggregations could be an important source of mortality contributing to the low f:m ratio seen in spawning aggregations of *P. areolatus.*

Routine monitoring

Long-term fisheries monitoring programs for Pacific Island nearshore fisheries have proven exceedingly difficult to maintain (e.g., Munro, l990; Munro and Fakahau, 1993). Indeed the latter authors state (p. 37), “Attempts have been made to establish statistical systems to monitor the artisanal fisheries in many tropical countries but we know of no example of successful sustained implementation.”

One objective of our research was to design a routine monitoring protocol to enable fisheries managers to operate simple, inexpensive, easily-taught monitoring programs for grouper spawning aggregations. The routine monitoring described in Appendix I is limited in both space and time (i.e., limited to spawning aggregation sites and to the peak months and peak days for those aggregations) and is simpler than other types of fisheries monitoring schemes that have been used in the region. Divers with no specialized training in marine biology were generally able to carry out accurate counts unassisted after a few days' practice. Nevertheless the complications associated with accurate estimates of spawning aggregation sizes turned out to be greater than we anticipated.

For example, being able to monitor only a portion of certain aggregations limited the types of data analysis we could do and the confidence we could place in the analyses we did do. Certain correlations that proved to be statistically significant were, nevertheless, inevitably less accurate than they would have been if all aggregations could have been monitored in total. In addition, even in as small an area as Palau, those months and days on which routine monitoring of grouper spawning aggregations were most appropriate for comparative purposes were found to differ for spawning aggregations of the same species at different locations. This is convenient insofar as it is difficult for one monitoring team to census more than one spawning aggregation per day. It is inconvenient, however, in that the timing at each spawning aggregation site must be determined by means of research monitoring for a considerable period of time - as we have done here - before one can identify the best months and lunar days for effective routine monitoring. Additional problems that arose are discussed in Appendix I and II.

Where underwater monitoring is too costly or too difficult, an alternative is to simply carry out gonad analyses of landed fish to establish the main spawning period. Under these conditions an initial research monitoring program such as ours would be unnecessary, but the degree of understanding of spawning aggregation dynamics would be greatly reduced.

What was done in Palau to protect spawning aggregations prior to our study was even less costly; it involved monitoring neither aggregations nor seasonality of gonad development. Restrictions on grouper fishing were implemented by the Palauan government simply in response to fishermen's descriptions of reproductive seasonality of groupers, their concern over the perceived declines in grouper stocks, and their requests for such restrictions (Johannes, 1991).

Elsewhere, however, fishers will not always be so conservation-minded, as willing to reveal their knowledge about spawning aggregations, nor as able to make satisfactory incomes from alternative fisheries during closed seasons.

One benefit of routine monitoring is that it requires fisheries personnel to be present on the spawning grounds when enforcement of fishing restrictions is most important; i.e., during peak spawning aggregation periods.

Other methods for monitoring spawning aggregations

As discussed earlier, many other tropical nearshore species aggregate to spawn at predictable times and places. Among them are: snappers (lutjanids), emperors (lethrinids), mullets (mugilids), jacks (carangids), barracuda (sphyraenids), milkfish (chanids); surgeonfish (acanthurids), rabbitfish (siganids), parrotfish (scarids), goatfish (mullids) and bonefish (albulids) (e.g., Johannes, 1978a; Thresher, 1984). These groups may pose monitoring and management challenges differing somewhat from those encountered in this study.

Many spawning aggregations consist of more or less densely packed individuals swimming in three dimensional schools off the bottom, such as mullets (e.g., Helfrich and Allen, l975), jacks (e.g., Johannes, l981); surgeonfish and unicornfish (e.g., Robertson, 1983; this study) and snappers (e.g., Domeier and Colin, 1997, this study). Here diver estimates of numbers will inevitably be less accurate. In fact, our experience with spawning aggregations of some snappers and surgeonfish consisting of several thousand individuals indicate that divers can do little better in such circumstances than to estimate their numbers simply as "several thousand" (see also, for example, Smith, 1972).

Hydroacoustic methods have been widely used for estimating numbers and biomass for continental slope and epipelagic fish spawning aggregations and the methodology is well established (Johannesson and Mitson, l983; MacLennan and Simmonds, l992). This approach may be adaptable for use with some aggregating reef fish species that are difficult to count using direct methods. Since spawning aggregations of some species are, unlike groupers, highly mobile, hydroacoustic methods should also be very useful in locating and characterizing them).

Some rabbitfish, such as *Siganus canaliculatus,* spawn in extremely shallow water (e.g., Hasse et al. 1977). Here neither UVC nor hydroacoustic methods seem practical. Portable towers might be used for monitoring such spawning aggregations where visibility permits counting fish from above the surface.

We found a good linear correlation between the CPUE of *E. polyphekadion* and our UVC estimates of abundance at Ebiil (Table 9). But we caution against assuming that CPUE will consistently show a significant linear correlation with abundance of aggregating spawners. As with any schooling fish, catch rates may not decline proprotionately as the aggregation shrinks because its density may not drop proportionately and fishermen may continue to make relatively good catches until the aggregation is almost exhausted.

In addition, our CPUE data was unusually accurate because of the controlled experimental nature of the fishing involved. The great majority of catch monitoring programs will not have that luxury. Getting good CPUE data from artisanal fisheries is often difficult because of the many boats, fishers and landing sites per unit of catch.

CPUE might sometimes be the only feasible option in the case of very diffuse spawning aggregations covering several square kilometers and where the fish are close to the bottom and thus not easily censussed using hyroacoustics, or UVC, such as *Epinephelus guttatus* (Beets and Friedlander, 1992; Shapiro et al. 1993), *Myctoperca microlepis* and *M. phenax* (Coleman et al. l996).

Two possible complications in using CPUE as an index of abundance are that some groupers cease to bite during or just prior to spawning (e.g., Randall and Brock, 1960; Carter, 1989), and, fishing over spawning aggregations of some species may be sexually selective (e.g., Moe, 1969; Thompson and Munro, l983)

Where the locations of spawning aggregations are unknown, or it is impractical to monitor them (e.g., if they are too far away to monitor easily, subject to frequent rough weather etc.) their seasonal timing, at least, may be determined by means of gonad analyses of portions of the catch.

Other species seen spawning

Various authors have noted that a variety of different reef fish species use common spawning sites (Randall and Randall, 1963; Johannes, 1968; Thresher, 1984; Colin and Bell, 1991; Colin, 1996). Among the species seen spawning at Ngerumekaol, Ebiil and/or Western Entrance (Table 10) is Palau’s single most important commercial reef fish, the bluespine unicornfish, *Naso unicornis* (**um**), and three more of the ten most common species in the commercial catches made between 1976 and 1990 (Anon, l993). These were the red snapper, *Lutjanus bohar* (**kedesau)**, the humpback snapper, *L. gibbus* (**keremlal)** and the bumphead parrotfish, *Bolbometapon muricatus* (**kemeduk**l).

Of the nine species or species groups Palauan fishermen listed in 1991 as declining seriously in numbers (Johannes, 1991) five spawn at one or more of our three study sites: *Epinephelus* species (**temekai**), *Plectropomus* species (**tiau**), bluespine unicornfish, *Naso unicornis* (**um**), bumphead parrotfish, *Bolbometapon muricatus* (**kemedukl**), and Napoleon wrasse, *Cheilinus undulatus* (**maml**).

Not all of the fish we observed spawning at our three study sites can be assumed to spawn selectively at these sites. But we did not witness any of the larger species spawning anywhere else during our many dives at locations other than our study sites during the three year study. Spawning of at least some of these species may thus be site-specific, although more research is needed to confirm this.

Most numerous among those species spawning in aggregations at our study sites were not groupers, but acanthurids, *Naso unicornis*(**um**), *N. literatus* (**cherangle**) and *Ctenochaetus striatus* (**masch**). These species were observed to spawn there while aggregating in thousands These aggregations were much shorter lived than those of the groupers; the fish left each day after they spawned. This may be one of the reasons that there are so many more references in the scientific literature to spawning aggregations of grouper than of acanthurids; typically aggregations of the latter, in our experience, do not last long enough to attract as much attention as do many groupers spawning aggregations [although some Palauan fishermen are well aware of them and exploit them (Johannes, 1981)].

Our observations were limited largely to mid to late afternoons from 10 days before new moon until about the day of new moon. Many reef fish species are known to spawn selectively at other periods of the day and other lunar periods (e.g., Johannes, 1978a; Robertson, 1983; Thresher, 1984). Thus it is probable that other species spawned at these aggregation sites unwitnessed by us, especially around the full moon period when many species spawn in Palau according to fishermen (Johannes, 1981) as well as in the morning (e.g., Robertson, l983; Colin and Bell, 1991; Colin and Clavijo, 1988), and possibly at night (see above).

As a means of estimating general abundance and diversity of coral reef fishes, UVC has become widespread in the past few years. The fact that many larger species of reef fish temporarily leave their usual habitats to spawn has important but rarely acknowledged implications for these activities. If UVCs are carried out during spawning migration or spawning aggregation periods, then the abundance of participating species will be underestimated - unless census-takers inadvertantly do their counts at spawning aggregation sites or on spawning migration routes. Then the general abundance of these species would be overestimated. Counts made during seasons and moon phases when spawning is occurring thus cannot be assumed to provide data that can be simply compared with counts made at the same locations at other times.

Management

Costs of research and monitoring for management

Here we briefly discuss the costs of research and monitoring of spawning aggregations. We do not provide detailed cost-benefit analyses because the specifics of our project are inevitably unique to a particular place, time and fishery. But it is instructive to consider in general terms the economics of research and monitoring that focuses on spawning aggregations.

The gross annual value of the commercial grouper fishery in Palau is roughly U.S.$100,000[33]). The net economic value of the fishery - i.e., after the costs of labor, equipment and fuel, research and management etc. are subtracted - will, of course, be much less. The true value of the fishery, which includes social benefits, is not easy to estimate.

The total cost of our three years' research was almost U.S.$300,000. It can be seen that, if our research served no other purpose than to support better management of Palau's grouper fishery, it would be hard to justify on economic grounds.

This research was funded very largely from sources outside Palau, as is the case with much of the fisheries research performed in the Pacific islands. But foreign economic assistance to the region is dwindling, and it cannot be taken for granted that the necessary sums would be readily available to determine grouper spawning aggregation dynamics elsewhere.

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33. Palau's commercial grouper fishery was worth about U.S.$20,000 in 1990, the latest year for which figures are available (Marine Resources Profiles of Palau, Forum Fisheries Agency Report # 95/12). It was estimated in 1992 that 60% of the catch was used for subsistence purposes, 20% for export, and 20% for local commercial sale (Palau Marine Resources Division, n.d.). If the these same proportions pertain to the grouper fishery, and if we assign the same value per unit weight for the import replacement value of the subsistence catch and sales within Palau, then Palau's total catch of groupers is worth about U.S.$100,000 annually.

