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Title: The effects of protected area management on the population biology and reproduction of *Lethrinus harak* from the inshore waters of Guam

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Protected area management has been considered a highly effective and practical fisheries management tool for tropical fish stocks. The success of this management option in facilitating recovery from heavy fishing has been well documented with numerous examples of increased abundance and biomass within well protected areas. Despite this, few studies have investigated the effects of protected area management on the age-based demographics of coral reef fish populations. In this study, population parameters of *Lethrinus harak* were compared among two marine preserves and two comparative fished sites on Guam. An optimal stratified-random visual survey design was used to estimate total abundance, density, and biomass within each site; standard ageing techniques using otoliths were used to compare age-based population parameters among sites; and histology was used to investigate the reproductive biology of *L. harak*. Total abundance and density were greater in the protected site for one preserve/non-preserve pair but not the other. Nevertheless, protected sites consistently had a greater mean size in all habitats and greater a total biomass. The difference in spawner biomass was outstanding as protected sites had 13 and 142 times the biomass of reproductive fish than their comparative fished sites. Protected sites consistently had a greater mean age as populations were comprised of a larger proportion of older individuals and were subject to lower rates of total mortality. Growth parameters varied significantly among sexes, sites, and protection status. Histological examination of gonads indicates that *L. harak* is a protogynous hermaphrodite. Size and age at female maturation did not differ according to protection status, but there was some indication that age at sex change occurred earlier in fished populations. Little insight into the seasonality of reproduction was achieved because of a low number of mature females in monthly samples. Historical catch data, yield-per-recruit stock assessment, and the discrepancies in population demography between protected and unprotected sites indicate that Guam's *L. harak* stock is subject to intense exploitation as the majority of individuals harvested had not reached reproductive maturity. These results suggest that Guam's marine preserve network has facilitated significant recovery and stability in the population structure of *L. harak*. However, additional management options including certain output controls would be of enormous benefit for the long-term sustainability of this species.

The effects of protected area management on the population biology and reproduction of *Lethrinus harak* from the inshore waters of Guam

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Chapter 1 Introduction

1.1 Coral reef fisheries

Managing coral reef fisheries is inherently difficult for several reasons. These include factors which are both biological and anthropogenic such as the high diversity of targeted species and the multiple gear types employed by fishermen (Russ 1991). On a typical Indo-Pacific coral reef, up to 300 species of fish may be harvested for food using a number of different gear types, which are both selective and unselective, targeting multiple species at varying rates (Munro 1980; Munro 1986; Munro & Smith 1984). Furthermore, some suggest that coral reef fish are more susceptible to overfishing than pelagic or temperate fishes because of certain life history characteristics (Russ 1991) that include small home ranges (Munro & Williams 1985), a high degree of habitat selectivity, complex sexual patterns and reproductive behaviors (Shapiro 1987; Fennessy and Sadovy 2002; Rhodes and Sadovy 2002), and for some species long life-spans (Choat and Axe 1996) and low rates of natural mortality and late-onset maturity (Manooch 1987; Pears et al. 2006). Assessing tropical fish stocks is further complicated by a lack of historical catch and effort data and species-specific biological parameters (Russ 1991; Adams 1996).

Options for the management of coral reef fisheries include one of two approaches or a combination of both; traditional fisheries methods like size restrictions and limiting effort or closed area management (Munro 1996). The first, which involves imposing output controls such as effort and size restrictions on subsistence fishermen, rarely works without considerable resources devoted to enforcement (Adams 1996; Russ 2002). Conversely, closing an area to fishing has been employed by many cultures for centuries facilitated under a system of common property management (often referred to as traditional community based management) (Ruddle 1996). This arrangement is now considered the most practical option for many Pacific Island nations looking to manage their fisheries because of the low-cost relative to the day-to-day costs of a government controlled system (Adams 1996).

The primary role of closed areas or Marine Protected Areas (MPA's) with respect to fisheries recovery are to increase density, mean size, biomass, and reproductive output of target species within the reserve compared to adjacent fished areas, while enhancing outside stocks via spillover of adult fish and increased recruitment (Bohnsack 1990). The success of MPA's in facilitating recovery of heavily fished stocks has been well documented throughout the tropics with numerous examples of increased density and biomass from within well protected sites and evidence of fishery enhancement via spillover (see Russ 2002). However, the effectiveness of MPA's largely depends on careful planning with respect to size and placement plus continued monitoring of their potential benefits over time (Hilborn et al. 2004).

Implementing a modern approach such as an MPA should not preclude continued research of the two major factors affecting reef fish population dynamics; their demography and reproductive biology. A common misconception amongst many fishery managers is the assumption that most reef fish species are demographically homogenous, whereby life history traits are similar over large spatial scales (Man et al. 1995; Gust 2004). In fact, growth, maturity, sex ratio, size-frequency, and reproductive patterns in tropical fish stocks can vary significantly over a broad range of spatial scales (Adams et al. 2000; Gust et al. 2002; Kritzer 2002; Williams et al. 2003; Gust 2004; Williams et al. 2006). This variability can arise from environmental conditions (e.g., density-dependent mortality), genetic differences (e.g., reproductively isolated stocks), or anthropogenic disturbances (e.g., fishing pressure or habitat degradation). Gust et al. (2002) found differences in growth and longevity for three scarid species and one acanthurid species between reefs ~20 km apart. This variation was strongly related to differences in mortality and it was concluded that density-dependent processes were responsible. At spatial scales of hundreds of kilometers a similar pattern was found whereby higher mortality rates led to faster growth and smaller mean sizes per age for *Lutjanus carponotatus* (Kritzer 2002). This pattern remained consistent for the Caribbean scarid, *Sparisoma viride* where large differences in growth were observed over 1500-2000 km (Choat et al. 2003). For *Lethrinus miniatus*, variability in growth parameters was observed at multiple scales (e,g, 20 km and ~600 km) (Williams et al. 2003). Capturing the large and small scale variability in these parameters is imperative as populations of reef fish with disparate life-history strategies such as growth rates and mortality respond to fishing pressure differently (Gust et al. 2002).

Like growth, the reproductive pattern observed in a species has a major impact on the way it responds to and recovers from heavy fishing pressure. Among exploited reef fish species, protogyny, in which functional females change sex to functional males, is the dominant sexual pattern (Sadovy de Mitcheson & Liu 2008). Sex ratios for protogynous species typically change from female-dominated to male-dominated as size increases (Kawaguchi & Marumo 1967; Warner 1975b). Therefore, males dominate the older age classes thereby constituting a small proportion of the total population. For protogynids, the selective nature of fishing leads to a disproportionate reduction in males as larger fish are more heavily targeted (Bannerot et al. 1987; Jennings & Lock 1996; Sadovy 1996). Consequently, heavy exploitation of a protogynous species, especially those that aggregate to spawn, can lead to the severe reduction of the male portion resulting in sperm limitation, and potentially recruitment failure (Coleman et al. 1996). Although considerable attention has been paid to the response of protogynous species to fishing pressure and to the development of management scenarios for their sustainable harvest, solutions remain complicated as sexual patterns of protogynids are increasingly shown to be complex (Buxton 1992; Sadovy & Figuerola 1992; Sadovy 1996; Armsworth 2001).

1.2 The family Lethrinidae

Species of the family Lethrinidae are abundant throughout reefs of the Indo-Pacific and constitute an important part of many commercial and recreational fisheries (Carpenter & Allen 1989). Compared with other highly exploited families such as the Serranidae, Lutjanidae, and Scaridae, much less is known regarding growth patterns and sexual development of lethrinids. *Lethrinus harak*, in particular, is a small-to-medium bodied emperor found on reefs throughout the Indo-Pacific region from the Red Sea to Samoa (Myers 1999). It is most commonly found on fringing reef flats where it feeds on mollusks and small fish. Protogyny has been established as the dominant sexual pattern in lethrinids (Young & Martin 1982; Ebisawa 1990, 1997, 1999, 2006; Bean et al. 2003). Although Ebisawa (2006) determined that *L. harak* from Japan is a protogynous hermaphrodite, sex ratio patterns from Kenya have suggested protandry (Kulmiye et al. 2002). While it is a favored fish in many inshore hook-and-line and gillnet fisheries throughout its distribution, few studies have been dedicated to understanding the population dynamics of this species.

On Guam, *L. harak* is listed as a 'species of greatest conservation need' (Bassler & Aguon 2006), as CPUE and harvest levels have declined considerably over the past two decades (Pitlik 1999). In addition, the relative importance of certain gear types used for harvesting *L. harak* have changed since 1984 and mean size of individuals caught by hook-and-line has significantly decreased over that time period. Approximately 61% of the total *L. harak* harvest (by numbers) on Guam since 1984 has been comprised of individuals below the size of reproductive maturity. Clearly, more research is needed to determine whether the observed reduction in mean size over time and the higher number of immature fish caught by fishermen are signs of overexploitation. Previous research efforts on Guam's *L. harak* fishery during the 1990's, which included a large-scale tagging program, produced limited data comprised only of length-frequency estimates over four years (Torres 1996). High tag-induced mortalities produced low tag-return rates of 0.84% (5 individuals from 595 tagged) which revealed little about their life-history characteristics. As a result large gaps remain in our understanding of the growth, local demography, and reproductive biology of this species.

The purpose of this study is to use a robust method for estimating population size, coupled with otolith analysis and histology techniques to analyze demographic and reproductive parameters of *Lethrinus harak* within and outside of selected marine preserves on Guam. Specifically, I set out to complete the following objectives:

1. Measure differences in abundance, density, and biomass of *L. harak* at habitat and site levels between two selected marine preserves and comparative fished areas using an optimal stratified-random sampling design (*sensu* McCormick & Choat 1987).
2. Compare age structures within and outside of marine preserves to establish if there has been a significant accumulation of older individuals in the preserves since their establishment eleven years ago, seven of which have been enforced.
3. Evaluate sex-specific and island-wide variation in growth using von Bertalanffy growth coefficients, to be incorporated into stock assessment models.
4. Determine the spawning season of *L. harak* on Guam using monthly gonadosomatic index estimates and changes in maturity status over a twelve month period.
5. Determine the sexual pattern of *L.* harak using histological examination and criteria developed for establishing protogynous hermaphroditism.
6. Determine the size/age at which *L. harak* matures and changes sex and establish a relationship between reproductive potential and body size and age.

Data from the DAWR long-term creel survey database will be used in conjunction with life-history data collected from this study to perform a yield-per-recruit stock assessment.

The output from these analyses will include certain biological reference points which in turn can be used by managers as benchmarks for developing management policies (Jennings et al. 2001).

1.3 Significance of study

The need for management of Guam's inshore fisheries has been recognized for decades as declines in CPUE and increases in fishing pressure were already evident in the 1970's (Amesbury et al. 1986; Hensley & Sherwood 1993). Katnik (1982) noted large differences in the abundance of many inshore reef fish, including *L. harak*, between comparative heavily and lightly fished areas. Unfortunately, the sites which were classified as lightly fished in Katnik's study are currently subject to greater fishing intensity coupled with environmental degradation from anthropogenic impacts. A reanalysis of long-term catch and effort data has shown the decline in Guam's reef fish stocks and the state of the fishery is much worse than was previously reported (Zeller et al. 2007).

This study will fill critical gaps in our knowledge of the biology of a locally important fish species. Knowledge of demographic variability around Guam will not only provide detailed insight into the current status of the *L. harak* stock, but will also give insight into the effectiveness of the current marine preserves in protecting larger mobile fish like lethrinids. Recommendations from this project can then be used to better manage this species through protection of critical juvenile habitat, by setting seasonal closures during spawning seasons or introducing minimum size limits based on size and age at first reproduction.

Chapter 2 Patterns of total abundance, density, biomass, and habitat preference of *Lethrinus harak* among protected areas and comparative fished sites

2.1 Introduction

In recent decades, there has been a vast quantity of literature emphasizing the effectiveness of marine protected areas (MPA's) and marine reserves as fishery tools, with much emphasis on their value to coral reef ecosystems (e.g., Polunin 1990; Bohnsack 1993; Dixon 1993; Roberts & Polunin 1993; Ballantine 1997; Bohnsack 1998; Roberts 1998; Roberts et al. 2001; Pauly et al. 2002; Roberts et al. 2005). The success of this management option in facilitating recovery of heavily fished stocks has been well documented, with numerous examples showing increased abundance and biomass of target species within protected areas that are enforced (summarized by Russ 2002). On coral reefs, protected area management is generally considered the most practical fishery management option, especially for small island nations where imposing size and effort restrictions on fisherman is largely ineffective (Russ 2002).

Protected area management provides many benefits to coral reefs. In a fisheries context, well-managed MPA's remove fishing pressure from targeted species, which reduces mortality rates and leads to an increase in the abundance, density, biomass, and mean size of the species (Roberts & Polunin 1991; Buxton 1993; Dugan & Davis 1993; Bohnsack 1993, 1996, 1998; Mosquera et al. 2000) This can, in turn, enhance fisheries through (1) density-dependent spillover of adult individuals from protected areas to areas open to fishing and (2) an increased reproductive potential yielding a greater larval supply and subsequent potential increase in recruitment to fished sites (Bohnsack 1990).

"Spillover" is defined as the net export of adult fish from within an MPA to an adjacent fished site. The process is generally linked to density-dependent effects (Sanchez Lizaso et al. 2000) where increasing numbers of fish leads to competition for space within MPA's, causing some individuals to move and spillover into adjacent areas open to fishing. Density-independent spillover has also been proposed (e.g., simple diffusion across density gradients), although such a process is often mediated by behavioral characteristics rather than MPA implementation (Abesamis & Russ 2005). Although logistically difficult, evidence of spillover from MPA's has increased in recent years for several species across broad spatial scales (Attwood & Bennett 1994; Russ & Alcala 1996; Zeller et al. 2003). For example, Alcala & Russ (1990) found that nearly twice the biomass could be harvested from Sumilon Island in the Philippines when 25% of the entire reef was a dedicated marine reserve. They attributed this extra biomass to spillover. McClanahan & Mangi (2000) measured emigration rates of reef fishes from an MPA in Kenya over seven years and found that spillover was most pronounced in important target species such as lethrinids, siganids, and acanthurids. Abesamis & Russ (2005) provide arguably the best evidence for density-dependent spillover from a marine reverse at Apo Island in the Philippines. They found densities of *Naso vlamingii* increased over time in a gradient outward from the reserve boundaries and related this to the increased frequency of aggressive interactions within the reserve, where densities of

N. vlamingii were at their highest. Spillover is an important process as it provides fisherman with a subsidy which offsets the loss of fishing grounds when MPA's are established.

The second major fishery benefit that MPA's provide is through increased larval supply as a direct result of having larger fish within an MPA. It has been demonstrated that MPA's aid in enhancing the reproductive potential of target species by increasing the density, mean size, and mean age of populations (Bohnsack & Ault 1996; Ault et al. 1997; Bohnsack 1998). Also, a population comprised of older individuals provides additional benefits through improved larval quality and survivorship of progeny from older females (Berkeley et al. 2004_b). However, what has not been demonstrated is whether this directly impacts the MPA and/or adjacent fished sites via subsequent increases in recruitment (Almany 2007). Recent studies have indicated that self-recruitment may be more prevalent in reef fish than previously perceived (Swearer et al. 1999; Cowen et al. 2000; Swearer et al. 2002; Jones et al. 2005). Nevertheless, evidence for the "recruitment effect" in relation to MPA's is almost non-existent.

To effectively evaluate the success of an MPA in providing fisheries-related benefits, detailed information on demographic parameters is required such as total abundance, density, and size frequency estimates for target species (Halpern 2003). Total abundance and biomass estimates in particular are a valuable starting point for managers as they are easier to interpret and compare between different time periods than catch data or mean densities (McCormick & Choat, 1987). However, robust population estimates of coral reef fish can be difficult to obtain due to the life history traits of some target species such as ontogenetic habitat shifts and can be further confounded by the presence and influence of habitat variability (Sale 1980; Sale & Sharp 1983; Fowler 1987; Choat & Ayling 1987; McCormick and Choat 1987; Friedlander & Parrish 1998).

Methods used for estimating total population size include mark-recapture, mark-resighting, and stratified-random visual survey designs (Zeller & Russ 2000). Mark-recapture and -resighting techniques rely on often unsubstantiated assumptions concerning immigration and emigration while the potential for tag loss creates difficulty with bias and precision (Arnason & Mills 1981). In addition, there is potential for further bias when habitat variability is not included in the distribution of sampling effort, a real problem for species with strong habitat affiliation. It is well known that habitat variability across reef environments influences the abundance and distribution patterns of reef fish (e.g., Hixon 1980; Kingett & Choat 1981; Russ 1985; Choat & Ayling 1987; Gust et al. 2001). For a species with a patchy distribution that is highly correlated with habitat type, stratified random sampling is the best method for estimating total population abundance with the highest confidence (McCormick & Choat 1987; Ault et al. 1999). Although, this method is labor intensive (large number of transects) and requires detailed prior knowledge of habitat types and distribution within the study area, it optimally minimizes the confidence intervals surrounding abundance estimates by allocating more effort to habitats of greater total area and higher variability in fish density (Siniff & Skoog 1964). Additional benefits of a stratified random sampling approach include enumeration of habitat-specific densities and size structures, total biomass estimates, and spawner biomass estimates when knowledge of size at sexual maturity for the species of interest is known (McCormick 1989).

Hence, in this chapter, I compare total abundance, habitat-specific density, size frequency, total biomass, and spawner biomass of *Lethrinus harak* between two marine preserves and two comparable fished sites on Guam. Given that landed biomass of *L. harak* has decreased significantly in the past decades despite increases in fishing intensity (Pitlik 1999), I set out to test whether (1) there are significant differences in the population parameters I measured and (2) whether these results correlate with the degrees of protection from fishing, afforded by Guam's marine preserves, which have been effectively enforced for over 7 years.

2.2 Methods

2.2.1 Study sites

This study was conducted at two marine preserves and two comparable fished sites on Guam (13°25' N, 144°45' E). Sites were selected based on three criteria to standardize comparisons: (1) all sites had to be roughly comparable in size, (2) each site had to contain a significant amount of seagrass habitat, and (3) comparative sites had to be near to one another. These criteria were used to standardize comparisons among sites. Based on these criteria, I selected Piti Marine Preserve and East Agaña Bay in the north as a protected area and comparative fished site, respectively (Figure 2.1). To the south, Achang Marine Preserve was selected as a protected site with Rios Bay the comparative fished site (Figure 2.1). All surveys were conducted on the reef flats at high tide where depth ranges from 0-3 meters. The only exceptions were some areas in Piti where reef flat habitat reached depths of up to ~10 meters.

Benthic maps from the Guam Coastal Atlas (Burdick 2006) were used to quantify the locations, proportions, and total areas of various benthic habitat types at each site. To optimize the allocation of transects to different habitat types, benthic classifications used by Burdick (2006) were compressed into six general categories (Table 2.4). Aggregate Reef Coral 10-90% (hereafter referred to as 'aggregate reef') consisted mainly of reef-building corals dominated by *Porites rus* and *P. cylindrica*, at depths of 1-10m. This habitat was found only at the Piti site. Pavement Coral 10-90% (hereafter referred to as 'coral pavement') consisted mainly of massive *Porites* spp. as well as other robust reef flat corals such as *Acropora pulchra*, *Pavona decussata*, and *Pocillopora damicornis* and occurred in depths of 1-3m. Pavement Macroalgae 10-90% (hereafter referred to as 'macroalgae') consisted of brown, thallate macroalgae such as *Padina boryana*, *Sargassum polycystum* and *S. cristaefolium*, and *Dictyota* spp. in depths ≤ 2m. Seagrass 10-100% (hereafter referred to as 'seagrass') consisted of stands of *Enhalus acoroides* in Piti, Achang, and Rios Bay and *Halodule uninervis* in East Agaña Bay in depths ≤ 2m. Pavement Turf 50-90% (hereafter referred to as 'turf pavement') consisted of rock or pavement substratum covered by algal turf communities and occurring in ≤ 2m. Sand Uncolonized 90-100% (hereafter referred to as 'sand') consisted of sandy habitats colonized by less than 10% algae (turf or macroalgae or both). The distribution of habitats within each site is shown in Figure 2.2. Although these habitat classifications are relatively broad, they are sufficient for the purposes of this study as a means to apportion sampling effort among zones of variable fish density.

2.2.2 Survey methodology

From June to October 2007, underwater visual surveys were conducted by snorkel following an optimal stratified-random sampling design (e.g., McCormick & Choat 1987). Strip transects, 50 x 5 m in dimension were selected as the optimal method for visual surveys on the reef flat. Although the point count method has been used in many studies, I found it to be less appropriate here because *L. harak* individuals are extremely wary, especially in areas where spear fishing is the most common fishing technique. The total number of transects per site were chosen to match the power obtained in McCormick & Choat (1987) in which ~1.5% of the total area was covered by transects. The allocation of transects among habitat types was determined using the following formula (Neyman 1934),

$$n_h = \frac{W_h * s_h * n}{\sum(W_i * s_i)},$$

where n_h represents the number of transects allocated to habitat *h*, W_h is the proportional habitat area, s_h is the estimated standard deviation of the mean fish density in habitat *h*, and n is the total number of transects at the site (Table 2.1). The model aims to optimize the total abundance estimate of *L. harak* by allocating sampling replicates using variance estimates and proportional habitat area. Standard deviations (s_h) used in the model were obtained during pilot surveys at each site, where five transects were performed in each habitat type (Piti, n=30, East Agaña, n=25, Achang, n=20, Rios, n=20). A minimum of five transects were carried out in each habitat regardless of how many were allocated using the Neyman formula. Individual *L. harak* were counted and allocated to 3 cm size classes along each transect. The observer trained in size estimation using wooden models that covered the entire size range of *L harak*. As among-observer error has been found to be the most common source of bias in visual surveys, all fish counts were conducted by one observer (Edgar et al. 2004). To minimize the influence of tide and time of day, all surveys were conducted during morning hours (~8:00am to ~11:00am) and within ±2 hours of the high tide.

The position of each transect was chosen randomly but constrained within the appropriate habitat type. A habitat map and handheld GPS unit (Garmin eTrex®) were taken into the field to assist in locating particular habitat types and to mark the beginning of each transect. These GPS coordinates were plotted onto a digital habitat map of each bay to ensure none of the transects overlapped.

2.2.3 Data analysis

The total population abundance of *L. harak* and associated variance was calculated for each site (Tables 2.2 and 2.3). In order to compare among sites of varying size, all counts were converted to densities. At each site a one-way analysis of variance (ANOVA) with a post-hoc Tukey test was used to detect differences in densities between habitats with the exception of East Agaña, where a Kruskal-Wallis test was used because assumptions of variance and normality were not met. Three-factor ANOVAs were used to test for differences in a) densities and b) mean fish size among habitats by protection status (preserve and nonpreserve) and geographic location (north and south). Only seagrass and macroalgae were used in the three-way comparison of densities as these habitats occurred in every site and were consistently utilized by *L. harak.* Variables were transformed using ln(x+1) transformation in order to meet assumptions of statistical tests. Biomass and spawner biomass was calculated for each site using individual fish weights computed from the length/weight relationship (Figure 3.2 in Chapter 3). Spawner biomass represents the sum of the total weight of all individuals >23.5cm, the size class in which *L. harak* approaches 100% reproductive maturity (Figure 4.7, Chapter 4). Mean biomass per transect was compared among sites using a one-way ANOVA with a post-hoc Tukey test.

2.3 Results

2.3.1 Patterns of abundance and density

The total number of *L. harak* counted on the transects were 243 (Piti), 667 (East Agaña), 772 (Achang), and 100 (Rios Bay). These values translated to total abundance estimates of 10720 (±1493 95%CI) at Piti, 18326 (±2584 95%CI) at East Agaña, 48561 (±3062 95%CI) at Achang, and 6876 (±2503 95%CI) at Rios Bay (Tables 2.2, 2.3, and 2.5). Achang Marine Preserve also had the highest density of individuals at 34.6 per 1000m^2, followed by the two fished sites, East Agaña and Rios, with densities of 10.6 and 7.4 per 1000m^2, respectively (Figure 2.3). Piti Marine Preserve had the lowest density at 7.0 per 1000m^2 (Figure 2.3). At the two sites with the highest densities (Achang and East Agaña), approximately 82% of all individuals occurred within seagrass habitat (Table 2.2).

The mean density of *L.* harak differed significantly among habitats within protected sites (Piti 2.6: $F_{5,84}$=3.95, P=0.003; Achang 2.6: $F_{3,79}$=52.15, P<0.001), although no differences were identified in unprotected sites (East Agaña [Kruskal-Wallis]: χ^2=7.84, df=4, P=0.098). At the habitat level, there was no significant difference in the mean density of *L. harak* in seagrass and macroalgae between protected and unprotected sites. However, the significant interaction suggests fish density responds differently to protection status between northern and southern sites (Table 2.6a). At the individual site level, Achang fish density in seagrass was significantly greater than at all other sites ($F_{3,139}$=8.17, P<0.001), while there were no significant differences among the other three sites (Figure 2.4).

2.3.2 Size frequency and habitat preference

A comparison of size frequency distributions revealed Piti Marine Preserve had a greater abundance of fish in the larger size classes (>20 cm) whereas its comparative fished site East Agaña Bay shows the opposite pattern (Figure 2.5a). At Rios, more than 60% of all *L. harak* observed were between 13 and 19 cm while Achang's population was dominated by fish between 5 and 25 cm (Figure 2.5b). These differences in the size frequency distributions between sites were also manifest in the mean size estimates. The mean size (cm) of *L. harak* at Piti was more than double that of East Agaña (21.2 cm ±0.43 SE and 9.8 cm ±0.17 SE). Achang and Rios were more similar at 14.3 cm (±0.26 SE) and 15.1 cm (±0.55 SE), respectively.

Because *L. harak* had its highest densities in seagrass habitats, separate size frequency distributions for this habitat were generated for each site (Figures 2.6, 2.7, 2.8, and 2.9). Despite the importance of other habitat types (e.g., macroalgae) outside of the marine preserves (Table 2.2), they contained few *L. harak* individuals. Consequently, these habitats were grouped together in East Agaña and Rios in order to produce an adequate sample size when plotting the size frequency distribution (Figures 2.7 and 2.9). At all sites, the smallest individuals occurred most frequently in seagrass. At Piti, aggregate reef, sand, macroalgae, and coral pavement habitats contained individuals larger than the mean size found in seagrass (Figure 2.6). To investigate this result further, size frequency distributions were compared between seagrass and all other habitats combined for every site (Figure 2.10). A clear pattern in habitat utilization is evident as fish <10 centimeters are rarely encountered outside of seagrass. There was one exception, East Agaña, where approximately 5 and 8% of all fish in other habitats were from the 5.5 and 8.5 cm size classes, respectively.

Comparisons of mean fish size within seagrass habitats at each location reveal a trend of increased size with marine preserve status (Figure 2.11). A significant interaction between protection status and habitat type (Status*Habitat) confirmed that the mean size of *L. harak* was consistently greater in the protected sites across all habitat types (Table 2.6b).

2.3.3 Patterns of biomass and spawner biomass

Despite having a total abundance nearly half the value of its comparative fished site, Piti Marine Preserve had a total biomass that was 4.2 times greater than that of East Agaña Bay (Table 2.5). Similarly, Achang Marine Preserve contained 8.3 times the biomass as Rios Bay. For spawner biomass, the effect of marine preserves was even more pronounced. Piti contained ~1136 kg in total, which was 142 times greater than East Agaña. Achang contained over 13 times the amount of spawner biomass than Rios Bay. When corrected for area, the discrepancies in total biomass and spawner biomass between preserve and nonpreserve sites are evident (Figure 2.12). The mean biomass per transect was significantly greater in protected sites than in unprotected sites ($F_{3,312}$=21.19, P<0.001) whereas no significant difference existed between protected sites (Tukey HSD: P=0.952) or between unprotected sites (Tukey HSD: P=0.999). For the protected sites, 46.7% of Piti's and 43.4% of Achang's total biomass is spawner biomass. For the fished sites, 27.6% of Rios's and only 1.4% of East Agaña's total biomass come from fish of a reproductive size.

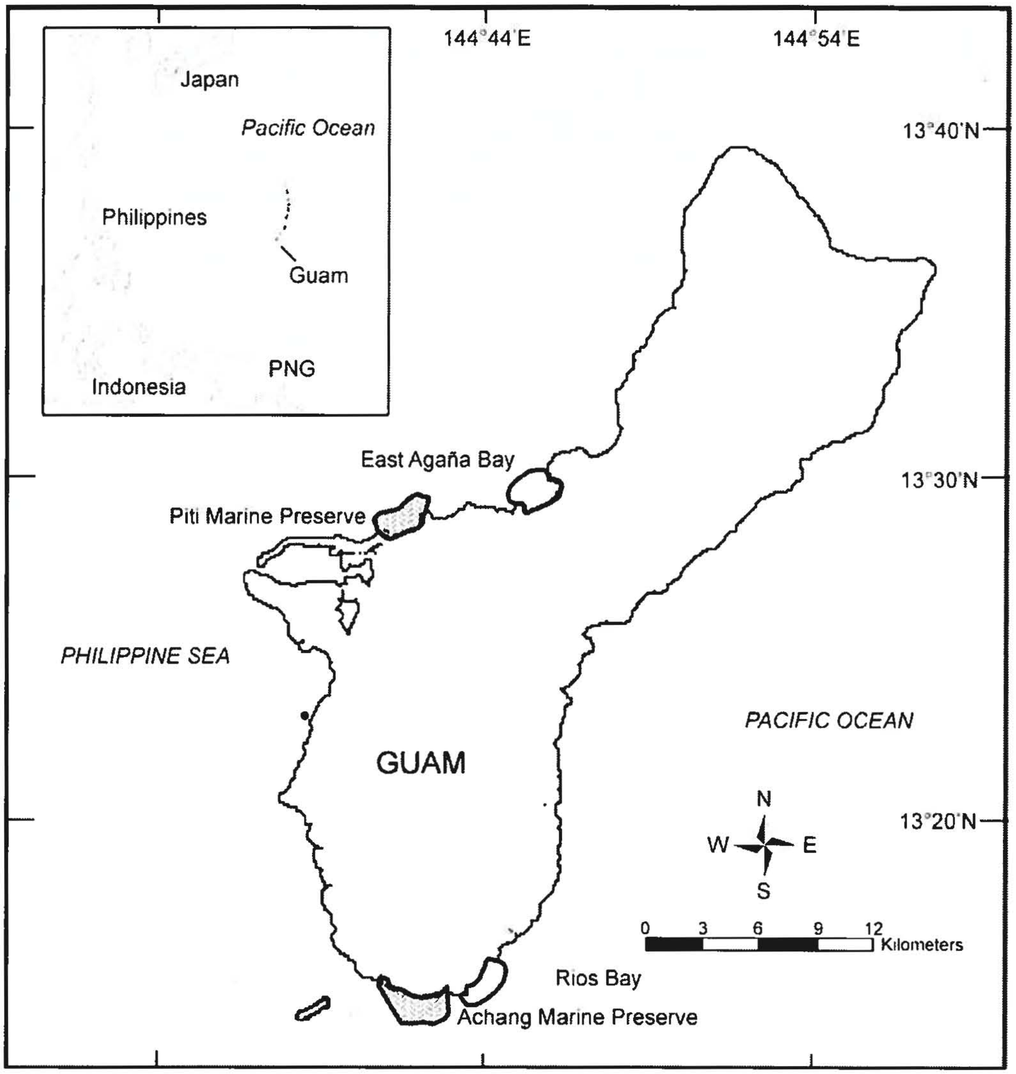

Figure 2.1. Map of Guam indicating the locations of the four study sites where visual surveys of *Lethrinus harak* were conducted.

