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### Reproductive dynamics of an exploited serranid on the Great Barrier Reef

by

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BSc (Hons) (Reading), MSc (Queensland)

A thesis submitted for the degree of Doctor of Philosophy in the School of Marine Biology and Aquaculture at James Cook University in March 2000

:

"At the time of coupling... their salacious propensities render them unguarded."

Attributed to Pliny the Elder, who was describing the spawning of mullet (1999 issue of Angler's Almanac).

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Melita Anne Samoilys

31 March 2000

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#### ABSTRACT

The reproductive output of exploited tropical reef fishes is intimately linked to factors such as mating patterns, sexual selection, sex ratios and reproductive success, because they affect an individual's fecundity and hence long term productivity. This information is critical to fisheries management, yet few studies have estimated annual fecundities and time schedules of egg production in exploited reef fishes. This study examines temporal and spatial patterns in egg production and spawning behaviour of an exploited serranid on the northern Great Barrier Reef.

Spatial patterns in the spawning behaviour of *Plectropomus leopardus* (coral trout) were examined by direct observations underwater at Scott and Elford Reefs for 3-4 years. A major (termed primary) spawning aggregation was located at each reef. These aggregations formed at the same sites each year, lasted for 5 days and formed around three consecutive new moons. Spawning occurred during a 33 min period spanning sunset. Maximum numbers of coral trout at these sites were 128 at Scott Reef, and 59 at Elford Reef. Smaller aggregations (termed secondary) were also located. Although their formation was inconsistent, their occurrence was also linked to the new moon phase.

The distribution and density of coral trout in a 6.5 km<sup>2</sup> area surrounding the primary site at Elford Reef was mapped using visual census surveys. Individuals were tagged within this area to estimate the catchment area of the aggregation site, and to track the movements of individuals to the site. The primary site was estimated to draw fish from a population of around 3,000 adults in an area of only 1.5 km<sup>2</sup>, and it was estimated that around 14% of the population used the aggregation site to spawn. However, certain individuals showed high site fidelity, returning to the site on subsequent evenings within a new moon phase, on subsequent new moons within the season, and the following year.

Temporal patterns in egg production were determined from histological sections of gonads. Annual fecundity was estimated from counts of hydrated oocytes (batch fecundity) and from the proportion of females with post-ovulatory follicles (spawning frequency). The time schedule of hydrated oocytes was closely linked to lunar phase and time of day. Spawning occurred predominantly during the new moon and first

quarter lunar phases at dusk. The average number of spawnings per female per year was estimated at 30, and annual fecundities of between 2.6 and 4.7 million eggs were obtained.

The results indicate that *Plectropomus leopardus* employs both aggregative and nonaggregative spawning. The high spawning frequency and range of mating mechanisms may maximise egg output. I suggest this flexibility in *P. leopardus* spawning strategies improves their resilience to exploitation.

#### ACKNOWLEDGEMENTS

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I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

31/3/00

31 March 2000

Melita Anne Samoilys

#### **Chapter One: GENERAL INTRODUCTION**

#### **1.1 Introduction**

The study of reproduction in marine organisms is both challenging and rewarding. Marine fishes are characterised by broadcast spawning, a lack of parental care and high fecundities (Sadovy 1996, Roff 1992, Thresher 1984) which make estimating reproductive output difficult. For coral reef fishes the study of reproductive events is doubly challenging due to the potential for extended spawning seasons and multiple and/or sequential spawning episodes by individual fishes (Robertson 1991).

Knowledge of the reproductive output of exploited fishes is critical for effective fisheries management, yet few studies have estimated annual fecundities or examined time schedules of egg production in tropical reef fishes. The reason for the paucity of studies is probably related to the logistics of studying fishes that have extended reproductive seasons, live in complex and variable environments, and are relatively mobile and wide ranging. This PhD project adopted a range of methods to address these difficulties for the common coral trout, *Plectropomus leopardus*, a large serranid, and the most heavily exploited fish on the Great Barrier Reef, Australia (Kailola et al 1993, Ayling and Ayling 1986). I examined temporal and spatial patterns in egg production and spawning behaviour both at the population and individual level, and tracked the spawning behaviour of known individuals. This combination of studies enabled me to provide the first estimates of annual fecundity for *P. leopardus*, and to demonstrate that the reproductive dynamics of coral trout are temporally and spatially complex, which has a significant bearing on the management of the coral trout fishery in Australia.

Fisheries management generally operates by controlling mortality. This requires an understanding of the demographic effects of fishing on fish populations. It has long been recognised that fishing has significant impacts on three major population processes (Russell 1931): recruitment, growth, and natural mortality, with fishing adding a fourth process – fishing mortality. Dynamic pool models incorporate these processes and thereby define mortality controls for optimal exploitation (Beverton and Holt 1957).

However, even recent, sophisticated dynamic pool models (eg. Goodyear 1993, Mace and Sissenwine 1993) fail to recognise the links between recruitment, growth and mortality, and reproductive output (Vincent and Sadovy 1998, Sadovy 1996). Reproductive output is intimately linked to factors such as mating patterns, sex ratios, sexual selection, and reproductive success because these affect an individual's fecundity and hence long term productivity (see Vincent and Sadovy 1998: Fig. 9-1). Fishing can directly impact on these factors, for example by selectively removing certain sex or size classes (Shapiro 1987). This can have severe consequences on overall fecundity since fecundity is size dependent (Roff 1992, Gladstone and Westoby 1988), or in terms of sperm limitation caused by removing males (Koenig et al 1996, Bannerot et al 1987). Understanding reproductive behaviour is therefore critical for devising appropriate fisheries management policies for exploited species, yet most fisheries stock assessment studies ignore reproduction or assume that reproductive output can be represented by a constant (Sadovy 1996). One notable exception is the use of minimum size limits which have been used as a standard fisheries management policy for decades (King 1995, Hill 1992). Size limits are set above the average size at first reproduction to allow females to spawn at least once. Limits may be increased when stocks require re-building (Appeldoorn 1996). Both size at first reproduction and fecundity are closely related to fish size (rather than age) due to the physiological and energetic constraints of producing eggs (Roff 1992), which illustrates the importance of size - based studies of reproductive behaviour.

The serranid Subfamily Epinephelinae, the groupers, are particularly important in the context of studying reproductive output and fisheries management. Firstly, most species of grouper occur on coral reefs (Heemstra and Randall 1993) where they are abundant and highly accessible to fishing. Ninety percent of the world's harvest of marine food is derived from artisanal fisheries of which groupers are a major component and are the most valuable in terms of dollars per kilo (Heemstra and Randall 1993). Secondly, serranid fishes have complex life histories being either protogynous or simultaneous hermaphrodites, rarely gonochoristic (Sadovy and Colin 1995, Shapiro 1987), and they spawn several batches of eggs in a season. Thirdly, serranids characteristically form well defined aggregations to spawn (Domeier and Colin 1997). Such aggregations have been described for *P. leopardus* (Samoilys and Squire 1994). Many fishes aggregate to spawn using predictable environmental signals. This can provide an ideal focus for

study and a tool for fisheries management (Sadovy 1996, Parrack and Huntsman 1982, Johannes 1980). In addition, aggregating fishes are particularly vulnerable to overfishing (Johannes et al 1999, Sadovy 1994b, Sadovy et al 1994a, Bohnsack 1989, Johannes, 1981).

Most reproductive work to date on exploited coral reef fishes has focussed on Epinepheline serranids particularly on the occurrence of spawning aggregations in the Caribbean (reviewed by Domeier and Colin 1997). However, basic biology and life history studies of Atlantic groupers are few (but see Koenig et al 1996, Sadovy et al 1994b). Therefore, it has been difficult to integrate information on spawning aggregations into population dynamics models for fisheries management purposes. For one serranid in Australia the reverse is true. Demographic studies on the common coral trout, Plectropomus leopardus, are well advanced (Russ et al 1996, Ferreira 1995, Ferreira and Russ 1995, 1994, Doherty et al 1994). However, information on their spawning behaviour, in particular that of forming aggregations to spawn is recent and sketchy (Samoilys and Squire 1994), and there is no information on their reproductive output. This is critical since coral trout are the dominant species in the commercial and recreational fisheries on the Great Barrier Reef (Kailola et al 1993). To assess the implications of spawning aggregations to fisheries management requires knowledge of the role aggregations play in a species' annual reproductive effort, and the overall dynamics of egg production.

This PhD project combined three approaches to define the reproductive biology of *P. leopardus* and to estimate their reproductive output. The three studies also combine to assess the significance of spawning aggregations to the reproductive dynamics of coral trout. The first approach involved an analysis of spatial patterns in the spawning behaviour of coral trout. An earlier pilot study demonstrated that *P. leopardus* formed spawning aggregations at specific sites (Samoilys and Squire 1994). Although spawning aggregations of epinepheline serranids have been widely documented from the Caribbean (reviewed by Domeier and Colin 1997), there have been no previous recordings of such events on the Great Barrier Reef. Further, spatial variation in female maturity and/or fecundity has been recorded in reef fishes (Jones 1991, Gladstone and Westoby 1988, Thresher 1985). The significance of aggregative behaviour to the reproductive dynamics of coral trout is not known. This study searched large areas of the

two study reefs looking for spawning behaviour and spawning aggregations of coral trout, and monitored their occurrence and timing both within and between years (Chapter Two).

Secondly, I monitored the behaviour of individual fish, to examine their spawning behaviour within both the spatial framework of the first study, and the temporal framework of the third study (see below). Thus, the movements of fish to spawning aggregations, their frequency of visits, and the frequency with which they spawned was investigated (Chapter Three). This investigation also aimed to determine what proportion of individuals were using the aggregation sites to spawn to assess the significance of aggregative spawning to the overall reproductive output of *P. leopardus*.

Thirdly, seasonal, lunar and diel patterns in egg production were examined to describe the temporal pattern of spawning. Most reproductive studies present seasonal data on gonad maturity gained through gonadosomatic indices or histological assessment, which simply provide the months of spawning (reviewed in Sadovy 1996, eg. Table 2.2). This information, however, tells us little about the actual frequency and duration of spawning and hence annual or lifetime reproductive output. For example, the basic reproductive biology of *P. leopardus* has been described by Ferreira (1995), who established that they spawn during the months of September to December on the northern Great Barrier Reef (GBR). However, Ferreira (1995) did not examine fine scale temporal patterns in spawning within the reproductive season. In contrast, a separate study detected lunar and diel patterns in the spawning behaviour of *P. leopardus* (Samoilys and Squire 1994). In the present study I examined schedules of egg production at broad and fine time scales using histological techniques on gonads sampled from two coral trout populations (Chapter Four). I also monitored the spawning behaviour of these two populations at the same time scales through direct observations (Chapter Two).

Ultimately, by measuring spawning frequency and batch fecundity using laboratory techniques on ovaries (Chapter Four), I was able to estimate annual fecundities. The significance of these estimates lies in the fact that they were derived within a temporal and spatial framework working at both the individual and population level. This combination of approaches provides one of the few comprehensive studies on the reproductive output of an exploited coral reef fish.

#### 1.2 Coral trout fishery

Species in the genus *Plectropomus*, coral trout, are members of the serranid Subfamily Epinephelinae, the groupers, rockcods, hinds and seabasses (Heemstra and Randall 1993). There are seven species of *Plectropomus* in the Indo-Pacific region, which includes the Red Sea (Heemstra and Randall 1993, Randall and Hoese 1986). *Plectropomus leopardus* (Lacépède 1802), the common coral trout, is the most abundant of the five plectropomids that occur on the Great Barrier Reef (Randall et al 1990, Ayling and Ayling 1986).

Coral trout are the dominant species in the commercial and recreational fisheries on the Great Barrier Reef, comprising up to 60% of the demersal reef fish catches landed by commercial fishers (Mapstone et al 1996, Turnbull 1996). They are also one of the four most targeted species by recreational anglers in north Queensland (Cormack 1997, Roy Morgan Research 1996), and the potential for increased fishing effort from the recreational sector is indicated by a five-fold increase in the number of privately owned vessels from 1968 to 1994 (Higgs 1996). Coral trout contribute close to 2,000 tonnes, or over A\$10 million in commercial catch, per annum (Mapstone et al 1996, Brown 1993, Trainor 1991). There is no published historic data on the coral trout fishery; the commercial fishery has existed at least since the 1950's (pers obs.). Commercial log book data has been collected since 1988 by the Queensland Fisheries Management Authority (QFMA). During that time the total catch of coral trout rose from 830 t in 1988 to a maximum of 1,760 t in 1996, and then declined to 1,500 t in 1998 (Samoilys and Williams in press). Unfortunately, commercial log books do not record fish sizes, only weights, therefore any changes in mean size of fish over time cannot be assessed. Management of the Queensland GBR line fishery is controlled by the QFMA with the following restrictions: a limited entry commercial fishery with two classes of fishing licences; a minimum legal size of 38cm total length; a recreational bag limit of 10 coral trout in possession/fisher; and hook and line gear restricted to a maximum of six hooks/line, three lines/fisher (QFMA 1996, 1999), all of which are designed to reduce catch and effort. The minimum size restriction, originally set at 35 cm TL, has been in place since at least 1984 (pers. obs., Hancock 1992), and was

designed to allow females the chance to spawn at least once before they enter the fishery (King 1995, Hill 1992). The coral trout fishery's minimum legal size is currently set at 38 cm TL (QFMA 1999). Reef closures through Great Barrier Reef Marine Park Authority (GBRMPA) protected areas legislation also has a direct impact on the fishery.

*Plectropomus* species are also important targets of the recent and burgeoning live reef fish trade in the Asia Pacific region (Richards 1993), and this has stimulated an increase in the demand for coral trout in Australia since 1995 (Elmer 1998). There has been a 30% increase in fishing effort recorded in 1996-8 (QFMA 1999), and 15% of the commercial coral trout catch is now taken as live product (Elmer 1998). In addition, the tourist industry in Queensland relies heavily on seafood, with an estimated \$35 million being spent on seafood meals in the Cairns region in 1993 and this demand is expected to double over the next 13 years (Geography Department, James Cook University 1993). In many countries the live reef fish fishery is actively targeting spawning aggregations (Johannes et al 1999, Johannes and Lam 1999). It has therefore become increasingly important to understand the biological mechanisms underpinning the formation of spawning aggregations in order to understand the significance of targeted fishing of such aggregations. Current management regimes in Queensland include minimum size limits (QFMA legislation) and fishery reserves - reefs that are closed to fishing (GBRMPA legislation). The latter represent approximately 20% of the Great Barrier Reef and were arbitrarily selected in the 1970s. The purpose of the present study was to provide biological information necessary to assess the relevance of current and proposed management regimes.

#### **1.3 Thesis outline**

This dissertation was designed to address the overall aim of the PhD Project: to describe the mechanisms of spawning aggregations, to assess the significance of aggregative behaviour to the reproductive dynamics of coral trout, and to estimate reproductive output. The project represents a case study on the reproductive dynamics of coral trout at a local scale and was conducted at two mid-shelf reefs, Scott and Elford Reefs off Cairns, to describe temporal and within-reef patterns in spawning behaviour over a four year period. Three integrated studies were designed to address the project's overall aim.

The specific objectives of these studies, presented in Chapters Two, Three and Four, are outlined below.

#### Chapter Two: Temporal and spatial patterns in coral trout spawning aggregations

The objectives of this study were to:

- (i) locate and record locations of spawning aggregations
- describe seasonal, lunar and diel patterns in the occurrence of spawning aggregations
- (iii) measure the magnitude and duration of spawning aggregations

## Chapter Three: Distribution and movement of coral trout in relation to spawning aggregations

The objectives of this study were to determine:

- (i) the distance travelled to aggregations or "source area" of a single aggregation
- (ii) the frequency of visits to aggregations by individual fish
- (iii) what proportion of the population aggregate to spawn

#### Chapter Four: Sexual maturation, spawning frequency and fecundity of coral trout

The objectives of this study were to:

- (i) describe seasonal, lunar and diel patterns in sexual maturity
- (ii) measure spawning frequency, batch fecundity and annual fecundity
- (iii) estimate minimum size at maturity and sex change

In Chapter Two I mapped the locations of coral trout spawning aggregations and monitored their occurrence using underwater visual census (UVC) surveys conducted over four years at Scott Reef and three years at Elford Reef. In Chapter Three I examined the significance of spawning aggregations to the local population, and the frequency with which individuals aggregate and spawn, by mapping the distribution and density of coral trout in a section of Elford Reef prior to spawning, and by tagging individuals to track their movements and behaviour. In Chapter Four I used gonadal histology to examine temporal patterns in reproductive maturity over two years to compare with the patterns of spawning observed through visual surveys during the same period at the same study sites. Gonads were also sampled to estimate spawning frequency and batch fecundity to determine the reproductive output of coral trout.

These three studies combine to assess the significance of spawning aggregations to the reproductive dynamics of coral trout and to estimate reproductive effort. Chapter Two defines the number of and use of aggregation sites on each of the two study reefs, and the numbers of coral trout using these aggregation sites. In Chapter Three the average density and distribution of coral trout prior to spawning is defined within a 6.5 km<sup>2</sup> area containing the main (primary) spawning aggregation site at Elford Reef. This information, combined with that from Chapter Two allows estimation of the proportion of the population aggregating to spawn. In Chapter Three the movement of known (tagged) coral trout in relation to the primary aggregation site on Elford Reef defines the use of aggregation sites by individual fish and the source or draw area of an aggregation. Tagged fish also provide information on the frequency of spawning whether individual coral trout use aggregation sites repeatedly through the season, whether individuals spawn more than once per night, and whether they spawn on several consecutive nights. This information relates to the reproductive maturity, spawning frequency and fecundity investigation of Chapter Four. The laboratory protocols used are described in detail in Appendix I. Chapter Four substantiates the temporal patterns of spawning detected in Chapter Two and describes the biological mechanisms on which this reproductive behaviour is based.