Whether a research program such as ours would be cost-effective in other locations would depend upon many factors, such as labor costs, the value of the resource, the efficacy of existing management, and the subsequent costs of indefinite routine monitoring.

The approximate costs per day of routine monitoring at one aggregation site in Palau would be roughly as follows:

Personnel (2 divers + 1 driver @ U.S.$50/person)	150
Fuel (12 gal @ U.S.$2/gal)	25
Boat and equipment (capital and maintenance)	50
Supplies	10
Administrative support	10
Recording, analyzing data obtained	20
TOTAL	U.S.$264

The number of days per year during which routine monitoring would be necessary would vary considerably with species and location. Monitoring a single aggregation for, say, 8 days per year, would cost roughly U.S.$2,100.

The costs of enforcement would vary greatly depending on a variety of circumstances and is not estimated here.

The above calculations, rough though they are, are sufficient to suggest that a research program such as ours, followed by indefinite routine monitoring along the lines described in Appendix I, is not generally justifiable economically. It could only be justified at a limited number of locations where the value of the relevant marine resources are greater than Palau's, or where the results are of sufficient generality that they can help in the development of management plans for similar fisheries elsewhere. We hope our research here will prove justifiable on the second of those grounds.

We believe that continued routine monitoring and further behavioral observation is justified at Ngereumekaol because:

1. the present study provides a good baseline for additional work, and
2. Ngerumekaol is the first reef fish spawning aggregation site to be protected by modern law in the entire Indo-Pacific. As such it should serve as a useful

source of knowledge and experience that can be judicially applied to other spawning aggregation sites in the region. For example, if a better understanding of the implications of the anomalous sex ratio of *P. areolatus* develops with further research at this site, it could have important implications elsewhere for the management of spawning aggregations of this species and perhaps others.

We believe that occasional monitoring of Ebiil and Western Entrance is justified for the first of the above reasons.

Precautionary management

Our studies provide a greater understanding of the dynamics of grouper spawning aggregations than appears to be available for any other grouper species or location in the Indo-Pacific with the possible exception of *Plectropomus leopardus* on the Great Barrier Reef (e.g., Samoilys and Squire, l994; Ferreira, 1995; Samoilys, 1997a; Zeller, 1997, 1998). Previous discussion should make it clear, however, that this is not enough information, even if coupled with routine monitoring in future years, to facilitate the rigorous quantitative management of Palau's grouper stocks.

Our research has revealed that, even within such a small area as Palau, different spawning aggregations of the same three species have different sex ratios (in *P. areolatus*, at least), different size distributions and intraspecific behavior, and somewhat different timing. Thus, we cannot predict these characteristics accurately for the other ten or so aggregation sites for these species that are known to exist in Palauan waters. Nor do we know enough about the population structures and dynamics of any of the three species to manage their stocks to achieve optimum yields or maintain optimum stock sizes (however 'optimum' might be defined).

It is becoming widely recognized that such finely tuned management is, in any event, quite impractical in coral reef fisheries (e.g., Russ and Alcala, 1996; Munro, 1996). A less sophisticated, but nevertheless invaluable and more economically realistic alternative exists, however - precautionary management. Precautionary management can mean managing without quantitative objectives other than that of protecting against the severe depletion or local extinction of a fishery (e.g., Johannes, 1998).

Lack of rigorous fisheries statistics is no excuse for not implementing this type of management when fisheries appear threatened. And we may take it for granted that spawning aggregations of food fish that fishermen know about and that are not protected are threatened. As Domeier et al. (l997) state, “Proactive rather than reactive management is called for when aggregation sites exist.”

There is nothing new about precautionary management. Its use, in fact, greatly predates that of classical Western style management. It has been employed for centuries, for example, by Pacific islanders, including Palauans (Johannes, 1978b, 1981). More recently it has also been used by many government fisheries managers. In this connection it has been used for at least a decade to protect grouper spawning aggregations by simply closing them to fishing for all or a portion of the spawning season (see review in the Introduction above).

Currently the most widely discussed precautionary management measure in coral reef fisheries is the marine protected area (e.g., Roberts and Polunin, 1993; Russ and Alcala, 1996; Hatcher et al. in press). Protecting spawning aggregations is another option. The two forms of protection coincide where a marine reserve encompasses important spawning sites.

Focusing protection exclusively on spawning aggregations may not always suffice if the bulk of the fishing pressure on a stock is not on them. The portion of the total annual Nassau grouper catch taken during the spawning season in various parts of the eastern Atlantic region, for example, ranges from 20 to 90% (Sadovy and Eklund, in press). As mentioned earlier, in Palau there has been a pronounced peak in landings of groupers during the spawning season (Kitalong and Oiterong, 1991), although the monitoring of fish landed was not inclusive enough to facilitate accurate estimates of the percentage of annual catch involved.

Aggregation-based management of groupers and other aggregating spawners in the tropics

As discussed earlier, in addition to groupers, many other coral reef fish species aggregate at the same sites in order to spawn. Our observations, like those of Randall and Randall (1963), Johannes (1981), and Thresher, (1984) emphasize the fact that the protection of major spawning aggregation sites can help protect many species simultaneously.

The reduction or elimination of fishing over a spawning aggregation will often be easier and simpler than alternative management measures. It could take any of the following forms:

1. Permanent reserve status for a spawning site (preferably with a buffer zone around it)

- especially useful for important spawning sites, i.e., those where large numbers of fish and/or many species spawn. Such a reserve could be part of a more general marine protected area.

2. Total closure of fishing for the target species during spawning months

-requires surveillance on fishing grounds or landing facilities

- fishing ground surveillance can be, however, circumscribed, i.e., limited to spawning aggregation sites

- is easier to accomplish where local marine tenure is strong

3. Closure during a portion of the spawning season

- If there is to be an open fishing period during the spawning season, it should probably be at the end of the season. At least some groupers tend to be less fertile then (e.g., Al-Thobaity and James, 1996) and fish that spawn in several different months during the season would have an opportunity to spawn before the fishery opened.

4. Closure of fisheries during the key lunar period(s) during the spawning season

- hard to enforce, difficult to educate fishermen about

- requires policing on the fishing grounds

- in areas where a live reef fishery exists, the law could be circumvented by catching fish during the closed periods and keeping them surreptitiously in holding cages until the season opened.

5. Banning of commercial sale during spawning months

- does not require fishing ground surveillance; can be monitored in markets, restaurants and export facilities

- may not be effective where subsistence fishing predominates

6. License limitation; village or regional government sells a limited number of licences for exploitation of a spawning aggregation, possibly using the proceeds to monitor the fishery

- much too complicated for most tropical small-scale multi-species fisheries, and too open to abuse

7. Quota system

- much too complicated for most multi-species artisanal fisheries (e.g., Munro, 1996)

8. Size limits

-impractical in multi-species artisanal fin-fisheries (e.g., Munro and Fakahau, 1993)

9. Banning grouper exports, including the live reef food fish trade during the spawning season

-the reasons for implementing recommendation no. 9 in Palau are discussed in the section on export controls below.

Some management issues

Where a species has only a few major spawning sites in a region, closures might be enforced on the spawning grounds. But not all spawning aggregations will be as accessible, nor as compactly constituted as those we studied. *Plectropomus leopardus*, for example, form smaller, more numerous spawning aggregations on the Great Barrier Reef (Samoilys and Squire, 1994). Palauan fishermen describe spawning aggregations of this species in their waters as having similar characteristics. It thus appears impractical to attempt regional control of the exploitation of such species by on-site enforcement of closures. But region-wide closures can be enforced to some degree through surveillance at commercial landing sites and markets.

Some species migrate to their spawning grounds in schools, following predictable routes along which fishermen intercept them with nets or fish fences. This includes mullets in Palau (Johannes, 1981), Tonga, Samoa, Solomon Islands, Hawaii (until the runs ceased), and Manus, Papua New Guinea (all Johannes, unpubl.) and bonefish in Kiribati (Johannes and Yeeting, ms). Here fishing closures and surveillance might be extended beyond spawning aggregation sites to the migration routes leading to them. Rabbitfish, *Siganus* *canaliculatus*, are similarly exploited during their migration to their spawning grounds in Palau (Johannes, 1981 and unpubl.) and in Bolinao, Philippines (McManus et al. 1992). As stated earlier, it appears as if fishermen at Ebiil exploit groups of female *P. areolatus* migrating to the spawning grounds. More information is needed to determine how widespread such practices are. Where they exist a complete prohibition on landing or holding (e.g., live in pens) the species involved during the spawning and migration season might be considered.

Where a large portion of the catch is used for subsistence purposes - a common situation in the Pacific islands (Dalzell et al. 1996) - control at the market may not suffice. Market surveillance may also be difficult where there are many small markets rather than one or two bigger ones to monitor, or where individual restaurants and hotels get their fish directly from fishers.

Social and legal circumstances will vary. Where customary marine tenure remains strong, as, for example, in parts of Melanesia, controls over exploitation of spawning aggregations are often easier to enforce; enforcement can be done by local traditional authorities provided that the regulations are made by them or with their active collaboration. The further the spawning aggregation is from the traditional owners of the fishing grounds, however, the harder such traditional enforcement at spawning aggregation sites is liable to be.

In situations where apprehension of law-breakers is difficult, fines should be very high so that, although the risk of being caught is low, the consequences are serious enough to act as a real deterrent.

Pros and cons of closing spawning aggregations to fishing

Advantages

1. Closures reduce chances of recruitment overfishing[34] by maintaining critical spawning stock biomass. As an indication of how vulnerable spawning aggregations can be to targeted fishing, the Ebiil spawning aggregation yielded 2,355 fish to line fishermen over the short period of our tagging exercise. (In this case all fish were released except for a few injured ones). Moreover, because of reef fishes' long pelagic larval period during which they are often dispersed offshore, such closures should provide a recruitment source for reefs throughout the archipelago, not just the area surrounding the spawning site. This is especially important for groupers in Palau because of

34. Reduction of spawning stock by fishing it down to the point where spawning is reduced so much that future recruitment will also be significantly reduced.

the decline and disappearance of their spawning aggregations in at least 5 other locations (see above). Since Palau is so far away from the nearest other reefs, it must rely for recruitment largely or entirely upon reproduction in its own waters.

2. Closures can prevent total management failure. If Palau's other grouper spawning aggregations continue to decline and disappear, the protected aggregations will help guard against the complete collapse of grouper stocks that has occurred in some other areas.

3. Closed areas are often simpler to implement than many other conservation measures. For example, closures of spawning aggregations to fishing can be limited in both time and space, facilitating relatively simple monitoring and surveillance. Where surveillance capabilities on fishing grounds are limited, some control can be effected by surveillance at landing sites and markets during spawning periods.