Table 2.1. A summary of how each of the six habitat types were apportioned using the Neyman model. W_h represents the proportional habitat area, s_h is the estimated standard deviation of the mean fish density in habitat h, and n_h is the number of transects allocated to habitat h. Modified from McCormick & Choat (1987).

Stratum $n=\frac{W_h*s_h*n}{\Sigma(W_i*si)}$	**Piti**				
	Area (m^2)	W_h	s_h	W_h*s_h	n_h
Aggregate Reef, Coral 10-90%	308341	0.2022	1.07	0.216	12
Pavement, Coral 10-90%	474762	0.3114	2.15	0.670	36
Pavement, Macroalgae 10-90%	37008	0.0243	6.01	0.146	8
Seagrass, 10-100%	85276	0.0559	6.73	0.377	20
Pavement, Turf 50-90%	431191	0.2828	0.43	0.120	6
Sand, Uncolonized 90-100%	187981	0.1233	1.07	0.132	7
Total		1.0000		1.660	89

Stratum $n=\frac{W_h*s_h*n}{\Sigma(W_i*si)}$	**East Agaña**				
	Area (m^2)	W_h	s_h	W_h*s_h	n_h
Pavement, Coral 10-90%	287399	0.1669	1.30	0.217	5
Pavement, Macroalgae 10-90%	830580	0.4824	1.73	0.837	10
Seagrass 10-90%	371768	0.2159	29.40	6.348	65
Pavement, Turf 50-90%	170694	0.0991	4.39	0.435	5
Sand, Uncolonized 90-100%	61351	0.0356	0.00	0.000	5
Total		1.0000		7.837	90

Stratum $n=\frac{W_h*s_h*n}{\Sigma(W_i*si)}$	**Achang**				
	Area (m^2)	W_h	s_h	W_h*s_h	n_h
Pavement, Macroalgae 10-90%	251940	0.1792	2.73	0.490	11
Seagrass 10-90%	486249	0.3459	4.01	1.388	31
Pavement, Turf 50-90%	597353	0.4250	3.83	1.629	36
Sand, Uncolonized 90-100%	70140	0.0499	4.30	0.215	5
Total		1.0000		3.721	83

Stratum $n=\frac{W_h*s_h*n}{\Sigma(W_i*si)}$	**Rios**				
	Area (m^2)	W_h	s_h	W_h*s_h	n_h
Pavement, Macroalgae 10-90%	647667	0.6968	1.34	0.934	17
Seagrass 10-90%	184470	0.1985	6.73	1.336	27
Pavement, Turf 50-90%	53805	0.0579	0.45	0.026	5
Sand, Uncolonized 90-100%	43553	0.0469	0.00	0.000	5
Total		1.0000		2.295	54

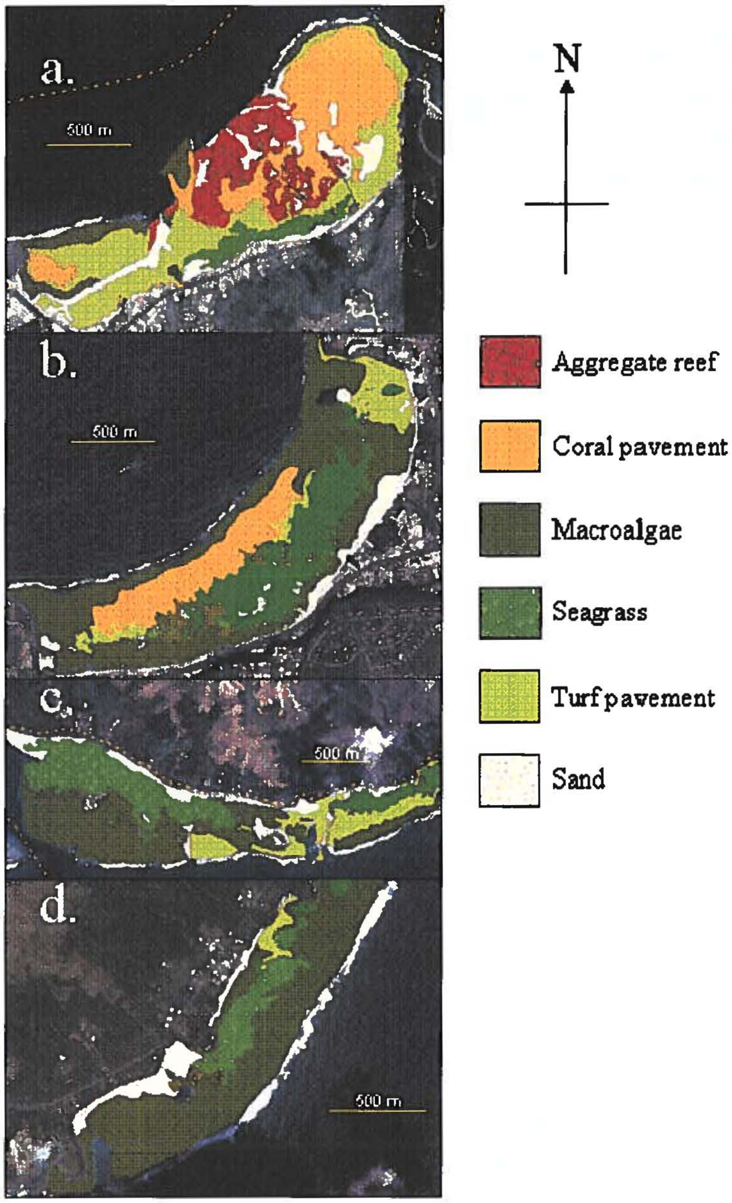

Figure 2.2. Satellite images of a) Piti Marine Preserve, b) East Agaña Bay, c) Achang Marine Preserve, and d) Rios Bay with overlayed distributions of each habitat type (see adjacent key). Images and habitat patterns modified from the Guam Coastal Atlas (Burdick 2006). Location of each site noted in Figure 2.1.

Table 2.2. Estimates of the total abundance and associated variance for each site using an optimal stratified-random sampling design. N_h represents the theoretical maximum number of transects that could be fit into habitat *h* without overlap, W_h is the proportional habitat area, n_h is the number of transects allocated to habitat *h*, x_h is the mean number of fish per transect for habitat *h*, s_h^2 is the variance surrounding the density estimate for habitat *h*, $s^2(x_{strat})$ is the variance surrounding the stratified mean density for the entire site, and $s(x_{strat})$ is the standard error of the stratified mean density for the entire site. Modified from McCormick & Choat (1987).

Piti Marine Preserve

Strata	Area (m^2)	% Area	N_h	W_h	n_h	x_h	s_h^2	$(W^2_h*s^2_h)/n_h$	N_h*x_h	% Fish
Aggregate Reef	308341	20.2	1233.4	0.202	12	2.25	1.603	0.00546	2775.07	25.9
Coral pavement	474762	31.1	1899.0	0.311	36	2.86	2.919	0.00786	5433.39	50.7
Macroalgae	37008	2.4	148.0	0.024	8	3.63	3.068	0.00023	536.61	5.0
Seagrass	85276	5.6	341.1	0.056	20	3.90	3.493	0.00055	1330.31	12.4
Turf pavement	431191	28.3	1724.8	0.283	6	0.00	0.000	0.00000	0.00	0.0
Sand	187981	12.3	751.9	0.123	7	0.86	0.690	0.00150	644.51	6.0
Totals	1524559		6098.2	1.000	89			0.01560	10719.88	

East Agaña Bay

Strata	Area (m^2)	% Area	N_h	W_h	n_h	x_h	s_h^2	$(W^2_h*s^2_h)/n_h$	N_h*x_h	% Fish
Coral pavement	287399	16.7	1149.6	0.167	5	1.20	1.304	0.00727	1379.52	7.5
Macroalgae	830580	48.2	3322.3	0.482	10	0.44	0.516	0.01202	1461.36	8.0
Seagrass	371768	21.6	1487.1	0.216	65	10.05	20.633	0.01480	14939.36	81.5
Turf pavement	170694	9.9	682.8	0.099	5	0.80	1.304	0.00256	546.22	3.0
Sand	61351	3.6	245.4	0.036	5	0.00	0.000	0.00000	0.00	0.0
Totals	1721793		6887.2	1	90			0.03664	18326.46	

Achang Marine Preserve

Strata	Area (m^2)	% Area	N_h	W_h	n_h	x_h	s_h^2	$(W^2_h*s^2_h)/n_h$	N_h*x_h	% Fish
Macroalgae	251940	17.9	1007.8	0.179	11	1.00	1.844	0.00538	1007.76	2.1
Seagrass	486249	34.6	1945.0	0.346	31	20.55	12.572	0.04853	39966.51	82.3
Turf pavement	597353	42.5	2389.4	0.425	36	2.40	4.347	0.02180	5734.58	11.8
Sand	70140	5.0	280.6	0.050	5	6.60	2.966	0.00148	1851.69	3.8
Totals	1405681		5622.7	1	83			0.07719	48560.54	

Rios Bay

Strata	Area (m^2)	% Area	N_h	W_h	n_h	x_h	s_h^2	$(W^2_h*s^2_h)/n_h$	N_h*x_h	% Fish
Macroalgae	647667	69.7	2590.7	0.697	17	1.94	3.944	0.11265	5028.95	73.1
Seagrass	184470	19.8	737.9	0.198	27	2.44	3.468	0.00506	1803.71	26.2
Turf pavement	53805	5.8	215.2	0.058	5	0.20	0.447	0.00030	43.04	0.6
Sand	43553	4.7	174.2	0.047	5	0.00	0.000	0.00000	0.00	0.0
Totals	929496		3718.0	1	54			0.11801	6875.70	

Table 2.3. Total population, variance, and 95% confidence limit estimates for each site with corresponding calculations. Refer to Table 2.2 for definitions of symbols.

	Piti	**East Agaña**	**Achang**	**Rios**
Population estimate				
$X=\Sigma x_h{*}N_h$	10720	18326	48561	6876
Variance of stratified mean				
$s^2(x_{strat})=\Sigma(W^2_h{*}s^2_h)/n_h$	0.01560	0.03664	0.07719	0.11801
95% confidence limits				
$X \pm t_{0.05}{*}N{*}s(x_{strat})$	±1493	±2584	±3062	±2503

Table 2.4. Habitat classifications from Burdick (2006) (left column) and how these classifications were condensed for the present study (right column).

Aggregate Reef, Coral 10 - <50%	Aggregate Reef, Coral 10 - 90%
Aggregate Reef, Coral 50 - <90%	
Scattered Coral, Rock 10 - <50%	
Pavement, Coral 10 - <50%	Pavement, Coral 10 - 90%
Pavement, Coral 50 - <90%	
Pavement, Macroalgae 10 - <50%	Pavement, Macroalgae 10 - 90%
Pavement, Macroalgae 50 - <90%	
Pavement, Macroalgae 90 - 100%	
Sand, Macroalgae 10 - <50%	
Pavement, Seagrass 10 - <50%	Seagrass, 10 - 100%
Sand, Seagrass 10 - <50%	
Sand, Seagrass 50 - <90%	
Sand, Seagrass 90 - 100%	
Pavement, Turf 50 - <90%	Pavement, Turf 50 - 90%
Rubble, Turf 50 - <90%	
Sand, Uncolonized 90 - 100%	Sand, Uncolonized 90 - 100%

Table 2.5. Estimates of total abundance, total biomass, and spawner biomass, with corresponding 95% confidence intervals.

Site	*Total Abundance*	*95% CI*	*Total Biomass (kg)*	*95% CI*	*Spawner Biomass (kg)*
Piti	10720	1493	2432	26	1136
East Agaña	18326	2797	575	21	8
Achang	48561	3062	5033	43	2182
Rios	6876	2503	604	25	167

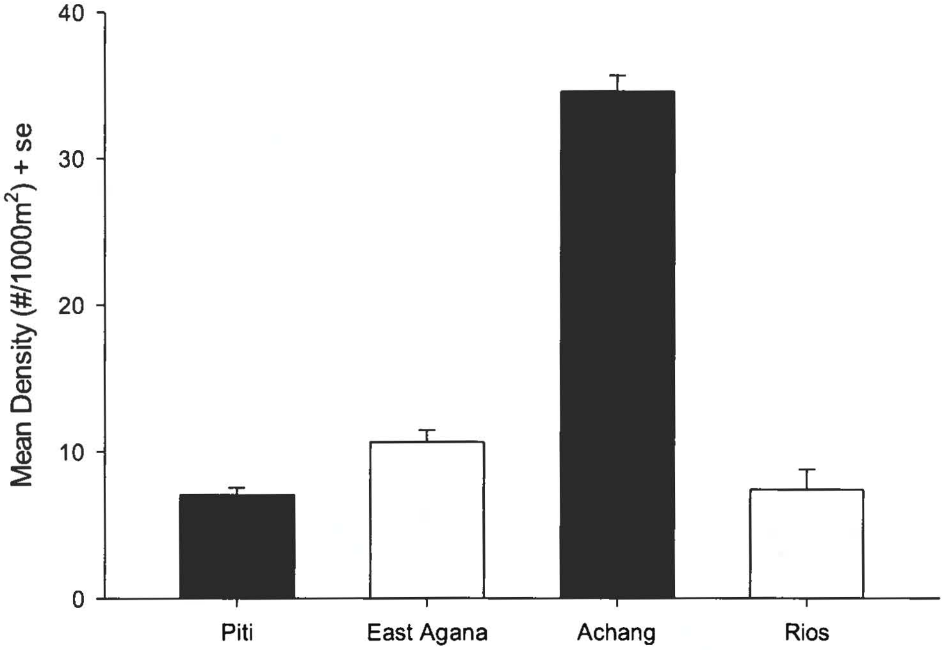

Figure 2.3. Comparison of overall mean densities of *Lethrinus harak* (+ s.e.) for each of the four study sites. Black bars represent marine preserves and grey bars represent fished sites.

Table 2.6. Three-factor analyses of variance comparing a) mean densities and b) mean sizes in seagrass and macroalgae (Habitat) between preserve and non-preserve sites (Protection status) to the north and south of Guam (Location). * indicates significance at <0.05, ** <0.01, * <0.001.

a) Mean density of *L. harak*

Source of variation	*DF*	*MS*	*F*	*P*
Protection status	1	398.2	2.19	0.141
Location	1	124.5	0.69	0.409
Habitat	1	1763.5	9.71	0.002**
Status*Location	1	791.8	4.36	0.038*
Status*Habitat	1	183.7	1.01	0.316
Location*Habitat	1	201.5	1.11	0.294
Status*Location*Habitat	1	1585.1	8.72	0.004**
Within	181	181.7		

b) Mean size of *L. harak*

Source of variation	*DF*	*MS*	*F*	*P*
Protection status	1	18.4	30.94	0.000***
Location	1	0.7	1.10	0.294
Habitat	3	29.7	49.87	0.000***
Status*Location	1	0.1	0.23	0.634
Status*Habitat	2	1.9	3.17	0.042*
Location*Habitat	1	0.02	0.04	0.847
Status*Location*Habitat	1	1.0	1.74	0.187
Within	1702	0.6		

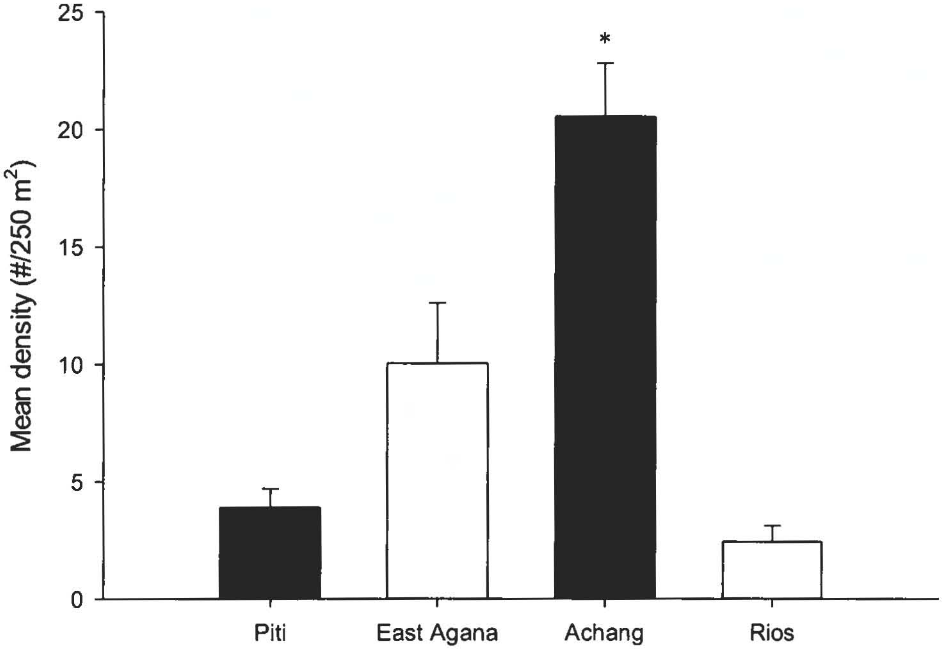

Figure 2.4. Mean density of *Lethrinus harak* in seagrass habitats for each site. * indicates significant difference from other sites.

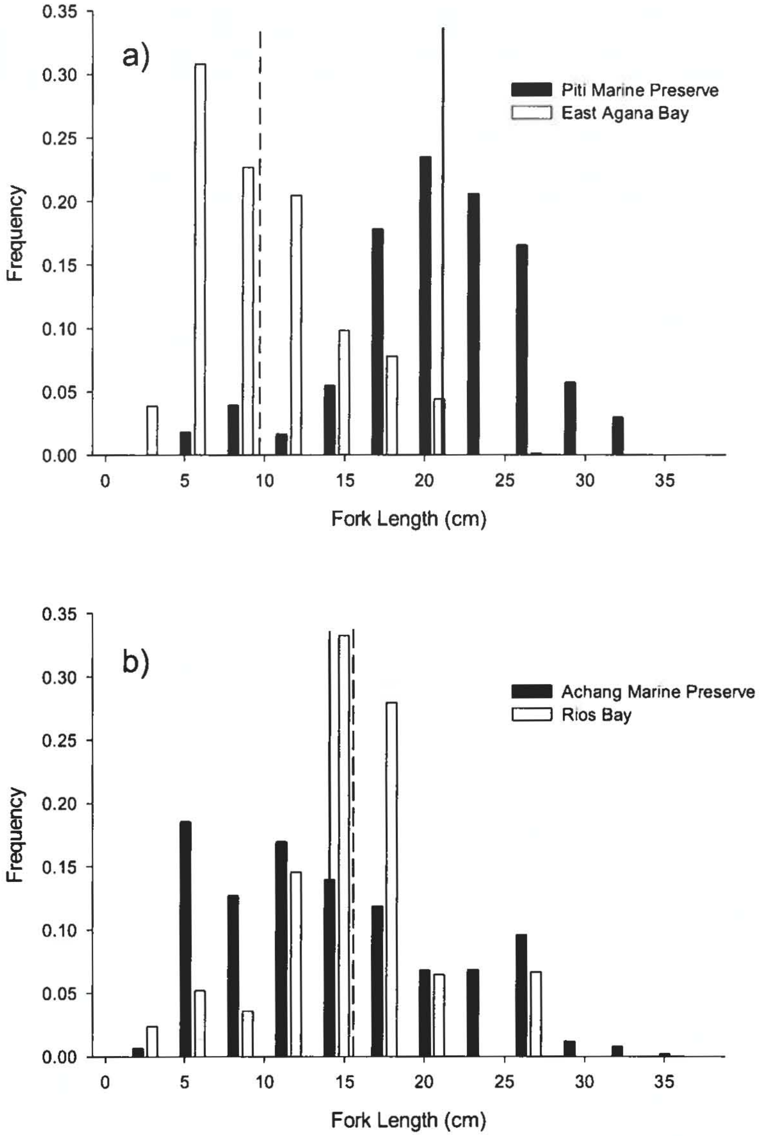

Figure 2.5. Comparison of overall size frequency distributions for a) Piti Marine Preserve and East Agaña Bay, and b) Achang Marine Preserve and Rios Bay. Dashed lines are mean sizes for a) East Agaña and b)Rios. Black lines are mean sizes for a) Piti and b) Achang.

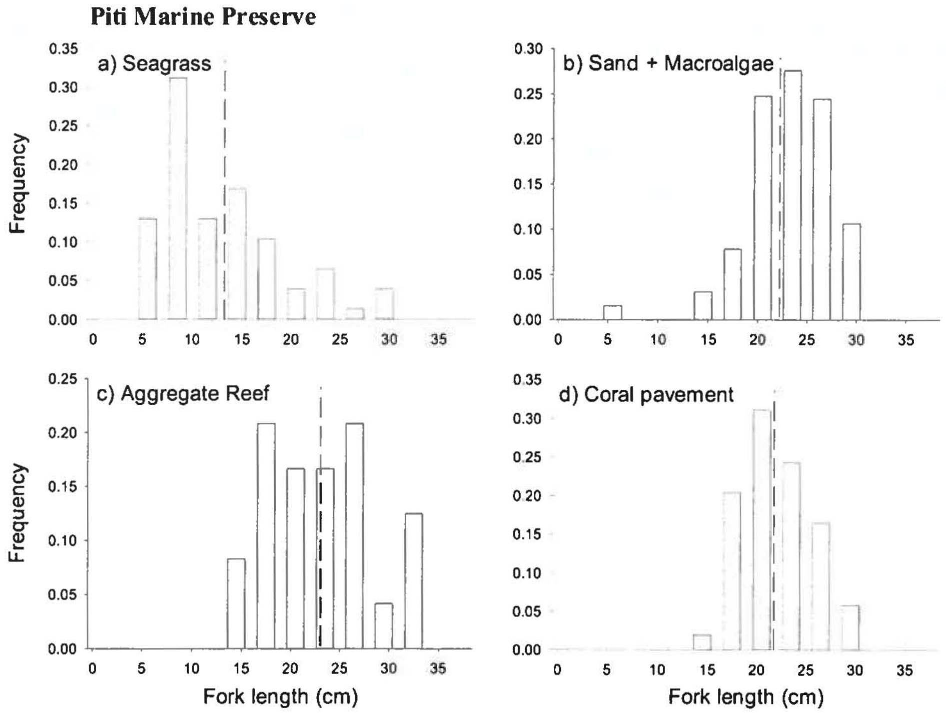

Figure 2.6. Size frequency distributions for habitats within Piti Marine Preserve. Dashed lines indicate the mean size for each habitat type.

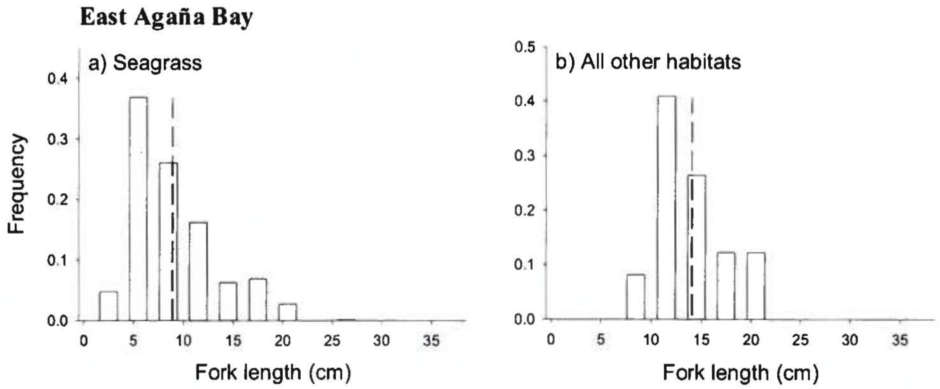

Figure 2.7. Size frequency distributions for habitats within East Agaña Bay. Dashed lines indicate the mean size for each habitat type. All habitats except seagrass were grouped due to the low occurrence of individuals.

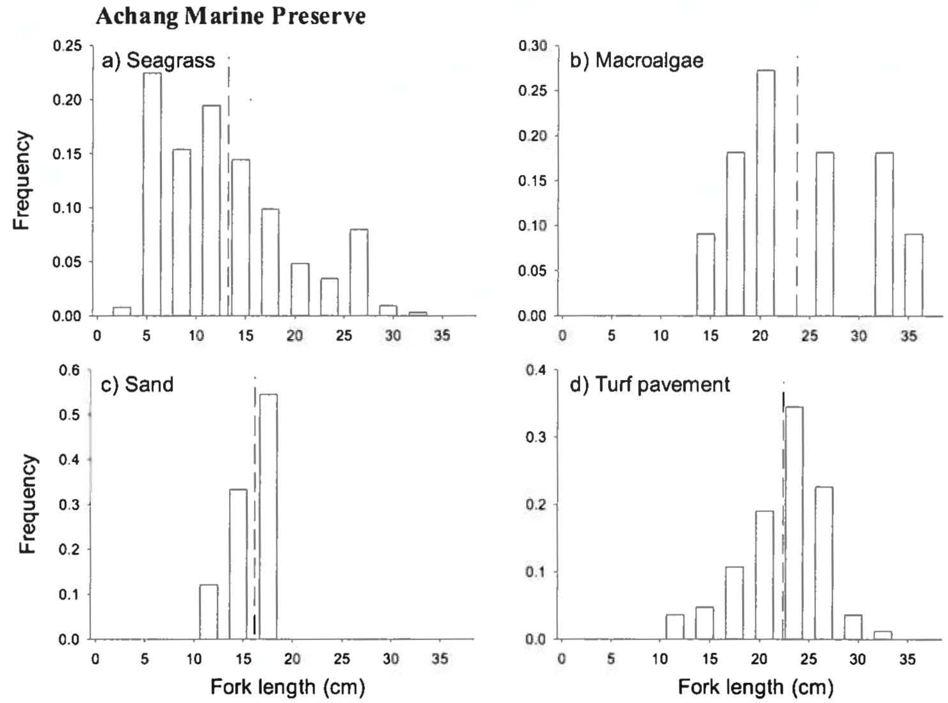

Figure 2.8. Size frequency distributions for habitats within Achang Marine Preserve. Dashed lines indicate the mean size for each habitat type.

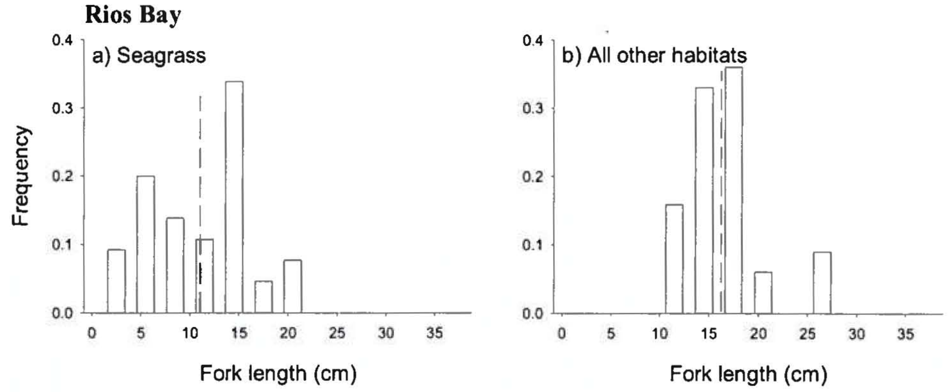

Figure 2.9. Size frequency distributions for habitats within Rios Bay. Dashed lines indicate the mean size for each habitat type. All habitats except seagrass were grouped due to the low occurrence of individuals.