#### **1.4 Publications arising**

- Samoilys, M.A. (1997) Periodicity of spawning aggregations of coral trout *Plectropomus leopardus* (Pisces: Serranidae) on the northern Great Barrier Reef. Mar. Ecol. Prog. Ser. 160: 149-159. From Chapter Two.
- Samoilys, M.A. and Roelofs, A. (in press) Defining the reproductive biology of a large serranid *Plectropomus leopardus*. CRC Reef Technical Manual. From Appendix I.

#### Chapter Two: TEMPORAL AND SPATIAL PATTERNS IN CORAL TROUT SPAWNING AGGREGATIONS

#### **2.1 Introduction**

A large number of epinepheline serranids aggregate to spawn, frequently at fixed sites, and their reproductive cycles are often correlated with lunar or semi-lunar rhythms (Sadovy 1996, Colin et al 1987, Shapiro 1987, Thresher 1984, Johannes 1981, 1980, 1978). Preliminary observations have shown that *Plectropomus leopardus*, the most abundant of the four plectropomid Serranidae found on the Great Barrier Reef, form distinct aggregations to spawn, at specific locations, and at specific seasonal, lunar and diel times (Samoilys and Squire 1994). Although spawning aggregations of epinepheline serranids have been widely documented from the Caribbean (reviewed by Domeier and Colin 1997), there have been no previous recordings of such events on the Great Barrier Reef. The significance of aggregative behaviour to the reproductive dynamics of *P. leopardus* is not known.

In the present study I conducted broad-scale surveys over large areas of two study reefs to look for spawning behaviour and spawning aggregations of *P. leopardus*, the common coral trout. I then monitored the occurrence and timing of spawning aggregations across a range of temporal scales to ascertain the frequency and significance of the spawning aggregations to coral trout reproduction. The purpose of the study was to: (i) locate and record locations of spawning aggregations; (ii) describe yearly, seasonal, lunar and diel patterns in the occurrence of spawning aggregations; (iii) measure the magnitude and duration of aggregations. The study was conducted at two mid-shelf reefs to provide some measure of inter-reef variation in the formation of spawning aggregations.

The importance of coral trout to the Great Barrier Reef fin fishery, and the resulting focus of research on the common coral trout, *Plectropomus leopardus* (Lacépède 1802) is evident in recent papers (Russ et al 1996, Ferreira 1995, Doherty et al 1994, Ferreira and Russ 1994, Rimmer et al 1994, Samoilys and Squire 1994). Understanding the reproductive behaviour of coral trout is important for two reasons. Firstly, coral trout are hermaphrodites (Ferreira 1995, Goeden 1978), and although evidence is still largely

circumstantial, it seems likely that sex change in *P. leopardus* is at least partly induced by social behaviour (Ferreira 1995, Samoilys and Squire 1994). Social regulation of sex change has long been recognised in some sequentially hermaphroditic fishes (Shapiro 1979, Robertson 1972, Fishelson 1970), and may be triggered during spawning aggregations (Shapiro et al 1993a, Shapiro 1987). Secondly, spawning aggregations are frequently targeted by fishers (eg. Johannes et al 1999, Sadovy et al 1994a), and elsewhere this has led to the disappearance of spawning aggregations and the subsequent collapse of groupers fisheries (Sadovy 1994a,b, Beets and Friedlander 1992, Bohnsack 1989, Olsen and LaPlace 1979). Anecdotal information indicates that the commercial fishery on the Great Barrier Reef (GBR), aided by the availability of global positioning system (GPS) equipment, is increasingly targeting spawning aggregations. To assess the implications of both these factors the reproductive behaviour of coral trout needs to be better understood.

To investigate spawning aggregations I surveyed reef sites at which seasonal aggregations of coral trout had been observed by local fishers (L.C. Squire pers. comm., Samoilys and Squire 1994). Preliminary investigations in 1990 at Scott Reef located one major aggregation site (Samoilys and Squire 1994), hereafter termed the "primary site". The present study focussed on the site identified at Scott Reef and a primary site located at neighbouring Elford Reef. This chapter presents three years (1991-1993) of monitoring the primary spawning aggregations at both reefs and for summary purposes includes results of the first year's (1990) pilot work presented in Samoilys and Squire (1994). The study also documented the simultaneous occurrence of spawning or aggregating trout on sites other than the primary sites.

#### 2.2 Methods

#### 2.2.1 Study Sites

The study was conducted at two midshelf reefs, Scott (17°05'S, 146°11'E) and Elford (16°55'S, 146°15'E) near Cairns, on the northern GBR (Figure 2.1). Aerial photographs of the two reefs purchased from AUSLIG, were referenced into a GIS package (ArcInfo) courtesy of the Great Barrier Reef Marine Park Authority, and subsequent

Figure 2.1 Location of study sites at Scott and Elford Reefs, south of Cairns, northern Great Barrier Reef.

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calculations of reef areas were done in ArcView 3.1. Aerial photographs were taken from an altitude of 1,524 m (5000 ft), giving a scale of 1:10000. Scott Reef represents a total area of 17.6 km<sup>2</sup>; this study focussed on an area of 4.6 km<sup>2</sup> (Figure 2.2). Elford Reef represents a total area of 21.3 km<sup>2</sup>; this study focussed on an area of 7.7 km<sup>2</sup> (Figure 2.3).

#### Primary aggregation sites

On each reef a primary aggregation site was defined as the area in which large numbers of coral trout were found aggregating during the spawning season. Such aggregations were defined as follows: the density of coral trout at the site was at least three times the average density of trout at the site outside the spawning season (see Data Analyses). Two primary sites were located, one in 1990 at Scott, and the other in 1991 at Elford. In the 1992 spawning season, broad-scale surveys of a section representing approximately 26% (Scott) – 36% (Elford) of the total reef area of each of the two study reefs were done on the new moon (when aggregations occurred, see Results), to search for other spawning aggregation sites (see also Secondary aggregation sites below). Due to the extensive size of the reefs, surveys could not cover all reef areas: the northern end of Scott Reef and the south-western and mid sections of Elford Reef were surveyed (Figures 2.2 and 2.3). No other primary aggregation sites were located.

After preliminary observations, the two primary aggregation sites were mapped and reproduced on underwater paper. During censuses the positions of individual fish were recorded on these maps. The site located at Scott Reef in 1990 measured 1700m<sup>2</sup> in area, and is described in Samoilys and Squire (1994). The primary aggregation site at Elford Reef was located in September 1991, measured approximately 3,200 m<sup>2</sup>, and was situated at the southern end of a channel which cut through the main reef from north to south. The channel was approximately 10m in width at the aggregation site, but opened into a much wider lagoon to the north (leeward). The site consisted of shallow reef top at approximately 3-6m depth on either side of the channel, with steep walls to seaward (south) dropping to a gently sloping sandy bottom at approximately 20-25m depth. To leeward the reef sloped at approximately 45° into the protected lagoon area which had a sandy bottom of approximately 16-20m depth. Moderate to strong tidal currents were encountered running either north or south in the channel.

Figure 2.2 Aerial photograph of Scott Reef. P = primary aggregation site. A1-3 = secondary aggregation sites; C1-3 = control sites for secondary aggregation sites. Dotted line represents total area that the broad scale surveys covered and encompasses all study sites.



Figure 2.3 Aerial photograph of Elford Reef. Abbreviations as in Figure 2.2.



#### Secondary aggregation sites

Broad-scale surveys of sections of the two study reefs were done on the new moon in the 1992 spawning season (Samoilys and Squire 1994), to search for other spawning aggregation sites. Sites were identified by the presence of groups of coral trout (~ 5 fish 25 m<sup>-2</sup>), because coral trout are generally solitary in their behaviour (Samoilys, 1987), or by coral trout exhibiting spawning related behaviour. Such behaviour was defined as any one of the following: males in spawning colours (darkened dorsal and caudal fin edges), courting, spawning rushes or male aggression (Samoilys and Squire 1994), and is hereafter, for convenience, referred to collectively as "spawning behaviour".

Searches were conducted on snorkel, and were confined to reef edges or slopes, both windward and leeward; lagoons were not included. Certain topographical features were targeted during searches such as channels through the reef crest, prominent spurs, and areas subjected to relatively strong currents. These features are characteristics of the primary aggregation sites and of spawning sites for many reef fishes (Johannes 1978). The locations of groups of coral trout or trout exhibiting spawning behaviour were noted and then re-surveyed after 1600 hrs on SCUBA (Samoilys and Squire 1994), to determine whether they represented spawning aggregation sites.

No other primary aggregation sites were found but smaller aggregation sites, termed secondary, were located. Consequently, three secondary aggregation sites (A1 - A3) and three corresponding control sites (C1 - C3) were established at each reef (Figures 2.2 and 2.3); they measured approximately 200m in length along the reef edge. At Elford Reef secondary aggregation sites were located at channels that divided the front (southern) edge of the reef which shelved steeply to 30 - 35m, similar to the structure of the primary site though the channels were much smaller in scale (Figure 2.3). The secondary aggregation sites at Scott Reef were submerged bommies with high coral cover away from the main reef on the northern and eastern edges of the reef (Figure 2.2). Control sites were established in the same general vicinity as the aggregation sites, in areas where no spawning behaviour or groups of coral trout had been observed. At Elford Reef they were located away from the channels, and at both reefs were located along a continuous reef edge.

#### 2.2.2 Censuses at primary aggregation sites

Numbers and sizes (in 5cm forklength, FL, size classes) of coral trout at the primary aggregation sites were counted by underwater visual census standardised to a fixed route and duration of 25 minutes, conducted by one of three trained observers on SCUBA. Training involved estimating fish size using wooden fish models (Samoilys 1997c, Bell et al 1985). The observer swam 1.5m off the bottom and searched a path width of approximately 10m, and limited observations to 20m depth due to repetitive diving constraints. The route was designed to allow the observer to view the whole aggregation site by the end of the census to give a total count. The location and size of each fish were marked on the topographical map. Further details of the methodology are given in Samoilys and Squire (1994). A total of 218 visual censuses were conducted between 1990 and 1993 (Table 2.1).

On each count water temperature was measured at 15m depth with a hand held mercury or red spirit thermometer, accurate to  $0.25^{\circ}$ C. Visibility was estimated by stretching a fibreglass tape measure between two divers. All counts were conducted in visibility > 7m. A crude relative index of current strength was estimated in the channel of each primary site by one of three observers: 1=negligible, 2=slight, 3=moderate, 4=strong. Tidal state of each census was defined by four phases: ebb, flood, high tide (slack water), and low tide (slack water), using published figures for Cairns (16°55'S, 145°47'E, Queensland Department of Transport Tide Tables 1990-1993), where slack water = within 60 minutes either side of low or high tide. Table 2.1Total number of visual census counts at primary aggregation sites on Scott and Elford Reefs, 1990-1993.Times are AEST and refer to the start of the 25 minute census.

SCOTT				ELFORD				
Time:	Dawn	0830-1230	1300-1600	Dusk	Dawn	0830-	1300-	Dusk
	<0600			>1630	<0600	1230	1600	>1630
1990	0	6	5	25	0	0	0	0
1991	0	7	4	21	0	7*	2	14
1992	0	9	5	21	0	8	6	22
1993	3	1	0	26 <sup>a</sup>	4	1	0	21
TOTAL	3	23	14	93	4	16	8	57

\* One count started at 1235.

<sup>a</sup> Includes one count in Jan 1994.

#### Seasonal periodicity

Regular monitoring of the aggregation sites throughout the year was conducted at dusk (>1630 AEST, Australian Eastern Standard Time) during a 5-6 day period around the new moon because preliminary observations found spawning behaviour and peak numbers of aggregating coral trout coincided with this period (Samoilys and Squire 1994). Four spawning seasons (1990-1993) were sampled at Scott Reef, and three spawning seasons (1991-1993) were sampled at Elford Reef (Table 2.2). Surveys were conducted monthly during the spawning season, August to December (Samoilys and Squire 1994). For the non-spawning season (January-July) counts were conducted every other month, starting in 1992, at both Scott and Elford Reefs. Bad weather disrupted this schedule of sampling at dusk around the new moon eight times in the non-spawning season and twice in the spawning season. For seven of these an alternative moon phase or month was sampled; for the other three times no census was obtained.

#### Lunar periodicity

During the spawning season, weekly or fortnightly censuses were conducted to sample the various stages of the lunar cycle. Each of the four phases of the moon was assigned a five day period, and each census was done according to lunar phase (Table 2.2). Lunar months ranged between 29-30 days thus the new moon period varied between 5 and 6 days. Lunar periods were based on moon light level records (data provided by the Anglo-Australian Observatory), which were divided arbitrarily into: 0.9-1.0 = Fullmoon; 0.0-0.1 = New moon; 0.4-0.6 = first and third quarters. The corresponding lunar day was calculated from lunar day 1 = 0.0 moonlight. The division also gave four interphase periods (Table 2.2). It was intended that the four major lunar phases would be sampled. The majority of UVC surveys fell within these periods, but bad weather resulted in some sampling in inter-phase periods. Table 2.2 summarises the number of lunar phases sampled per month and year. **Table 2.2** Total number of visual census counts conducted at dusk on the primary aggregation site summarised by lunar phase and year at Scott and Elford Reefs. d = lunar day, where 1 = new moon. Actual dates of censuses are shown in Figure 2. January 1994 is included in the 1993 year. \* indicates one count conducted in the afternoon (1300-1600) not dusk.

REEF &	NEW		1ST		FULL		3RD QUAR	
YEAR	MOON	4-5 d	QUAR	11-13 d	MOON	19-20 d	21 <b>-</b> 25 d	26-27 d
	28-3 d		6-10 d		14-18 d			
Scott 1990	4	-	3	1	3		3	1
Scott 1991	4	1	3*	3	5*	-	-	=
Scott 1992	8	-	-	-	5	-	-	-
Scott 1993	8	3	4	-	5	-	2	-
Elford 1991	3	-	1	1	4	-	-	-
Elford 1992	7	1	2	-	4	-	-	1
Elford 1993	6	1	3	-	3	-	1	_

#### Diel periodicity

To standardise counts for intra and inter-annual and inter-reef comparisons, the majority of censuses were completed at dusk because spawning occurred at sunset (Samoilys and Squire 1994). To determine any daily patterns in the aggregations a set of censuses were conducted during other times of the day (Table 2.1).

#### 2.2.3 Censuses at secondary sites

Secondary aggregation sites and their corresponding control sites were surveyed for two years, during the spawning season, to examine possible spawning behaviour at sites other than the primary sites, and to monitor coral trout densities at such sites. Secondary sites were censused for 15 minutes, on SCUBA, which involved a search path of approximately 20 m width over a depth range of 2 m to 20 m, first swimming along the deeper level, then returning along the shallower level, giving a total census area of  $\sim$ 4000 m<sup>2</sup>. Censuses recorded the number of coral trout, their lengths, any spawning behaviour, and were conducted by trained observers. Counts were limited to a depth of 20 m because of repetitive SCUBA diving, however, trout were observed at these sites below this depth. Thus, observations were consistently biased towards fish at < 20m depth, as at the primary site (see above). Censuses were conducted at dusk (after 1630 hrs) to monitor both primary and secondary sites simultaneously when sufficient divers were available. When this was not possible censuses were conducted after 1330 hrs, by which time coral trout have established their aggregations (see Results). In 1992 secondary sites were surveyed on the new and full moons during the spawning season, on the same days that the primary sites were surveyed. In each lunar phase two counts were done at each site (2 evenings). This was repeated in 1993, but only two of the aggregation sites and two of the control sites were censused; again censuses were conducted on days that primary sites were surveyed.

#### 2.2.4 Spawning behaviour

Spawning behaviour of *P. leopardus* on aggregation sites involves elaborate courtship behaviour from males which culminates in spawning rushes by male and female pairs (Samoilys and Squire 1994). The behaviour of coral trout was monitored for 30 minutes after each visual count to record spawning rushes. To confirm that gametes were released during spawning rushes I attempted to catch gametes from a spawning rush to identify the eggs, and rear them to larvae in the laboratory. A plankton net, approximately 1m<sup>2</sup> and 0.7mm mesh size, was dragged by two divers through the water at the point at which a spawning pair of fish had turned at the peak of their spawning rush. Newly released eggs measure 0.81mm in diameter (R. Garrett pers. comm.). The divers returned immediately to the boat to search the contents of the net for eggs, which were then flushed into a 2 litre insulated container filled with seawater. The container was aerated and transported back to the laboratory in Cairns (ca 3 hrs). Eggs were incubated in the Northern Fisheries Centre (NFC) hatchery facilities. The morphology of embryos and larvae was compared with a NFC photographic record of development of laboratory hatched *P. leopardus* eggs (Rimmer et al 1994).

#### 2.2.5 Data Analyses

For present purposes I defined a primary spawning aggregation as follows: an aggregation was present at the primary aggregation site if the density of coral trout at the site during the spawning season (August-December) was at least three times the average density of trout at the site during the non-spawning season (January - July). This aggregation mean density was at least ten times the standard deviation of the density of trout at the site during the non-spawning season.

#### Diel periodicity

Visual counts from different times of the day at the primary aggregation site (Table 2.1) were compared using paired t-tests (Zar 1996) on data from days on which trout had aggregated at dusk. Data were the differences between pairs of counts from days on which two counts were done: morning and dusk, or afternoon and dusk, or dawn and dusk. Thus, the dusk counts were split into three respective groups for analyses. Data

were pooled across all years. The data sets were small and normal probability plots were difficult to interpret, however, paired t-tests are robust to departures from normality (Zar 1996). Analyses involved three multiple t-tests therefore a Bonferroni adjusted critical significance level was used (p<0.017, Siegel 1992).

#### Secondary sites

Counts of numbers of coral trout at the secondary aggregation sites during new and full moon phases were compared using a fixed two-way model Analysis of Variance (Zar 1996, Underwood 1981), with lunar phase and site type (aggregation or control) as factors and the six sites as experimental units. An analysis was run for each reef and each year since the number of sites varied between years. Homogeneity of variances was examined using plots of residuals; all data were transformed by:  $\sqrt{(x + 0.5)}$ .