4. Appropriately timed closures can protect fish migrating to and from spawning aggregation sites as well as fish at the aggregation site. As discussed above, fishermen sometimes target fish on their spawning migrations as well as on the spawning grounds.

5. Protecting spawning aggregations is not just easily understandable and more accepted by fishermen than some other management measures, but was also a traditional practice in many Pacific island cultures (e.g., Johannes, 1978b). A traditional management precedent such as this makes its contemporary use more acceptable to fishermen. Indeed, it was fishermen who asked the Palauan government to employ this approach as a conservation measure[35] (Johannes, 1981).

6. If fishing on spawning aggregations is prohibited, but research on them is permitted, the aggregations can provide invaluable controls or baselines for comparison with aggregations in fished areas.

7. Areal closures can be used to maintain undisturbed habitat.

8. Some spawning grounds are used by many species (see above), and spawning seasons of many species are similar (reviewed by Johannes, 1978a). Area closures can thus facilitate multi-species protection.

9. Seasonal closures help avoid seasonal gluts in catches of aggregating species.

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35. Similarly the majority of commercial, charter boat and recreational fishermen on the Great Barrier Reef support the concept of spawning closures (Turnbull and Samoilys, 1997). It is perhaps worth pointing out that the worship of fertility is an almost universal human trait. Perhaps it is for this reason that protecting spawning aggregations seems to strike a responsive chord in fishermen who are sometimes less inclined to accept other kinds of control.

Disadvantages

1. Closures are not useful for species which do not form large spawning aggregations at predictable times or places.
2. Closures would not protect stocks adequately if they simply displaced catches to the open season. (Since fish in spawning aggregations are typically much easier to catch than they are at other times, this may not often be a problem).
3. Closures would probably be unpopular with fishermen in areas where most of their annual catch of a species comes from its spawning aggregations, the species is not readily caught at other times or locations and/or alternative resources are not readily available to exploit.
4. Closures will probably result in increased effort in other fisheries. Whether this creates a problem or not would depend, of course, on whether these alternative fisheries could sustain the increased effort.
5. Closures would publicize the locations of key spawning aggregation sites and thus risk attracting poachers.
6. Areal closures are difficult to enforce if spawning aggregations are numerous and small [as with *Plectropomus leopardus* on the Great Barrer Reef (Samoily and Squire, l994) - as well as in Palau according to Palauan fishermen] - rather than large and relatively few, as with the three species examined in this study.
7. Closures decrease short-term fishing efficiency.

Improving precautionary management in Palau

As discussed above, precautionary management of grouper fisheries was already practiced in Palau before our research began - long before in the case of traditional **bul**. More recently the national closure of grouper fishing from April through July has been put into effect, as well as the national law helping to protect the Ngerumekaol spawning aggregation.

During the course of our work in Palau we found that local people were genuinely interested in getting outside opinion on the appropriateness of their locally generated marine resource management efforts. We were pleased to be able to tell them that not only were they on the right track, but that they set a very good example for many countries possessing far more formal scientific expertise. They are, in fact, well ahead of the rest of the entire Indo-Pacific, including developed western countries such as Australia, in their efforts to protect their spawning aggregations. But what improvements in their management might be implemented as a result of our research?

Ngarchelong and Kayangel States are clearly interested in, 1. conserving their reef fish resources, and 2. ensuring that an adequate share of the benefits from those resources accrue to themselves. For these reasons, we recommend establishing one or more no-fishing zones in their waters. Kayangel has already created one at remote Ngaruangel atoll, where fishing has been prohibited for three years while the state decides how best to manage the atoll for the long term.

Whereas criteria for choosing protected sites are many, and their general discussion beyond the scope of this report, the reef surrounding Western Entrance has several attributes that might make it ideal. First, the spawning aggregation of *P. areolatus* at Western Entrance is Palau's only aggregation of this species known-to be in good shape (i.e. without disturbingly low f:m ratios). And, as mentioned earlier, a number of other grouper spawning aggregations in Palau are known to have disappeared entirely. That alone is reason enough to protect it in our opinion.

Second, in terms of prevailing currents. Western Entrance is at the upcurrent end of Palau. In theory, then, the larvae of groupers and other reef fish and invertebrates produced there would therefore have a greater chance of returning to Palau's reefs that those originating further downcurrent in Palau.

Finally, since Western Entrance is relatively remote, fishing pressure there appears to come less from the reef's owners, i.e. the fishermen of Ngarchelong and Kayangel, than it does from those with large boats and deep pockets to cover fuel costs, i.e., the commercial and recreational fishermen based in Koror.

Some recreational fishing by outsiders may be in the interest of Ngarchelong and Kayangel, but only to the extent that residents can control it and benefit from it. Both states are already planning a tourist-based inshore sportfishery (Anon., 1996). Such a fishery will require areas of abundant fish, a high degree of control of the fishery, as through permits and surveillance, by the states, and participation by the fishermen of Ngarchelong and Kayangel as guides.

The reefs on either side of Western Entrance, Ngarael to the northeast and Ngebard to the south, provide some of the best inshore sportfishing in Palau. Protection of part of this reef system - such as through establishment of a reserve in which only catch-and-release fishing is allowed - would both protect important spawning aggregations and conserve the reef fish that are necessary to support a potentially profitable sportfishing industry. Development of a sportfishery would also help justify the costs of enforcing such a protected area.

a. Ngerumekaol

As stated above, we propose that ripe *P. areolatus* that are unable to spawn during the annual aggregation period(s) may continue to return to the spawning site at subsequent new moon periods in an attempt to spawn even though the typical spawning season has passed. The physiological basis for such behavior is unclear but may be linked to a feedback mechanism whereby, if spawning cannot or does not occur, the imperative to spawn persists and the animal returns repeatedly (and stays longer) in response.

These facts, plus the following combination of factors, clearly argue for stronger management, even though the available data are insufficient to prove the case with scientific rigor[36]:

1. the spawning aggregation of *P. areolatus* at Ngerumekaol is almost certainly much smaller than it was in the 1970s[37],
2. recorded grouper catches in Palau between 1980 and 1993 dropped by 75%,
3. a number of other grouper spawning aggregations in Palau have disappeared, apparently due to overfishing,
4. a relationship has been reported elsewhere between declines of grouper spawning aggregations and the collapse of associated stocks, (reviewed above in the Introduction),

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36. Although catch statistics for Palau show that grouper catches dropped steadily for at least 13 years, the species are lumped, only a portion of the catch was monitored, and the data pertain to catches from throughout Palau, not those from a particular spawning aggregation. In addition, no fishing effort data are available. Moreover, during this period the private fish-selling sector expanded while the landing data came from only two major markets, thus the recorded catch would probably have decreased even if the catch stayed constant. In short, it is not possible to interpret these data conclusively.

37. This statement is based in part on the widely held opinion of Palauan fishermen. Kitalong and Oiterong (1991, p. 4) state for example, that "(spear) fishermen said that you could easily push smaller groupers aside (in the Ngerumekaol aggregation) and search through a pile for larger-sized fish in the late 1970s". There are also REJ's field notes, based on observations made on the day before new moon in June of l976; although he did not count the fish, he observed that aggregating *P. areolatus* occupied the entire length of Ngerumekaol Channel, whereas they were never observed to occupy more than about the seaward half of it during the three years of the present study.

5. the closure of spawning aggregations of groupers to fishing will also protect the spawning sites of a number of other important food fish whose catches are decling seriously according to fishermen.

It should be stressed that the degree of protection the Ngerumekaol spawning aggregation was given, although not adequate, nevertheless may well have prevented it from disappearing altogether. As noted above, some groouper spawning aggregations have already disappeared in Palau, and the Ngerumekaol spawning aggregation is situated a mere half hour boat ride from Koror where the bulk of Palau's human population resides.

An estimate of the number of fish below which such an spawning aggregation should not be allowed to drop without taking immediate remedial measures might be achieved through the use of Population Viability Analysis (e.g., Gilpin, 1989). Such an analysis would, however, take us well beyond the limits of the present investigation and, as Vincent and Sadovy (1998) have pointed out, has its own significant limitations when applied to fish populations.

The current ban on fishing at Ngerumekaol extends from April 1 through July 31. Significant spawning aggregations of *E. polyphekadion* and *E. fuscoguttatus* formed in August, a month after the ban was lifted. Moreover, *P. areolatus* formed spawning aggregations every month for the last 2 months of our study. Clearly then, the four-month seasonal closure does not fully protect the spawning aggregations of any of the three species.

As discussed earlier, we strongly recommend continued routine monitoring of the Ngerumekaol spawning aggregations and periodic monitoring of Ebiil and Western Entrance. For Ngerumekaol, under normal circumstances, we recommend monitoring from May through July, with April monitoring in the years when a new moon occurs during the last half of April. However, because of the current aberrant seasonal timing of *P. areolatus* spawning aggregations we recommend at least one day of monitoring per month (the 2nd, 3rd or 4th day before new moon) from August through April as well - at least until such time as the seasonal timing of these aggregations reverts to the typical pattern.

Spawning aggregation sites are sometimes the focus of recreational diving. Ngerumekaol in Palau, and the Cod Hole on the northern Great Barrier Reef, for example, are spawning aggregation sites that are widely promoted by both international dive magazines and local dive tour operators as prime dive sites.

However, our observations suggest that recreational divers, especially those in groups, may disrupt spawning aggregations and possibly inhibit spawning. (Zabala, et al. [l997b] observed the grouper *Epinephelus marginatus* being attracted to divers, but this was in a marine reserve where recreational divers were allowed to feed these fish).

No terrestrial nature reserve manager would encourage visitors to walk through rookeries full of nesting birds. River rafting during the salmon spawning season is prohibited on a number of U.S. rivers. Similarly, we believe that, as a precautionary measure, recreational diving groups should be discouraged from investigating reef fish spawning aggregations.

Because Ngerumekaol is an important recreational dive site, Koror State may wish to allow some diving in the area. It is not clear if, or to what degree recreational divers might interfere with grouper spawning activities. But diving during the days of the lunar month when grouper spawning aggregations are absent would likely have little negative impact (unless physical damage to the site, such as anchor damage, were to occur).

Koror State might allow diving by special permit only, thereby controlling exactly when and how many people dive there. A special guide - be it a Koror State employee or not - might be required to accompany the divers. This would simplify enforcement as well a help ensure that diving activities remain within Koror's guidelines. The permit system could also serve to generate revenues needed for monitoring and management of Ngerumekaol. At the same time, Ngerumekaol could be made into a showcase dive site, especially if guides provided interpretive services.

As discussed earlier, the existing law banning fishing at Ngerumekaol is not very specific in describing the restricted area. For legal purposes, and to ensure universal understanding of restrictions, the geographic limits of those restrictions should be clearly defined and marked. For reasons discussed earlier, it would also be desirable to expand the area in question. (We have discussed in detail with Koror State where these boundaries might best be placed).