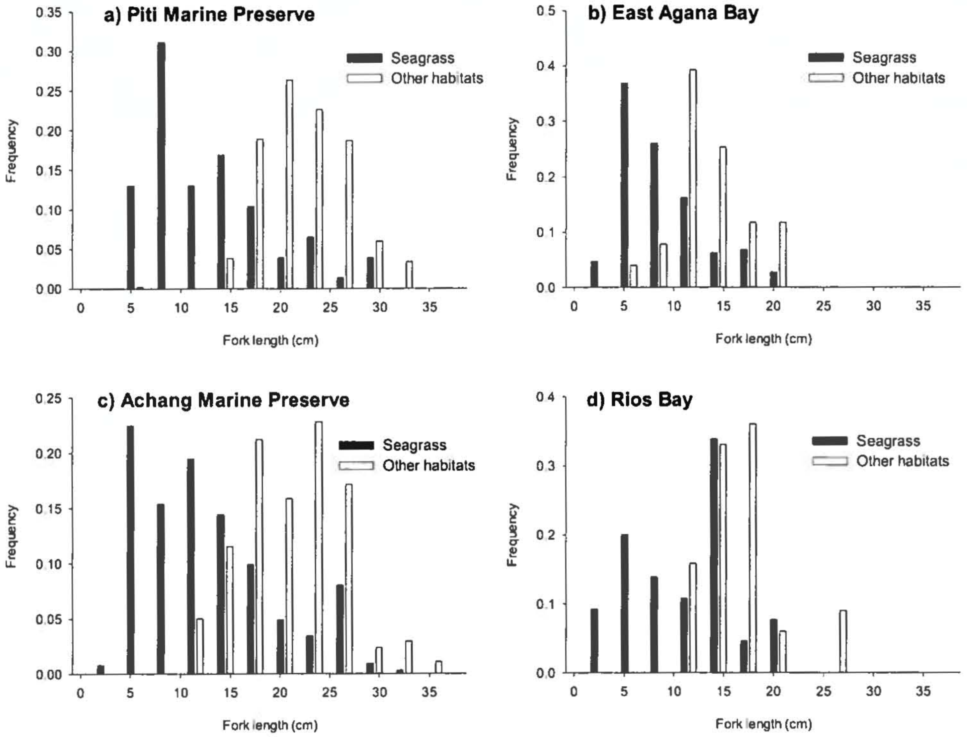

Figure 2.10. Size frequency distributions for seagrass and all other reef flat habitats in a) Piti Marine Preserve, b) East Agaña Bay, c) Achang Marine Preserve, and d) Rios Bay.

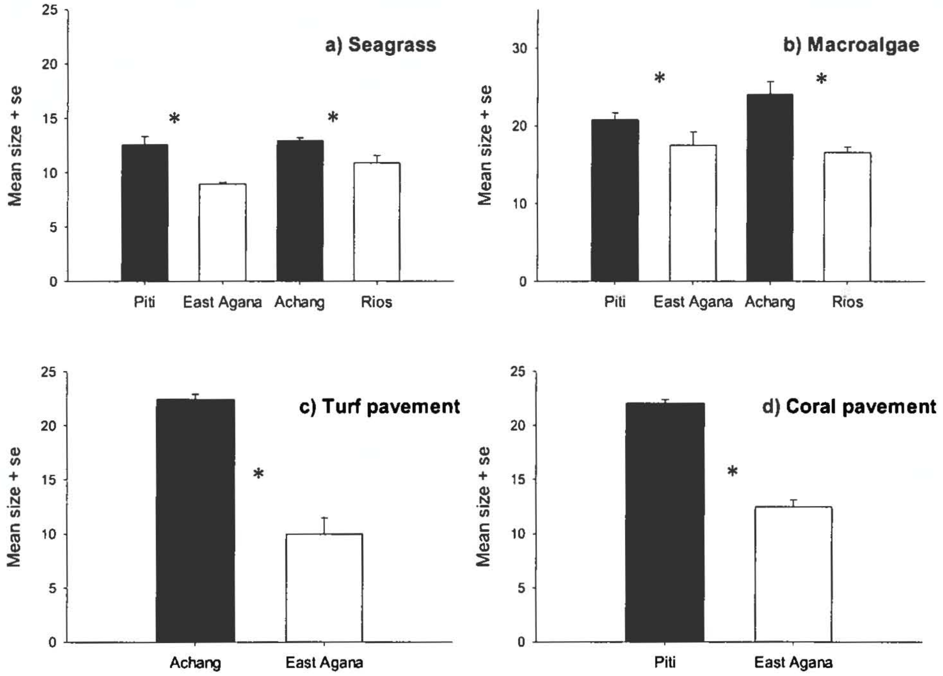

Figure 2.11. Comparison of mean sizes (cm) of individuals from a) seagrass, b) macroalgae, c) turf, and d) coral pavement habitat types within each site. Black bars represent marine preserves while grey bars represent fished sites. * indicates significant difference between comparative protected and unprotected site.

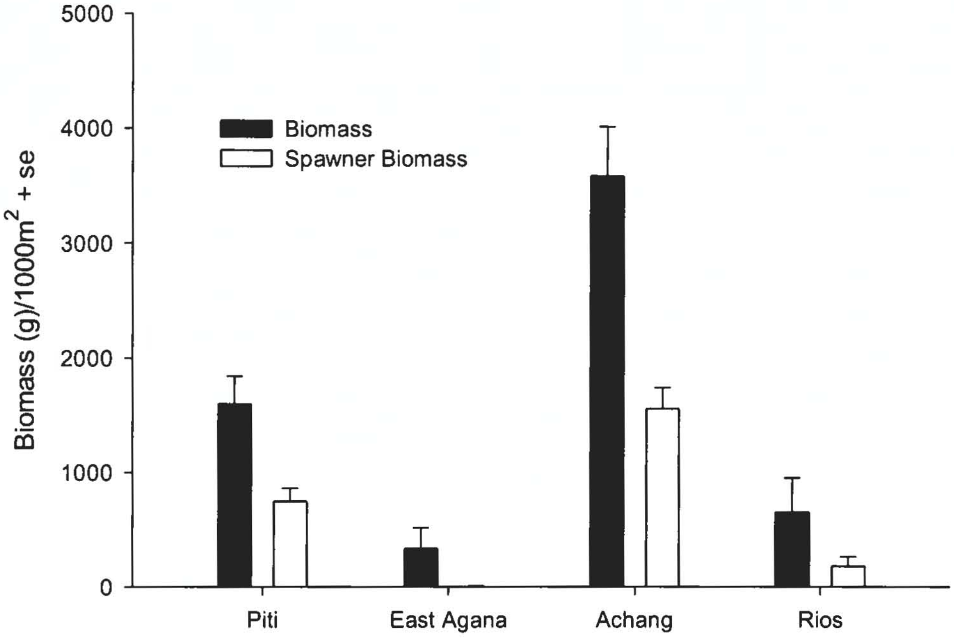

Figure 2.12. Comparison of total biomass and spawner biomass (fish ≥23.5 cm) (+ s.e.) of *Lethrinus harak* at each of the four study sites.

2.4 Discussion

Achang Marine Preserve and its comparative site Rios Bay are similar in the types of habitat present, have the same orientation (i.e., southwards), and are separated by a distance of only 550 m. However, there were vast differences in the total abundance and density of *L. harak* between the two sites. Because fishing for *L. harak* is banned, the overall density in Achang was over 4.5 times greater than that of Rios, which is near the average comparison between protected and unprotected sites of 3.7, determined through meta-analyses by Mosquera et al. (2000). On the other hand, it was unexpected that East Agaña Bay had a greater density of *L. harak* than Piti Marine Preserve. The greater total abundance and density in East Agaña does not fit the trend commonly documented in the literature of higher fish numbers inside protected sites. However on closer examination, it is clear that the population structure of *L. harak* within Piti is very different to that of East Agaña which is comprised almost entirely of juvenile fish (~99%). A number of factors are driving the unexpected difference in density. First of all, high densities of very small *L. harak* in East Agaña indicate potentially high levels of recruitment as over 50% of the individuals counted on transects were less than 10 cm. Conversely, Piti appears to experience lower levels of recruitment but greater survivorship as nearly 70% of individuals are greater than 20 cm. While East Agaña contains high numbers of juvenile fish, high mortality rates most likely from fishing (see Chapter 3) hinder the vast majority from reaching a reproductive size.

It is clear that seagrass is a preferred habitat of both juvenile and adult *L. harak* as it had the highest densities compared with any other habitat type, a pattern consistent across sites. This concurs with Dorenbosch et al. (2005) who classified *L. harak* as a 'seagrass generalist.' However, densities of fish within seagrass habitat among sites did not follow an expected pattern. Densities were shown to respond differently to the reduced fishing pressure in the preserves between northern and southern sites, which could be a result of differences in seagrass species among the sites. Another influence was the difference in proportional habitat area between the marine preserves; in Piti, seagrass comprised only 5.6% of the total area, while it accounted for 34.6% in Achang (Table 2.2). Because habitat preference for this species was demonstrated by the present study to change with size, the differences in the size frequency distributions between northern and southern sites may also have contributed. The fact that over 80% of Achang's and East Agaña's *L. harak* population is found in seagrass is an interesting result. Nevertheless, care should be taken not to interpret the '% Fish' column of Table 2.2 as habitat importance because the values are highly dependent on the proportion of each habitat present as well as the presence of other habitats in a site.

One of the benefits of using underwater visual census techniques (fishery independent data) to estimate size frequencies and mean sizes is that it eliminates the bias associated with gear selectivity (fishery dependent data) (Samoilys & Carlos 2000). I took this one step further by stratifying the surveys according to different habitat types, which allowed for a detailed comparison of size frequency and mean size at the habitat level plus an estimate of the total abundance of *L. harak*. Furthermore, focusing only on one species ensured the adequate representation of different life stages (i.e., adults and juveniles), which is a problem that commonly confounds data sets on reef fish assemblages. On

Guam, vast differences in the overall size structure between protected and unprotected sites are evident as unprotected sites consistently showed truncated size frequency distributions characteristic of the effects of intense fishing pressure. On the other hand, the marine preserve sites, where fishing for *L. harak* is banned, had a greater proportion of large individuals. If an MPA is to facilitate stock recovery, the size of the MPA must match the magnitude of movement of target species (Norse et al. 2003). Although little is known of the movement of *L. harak*, the concurrent buildup of larger fish and total biomass indicates that Guam's marine preserves are achieving their primary objective of allowing fish stocks to recover in the absence of fishing.

At the habitat level, size distributions among habitats indicate a clear ontogenetic shift in habitat preference. It appears that *L. harak* does not utilize any habitat type except seagrass until it reaches 10 centimeters. For *Epinephelus striatus*, ontogenetic shifts in habitat are a trade-off, driven by the potential for greater growth rates at the expense of higher mortality (Dahlgren & Eggleston 2000). The same might be said for *L. harak*. Larger individuals feed more commonly in habitats outside of seagrass (B.M. Taylor, pers. obs.), whereas small individuals find greater protection within dense areas of seagrass. A separate study comparing gut-content analysis with size frequency distributions might help tease these factors apart. Numerous studies have highlighted the importance of seagrass as a nursery habitat for juvenile reef fish of many species, including lethrinids (e.g., Parrish 1989; Connolly 1994; Hannan & Williams 1998; Nagelkerken et al. 2000; Dorenbosch et al. 2005). Numerous hypotheses have been proposed to explain the utility of seagrass beds as nursery habitats. Three major hypotheses are (1) structural complexity of seagrasses offer optimal protection against predators (Parrish 1989), (2) seagrasses are located in reef zones that are frequented less by predators (Shulman 1985), and (3) seagrasses offer a greater source of food for juvenile fish than other habitats (Odum & Heald 1972; Carr & Adams 1973).

It was clear from these results that fishing had directly impacted fish stocks, evidenced by the reduced mean size in the non-protected sites. An increase in the mean fish size is often considered an indicator of a stock that is in recovery and, hence, is one of the proposed benefits of MPA's. However, the results of this study serve as an example of why and how interpretation of mean sizes should be done carefully. Such statistics can easily be confounded by habitat variability and the inherent size structure of a population. For example, the greater mean size of *L. harak* within Achang Marine Preserve was only evident because surveys were stratified by habitat types. When all habitats are pooled, the overall mean size in Achang (14.3 cm) was lower than that of Rios (15.1 cm) (Figure 2.5b). However, this lower value is a direct result of a greater total number of juvenile individuals within the site. At the habitat level, the mean size was consistently larger in Achang than in Rios (Table 2.6). Reduced mean fish size is a common indicator of overfishing because fishing selectively targets the larger individuals in a population (Munro 1996; Ault et al. 2005).

One of the most outstanding results from this study was the enormous difference in the spawner biomass between areas open and closed to fishing. This was the most obvious at East Agaña Bay where overfishing has reduced the biomass of reproductive individuals to approximately 8 kg. This equates to approximately 20 to 30 individuals capable of reproducing within the entire bay. The maintenance of a reproductively viable stock is extremely important in fisheries science and the benefits that marine protected areas can provide have been demonstrated here and in other studies (Bohnsack & Ault 1996; Bohnsack 1998; Guenette & Pitcher 1999). In addition, the ratio of spawner biomass to total biomass differed enormously between protected and unprotected sites. This difference indicates that intense fishing activity is likely the major factor reducing the population size in areas outside of marine preserves. The lack of adults in East Agaña is probably a result of the nonrandom selectivity of fishing, which targets larger size classes. Measures of spawner biomass ratios could potentially be used as a measure of stock health for other species on Guam. However, this can only be done if the whole population is surveyed using a similar approach to the one employed here, which includes both juvenile and adult habitats.

One potential bias in the sampling design in this study was the habitat classifications. There was some variability in the species composition of certain habitat types among each of the sites. For example, although seagrass was classified as one habitat type, two seagrass species were found to occur: *Enhalus acaroides* occurred in Piti, Achang, and Rios and *Halodule uninervis* in East Agaña. The blades of *E. acaroides* are longer and thicker than *H. uninervis* which reaches a maximum size of only ~15 cm. This morphology may influence the densities and size distributions of the *L. harak* individuals within. Similarly, macroalgal assemblages varied among sites, although like the seagrass it was classified as one habitat type. For instance, *Caulerpa racemosa* was the dominant species in Achang, *Halimeda macroloba* in East Agaña, *Padina boryana* in Piti, and many species were patchily distributed within Rios. It is likely that *L. harak* interacts differently with different macroalgae communities. These interactions might bias results as all macroalgae habitats were grouped together. Such bias would affect comparisons between sites more so than estimates within a certain site. Nevertheless, the major purpose of the habitat classification was to separate regions of variability in fish density to obtain a more accurate total estimate. This purpose was achieved in the present study.

In conclusion, the present study has demonstrated that Guam's marine preserves are supporting healthier *L. harak* populations compared with sites open to fishing with greater representation of the larger size classes. Increased abundance, density, mean size, total biomass, and spawner biomass of a highly targeted species have been demonstrated from within protected areas and equally important is the identification of seagrass as a critical habitat, particularly for juveniles. Enormous disparities between protected and unprotected sites emphasize the impact intense fishing pressure has had on this species on Guam and stress the importance of strong fisheries management for maintaining a healthy stock. The patterns observed in this chapter have also been documented for other systems where marine protected areas benefit local fish populations which are reviewed by Russ (2002). However, to fully assess the efficacy of Guam's marine preserves as a fisheries management tool, additional age-based demographic data such as age structures, growth parameters, and mortality estimates are needed. These parameters are explored in Chapter 3.

Chapter 3 Age-based demography of *Lethrinus harak* among protected areas and comparative fished sites

3.1 Introduction

As recently as the mid-1990s, many fisheries biologists believed that annual increments do not form on the otoliths of fishes from low latitudes; thus, suggesting tropical fishes could not be accurately aged using otoliths (Munro & Williams 1985; Polunin et al. 1996). It was believed that the lack of seasonal climatic variation at low latitudes caused the formation of incremental bands in otoliths at non-annual or highly variable rates. Although the interpretation of sectioned otoliths in tropical fishes is undeniably more difficult when compared to those from temperate regions, it is now established that tropical fishes of virtually all families deposit annual increments (Lou 1992; Fowler 1995; Choat & Axe 1996; Newman et al. 1996). Consequently, otolith ageing has led to major advances in tropical fisheries biology and management in the past decade.

Age-based demographic information is the cornerstone of population dynamics (Cole 1954). The benefits to population modeling gained from age information are very large. Age frequency distributions and longevities derived from the random sampling of a population give an indication of exploitation levels. In addition, age-based catch curves are perhaps the simplest and most accurate estimates of total mortality rates (Beverton & Holt 1957; Hilborn & Walters 1992). Length-at-age data can be used to infer growth trajectories, while variation in growth parameters among sampled populations can be used to investigate localized responses to environmental gradients (Gust et al. 2002). Additionally, this variation might have a genetic basis, which indicates possible geographic or reproductive isolation, and hence could be used for stock identification (Begg et al. 1999). Furthermore, age at recruitment, maturation, and sex reversal are all important biological reference points while age frequency analysis can provide insight into temporal variability in recruitment when one dominant age class is preserved in the population structure over time (Russ et al. 1996). The utility of age-based analysis makes it a perfect tool for understanding the dynamics of exploited reef fish populations and their responses to various management scenarios.

While there is less age-based information available for coral reef fish species compared to temperate species, recent work has revealed coral reef fishes exhibit greater longevities and more regional variability in growth parameters than previously believed (Choat & Robertson 2002). Because coral reefs are highly competitive and diverse ecosystems, many believed tropical reef fish were subject to high turnover rates resulting in short-lived species that exhibited fast growth (Pauly 1994). This was however shown to be false with species from families such as the Serranidae (Moe 1969; Manooch & Haimovici 1978; Nagelkerken 1979; Matheson & Huntsman 1984), Lutjanidae (Newman et al. 1996), Scaridae (Choat et al. 1996), Lethrinidae (Pilling et al. 2000), and Acanthuridae (Choat & Axe 1996) all shown to exhibit long life spans and, for some, slow growth rates. Many acanthurid species, for example, have very fast initial growth rates, but total lifespans extending nearly 50 years (Choat & Axe 1996).

While the plasticity in regional growth parameters is a direct result of the dynamic environment in which coral reef fish live, it is further complicated by the effects of fishing (Rijnsdorp & van Leeuwen 1992). For example, as the mean maximum size achieved by the Caribbean scarid *Sparisoma viride* was found to increase with latitude, fishing intensity also influenced growth patterns over a similar spatial scale (Choat et al. 2003). Growth trajectories and mean maximum sizes for three scarid species and one acanthurid on the Great Barrier Reef (GBR) were found to differ between mid-shelf and outer-shelf reefs as a result of differences in density-dependent processes such as mortality (Gust et al. 2002). Demographic plasticity has also been demonstrated in the emperor reef fish *Lethrinus miniatus* with differences in growth over various spatial scales (20 km and 600 km) which were attributed to historic differences in fishing pressure and regional differences in productivity (Williams et al. 2003). Growth parameters have a major effect on the way fish populations respond to fishing pressure (Hilborn & Walters 1992) and potential yield from a population may vary spatially as a result of demographic variability (Caddy 1975). Hence, it is important to identify the spatial scales of demographic variability in reef fish stocks to understand which scales are of significance to assessment and management (Sale 1998). Therefore, the accumulation of age-based parameters for reef fish over broad spatial scales is merited (Choat & Robertson 2002).

Of recent interest is the effect of closing areas to fishing and the possible flow-on effects of reduced fishing pressure on the age-based demographics of targeted reef fish populations (Ferreira & Russ 1995; Choat et al. 2003). Marine protected areas (MPA's) have become an important management tool for conservation of targeted fish stocks (Roberts et al. 2005). If effectively enforced, MPA's have been shown to increase abundance, biomass, mean size, and reproductive potential of fish populations. However, to fully evaluate the effectiveness of MPA implementation, additional life-history data, like age-based demography is equally important. Differences in growth parameters, age structures, longevities, and mortality rates between protected and comparative unprotected sites can provide additional insight into the effectiveness of an MPA beyond simple enumeration of abundance and biomass. While there have been many studies quantifying fish abundance, density, and mean size between MPA's and comparative fished sites (summarized by Russ 2002), few have provided detailed investigations of age-based demographic parameters. Ferreira & Russ (1995) compared populations of *Plectropomus leopardus* from reefs on the GBR that were fished and others that had been closed to fishing for 3-4 years. They found no significant difference in mean size or age between reefs of differing protection status but determined that age comparisons were more effective comparisons than mean length because age and length in reef fish is decoupled (Choat & Robertson 2002). Failure to detect significant differences were attributed to the short time period in which the reefs had been protected, not allowing a long enough time for the older age classes to accumulate. Similarly, Adams et al. (2000) made demographic comparisons of the same species on the GBR but at two spatial scales and on reefs that had been closed to fishing for a period of 8-10 years. Females were significantly larger and older on protected reefs and those above the minimum size limit comprised a greater proportion of the population. As *Plectropomus leopardus* is a protogynous hermaphrodite, the authors suggested reef closure status may have had a positive effect on sex ratios, but regional variation in reproductive biology made this difficult to determine. Clearly, more age-based analyses of reef fish species in sites open and closed to fishing are needed. Understanding demographic variability and the response to fishing pressure will allow for the development of detailed models which estimate the potential of an MPA for improving or maintaining fishery yields.

Although it is one of the most abundant medium-sized piscivores found on Guam's inshore reefs, there is little demographic information available for *Lethrinus harak*. I utilize information gathered from analysis of otoliths to compare growth parameters, age structures, mortality rates, and longevities of *L. harak* between two marine preserves and two comparative fished sites on Guam. Such comparisons will indicate whether there has been a significant accumulation of older individuals in the preserves during their seven year closure and whether or not protection status has had a significant effect on the mortality rates at each site. In addition, a yield-per-recruit model is used to assess the current state of the *L. harak* stock on Guam and to advise future management efforts.

3.2 Methods

3.2.1 Sample sites

Specimens of *L. harak* were sampled from various sites around Guam from June 2007 to June 2008. Sampling was fishery-independent and done using either hook-and-line or by spearfishing. To make a comparison of the demographics of *L. harak* between marine preserves and fished sites, the collections were concentrated at the same four sites outlined in Chapter 2: Piti Marine Preserve and East Agaña Bay represented protected and fished sites to the north, and Achang Marine Preserve represented the protected site to the south. However, because of the low abundance of fish in Rios Bay, it was difficult to obtain an adequate sample size. Therefore, Cocos Lagoon was used as the comparative fished site in the south (Figure 3.1). This site contains similar habitats as the other four sites, including a large proportion of seagrass as described in Chapter 2 and is directly adjacent to Achang. Additional samples (n=120) were obtained from eight other sites on Guam which were included the in age-based analyses but only to estimate overall growth parameters, age structures, and mortality rates that were not site-specific.

3.2.2 Otolith processing and age determination

Upon collection, all samples were placed on ice and immediately returned to the lab for processing. Measurements were taken of fork and total length (mm), and total and gutted weight (g). The sex of individual fish was determined macroscopically using the criteria outlined in Table 4.4 (Chapter 4). Sagittal otoliths were removed, cleaned in ethanol, and stored dry until sectioning. One of the sagittal otoliths was chosen at random, weighed to the nearest 0.001 g and total length was measured using digital calipers to the nearest 0.01 mm.

One sagittal otolith from each fish was mounted in Crystalbond on the edge of a slide and ground to the nucleus using 600-grit waterproof sandpaper mounted on a GEMMASTA™ grinding wheel (Choat et al. 2003). The sagitta was then cemented with the flat side down and the opposite side ground to produce a thin transverse section approximately 300 μm thick (Choat & Axe 1996). The section was covered in

Crystalbond and placed on a hotplate at 230°C for approximately 30 minutes to improve the visibility of annual increments. Transverse sections of the sagittae of *L. harak* were used to assign ages to individuals by counting annual increments using transmitted light with a low power microscope (20-40x). Annuli were represented by alternating translucent and opaque increments which were counted along a consistent axis on the ventral face of the section. Blind readings were made on three separate occasions 1-2 weeks apart and the final age (in years) of an individual was determined when two or more counts agreed (Choat & Axe 1996). For fish younger than one year, the size of the otolith from the sulcus groove to the furthest tip was measured using image analysis. Daily rings were counted back from the one year increment on an older fish until they reached the size of the younger fish to estimate the approximate age of those younger than one year.

3.2.3 Age structure

Once ages were determined, they were combined with size-frequency distribution data from the underwater visual surveys (Chapter 2) to estimate the age structure for each site (Grandcourt et al. 2006). To do this, an estimate of the total abundance from each site was broken down by size class and ages were distributed proportionally using an age-length key (Figure 3.3). This was extrapolated over the total size frequency of individuals recorded during surveys to produce an estimate of the age frequency distribution for each site. For Cocos Lagoon, however, where visual surveys were not done, the age structure was derived using only the fishery-independent data. Although gear selectivity might influence the observed age structure and mean age in Cocos by under-sampling the younger age classes it will have no affect on the mortality rates estimated using age-based catch curves.

3.2.4 *Growth, mortality, and data analysis*

The von Bertalanffy growth function (VBGF) was fitted to length-at-age estimates using non-linear least squares estimation (von Bertalanffy 1938). The VBGF is represented by,

$$L_t = L_\infty(1-e^{-K(t-t_0)}),$$

where L_t is the fork length of a fish at age t, L_∞ is the mean asymptotic fork length, K is the growth coefficient which describes the rate at which fish grow towards L_∞, t is the age of the fish, and t_0 is the theoretical age at which fork length equals zero, as described by the growth rate. The VBGF was also fitted to weight-at-age estimates for use in stock assessment modeling (see below). Growth parameters were compared between sexes and among sites using bivariate 95% confidence ellipses surrounding the K and L_∞ estimates (Kimura 1980) and the analysis of residual sums of squares (ARSS) method (Chen et al. 1992). In addition, one-way analyses of variance (ANOVA) were used to compare mean size-at-age among sites and between sexes. Variables were transformed using either ln(x+1) or squareroot transformations to meet assumptions of normality and homoscedasticity.

Estimates of total mortality were obtained using age-based catch curves from the estimated age structures for each site (Grandcourt et al. 2006). The natural logarithm of the number of fish in each age class was plotted against its corresponding age and total mortality (Z) was estimated as the slope from a fitted line (Beverton & Holt 1957; Ricker

1975). For Piti and Achang Marine Preserves where *L. harak* populations are not fished, estimates of natural mortality (M) were considered equal to fishing mortality (F). Fishing mortality (F) was estimated for the non-protected sites by subtracting M (the mean Z from protected sites) from Z (total mortality calculated for respective non-protected site). For comparison, estimates of M were also calculated for each site using the general equations of Pauly (1980),

$$\log_{10}M = -0.0066 - 0.279*\log_{10} L_\infty + 0.6543*\log_{10}K + 0.4634*\log_{10}T,$$

which is based on VBGF parameters (L_∞ and K) and mean seawater temperature (T), and Hoenig (1983), where $\log_e Z = 1.46 - 1.01*\log_e t_{max}$. Analysis of covariance (ANCOVA) was used to compare the slopes of age-based catch curves, age by otolith weight regressions, and length-weight regressions between sexes and among sites. Length and weight variables were natural log-transformed to linearize the power relationship between the variables. Sex ratios in the age classes were compared using χ^2 goodness of fit and between protected and unprotected populations using a replicated G test of goodness of fit (Sokal & Rohlf 1995).

3.2.5 Assessment of fishery

Growth parameters K, W_∞, and t_0 from the VBGF (fitted to weight-at-age data) and the natural mortality rate (M) were used to construct a yield-per-recruit (YPR) stock assessment model for *L. harak*. This analysis aims to maximize yield from each cohort by altering fishing mortality and the age at first capture (Beverton & Holt 1957). The model follows King (2007):

$$F\exp[-M(t_c - t_r)]W_\infty \qquad (1)$$

$$1\exp[-0K(t_c - t_0)]/(M+F+0K) \tag{2}$$

$$-3\exp[-1K(t_c - t_0)]/(M+F+1K) \tag{3}$$

$$3\exp[-2K(t_c - t_0)]/(M+F+2K) \tag{4}$$

$$-1\exp[-3K(t_c - t_0)]/(M+F+3K) \tag*{(5),}$$

where yield-per-recruit is equal to (1) multiplied by the sum of (2), (3), (4), and (5). In the equations, F represents fishing mortality, M is natural mortality, t_c is the age at first capture, t_r is the current age at recruitment to the fishery, W_{∞} is the mean asymptotic gutted weight, K is the growth coefficient which describes the rate at which fish grow towards W_{∞}, and t_0 is the theoretical age at which gutted weight is equal to zero. Historical catch data for *L. harak* on Guam was obtained from the Guam Division of Aquatic and Wildlife Resources (DAWR) to analyze trends in gear utilization and potential changes in mean sizes for each gear type over time.

3.3 Results

Between 5 June, 2007 and 20 June, 2008, a total of 415 *L. harak* were collected from sites on Guam ranging in size from 83 to 327 mm FL (Table 3.1). These individuals ranged in age from <1 to 13 years. Of these, 328 were identified as females, ranging in age from <1 to 11 years, while 85 were identified as males ranging in age from 3 to 13. Both sexes showed a very strong relationship between length and weight (Figure 3.2). The slopes of the length-weight regressions did not differ significantly between sexes (ANCOVA; $F_{1,405}$=0.267, P=0.61) or among sites (ANCOVA; $F_{3,286}$=0.573, P=0.63). However, females on average (185 mm) were considerably smaller than males (240 mm) (Table 3.1).

3.3.1 *Otolith interpretation*

Annuli were identified in 410 of the 415 otoliths examined (99%) (Figure 3.3). These increments were easier to interpret in older fish (>4 years) compared with younger fish (<1 to 4 years) which were more difficult. Otolith weight proved to be a good predictor of age for both males (r^2=0.79, $F_{1,83}$=306, P<0.001) and females (r^2=0.87, $F_{1,318}$=2086, P<0.001) (Figure 3.4). This relationship strengthened at the site level as r^2 values for Piti, East Agaña, Achang, and Cocos were 0.91, 0.85, 0.88, and 0.86, respectively (Table 3.2). However, the slopes of age by otolith weight regressions differed significantly between sexes (ANCOVA; $F_{1,393}$=12.97, P<0.001) and among sites (ANCOVA; $F_{3,287}$=3.21, P<0.05).

3.3.2 *Age structure*

To compare the demographic makeup of areas open and closed to fishing, paired sites consisted of 1) Piti (protected) and Cocos (fished) and 2) Achang (protected) and East Agaña (fished). This pairing was done based on similarities in habitat; Piti and Cocos both have considerable deeper lagoonal habitat, with a small area of seagrass and Achang and East Agaña are comprised primarily of a shallow reef flat dominated by seagrass beds.

The age frequency distribution for Piti describes a slow, steady decline in numbers of individuals with age until a maximum age of 13 years is reached (Figure 3.5a). At this site, fish of ages 8-13 make up nearly 13% of the total population. By comparison, the older age classes (>7 yrs) from Cocos Lagoon are heavily truncated and represented by only three individuals. Unlike Piti and Cocos, the age structures of Achang and East Agaña are dominated by individuals from the first four year classes (Figure 3.5b). However, East Agaña has no *L. harak* individuals older than 6 years, while Achang contains individuals in all age classes up to 13 years.