#### 2.3 Results

#### 2.3.1 Primary aggregation sites

#### Seasonal periodicity and fish sizes at primary sites

The mean number of coral trout observed at the primary aggregation sites during the non-spawning period (Jan-Jul) was  $6.58 \pm 1.09$  SE (n=12) at Scott Reef and  $6.67 \pm 1.38$  SE (n = 6) at Elford Reef (densities of 3.87 fish/1000m<sup>2</sup> and 2.08 fish/1000m<sup>2</sup>, respectively). Thus, an aggregation was defined as >20 coral trout on each primary site (see data analyses). The number of coral trout observed at the primary sites increased dramatically during the spawning season (Figure 2.4). Coral trout aggregated and spawned intermittently over an average period of 68.5 days ( $\pm$  10 SE, n=4 years) each year, with a range of 43 (1990) to 90 (1992) days. The timing of the formation of spawning aggregations on the two reefs was similar, generally occurring from September to November, with some variation in the onset and cessation of the spawning season. For example in 1992, the season started earlier, in August, and in 1991 the season extended into December (Figure 2.4). Note that counts at Elford Reef in 1991

**Figure 2.4** Number of coral trout at dusk at Scott and Elford Reef aggregation sites, 1991-1993. Shaded area represents total number of fish; solid line represents water temperature at 15m depth. Dark squares indicate dates of census counts. Boxed areas are dates on which spawning rushes were seen. The year of each graph refers to the spawning season, August-December. Counts continued into the new year and are presented on the same graph to demonstrate the dispersal of the aggregations.


## **ELFORD REEF**



did not start until October. Additional surveys in the 1994 spawning season, though not replicated, found spawning aggregations present in October, November and December at Elford Reef, and in October and November (December not checked) at Scott Reef (Roelofs unpublished data).

At Scott Reef maximum numbers of fish in the aggregations ranged from 63-90 in 1990 (Samoilys and Squire 1994), 73-74 in 1991, 97-128 in 1992 and 83-99 in 1993 (Figure 2.4). These peaks in trout density represented magnitude changes from pre-spawning densities of up to 13-fold in 1990, 19-fold in 1991, 16-fold in 1992, and 14-fold in 1993. At Elford Reef maximum numbers of fish in the aggregations ranged from 44 to 53 in 1991, 55 to 57 in 1992, and 50-59 in 1993 (Figure 2.4). These numbers represented magnitude changes from pre-spawning densities of up to 6-fold in 1991 and 8-fold in 1993.

In September 1992 and in August and September 1993, coral trout were tagged at the primary site on Elford Reef (Chapter Three, Samoilys unpublished), which may have affected the behaviour of fish, thus some of the visual counts during those months are not strictly comparable with data from other years, or with data from Scott Reef. Nevertheless, in both years the seasonal aggregation cycle at Elford Reef closely resembled that seen at Scott Reef. However, the maximum number of fish in the aggregations differed considerably between Scott and Elford reefs, with consistently greater numbers recorded at Scott Reef. The highest number recorded was 128 trout at Scott, in 1992, and the highest number at Elford was 59, in 1993 (densities of 75 fish/1000m<sup>2</sup> and 18 fish/1000m<sup>2</sup>, respectively).

Three successive aggregations formed at each primary site during each spawning season. The magnitude of different aggregations varied within a season, with predominantly two major aggregations and one smaller aggregation per year (Figure 2.4). This contrasts with the first year of monitoring in 1990 at Scott Reef in which one major aggregation was recorded, though a small aggregation was detected in November, and 20 trout were observed in September (Samoilys and Squire 1994).

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The majority (68%- 82%, Scott and Elford Reefs, respectively) of spawning aggregations at dusk occurred on a flooding tide, and current strength was generally low at this time (Table 2.3).

**Table 2.3** Current strength and tidal state during visual counts at dusk which recorded aggregation numbers of coral trout at Scott and Elford Reef primary sites, 1990-1993. Values represent percentages of the total number of counts. For tidal state n=37 and 22 for Scott and Elford Reefs, respectively. For current strength n=30 and 16, respectively.

	SCOTT	ELFORD
CURRENT		
Negligible	33.3	62.4
Slight	40.0	18.8
Moderate	16.7	18.8
Strong	10.0	0.0
TIDE		
Ebb	13.5	9.1
Flood	67.6	81.8
High tide	10.8	0.0
Low tide	8.1	9.1

The sizes (visual estimates) of coral trout found at the primary site when fish were aggregating ranged from 16 cm to 76 cm forklength at Scott Reef and from 16 cm to 61 cm at Elford Reef (Table 2.4). There was no evidence of a decline in average size of fish over years at either reef; the median size class was 41-45 cm FL in all years at both reefs. Similarly, the quartiles indicate the size structure of the aggregating populations was consistent over years at both reefs. However, large individuals (> 65cmFL) were only observed at Scott Reef (Table 2.4). Numbers of sub-adult fish (16 cm and 21 cm size classes) were low (1-5), and showed little variation among years at either reef except for 1991 in which there was an absence of young fish at both reefs.

**Table 2.4**. Size classes of aggregating coral trout at the primary sites at Scott and Elford Reefs. Sizes are forklengths measured in 5cm increments, thus 41 = 41 - 45 cm, 36 = 36 - 40 cm etc.

Year	SCOTT REEF					ELFORD REEF			
	n	Median	Quartiles	Range	n	Median	Quartiles	Range	
1990	680	41	36,51	16-76	-	-	-	-	
1991	395	41	36,51	26-61	229	41	36,41	31-61	
1992	652	41	36,46	16-76	388	41	36,46	16-61	
1993	452	41	36,46	21-66	241	41	36,46	21-51	

## **Temperature**

In general, the first increase in trout numbers of the season corresponded with water temperatures rising above 24.00°C as the season of southerly winds ("winter") came to an end (Figure 2.4). In 1990, 1991 and 1993 this occurred in September. In 1992 when water temperature rose earlier in August, the first aggregation of the season occurred in August (Figure 2.4). The minimum temperature of the spawning season (corresponding with the peak of the first spawning aggregation) was 24.25°C (Table 2.5). The maximum temperature of the spawning season was 28.50°C (Table 2.5), which was below the summer maximum (30°C, Australian Institute of Marine Science records, Miles Furnas pers. comm.) for the region in 1992-1993. Maximum temperature readings for the spawning season at Elford reef were 1.75 - 2.00°C lower than those at Scott Reef in 1992 and 1993 (Table 2.5); though temperatures reached 28.00°C again during the 1994 spawning aggregations (Roelofs unpublished data). The results suggest a temperature threshold of 24.00°C for the onset of spawning.

**Table 2.5** Water temperature (at 15 m depth) at the first aggregation (min) and last aggregation (max) of the spawning season (see Figure 2.4) for Scott and Elford Reef primary aggregation sites. Monitoring at Elford Reef in 1991 started late, hence the minimum temperature was not recorded. Temperature readings between reefs were taken within 1 - 4 days of each other.

	SCO	DTT	ELF	ORD
	Min <sup>o</sup> C	Max <sup>o</sup> C	Min <sup>o</sup> C	Max⁰C
1990	25.00	27.00	-	-
1991	25.00	28.50	?	28.50
1992	24.25	28.00	24.75	26.00
1993	24.50	28.00	24.50	26.25

## Lunar periodicity

The formation of coral trout aggregations was closely correlated with the new moon phase (Figure 2.4), which corresponds to lunar days 28 to 3 (Table 2.2). Occasionally aggregations persisted to lunar day 4, but numbers of coral trout were dropping by the first quarter moon phase (lunar days 6-10). If data from both reefs and three years are combined, new moon aggregations persisted for a mean of 4.7 (+ 0.45 SE, n=10) days, with a maximum of 7 days. These values are approximate due to incomplete sampling. The lunar pattern was particularly evident in the dispersal of aggregations and their subsequent re-formation through the season. The first aggregation of the season, however, was not closely confined to the new moon phase. At this time, numbers of coral trout typically began increasing on the full moon, continued to rise through the third quarter, and reached peak densities around the new moon. This pattern was seen in 1990 at Scott Reef (Samoilys and Squire 1994), in 1991 at Scott Reef, and at both reefs in 1992 (Figure 2.4). Subsequent aggregations of the season were then closely correlated with the new moon period. The lunar pattern described here cannot be more precisely defined because it was not possible to sample every day at both reefs throughout the spawning season, and sampling was not evenly distributed.

#### Diel periodicity

The data sets were small and therefore comparisons between numbers of coral trout aggregating at different times of the day should be treated cautiously. Trends in the data suggest that aggregations were not well established at dawn or through the morning, but had stabilised by the afternoon (Figure 2.5). Numbers of coral trout differed significantly at Scott Reef between morning and dusk counts (t = -4.29; df.=9;p=0.002, Figure 2.5), but differences were not significant between dawn and dusk counts (t =-4.18; df=2; p=0.05). As indicated in Figure 2.5 the aggregations had stabilised by the afternoon, and there was little difference in the total number of fish between afternoon and dusk periods (t = -0.98; df=7;p=0.36). Fewer data were available for Elford Reef because counts conducted on 6 days in 1992 when coral trout were tagged for a separate study were not used. Nevertheless, a similar trend in the data was found (Figure 2.5), though numbers of fish were not significantly lower earlier in the day compared with at dusk (morning: t = -3.31; df=4;p=0.03; dawn: t = -3.95; df=3; p=0.03 [Bonferroni adjustment gives p<0.017, see Data Analyses above]). As at Scott, the aggregation had stabilised by the afternoon and numbers of coral trout were not different to those recorded at dusk (t = 0.28; df=3; p=0.8, Figure 2.5).

#### Spawning behaviour

Spawning rushes (Samoilys and Squire 1994) between a male and female pair were observed at both primary aggregation sites in all years (Figure 2.4). A total of 94 rushes were observed; these occurred during a 22-52 minute period (average duration =  $33 (\pm 4 \text{ SE})$  min) prior to darkness, from 41 minutes prior to sunset to 19 minutes after sunset. These times are not definitive since behavioural observations were only conducted for 30 minutes after a census and observations at other times were opportunistic. However, we have never observed spawning rushes at other times of the day (Samoilys and Squire 1994, Samoilys unpublished data). Searches with torches in the dark failed to locate coral trout out in the open. Since coral trout are diurnally active and rest hidden within the reef at night (Samoilys 1987) it seems unlikely that the coral trout spawned at night. Rushes were only seen in aggregations at the primary sites (Figure 2.4), with three exceptions (see below). As rushes are completed in a matter of

seconds in low light conditions they are difficult to see. On three occasions spawning rushes were observed at the primary sites when coral trout were not aggregating: at Scott Reef in September 1991 (11 fish present at the primary site); and at both Scott Reef (3 fish) and Elford Reef (15 fish) in December 1993 (Figure 2.4). These observations did, however, all occur during the new moon phase. In fact all spawning rushes occurred around the new moon period (lunar days 28 to 3; rushes were also observed on lunar days 4 and 5 in October 1992 and 1993, Figure 2.4), with one exception. The exception was during the full moon of September 1992: spawning rushes were observed at both primary sites and the coral trout were also aggregating (Figure 2.4, see 'lunar periodicity' above). Predation on the eggs by planktivorous fusiliers (Caesio spp.) was observed 7 times during 72 spawning rushes in 1991-1993. This is a minimum estimate since this activity was not systematically recorded during these years and it is less than half the predation rate of 27% observed in 1990 when this activity was carefully recorded (Samoilys and Squire 1994).

At 1830 on 18 October 1993 approximately 20 eggs were collected by net after a single spawning rush. There were no other species spawning in the same location at that time. The eggs were returned to the laboratory where they were maintained at 27.0 - 30.5°C. By 1530 the following afternoon 10 embryos remained alive. By 0930 the next day 5 of the embryos had hatched into yolk sac larvae. Most of the yolk had been absorbed, and the larvae resembled 16 hr old coral trout larvae illustrated in NFC photographic records (M. Pearce pers. comm.). This indicates hatching occurred 23hr after spawning which corresponds with previous reports on the duration of development to hatching (18-24 hr, Rimmer et al 1994). A further three embryos hatched approximately 24 hr later giving a total of 8 larvae. On the fourth day after spawning the 8 larvae all looked the same and resembled three day old coral trout larvae with well developed eyes, chromatophores present on the myomeres at the ventral posterior end, and a distinctly large but short gut, however, the oil globule was much reduced in size (M. Pearce pers. comm., Leis 1986).

## 2.3.2 Secondary aggregation sites

Aggregations are short term behavioural phenomena, and therefore the time course of an aggregation should be monitored. This was done for the primary sites (see above),



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Figure 2.5 Mean number of coral trout on (a) Scott and (b) Elford Reef aggregation sites during aggregation periods (new moon), at different times of the day, pooled across all years. Error bars are standard errors. Dawn = <0600hrs; Morning = 0830-1230hrs; Afternoon = 1300-1600hrs; Dusk = >1630hrs.

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however, for logistic reasons it was not possible to simultaneously monitor an additional 4 -6 sites on each reef over several days. Consequently, monitoring data from the secondary sites was restricted to new or full moon periods, and then only for 2 days, though these days did coincide with days on which the primary sites were surveyed. The surveys did not reveal clear patterns in coral trout densities in relation to secondary sites. Numbers of trout were not consistently greater at the aggregation sites compared with the control sites during the new moon, as might be expected of aggregative behaviour (Tables 2.6 and 2.7). Significant differences in numbers of coral trout between secondary sites were only found at Elford Reef in 1993, where a significant interaction between site type and lunar phase was detected (Table 2.8). As was expected, the density differed between the aggregation and control sites during the new moon phase but not during the full moon (LSD pairwise comparisons, p<0.05). This provides evidence for new moon aggregative behaviour at two secondary sites at Elford Reef. It would appear that generally the secondary site definitions, aggregation and control, are not well supported by the results.

The secondary site surveys do, however, provide strong support for the new moon timing of spawning behaviour. Spawning behaviour was never seen during the full moon phase at any of the sites with one exception (Tables 2.6 and 2.7). The latter constituted one evening during the full moon in November 1993 at Scott Reef. Actual spawning rushes were never seen at the secondary sites (Tables 2.6 and 2.7). It should be noted, however, that spawning rushes are very rapid and at the primary sites were only observed after censuses during behavioural observations. The latter were not conducted at secondary sites.

**Table 2.6**Mean number (no fish 4000m<sup>-2</sup>) of coral trout at secondary sites(standard errors in parentheses) at Elford Reef. n = number of visual counts. Spawningbehaviour:  $\checkmark$  = male colours or male aggression observed;  $\checkmark$   $\checkmark$  = courtship observed, $\thickapprox$  = no spawning behaviour observed. Censuses were conducted >1630 hr.

ELFORD 1992		NEW MOON			FULL MOON			
	Site no.	n	Mean no. (SE)	Spawning behaviour	n	Mean no. (SE)	Spawning behaviour	
	1	4	9.0 (3.5)	~ ~	2	6.5 (0.5)	×	
Aggregation	2	4	2.8 (1.4)	~	4	2.0 (0.4)	×	
sites	3	3	3.0 (2.5)	~~	2	4.0 (0.0)	×	
	1	2	4.0 (0.0)	×	2	5.0 (1.1)	×	
Control sites	2	4	5.0 (1.9)	×	4	2.0 (0.4)	×	
	3	3	2.7 (0.9)	~ ~	2	0.5 (0.5)	×	

ELFORD 1993		NEW MOON			FULL MOON			
	Site	n	Mean	Spawning	n	Mean	Spawning	
	no.		no. (SE)	behaviour		no. (SE)	behaviour	
Aggregation	1	6	10.0 (3.2)	~ ~	3	4.0 (1.0)	×	
sites	3	4	11.8 (3.5)	~ ~	3	2.7 (1.4)	×	
Control sites	1	6	0.8 (0.7)	×	3	1.7 (0.3)	×	
	3	4	2.8 (1.1)	~ ~	3	1.3 (0.3)	×	

**Table 2.7**Mean number (no fish 4000m<sup>-2</sup>) of coral trout at secondary sites(standard errors in parentheses) at Scott Reef. n = number of visual counts. Spawningbehaviour:  $\checkmark$  = male colours or male aggression observed;  $\checkmark$   $\checkmark$  = courtship observed, $\thickapprox$  = no spawning behaviour observed. Censuses were conducted >1630 hr.

SCOTT 1992			NEW MO	DON	FULL MOON			
	Site no.	n	Mean no. (SE)	Spawning behaviour	n	Mean no. (SE)	Spawning behaviour	
	1	3	6.0 (1.2)	×	2	9.0 (5.0)	×	
Aggregation	2	3	2.7 (0.9)	×	2	5.5 (0.5)	×	
sites	3	2	7.5 (5.5)	~ ~	2	4.0 (0.0)	×	
	1	4	3.5 (0.3)	×	4	3.0 (1.5)	×	
Control sites	2	4	3.3 (0.9)	~ ~	4	2.3 (0.8)	×	
	3	2	8.5 (4.5)	×	2	4.0 (2.0)	×	

SCOTT 1993			NEW MC	DON	FULL MOON		
	Site no.	n	Mean no. (SE)	Spawning behaviour	n	Mean no. (SE)	Spawning behaviour
Aggregation	1	8	11.4 (3.0)	~ ~	4	7.3 (1.4)	~ ~
sites	3	7	6.0 (1.1)	~	3	3.3 (2.0)	×
Control sites	1	8	3.6 (1.0)	×	5	7.0 (0.7)	×
	3	8	2.6 (1.0)	×	3	5.7 (1.5)	×

**Table 2.8**Results of mixed model analyses of variance (ANOVA) tests for theeffects of lunar phase (A) and site type (aggregation or control, B) on numbers of coraltrout. Probability: NS = not significant (p > 0.05), \* = p < 0.05.