Law enforcement also needs improvement. Although patrolling at Ngerumekaol or elsewhere is probably best done somewhat unpredictably in order to keep prospective poachers guessing, it would be advisable to concentrate management efforts in the periods during which grouper spawning aggregations are largest and poachers are likely to be able to remove the greatest numbers of fish, namely between the lst and 10th day before the new moon. One way of cutting down management costs would be to combine enforcement and management activities when practical, by training and using enforcement personnel in monitoring.

Although substantial increases in the abundance of groupers and other large carnivores have been observed after the implementation of marine reserves (reviewed by Russ and Alcala, 1996), it may take five or more years before such effects become obvious. As these authors state (p. 959), such results place “a great onus on fisheries and community management for long-term enforcement (scales of 5-10 yr) to ensure usefulness of marine reserves as potential sources of recruitment to fished areas.”

To summarize our recommendations with regard to the Ngerumekaol spawning aggregation:

1. Establish a year-round, no-fishing zone encompassing Ngerumekaol Channel and a well-marked and substantial buffer zone around it. (At this writing, legislation to act on this recommendation had been introduced in the Koror State Legislature).
2. Ban recreational diving at Ngerumekaol between full moon and new moon throughout the year[38] and establish a permit and guide system for recreational diving at other times.
3. Step up surveillance and enforcement, concentrating on the lst to 10th days before new moon when aggregation counts are highest
4. Carry out routine monitoring indefinitely.

b. Ebiil and Western Entrance

At Ebiil and Western Entrance significant spawning aggregations occurred in August and early September, as much as 6 weeks after the end of the **bul**. Thousands of fish were taken from the Ebiil spawning aggregation by fishermen during this period in l996. For this reason we recommend that the **bul** be extended through August. For the same reason we also recommend that the national closed season for grouper fishing in Palau be extended through August.

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38. In 1996 and 1997 Koror State declared Ngerumekaol off-limits to diving from April through July.

Export controls

Current Palauan national law prohibits fishing for five grouper species, including the three studied here, from April through July each year, with the intent of protecting them while they aggregate to spawn.

Another nationwide control recommended here is to ban the export of these and other reef food fish species from Palau. Export restrictions have the advantages of ease of enforcement and not hindering fishing efficiency. Palau already prohibits the export of Napolean wrasse, bumphead parrotfish, and a number of marine invertebrates, and has an active enforcement program at the Palau airport.

Given Palau's growing domestic demands for reef fish, a year-round export prohibition on not just groupers, but all reef food fish, should be considered [see Birkeland (1996) for an extended argument in favor of banning reef fish exports in general]. Palau's population has been forecast to grow from its current 18,000 to between 29,000 and 70,000 by the year 2020 (Anon., 1997). Add to that the rapidly growing number of visitors, with their additional demand for seafood as well as for diving and sportfishing, and it becomes apparent that exporting reef fish not in Palau's best interests.

Palau has been exporting as much as one third of its total reef fish catch of roughly 1,500mt per year. Most exports have gone in fresh and frozen form to Guam and Saipan, while live reef fish exports to Asia have occurred intermittently (about 54 tonnes from 1984 through 1988, and 35 - 75 tonnes from 1994 through 1996).

Most of the live reef fish exported have been grouper, as is typical of the region's live reef food fisheries supplying Chinese markets in Asia. Live reef fish fisheries bring special concerns, and in general, the problems they create for small island countries can easily outweigh the benefits (Johannes and Riepen, 1995; Johannes and Lam, l999). One of these problems is that they often target spawning aggregations, as they did in Palau in the 1980s, when at least one spawning aggregation was virtually eliminated.

The live reef food fish trade prefers plate-sized groupers to larger ones, which means, in essence, that they ship mainly females. Exporters of live reef fish have told LS and REJ that gravid female groupers do not withstand stress as well as male fish or females not in spawning condition. They also note that gravid females that are anaesthetised for transport die at dosages otherwise considered safe. In addition, they tend to release their eggs spontaneously in the holding facilities. Once a few fish die or release their eggs in a high-density tank environment, the degradation in water quality often soon kills all the remaining fish (see also Bentley, in press).

In summary, it is recommended that all exports of reef food fish from Palau be banned.

Research prohibition

Palau's ban on fishing on grouper spawning aggregations lacks any exemptions for research. There is nothing to gain and possibly much to lose by preventing researchers from sampling fish from spawning aggregations. It severely constrains research, such as that described here, that is directed toward better fisheries protection. For most grouper species, moreover, it is not possible for divers to determine sex reliably by sight. An aberrant sex ratio for such species can therefore not be detected without obtaining some fish from a spawning aggregation for gonad analyses.

Moreover, if Palau is to provide eggs or broodstock for aquaculture, which is a potential future prospect (see below), then access to spawning aggregations - such as has been available for this purpose in the past in Palau (e.g., Tamaru et al. 1996; Tucker and Fitzgerald, l994) - is essential.

The Ngerumekaol spawning aggregation may now be the most studied coral reef spawning site in the world, and certainly one of the most readily accessible. We have not only the detailed information on spawning aggregations of three species of grouper, but we also have preliminary information on the spawning of more than 50 other species at the site. This site thus has great future research potential. For this reason we recommend that efforts be made to encourage further research there in future years.

Spawning aggregations and marine reserves

Russ and Alcala (l996, p. 947) state, "A major objective of the use of marine reserves in protection of coral reef fisheries is protection of a critical spawning stock biomass to ensure recruitment supply to fished areas via larval dispersals." (For similar assertions see Bohnsack, l998; Roberts and Polunin, l993). Clearly for that reason, the boundaries of coastal marine reserves in the tropics should, wherever possible, encompass spawning aggregation sites. Moreover, the presence of an important spawning aggregation site is in some cases justification in itself for the establishment of a marine reserve. Such is the main reason for the Palauan government's declaring Ngerumekaol a marine reserve. Yet there is little evidence in the literature indicating spawning aggregation sites were given any consideration when the boundaries of most marine reserves were drawn throughout the Indo-Pacific. Palau's Ngerumekaol Spawning Area appears to be in fact, the first example of a modern coral reef reserve in the Indo-Pacific created specifically to protect spawning aggregations of reef fish. In contrast, in the Caribbean as described earlier, protecting of grouper spawning aggregations is taken seriously in many contries. Beets and Friedlander (1998) have recently demonstrated that the closure of a Caribbean grouper spawning aggregation led to a significant increase in the mean size of aggregating fish and a much less skewed sex ratio. (Changes in absolute numbers of fish were not assessed).

As discussed earlier, many larger reef foodfish tend to spawn at the same locations at or near the outer reef drop-off in both the Caribbean and tropical Pacific (e.g., Randall and Randall, 1963; Munro, 1974; Loubens, 1980; Johannes; l981; Colin and Clavijo, 1988). Fishers generally know far more about the location and timing of such spawning aggregations than researchers. Indeed many researchers have acknowledged that they first learned of the existence of particular spawning aggregations from fishers (e.g., Catala, 1957; Craig, 1969; Smith, 1972; Bagnis et al. 1972; Hasse et al. l977; Cross, 1978; Olsen and LaPlace, 1978; Johannes, 1981 and 1989; Thompson and Munro, 1983; Johannes and Squire, 1989; Colin et al. 1987; Aguilar-Perera, 1993; Coleman et al. 1996; Aguilar-Perera and Aguilar-Davila, 1996; Koenig et al. 1966; Passfield, 1996; Gobert, 1997; Domeier and Colin, 1997; Beets and Friedlander, 1998; Sadovy and Eklund, in press; Samoilys and Squire, 1994). This is just one of many reasons why fishers should be involved from the start in the design and planning of marine reserves.

Marine reserves would protect spawning aggregations more effectively if members of such aggregations did not sometimes move to other spawning aggregations outside the reserve. Our tagging work provided no evidence that any fish moved from one spawning aggregation to another. Zeller (1998) found that all 35 *Plectropomus leopardus* he tagged and tracked ultrasonically displayed spawning aggregation site fidelity over a two-month spawning season. Luckhurst (in press) reported similar results for tagged *Epinephelus guttatus*.

Grouper spawning aggregations as an aquaculture resource

Grouper aquaculture is a growing business, especially in Southeast Asia (e.g., Johannes and Riepen, 1995; Nelson, 1996). Knowledge of the timing and location of spawning aggregations of groupers could facilitate access to broodstock for the purpose. According to Kuo (1995, p. 307), "The availability of mature males and females (groupers), at the same time, has been the major bottleneck of natural spawning in captivity. However, acclimation of wild-capture males with the female broodstock for a period of time makes natural spawning possible. . . . This has become the most commonly practiced method in recent years, and is the most practical way to produce fertilized eggs consistently in a commercial operation."

Wild males are used because they are typically several years older than females and thus take longer to produce in captivity. However, wild grouper broodstock are reportedly hard to obtain in southeast Asia. Aquaculturists in Taiwan, for example, are often forced to grow out their own (Nelson, 1996).

As work sponsored by the Guam Aquaculture Development and Training Center has shown, it is possible in Palau to catch gravid groupers with baited lines while they are in their spawning aggregations and to strip these fish and airship their fertilized eggs without unacceptable mortality (Tamaru, 1993). Rimmer et al. (1994) also succeeded in spawning wild coral trout, *Plectropomus leopardus*, captured with hook and line while in their spawning aggregations on the Great Barrier Reef.

The provision of broodstock or fertilized eggs from wild Pacific Island stocks for aquaculture may have some economic potential. Fertilized eggs from wild stock should fetch premium prices[39]; they are reported by Asian aquaculture researchers to achieve higher hatching rates and produce more robust larvae than eggs from farm stock (Johannes and Riepen, 1995).

Methods for wild stock enhancement with hatchery-produced groupers are currently under development in Bahrain (Uwate and Shams, 1997) and Okinawa. If sea ranching of groupers proves feasible, wild broodstock will prove especially valuable since hatchery broodstock are selected for adaptation to hatchery conditions and their progeny are thus less likely to perform well in the wild.

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39. This is similar to shrimp aquaculture, where wild broodstock of certain shrimp may be worth hundreds of times as much as cultured broodstock.

Colin (l982) found that cross-fertilization of different surgeonfish species was possible, even with speared fish. The possibility of producing hybrids with improved aquaculture characteristics should be explored. In Palau both *Epinephelus* species we studied spawned within a day or two of one another at the same locations, facilitating the possible production of hybrids.

In various Pacific island countries the precise locations and timing of spawning of various species of grouper are well-known. Selling wild-caught fish as brood-stock need not threaten local stocks because a few females can provide millions of eggs.

Destructive fishing practices and spawning aggregations

Explosives, although used only occasionally for fishing in Palau, are widely used for the purpose in other parts of the Indo-Pacific, notably in the Philippines, Indonesia and Chuuk. The immediate damage to spawning aggregations that will occur when subject to dynamite fishing is obvious. But there is also a possibility that explosives may also render aggregation sites unusable by future generations of fish.