There was considerable overlap between male and female age distributions, although males had a significantly greater mean age (6.2 vs 3.2 yrs; $F_{1,407}$=179.0, P<0.001) (Figure 3.6). The sex ratio in all age classes was significantly different (χ^2=235, df=13, P<0.001). In protected sites, females ranged from <1 to 11 years while males ranged from 3 to 13 years (Figure 3.7a). Individuals ≥8 years represented nearly 8% of the female population while those ≥10 years represented 16% of the male population. In unprotected sites, females ranged from <1 to 8 years while males ranged from 3 to 11 years old (Figure 3.7b). Individuals ≥8 years represented less than 1% of the female population while those ≥10 years comprised less than 4% of the male population. A replicated G test of goodness-of-fit determined that sex ratios by age were not significantly different between protection status as sample sizes for older ages were inadequate to detect differences (G=4.963, df=7, p=0.664).

3.3.3 Growth

The VBGF was fitted to length-at-age data for all specimens combined and then for separate sexes (Figure 3.8, Table 3.4). *Lethrinus harak* is relatively fast-growing with approximately 75% of the mean asymptotic length achieved in the first three years. The overall K and L_{∞} estimates for this species were 0.414/yr and 259.8 mm, respectively (Table 3.4). Male and female growth was shown to be significantly different (ARSS $F_{3,397}=13.0$, $P<0.001$) supported by non-overlapping bivariate 95% confidence ellipses surrounding the parameters K and L_{∞} (Figure 3.8). Although the mean size-at-age for males and females was significantlydifferent for only ages 3 and 7 (ANOVA; $F_{1,98}=7.27$, $p=0.008$ and $F_{1,25}=5.79$, $p=0.02$), there was a general trend whereby males reached a greater mean size at age compared with females (Figure 3.9).

Growth rates differed among the sites, though not between Piti and Cocos (ARSS; $F_{3,151}=0.287$, $P=0.83$) (Figure 3.10). Results from the ARSS method were consistent with the plots of the bivariate confidence ellipses for each comparison. East Agaña had the fastest initial growth rate with a $K=0.560$ (Table 3.4). These growth rates were further examined using the mean size-at-age for ages 1 through 5. An ANOVA with a post-hoc Tukey test suggested that the mean size of two year old *L. harak* in East Agaña was greater than that of all other sites ($F_{3,53}=14.1$, $p=0.001$). For age three, the mean size in Piti was significantly greater than those of Achang and Cocos ($F_{3,59}=5.72$, $p=0.002$). For ages four and five, the mean size in Cocos was significantly greater than that of Achang ($F_{3,54}=5.98$, $p=0.001$ and $F_{3,31}=3.13$, $p=0.04$, respectively). When plotted, it is clear that between the ages of one and five, individuals in East Agaña consistently maintain a larger mean size than those in Achang (Figure 3.11). Two year old fish in East Agaña were on average 4 cm larger than fish of the same age in Achang (Figure 3.11). Across all age classes, fish from Achang were consistently smaller.

Differences in growth parameters were evaluated for populations within and outside of protected sites and for fish at differing geographic locations ('north' and 'south' sites). Significant differences in growth were identified (ARSS; $F_{3,278}=8.18$, $P<0.001$) with populations from protected sites having a slower growth rate and a greater mean asymptotic size (Figure 3.12). Growth was also found to differ between populations to the north and south (ARSS; $F_{3,320}=12.93$, $P<0.001$) with those in the south growing slower and reaching a smaller asymptotic length (Figure 3.13).

3.3.4 Mortality

Age-based catch curves predicted lower total mortality rates (Z) of 0.284 and 0.300 for protected sites Piti and Achang compared with 0.504 and 0.792 for fished sites Cocos and East Agaña (Table 3.5). The slopes of catch curves differed significantly between paired protected and unprotected sites (ANCOVA; $F_{1,15}=359$, $P<0.001$ and $F_{1,16}=165$, $P<0.001$) (Figure 3.14). Estimates of M derived from Pauly's (1980) and Hoenig's (1983) equations for all sites exceeded the estimates of Z and should therefore be treated with caution (Table 3.5). The estimates of F and E were considerably higher in East Agaña, suggesting greater fishing intensity. Overall, populations within protected areas are not exploited, whereas unprotected populations have an exploitation ratio of 0.49 (Table 3.5).

3.3.5 Assessment of fishery

The YPR stock assessment model revealed optimal yield is achieved at a high fishing mortality ($F>0.6$) but only when age at first entry is a considerable seven years (~245 mm

FL; Figure 3.15). Yield decreased when t_c is extended beyond seven years. Based on catch data from DAWR creel surveys, the current t_c is approximately one year. At current rates of F and t_c (F=0.50 for East Agaña and F=0.21 for Cocos), East Agaña is suffering from growth overfishing while Cocos is just beyond optimal yield for that age (Figure 3.15). However, the yield-per-recruit for Cocos still only represents a yield that is approximately 60% of the optimal when t_c=7. An increase in t_c to match the age at 50% maturity (t_{50}) would be associated with an increase in yield-per-recruit by approximately 140% in East Agaña and 45% in Cocos at current fishing mortalities. In addition, this would ensure that ~50% of *L. harak* would have the opportunity to spawn once before being harvested. At both sites, yield-per-recruit would be greatly increased if the age at first capture was increased and fishing pressure reduced considerably. Of the estimated total *L. harak* catch (by number) from 1984 to 2008, 61% is comprised of immature individuals (Figure 3.16). In the last four years (2005 through 2008), this number increased dramatically to 84%.

Hook-and-line, snorkel spear, and gill netting are the three most common techniques for harvesting *L. harak* on Guam. They comprised between 77 and 98% of the total catch from 1984 to 2008 (Mean = 91%). Hook-and-line has accounted for a significantly increasing proportion of the total catch over time (>80% in recent years; Regression: $F_{1,6}$=39.57, P<0.001). Over the same time period, proportions of snorkel spear and gill netting have significantly declined (Regression: $F_{1,6}$=7.00, P<0.05; $F_{1,6}$=19.36, P<0.01) (Figure 3.17). There has also been a significant reduction in the mean size of *L. harak* harvested using hook-and-line from 220 mm to 185 mm, whereas this trend is not apparent for the other two methods (Regression: $F_{1,6}=6.15$, $P<0.05$; Figure 3.18). To explore the differences in fishing mortality between the fished sites Cocos and East Agaña, historical catch data was broken down by location. Highly unselective netting techniques (gill net, surround net, and drag net) comprised 19% of Cocos's *L. harak* harvest from 1984 to 2008 while these techniques comprised 50% of the harvest from East Agaña. Cocos had a greater mean fish size for every method, though no comparison was statistically significant. In addition, creel survey data reveal no individual *L. harak* above reproductive maturity from East Agaña collected since 2003.

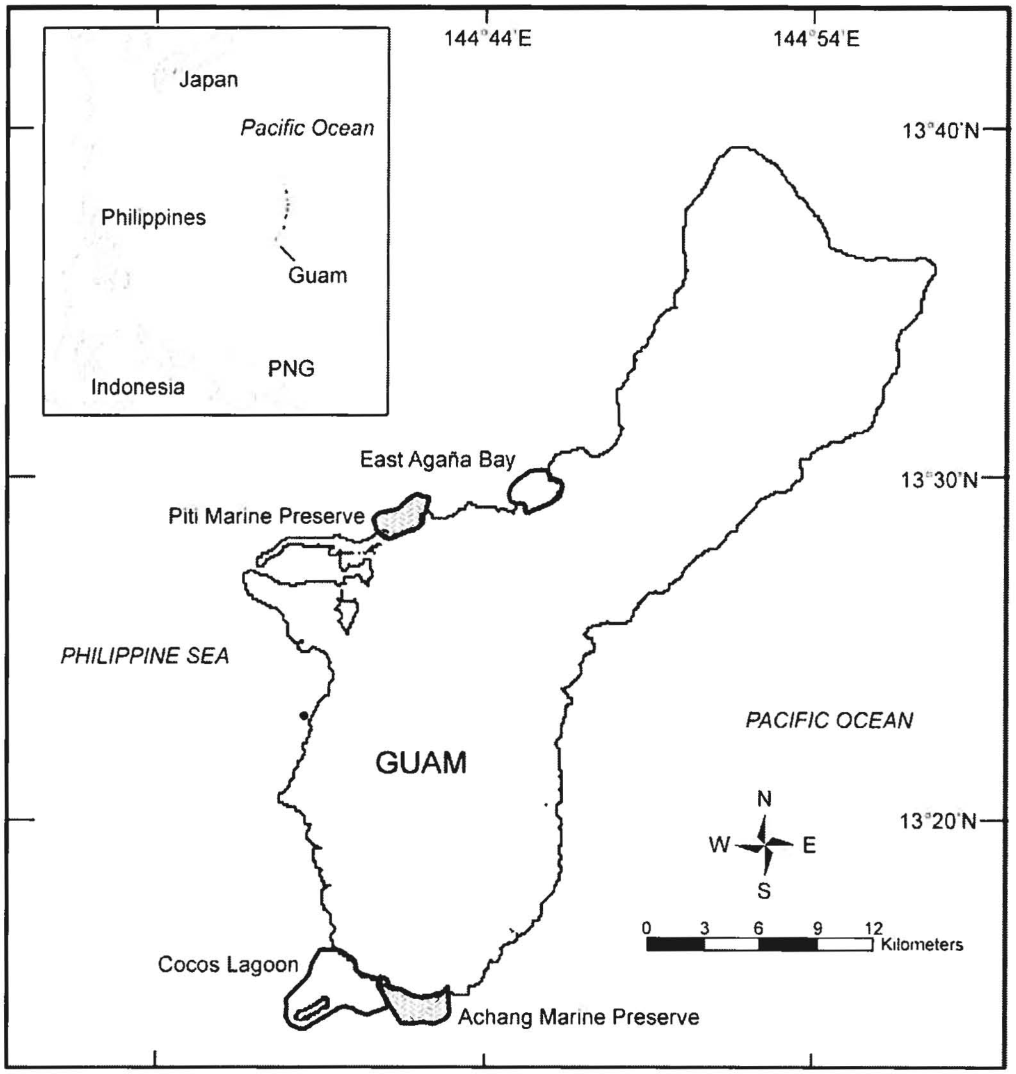

Figure 3.1. Map of Guam indicating the four study sites.

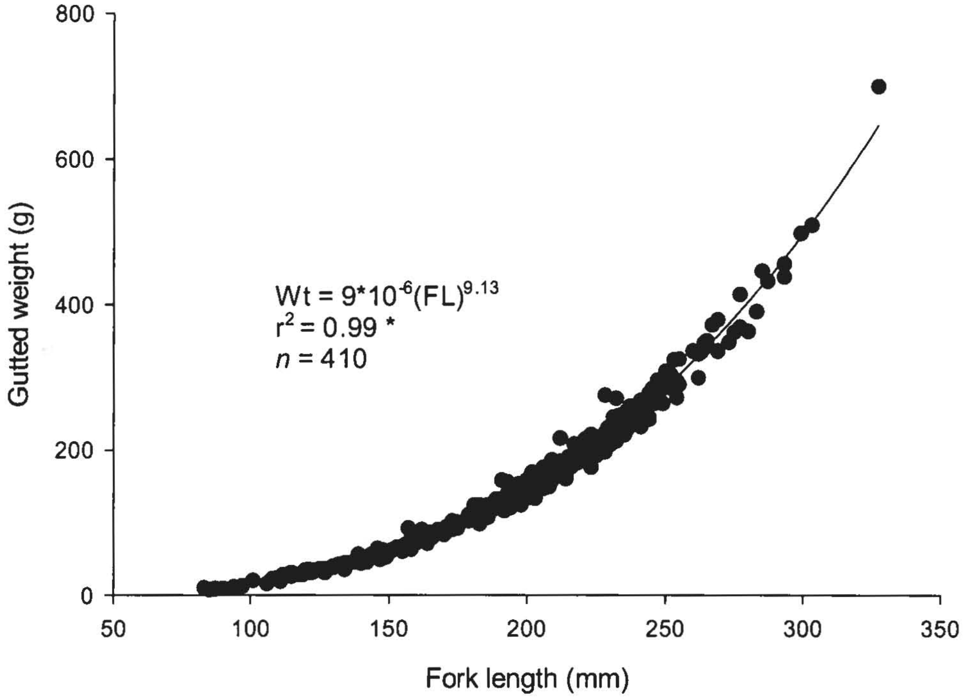

Figure 3.2. Relationship between fork length and total weight for all *Lethrinus harak* sampled during the study period. * indicates significance.

Table 3.1. Results of the regression analysis describing the length-weight relationships for each of four sites and both sexes. FL= fork length (mm), GW= gutted weight (g).

Parameter	*n*	*a*	*b*	r^2	$FL_{min-max}$	$GW_{min-max}$	Av FL (SE)	Av GW (SE)
Site								
Piti	68	$2x10^{-5}$	2.982	0.992	110-327	24-700	211.1 (5.7)	193.7 (15.4)
East Agana	76	$2x10^{-6}$	3.396	0.995	85-254	7.6-292	171.5 (4.7)	105.7 (7. 6)
Achang	63	$2x10^{-5}$	2.993	0.995	108-251	22-298	180.5 (5.0)	125.1 (9.1)
Cocos	89	$2x10^{-5}$	2.995	0.993	112-265	26-350	200.5 (4.5)	165.7 (9.2)
Sex								
Females	328	$8x10^{-6}$	9.163	0.990	83-327	7.6-700	185.4 (2.3)	134.2 (4.7)
Males	85	$2x10^{-5}$	2.959	0.950	179-293	110-456	239.6 (2.6)	257.5 (8.3)

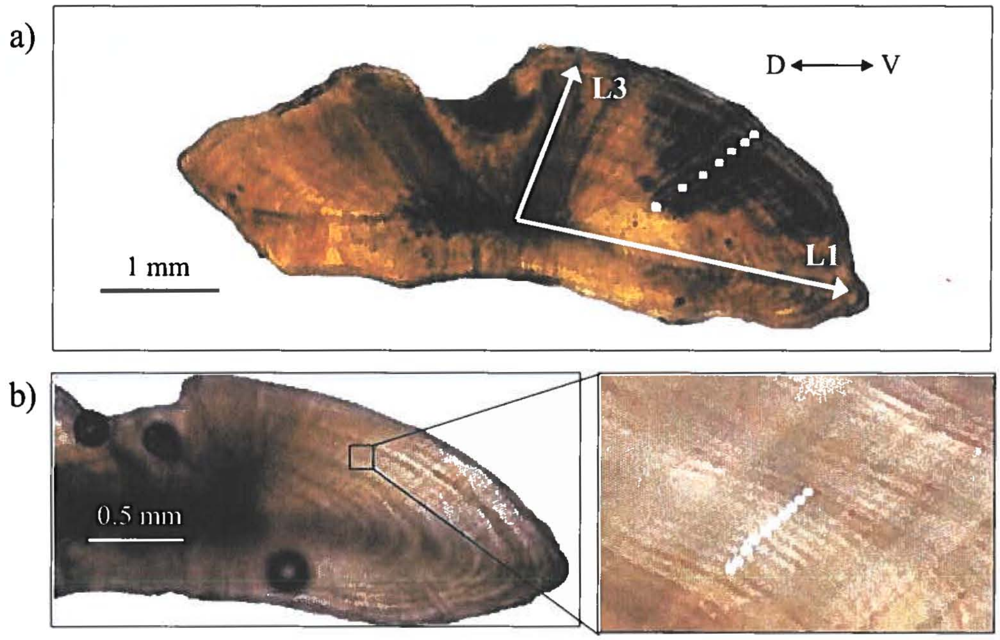

Figure 3.3. Transverse sections of otoliths from *Lethrinus harak*. a) Seven year-old individual with arrows indicating the ventral L1 and sulcal L3 axes for counting annuli. b) Individual less than one year old displaying clear daily rings under high power magnification. D = dorsal, V = ventral.

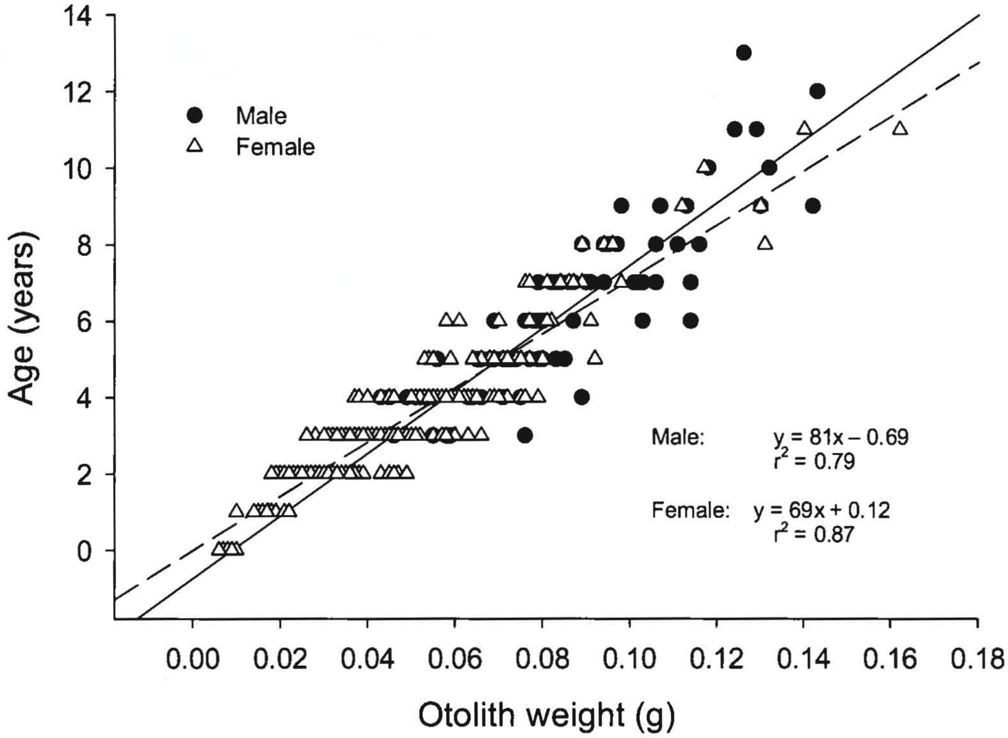

Figure 3.4. Relationship between otolith weight and age for *Lethrinus harak*. Age was derived from counting annual increments in transverse sections of otoliths.

Table 3.2. The relationship between age (years) and otolith weight (g) for *Lethrinus harak* at each of four sites and for both sexes.

Parameter	*n*	Equation	r^2	*P*
Site				
Piti	68	Age= 85.9(OtoWt) - 0.66	0.91	<0.001
East Agana	75	Age= 80.2(OtoWt) - 0.21	0.85	<0.001
Achang	63	Age= 74.0(OtoWt) - 0.16	0.88	<0.001
Cocos	87	Age= 75.1(OtoWt) - 0.17	0.86	<0.001
Sex				
Females	320	Age= 68.7(OtoWt) + 0.12	0.87	<0.001
Males	85	Age= 81.3(OtoWt) - 0.69	0.79	<0.001

Table 3.3. The observed age-length key for *Lethrinus harak* from a) the otolith study and b) the estimated age distribution from surveys at all sites combined. The sizes are midpoints of 30 mm size classes.

Size class (mm)	Age (years)														Total
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	
a) The otolith study															
25															0
55															0
85	10														10
115	2	20	2												24
145		8	30	6											44
175			31	34	7										72
205			11	47	49	9	1	1							118
235				9	22	29	12	17	5	2					96
265					1	2	5	7	4	3	3	2		1	28
295								1	2	2		1	2		8
325												1			1
Total	12	28	74	96	79	40	18	26	11	7	3	4	2	1	401
b) The estimated age distribution from surveys at all sites															
25	46														46
55	414														414
85	294														294
115	22	223	22												267
145		37	139	28											204
175			82	90	18										190
205			12	50	52	10	1	1							126
235				9	21	28	12	16	5	2					93
265					4	8	19	27	15	11	11	8		4	107
295								3	5	5		3	5		21
325												9			9
Total	776	260	255	177	95	46	32	47	25	18	11	20	5	4	1771

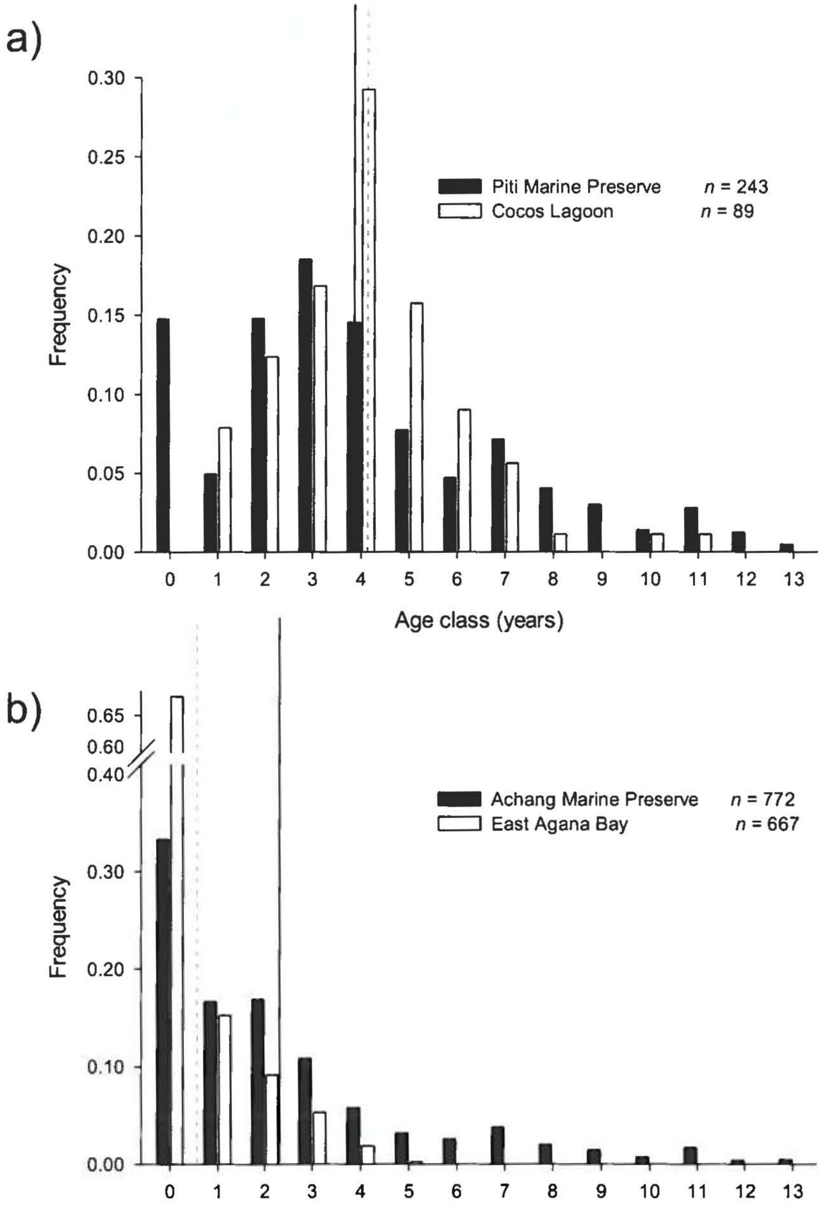

Figure 3.5. Age frequency distributions of *Lethrinus harak* from a) Piti Marine Preserve and Cocos Lagoon and from b) Achang Marine Preserve and East Agaña Bay. Note the differences in y-axes. Data for Piti, Achang, and East Agaña derived from catch curves using length-frequency data extrapolated using an age-length key. Data for Cocos Lagoon are derived from fishery-independent samples. Lines indicate mean size for each population.

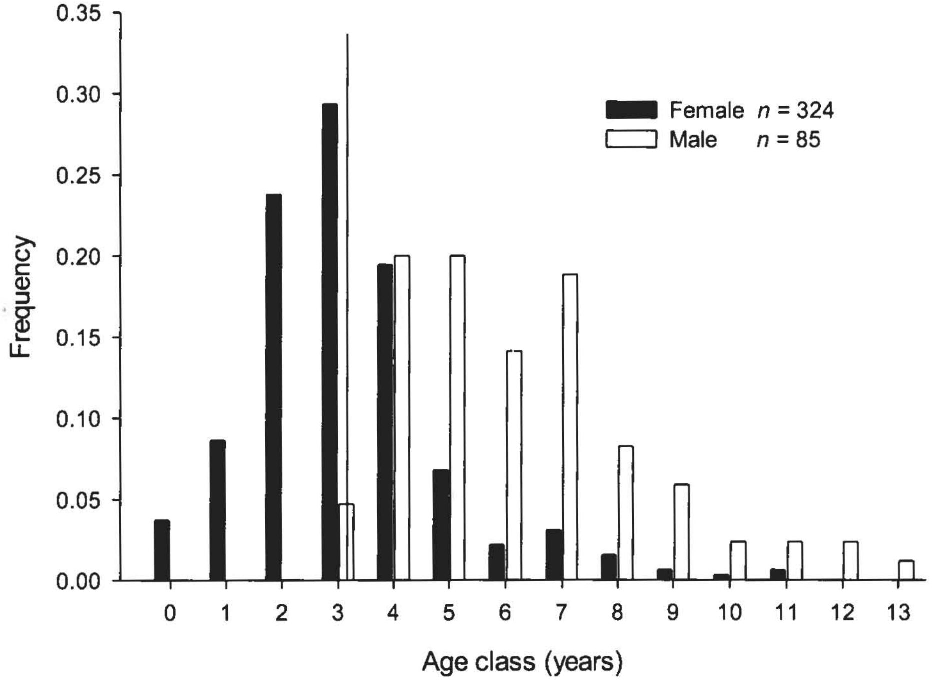

Figure 3.6. Male and female age frequency distributions for *Lethrinus harak* from all sites sampled using fishery-independent methods. Lines indicate mean size for each population.

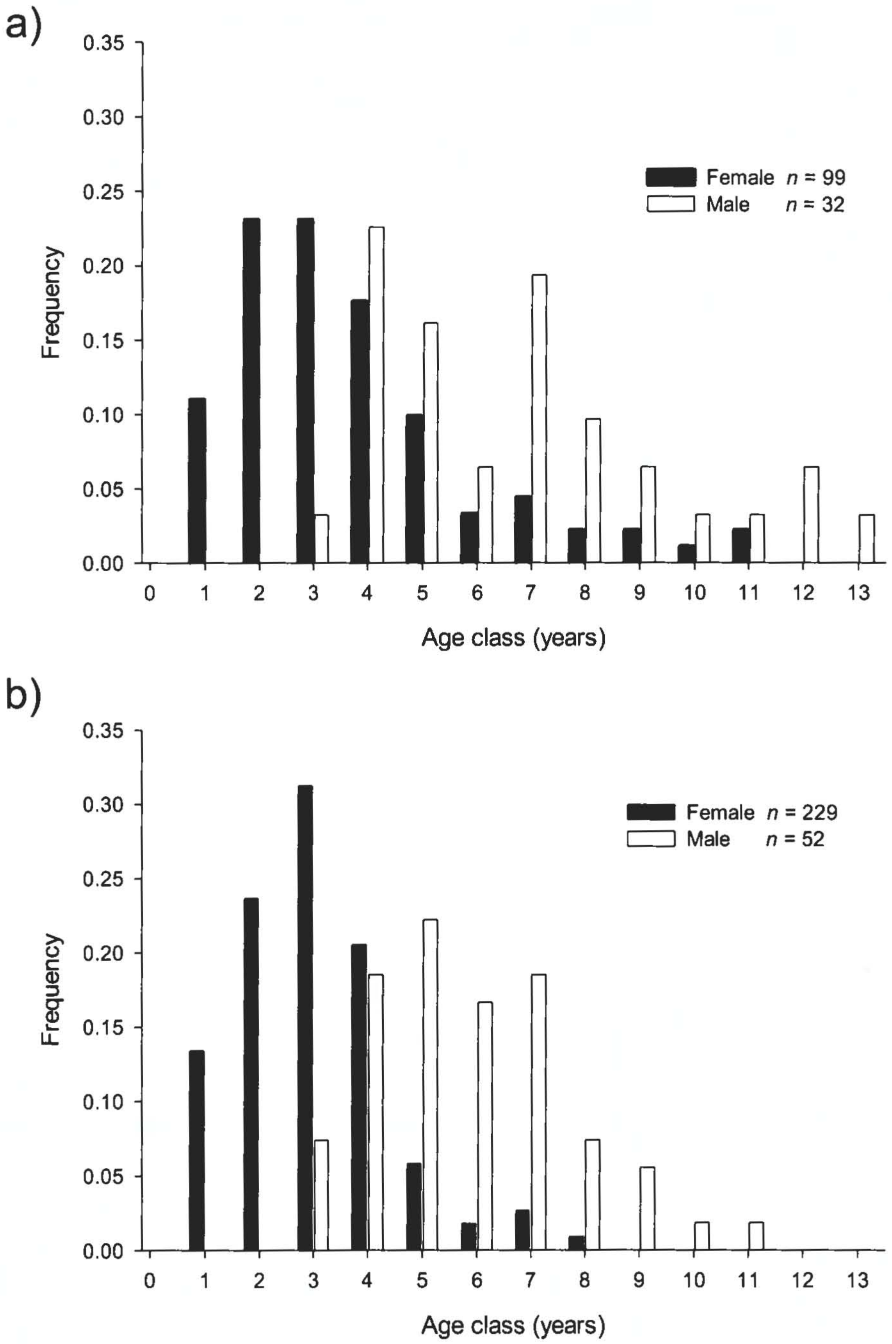

Figure 3.7. Sex-specific age frequency distributions of *Lethrinus harak* from a) protected (Piti and Achang) and b) unprotected sites (East Agaña, Cocos, and others) on Guam.