Reef and year	Source of	Degrees	Mean	F Ratio and
	variation	of	Square	probability
		Freedom		
Elford 1992	A. Lunar phase	1	0.6845	0.69 NS
	B. Agg/Control	1	0.4361	0.44 NS
	AxB	1	0.1566	0.16 NS
	Residual	8	0.9895	
	Sampling error	25	0.4549	
Elford 1993	A. Lunar phase	1	3.4426	8.59 *
	B. Agg/Control	1	12.5009	31.20 *
	AxB	1	4.7674	11.90 *
	Residual	4	0.4006	
	Sampling error	28	0.6942	
Scott 1992	A. Lunar phase	1	0.2435	0.46 NS
	B. Agg/Control	1	1.9051	3.57 NS
	A x B	1	1.1485	2.15 NS
	Residual	8	0.5340	
	Sampling error	22	0.5583	
Scott 1993	A. Lunar phase	1	0.3240	0.29 NS
	B. Agg/Control	1	5.3659	4.88 NS
	A x B	1	5.2853	4.81 NS
	Residual	4	1.0998	
	Sampling error	38	0.5981	

## **2.4 Discussion**

A consistent spatial and temporal pattern in the formation of coral trout spawning aggregations was observed at both Scott and Elford reefs. A primary aggregation site was located at each reef, defined as a site at which coral trout aggregated in large numbers. Surveys of surrounding reef areas at Scott and Elford (that covered 26-36% of each reef) failed to locate other primary aggregation sites, though apparent smaller, secondary sites were found. The surveys of secondary sites revealed that the definition secondary "aggregation site" was not well supported. Spawning behaviour was observed at both secondary aggregation sites and control sites, densities were generally not greater at aggregation sites during the new moon phase, and the densities of coral trout never exceeded average densities recorded at each reef during the non – spawning season, therefore they could not be defined as aggregating. The maximum number of coral trout seen at one of the secondary aggregation sites was 20, equivalent to 5 fish  $1000 \text{ m}^{-2}$ . However, the surveys do confirm the occurrence of spawning behaviour outside the primary aggregation sites. Samoilys and Squire (1994) suggested that P. leopardus spawns at multiple sites, based on the relatively low numbers of coral trout that aggregated at the primary site on Scott Reef in 1990, but that reefs off Cairns may support only one or two primary sites, with several secondary sites. The results of the present study suggest that the spawning activity of P. leopardus was concentrated at primary sites where coral trout employ aggregative spawning, but spawning also occurs at other, secondary sites, though the stability of these sites remains questionable.

The location of each of the two primary spawning aggregation sites was stable over four and three years, respectively. At both reefs the primary sites were well defined areas which did not vary either intra- or inter-annually. This contrasts with another serranid *Epinephelus guttatus*. In Puerto Rico that species has a large and well defined aggregation site, but specific spawning sites within the area varied both intra- and interannually (Sadovy et al 1994b, Shapiro et al 1993a). The aggregation pattern at the primary sites was highly consistent, both spatially and temporally. I suggest that this may reflect physical parameters that govern a site's suitability for spawning, such as reef slope profile, current regime, and the location of the site in relation to the rest of the reef and deep water. The primary sites may represent a combination of several of such physical factors, and are therefore highly preferred or suitable spawning sites. Both the primary sites in the present study are relatively exposed reef edge sites neighbouring deep water. Such physical characteristics have been discussed in relation to spawning sites previously (eg. Johannes 1978), but, as in the present study, they have not been systematically quantified (but see Shapiro et al 1988). This is difficult to do but is necessary if we are to test the hypothesis that such physical parameters are important factors for spawning fishes.

A consistent temporal pattern in the formation of spawning aggregations at the primary sites was recorded at Scott and Elford reefs, from 1991 to 1993. P. leopardus aggregated and spawned around three consecutive new moons within the period August-December, each year, and consistently during the new moon of October. In addition, all spawning behaviour at secondary sites was observed during the new moon phase with one exception. The results do not preclude spawning at other times. Though rare, spawning rushes were observed at the primary sites during an aggregation that occurred around a full moon and when there was no aggregation present. In addition, there was evidence that the first aggregation of the season was less tightly coupled with the new moon phase; the reason for this remains unclear. Nonetheless, the new moon timing of the primary spawning aggregations was unmistakable. Lunar periodicity of spawning is common in a number of reef fish species, but appears largely limited to pelagic spawners that migrate to spawn (Sadovy 1996). For example, the Nassau grouper, probably the most studied epinephelid, aggregates and spawns on two consecutive full moons each year in the Bahamas (Colin 1992). The possible advantages of lunar spawning cycles for larval survival (Shapiro et al 1988, Johannes 1978), synchronising adult behaviour (Robertson 1991, Robertson et al 1990, Colin et al 1987), and the proposal that aggregations may play an important role in mate selection and sex change (Shapiro et al 1993b) have been discussed for *P. leopardus* (Samoilys and Squire 1994). The relevance of such hypotheses to the reproductive strategies of coral trout requires further investigation into the mechanisms of sex change.

Although the present study does not unequivocally show that spawning only occurred around the new moon, the majority of observed spawning events were clearly linked to the new moon phase. Lunar periodicity of spawning was not detected in histological

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examinations of gonads of P. leopardus (Ferreira 1995) or the congeneric P. maculatus (Ferreira 1993). Ferreira (1995) suggested that the new moon may represent a peak in spawning activity, but that coral trout spawn throughout the three month season. In her analysis Ferreira (1995) used standard histological staging, and defined spawning females as those with 'oocytes in late stages of vitellogenesis from tertiary yolk globule stages to hydrating stages'. I suggest that this stage probably combines both imminent spawners and pre-spawners (Chapter Four). Furthermore, Ferreira (1995) noted that 'running ripe' females (imminent spawners) were only found late in the afternoon during the new moon phase, although she was unable to quantify the observation. Unlike the present study, Ferreira (1995) was unable to stratify her sampling temporally within the reproductive season because she largely relied on fishers's catches. Sadovy et al (1994b) showed that although mature (ripe) ovaries of Epinephelus guttatus were present for a three month period, spawning activity was limited to only about two weeks, based on the frequency of hydrated oocytes. New moon spawning in coral trout is further corroborated by the timing of larval supply and juvenile settlement of P. leopardus measured in 1990 at a neighbouring reef (Doherty et al 1994). Larval abundance peaked around the new moon, mean pelagic larval duration was 25 days indicating birth was close to the previous new moon, and back-calculated ages from juvenile coral trout confirmed both these birth and settlement times (Doherty et al 1994).

The present study was unable to verify whether all coral trout used aggregations to spawn. Observations of spawning behaviour likely to result in spawning rushes (though these were never seen) at secondary sites where coral trout did not aggregate, suggests that aggregative spawning at primary sites is not the only mode of spawning. Further, the low numbers of coral trout in primary aggregations suggests that only a small proportion of the population are using these primary sites. This question was investigated in Chapter Three. In addition, although the new moon timing of aggregative spawning is clearly demonstrated here, coral trout may employ other spawning mechanisms at other times which would not have been detected in the present study. This question was examined through histological examination of gonadal maturity and development (Chapter Four). Spawning occurred in water temperatures between 24.25°C and 28.50°C; the annual range of the region is 22 - 30°C (Australian Institute of Marine Science records, Miles Furnas pers. com.). Possibly the increase in water temperature after austral winter triggers the onset of the spawning season in *P. leopardus* on the Great Barrier Reef. Evidence from the western Atlantic, Caribbean and Bahamas suggests the timing of spawning in serranids is temperature related (Sadovy 1996). Those species that occur in cooler latitudes tend to spawn during summer, whereas those in warm or low latitudes spawn at temperatures below the annual maximum (Sadovy 1996, Thresher 1984). Further evidence of the role of temperature is seen in the spawning of *Epinephelus striatus*, the Nassau grouper, which is confined to a narrow temperature range of 25-26°C (Tucker et al 1993, Colin 1992).

Aggregations of coral trout persisted for around five days over the new moon, although the fish were not at the primary sites throughout the day. Numbers of coral trout were low in the morning, and the aggregations did not stabilise until after 1300hrs, therefore surveys to monitor spawning aggregations must be conducted in the afternoon. The low numbers of fish at the primary sites in the morning suggest that the coral trout may hide within the reef, or disperse during that time. Since aggregations occurred for several days and feeding studies have shown that coral trout consume an average of one prey item daily (St John 1999), I propose that the fish disperse to feed. This assumes that aggregations on successive afternoons consist of the same individuals. Tagging at Elford Reef provides some evidence for this (Chapter Three). Numbers of fish were also lower at dawn compared to dusk. Coral trout are diurnally active and rest hidden within the reef at night (Samoilys 1987). Since the fish were aggregating until dark they were likely to remain at the aggregation site over night, and therefore would not start to disperse until daylight. No spawning behaviour was seen at dawn; dawn spawning is not known in serranids (Sadovy 1996).

Spawning rushes were only observed during a discrete (~30 min) period spanning sunset. Ferreira (1995) noted that 'running-ripe', i.e. ovulated female *P. leopardus* were only captured late in the afternoon. A similar narrow time frame (20 min) around sunset is reported for *Epinephelus striatus* (Colin 1992) and for *Mycteroperca tigris* (Sadovy et al 1994a). Dusk spawning is common in many pelagic species especially the Serranidae

(Sadovy 1996). Reduced risk of predation from diurnal egg-predators has been proposed to explain dusk spawning, but this has not been tested (Robertson 1991, Shapiro et al 1988). Planktivorous fusiliers (Caesio spp.) were observed feeding on eggs from coral trout spawning rushes up to 27% of the time. It is possible that changing light levels might provide cues for the initiation of the final stages of egg maturation (Hoffman and Grau 1989). The behaviour of the coral trout indicates it may be the females that govern the timing of spawning because the males court over a large portion of the day but females only respond at dusk (Samoilys and Squire 1994, Chapter Four). The dusk spawning pattern suggests tide is not a significant environmental cue for coral trout spawning. In contrast, diurnal spawners, such as parrotfishes, wrasses and surgeonfishes, often vary their time of spawning in response to tide (Colin and Bell 1991, Hoffman and Grau 1989, Thresher 1984, Choat and Robertson 1975). Strong ebbing tidal currents have been considered important for carrying eggs away from the reef to avoid predation (Johannes 1978), though this has not been demonstrated empirically (Shapiro et al 1988). Although spawning aggregations of coral trout occurred during the relatively stronger spring tides (new moon), the majority of spawning events occurred on flooding tides and when currents at the primary sites were rarely strong.

The rearing and identification of coral trout larvae from gametes collected from a pairspawning rush supported the assumption that coral trout spawning rushes are spawning events (Samoilys and Squire 1994). In some fishes spawning behaviour does not always result in the emission of gametes. Such "pseudospawning" has been observed, though infrequently, in the demersal spawner *Stegastes partitus*, possibly to test male readiness, or as a means of gaining access to an egg meal (Cole and Sadovy 1995, Cole pers. comm.). It seems unlikely that the spawning rushes of coral trout were "pseudorushes" because they were only observed within a narrow time frame at sunset, whereas males courted over a large portion of the day. The occurrence of planktivorous caesionids feeding at the peak of some spawning rushes also confirms the presence of eggs.

Both reefs in the present study are open to fishing and therefore fishing may have affected the spawning aggregations, particularly the numbers of coral trout aggregating. This is impossible to assess properly without data collected prior to fishing or over many years; the following conclusions are therefore tentative. Despite local concern that fishing pressure was escalating, particularly in response to the new live fish export fishery (Elmer 1998, Richards 1993), numbers of coral trout aggregating at Scott Reef did not decline over four years, with similar densities measured for a fifth year in 1994 (Roelofs, unpublished data). In addition, there was no decline in average size of fish or numbers of large individuals over time. Nor was there any evidence of an increase in numbers of small individuals over time. However, the difference in the density and maximum size of aggregating coral trout between the two reefs was notable, with numbers and maximum sizes consistently lower at Elford Reef. Clearly nothing can be concluded in a comparison between two reefs. However, general observations of local fishing practices at Elford Reef, including the occurrence of commercial fishing boats at the primary site, suggested that this site was targeted more than that at Scott Reef, which could explain the lower densities and lack of large (>65cmFL) fish at Elford Reef. Subsequent monitoring of the two primary aggregations on the October new moon only has shown that the aggregation at Elford Reef did not form at all from 1996 -1998, with targeted commercial fishing implicated as the cause (Samoilys et al in prep.).

#### **Management Implications**

From a fisheries management perspective, it is clear that the temporal and spatial predictability of coral trout spawning aggregations makes them potentially vulnerable to overfishing, a feature that has led to the disappearance of plectropomid spawning aggregations in the Pacific Islands (Johannes et al 1999). Fishing effort on the Great Barrier Reef is likely to be increasing through new commercial ventures, an increasing recreational sector, and improved navigational equipment. Possibly *P. leopardus* is less vulnerable to over-fishing from targeting spawning aggregations compared with other groupers (eg. *P. areolatus*, Johannes et al 1999; *Mycteroperca tigris*, Sadovy et al 1994a; *Epinephelus striatus*, Sadovy 1994b), because aggregations are relatively small in magnitude, they appear to disperse in the mornings, and spawning does occur at other sites. The importance of spawning at sites other than the primary sites to the overall

present dataset. Chapters Three and Four address this question. It has been argued that the protogynous reproductive strategy of groupers makes them more resilient to exploitation than gonochoristic populations. The suggested compensatory mechanism is a flexible social organisation which enables them to maintain high spawning success, for example by changing sex earlier (Bannerot et. al 1987). However, even if these mechanisms exist they evidently break down at high fishing mortalities because sperm becomes limited (Vincent and Sadovy 1998, Koenig et al 1996, Bannerot et al 1987, Garratt 1986). The existence of a considerable and apparently non-declining commercial fishery of coral trout on the GBR (Mapstone et al 1996) supports the notion that coral trout stocks may be resilient to fishing pressure. However, our understanding of compensatory mechanisms is still poor. Sadovy (1996) suggests that the social cues required to effect compensatory sex change may be disrupted when spawning aggregations are targeted by fishers. Although the temporal and spatial predictability of coral trout spawning aggregations makes them vulnerable to fishing, it allows for specific seasonal or spatial closures and the opportunity for precautionary management strategies that simultaneously protect aggregations for further research.

# Chapter Three: MOVEMENT AND DISTRIBUTION OF CORAL TROUT IN RELATION TO SPAWNING AGGREGATIONS

## **3.1 Introduction**

The regular and predictable occurrence of seasonal spawning aggregations of *P. leopardus* over a 3-4 year period on the northern Great Barrier Reef has been clearly demonstrated (Chapter Two, Samoilys and Squire 1994). A separate study has determined that these aggregations have persisted at the same sites for at least 9-10 years (Samoilys et al in prep.). However, despite a considerable body of work on the biology of *P. leopardus* (Russ et al 1996, Ferreira 1995, Ferreira and Russ 1995, 1994, Doherty et al 1994), the role that spawning aggregations play in the species' annual reproductive effort is not known.

Of particular interest in terms of assessing the significance of targeted fishing on aggregations, is what proportion of the coral trout population use spawning aggregation sites to spawn, particularly since the numbers of coral trout aggregating per site are not great (44-128 fish, Chapter Two). If the latter is an indication that only a small proportion of the population aggregates to spawn, then the protection of spawning aggregations as a means of protecting spawning stock biomass (i.e. reproductive potential) is probably less critical. Similarly, little is known of the catchment or source area of aggregations, ie. how far coral trout will migrate to an aggregation site (but see Zeller 1998). In addition, the frequency with which individual fish revisit the aggregation sites is poorly understood. For example, do individuals use aggregation sites repeatedly through the season? Do individuals spawn more than once per night, and do they spawn on several consecutive nights? These questions address the issue of how important a particular spawning site is to the local population, and hence how it should be managed. Zeller (1998) has provided preliminary information to these questions by tracking the movement of eight male and four female P. leopardus in relation to spawning aggregation sites at Lizard Island on the northern Great Barrier Reef. The management of spawning aggregations, both spatially and temporally,

requires a thorough understanding of these issues, especially the significance of aggregative spawning to the coral trout population as a whole.

To examine the significance of spawning aggregations to the local population, I mapped the distribution and density of coral trout in a section of Elford Reef containing the primary aggregation site (Chapter Two) prior to the spawning season. This information, combined with the regular monitoring of densities of coral trout in aggregations (Chapter Two) provided an estimate of the proportion of the population aggregating to spawn. To address the questions of catchment area, movement and visitation to spawning aggregations by *P. leopardus* I tagged individuals at Elford Reef to track their movements and behaviour.

#### 3.2 Methods

There were two components to the study. Firstly, the density and distribution of coral trout trout in a 6.5km<sup>2</sup> area surrounding the primary site, was mapped to determine what proportion of the coral trout population on Elford Reef was aggregating at the primary aggregation site (section 3.2.2). Secondly, a tagging program was established within this area, to track the movements of coral trout in relation to the primary aggregation site (section 3.2.3). This estimated the distance travelled to aggregations or "source area" of a single aggregation, the frequency of visits to aggregations by individual fish, and provided information on individual spawning behaviour.

## 3.2.1 Study sites

Investigations into the distribution of coral trout in relation to spawning aggregations and the movements of individuals to aggregation sites were conducted on Elford Reef (16°55'S, 146°15'E), in conjunction with observations of spawning behaviour (Chapter Two). Tagging was focussed on and around a major (primary) spawning aggregation site. Full details of the primary site are given in Chapter Two. For control purposes this work was not conducted at Scott Reef because of the manipulative nature of tagging.

## 3.2.2 Distribution and density mapping

I mapped the distribution and density of coral trout over a 6.5 km<sup>2</sup> area surrounding the primary site at Elford Reef in August 1993 prior to the spawning season (Figure 3.1). The reef area was mapped from aerial photographs taken from an altitude of 1,524 m (5000 ft), giving a scale of 1:10000. Photographs were purchased from AUSLIG and referenced into a GIS package (ArcInfo), courtesy of the Great Barrier Reef Marine Park Authority. Calculations of reef areas were done in ArcView 3.1. The study area represented approximately 31% of Elford Reef and consisted of submerged reef habitat to 20m depth (Figure 3.1). The study area was categorised according to dominant strata based on orientation to the dominant trade winds, and habitat type (e.g. lagoon, slope). Habitats were further categorised into sub-habitats based on reef structure, which related to channels or breaks in the reef slope (Table 3.1, Figure 3.1). Thus, the survey area was prepared for stratified random sampling (e.g. McCormick and Choat, 1987) using underwater visual census surveys.