As we noted earlier, when disturbed on spawning sites *E. fuscoguttatus* often sought shelter in large holes and overhangs, and female *P. leopardus* sometimes sought shelter on the sites in smaller crevices and overhangs and under coral heads. Beets and Friedlander (l998) also noted the association between spawning aggregations of *Epinephelus guttatus* and structurally complex habitat. Use of explosives in such habitats would destroy such shelter and might thus render the area unacceptable to spawning fish.

The uncontrolled harvesting of reef fish spawning aggregations by the live reef food fish trade is widespread. Cyanide is often used for the purpose. Cyanide not only stuns the target fish, but also kills corals (Jones, 1997), and, over a period of years these corals disintegrate. Like dynamiting, therefore, cyanide degrades spawning habitat.

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APPENDIX I. ROUTINE MONITORING

Choosing the best months for monitoring

As described earlier, to compare the size of a spawning aggregation from month to month we chose the peak counts in a given month as the descriptor. In order to follow spawning aggregation dynamics through an entire spawning season it would be desirable for two reasons to monitor at least one month both before and after the months of any significant aggregation activity. First, the finding of non-existent or small spawning aggregations before and after the peak months will confirm that the seasonal peak has indeed been observed. Second, measurement of the length and timing of the spawning aggregation season will allow identification of any inter-annual differences in this timing. Such changes may reflect potentially important changes in other stock characteristics such as sex ratio, as discussed earlier.

If such extensive monitoring is deemed impractical[40], there is a more limited alternative. It may be possible to detect substantial inter-annual changes in spawning aggregation size and sex ratio over a number of years by monitoring for four months a year at each site. In general, May through August encompasses the seasonal peak in aggregation sizes of two, and perhaps all three species at all three sites (we know nothing about what happens at Western Entrance after August). It should be noted, however, that the largest annual spawning aggregation counts for *P. areolatus* at Ngerumekaol occurred in March in l995 and February in l996 (Fig. 4) - an apparently anomalous situation as discussed earlier.

We remind the reader that we are focussing only on groupers here. The fact that other important food fish spawn at these aggregations may suggest, on further investigation, that it would be desirable to monitor during other months and lunar periods as well.

Choosing the best days of the lunar month for monitoring

To determine the most probable days of the lunar month on which monitoring will record peak numbers, it must first be established whether the monthly timing of the peak count of the aggregations varies in anything but a random fashion with respect to the lunar calendar. If, for example, the day of the peak

40. It may not be cost effective, as discussed earlier in this report.

was found to occur later in the lunar month over the course of this study, it would be inappropriate to use a static routine monitoring protocol; extensive monitoring would have to be carried out indefinitely in order to keep track of the timing. If the lunar monthly timing was found to vary seasonally, but not over the long-term, this would also complicate the protocol design, although less so.

In order to determine whether the lunar monthly timing of the spawning aggregations varied in anything but a random manner with respect to the seasons and over the course of this study, we computed correlation coefficients between the number of days before new moon on which the peak counts of fish occurred, as well as each of:

1. the calendrical month of the year (1-12) and
2. the lunar month within the study period (1-28)

Coefficients vary between -1 and 1. The closer to zero the coefficient, the less likely there is a real relationship between the two factors. The following table shows the correlation coefficients for each species at each site.

Table 14. Correlation coefficients for relations between peak spawning days and months of the year (seasonal) and months of the study period (long term). (Number of cases shown in parentheses)

	Ngerumekaol		Ebiil		Western Entrance	
	Long term	Seasonal	Long term	Seasonal	Long term	Seasonal
P. areolatus	-0.42 (11)	-0.51 (11)	-0.33 (13)	-0.10 (13)	-0.21 (7)	0.68* (7)
P. areolatus sex ratio	-0.53 (10)	-0.75* (10)	-0.21 (11)	0.00 (11)	-0.18 (7)	0.68* (7)
E. fuscoguttatus	-0.16 (7)	-0.46 (7)	0.11 (6)	0.82* (6)	0.56 (3)	0.50 (3)
E. polyphekadion	-0.62 (4)	-0.52 (4)	0.33 (4)	0.30 (4)	0.37 (4)	0.45 (4)

* indicates that the correlation is significant at the 95% confidence level.

There were no significant correlations between the lunar days of peak counts and months of the study period (long term) at any of the three sites (Table 3). There were two cases in which month of the year ("season") was significantly correlated with the day of peak fish count (and with sex ratio). At Ngerumekaol, the peak sex ratio of *P. areolatus* occurred slightly later in the month as the year progressed, while at Western Entrance it occurred slightly earlier in the month as the year progressed.

It appears from these limited results that the possibility of seasonality of reproduction changing through the long term is not a critical concern. It also seems unlikely in view of the well-documented regular seasonality of reproduction of many marine organisms. Also, knowledge concerning the usual seasonal timing of these aggregations has been passed down by generations of Palauan fishermen. Our contrary finding with *P. areolatus* at Ngerumekaolappears to be unusual, as discussed above.

We determined the best lunar days on which to monitor in order to determine peak counts. If one could monitor every day of the month, the problem would be simple. But since cost is an important consideration in the design of a long-term monitoring program, monitoring should be limited to as few days per month (and as few months per year) as are practical.

One strategy would be to determine which day of the lunar month the peak size is most likely to occur, and monitor on that day each month. This can be done by simply computing the average day of the peak for each species at each site. These averages, along with their standard deviations, are shown for each of the species and sites in Table 3.

The average peak days shown in Table 3 represent those days of the lunar month on which one is most likely to observe the peak spawning aggregation size for that month. The standard deviation of the mean is a measure of how likely the peak is to occur on that day. These statistics give no indication of how far off the peak (in numbers of fish) the count on that day is likely to be if the peak does not in fact occur on that day.

However, the shapes of the temporal trends in spawning aggregation size for each site, species, and month do give an indication of this. Typically, over the course of the two weeks or so preceding a new moon, the number of fish tends to increase gradually, eventually levelling off at or near the peak for a few days, then rapidly decreasing to or near zero in just a few days (e.g., Fig 2). It is evident, then, that if one monitors too early at Ebiil or Ngerumekaol - one or two days before the peak - the count will likely be only slightly lower than the peak count. But if one monitors too late, i.e., one or two days after the peak, the count may be substantially lower than the peak count. Clearly, therefore, the strategy of trying to monitor on a single day to "capture" the peak, is risky.

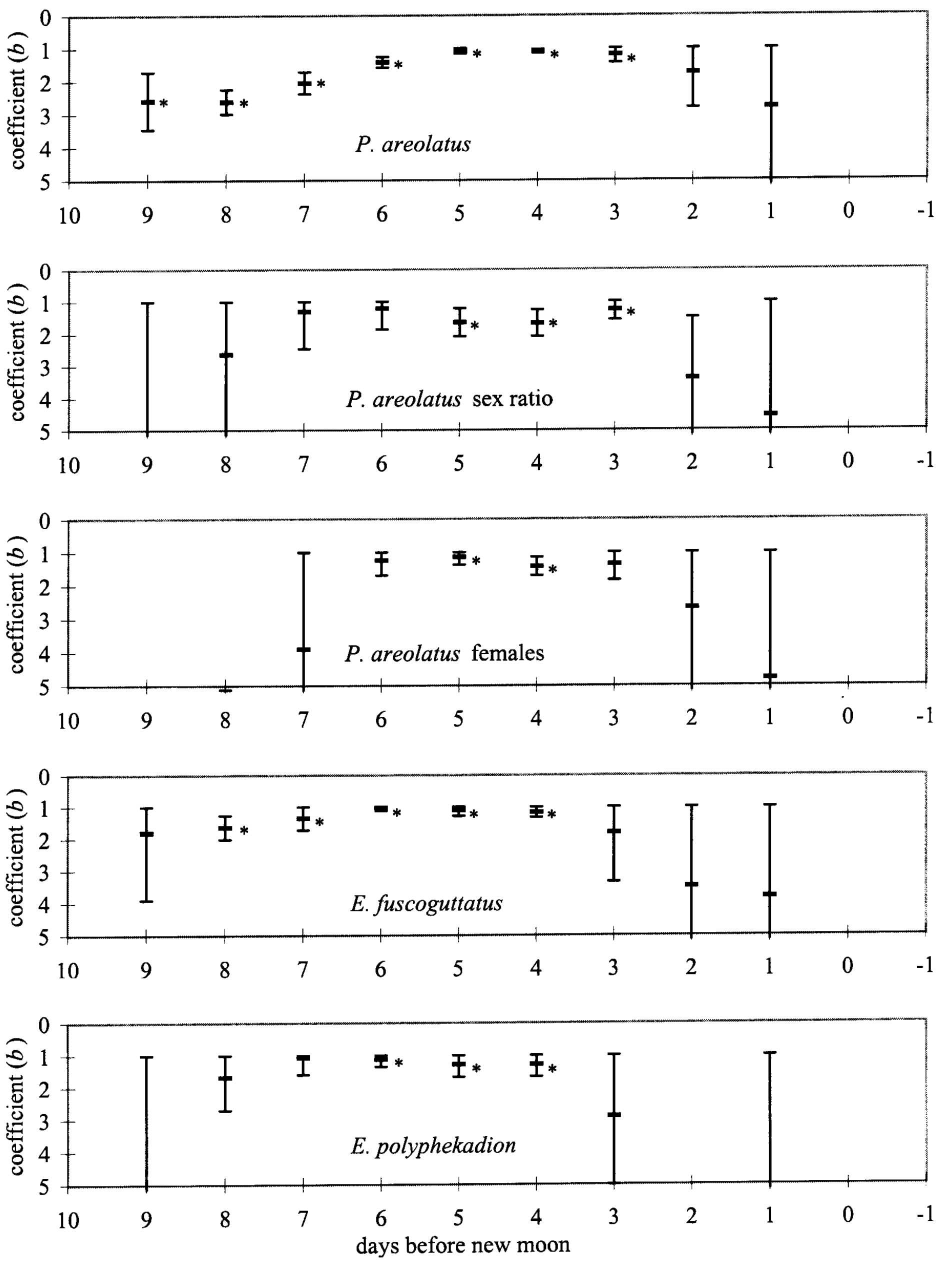
A better approach is to monitor several days before, during, and after the most likely day of the peak. For each site and species, the best combination of days to monitor for a given level of confidence in capturing the peak can easily be determined. For example, if one is satisfied with a 95% level of confidence in capturing the peak, the number of days of monitoring needed to attain that level, and which days are best, can be computed, and monitoring can be carried out on those days. But this approach is not recommended for long term monitoring because a strict adherence to the chosen protocol would be necessary in order to attain the desired level of confidence in the results. If, for example, four particular monitoring days are called for but one day is missed because of bad weather, the level of confidence in having captured the peak may be seriously compromised. A much greater degree of flexibility is needed in order to cope with the inevitable difficulties in planning and executing monitoring.