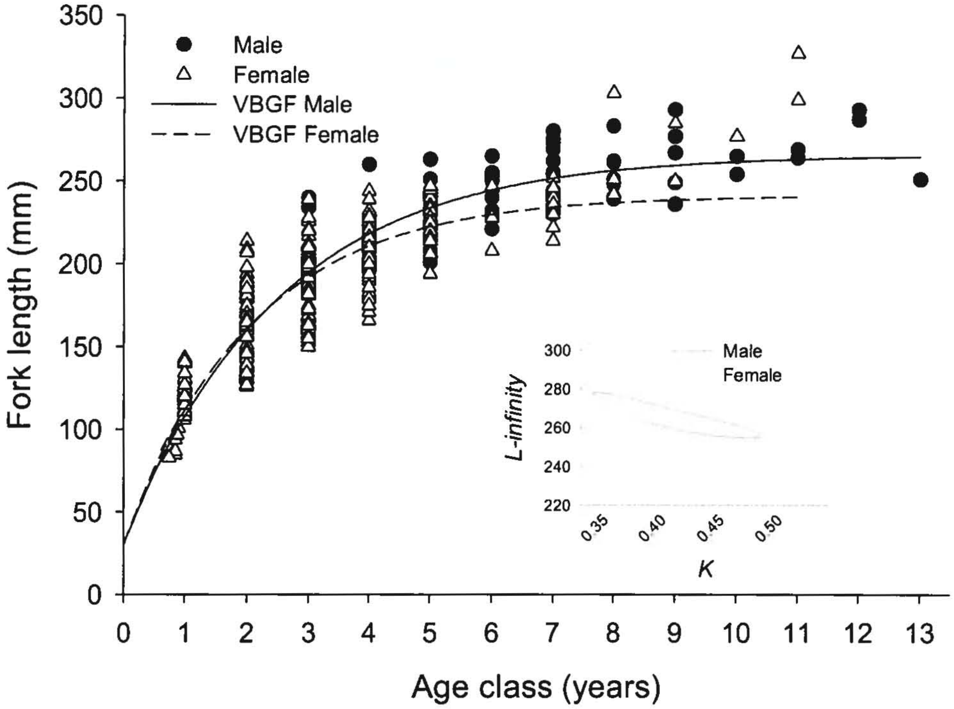

Figure 3.8. Von Bertalanffy growth (VBGF) curves fitted to length-at-age data for male and female *L. harak* from Guam with corresponding bivariate 95% confidence ellipses surrounding the parameters *K* and *L*,. Includes additional samples (n=120) from areas outside the main four study sites.

Table 3.4. Estimates of growth parameters (±95% CI) for *Lethrinus harak* for all sites and populations examined, using length-at-age data.

Locality	*n*	L_∞	CI	*K*	CI	t_0	r^2
Site							
Piti	68	273.0	260.1, 286.0	0.354	0.313, 0.407	-0.329	0.87
East Agaña	76	233.4	216.5, 255.0	0.560	0.459, 0.678	-0.245	0.81
Achang	63	250.9	230.1, 272.2	0.360	0.303, 0.448	-0.354	0.85
Cocos	89	267.6	252.3, 284.7	0.361	0.313, 0.420	-0.329	0.86
Status							
Protected	131	271.2	260.5, 277.1	0.328	0.318, 0.370	-0.357	0.85
Unprotected	165	252.0	240.0, 264.8	0.445	0.395, 0.504	-0.285	0.85
Location							
North	144	268.7	258.6, 278.2	0.395	0.364, 0.437	-0.300	0.86
South	152	262.4	248.0, 276.4	0.357	0.318, 0.412	-0.340	0.84
Sex							
Males	85	265.8	254.8, 278.1	0.400	0.340, 0.485	-0.300	0.50
Females	325	241.1	228.6, 253.6	0.485	0.436, 0.535	-0.273	0.78
Combined	410	259.8	252.0, 266.6	0.414	0.389, 0.450	-0.296	0.81

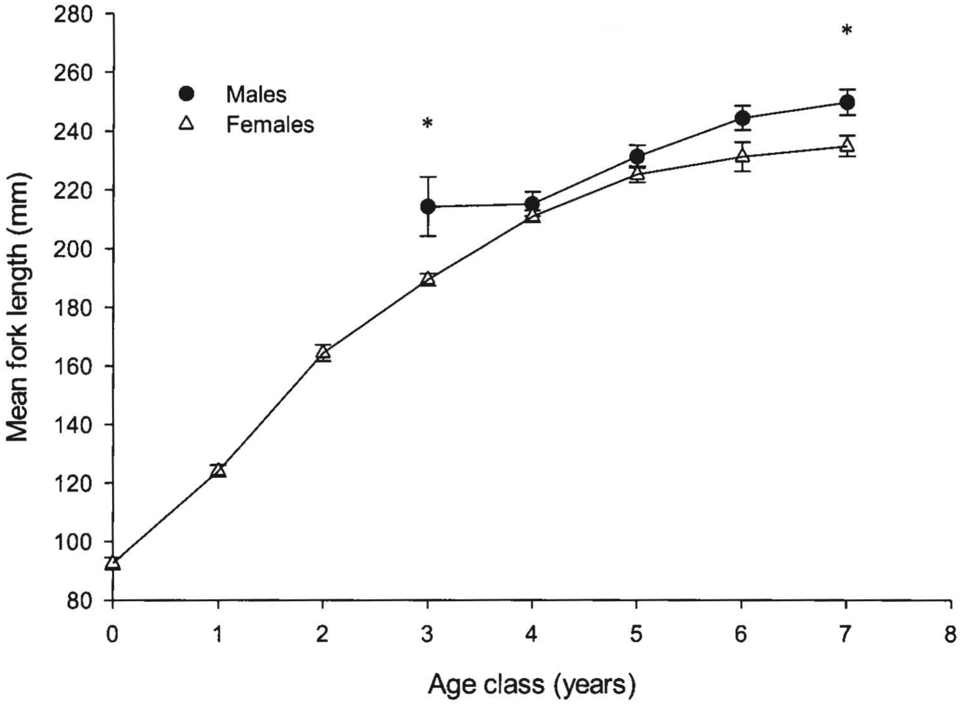

Figure 3.9. Mean size-at-age for males and female *Lethrinus harak* with corresponding standard errors. * Indicates significant difference.

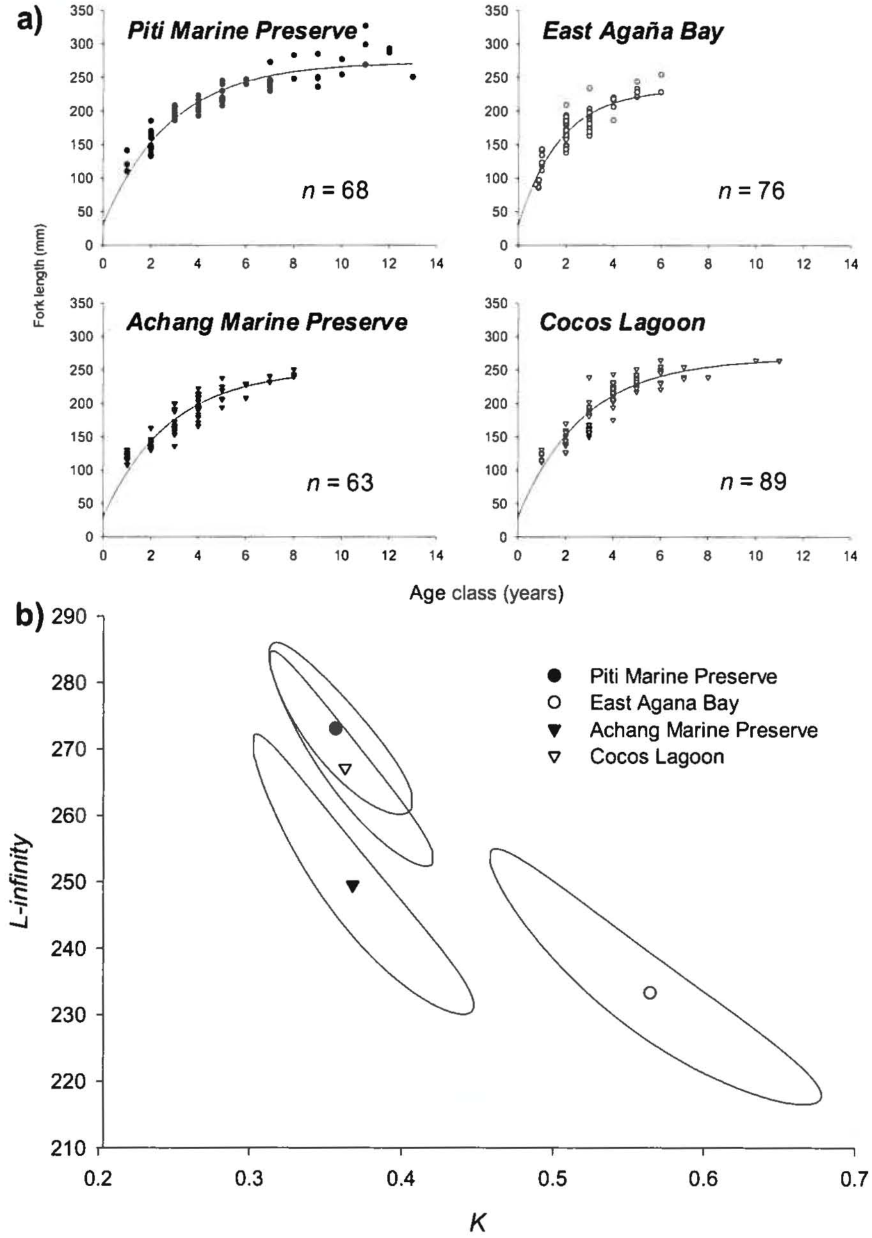

Figure 3.10. *Lethrinus harak*. a) Von Bertalanffy growth curves fitted to length-at-age data from Piti Marine Preserve, East Agaña Bay, Achang Marine Preserve, and Cocos Lagoon. b) Comparison of growth among these four populations using bivariate 95% confidence regions surrounding estimates of *K* and *L*,.

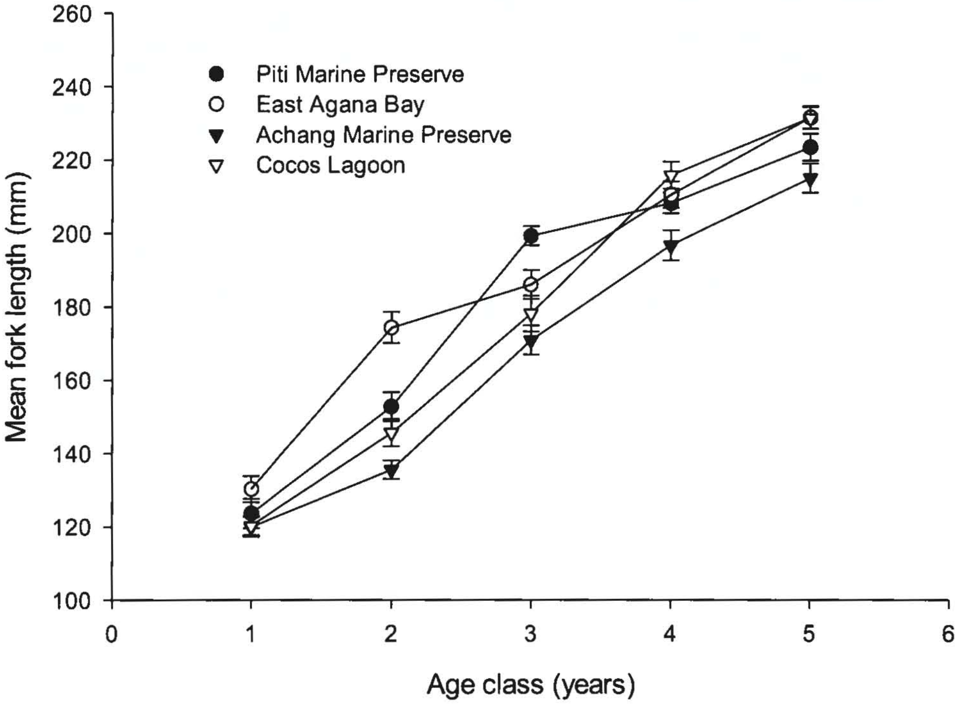

Figure 3.11. Mean size-at-age for *Lethrinus harak* from age 1 to 5 at four sites on Guam (error bars indicate standard error).

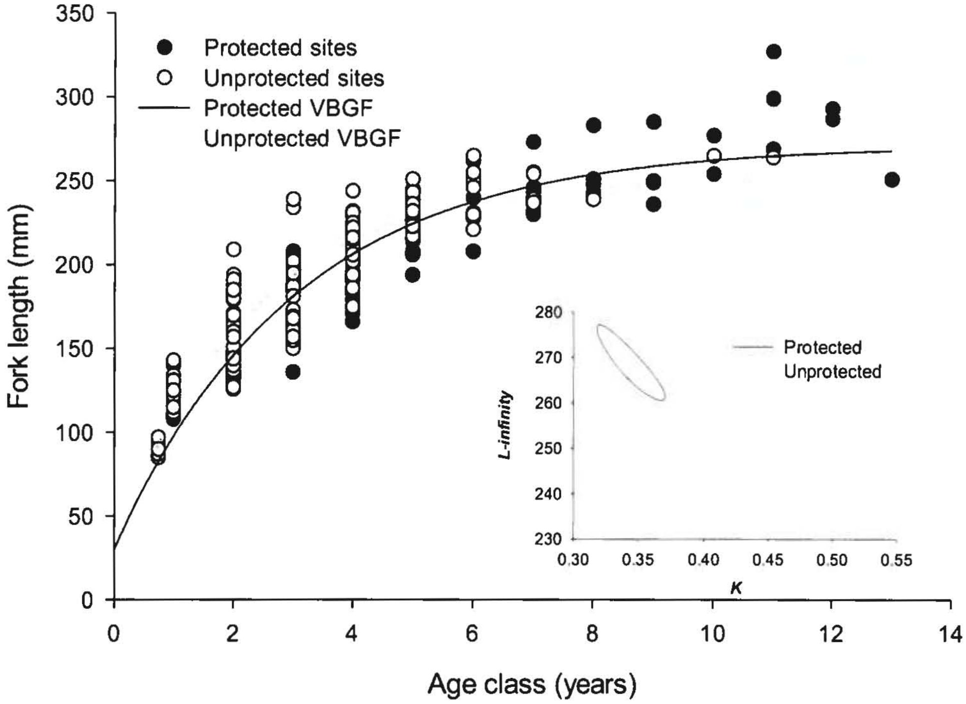

Figure 3.12. Von Bertalanffy growth (VBGF) curves fitted to length-at-age data of *Lethrinus harak* from protected and unprotected sites on Guam with corresponding bivariate 95% confidence regions surrounding estimates of *K* and L_{∞}.

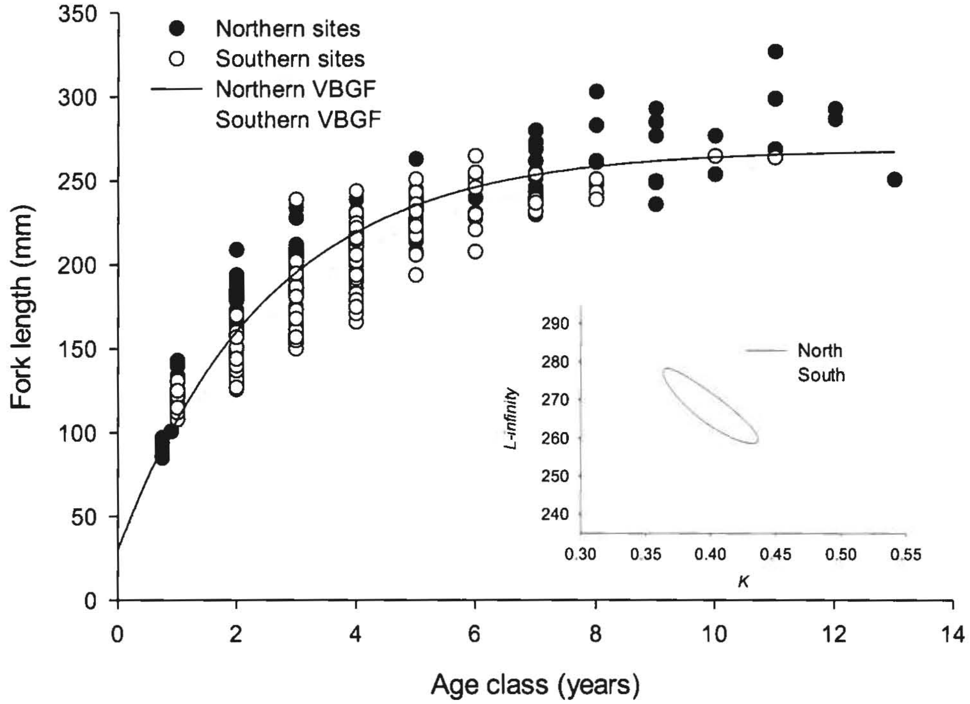

Figure 3.13. Von Bertalanffy growth (VBGF) curves fitted to length-at-age data of *Lethrinus harak* from northern and southern sites on Guam with corresponding bivariate 95% confidence regions surrounding estimates of K and L_∞.

Table 3.5. Estimates of instantaneous rates of total mortality (Z), natural mortality (M), Pauly's (1980) estimate of M, Hoenig's (1983) estimate of M, fishing mortality (F), and exploitation (E) for *Lethrinus harak* in each of four sites on Guam.

Parameter	ABCC Equation	r^2	Z (yr^{-1})	Pauly's M (yr^{-1})	Hoenig's M (yr^{-1})	F (yr^{-1})	E
Site							
Piti	y= -0.284x + 3.6	0.86	0.284	0.489	0.323	-	-
East Agana	y= -0.792x + 3.9	0.99	0.792	0.690	0.705	0.500	0.63
Achang	y= -0.300x + 3.18	0.89	0.300	0.506	0.527	-	-
Cocos	y= -0.504x + 5.0	0.88	0.504	0.498	0.382	0.212	0.42
Status							
Protected	y= -0.293x + 4.0	0.93	0.293	0.466	0.323	-	-
Unprotected	y= -0.573x + 6.2	0.97	0.573	0.581	0.382	0.280	0.49
Overall	y= -0.451x + 6.0	0.97	0.451	0.549	0.323		

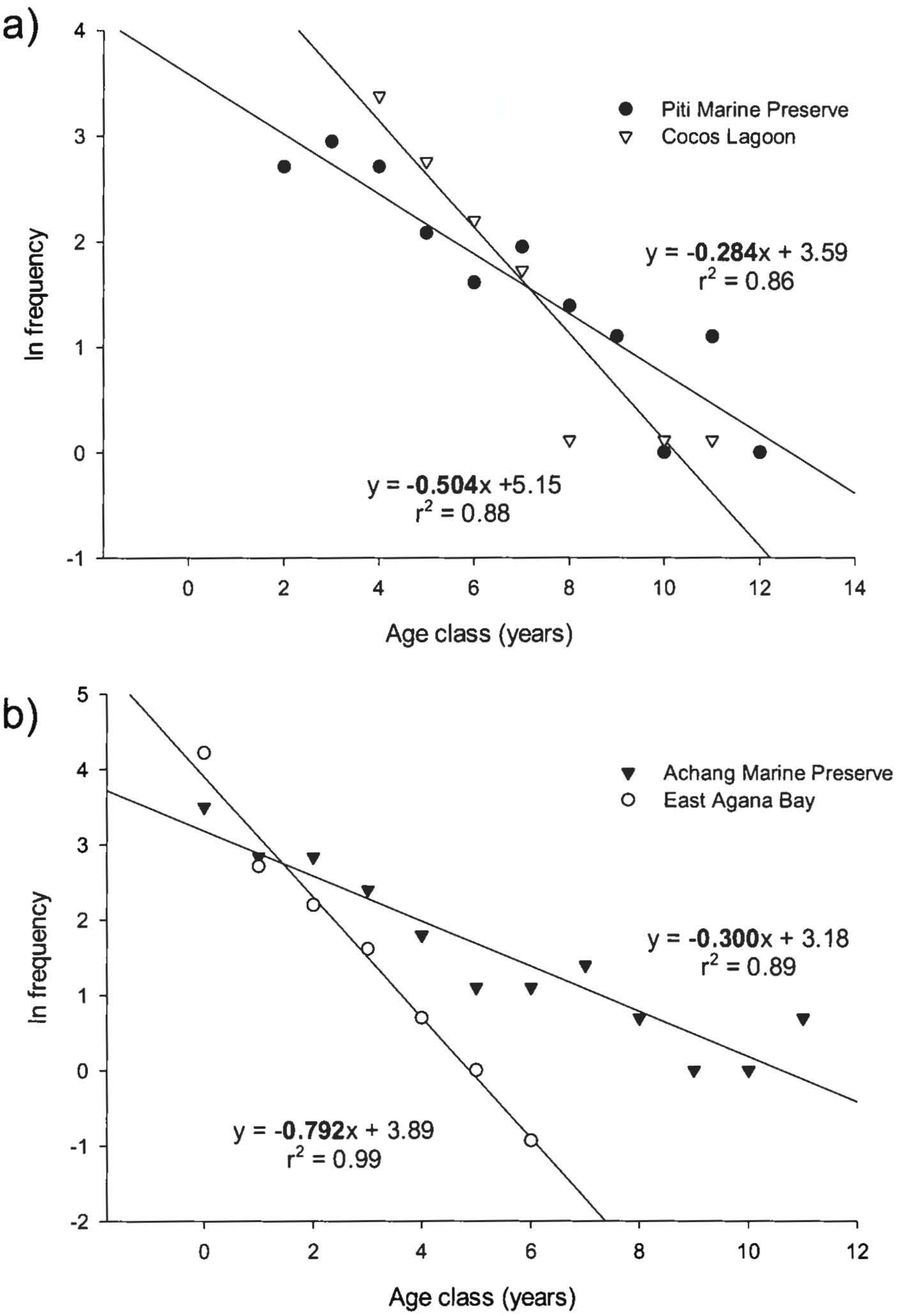

Figure 3.14. Age-based catch curves representing instantaneous total mortality rates for *Lethrinus harak* populations in a) Piti Marine Preserve and Cocos Lagoon and in b) Achang Marine Preserve and East Agaña Bay.

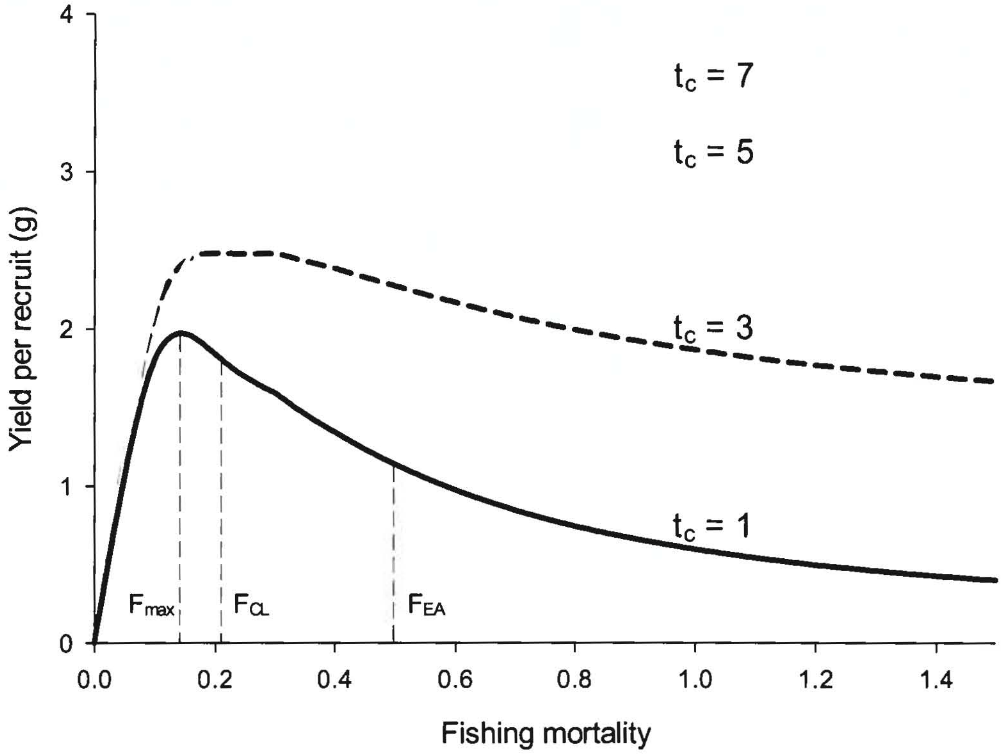

Figure 3.15. Yield-per-recruit model trajectories for various ages at first capture (t_c) for *Lethrinus harak* on Guam. Dashed lines indicate the current exploitation scenarios for Cocos (F_{CL}) and East Agaña (F_{EA}) as well as the maximum yield-per-recruit (F_{max}) for t_c = 1.

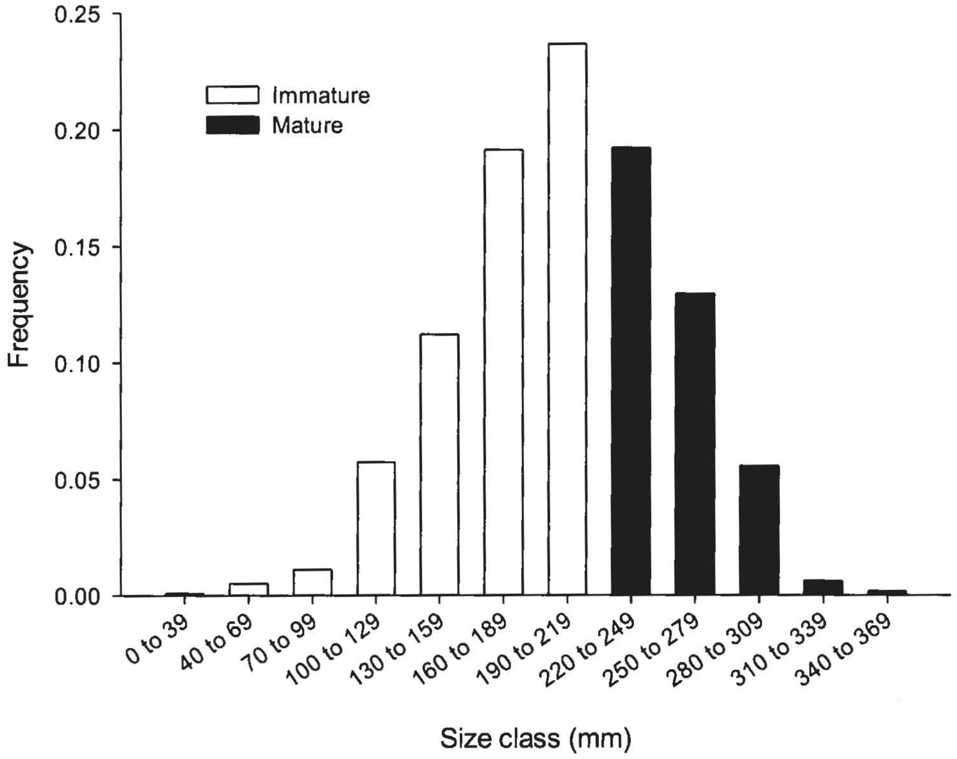

Figure 3.16. Grouped length frequency data of *Lethrinus harak* harvested on Guam from 1984 to 2008 for all gear types. Gray bars represent reproductively immature size classes and black bars represent reproductively mature size classes. Data from creel surveys conducted by Guam Division of Aquatic and Wildlife Resources (DAWR).

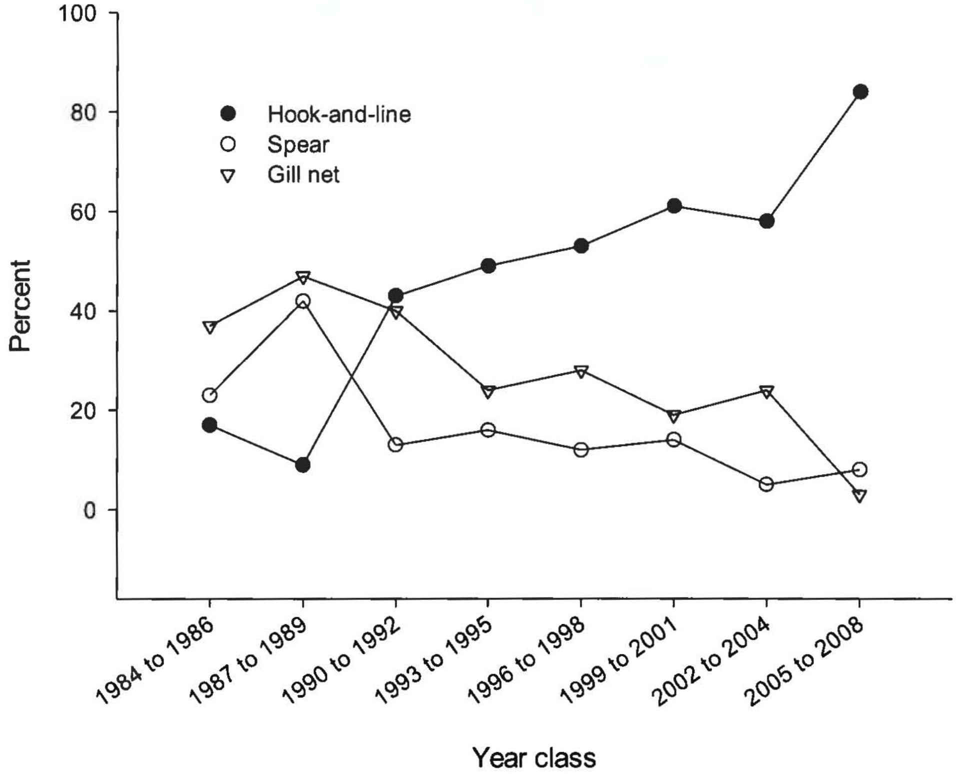

Figure 3.17. Percent of the total estimated *Lethrinus harak* harvest on Guam represented by hook-and-line, snorkel spear, and gill netting techniques from 1984 to 2008. Data from creel surveys conducted by Guam Division of Aquatic and Wildlife Resources (DAWR).