Coral trout densities in the 6.5km<sup>2</sup> study area were surveyed using stationary point counts for their ease and speed of deployment (Samoilys and Carlos 2000, Samoilys 1997b, Bohnsack and Bannerot 1986). Stationary point counts are a standard technique for estimating densities of reef fishes, and have been shown to be as accurate and as powerful as strip transects for serranids (Samoilys and Carlos 2000). Coral trout are not diver-shy on the Great Barrier Reef, and are easily counted, though, as with most visual census methods, their densities are underestimated (Samoilys and Carlos 1992). The sampling design (Table 3.1) fell between the simple random and optimal (or Neyman) sampling designs described by McCormick and Choat (1987). Optimal sampling requires prior knowledge of coral trout density per habitat category, and the proportion each habitat contributes to the total survey area. In this case the latter was known but not the former. The sampling design in Table 3.1 involves more sampling effort in the more complex habitat strata (Front Slope), but within the smallest habitat unit, sampling effort was equal with two study sites (a,b) and 12 replicates. The two study sites in each habitat type were Figure 3.1 Visual census sites at Elford Reef for mapping density and distribution of coral trout prior to spawning. FSPb etc = site names which relate to habitat strata(see Table 3.1): F=reef front (windward), B=reef back (leeward), M=mid reef, C=channel, B=bommies, L=lagoon, S=slope, P=spur (on slope). a & b = replicate visual census sites. FC = primary spawning aggregation site.



located haphazardly. Sites were approximately 200-300m long, and limited to 60m in width and were marked with surface buoys (Figure 3.1). The channel site listed as FC is the primary spawning aggregation site that had been monitored for total numbers of coral trout since 1991 (Chapter Two). Since there was only one study site in the front channel habitat, this site was surveyed twice. The level of replication for *P. leopardus* was determined from a previous study (Samoilys and Carlos 2000). Thus, a total of 180 censuses across 15 sites in the 6.5 km<sup>2</sup> area was used to estimate coral trout population size.

A 10m radius census area for the point counts was selected, because water visibility and the visibility of coral trout both allow a large census area, which is preferable considering the size of coral trout, their relatively low density, and patchy distribution (Samoilys and Carlos 2000, Samoilys 1997b). The method used followed that of Samoilys and Carlos (2000): the census area was estimated by eye and then two radii were measured after each count to derive the actual area censused; census counts were standardised to 2 minutes, and no fish that entered the census area after the start of the census were counted. Replicate census counts were placed haphazardly within each site, using two separate anchorages. i.e. six point counts per anchorage, and distances between counts were swum using a randomly selected time. Census sites varied in depth from 2 m to 22 m. All coral trout were counted, and their forklengths (FL) estimated in 5cm size classes.

All censuses were conducted in the day between 0900 and 1700. Sites were surveyed between 17<sup>th</sup> and 25<sup>th</sup> August 1993, prior to the onset of the spawning season in September, as determined from monitoring coral trout densities at the primary site (Chapter Two) and gonad assessment (Chapter Four). Where census sites corresponded with tagging locations (see section 3.2.3), surveys were conducted prior to tagging except at the primary aggregation site. Here, point counts were done one week after tagging, which was considered to be enough time to allow the fish to settle after the disturbance of fishing.

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**Table 3.1** Sampling design and sites used in pre-spawning visual surveys of coral trout density and distribution at Elford Reef (see also Figure 3.1).  $1^{\circ}$  = primary spawning aggregation site.

STRATA	Front (F)					Back	к (B)			Mid	(M)				
HABITAT	Slope (S)		Channel (C)	Slope (S)		Bommies (B)		Lago (L)	oon	Flat	(F)	Chanr	nel (C)		
SUB-HABITAT	Slope	e (S)	sPur	(P)											
SITES	a	b	a	b	1°	a	b	a	b	a	b	a	b	a	b
REPLICATES	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12

## 3.2.3 Tagging

Coral trout were tagged at the primary site at Elford Reef in 1993 (Figure 3.2). Three different tags were used: conventional blue plastic dart tags, blue plastic T-bar tags (developed by the clothing industry), and freeze brands. The latter enable fish to be recognised individually underwater, though are only effective for periods of up to around 4 months (Samoilys 1997a). They were used in this study for intra-annual returns over the spawning season (~3 months). Previous tagging work on coral trout at the Northern Fisheries Centre has found dart tags to be effective for at least 1-2 years (pers. obs.). These tags were therefore used for longer – term returns. T-bar tags (used in the clothing industry), another effective tag for coral trout (Davies 1996) were used as a back-up. Both the dart and T-bar tags also provided a means of assessing tag loss in the freeze brands, which was found to be negligible during the four month period in which the underwater resightings were obtained.

Tagging was conducted mainly at the primary site on Elford Reef, however, a small number of fish was also tagged at sites approximately 0.5km and 1.0km distant from the primary site, on the windward face of the reef (Figure 3.2), in an attempt to obtain additional information on fish movements around the primary site. Tagging took place between 16/08/93 and 4/10/93 and coincided with the start of the spawning season on the new moon in September (14/09/93).

## **Resightings**

Resightings of tagged fish were obtained either from visual census counts at the primary site conducted weekly or fortnightly from August to December (see Tables 2.1 and 2.2 in Chapter Two) or during searches on snorkel and SCUBA in other areas. These included those sites where fish were tagged, including the primary site (Figure 3.2), and in areas radiating out from the primary site, such as the front slope (FS), channel (MC), the mid flat (MF) and the lagoons (ML, Figure 3.1); a total area of 2.4 km<sup>2</sup>. Searches were conducted over a two – month period (13<sup>th</sup> October – 14<sup>th</sup> December 1993), for 16 days. Searches were spread across the new moon periods of October (4 days), November (4 days) and December (3 days), with additional searches conducted during the full moon period in October (3 days), and the first and third quarter periods in

**Figure 3.2** Tagging locations at Elford Reef. Nomenclature refers to locations in relation to the primary aggregation site, P. FSW0.5 = front slope 0.5 km west of P; FSE1 = front slope 1km east of P, etc.



November (2 days). A small number of tag returns were also obtained from fishers. On 15<sup>th</sup> November 1993 (new moon) five tagged fish were speared on the primary site because their brands had faded beyond recognition and positive identification was required for any previous resightings of these individuals. In addition 17 fish were recaptured between September and December 1994, 10 by fishers and the rest for gonad assessment (Chapter Four). Searches for tagged fish at the primary site were conducted over 3 days during each new moon period in October – November, when aggregations lasted for an average of 4.7 days (Chapter Two). The searches at the primary site were the only searches with which a lack of observation is strong evidence the fish was not at the primary site. The same cannot be said for the other searches which were over much larger areas and were made largely on snorkel.

There is an important distinction between tag resightings and conventional fisheries tag returns. The latter usually represent a dead fish and therefore there can only be one return per individual. All "tag returns" in this study are referred to as resightings, and those individuals that were fished and killed are specified. Thus resighting data can represent multiple resightings of the same individual.

The catchment or source area of the aggregation site was estimated by joining the most distant location points of resighted tagged fish to form a convex polygon which was then measured in ArcView. The convex polygon method is a standard method for calculating home ranges (Samoilys 1997a, Sukumar 1989, Anderson 1982, Odum and Kuenzler 1955). Assumptions followed those of Samoilys (1997b) in an earlier study on coral trout movement: resighting locations were considered to be independent (Swihart and Slade 1986) and all resighting locations were used (cf. Schoener 1981). In the present situation the convex polygon method estimates the largest area from which coral trout were drawn to the primary site, as indicated by resighting locations, and is limited more by search effort and ability to locate tagged fish than the method's assumptions.

## 3.2.4 Spawning behaviour of known individuals

The behaviour of branded individuals was noted whenever SCUBA observations were conducted on the primary site, predominantly during visual census surveys (Chapter Two), but also during the searches of the present study. After the visual census surveys, a branded individual was located and then its behaviour observed for 15 minutes. The occurrence of spawning behaviour (eg. males in spawning colours, courting, rushes or male aggression, see Samoilys and Squire 1994) was noted together with the time. In addition, the location and size of male territories were marked on underwater maps of the primary site (see Chapter two).

#### 3.2.5 Data analyses

Densities were scaled up to per  $1000m^2$  from the point count census area of  $\sim 314m^2$  for ease of presentation, and because each census area varied slightly since radii were estimated visually and then measured after the count (see above, Samoilys and Carlos 2000). The densities of coral trout in the eight habitats were compared using a One-Way Analysis of Variance (Zar 1996, Underwood 1981), with replicate sites (a and b) within habitat blocked as experimental units. Homogeneity of variances was examined using plots of residuals; data were not transformed.

#### **3.3 Results**

## 3.3.1 Distribution and density of coral trout

The density of coral trout varied considerably between habitat types in the 6.5 km<sup>2</sup> survey area (Figure 3.1), ranging from 0.8 to 4.1 fish  $1000m^{-2}$  (Table 3.2); and a size range of 16 - 65 cm FL.

**Table 3.2** Density and total number of coral trout in the survey area surrounding the primary aggregation site at Elford Reef in August 1993. Habitat abbreviations as in Table 3.1 and Figure 3.1. Note FC is the primary aggregation site. Adult coral trout refers to fish >25cm FL (> 50% of females are reproductively mature, see Chapter Four). Standard deviations are given in parentheses.

Habitat	Mean no	Mean no adult	Habitat	Total no	Total no
stratum	trout	trout	area	trout	adult trout
	$(1000 {\rm m}^{-2})$	$(1000 {\rm m}^{-2})$	(km <sup>2</sup> )	per habitat	
BB	2.26 (2.20)	2.12	0.9	2030	1911
BS	0.80 (1.69)	0.80	1.06	844	844
MF	2.79 (2.87)	2.26	2.56	7134	5775
ML	2.65 (3.60)	2.39	1.17	3105	2795
MC	1.19 (2.06)	1.06	0.32	378	336
FSS	4.11 (3.32)	3.32	0.45	1851	1493
FSP	3.85 (3.38)	3.58	0.04	154	143
FC	1.73 (2.09)	1.73	0.0032	6	6
total			6.5	15502	13303

Despite high variation in density between habitats (Table 3.2), these differences were not significant at p < 0.05 (Table 3.3). High residuals caused by some replicate counts in the front slope and mid lagoon habitats (illustrated by high standard deviations, Table 3.2) are likely to be the reason for the lack of statistical significance. The purpose of the pre-spawning surveys, which were stratified by habitat, was to estimate the total population of coral trout in the study area surrounding the primary site. Therefore, mean densities were calculated by averaging across both replicate study sites (n=24 replicates, see Table 3.1) to obtain the best available estimate of total population size per habitat. This gave a total population for the whole area of 15,500 coral trout or 13,300 adult coral trout (Table 3.2). It should be noted that these calculations assume habitat homogeneity within the strata defined and that the number of study sites (two) per habitat type was adequate. Since intra-habitat type variation in coral trout density was high, at least in certain habitat types (Table 3.2), the derived total population densities are likely to be approximate.

**Table 3.3** Results of One –way ANOVA comparing coral trout densities between habitat types. See Table 3.1 for habitats. NS = non significant (p > 0.05).

Source of	Degrees of	Mean Square	F Ratio and
variation	freedom		probability
Habitat	7	33.42	2.24 NS
Residual	8	14.90	
Sampling error	176	7.16	

Broad-scale searches for spawning aggregation sites in 1992 which covered the  $6.5 \text{km}^2$  study area (see Chapter Two, Figure 2.3) did not locate any other primary aggregation sites. In addition, searches for tagged fish (see below) did not locate any other spawning aggregations in the 6.5 km<sup>2</sup> study area, except for one apparent site at FSE0.9 – approximately 900 m east of the primary site on the front edge of the reef (Figure 3.2). On 14<sup>th</sup> November (new moon) 16 trout were counted at this location, and courting and male aggression were observed. Two tagged fish were also seen in this group of coral trout. These results indicate that the primary aggregation site identified at Elford Reef was probably the only primary site in an area which contained 13,300 adult coral trout.

## 3.3.2 Intra-annual resightings of tagged coral trout

One hundred and thirty three coral trout were tagged in 1993. Four fish died during or shortly after tagging giving 129 fish released successfully. Of the 129 fish that were successfully tagged and released in 1993, 83 were resighted and positively identified (Table 3.4), an overall recapture rate of 64%.

**Table 3.4** Location, date and the number of coral trout tagged and released, andresighted in 1993 at Elford Reef. The number of individual fish resighted at the primarysite is a sub-group of the total number of fish resighted.

Tagging	Number	Dates tagged	Number of fish	Number of
location	of fish		resighted	fish resighted
	tagged		(recapture	at primary
			rate)	site
Primary site	79	17,24-26 Aug; 15-17	55 (70%)	55
	-	Sep		
FSE.5	15	16, 25 Aug; 16 Sep; 4	7 (47%)	0
		Oct		
FSE1	7	16 Sep; 4 Oct	6 (86%)	5
FSW.5	11	17,25 Aug; 15 Sep; 4	4 (36%)	2
		Oct		
FSW1	17	25 Aug; 14,15 Sep; 4	11 (65%)	5
		Oct		
TOTAL	129	17 Aug – 4 Oct	83 (64%)	67

A total of 194 tag resightings of the 83 fish (Table 3.4) were recorded between  $17^{th}$  August and  $14^{th}$  December 1993; a further 10 were obtained in September – December 1994. There were 137 resightings of the 55 fish that had been tagged at the primary site, of which 10 resightings were not at the primary site (Figure 3.3). Fifty-three of these fish were only resighted at the primary site. This suggests that, despite intensive searches for tagged fish in an area of 2.4 km<sup>2</sup> around the site (see Methods), the fish either moved beyond this area, were missed during the searches, or both. The 10 resightings that were not at the primary site were of seven fish; one fish was resighted at three different locations (brand # 85). Five of these seven fish (including # 85) were also resighted at the primary site.

**Figure 3.3** Location of resightings of coral trout tagged at the primary spawning aggregation site at Elford Reef in 1993. Fish tagged at other locations that were resighted at the primary site at least once are also shown (see source area estimation in text). P = primary spawning aggregation site; FSW1, FSW.5 etc = other tagging locations (see Figure 3.1). Fish tagged at different sites were colour coded as follows: P = Red; FSW1 = Pink; FSW.5 = Brown; FWE1 = Yellow. Numbers of fishes resighted were coded using the following symbols:  $\Omega = 1$  fish;  $\Box = 2$  fish; O = 5 fish;  $\swarrow = 50$  fish.


## Other tagging locations

There were 18 resightings of the seven fish from tagging site FSE.5, none of which were at the primary site. Thirteen resightings of four fish were at the tagging site, FSE.5, and each fish was seen during both new moon and full moon periods. The other five resightings of three individuals were at different locations. A maximum distance travelled of 0.5 km was recorded. No spawning behaviour was observed at FSE.5. The only spawning behaviour seen in this group of fish was in brand # 31 which was seen in male colours and courting (see Chapter Two for definitions) at a location 200m east of FSE.5.

There were 17 resightings of the six fish from site FSE1, of which all but one were at different locations. Notably, 13 resightings were at the primary site, represented by five individuals (Figure 3.3). A maximum distance travelled of 2.1 km was recorded.

There were four resightings of the four fish from site FSW.5, of which two were at the primary site (Figure 3.3), and two were at the tagging site representing a maximum distance travelled of 0.5 km.

There were 29 resightings of the 11 fish from site FSW1, of which 16 (five fish) were at the primary site (approximately 1 km distance, Figure 3.3), three were 100 m west of FSW1, and two individuals were resighted 1.4 km and 5.1 km from FSW1 (not shown in Figure 3.3 because they were not resighted at the primary site).

Since this study was concerned with the movement of coral trout in relation to the primary spawning aggregation site and their use of that site, the following analyses focus on those fish that were located at the primary site at least once (Figure 3.3). Thus, those fish that were tagged elsewhere but were resighted at the primary site are included in these analyses: five fish from FSE1, two fish from FSW.5, and five fish from FSW1, giving a total of 67 fish (Table 3.2).

The locations of the resightings of the 67 fish that moved either to or from the primary aggregation site represented an area around the primary site of 1.5 km<sup>2</sup>, approximately 7% of Elford Reef, and a maximum distance travelled to/from the primary site of 1,200m (Figure 3.3). Estimation of this catchment area was constrained by search effort and ability. Since the search area was larger (2.4 km<sup>2</sup>) than the catchment area, the result suggests that search effort was spread over an area large enough to incorporate the catchment area. However, the catchment area calculation also assumes that search effort was equal in those areas where the most distant locations were recorded (it is irrelevant that effort was greater at the primary site within the catchment area, since this location does not define the catchment area). The most distant locations were recorded at: (i) the front slope, both west and east of the primary site; (ii) the mid channel; (iii) the western mid flat; and (iv) the eastern mid lagoon (Figure 3.3). Although search effort was similar in the front slope and channel habitats, it was less in the mid flat and lagoon habitats (Table 3.5). Further, search effort was minimal in the eastern mid flat and the back slope habitats and no searches were conducted in the back bommies and western mid lagoon. Thus, despite the search area being larger than the estimated catchment area, it is quite possible that tagged coral trout from wider or different habitats were not detected. Further, the catchment area was necessarily limited by the extent of the search area, and fish may have moved beyond the search area.

**Table 3.5** Search effort expended for locating tagged coral trout, by broad habitat area. Search effort is given as a percentage of the total search effort . FS = front slope, MC = mid channel, MF = mid flat, ML = mid lagoon, BS = back slope (see Table 3.1 and Figure 3.1 for habitat definitions). West and east are in relation to the primary aggregation site, P.