Instead of ensuring that the fish are counted on the day on which the counts peak, the protocol can be designed to ensure that the fish are counted on a day that gives a reasonable ability to predict the peak count. This allows any of several days to be monitored, and a reasonably reliable prediction of the peak may be obtained after only one or two days of monitoring. If cost and flexibility are important considerations, this is clearly the superior strategy. Figs. 10 - 12 illustrate this strategy for all three sites.

For each site, species, and day before new moon, a separate regression function was computed. The function was of the linear form: monthly peak = b*count, where "count" was the number of fish counted on that day of the month. The number of observations used to estimate the coefficient b is the number of times (months) during which that day was monitored during this study.

In the graphs on the following pages (Figs. 10 - 12), the coefficient b is plotted against the day of the month. The closer b is to 1, the closer that day is to the average peak day. To reiterate, the goal is not to choose that peak day, but rather to choose the day when the ability to predict the peak count is maximized. That ability is reflected in the confidence limits associated with b.

Figure 10. Coefficients to estimate monthly spawning aggreagation peaks, Ebiil



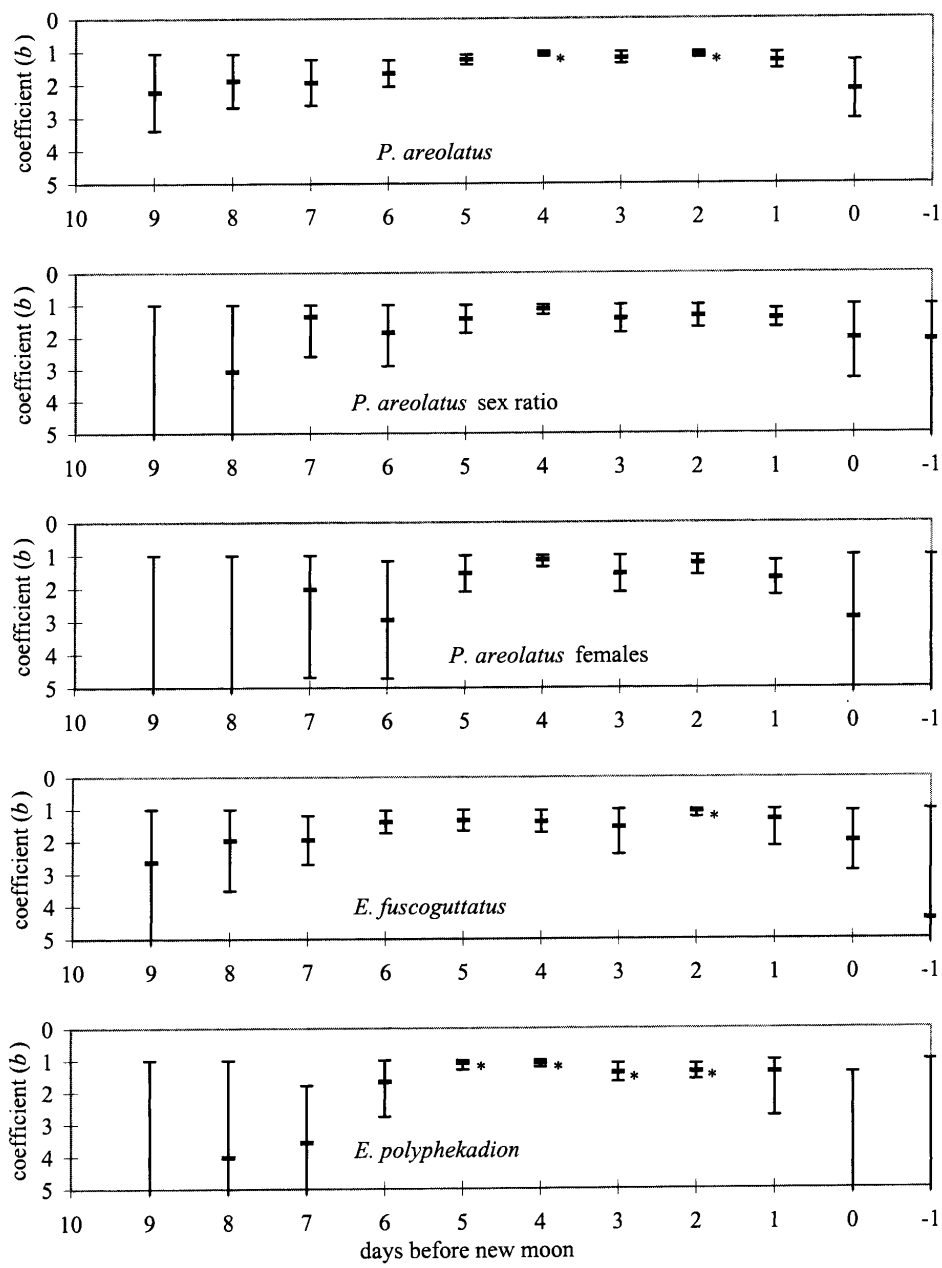
footnotes:

Monthly peak =*b*(count). Error bars indicate 95% confidence limits

* indicates function for which *F* was significant at the 95% confidence level

Only 1 through 9 DBNM computed and graphed here

Figure 11. Coefficients to estimate monthly spawning aggregation peaks, Ngerumekaol



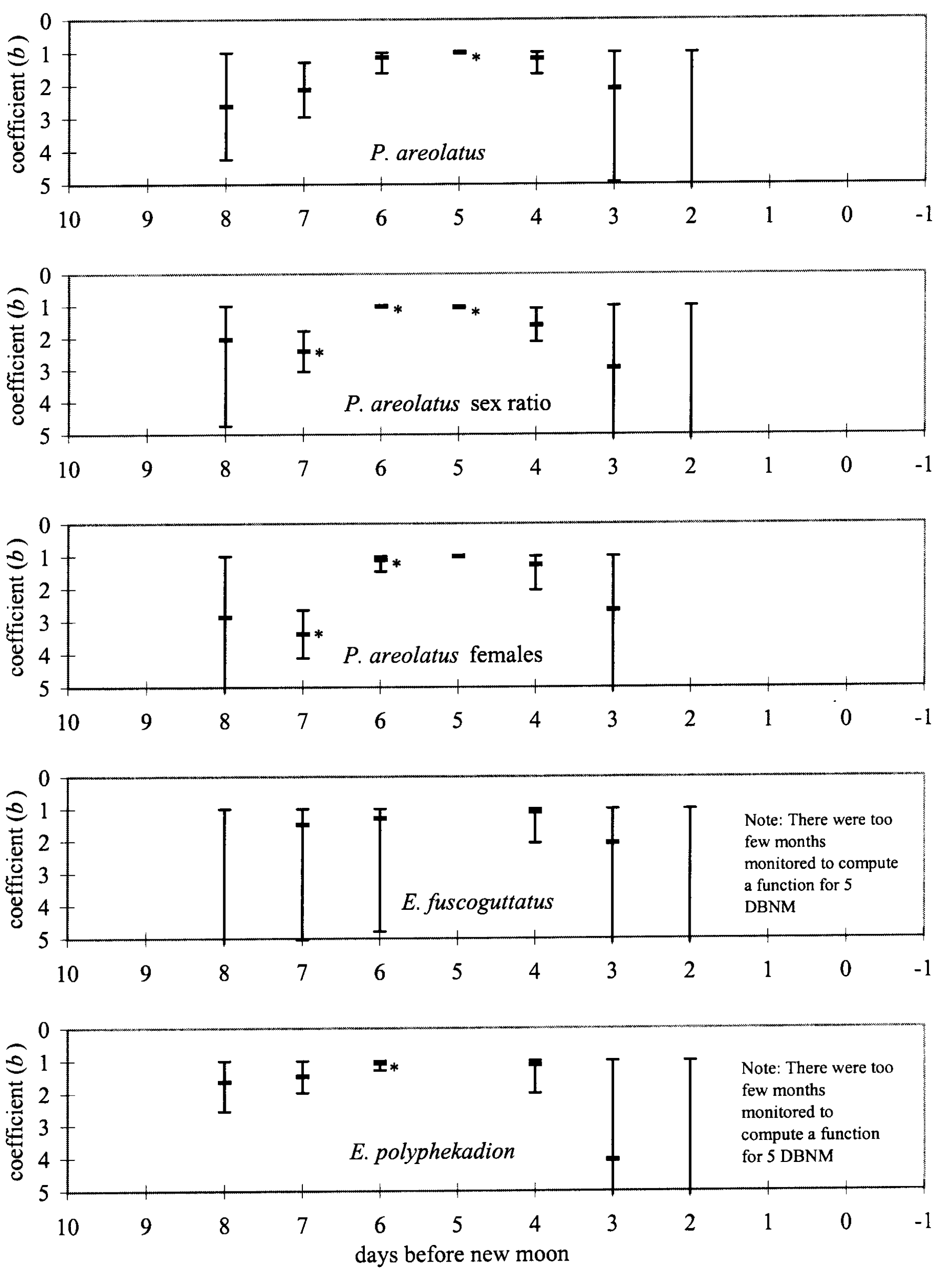
footnotes:

Monthly peak =*b*(count). Error bars indicate 95% confidence limits

* indicates function for which *F* was significant at the 95% confidence level

Only l through 9 DBNM computed and graphed here

Figure 12. Coefficients to estimate monthly spawning aggregation peaks, Western Entrance



footnotes:

Monthly peak =*b*(count). Error bars indicate 95% confidence limits

* indicates function for which *F* was significant at the 95% confidence level

Only 2 through 8 DBNM computed and graphed here

The error bars shown on the graphs are 95% confidence limits. Looking at *P. areolatus* at Ebiil, for example, one can be 95% confident that the mean of *b* for all possible hypothetical counts made 2 DBNM is between 1.00 and 2.81. This range is much larger than that required to predict the peak with a reasonable degree of reliability. The day with the narrowest confidence interval is 4 DBNM ($1.04 < b < 1.15$), followed by 5, 6, and 3 DBNM.

In order to put this strategy into practice, first, monitoring would be routinely done on the day or days where *b* has the narrowest confidence interval (favoring the earlier acceptable days in order to have some flexibility to accommodate bad weather, etc.). Second, after monitoring, the month's peak count can be predicted by multiplying the count for a given day by the coefficient, *b*, for that day. If more than one day was monitored during the month, an average of the several predictions can be computed, weighted according to the narrowness of the confidence intervals associated with each day's coefficient.