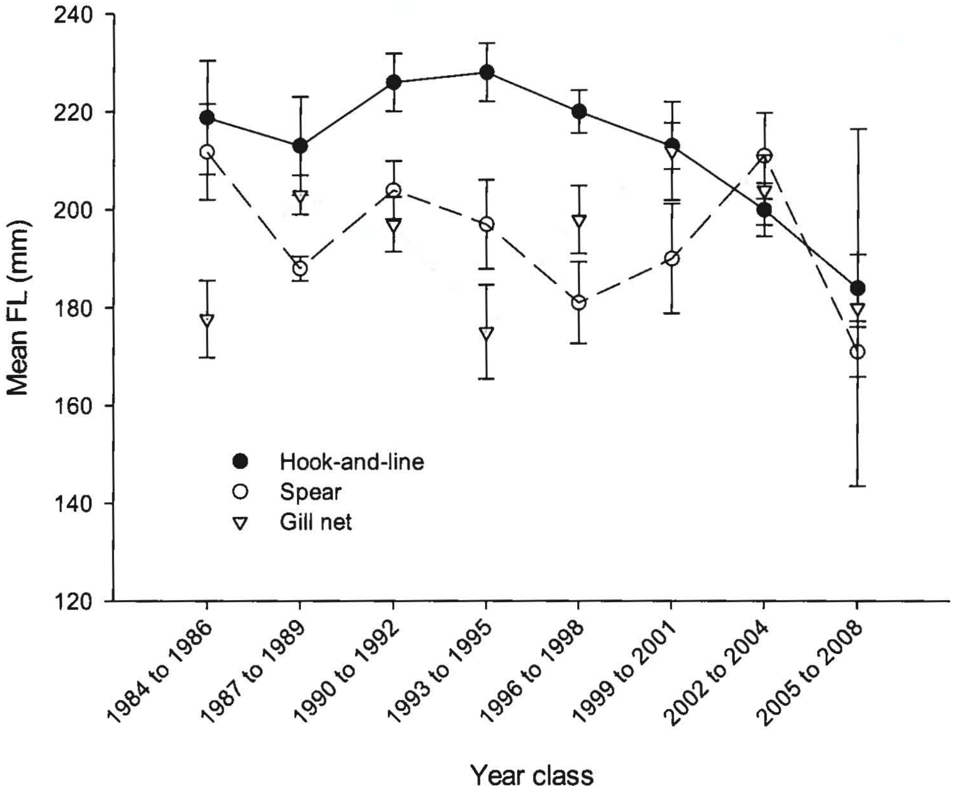

Figure 3.18. Mean size of *Lethrinus harak* harvested on Guam using hook-and-line, snorkel spear, and gill netting techniques from 1984 to 2008. Data from creel surveys conducted by Guam Division of Aquatic and Wildlife Resources (DAWR). Error bars indicate standard error.

3.4 Discussion

The reduction in fishing pressure within Guam's marine preserves has had a major impact on the demography of *L. harak*. Age structures, growth, and mortality rates varied between populations from protected and unprotected sites which, in turn, severely affected the longevities of both sexes within these sites. The significance of the differences between protected and unprotected sites is that fishing pressure affects the population structure of this species in a nonrandom pattern. The resultant skewed population structure could lead to inadequate population processes yielding sperm or egg limitation, recruitment failure, and a collapsed stock (Bannerot et al. 1987).

Age frequency distributions followed a consistent pattern in which non-fished sites had a much larger proportion of older individuals than comparative fished sites. In addition, the similarity in age structures between Piti and Cocos and between Achang and East Agaña suggested that coupling these sites by habitat composition rather than geographic location made for much better comparisons. This increased the likelihood that observed variability was a result of external influences such as fishing pressure or habitat degradation rather than differences in habitat types.

One of the most important results was the differences in proportional composition of older individuals. Whereas Achang had individuals of all age classes up to an estimated 13 years, no individuals in East Agaña were over 6 years old. For Piti, the proportion of 10-13 year old *L. harak* was much greater than that of Cocos. These results highlight two major points: (1) fishing is selecting larger, older fish from unprotected sites while significantly reducing the maximum longevities in some instances and (2) populations within protected areas are much more stable, have higher reproductive potentials, and therefore are probably more resilient to environmental change. The maximum age of 13 years for *L. harak* identified in this study is similar to that of Lassi (2003) from Fiji and Hilomen (1997) from the GBR of 12 and 15 years, respectively. Adams et al. (2000) found similar but less pronounced differences in age structures for *Plectropomus areolatus* at comparative sites open and closed to fishing on the GBR. They found that although mean size and age of males did not significantly differ between sites open and closed to fishing, those of females were consistently greater in protected reefs. In the present study, protected sites had a much greater proportion of older males and females than unprotected sites, indicating that the protected sites have accumulated older fish during their seven year closure. The discrepancy in maximum female ages may also indicate that female fish are changing sex to males earlier when under more intense fishing pressure. This is examined in further detail in Chapter 4.

Growth parameters from the present study differed greatly from previous estimates of *L. harak* growth. From the GBR, Hilomen (1997) estimated K and L_∞ values at 0.313/year and 285 mm FL. However, the age and size at recruitment in Hilomen's study was -2 years and approximately 90 mm, suggesting that not only were ages of young fish underestimated but also that the VBGF was poorly fitted to the length-at-age data. From Fiji, Lassi (2003) estimated K and L_∞ values at 0.9/year and 285 mm TL (~259 mm FL). There is evidence that this latter study also underestimated ages, which would lead to such a high intrinsic growth rate. For instance, individuals that were larger than the mean asymptotic size were encountered at age two. In addition, there was a lack of smaller individuals in the data set (the smallest fish aged was over 200 mm TL).

Growth parameters for *L. harak* on Guam varied between protection status, geographic locations, sexes, and among sites. Sexual dimorphism in body size and growth rates is common in tropical reef fishes. Although the growth patterns for *L. harak* males and females are significantly different, the differences are not pronounced. Mean size-at-age was significantly different for only two age classes (3 and 7 years). In addition, growth patterns may be confounded because the comparisons were made across age classes where *L. harak* changes sex (see Chapter 4). The effect of sexual dimorphism on growth is of increasing concern in fisheries management because an increasing number of protogynous species are being shown to exhibit sex-specific growth patterns (Munday et al. 2004). When this effect is not taken into account, VBGF parameters are inaccurate; which in turn, causes stock assessment models to be misdirected (Bannerot et al. 1987; Appeldoorn 1996).

At different sites, variability in growth can be caused by a number of factors. There have been an increasing number of recent studies investigating regional differences in growth and the causes of such variability (Gust et al. 2002; Kritzer 2002; Choat et al. 2003; Williams et al. 2003). Most of these studies attribute differences in growth patterns to density-dependent processes such as mortality and competition. However, the increased mortality as a result of fishing pressure has rarely been examined as a cause of variability in growth because the majority of these studies have been done in areas with low fishing pressure or on species that are not targeted by fishers. It is for this reason that growth comparisons between protected and unprotected sites is of interest.

Significant differences in growth rates should be interpreted carefully because the VBGF is very sensitive to sample size. For East Agaña and Achang, failure to sample fish in the largest size classes caused low estimates of L_{∞}. In East Agaña, *L. harak* of these size classes are effectively nonexistent (see Chapter 2) and full growth potential is likely never reached within this population. East Agaña's *L. harak* population does, however, exhibit a faster intrinsic growth rate in the early years of life when compared to all other sites. Unfortunately, without an adequate sample size of older individuals, this result can be questioned because of the VBGF's sensitivity to sample size and age distribution. Kritzer et al. (2001) suggests a general rule of obtaining at least 7-10 individuals per age class to effectively estimate growth parameters.

Significant variability in growth is also evident between grouped populations within protected and unprotected sites on Guam. *Lethrinus harak* populations in unprotected sites have a faster growth rate and reach a smaller mean asymptotic size. This trend follows that of other studies where individuals from populations with higher mortality rates grow faster but to smaller maximum sizes (Gust et al. 2002; Kritzer 2002; Choat et al. 2003). Despite finding differences in growth patterns, it remains very difficult to identify the environmental, biological, and physiological processes that underlie variation in growth. What is shown here, however, is that marked variability in growth parameters occurs over very small spatial scales (<1 km). The significance of this is that coral reef fisheries managers must understand that such variability can occur, and that it would be prudent to consider this when managing resources based on limited biological data.

The integration of fishery-independent catch-at-age data with intensive optimally-stratified visual surveys where fish of all size classes were recorded allowed for age-based catch curves which provided very accurate estimates of total mortality at each site. In addition, the ecology of *L. harak* makes it ideal for such a technique because adults, subadults, and juveniles all co-occur in similar habitats, individuals are easily visible (non-cryptic), and the species is highly habitat dependent. These characteristics should add confidence to estimates wherever accurate benthic data is available. The high rates of total mortality estimated for unprotected sites indicate a rapid exponential decay of individuals with age as a result of fishing intensity. If total mortality rates in protected sites represent the natural mortality in comparative fished sites, then Cocos and East Agaña have fishing mortality (F) estimates of 0.212 and 0.500 year^{-1}, respectively (Table 3.5). These very large differences in total mortality rates indicate that there is potentially little exchange of individuals between protected and fished sites, because exchange would cause age-based catch curves to be more similar.

The general equations of Pauly (1980) and Hoenig (1983) typically produced higher estimates of M than the age-based catch curves. Pauly's equation is sensitive to changes of parameters within the VBGF (Pauly 1980). Therefore, an adequate sample size is critical to producing precise and accurate results (Kritzer et al. 2001). Hoenig's equation appears to have produced more accurate estimates of M for *L. harak* on Guam, but this equation is based entirely on the maximum age encountered and is probably not suitable to use in areas of intense fishing pressure where the maximum longevities are reduced by fishing activity.

The inclusion of minimum size limits to the current management scenario for *L. harak* is highly recommended. Output controls such as minimum size limits can be used to control fishing effort and to help maintain a healthy spawning stock biomass (Jennings et al. 2001). Roberts et al. (2005) encourage the combination of protected areas with conventional fisheries management tools and propose that they complement each other and allow for more stable population structures which sustain higher rates of reproduction. Currently, catch data strongly suggests that growth overfishing is occurring as a result of the overwhelming harvest of juvenile fish as well as via unsustainable netting techniques. Size limits would not be an applicable tool if the majority of juvenile fish were harvested using unselective techniques (i.e., netting), however, evidence suggests this is not the case for *L. harak* on Guam. In recent years (2005-2008), hook-and-line and snorkel spear methods comprised 92% of the *L. harak* catch, but an estimated 84% of the catch was juveniles, indicating that fisherman are selectively harvesting immature fish. Between Cocos and East Agaña, trends in catch data suggest that a greater use of unselective netting techniques increases fishing mortality and has a detrimental effect on population structure and sustainable harvest. Subsequently, this practice yields growth overfishing.

Of equal importance as fishery yield is the ability for a stock to maintain reproductive success. The successive elimination of older, more fecund fish in a population via intense fishing activity has been termed "juvenescence," where reduction in the mean fish size over time concurrently reduces the mean age of the population (Ricker 1963). This effect has been well-documented for several coral reef fish stocks of South Florida. Fishing pressure has reduced the spawning potential ratio (SPR) to below United States federal "overfishing" standards for the majority of targeted species (Ault et al. 1998). Results from the present study reveal the spawning potential of *L. harak* on Guam is severely reduced in sites open to fishing. This problem could be addressed by increasing the age at first capture (t_c) to above age at 50% maturity (t_{50}) which would not only increase yield-per-recruit but also ensure greater reproductive output across the island.

The very large differences in *L. harak* population structure and mortality between protected and fished sites on Guam indicate that Guam's marine preserve network is effective in building up reef fish populations within their boundaries and could, in turn, be supplying the local fishery with increased egg production or increased yield via spillover. However, the differences documented in this study also paint a bleak picture for Guam's fisheries; highlighting the intense levels of fishing that have decreased fish populations in certain areas to the point that reproductively mature individuals are rare. In order to turn this situation around, it is recommended that in addition to maintaining the current marine preserves, other management options are adopted in the form of output controls which are designed to significantly reduce fishing mortality.

Chapter 4 Reproductive biology of *Lethrinus harak* on Guam

4.1 Introduction

Reproductive patterns in coral reef fish have increasingly been shown to be complex as over 50% of exploited species are now recognized as hermaphroditic (Sadovy 1996; Sadovy & Domeier 2005). Fish that undergo ontogenetic sex change can exhibit protandry (functional males switch to functional females), protogyny (functional females switch to functional males), or simultaneous hermaphroditism (reproductive material of both sexes exists simultaneously) (Atz 1964). For coral reef fish, protogyny has been established as the dominant sexual pattern in families like the Serranidae, Pomacanthidae, Lethrinidae, Labridae, and Scaridae, among others (Sadovy de Mitcheson & Liu 2008). Sex ratios for protogynous species typically change from female-dominated to male-dominated as size increases. However, many factors can alter sex ratios (e.g., size dimorphism, differential growth rates, differential mortality, and spatial segregation by sex), so they should only be used as a guide and not a prognosis of sexual pattern (Kawaguchi & Marumo 1967; Warner 1975b; Sadovy & Shapiro 1987). Instead, histological examination of gonads is required to identify key criteria which indicate protogynous hermaphroditism. Sadovy & Shapiro (1987) outline these criteria as follows: 1) membrane-lined central cavities in testes; 2) presence of transitional individuals; 3) atretic bodies of yolked oocytes within testes; and 4) sperm sinuses in the gonadal wall. These features are highly indicative of protogyny and can only be determined microscopically.

Protogynous species can be monandric (exhibiting one developmental pathway; i.e., all males derived from females) or diandric (two male pathways; i.e., primary males develop from an immature stage and secondary males develop from mature females) (Reinboth 1967). Many studies have investigated developmental pathways in detail, though pathways appear to be diverse within and among families (Liu & Sadovy 2004b). For example, in some labrids and scarids, no clear differences exist in the testicular morphology between primary and secondary males (Smith 1965; Sadovy & Colin 1995). In the genus *Cephalopholis*, spermatogenic tissue is distributed throughout juvenile gonads as well as in all functional female stages (Smith 1959, 1965; Siau 1994; Chan & Sadovy 2002). As a result, Liu & Sadovy (2004b) highlight the importance of sampling all possible size classes to understand male developmental pathways, as sexual pattern can influence the way a species or population responds to fishing pressure.

Fishing alters the size and age structure of a population non-randomly as larger fish are more vulnerable to fishing gear and therefore more heavily targeted by fisherman (Munro 1996). For protogynids, this non-random selectivity leads to reduction in males (the largest/oldest individuals), which often constitute a smaller proportion of the total population (Bannerot et al. 1987; Jennings & Lock 1996; Sadovy 1996). This becomes problematic in fisheries biology because a significant loss of males within the population can lead to sperm limitation, greatly reducing the reproductive capacity of the population which can ultimately lead to recruitment failure (Coleman et al. 1996). Most current measures of spawning potential consider reproductive capacity to be highly correlated with spawner biomass (the total biomass of sexually mature fish; Coleman et al. 2000). While this relationship is accurate for gonochorists, Coleman et al. (2000) proposed that a 90% reduction in the number of male fish (potentially constituting only a small proportion of a protogynous population) would result in a 90% reduction in the reproductive capacity of the population. In addition, because male and female reproductive capacity increases exponentially with fish size (Berkeley et al. 2004a, 2004b; Pears et al. 2006), the magnitude of reproductive decline would be even greater as the largest males and females within a population are removed via selective fishing practices.

Exploitation has also been found to alter the size or age at sex reversal for reef fish. Mechanisms causing sexual transition in reef fish can be either endogenous (strict internal schedule such as absolute size or age) or exogenous (social characteristics such as sex ratio or relative size of other fish) (Thompson & Munro 1983; Shapiro 1989; Ross 1990; Buxton 1993). The latter often function as compensatory mechanisms for heavy fishing pressure by accelerating processes in order to maintain population sex ratios. Such mechanisms include accelerated maturation, accelerated sex reversal, accelerated growth, and combinations thereof (Huntsman & Schaaf 1994; Huntsman et al. 1999). Accelerated maturation has been demonstrated for the gag *Mycteroperca microlepis* where females matured at a smaller size during 1994-1995 than during 1976-1982 (McGovern et al. 1998). Accelerated sex reversal has been demonstrated for many species by experimentally manipulating social structures (Ross 1981; Shapiro 1981; Nemtzov 1985; Liu & Sadovy 2004a). When sex change patterns and compensatory mechanisms go unaccounted for, the applicability of stock assessment models becomes problematic because the assumption of constant recruitment may fall short for protogynids (Buxton 1992; Sadovy & Figuerola 1992). This occurs because the reduction in reproductive potential from fishing is amplified for protogynids by the specific targeting of one sex (males). In turn, this further complicates the management of protogynous reef fish populations.

In the genus *Lethrinus*, gonochorism has been suggested for *L. atkinsoni*, *L. nebulosus*, and *L. obsoletus* (Ebisawa 1990, 1999, 2006). However, protogyny appears to be the dominant sexual pattern having been confirmed for six species (*L. atkinsoni*, *L. genivittatus*, *L. lentjan*, *L. miniatus*, *L. rubrioperculatus*, and *L. variegatus*) and suggested for many others, including *L. harak* (Young & Martin 1982; Ebisawa 1997, 2006; Bean et al. 2003; Sumpton & Brown 2004; Sadovy de Mitcheson & Liu 2008). The reproductive biology of *L. harak* has been studied at least four separate times prior to this study albeit from different locations in the Indo-Pacific. Hilomen (1997) and Lassi (2003) studied the size and age at sexual maturity of *L. harak* from the GBR and Fiji, respectively, and both suggested protogyny while Kulmiye et al. (2002) suggested protandry based on size-frequency distributions of fish from Kenya. All three studies used histology although none attempted to define sexual pattern based on key criteria. A more comprehensive investigation of *L. harak* reproductive biology from Japan based on histological evidence suggested protogyny (Ebisawa 2006).

In this chapter, I investigate the reproductive biology of *L. harak* on Guam with respect to size and age at maturation and sex reversal, spawning periodicity, and sexual pattern. These parameters are compared for populations from protected and unprotected sites to determine whether fishing has affected reproductive biology. Such information has important management implications for ensuring reproductively viable populations.

4.2 Methods

4.2.1 Processing of gonads

Specimens of *L. harak* were sampled from various sites around Guam from June 2007 to June 2008 following the same protocol as in Chapter 3. Whole gonads removed from each individual were blotted dry, weighed to the nearest 0.001 g, sexed and staged macroscopically, and immediately preserved in FAACC (formaldehyde 4%, acetic acid 5%, calcium chloride 1.3%; Samoilys & Roelefs 2000) for histological processing. Sections of gonads embedded with paraffin wax were mounted on glass slides and stained with Haematoxylin and Eosin. These sections were viewed under a high powered microscope (40x) to confirm previously assigned macroscopic sexes and stages, and to determine reproductive ontogeny. Individual female oocytes were classified into five stages following West (1990): chromatin nucleolar (CN), perinucleolar (PN), cortical alveoli (CA), vitellogenic (V), and ripe (R) (Figure 4.1). To explore the relationship between female body size, age, and reproductive contribution, ovary weight was used as a proxy for reproductive capacity and overlaid on the VBGF for immature and active females (excluding resting and spent individuals) following Pears et al. (2006). Relative reproductive contribution (reproductive output by body size) was examined by plotting the mean cumulative gonadosomatic index values by size and age class. In addition, the age and size at first capture (t_c and L_c, respectively) were compared with the age and size at 50% maturity (t_{50} and L_{50}) and the optimal age and size at first capture (t_{opt} and L_{opt}) which are based on the yield-per-recruit model from Chapter 3.

4.2.2 *Maturation and sex reversal*

Age and size of female maturation and age and size at sex reversal were explored by plotting the proportion of mature individuals or males over the various size or age classes, respectively. A sigmoidal curve was fitted to the data by minimizing the sums of squares using the variables a, b, and x_o, where

$$y = \frac{a}{1 + e^{-((x-x_o)/b)}}.$$

Ages and sizes of maturation and sex reversal were compared graphically between populations within and outside of marine preserves. Sex ratios by age were compared between these populations using a replicated G test of goodness-of-fit (Sokal & Rohlf 1995).

4.2.3 *Reproductive seasonality*

Seasonality in the reproductive biology of *L. harak* was investigated using monthly gonadosomatic indices (GSI). GSI plots represent the proportional relationship between gonad weight and body weight and follow the assumption that gonad weight increases during times of spawning as a result of the swelling and ripening of oocytes. The frequency and occurrence of transitional individuals, spent individuals, and resting individuals were related to the GSI and to the mean monthly sea surface temperatures (SST) during the months fish were sampled. Transitional individuals contain proliferating reproductive male tissue (spermatogonia) and degenerative female tissue (vitellogenic oocytes). Sexual transition in some families of tropical reef fishes like the Serranidae, Sparidae, and Labridae occurs rapidly and directly after spawning (Reinboth 1962; Chan & Phillips 1967; Bruslé & Bruslé 1975; Fishelson 1975; Warner 1975a; Dipper & Pullin 1979; Jones 1980). Hence, periodicity in sexual transition was considered as offering potential insight into spawning seasonality.

4.2.4 Reproductive development

Histology sections of *L. harak* gonads over a wide range of body sizes and ages were examined to confirm whether or not *L. harak* is a functional hermaphrodite. Evidence indicative of protogyny, protandry, and sequential hermaphroditism as outlined in Sadovy & Shapiro (1987), were used to infer the sexual ontogeny of this species.

4.3 Results

The gonads of 414 *L. harak* individuals were examined, ranging in size from 83 to 327 mm FL. The overall sex ratio was M:F = 1: 3.80, though this varied considerably with size and age (Tables 4.1 and 4.2). Females were the dominant sex in the smaller size and younger age classes whereas the bigger and older fish were predominantly males. Interestingly, the three largest fish were all female (299, 303, and 327 mm FL). High variability in sex ratios among monthly samples may be an artifact of sampling, particularly for those months when the sample size was small (Table 4.3).

4.3.1 Ovarian stages

The ovarian development and maturation of female *L. harak* was classified into six stages: immature; maturing; mature, resting; mature active; ripe; and spent (Table 4.4). An additional stage, 'running ripe,' is often included in studies of reef fish reproductive biology (West 1990), however, no individuals matching this criteria were encountered. It is highly likely, however, that *L. harak* undergoes a running ripe condition just prior to spawning. Of 327 females examined, 64% were classified as immature, 3.7% maturing, 5% resting mature, 17% mature active, 9.5% ripe, and 1% spent. Immature individuals comprised the majority of the female catch for 10 of the 13 months (Figure 4.2). Maturing females were rare and were found within the 185 to 235 mm size range (Figure 4.3). Macroscopic staging and sex determination proved to be variable with female staging agreeing only 76% of the time and sex determination agreeing 92% of the time with histological techniques. Therefore, sexes and stages determined through histology were used in all plots and analyses.

There was a strong linear relationship between female gonad weight and fork length when gonad weight was log-transformed (Figure 4.4a). Similarly, there was a strong logarithmic relationship between female gonad weight and age when gonad weight was log-transformed (Figure 4.4b). Plotting ovary weight as a proxy for female reproductive capacity with the VBGF demonstrated the increase in reproductive potential for females with greater size and age (Figure 4.5). The largest female sampled had a whole gonad weight of over 45 grams and a GSI value which was 4.6 times greater than the mean GSI for all other active females. There was a strong relationship between cumulative GSI value and size and age (Figure 4.6). It is evident from Figure 4.6 that *L. harak* is being harvested at a size and age well below those of reproductive maturity and optimal yield.

4.3.2 Size and age at female maturation and sex reversal

Female *L. harak* mature over a relatively narrow size range, where the length at 50% maturity (L_{50}) was 208 mm FL (Figure 4.7a). The age at 50% maturity (t_{50}) was 3.8 years (Figure 4.7b). When comparisons were made between populations within and outside of marine preserves on Guam, size and age at maturity differed only slightly, and may be explained by variability in sample size (Figure 4.8). The length and age at 50% sex reversal was 241 mm FL and 5.38 years, respectively (Figure 4.9). The length at sex reversal differed only slightly between protected and unprotected populations (Figure 4.10a), but the difference for age at sex reversal was more pronounced. Male-female sex ratios were 1:1 at an estimated 5.2 years of age outside of marine preserves compared to an estimated 6.1 years within (Figure 4.10b). A replicated G test of goodness-of-fit determined that sex ratios by age were not significantly different between protection status as sample sizes for older ages were inadequate to detect differences ($G=4.963$, $df=7$, $p=0.664$).

4.3.3 Seasonal variability

Unfortunately, a low monthly sample size of mature females made it difficult to discern seasonal patterns from the GSI plot. The minimum sample size for mature females for this study was set at 15 per month. This was only achieved for two of the thirteen months

(Figure 4.11). However, the monthly GSI plot does suggest the period of highest reproductive activity is between October and January (Figure 4.11). There is some correspondence between the monthly GSI values and the mean monthly sea surface temperature (SST) on Guam, indicating that the spawning season begins as the SST decreases from the annual peak of 30.5°C (Figure 4.12). In addition, resting individuals were encountered during months of low GSI values. The most convincing evidence of spawning periodicity is that transitional individuals were only encountered in seven months of the year, six of which were consecutive. One transitional individual was encountered in February and nine others were encountered from May to October. Except for the occurrence in February, the presence of transitional fish corresponded with the yearly increase in mean SST (Figure 4.13). If sex reversal occurs after spawning periods for *L. harak*, this may be evidence for a defined spawning season on Guam.

4.3.4 Developmental ontogeny

A total of ten transitional *L. harak* individuals were identified from the 414 gonads examined microscopically. Transitional individuals contained degenerative atretic oocytes in the presence of mature or maturing male reproductive material (Figure 4.14). All individuals were above the size and age at female maturation. Other features that were common in male *L. harak* gonads that are indicative of protogynous hermaphroditism included peripheral dorsal sperm sinuses and a remnant ovarian lumen (Figure 4.15). The presence of sperm crypts among degenerative female tissue suggests that *L. harak* male and female tissues are not delimited by connective tissue during sex reversal which is common in both serranids and scarids (Smith 1965; Choat and Robertson 1975; Figure 4.16).

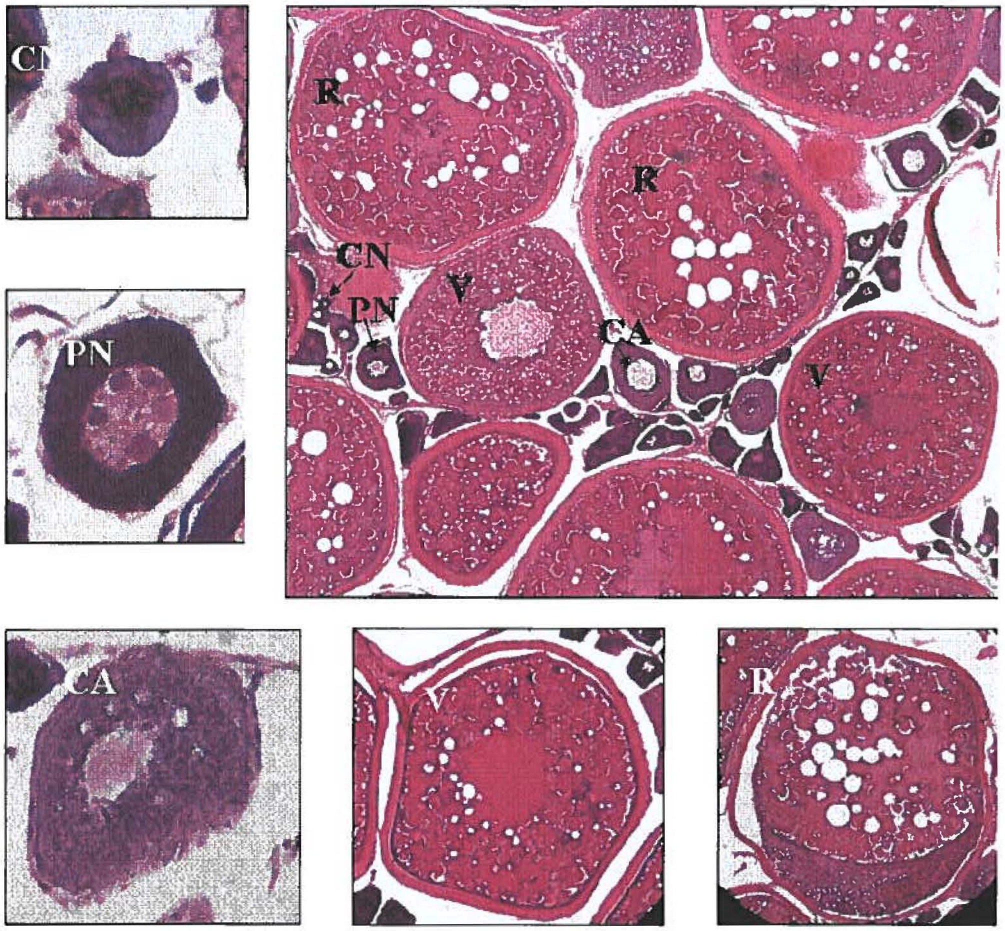

Figure 4.1. Stages of oogenesis in a ripe female *Lethrinus harak* following criteria outlined in West (1990). CN= chromatin nucleolar, PN= perinucleolar, CA= cortical alveoli, V= vitellogenic, and R= ripe.

Table 4.1. Sex ratios and total sample sizes of *Lethrinus harak* by size class.