	FS west	FS east	MC	MF west	MF east	ML east	BS east
Search effort	23%	27%	20%	14%	5%	9%	2%

If the coral trout population densities per habitat type (section 3.3.1) are applied to the  $2.4 \text{ km}^2$  search area around the primary aggregation site, there were an estimated 6,023 coral trout, or 4,986 adult coral trout in the area searched. Using similar calculations, the catchment area of  $1.5 \text{ km}^2$  equates to an estimated total population of 3,828 coral trout or 3,169 adult coral trout.

#### Frequency and timing of resightings

The frequency of resightings per individual ranged from 1 to 12 for fish tagged and resighted at the primary site (Figure 3.4). A similar range of resightings, from 1 to 11 was obtained from the 12 fish resignted at the primary site but tagged elsewhere (Figure 3.4). The mode of resightings was 1 rather than 0, which indicates a degree of fidelity to the primary site. The large percentage of resignted fish that were only seen once suggests that there was considerable turn-over at the aggregation site and that coral trout were not likely to be seen on more than one occasion. It is possible that the high frequency of individuals resignted only once and the timing of these resigntings may have been related to the date of tagging since coral trout were tagged at the primary site both in August (prior to the spawning season) and in September (at the start of the spawning season), see Table 3.4. However, fish tagged at both times were both resighted once and several times: fish tagged in August (n=12) were resighted from 1 to 6 times; fish tagged in September (n=43) were resignted from 1 to 12 times. In addition, fish that were resighted only once were seen throughout the season (August -November), not just soon after tagging, including 5 individuals that were resignted in the 1994 spawning season (September - December). Interpretation of the frequency of resightings (Figure 3.4) is also based on the assumption that resightings were distributed throughout the four month period, and were not, for example, aggregated in September which would explain the high percentage of single resightings. The frequency of resightings per individual by month was as follows: August: 1 - 2 resightings, four fish; September: 1 - 3 resightings, 16 fish; October: 1 - 6 resightings, 31 fish; November: 1 - 5 resightings, 25 fish; December: 1 resighting, four fish. Note that the same individuals may occur in the different months as they were resignted throughout the spawning period.



Figure 3.4 Number of resightings per tagged coral trout at Elford Reef in 1993. P = primary aggregation site, O = three other tagging locations (FSW1, FSW.5, FSEI1). Note: the zero resightings are not relevant to the 12 fish from the O sites (see text for explanation).

Despite the mode of one resighting per individual, certain individuals were resighted at the primary aggregation site many times during the four month period (see below). Unfortunately, it is not possible to sex coral trout without examining their gonads unless they are engaged in spawning behaviour (Chapter Two), and therefore it is difficult to determine whether there was a gender bias in the resightings since the sex of the resighted fish was often unknown. However, size provides some indication of sex, since the average size at sex change was 46 - 50 cm FL (Chapter Four). The size structure of the resighted fish tagged at the primary site (Figure 3.5) does not provide any evidence of a gender bias in the fish that were resighted several times. All size classes were represented in each category of resightings, except the smallest size class, 26-30 cm. Fish that were almost certainly male (  $\geq 46$ cm) and fish most likely to be female (26-40 cm) were resighted both once and several times (Figure 3.5).

## Frequently (> 4) resignted fish at the primary site

The combination of visual census counts (Chapter Two) and searches at the primary site (see section 3.2.2) ensured that the site was monitored for 79% of the time that aggregations were present during October – December. Therefore resightings data provide a reasonably accurate representation of the presence or absence of known individuals at the primary aggregation site.

The most frequently resighted individual (n=12), brand # 75, tagged at the primary site during the new moon phase on 16<sup>th</sup> September, was resighted only at the primary site. It was first seen during the following new moon on 16<sup>th</sup> October, and then during the new moon phases of November and December. It was also seen during the the full moon phase of October and the 1<sup>st</sup> quarter moon phase of October and November. The fish was 27 cm FL, and therefore almost certainly female and possibly immature (Chapter Four). No spawning behaviour was observed in this individual. The combination of small size and regular resightings during different lunar phases (indicating that this fish did not leave the primary site) suggest that this female may have been a resident of the spawning site, since coral trout are site attached (Samoilys 1997a).



**Figure 3.5** Size structure of the resignted fish tagged at the primary site. 26, 31 = 5 cm size classes (26 = 26-30 cm, 31 = 31-35 cm, etc.).

The second most frequently resighted fish (n=11), # 42, tagged at FSW1 during the new moon phase on 14<sup>th</sup> September, was resighted only at the primary site. It was resighted only during the new moon phase, and this occurred in October, November and December. This fish was 52.5 cm FL, male, and was seen patrolling a territory, courting and spawning. This male was seen at the spawning site at dusk for three consecutive evenings in October (14-16<sup>th</sup>), three evenings in November (12<sup>th</sup>, 13<sup>th</sup>, 15<sup>th</sup>) and two evenings in December (13<sup>th</sup>, 14<sup>th</sup>). It was also observed in the afternoon on 13<sup>th</sup> and at dawn on 15<sup>th</sup> November. The latter suggested that this individual was present at the site on the evening of 14<sup>th</sup> (see Chapter Two), however no evening survey was conducted on that date. This male defended the same territories were consistently observed during the three years of monitoring (Chapter Two). The results suggest that this male travelled to the spawning site to spawn, was one of the dominant males at the site, and was resident elsewhere, possibly in the vicinity of FSW1.

Fish # 74, tagged at the primary site during the new moon phase on 15<sup>th</sup> September, was resighted eight times, only at the primary site. It was resighted only during the new moon phase, in September, October, and November. This fish was 40.5 cm FL, female, and was seen being courted by male # 42 in October and November. This female was seen at the spawning site at dusk for one evening in September (15<sup>th</sup>) and then in the morning on 17<sup>th</sup> September; three consecutive evenings in October (14-16<sup>th</sup>), and in the afternoon and evening of 15<sup>th</sup> November. Since this fish was not observed at the spawning site during other lunar phases, the results suggest it travelled to the site to spawn, for three consecutive new moons periods.

Fish # 024, tagged at FSE1 on 4<sup>th</sup> October during the 3<sup>rd</sup> quarter lunar phase, was resighted eight times, only at the primary site during the new moon phases of October, November and December. This fish was 51.0 cm FL, male, and was seen courting and patrolling a territory. The results suggest the male travelled to the primary site to spawn, was one of the dominant males at the site, and was resident elsewhere, possibly in the vicinity of FSE1.

Fish # 008, tagged at the primary site during the new moon phase on 17<sup>th</sup> September, was resignted six times, only at the primary site. It was resignted only during the new

moon phase, in October, November and December. This fish was 44.0 cm FL, female, was seen being courted by male # 42 in September and December, and spawned with this male in December. This female was seen at the spawning site at dawn and dusk on 16<sup>th</sup> September; on three days in October (12<sup>th</sup>, 14<sup>th</sup>, 15<sup>th</sup>), though only in the evening of 12<sup>th</sup>, and then in the evening of 14<sup>th</sup> December. Since this fish was not observed at the spawning site during other lunar phases, the results suggest it travelled to the site to spawn, for three consecutive new moons periods.

Fish # 17 was resighted six times, was 30.0 cm FL, and was seen during the new moon periods of September and October, the full moon of October, and the 1<sup>st</sup> quarter lunar phase in November. Spawning behaviour was never observed in this fish. These observations suggest that the fish may have been a resident of the aggregation site and not yet reproductively mature.

Of the fish that were resighted five times, two were seen only during the new moon period and were female (# 80, 47.5cm FL; #016, 42.0cm FL), and two were seen during various lunar phases and did not exhibit any spawning behaviour (#11, 45.5 cm FL; #02, 36.0 cm FL). These observations provide further support for the suggestion that individuals that were reproductively active moved to the primary site during the new moon phase, and that the non-active fish may have been residents of the site.

 Table 3.6
 Number of resightings of tagged fish at the primary site by lunar phase

 during Aug – Dec 1993.
 Lunar phases represent 5-6 day periods, as defined in Chapter

 Two.
 Adjusted number of resightings represents standardised resightings by adjusting

 for search effort.

Lunar	No. days searched	No. resightings	Adjusted no. resightings
phase			
New	20	129	6.45
1 <sup>st</sup>	8	11	1.38
Full	8	12	1.5
3rd	4	3	0.75

There were at least four times the number of resigntings of tagged fish at the primary site during the new moon phase compared with other lunar phases (Table 3.6).

## Male territories

A maximum of six dominant males were observed at Elford Reef in 1993. These males are termed dominant because they defended specific territories at the primary site (Samoilys and Squire 1994). Thus, a maximum of six male territories were identified. These males returned to the same territory at the primary site at Elford Reef on subsequent days within the new moon period, and on subsequent new moons (e.g. tag # 42, #024, see above). The territories ranged from approximately  $50 - 250 \text{ m}^2$  in size. Females appeared to move throughout the spawning site during the late afternoon, and then settled within a male territory around 17:15 - 17:30 hr.

#### 3.3.2 Inter-annual returns

Twenty-nine fish that had been tagged in 1992 as part of a separate study in which 102 coral trout were tagged at the primary site (Samoilys unpublished), were resighted and positively identified in subsequent years: 22 in 1993 and 7 in 1994. These fish were identified by a dart and/or T bar tags because they were captured and killed (12 fish) by us or by fishers (commercial or recreational), or were captured and released (17) during the 1993 tagging exercise (see Methods). A further 18 resightings of fish tagged in 1992 were recorded (in 1993) but these fish could not be individually recognised underwater. A minimum of four could be separately identified by the presence of a) both tags, b) dart tag only, c) T - bar tag only. Thus, at least 33 (32%) of the fish tagged in 1992 were resignted in subsequent years at the primary site, providing some evidence of primary site fidelity between years.

### 3.4 Discussion

Based on the resightings of 67 fish observed at the primary spawning aggregation site at Elford Reef, this study estimated that the spawning site drew fish from a population of 3,000 adults in an area of  $1.5 \text{ km}^2$ , or a maximum distance of 1,200 m. This may be an underestimate since few fish were located off the primary site, and therefore searches may not have been wide enough. However, the results are consistent with Zeller (1998) who, using ultrasonic tracking on thirteen *P. leopardus* at Lizard Island in the far northern GBR, recorded a mean distance travelled to spawning aggregations from home ranges of 912 m (range: 220 - 5210 m). In the present study searches for tagged fish were strongly constrained by the logistics of snorkeling over large areas of coral reef. This highlights the advantages of ultrasonic tracking for such investigations, though the latter are limited by sample size (e.g. Zeller 1998: 8 males, 4 females, 1 unknown). *P. leopardus* is capable of intra-reef movements of several kms with maximum distances of 7.5 km (Samoilys 1997a) and 5.2 km (Zeller 1998) recorded in previous studies, and 5.1 km in the present study.

The significance of the estimated catchment area can be considered in the following context. The primary aggregation site was the only primary site located in  $36\% (7.7 \text{km}^2)$  of Elford Reef (Chapter Two), and it was estimated to draw coral trout from a mere 7% (1.5 km<sup>2</sup>) of the total reef area for Elford Reef. In contrast, Zeller (1998) tracked *P. leopardus* in an area of approximately 9 km<sup>2</sup>, and located four major aggregation sites. Although, searches for tagged coral trout in the present study only covered 2.4 km<sup>2</sup> around the primary site, no other primary sites were located within the 6.5 km<sup>2</sup> study area (Chapter Two). This implies that the primary aggregation site at Elford Reef was only used by coral trout resident within ~ 1km of the site. Assuming the sampling protocol did not fail to detect other primary sites within the study area, the results suggest that a large proportion of coral trout on Elford Reef may not use the primary aggregation site as their principal site for spawning. Alternatively, they may have been using primary sites located just outside the 6.5 km<sup>2</sup> area, which were not detected by the present study.

Certain tagged individuals were resighted many times at the primary aggregation site during the four month observation period indicating strong site fidelity. Behaviour observations indicated that some of these were large dominant males that established spawning territories at the aggregation site (Samoilys and Squire 1994). These males repeatedly returned to the aggregation site during subsequent new moon periods, and were present at the site on consecutive evenings during each new moon period. This concurs with the fact that male coral trout were in spawning condition throughout August – December (Chapter Four). Zeller (1998) also found male *P. leopardus* showed fidelity to one spawning aggregation site and visited it repeatedly through a two month tracking period. Similar site fidelity in males has been reported for the con – specific *P. areolatus* in Palau (Johannes et al 1999).

Not all individuals that were resighted many times during the four month period were males. Several females were observed revisiting the primary site on separate occasions within a new moon period, and also on subsequent new moons. In addition, site fidelity over 2- 3 years was also demonstrated, by inter-annual tag resightings. However, almost 50% of the fish sighted at the aggregation site were never seen again suggesting considerable turn over of individuals at the aggregation site. Further, fish resighted only once were seen throughout the season, including 1994, which suggests individuals used the primary aggregation site infrequently, but retained a link with it over time, at least from one year to the next. The reason for the difference between visitation rates between individuals is not clear. Further, this study cannot assess whether those individuals that used the aggregation site frequently represent fish that spawn more frequently than individuals that spawn elsewhere, because detailed behavioural observations of the latter are lacking. It is possible that aggregative spawning represents the greatest spawning activity, and hence contributes disproportionately more to the reproductive output of coral trout.

In 1993 the mean number of coral trout in any one aggregation was only 40 fish (Chapter Two). This raises questions about individual turn-over and the proportion of the local population of 3,000 fish using the spawning site. The frequency of resightings and the behaviour of known coral trout at the site (this Chapter), together with the duration of aggregations through the season based on visual monitoring (Chapter Two) and gonadal maturity schedules (Chapter Four), provide some insight to these questions,

and can be interpreted through the following steps. Combining the results of Chapters Two and Four, spawning aggregations last an average of 5 days each new moon period, for three months, for a total of 15 days per spawning season. With the assumption that our search effort at the site was 100 % during aggregation periods (actual effort was  $\sim$ 80%), two hypothetical scenarios can be envisaged. Firstly, if 100 % turn-over at the aggregation site is assumed, then 40 fish will visit the site on 15 evenings, i.e. equivalent to 20% of the population, or 600 fish in the source area. Secondly, the opposite case, assuming 0% turn-over, then only 40 fish from the source population use the aggregation, i.e. 1.3 % of the population. The second scenario is not supported by the high proportion (47%) of tagged fish (26 individuals) that were only resignted once at the spawning site. The remaining 53% of tagged fish (29 individuals) that were resighted 2-12 times at the primary site support a scenario somewhere between the two hypothetical scenarios. Although these calculations are extremely crude, they suggest that > 1.3 % and < 20 % of the 3,000 coral trout in an area of 1.5 km<sup>2</sup> around the primary site were using the site to spawn. Zeller (1998) inferred 31% of the population used spawning aggregations from the proportion of tagged coral trout (13 out of 35) that were tracked to spawning aggregations. The result is not surprising considering the small numbers of coral trout in an aggregation (Chapter Two). The results indicate that a significant portion of the population did not spawn in aggregations that we sampled or that we were aware of. One way to measure the total number of coral trout using an aggregation site would be to fish it down throughout the season; but this is unlikely to be allowed on the Great Barrier Reef under current permitting regulations. However, grouper fisheries based on targeting spawning aggregations in the Pacific can provide these data. For example, for the con-specific, P. areolatus in Solomon Islands, the maximum number extracted from one site was recorded at between 1,600 and 2,500 individuals (Johannes and Lam 1999).

The possible benefits of aggregative spawning have been discussed at length (Samoilys and Squire 1994). The results of the present study indicate that less than a quarter of the population is able to access this mode of spawning. The reasons for this may lie in the territorial behaviour of males at the spawning sites, and the fact that *P. leopardus* pair-spawns, which effectively limits the carrying capacity of the site. The males established adjoining territories within spawning sites which they guarded aggressively against other males; females spawned within these territories. Only six territories were observed

at the Elford Reef primary site, which would limit the number of dominant males on the site to six. Although sneaky male spawning was observed, it was very rare (Samoilys and Squire 1994). Since the average sex ratio (male:female) in *P. leopardus* was 1:1.83 (Chapter Four), a large number of males were excluded from the primary aggregation site and must presumably use other areas to spawn. Possibly these males spawn in single pairs or small groups (Chapter Two). Alternatively, these males may be non-reproductive, roaming males. In contrast, the con-specific *P. areolatus* forms aggregations of 200-300 fish (Johannes 1988), yet the males also establish territories and are aggressive towards each other (Johannes et al 1999). Other epinepheline serranids such as *Epinephelus striatus* (Colin 1992) are group spawners where males spawn together with females in large numbers. However, for many of the epinepheline serranids reported to form spawning aggregations it is not known whether they pair or group spawn (Domeier and Colin 1997).

In summary, the present study has revealed some unexpected results in terms of the dynamics of the primary spawning aggregations that coral trout establish. Only a small proportion of the population appear to use such aggregative spawning, and therefore the implications of targeted fishing of P. leopardus spawning aggregations will be less critical if they do not represent a major component of the species' annual reproductive output. However, the relative contribution to overall egg production of primary site spawners remains unknown. Many individuals that do use the aggregation site, do so repeatedly through the season, suggesting that during the new moon period this is their preferred spawning mechanism. The reasons for these apparent differences between individuals is unclear. Certainly with the males, there is a strong indication that only large dominant males can establish territories at the site which they defend throughout the season when the aggregations are formed. Presumably access to females is maximised in such aggregations. The reason large numbers of females do not use the aggregation site may be linked to energetic costs and/or predation risks involved in moving from their home sites (Samoilys 1997a) to the spawning site. The results show clearly that spawning mechanisms in P. leopardus are not straightforward, and are possibly not typical of other serranids (Domeier and Colin 1997, Sadovy 1996, Shapiro 1987). This may be the clue to their success as a numerically dominant, yet highly exploited, piscivore on the Great Barrier Reef.