Finally, the confidence associated with the final prediction of the monthly peak can be computed. It can be expressed as a prediction interval equal to the product of the standard error of the prediction and the critical value of *t* at the desired level of confidence. If more than one day was monitored, computation of the standard error of the prediction is not simple, but if only one day was monitored, it is straightforward:

$$\text{Prediction interval} = +/- = +/- t_{(.95,n-1)} * (\text{MSE}(1+(X^2/\text{sum of } x^2)))^{1/2}$$

where X is the count, and MSE (mean squared error), sum of x^2 (sum of squared values of sample counts), n (number of cases) and *b* are given in Table 15. Values for $t_{(.95,\ n-1)}$ can be found in most statistical textbooks.

Table 15. Statistics for the predictive regression model, monthly peak = *b*(count)

Ebiil: *Plectropomus areolatus* counts

DBNM	n	*b*	S.E (b)	Sign.(*F*)	*p* value (*b*)	MSE	Sum x^2 95% (*b*)	Lower 95% (*b*)	Upper 95% (*b*)
9	9	2.58	0.38	0.0071	0.0001	8432.7	59409	1.71	3.45
8	12	2.61	0.17	0.0000	0.0000	3691.1	128549	2.24	2.98
7	12	2.05	0.15	0.0000	0.0000	4733.0	205875	1.71	2.38
6	12	1.42	0.07	0.0000	0.0000	2417.6	441568	1.26	1.58
5	13	1.08	0.04	0.0000	0.0000	1336.1	859853	1.00	1.16
4	13	1.09	0.03	0.0000	0.0000	550.2	846136	1.04	1.15
3	13	1.19	0.12	0.0004	0.0000	8856.9	648544	1.00	1.44
2	12	1.74	0.49	n.s.	0.0044	42743.5	181007	1.00	2.81
1	11	2.79	1.70	n.s.	0.1313	70969.6	24592	1.00	6.58

Ebiil: *Plectropomus areolatus* sex ratios (F/100M)

DBNM	n	*b*	S.E (*b*)	Sign. (*F*)	*p* value(*b*)	MSE	Sum x^2 95% (*b*)	Lower 95%(*b*)	Upper 95%(*b*)
9	9	7.06	4.29	n.s.	0.1382	1772.4	96	1.00	16.95
8	11	2.63	1.51	n.s.	0.1128	1545.4	675	1.00	6.00
7	11	1.30	0.52	n.s.	0.0316	1239.8	4599	1.00	2.45
6	11	1.20	0.29	n.s.	0.0022	753.7	8691	1.00	1.86
5	11	1.65	0.20	0.0069	0.0000	250.9	6497	1.21	2.08
4	11	1.67	0.18	0.0034	0.0000	217.5	6422	1.26	2.08
3	11	1.25	0.14	0.0047	0.0000	232.3	11308	1.00	1.57
2	10	3.38	0.84	n.s.	0.0029	782.1	1118	1.49	5.28
1	9	4.56	1.78	n.s.	0.0338	1208.1	380	1.00	8.67

n.s. = not significant
DBNM = Days before new moon
S.E. = standard error
Sign. = statistical significance

In order to put this strategy into practice, the following three steps can be taken:

1. Monitor on the best day(s): From the appropriate graphs, choose the day or days where *b* has the narrowest confidence interval. This will require making tradeoffs among the three species. Those days indicated with an asterisk (*) are those for which the F statistic of the regression function was significant; that is, for which we have 95% confidence that the function describes a real relationship. Monitoring should be limited to those days. Because of unpredictable weather, it is best to place some preference on the earlier acceptable days. Thus, if the initial plan does not work out, there will still remain some acceptable monitoring days before confidence declines rapidly.
2. Compute the predicted peak count: After conducting the count, using Figs. 10 - 12, listing the coefficients, *b*, to compute the predicted peak count by multiplying the count by *b*.
3. Compute the confidence associated with the peak count: The confidence associated with the above prediction can be expressed by a prediction interval equal to the product of the standard error of the prediction and the critical value of *t* at the desired level of confidence. Use the following formula and Table 15 to compute the 95% confidence interval associated with the predicted peak count.

An example: if 100 *P. areolatus* are counted 4 DBNM at Ebiil, the predicted peak count for the month is bX = 109 fish. The prediction interval is +/- 51 fish. Since the peak cannot be less than 100, one can be 95% confident that the actual peak count for the month is between 100 and 160 fish.

A critical assumption in this approach is that, as discussed above, the lunar timing of the spawning aggregations does not change seasonally or over the long term. Computation of the confidence intervals associated with the coefficient *b*, is based on the assumption that the sample (the months of this study) is representative of the entire population of months (all future months). The analysis summarized in Table 14 indicates that this is a reasonable assumption. But to ensure that it holds, it would be desirable to conduct more intensive monitoring over the course of a month occasionally—ideally monitoring enough days to capture both the peak and the drop-off to near-zero numbers.

Choosing the best time of day for monitoring

In determining what time of day to conduct routine monitoring, one ideally wants to keep the most important variables constant, or at least to account for their effects. Some of the non-temporal variables that affect a given fish count (described more in Appendix II) include water visibility and current speed. Although we did not assess the effects of these and other variables rigorously, we suggest that these two, together with time-of-day, are the variables with the greatest influences on a given count (aside from day of the month and month of the year).

The effect of time-of-day must inevitably be a function of the day of the lunar month. Since we saw fish both arriving and departing during the course of day it is inevitable that there was, in fact, daytime variation in aggregation numbers despite the fact that our limited attempts to demonstrate it failed.

During the gradual build-up in numbers in the first stages of an aggregation, we might expect counts later in the day to be greater than those early in the day. The opposite would occur during the days when an aggregation is dispersing. This rate of change would probably be greater during the dispersion period than during the build-up period, as aggregation numbers decline much more rapidly than they increase (Fig. 2).

The effects of water visibility and current speed on our fish counts were not empirically examined, but the observers made educated guesses of those effects (described in Appendix II). It was estimated that each of the two factors could affect a given count by as much as 30%, and together, as much as 50% (i.e., 100 fish counted under “ideal” conditions would be counted as 50 fish under “worst” conditions).

At our study sites, visibility is primarily dependent on current direction which is primarily dependent on semidiurnal tidal phase and secondarily on lunar tidal phase and wind and wave conditions. Current speed is also dependent on both the semidiurnal and lunar tidal phases.

At Ngerumekaol, for example, the top of the incoming tide generally yields the clearest water and slowest current.

Therefore, at locations where visibility and current speed are highly variable yet predictable, aiming to monitor during a particular tidal phase rather than particular time of day is probably the best approach. This was the approach tried initially at all three sitess, but at Ngerumekaol, it was found that current direction and speed were not predictable enough: weather conditions and unknown factors influenced them too much to make the method practical. The alternative was then adopted of monitoring during a certain time of day, regardless of other conditions. The constant-time-of-day approach also has the advantage of being easier to plan for and establish a routine. Whichever approach is taken, the effects of the most important factors that cannot be kept constant - in our case visibility and current speed - can be corrected for after their effects are estimated.

The most important monitoring days at our study sites were the few days before new moon. An advantage of monitoring in mid to late afternoon during those days was that this was the time most likely to yield clear waters on an incoming current with the sun behind the swimmer.

APPENDIX II. FACTORS AFFECTING ACCURACY AND PRECISION OF FISH COUNTS

Here we discuss how we estimated fish counting errors. The discussion is necessarily fairly complex. We want to reassure the reader at the outset that we are not recommending such elaborate data-treatment for purposes of routine monitoring. The approximations afforded by untransformed counts may suffice for routine management needs.

Measurable sources of variation

Sources of variation in counts due to variations in visibility and current speed can be estimated (and we estimated them during each count). But their effects on the fish counts are not easily measured. In order to measure the effects of one, the others must be kept constant. But in an observational (i.e., non-experimental) investigation such as this one, this is not possible. Therefore, measuring accurately the effects of what are likely to be important factors, such as visibility and current speed, are nearly impossible to do empirically.

One alternative is to lump them together as “sampling error.” A second alternative, which is used here, is to use the “expert opinion” of the most experienced visual census observers to estimate the effects of these environmental factors. These observers simply made quantitative estimates of the effects of each of these factors.

We recognize that some readers may take a dim view of such "unscientific" estimates. But if they are made by divers highly experienced at monitoring these species and sites, they are helpful in indicating the magnitude of counting errors posed by currents and visibility. Monitors were highly familiar with monitoring conditions at these sites, having spent 170 site-days (LS) and 150 site-days (TG) counting and observing the three species over a three year period. In addition, prior to this study LS had made several hundred dives on spawning aggregations of groupers on the Great Barrier Reef (e.g., Johannes and Squire, 1988; Samoilys and Squire, 1994)

As discussed above, the monitoring method we used helped minimize the effects of these environmental factors. It involved not a timed swim nor a swim along a particular route, but a count of all visible fish within a particular area. If visibility was poor, for example, the observer spent more time covering the site, often swimming more of a zig-zag rather than a straight line across the site.

Current speed and visibility were estimated by the observer after each count. Current speed was recorded on an eight-point scale, ranging from "no current" up to "very fast current" (about 2 knots). Visibility was recorded on a seven-point scale and ranged from "very poor visibility" (approximately 5-7m) up to "excellent visibility" (approximately 20m or greater). The "visibility" factor measured here was considered to incorporate all the factors affecting the ability to see fish (e.g., cloud cover, turbidity, position of the sun), so those factors did not need to be examined separately. The average visibility score over the course of this study was 4.6, or between "moderate" and "moderate-good." The average current speed score was 3.1, or just faster than "moderate-slow."

There was consensus among the observers that "visibility" affected only the observer and did not affect the fish to a detectable degree (e.g., the fish did not tend to stay lower or hide in the rocks when the water was more turbid). Current speed, in contrast, affected both the behavior of the fish and the abilities of the observer to count them. For *P. areolatus*, for example, the stronger the current, the greater the tendency for the fish to stay closer to the bottom and in holes, making them more difficult to count. The effect was somewhat less important at the northern sites than at Ngerumekaol because at the northern sites the fish aggregated in areas with less cover. Less important than the effects on the fish, but also affecting the counts, was the increased difficulty in counting fish while the monitor was swimming either against or with strong currents.

Notwithstanding these differences in the "visibility" and "current speed" effects among the three species and three sites, rough estimates of the two effects that can be applied to all species at all sites are the following: 70% of the fish counted under conditions of no current would be counted if there was a very strong current.

Seventy percent of the fish counted under "very good visibility" would be counted if there was "very poor visibility." As already noted, these figures are simply estimates made by the observers during the course of the study.

Combining the effects of the two factors, under the worst conditions (i.e., very poor visibility and very fast current) one would expect to count 70%*70% = 49% of the fish that would have been counted under ideal conditions. Under moderate conditions, one would expect to count 85%*85% = 72% of the fish that would have been counted under ideal conditions. As it turned out, the combined current and visibility conditions did not vary dramatically over the course of the study. It was determined that adjusting the raw counts to account for the visibility and current effects would have only minor effects on the results. Therefore, the results presented throughout this report are based on raw, unadjusted counts.