Size class FL (mm)	*Total*	*Unsexed*	*Males*	*Females*	*% males*	*Sex ratio M:F*
80-99	10	0	0	10	0.0	
100-119	12	0	0	12	0.0	
120-139	28	0	0	28	0.0	
140-159	33	0	0	33	0.0	
160-179	43	0	1	42	2.3	1 : 42.0
180-199	68	0	2	66	2.9	1 : 33.0
200-219	85	0	15	70	17.6	1 : 4.67
220-239	70	0	24	46	34.3	1 : 1.92
240-259	40	1	24	16	61.5	1 : 0.63
260-279	16	0	15	1	93.8	1 : 0.07
280-299	7	0	5	2	71.4	1 : 0.40
300-319	1	0	0	1	0.0	
320-339	1	0	0	1	0.0	
Total	414	1	86	328	20.8	1 : 3.80

Table 4.2. Sex ratios and total sample sizes of *Lethrinus harak* by age class.

Age class (years)	*Total*	*Unsexed*	*Males*	*Females*	*% males*	*Sex ratio M:F*
1	28	0	0	28	0.0	1 :
2	77	0	0	77	0.0	1 :
3	99	0	5	94	5.1	1 : 18.8
4	80	0	17	63	21.3	1 : 3.71
5	40	1	17	23	43.6	1 : 1.29
6	19	0	12	7	63.2	1 : 0.58
7	26	0	16	10	61.5	1 : 0.63
8	12	0	7	5	58.3	1 : 0.71
9	7	0	5	2	71.4	1 : 0.40
10	3	0	2	1	66.7	1 : 0.50
11	4	0	2	2	50.0	1 : 1.00
12	2	0	0	2	0.0	1 :
13	1	0	0	1	0.0	1 :
Total	398	1	83	315	20.9	1 : 3.78

Table 4.3. Sex ratios and total sample sizes from monthly samples of *Lethrinus harak* from Guam.

Month	***Total***	***Unsexed***	***Males***	***Females***	***% males***	***Sex ratio M:F***
June 07	30	0	4	26	13.3	1 : 6.50
July	20	0	9	11	45.0	1 : 1.22
Aug	24	0	7	17	29.2	1 : 2.43
Sept	33	0	7	26	21.2	1 : 3.71
Oct	33	0	5	28	15.2	1 : 5.60
Nov	17	0	3	14	17.6	1 : 4.67
Dec	36	0	2	34	5.6	1 : 17.0
Jan 08	17	0	2	15	11.8	1 : 7.50
Feb	20	0	4	16	20.0	1 : 4.00
Mar	13	0	5	8	38.5	1 : 1.60
Apr	96	0	17	79	17.7	1 : 4.65
May	35	1	10	24	29.4	1 : 2.40
June	31	0	10	21	32.3	1 : 2.10
Total	405	1	85	319	21.0	1 : 3.76

Table 4.4. Descriptions of stages of ovarian development in *Lethrinus harak*.

Lethrinus harak	Ovary	
	Macroscopic	Microscopic
Stage 1 - Immature	Gonad lobes thin and cylindrical, transparent and often with a pinkish tint. Lobes typically ≤ 2cm in length. Oocytes and ovigerous folds not discernible.	Densely packed primary oocytes in the chromatin nucleolar and perinucleolar stages. Thin gonad wall and tight ovigerous folds.
Stage 2a - Maturing	Similar to stage 1 but more dense and usually with blood appearing. Slightly orange or yellow in color.	Ovaries tightly packed and dominated by perinucleolar stage oocytes that have multiple nucleoli easily visible at the periphery of the nucleus. Many cortical alveoli stage oocytes present which are ≥4 times larger than other previtellogenic oocytes. Thin gonad wall.
Stage 2b - Mature, resting	Long and slightly flaccid with blood vessels fading. Orange-brown in color and translucent towards edges.	Ovary dominated by previtellogenic primary oocytes (chromatin nucleolar and perinucleolar) but also with a large proportion of cortical alveoli stage oocytes. Gonad wall very thick and presence of brown bodies and atretic oocytes common.
Stage 3 - Mature active	Gonad lobes thick and peach in color. Blood vessels usually prominent. Oocytes and ovigerous folds visible through gonad wall.	Ovary dominated by vitellogenic yolk stage oocytes but with previtellogenic oocytes present in various proportions. Ovigerous lamellae disappearing or gone.
Stage 4 - Ripe	Gonad lobes thicker and more tightly packed than stage 3. Color can vary from brown-orange to light peach. Blood vessels often depressed but not always. Large oocytes visible through gonad wall but ovigerous folds not discernible.	Vast majority of oocytes in vitellogenic or ripe stages and densely packed. Gonad wall relatively thin and ovigerous lamellae not discernible. Large yolk proteins apparent in oocytes and nucleus often broken down.
Stage 5 - Spent	Gonad lobes completely flaccid and transparent. Often brown-orange in color. Blood vessels slightly visible.	Thick gonad wall that lacks definitive shape. Numerous previtellogenic stage oocytes scattered throughout and an abundance of brown bodies.

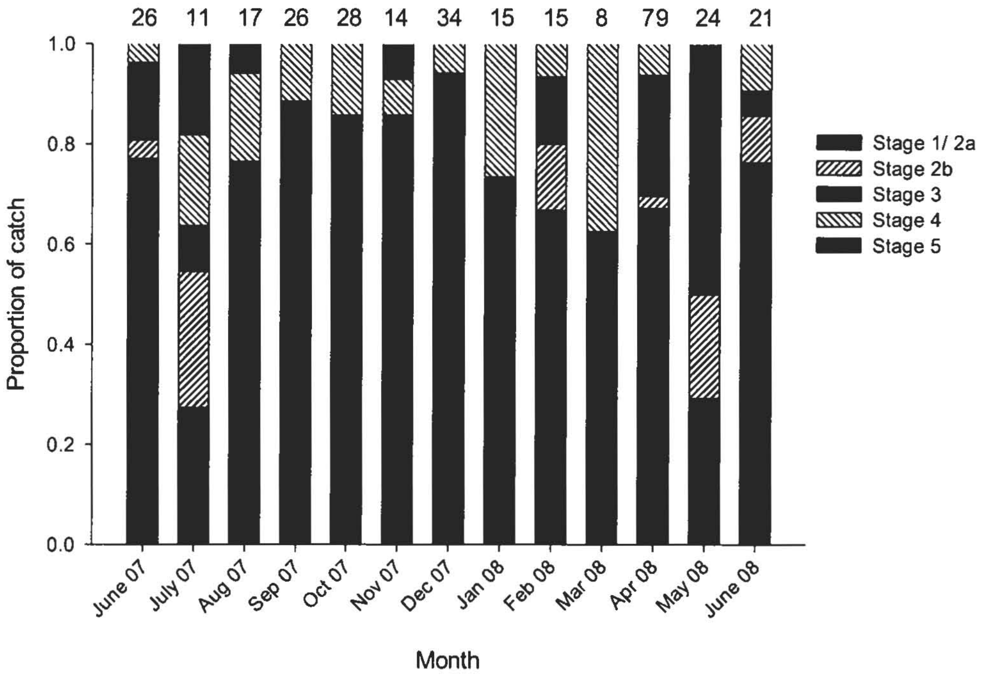

Figure 4.2. Proportions of ovarian stages of females in monthly samples of *Lethrinus harak* from Guam. Numbers over bars refer to numbers of samples collected in that month.

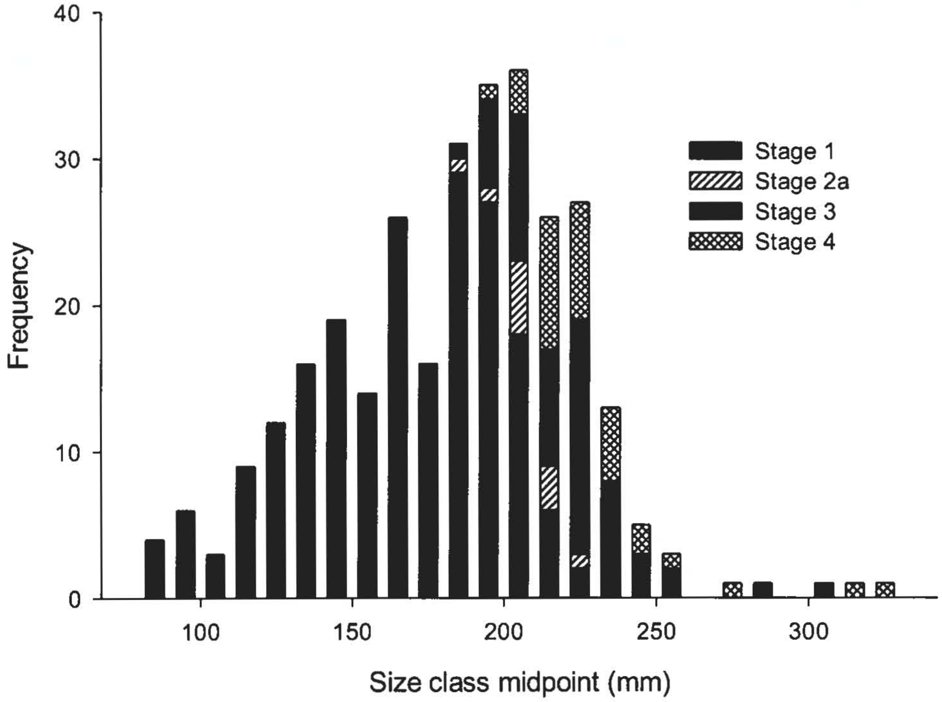

Figure 4.3. Size frequency distribution of female *Lethrinus harak* collected from June 2007 to June 2008 with proportions of ovarian stages by size class.

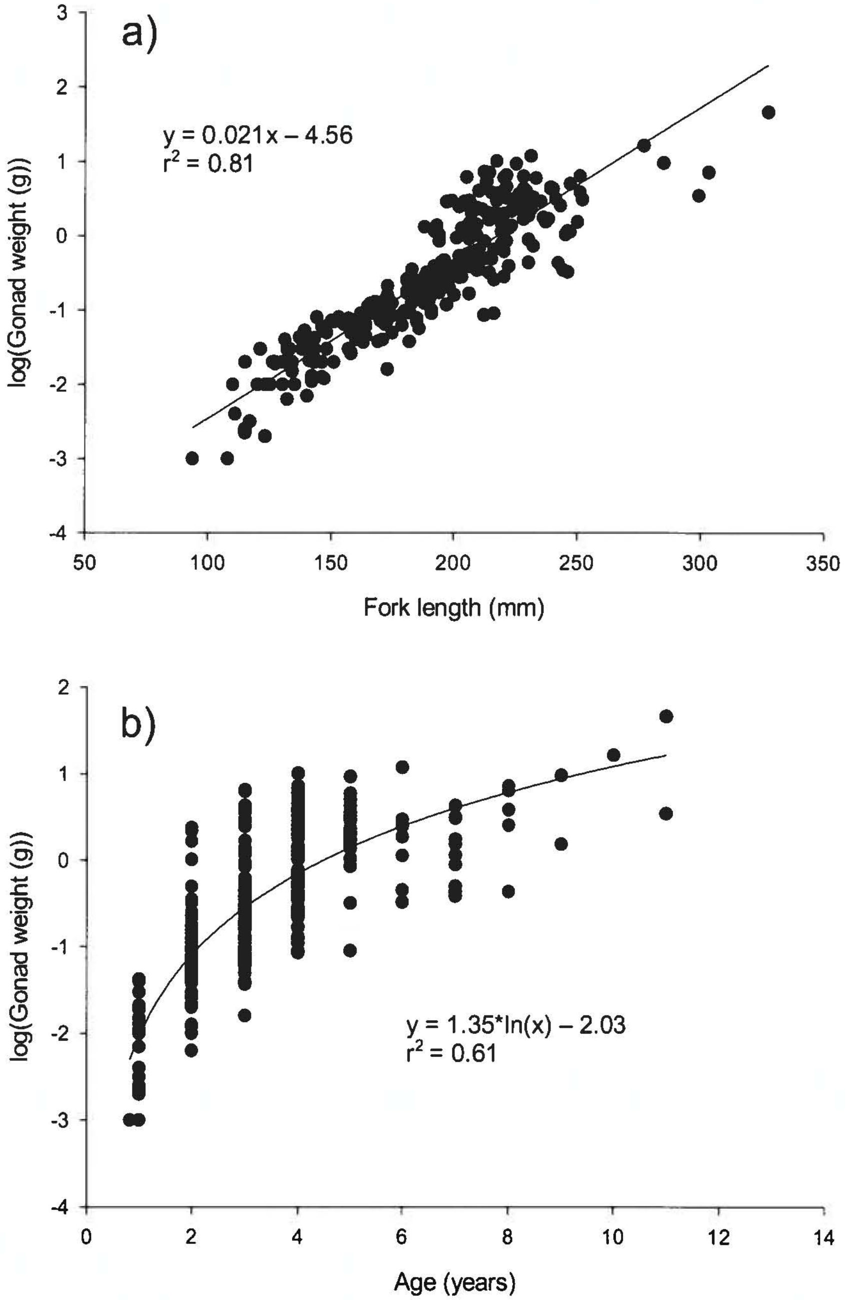

Figure 4.4. Relationship between a) fork length and gonad weight and b) age and gonad weight for reproductively mature *Lethrinus harak* females on Guam.

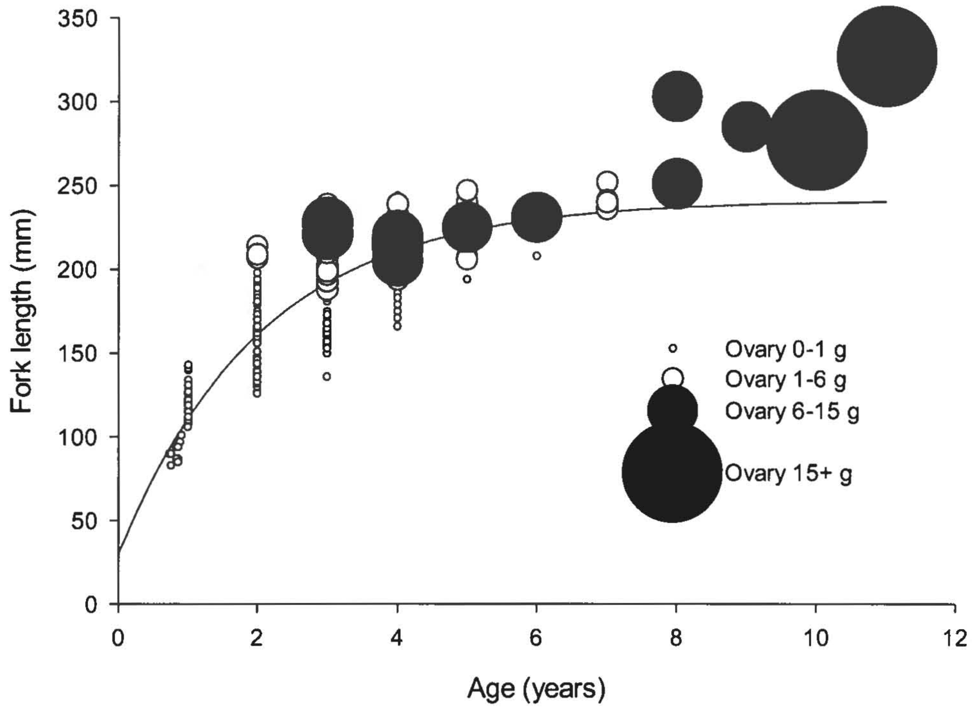

Figure 4.5. Ovary weight plotted over length-at-age data and VBGF for immature and active female *Lethrinus harak*.

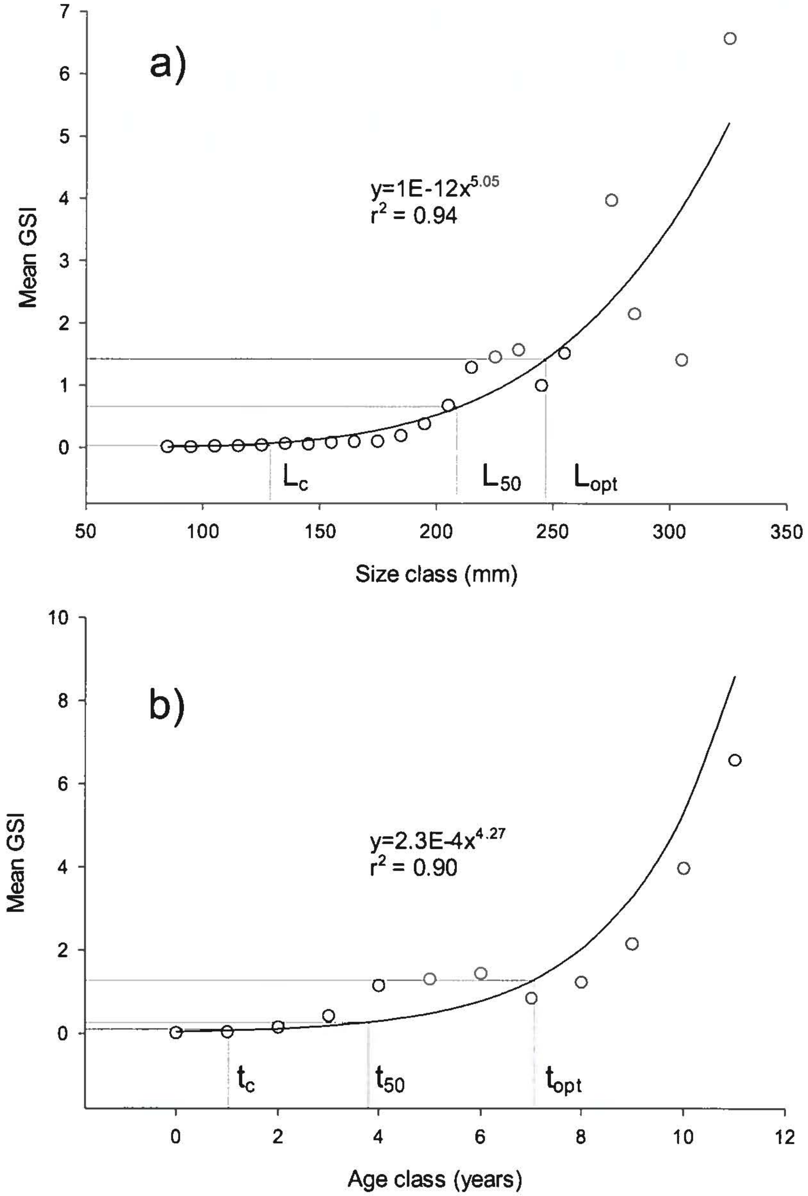

Figure 4.6. Mean cumulative GSI by a) size and b) age class for female *Lethrinus harak*. L_c=current length at first capture, L_{50}=length at 50% maturity, L_{opt}=optimal length at first capture based on yield-per-recruit model, t_c= current age at first capture, t_{50}=age at 50% maturity, t_{opt}=optimal age at first capture based on yield-per-recruit model.

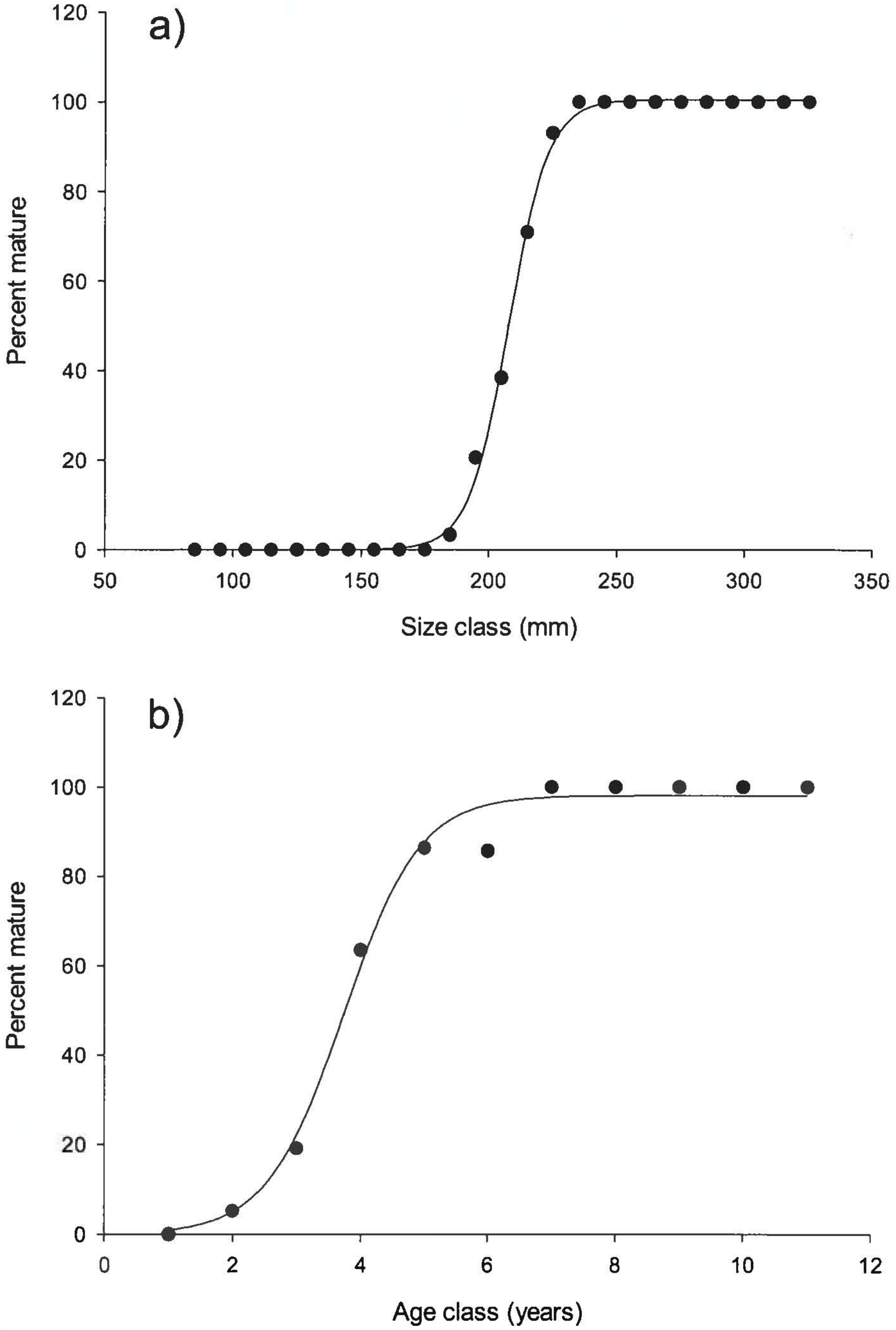

Figure 4.7. a) Length and b) age at female maturation represented by the proportion of mature individuals by size and age class.

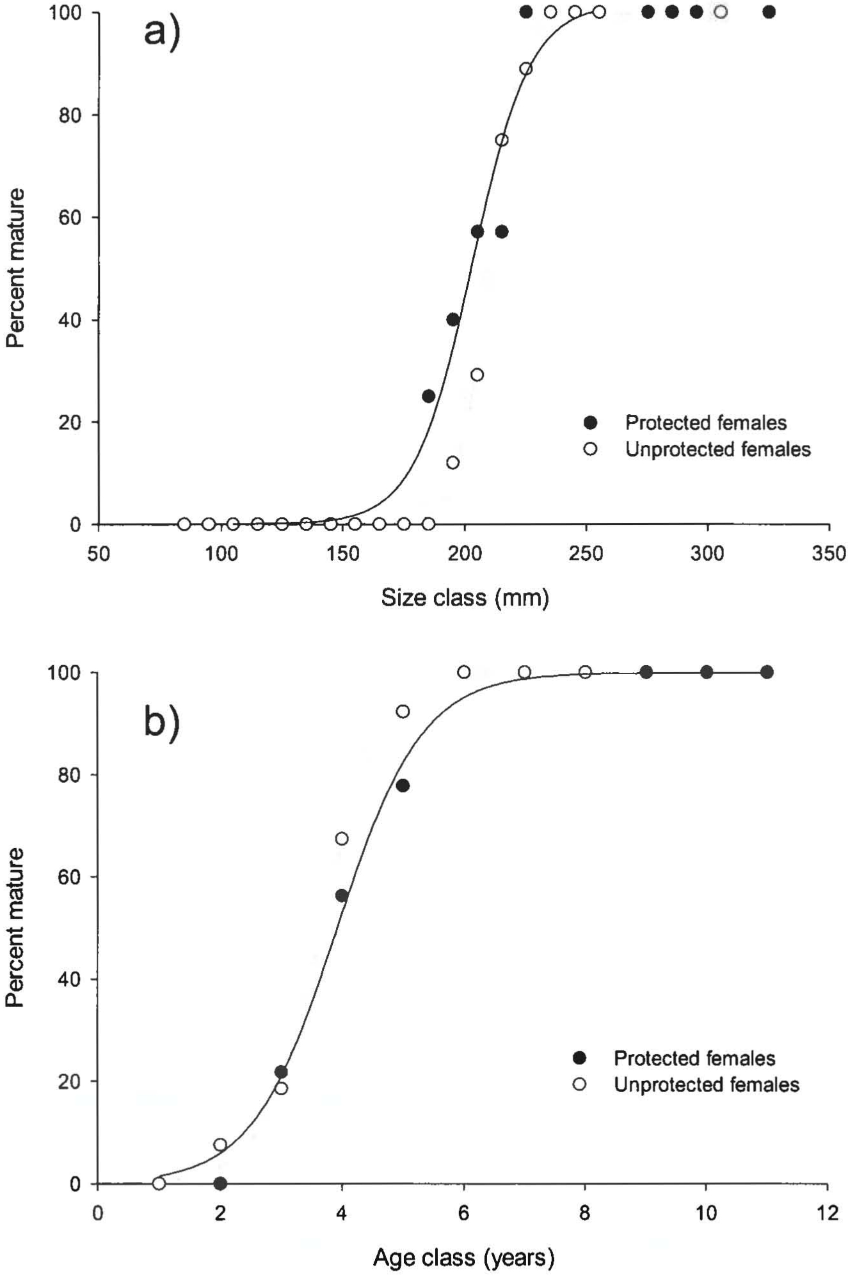

Figure 4.8. Comparisons of a) size and b) age of female maturation for *Lethrinus harak* between populations within and outside of marine preserves.

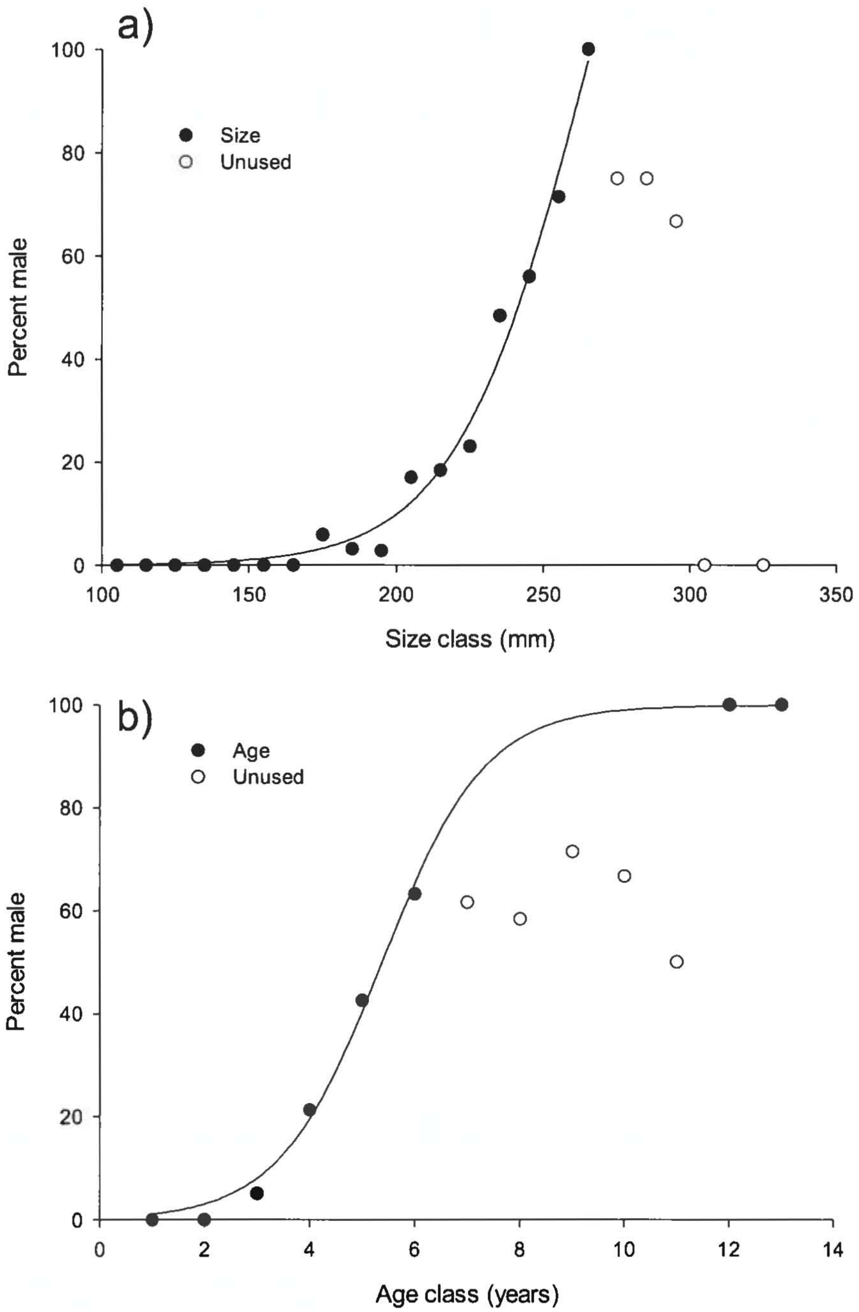

Figure 4.9. a) Length and b) age at sex reversal for *Lethrinus harak* represented by the proportion of males by size and age classes.