# Chapter Four: SEXUAL MATURATION, SPAWNING FREQUENCY AND FECUNDITY OF CORAL TROUT

## **4.1 Introduction**

Spawning frequency, duration, and fecundity have rarely been measured in exploited coral reef fishes, and have not been estimated for any of the target species of the hook and line fishery on the Great Barrier Reef (GBR). The frequency and duration of spawning are key elements in the estimation of lifetime fecundity (Sadovy 1996). Estimating this value is an important goal in fisheries management because of the close links between fecundity and recruitment, and hence stock productivity (Cushing 1996). In addition, reproductive output can be directly affected by fishing, for example through the selective removal of certain size classes or sexes, or through disrupting spawning behaviour (Beets and Friedlander 1999, Vincent and Sadovy 1998, Bohnsack 1996, Sadovy 1996, Shapiro 1987, Bannerot et al 1987).

The few studies that have investigated patterns in egg production in exploited tropical reef fishes have revealed that spawning is often restricted to a narrow window of time within a relatively protracted spawning season (Koenig et al 1996, Sadovy et al 1994b), but spawning frequency may nevertheless be quite high (Collins et al 1993, Davis and West 1993). The present study aimed to determine reproductive output in the epinepheline serranid, *Plectropomus leopardus*. Like many finfish on the GBR, *P. leopardus* spawns in spring – early summer: September – December in the north (Ferreira 1995) and October – January in the south (Goeden 1978). The reproductive mode of protogynous hermaphroditism and indeterminate spawning (*sensu* Hunter et al 1985) has been documented by Ferreira (1995) for *P. leopardus*; however gonad maturity schedules were only studied at monthly intervals, and fecundity was not measured.

The present study examined patterns in egg production at seasonal, lunar and diel time scales, from which spawning frequency could be determined and hence annual fecundity. *P. leopardus* forms seasonal spawning aggregations that have lunar and diel

cycles (Chapter Two, Samoilys and Squire 1994). By examining gonad maturity on the same time scales, the present study was designed to assess the contribution of the aggregative mode of spawning to the overall reproductive output of *P. leopardus*. In addition, average size at maturity and sex change, and sex ratios were estimated. These parameters, particularly in protogynous hermaphrodites, can be directly affected by fishing; they therefore provide useful indicators of fishing impacts (Koenig et al 1996, Sadovy 1996, Roff 1992, Bannerot et al 1987).

A combination of approaches was employed to overcome some of the difficulties usually associated with studying reproductive output in the generally larger, more mobile fishes taken in coral reef fisheries. Patterns in egg production were studied throughout two spawning seasons, at which time spawning aggregations and spawning behaviour were also monitored underwater (Chapter Two). I was able to structure the sampling at three temporal scales (season, moon phase, time of day), which is often difficult in fisheries research because it typically relies on samples taken by the fishery. In this way it was possible to obtain realistic estimates of fecundity. Standard histological techniques for gonad maturity staging and whole oocyte counts for fecundity estimation were employed. Full details are provided in Appendix I. The study focussed on female reproduction because the production of eggs has greater physiological and energetic constraints compared with the production of sperm, and therefore it is usually females that dictate the timing of spawning (Roff 1992). Spawning periodicity was determined by analysing the temporal distribution of hydrated oocytes and early and late-stage postovulatory follicles (Melo 1994, Davis and West 1993, West 1990).

*P. leopardus* is a multiple sequential spawner. Oocytes, in several stages of development, including atresia, occur simultaneously in reproductively active ovaries (Appendix I, Ferreira 1995), a condition termed asynchronous oocyte development (Wallace and Selman 1981). For such species, it is impossible to identify a predetermined annual spawning batch. Consequently, the number of eggs produced in a single spawning batch (batch fecundity) is estimated (Hunter et al 1985), and the annual fecundity calculated from the number of spawnings per year, or spawning frequency, as determined by post-ovulatory follicles (Hunter and Macewicz 1985), (Davis and West 1993, Hunter et al 1985).

I used multiple approaches to estimate fecundity. Annual fecundity was estimated in 1993 by measuring batch fecundity from preserved samples, and by measuring spawning frequency from histological sections of ovaries collected from the same period. In 1994, an alternative technique using fresh samples was trialed. The latter are useful only as corroboration because of the small sample size and the different sampling period. To estimate annual fecundity spawning frequency and batch fecundity should be measured from samples taken during the same year (Hunter and Macewicz 1985). A third measure of egg output was also trialed in 1993, when the number of eggs spawned by a female in one evening was estimated. The eggs were obtained by stripping the females (Appendix I, Rimmer et al 1994), thereby obtaining ovulated eggs in the lumen. Thus, egg counts represented the potential total number of eggs to be spawned that evening based on the assumption that all ovulated eggs would be released via one or more spawning rushes (Chapter Two).

There are a number of factors that affect fecundity to a greater or lesser extent, such as fish size, weight, and age. Fecundity also varies between individuals, between years, within spawning seasons and due to such factors as missed seasons and seasonal shifts in somatic growth (Sadovy 1996, Cole and Sadovy 1995, Roff 1992, PDT 1990, Hunter and Macewicz 1985, Bagenal 1966). It is therefore notoriously difficult to measure. However, by adopting a combination of methods this study provides the first estimates of fecundity for *P. leopardus* on the GBR. In addition, patterns in egg production were determined to assess the significance of aggregative behaviours in spawning.

## 4.2. Methods

### 4.2.1 Study sites and field sampling

Sampling of coral trout gonads took place over two years (1992-1993) and at two reefs (Scott and Elford Reefs). Small spawning aggregations, termed secondary sites, were located at each reef during visual surveys (see Chapter Two for definitions). At both reefs three secondary aggregation sites and three corresponding control sites were established for both collection of gonad samples and for visual estimation of population

Figure 4.1 Sampling sites for coral trout gonads at Scott Reef in 1992 & 1993. A1-3 = secondary aggregation sites; C1-3 = control sites for secondary aggregation sites. Other sites sampled: B = bay; L = lagoonal area; N= northern area; O = outer deep "bommies" (1992 only); R = patch reef (1993 only). P = primary aggregation site (no fish were taken from this site).



Figure 4.2 Sampling sites for coral trout gonads at Elford Reef in 1992 & 1993. P =
Primary aggregation site; A1-3 = secondary aggregation sites; C1-3 = control sites for
secondary aggregation sites; B = back "bommie" field site. Other sites sampled in 1992: WC
= western channels; WS = western slope; DL and SL = deep and shallow lagoon, respectively.



abundance (Chapter Two). Coral trout were also collected from five additional sites at each reef to increase the total sample sizes (Figures 4.1 and 4.2). During 1993 sampling from secondary aggregation and control sites was discontinued to avoid the conflict between destructive sampling and visual surveys. Fish were mostly taken from other sites, with the exception of control site 3 at Scott Reef where sampling continued in 1993. In addition, a small number of fish were obtained from the primary aggregation site at Elford Reef.

Coral trout were primarily (81%) captured by speargun, supplemented by hook and line fishing (19%). Spearfishing was conducted in a consistent manner across all sampling scales (times, locations etc). To avoid bias in sampling, the spearfisher targeted individuals on sight. Sampling was thus haphazard, although there may have been some bias towards larger fish (Welch 1998). Where possible, fish exhibiting courtship behaviour were captured and their behaviour noted with the gonad sample. Line fishing was conducted opportunistically, and will have been biased towards larger fish due to gear selectivity (Ferreira and Russ 1994, Welch in prep.).

Sampling was done between September 1992 and January 1994 which spanned two spawning seasons and one non-spawning season (Chapter Two). In the spawning seasons, sampling was stratified temporally to obtain gonads: (a) monthly (September -December); (b) from four lunar phases (new, full, first and third quarter, defined in Chapter Two); and (c) from four time periods (0700-0900, 1000-1230, 1330-1545, 1630-1830). During the non-spawning season, fish were obtained in the following months: January, March, June, July and August, during the new moon phase and during daytime periods (1000-1230; 1330-1545). A larger total sample size was obtained from Scott Reef because tag-resightings (Chapter Three) dominated field work at Elford Reef. A total of 772 gonads were collected: 270 in 1992, 490 in 1993 and a further 12 in January 1994; 628 by spearfishing and 144 by line fishing, of which 752 were processed histologically.

For each sampling period, a minimum sample size of 10 gonads was sought. This is the sample size recommended by Hunter and Macewicz (1985) for estimating spawning frequency using postovulatory follicles. The optimal sampling strategy of every 1-2 hrs over 3-4 days was impossible because coral trout are diurnal (Samoilys 1987).

Date, time and site of capture were recorded for each animal. Fork length was measured to 0.5cm and whole fish weight was measured to 0.1 kg. Fresh wet gonad weight was obtained but these were approximate because the fine balance did not operate well at sea. Comparisons between fresh and preserved weights were significantly different (p<0.05; paired t-test; n=135) and therefore all subsequent analyses and discussion refer to preserved weights measured in the laboratory.

Gonads were fixed within 2 hours of capture, and gonads >1cm in diameter were injected to ensure rapid fixation. For most samples, FAACC (Formaldehyde 4%, Acetic Acid 5%, Calcium Chloride 1.3%; Winsor 1991, 1994) was used as the fixative and preservative (Appendix I). Other samples were fixed in 10% formalin buffered in sea water.

There is a possibility that the histological information gained may not reflect the gonad development of primary site spawners (defined in Chapter Two) since very few gonads were collected from the primary site. However, destructive sampling on the primary site would have seriously compromised all other data (visual observations, Chapter Two, and movements, Chapter Three), and this was considered a higher priority. However, a total of 23 fish were speared at the Elford Reef primary site to assess possible differences in reproductive maturity of primary site spawners: 7 in September – October 1992, 4 in March 1993, and 12 at the end of the spawning season in November 1993. Several of these fish were tagged individuals (Chapter Three). Another seven gonads of known individuals were obtained in 1994 from the primary site.

## 4.2.2 Histology

A descriptive approach using standard histological techniques on preserved gonads was used to determine: (i) gonad maturity (ontogenetic and seasonal development) and (ii) spawning frequency (full details are reported in Appendix I). The right lobe (no difference between right and left lobes were detected by Ferreira 1995) of each preserved gonad was embedded in paraffin, and mid, proximal and distal portions were sectioned transversely at 5 µm thickness and stained with Mayer's haematoxylin-eosin for histological examination. Three sections were taken to examine differential

development along the length of the gonad. Ovary and testis stages were defined (Appendix I) based on Ferreira's (1993, 1995) and Adam's (1996) stages for *Plectropomus* spp., schemes developed for other tropical Pacific species (McPherson 1991, Ebisawa 1990) and reviews (West 1990, Sadovy and Shapiro 1987, Hunter and Macewicz 1985, Smith 1965). Ovaries were classified qualitatively by the most advanced oocytes present, including the presence of postovulatory follicles, regardless of how many there were. This is the simplest and most widely used approach, and was considered preferable since quantitative approaches are much more time consuming, complex to analyse because of the size difference between oocytes of different development stages, and are not demonstrably superior in their results (West 1990). Histologically prepared slides (Appendix I) were read in random order with no prior knowledge of the date, time, or fish size of the sample to minimise bias in interpretation.

#### 4.2.3 Fecundity

Counts of whole hydrated oocytes were conducted to measure batch fecundity (Appendix I). Selection of hydrated ovaries from the 1993 collection of gonads was based on the following sample criteria: (a) lunar phase: new moon (days 28 to 3); time of day: >1300 hr; month: September, October or November, to maximise the selection of running ripe females (Chapter Two). Thirty gonads in a hydrated but not ovulated state (Appendix I) were selected for oocyte counts.

Hydrated oocytes were counted gravimetrically (Appendix I, Hunter et al 1985). However, it was difficult to separate the eggs from the stromal tissue of preserved gonads. Consequently, a further ten ripe or running-ripe (hydrated) ovaries were collected during the following spawning season (1994) to trial an alternative technique using fresh samples (Appendix I, Lowerre-Barbieri and Barbieri 1993). Coral trout suspected to be female were collected from known aggregation sites (Chapter Two) during new moon periods in November and December 1994. Ovaries were examined for ripeness (hydrated oocytes) within 1 hour of capture. Ripe ovaries were dissected and placed on a bed of ice. Date, time of capture, and site of capture details were recorded for each animal. Fork length was measured to 0.5 cm and whole fish weight was measured to 1.0g. Hydrated oocytes of fresh gonads were counted volumetrically

(Appendix I, Hunter et al 1985). Counts represented hydrated oocytes both within the lamellae and those that had been released (ovulated) into the lumen.

To estimate the number of eggs in one spawning, females were captured by speargun in the late afternoon of the October - November new moons in 1993. Firm hand pressure was applied to the abdomen to strip eggs from the ripe gonad. Oocytes were preserved in 10% buffered formalin and later counted volumetrically using the hydrated oocyte method (Appendix I, Hunter et al 1985). The eggs and the preserved gonad were also processed histologically (see above) to verify the maturity stage of the oocytes.

Spawning frequency was determined histologically by counting the proportion of mature females with post-ovulatory follicles, considered equivalent to the fraction of females spawning per day (Hunter and Macewicz 1985).

## 4.3 Data Analysis

Data were only collected from two reefs and for two years, therefore comparisons between reefs and years were done graphically. Statistical analyses focussed on within year and within reef comparisons.

The results from the histological staging were presented as a percentage or proportion of fish in each stage (West 1990). Since the data were ranked on an arbitrary scale, Chi-square ( $\chi^2$ ) analyses were used for comparisons (Zar 1996, West 1990).

## Size at maturity

Size at first maturity was defined as the length at which half of the females were sexually mature (King 1995), and samples were pooled from both reefs and years. Previous workers recommend using samples taken only during the spawning season because of the difficulties of distinguishing between resting and immature females (Davis and West 1993, Ferreira 1993, 1995). However, the error rates for misidentification were low in the present study (3%, Appendix I ) and therefore all females were used to maximise the sample size.

## Season

The seasonal development of ovaries was described by plotting the percentage occurrence of mature females in a vitellogenic state against month, and by plotting gonadosomatic index (GSI) against date, with all subsamples (eg. sites, times) pooled. In each case immature individuals were excluded.

GSI is a reasonable approximation of reproductive effort in fishes with no parental care (Roff 1991), and was calculated as:

GSI = Gonadal Weight

Gonadal Weight + Somatic Weight

for mature females (i.e. immature individuals were excluded) and for males.

## Month

Monthly variation in maturity during the spawning season was examined by plotting the frequency of pre-vitellogenic (excluding immature individuals) and vitellogenic females by month with either reefs or years plotted separately.

# Lunar phase and time of day

Lunar periodicity was examined by plotting the percentage occurrence of each vitellogenic stage, and the proportion of females with post-ovulatory follicles, against month for the spawning season, with reefs and years pooled.  $\chi^2$  analysis (Zar 1996) was used to compare frequencies between lunar phases. The same procedures were used to examine diel periodicity by plotting the percentage occurrence of each vitellogenic stage against time period during peak spawning months, and comparing frequencies with a  $\chi^2$  analysis.

## Spawning frequency

The mean number of spawnings per female was estimated by calculating the proportion of vitellogenic females with post-ovulatory follicles (POFs) during two lunar periods and two two-month periods. The lunar periods were days 28 to 10 (from new moon to  $1^{st}$  quarter) and days 14 to 25 (from full moon to  $3^{rd}$  quarter), which represented the highest and lowest peaks in spawning activity, respectively, during the spawning season (see Results). The two two-month periods were: September – October, and November – December, which also represented the highest and lowest peaks in spawning season (see Results). This provides the best estimate of the average daily spawning frequency from this data set because fish were not sampled every day throughout the spawning season.

## Annual fecundity

Annual fecundity was calculated as the product of spawning frequency and batch fecundity (Hunter et al 1985).

#### 4.4 Results

#### 4.4.1 Histology

Gonads from 752 individuals, taken from Scott and Elford Reefs from September 1992 to January 1994, ranging in size from 23.0 to 65.5 cm FL (19.0 – 58.0 cm SL), and in weight from 0.15 to 4.875 kg, were staged histologically. Histological readings confirmed the diagnosis of previous studies (Goeden 1978, Ferreira 1995) that *Plectropomus leopardus* is a protogynous hermaphrodite. Male gonads featured a central cavity that represents a remnant of the ovarian lumen, and mature gonads undergoing sexual transition from female to male were identified. The sex structure of the sample was as follows: 445 females (35 immature), 243 males, 61 transitionals and 3 bisexuals (simultaneous hermaphrodites in which sexually mature male and female cells co-occurred). Assuming that the sampling was not biased (see section 4.2.1) this gives a female:male sex ratio of 1.83:1. The reason for the difference in proportion of

transitionals between 1992 and 1993 (Table 4.1) is not clear. Average sizes of the sexes are shown in Table 4.1. A detailed description of the histological stages is given in Appendix I.

Readings of proximal, medial and distal sections differed for 129 fish (17%) and only 10 of these differed with respect to sex; these all related to transitionals (Appendix I). The error rate for both sexing the population and for staging females by medial section alone was 0.7%. (Full details of the differences and their associated error rates are given in Appendix I, section 3.1.3.) On the basis of these negligible differences between section readings, the medial section was taken as the standard reading to conform with other studies.

## Size/sex frequency

The size frequency plot for the whole sample was bimodal which corresponded with sex (Figure 4.3). Females were on average smaller than males, but there was a broad overlap in size between the two sexes. Similarly, transitionals occurred across a broad size range, from 27.5 cm to 55.5cm forklength (FL). Mean sizes for juveniles (immature females), females, transitionals and males are given in Table 4.1. In addition, three bisexual individuals were identified of 43.0 cm, 43.5cm and 50.0 cm FL (Figure 4.3). These fish had both hydrated oocytes and dorsal and central sperm sinuses filled with spermatozoa, and are described in Appendix I.

**Table 4.1**. Mean size (cm FL, standard errors in parentheses) of females, males and transitionals; and sex ratios. Sample sizes are also shown. 12 fish taken in January 1994 at Scott Reef are included in the 1993 sample.