Relationships between spawning aggregation counts and true aggregation size

Not all fish at a spawning aggregation site will be counted during a given census. First, some fish may be outside the boundaries of the count area. Second, some fish inside the count area will be missed by monitors.

As stated earlier, a core area for monitoring was delineated at each of the three study sites so as to capture the largest proportion of the aggregation that it was possible to count in about an hour. At Western Entrance the entire aggregation was always contained within the core area. But during peak aggregation periods at Ngerumekaol and Ebiil the aggregation expanded beyond the core area, so our counts were lower than the total numbers of groupers present. The percentages of the aggregations that were found inside the monitored areas at peak aggregation size were roughly estimated by primary monitors (LS and TG) (Table 16).

Table 16. Percentage of fish in spawning aggregations that were found inside the monitored areas during peak spawning aggregation periods

	Ngerumekaol	Western Entrance	Ebiil
P. areolatus	80%	100%	90%
E. fuscoguttatus	85%	100%	80%
E. polyphekadion	95%	100%	35%

Because we were able to monitor only an estimated 35% of the *E. polyphekadion* spawning aggregation at Ebiil, the uncertainty associated with those counts is necessarily large relative to count of other species and at other sites.

Not all fishes inside the count area are necessarily counted, whereas some fish may be counted twice. Following are estimates (by LS and TG) of the average number of groupers of each species, based on their different behaviors, that are counted in ideal counting conditions (i.e., no current, good visibility) at each site.

Plectropomus areolatus

Typically, 90% of the aggregating fish are on or near the bottom, while 10% are up and moving about in the water column. About 40% of the 10% in the water column will be double-counted and a similar percentage missed. About 5% of the 90% on the bottom will be missed because they are hidden in the rocks. Therefore, of 100 fish in the count area, about 95 can be expected to be counted with ideal visibility and current conditions.

Epinephelus polyphekadion

About 5% of the fish will be missed because they are hidden in the rocks. Another 5% of visible fish will be missed, while 5% are double-counted. These two effects occur in part because of the occasional tendency for groups of these typically immobile fish to suddenly move rapidly in unison and “shuffle” themselves into new positions. So, for each 100 fish in the count area, approximately 95 can be expected to be counted under conditions of ideal visibility and current conditions.

Epinephelus fuscoguttatus

About 5% of the fish will be missed because they are hidden in the rocks. About 3% of the visible fish will be double-counted (these fish tend to bolt out and swim ahead of the observer, in effect being "herded" by the observer). Therefore, for each 100 fish in the count area, approximately 98 can be expected to be counted with ideal visibility and current conditions.

Sex ratio estimates

Estimation of sex ratios of *P. areolatus* at spawning aggregations during UVC is subject to two potential biases:

1. Since the females move under coral ledges more than males, female numbers may be underestimated.
2. Sex ratios might differ between the portions of the aggregations we monitored and those that we could not monitor (see discussion above of instances where we could not monitor the entire aggregation).

The above biases in sex assessment appear to be small. Therefore estimated sex ratios in different aggregations appear amenable to comparison.

Effects of SCUBA versus snorkel

At Ebiil and Western Entrance, the counts were always performed using SCUBA. At Ngerumekaol, SCUBA was always used near the channel mouth and in the middle of the channel. But snorkel was used during the first year along the channel walls. The team then switched to SCUBA along the deeper of the two channel walls. On 66 occasions, the counts were performed along this wall twice within an hour or so, once on SCUBA and once on snorkel. This was done in order to see whether there was a difference between the counts of the two methods.

A model of the form (SCUBA count) = a + b*(snorkel count) would be the simplest way to examine the effect of counting method. But this could be complicated by the fact that three additional factors might affect the relationship between "SCUBA count" and "snorkel count": visibility, current speed, and which count was done first. For example, in high visibility conditions one might be able to count as well from the surface on snorkel as on SCUBA, but not necessarily so with poor visibility. The second count might be affected if the first count scared fish into hiding or out of the transect. Effort was made to keep the two counts close enough together to maximize the likelihood of having the same fish on the site, but far enough apart to give the fish time to settle back down after the first count. The average wait between counts on these 66 occasions was 40 minutes.

It was found that the error variance in a simple model was not constant with increasing counts: the higher the count, the higher the error term in a model of the above form. Therefore, a more complex model had to be used, and it was found that the following form met the necessary assumptions more satisfactorily:

$(\text{SCUBA count})^{1/2} = a$

$+\ b$*(snorkel count)$^{1/2}$

$+\ c$*(visibility)*(snorkel count)$^{1/2}$

$+\ d$*(current speed)*(snorkel count)$^{1/2}$

+ e*(SCUBA count done first?)*(snorkel count)$^{1/2}$

Current speed was recorded on an eight-point scale from 0 (no current) up to 7 (very fast current). Visibility was on a seven-point scale from 1 (very poor visibility) up to 7 (very good visibility). “SCUBA_count_done_first?” was a yes/no indicator variable. The model statistics for each species follow:

***P. areolatus F* = 77.4; adj. R^2 = 0.82; n =66**

	Coefficient	Standard Error	Null Hypothesis	*t* statistic	*p*-value
a	0.28	0.30	$a = 0$	0.92	0.36
b	1.18	0.10	$b = 1$	1.76	n.s.
c	-0.042	0.017	$c = 0$	-2.51	0.017
d	-0.001	0.012	$d = 0$	-0.10	0.92
e	-0.009	0.053	$e = 0$	-0.18	0.86

E. fuscoguttatus ***F*** **= 57.7; adj. R^2 = 0.78; n = 66**

	Coefficient	Standard Error	Null Hypothesis	*t* statistic	*p*-value
a	0.93	0.20	*a* =0	4.63	<0.0001
b	1.17	0.17	*b* =1	1.00	n.s.
c	-0.066	0.032	*c* = 0	-2.07	0.04
d	-0.014	0.025	*d* = 0	0.58	0.57
e	0.141	0.095	*e* = 0	1.48	0.15

E. polyphekadion ***F*** **= 72.0; adj. R^2 = 0.81; n = 66**

	Coefficient	Standard Error	Null Hypothesis	*t* statistic	*p*-value
a	0.04	0.18	*a* = 0	0.19	0.85
b	1.79	0.26	*b* = 1	3.06	sign.
c	-0.125	0.058	*c* =0	-2.15	0.03
d	-0.050	0.030	*d* = 0	-1.69	0.10
e	0.03	0.14	*e* = 0	0.25	0.81

n.s. = not significant

In general, the SCUBA counts were higher than the snorkel counts, but the difference depended strongly on the visibility. Neither current speed nor which count was done first affected the difference. For all species, it was found that the better the visibility, the less the difference between the SCUBA and snorkel counts, as expected. For *P. areolatus*, the SCUBA count was as much as 35% higher than the snorkel count in poor visibility. For *E. fuscoguttatus*, the difference was as much as 80%, and *E. polyphekadion*, as much as 130% (but only a small portion of the site's *E. polyphekadion* were typically in this transect). The effect of visibility for *P. areolatus* counts was roughly 10% of the snorkel count for every 3m increase in visibility. For *E. fuscoguttatus* the figure was 13%, and for *E. polyphekadion*, 31% (estimated from simplified models not shown here).

Although the differences between the two counting methods were sometimes substantial, they applied to only one of eight transects that made up the total count at the site. It was therefore deemed unnecessary to make adjustments to the snorkel counts made during the first year of this study, and the analyses presented throughout this report are based on the original, unadjusted counts.

Calculating adjustment-related error in early counts

Earlier we described how, starting in June 1995, we expanded the area at Ngerumekaol in which the fish were counted. Below are the regression functions showing the ajustment-related error in the earlier counts. It can be seen that the 96% confidence limits for the adusted counts are encouragingly small.

P. areolatus (male)
adjusted count = 1.71*raw count
n = 107
adjusted $R^2 = 0.93$
$F = 1743.5$ ($p = 3*10^{-67}$)
95% confidence interval for coefficient: 1.67 - 1.75

P. areolatus (female)
adjusted count = 1.49*raw count
n = 107
adjusted $R^2 = 0.90$
$F = 1118.5$ ($p = 8*10^{-58}$)
95% confidence interval for coefficient: 1.43 - 1.55

E. fuscoguttatus
adjusted count = 2.82*raw count
n = 107
adjusted $R^2 = 0.84$
$F = 850.5$ ($p = 1*10^{-44}$)
95% confidence interval for coefficient: 2.65 - 2.98

P. polyphekadion adjusted count = 1.63*raw count
n = 107
adjusted R^2 = 0.98
F = 9345.6 ($p = 2*10^{-104}$)
95% confidence interval for coefficient: 1.60 - 1.66

Unmeasurable sources of variation

After accounting for all sources of variation that can be identified and measured, what is left over can be called "counting error". Because time was such an important factor on the scale of both seasons and days, and because its effects were not linear or simple, it was difficult to separate counting error from temporal effects. The approach used here was to analyze the variance in counts on those occasions when replicate counts were made within a period of no more than one hour.

There were two sets of data available to do so:

1) the counts in the channel of Ngerumekaol performed on both SCUBA and snorkel (by the same observer), and

2) counts made by two observers at the same time (using the same method).

The variability left over after accounting for the method used (in the first data set) or the observer (in the second data set) can be considered an estimate of the counting error. The standard error of the residuals in the regression models described above is just that.

Not surprisingly, examination of the simple regression models described above reveals that the error variance increased with increasing counts; the higher the count, the greater the error (in numbers of fish). Thus, the error cannot be described in terms of a standard number of fish. Neither can it be described as a percentage of the count. It is, in fact, a somewhat complex function of the number of fish counted. As a simple solution, separate standard errors were computed for each of several ranges of counts within the overall range of counts. From those, it was determined (necessarily with some degree of subjectivity) that the minimum counting error (i.e., for very low counts) was about 3 fish. As a percentage of the count, the error gradually decreased with increasing counts, ultimately levelling off at about 15% of the count. This description can be reasonably expressed with the following function:

$$\text{Standard error} = 3 + 0.15*\text{count} - 0.16*\text{count}^{1/2}$$

This estimate of counting error can be used to evaluate comparisons of counts on the scale of days, months, and years. The 95% confidence interval for a given count is approximately plus or minus two times the standard error (the 2-tailed t statistic is equal to 1.96 for n = 8). The interval for 100 fish would therefore be 67 to 133 fish.

During this study, counts were performed by: 1. a highly experienced UVC observer (performing most counts at Ebiil and Western Entrance), and 2. previously inexperienced observers who gained much experience during the study (performing most of the counts at Ngerumekaol). It should be noted that the error estimates given above were derived from data sets made up of observations by the second category of observers. Assuming the counting error to be somewhat a function of observer experience, it is probable that the counts at Ebiil and Western Entrance were more precise than is suggested by the counting error estimated above.