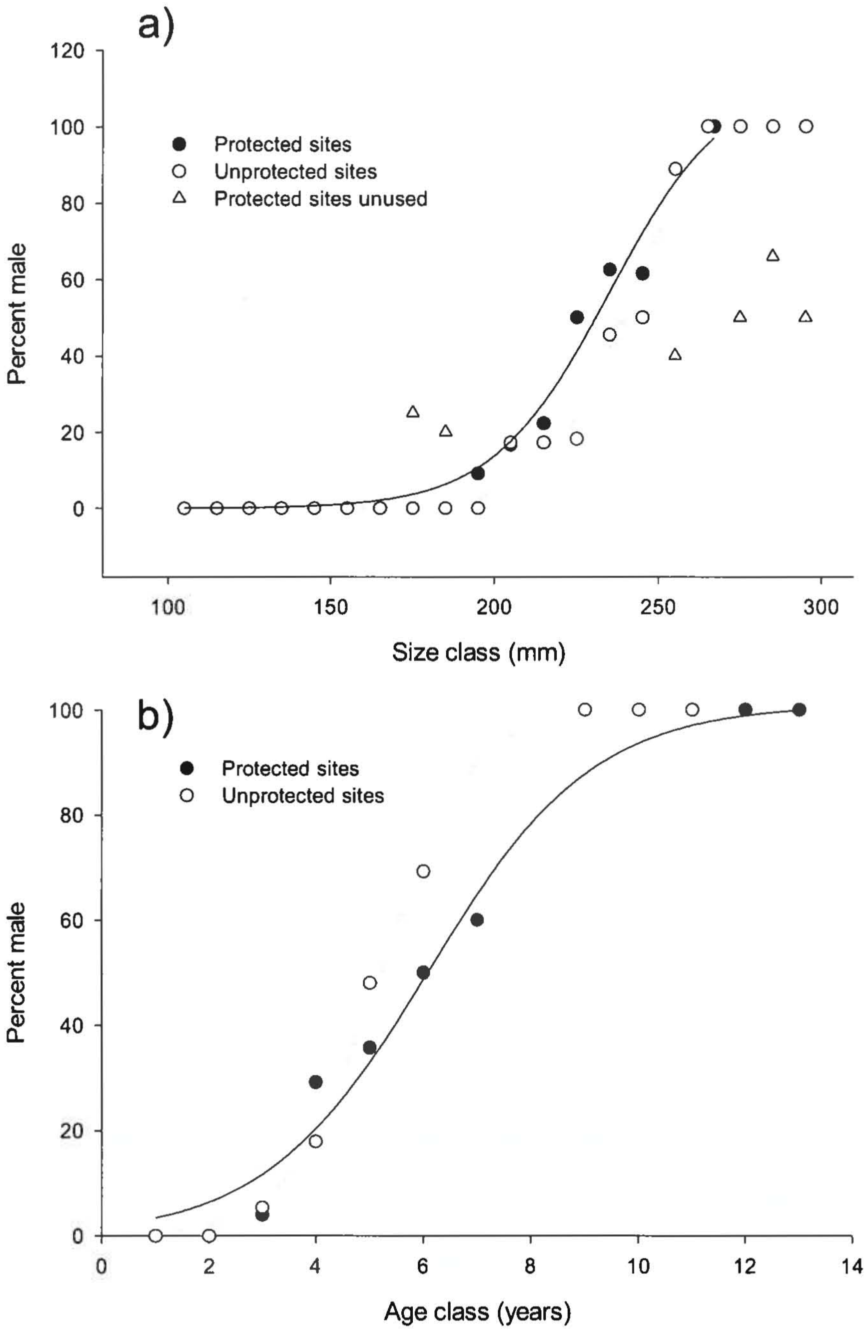

Figure 4.10. Comparisons of a) size and b) age of sex reversal for *Lethrinus harak* between populations within and outside of marine preserves.

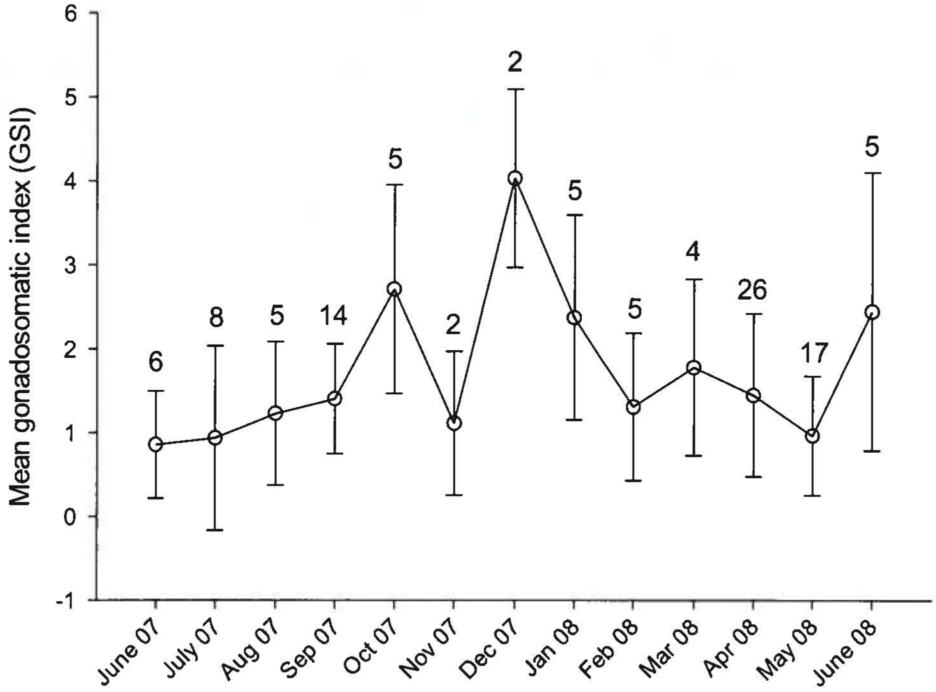

Figure 4.11. Mean monthly gonadosomatic indices for mature female *Lethrinus harak* from Guam. Error bars represent the standard error about the mean and numbers indicate sample size.

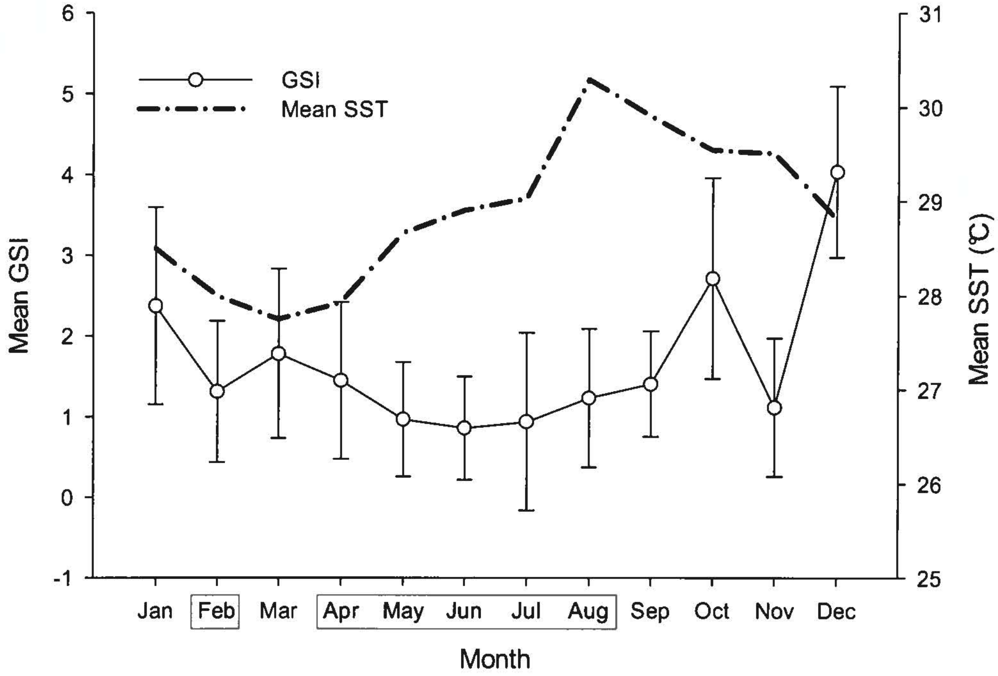

Figure 4.12. Plot of monthly gonadosomatic index values (+SE) for *Lethrinus harak* and mean sea surface temperatures over the period when sampling occurred. Grey boxes surround months in which resting mature females were encountered.

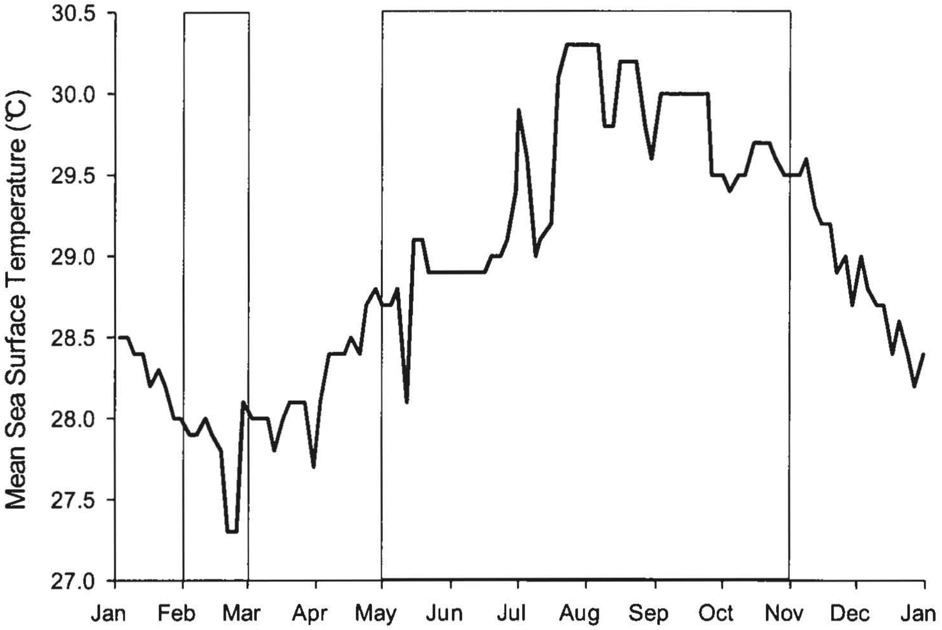

Figure 4.13. Mean sea surface temperature on Guam taken every three to four days and months in which transitional *Lethrinus harak* individuals were encountered (indicated by grey area).

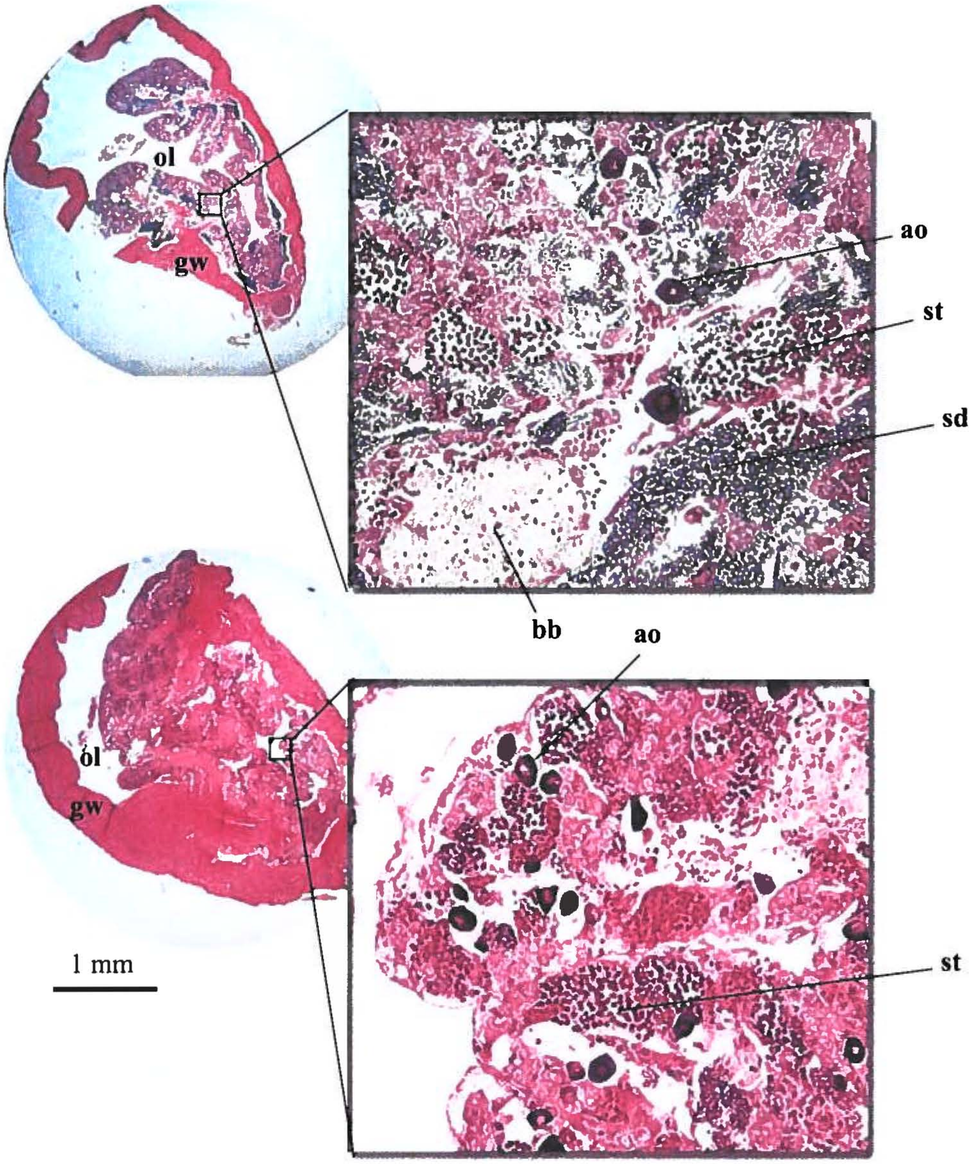

Figure 4.14. Pictures of two transitional *Lethrinus harak* (243 mm FL, 7 years old and 248 mm FL, 8 years old) at 4x (circle) and 40x (square) magnification. ao = atretic oocyte, bb = brown body, gw = gonad wall, ol = ovarian lumen, st = spermatocytes, sd = spermatids.

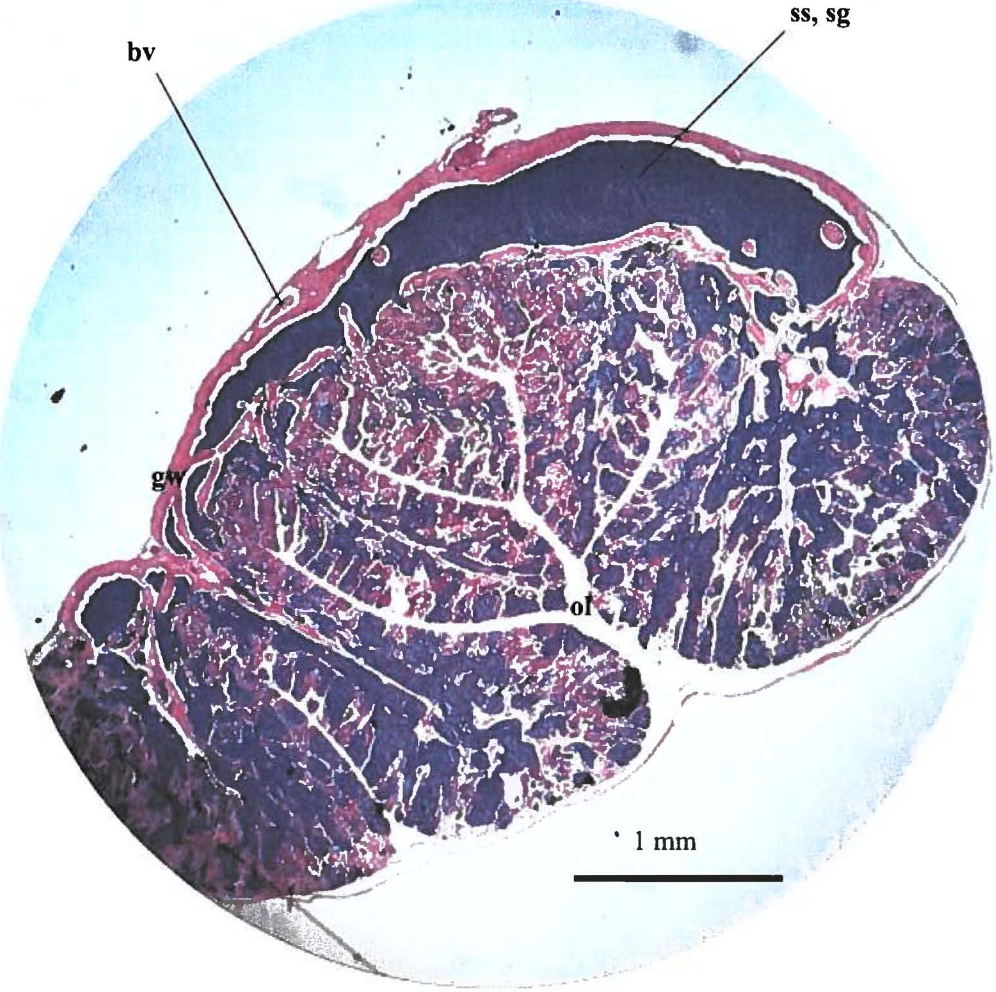

Figure 4.15. Picture of a mature functional male *L. harak* (225 mm FL, 5 years old) displaying a peripheral dorsal sperm sinus filled with mature spermatogonia and a remnant ovarian lumen. bv = blood vessel, gw = gonad wall, ol = ovarian lumen, sg = spermatogonia, ss = sperm sinus.

Figure 4.16. Picture of an immature male *L. harak* (186 mm FL, 3 years old) with atretic oocytes and developing sperm crypts. ao = atretic oocyte, gw = gonad wall, sc = sperm crypt.

4.4 Discussion

Evidence from this study indicates that *L. harak* is a protogynous hermaphrodite, which is consistent with conclusions from Ebisawa (2006) and with other species in the family Lethrinidae (Young & Martin 1982; Ebisawa 1990, 1997, 1999; Bean et al. 2003; Sumpton & Brown 2004; Ebisawa 2006; Sadovy de Mitcheson & Liu 2008). Ten transitional individuals were identified, all of which were larger than L_{50} and four were within the size range at which 100% of the female population was mature. Other features which suggest protogyny were the presence of peripheral dorsal sperm sinuses and a remnant ovarian lumen in mature male testes. In addition, sex-specific length and age frequency distributions follow the pattern typical of protogynous hermaphrodites in which males dominate the higher length and age classes and females dominate the lower. Sadovy de Mitcheson & Liu (2008) noted that many lethrinid species show size-specific sex ratios indicative of monandric protogyny but primary males may exist having male testes with the structure of an ovarian lumen. Evidence of this for *L. harak* exists in that five male individuals were identified below the size of 50% female maturation. All five had the structure of an ovarian lumen, three of these contained mature spermatozoa, and another contained sperm crypts in the presence of atretic oocytes. This merits a more detailed investigation of the early gonadal development of the species.

The data supports conclusions concerning sexual ontogeny, female maturation, and sex reversal. However, the sample size of larger, older fish was inadequate for exploring questions regarding seasonality in reproduction and comparisons of size/age at sex reversal. The vast majority of *L. harak* specimens collected were immature. Obtaining an adequate number of large mature individuals each month proved to be difficult as populations outside of marine preserves were comprised of smaller and younger fish at reduced densities (see Chapter 2). Permits to collect specimens within two marine preserves were not issued until the last months of the study period (April - May 2008). The greater abundance of mature female *L. harak* in Guam's marine preserves is reflected in the monthly sample sizes for the GSI plot, in which these two months had the largest sample sizes (Figure 4.11). The study would have benefited if marine preserve collection permits had been obtained earlier and sampling from these sites was spaced out over the duration of the study period to ensure an adequate monthly sample of functionally mature females.

There was little discrepancy in size and age at female maturation between protected and unprotected *L. harak* populations. However, there was a distinct difference in the age at sex reversal between protection statuses although this was driven by a higher abundance of older females within protected areas. Fishing non-randomly selects for larger and older individuals in a population and therefore truncates the size and age structures (Ricker 1969; Miranda et al. 1987). Over time this affects a population's reproductive potential and if fishing persists at unsustainable levels, the stock will collapse through recruitment overfishing (Bannerot 1987). Reef fish populations have been shown to have a decreased size at maturity in response to heavy fishing pressure (McGovern et al. 1998) and accelerated sex reversal has been demonstrated by manipulating social structures (Ross 1981; Shapiro 1981; Nemtzov 1985; Liu & Sadovy 2004a). Such changes in population processes can act as compensatory mechanisms when faced with a declining reproductive potential (Huntsman & Schaff 1994). Despite vast differences in sex ratios between protected and unprotected sites for ages 9 through 11 in the present study, these differences were not significant because of low sample sizes of old individuals in areas open to fishing. With increased sampling, it is likely that these differences would be significant, indicating that females from protected sites are delaying sex change to male until a later age because of a greater abundance and longevity of males within the protected population.

The small proportion of resting, inactive *L. harak* females in this study (~15% of mature females) coupled with their discrete seasonal occurrence creates confusion regarding the reproductive seasonality for the species on Guam. Anecdotal reports from Palau suggests individuals of this species are seen forming spawning aggregations in lagoons throughout the year (Johannes 1981). Other studies have produced equally unclear GSI plots similar to the present study. From Fiji, Lassi (2003) suggests that *L. harak* spawns year-round with the strongest GSI peaks occurring from August to October. In Kenya, the spawning season has been reported to be from October to February (Kulmiye et al. 2002). In the Ryukyu Islands of Japan, it has been reported to spawn from April to July (Ebisawa 2006). It seems most likely that *L. harak* spawns throughout the year across its distribution with varying seasonal intensity depending on location. Some authors suggest that smaller *Lethrinus* species, similar to *L. harak*, have longer spawning seasons whereas larger species tend to have shorter seasons (Loubens 1980; Brown et al. 1994; Sadovy 1996). Furthermore, the difference in mean GSI values among ovarian stages in *L. harak* is unpronounced and active individuals are encountered throughout the year, so it would require a very large monthly sample size of active females to get a definitive picture of spawning seasonality.

Like many marine teleosts, *L. harak* exhibits a strong pattern of increased female reproductive potential with size and age. Reproductive capacity increases throughout an individual's lifetime as less energy is used for growth and more resources are allocated to reproduction (Roff 1984). In addition, improved larval quality and survivorship has been linked to increased age in females (Berkeley et al. 2004a; Bobko & Berkeley 2004). Therefore, the importance of larger, older individuals in a population is obvious and it is imperative that a proportion of these individuals are preserved in the population. It is clear from previous chapters that Guam's marine preserve network is effective in allowing and accumulation of older age classes for both males and females which, in turn, has a positive effect on the total reproductive potential. For protogynous populations in which reproductive capacity is heavily influenced by social structure, MPA's provide a unique form of protection from fishing pressure as entire communities are potentially undisturbed (Roberts et al. 2005). Wherever fishing occurs, large females, which are disproportionately important to reproductive processes, become rare and the mean size, age, and proportion of males is generally reduced, ultimately leading to sperm limitation (Smith 1982). For this reason, size limitations in addition to protected area management would be beneficial to Guam's *L. harak* fishery. This is further emphasized when female reproductive contribution is compared between reference points as in Figure 4.6. It is apparent that *L. harak* on Guam is being exploited at sizes and ages well below maturation and optimal yield, but it is also being exploited long before individuals can make a significant reproductive contribution.

In conclusion, the only current management regulation protecting *L. harak* on Guam is the network of marine preserves. This and previous chapters have highlighted the efficacy of preserves in increasing the reproductive biomass and creating larger and older population structures within protected sites. The vast differences suggest that protected sites on Guam contribute disproportionately to the total reproductive capacity of the island-wide *L. harak* population.

Chapter 5 General Discussion: The importance of marine preserves in maintaining the *Lethrinus harak* population on Guam

5.1 The significance of demographic variability

The impacts of fishing on Guam's *L. harak* population have been clearly demonstrated. Marine preserve sites contained more intact populations than comparative fished sites with a higher proportion of larger, older individuals with greater reproductive potential. Chapter 2 highlighted the significant differences in abundance, size structure and standing spawner biomass of *L. harak* between protected and unprotected sites while Chapter 3 highlighted the differences in age structure and male and female longevities. Chapter 4 confirmed protogyny as the sexual pattern of *L. harak* and emphasized the importance of maintaining the older age classes in a population as they make a disproportionate contribution to spawning output. Although *Lethrinus harak* represents only one heavily targeted species in a multi-species coral reef fishery, it has served as an ideal model in demonstrating the efficacy of Guam's marine preserves. Despite the lack of detailed demographic data prior to their establishment, results from this study suggest the preserves are beneficial in maintaining "healthy" reef fish populations which probably sustain the adjacent fished areas. The vast differences in demographic parameters and reproductive potential between areas open and closed to fishing indicate the preserves are a vital tool in managing Guam's nearshore fishery.

It is imperative however to determine whether the higher spawner biomass inside the preserves are providing a recruitment subsidy for the remainder of Guam's reefs open to fishing. Although there is little empirical evidence for this recruitment effect, some recent studies have shown that a greater proportion (up to 50%) of newly settled larvae originate from the parent reef than was previously thought (Swearer et al. 1999; Cowen et al. 2000; Swearer et al. 2002; Jones et al. 2005; Almany et al. 2007). This high degree of self-recruitment is facilitated by a number of factors including local entrainment via nearshore eddies and the ability of late-stage larvae to detect reefs from considerable distances (Leis 2002; Paris and Cowen 2004). Despite low spawner biomass outside the preserves, many areas like East Agaña Bay contain large numbers of juveniles as a direct result of significant recruitment events. The only explanation for high levels of recruitment in the face of reduced reproductive effort is that Guam's marine preserves are the largest source of larval supply.

When making comparisons of age-based demography between sites open and closed to fishing, two assumptions are made regarding protection status and the movement patterns of reef fish (Ferreira & Russ 1995). First, it is assumed that no fishing occurs in the protected areas. Second, it is assumed that fish movement across protected area boundaries does not obscure the effects of no fishing on the population structures. While poaching is common in Guam's marine preserve network, data from confiscated catch indicates that *L. harak* is rarely targeted by illegal poachers (R.B. Tibbatts, pers. comm.). This is due to the behavior of both *L. harak* and the poachers. Most poaching is done by spearfishing at night to avoid detection. *Lethrinus harak* is difficult to spear at night as it tends to leave the reef flat probably moving into deeper water on the reef slope or adjacent channels (B.M. Taylor pers. obs.). As for the second assumption, little is known of the daily or seasonal movement patterns of *L. harak*, although studies of home ranges in other reef fish species have revealed high site fidelity (Holland et al. 1996; Zeller 1997; Parsons et al. 2003). Violation of this latter assumption may yield no difference in population structure between protected and unprotected reefs; however, this is not an issue in the present study because a consistent preserve effect was observed for *L. harak* populations on Guam. Additionally, when interpreting differences in population structure between areas open and closed to fishing, it is important to realize that the variability observed (or lack of) is not only influenced by the reduction in fishing mortality, but also by the level of exploitation in unprotected sites, the duration of protection, and the degree of habitat variability among sites. Knowledge of these factors is important when evaluating the effectiveness of protected area management.

The wide-ranging benefits make protected area management a valuable tool for managing artisanal multi-species, multi-gear fisheries such as the coral reef fishery of Guam (Hawkins & Roberts 2003). This is especially the case for those that target protogynous hermaphrodite species. The effects of exploitation on protogynous populations are amplified because the selective removal of larger (and older) individuals disproportionately decreases the reproductive capacity of the population and alters size- and age-specific sex ratios (Bannerot et al. 1987; McGovern et al. 1998). The use of protected area management for maintaining "healthy" protogynous populations is highly recommended as it allows populations to maintain natural age structures in the absence of fishing pressure (Berkeley et al. 2004b; Birkeland & Dayton 2005; Roberts et al. 2005). The present study, which demonstrated a build-up of older age and size classes and a greater proportion of large reproductive females within Guam's marine preserves, further supports the idea that protected area management is enormously beneficial for protogynous species.

5.2 Directions for the future

This study has provided a rare insight into the effects of protected area management on the age-based demography of an exploited reef fish species. It offers further evidence that protogynous reef fish are extremely susceptible to high levels of fishing pressure and that protected areas facilitate population recovery and stability and assist in restocking fisheries (Roberts et al. 2001). However, this study also uncovers additional questions concerning the efficacy of Guam's marine preserve network. The discrepancies in mortality rates and age structures between preserves and non-preserves might be interpreted as a lack of spillover, which might otherwise produce a more homogenous demography across sites. Clearly a better understanding of *L. harak* movement patterns is needed to provide insight into the magnitude of spillover which may or may not be occurring. The spillover of adult fish from closed areas is an important process as it provides fisherman with a subsidy which offsets the loss of fishing grounds when preserves are established.

It is hoped that data and conclusions from this study will be used to better manage the *L. harak* population on Guam. The implementation of minimum size limits is highly recommended for improving the reproductive viability of populations in unprotected sites. The combination of protected area management and conventional management techniques like size limits has been supported by many fisheries researchers (Hilborn et al. 2004; Roberts et al. 2005; Almany et al. 2007). Although considered essential components of management programs, MPA's are not sufficient when used as the only management tool (Allison et al. 1998). It is proposed that data from this study will be used to model the effects of protection status on the population biology of *L. harak* on Guam. Such a model will give detailed insight to the effects of fishing pressure on the population and the role of Guam's marine preserves in offsetting these effects.

In conclusion, this study has provided evidence of the vulnerability of a protogynous species to intense fishing pressure and the effectiveness of protected area management in restoring and maintaining a healthy reproductive population. Differences in abundance, mean size, density, biomass, spawner biomass, size structure, age structure, growth, sex ratio, and age at sex reversal were identified in *L. harak* populations between protected and unprotected sites suggesting that eliminating fishing mortality facilitates improvement and stability of the population structure. This evidence comes after only seven years of enforced protection which suggests that additional fishery improvement will only occur if the status of Guam's marine preserve network is maintained. However, additional management measures will be necessary for stocks to recover to the point where the island-wide population is reproductively viable. It is imperative that such information is distributed amongst the various stakeholder groups who are also included in discussions of future management of Guam's marine resources.

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