	Scott	Scott	Elford	Elford	Reefs &
	1992	1993	1992	1993	Years
					combined
Immature	27.8	30.3	-	29.2	30.0
Females	(4.75)	(0.39)		(0.99)	(0.41)
	n = 2	n = 28	n = 0	n = 5	n = 35
Mature	42.6	35.6	40.2	39.1	39.0
Females	(0.78)	(0.56)	(0.60)	(0.45)	(0.33)
	n = 97	n = 137	n = 74	n = 102	n = 410
Males	48.7	43.4	49.4	45.9	46.0
	(1.26)	(0.94)	(1.08)	(0.65)	(0.48)
	n = 39	n = 74	n = 32	n = 98	n = 243
Transitionals	37.4	39.7	38.0	39.9	39.6
	(2.76)	(1.39)		(1.17)	(0.84)
	n = 5	n = 28	n = 1	n = 27	n = 61
Female:Male	2.54:1	2.23:1	2.31:1	1.09:1	1.83:1
sex ratio	n = 138	n = 239	n = 106	n = 205	n = 688

## Size at first maturity and size at sex change

The average size at first maturity (50% of females mature) was estimated at 21-25cm FL (Figure 4.4). However, this was the smallest size class of fish that was collected and therefore I cannot be certain that individuals < 21cm were not mature. In addition, only 4 fish were collected in this size class which is clearly inadequate for calculating average size at first maturity with confidence.



Figure 4.4 Size (forklength) distribution of female maturity stages showing size at first reproduction. Years and reefs combined. Stages: IM = immature; RE = resting; RI = ripe; RR = running ripe; SP = spent. Sizes are in 5cm classes (21 = 21-25, 26 = 26-30, etc). Sample sizes are given above each histogram.



**Figure 4.5** Size distribution of females and males showing average (50%) size at sex change. Abbreviations as in Figure 4.4. Sample sizes are given above each histogram.

At 41-45cm FL, 41% of the sample was male, and at 46 - 50 cm FL, this increased to 64% (Figure 4.5). There was a broad overlap in size range between the sexes, and males were found as small as 26 - 30 cm FL (Figure 4.3). 46 - 50 cm FL estimates the average size at sex change (50% of individuals are male).

### Season

A September-December (Australian spring-early summer) spawning season for *Plectropomus leopardus* was clearly illustrated by the occurrence of mature females in a vitellogenic state of maturity (Figure 4.6a) and the change in female gonadosomatic index (Figure 4.6b). (The difference in sample size between the two indices reflects missing fish weight data.) All vitellogenic females were found from September to December, except for two individuals: one collected in August and the other in January. Mature females may be pre-vitellogenic (resting), vitellogenic (ripe) or atretic (spent) (Appendix I). Only vitellogenic females are plotted in Figure 4.6. Although sampling was conducted in June, unfortunately only four fish were captured, none of which were mature females; three were immature and one was transitional.

Three gonad maturity stages were defined in males: ripe, resting and spent, with resting defined as either pre-spawning or post-spawning (Appendix I). In contrast to females, ripe males were captured throughout the year (Figure 4.7), including March and July which are well out of the spawning season, as defined by female vitellogenesis schedules (see above) and by visual observations of spawning behaviour (Chapter Two). There was an increase in the proportion of spent or resting males in December, January and March (Figure 4.7) which coincided with the end of the spawning season. The Spent and Resting stages proposed here (see Appendix I for full details and Plates) are based on relatively small sample sizes and require further testing. For example, the unexpected occurrence of Spent males in August and September suggests that the criteria require further evaluation. The issue of defining male maturity stages is further discussed in Appendix I. Although sampling was not continuous throughout the non-spawning period (Jan – July), missing or scant data (Figure 4.7) reflect that few or no males were captured in the sample.





**Figure 4.6** The timing of occurrence of sexually active females from September 1992 to January 1994. a) Percentage of mature females in a vitellogenic state of development; b) mean gonadosomatic index (GSI), error bars are standard errors.



**Figure 4.7** The timing of occurrence of male maturity stages from September 1992 to December 1993. Stages: C + I = Complete and Incomplete ripe males, R1 = Resting post-spawn males, R2 = Resting pre-spawn males, S = Spent males. Sample sizes are given above each histogram.

## <u>Month</u>

During the spawning season, the proportion of mature females in a vitellogenic state ranged from 93-100% in September and October at both reefs in 1992; but the proportion was lower (66% to 82%) in 1993 (Figure 4.8). This pattern shifted later in the season with 58 - 61% of females in a vitellogenic state in November, and only 9-26% of mature females in a vitellogenic state in December. The pattern suggests September-October was the main period of spawning in 1992-1993 in this northern region of the Great Barrier Reef, although spawning continued through November and December. Vitellogenic females were rare outside this four month period, with one individual found in August 1993 and one in January 1993; although sampling in 1992 did not begin until September.

Spent females, those in a pre-vitellogenic state but with atretic oocytes, first appeared in October and were then seen in subsequent months, except in December 1993 (Figure 4.8). The latter is surprising, since it is unlikely that spent females were not present at the end of the spawning season in that year. However, numbers were never great, the highest proportion being 22% in October 1993. Atretic females were not seen outside the period October – December, with one anomalous exception. One female with atretic oocytes (of all three ages/stages) was collected in August 1993. However, apart from the atretic oocytes, the gonad typified a resting stage rather than spent stage because the lamellae were not disrupted or disorganised and the ovary was not vascularised or vacuolated (Appendix I).

## Lunar phase

A lunar pattern was evident in the sexual development of female coral trout: the frequency of the four female maturity stages was not independent of lunar phase ( $\chi^2 = 51.52$ , df = 9, p<0.001). Maximum spawning activity occurring around the new moon and first quarter lunar periods (Figure 4.9). This pattern was seen in both measures of spawning activity: the presence of hydrated oocytes and the presence of post-ovulatory follicles (POFs, Figure 4.9). A sample of 21 fish collected on lunar day 4 in Sept – Oct, just outside the new moon period (lunar day 28 to 3, see Methods) was included in the







**Figure 4.9** Proportion of vitellogenic stages and proportion of mature females with postovulatory follicles (POFs) by lunar phase during the spawning season (Sep-Dec). Reefs (Scott and Elford) and years (1992 and 1993) combined. Lunar phases: NM = new moon;  $1^{st}$  = first quarter; FM = full moon;  $3^{rd}$  = third quarter. Vitellogenic stages: YV = yolk vesicle; YG = yolk globule; MNS = migratory nucleolus; HY = hydrated. Sample sizes are given above each histogram.
new moon sample to boost the sample size. The greatest proportion of females with hydrated oocytes (57-64%) and the greatest proportion of females with POFs (52-54%) was seen during the new moon and first quarter phases (Figure 4.9). If the migratory nucleolus and hydrated stages are considered together, 82% of females were in imminent spawning condition ("running-ripe") in the new moon phase, 67% in the 1<sup>st</sup> quarter, versus 42% during the full moon phase and 0% in the 3<sup>rd</sup> quarter. The results suggest females reduce their spawning activity during the full moon and probably the third quarter periods, as seen by the low proportion of hydrated oocytes and POFs at that time. Unfortunately, confidence in the results for the third quarter phase is low because sample size was very small. Although ten females were taken at this time, sampling was biased towards November and only five of the females were vitellogenic; the others were either resting or immature.

Samples sizes were too small during the first quarter moon phase for a formal analysis of size structure differences in hydrated females between the new moon and first quarter, but there is some suggestion that it is primarily the larger females that spawn during the first quarter phase (Figure 4.10). Although the size range was the same (28.0 -59.0 cm FL during the new moon, and 26.5 - 52.0 cm FL during the first quarter), the modal size classes shifted from 31-35 cm and 36-40 cm FL during the new moon, to 41-45 cm and 46-50 cm FL during the first quarter.

## Time of day

Sampling at different times of the day was conducted in October and November in both years during the new moon. A diurnal pattern was evident in the sexual development of female gonads: the frequency of the four ripe stages was not independent of time period ( $\chi^2 = 26.99$ , df = 9, p<00.1). The greatest proportion of females with hydrated oocytes (76%) was seen during the afternoon period (1330-1545), as shown in Figure 4.11. There were no differences in the proportion of females with hydrated oocytes between the early morning (0700-0900), mid morning (1000-1230) and early evening (1630-1830) periods. Proportions ranged from 35 to 52%. The presence of migratory nucleolus stage oocytes was greatest (43%) during the early morning (0700-0900). This is the first stage of hydration, when the nucleus migrates to the pole and the yolk globules begin to fuse (West 1990, Appendix I). The shift from 43% of females with migratory nucleolus



**Figure 4.10** Size frequency plot of females with hydrated oocytes during new moon (n=68) and first quarter (n=12) moon phases. Smaller fish = 26-40 cm. Larger fish = 41-55 cm in  $1^{st}$  quarter and 41-60 cm in new moon.



Figure 4.11 Proportion of vitellogenic stages in females at four different times of the day in October-November, during the new moon. Reefs (Scott and Elford) and years (1992 and 1993) combined. Abbreviations as in Figure 4.9.

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stage oocytes in the early morning to only 15% in the afternoon, with a concurrent increase from 43% to 76% of females with hydrated oocytes (Figure 4.11), provides an indication that hydration takes between 4 - 9 hrs to complete. The decline in frequency of hydrated females from the afternoon to early evening (76% to 35%, Figure 4.11) indicates the completion of spawning, i.e. when hydrated oocytes are released, and it is accompanied by an increase in the frequency of the youngest post-ovulatory follicles (POFs, see below).

To calculate the duration of POFs to estimate the timing of spawning in *P. leopardus*, vitellogenic females sampled throughout the spawning season (September – December) were examined. Out of a total of 240 vitellogenic females, 103 had POFs. The occurrence of each of the four stages ( = ages) of the POFs (see Appendix I) showed a diurnal pattern that clearly indicated spawning occurred in the early evening (Figure 4.12). Very early and early POFs were only seen in the early evening period (1630 – 1830 hr), and these were found in hydrated (n = 7), migratory nucleolus (n = 1) and yolk globule (n = 8) stage females. The time at which the frequency of the youngest POFs increases and the frequency of hydrated oocytes decreases provides a measure of peak spawning time (Melo 1994, Parrish et al 1986, Hunter and Macewicz 1980). Figures 4.11 and 4.12 demonstrate that this occurred between the afternoon and early evening periods.

Since sampling was not conducted through the night, the lifetime of the two early stage POFs cannot be determined, though it is clearly less than 15 hr since they were not found at 0700 hr the following day (Figure 4.12). Since they both occurred in the same time period this suggests degeneration from very early to early POF took up to 2 hr. In addition, three females had both very early and early POFs (Figure 4.12) suggesting these two stages are close in time and that the females spawned on two occasions in the one evening. Since spawning rushes were only observed over a mean period of 33 min (Chapter two), it is probable that degeneration from the very early to early POF stage takes around 30 min.

Late POFs were found throughout the day including the early evening. This suggests two things: (i) that they last for around 24 hr, possibly longer; although sampling through the night would be necessary to determine when late POFs are no longer found;



**POF** stage

**Figure 4.12** Time course of post-ovulatory follicles (POFs) in *P. leopardus* during the spawning season (September – December). Occurrence of POF stage (late, mid, early and very early) is plotted against time of day. Full details of POF stages are provided in Appendix I. n = number of females with POFs collected in each time period. NB: Six females that were collected  $\leq 15$  minutes outside a time period were added to the closest time period (4 in 1330-1545 [collected at 1600] and 2 in 1630-1830 [collected at 1615]). Symbols represent females with >1 stage of POF:  $\bullet = 8$  females with mid and late;  $\blacklozenge = 3$  females with very early and late;  $\blacklozenge = 3$  females with very early and early;  $\bigstar = 2$  females with early and mid.

(ii) that POFs degenerate to a late stage in <15 hr and that this late stage can persist for 12 hr. Mid POFs showed a similar pattern to late POFs suggesting that the two stages are close in time and that the process of degeneration from mid to late was up to 2 hr. In addition, eight females with both mid and late POFs were found, which also indicates the two stages are close in time (Figure 4.12). The late POFs seen throughout the day are unlikely to have resulted from spawning over a combination of evenings (i.e. POFs are >24 hr old, as in temperate species) because the spawning frequency calculations suggest *P. leopardus* spawn every second evening (see Section 4.4.2 below). However, three females with both very early and late POFs and two females with both early and mid POFs were found in the early evening (Figure 4.12). These presumably represent individuals that have just spawned and also spawned the previous evening.

#### Spatial patterns

To examine spatial patterns in the spawning activity of *P. leopardus* the proportion of the different vitellogenic stages were examined during periods in which hydrated oocytes peaked, with the early evening time period excluded (see above). Spatial patterns were investigated with respect to secondary aggregation sites and control sites (see section 4.2) in 1992. The results provided no clear indication that spawning activity differed in a consistent pattern between aggregation and control sites (Figure 4.13). The proportion of hydrated and migratory nucleolus stages pooled together ranged from 74-92% at the aggregation sites, and from 63 - 100 % at the control sites (Figure 4.13).

The few samples (14 females, 1 transitional and 8 males) collected from the primary site at Elford Reef do not suggest differences in maturity schedules at this site. Twelve of the females were collected on the new moon in September 1992 or November 1993, and all were hydrated. One hydrated female was collected on the full moon in September 1992, and a resting female was collected in March 1993. Six of the males were collected during the spawning season and all were ripe. The other two were collected in March 1993 and they were resting.



**Figure 4.13** Proportion of vitellogenic stages in females from aggregation (agg) and control (con) sites during September – December new moon and 1<sup>st</sup> quarter moon phases. Data combined from Elford and Scott Reefs for 1992. Lunar days 4 and 5 (in between new moon and 1<sup>st</sup> quarter phases) and 27 (one day prior to new moon phase) have been included. Abbreviations as in Figure 4.9. Sample sizes are given above each histogram.

## 4.4.2 Fecundity

## Spawning frequency

The proportion of vitellogenic females with POFs ranged from 0% during November – December full moon and third quarter lunar phases, to 63% during the September – October new moon and first quarter moon phases (Table 4.2). The latter represents a spawning frequency of 1.59, i.e. females spawn on average every one and a half days. Since spawning only occurs during a short period in the early evening (see above) the results suggest females generally spawned every other day but may also spawn every day, during the peak spawning months of September and October. Though rare, the occurrence of both early and late POFs in some females (5 out of 103, see above) supports the conclusion that females can spawn every day. The spawning frequency dropped substantially during the Full moon/3<sup>rd</sup> quarter lunar phases and during the months of November and December (Table 4.2).

**Table 4.2** Proportion of vitellogenic females with post-ovulatory follicles (% POFs) and corresponding total number of days spawning (Spawns) and spawning frequency (Freq.). New moon to first quarter period = lunar days 28 to 10; Full moon to third quarter period = lunar days 14 to 25.

	New moon – first quarter				Full moon – third quarter			
	POFs	Spawns	Freq.	n	POFs	Spawns	Freq.	n
	(%)				(%)			
Sept –	63	17	1.59	123	26	6	3.8	57
Oct								
Nov –	27	7	3.7	45	0	0	0	13
Dec								

### Batch fecundity

A mean batch fecundity of 87,244 eggs was estimated for the 1993 preserved gonads, and of 157,702 eggs for the 1994 fresh gonads (Table 4.3). These values represent the number of eggs spawned per batch through the season. The mean number of ovulated eggs from the stripped females was 22,928. The count of ovulated eggs does not represent a batch fecundity estimate. Since the 1993 samples represented hydrated oocytes in the lamellae (pre-ovulation) and the stripped samples represented ovulated eggs in the lumen (see section 4.2), their sum is equivalent to the 1994 samples which represented both hydrated oocytes within the lamellae and ovulated eggs in the lumen. However, the low counts of ovulated oocytes (Figure 4.14) indicate that the stripping technique may not have been very effective in removing all ovulated eggs. In addition, histological examination of the stripped females found that oocytes were either hydrated (n=6), or yolk globule (n=2) stage, which suggests that two females may not have ovulated.

Variation in the estimates was extremely high, as illustrated by the standard deviations (Table 4.3). Egg counts varied widely between fish of similar sizes or weights (Figure 4.14). Though sample sizes were small, the fresh gonads of 1994 gave higher estimates in all size classes than the preserved 1993 samples, and are likely to provide a more accurate estimate of batch fecundity for *P. leopardus* (see Methods, section 4.2.3). Surprisingly, both the 1993 and the 1994 estimates of batch fecundity showed a drop in fecundity in the largest size class of fish, though sample sizes were very small for this size class (Table 4.3).

As expected, batch fecundity in 1993 did increase with fish size and weight, but the relationship was weak though significant (size:  $r^2 = 0.33$ , p=0.004; weight:  $r^2 = 0.37$ , p=0.017 (Figure 4.14). A regression was not plotted for the 1994 data due to low sample size. The data show a rather different relationship, with batch fecundity rising steeply with increasing size and weight (Figure 4.14). This could be a function of greater variability between individuals and a smaller size/weight range in the sample.



**Figure 4.14** Batch fecundity (BF) versus size and weight of *P. leopardus*. Regression slopes are plotted for the 1993 data (n=26).

# Annual fecundity

The average estimated total number of spawnings per year per female was 30. This value is the total number of spawns in the period September – December (Table 4.2), based on the proportion of females with post-ovulatory follicles (see section 4.3, Data Analysis). If the mean batch fecundity from the 1994 samples of 157,702 eggs (Table 4.3) is taken as the best estimate for batch fecundity, the annual fecundity was 4.7 million eggs spawned per female per year. However, annual fecundity should be calculated from batch fecundity and spawning frequency measured in the same year (Hunter et al 1985). Therefore the more correct estimate of annual fecundity for *P. leopardus* was 2.6 million eggs spawned per female in 1993.

**Table 4.3** Mean batch fecundity for 1993 and 1994, and mean number of ovulated eggs (stripped females), per size class, and for all samples. Standard deviations are in parentheses.

Fish size class	1993 samples	1994 samples	stripped samples				
(cm FL)							
<u>&lt;</u> 35.0	37,867	101,095	59,715				
	(28,180)	(46,025)	(12,208)				
	n=7	n=3	n=2				
35.1 - 45.0	109,109	186,794	10,895				
	(45,861)	(43,944)	(11,363)				
	n=16	n=6	n=5				
> 45.0	85,848	152,972	9,520				
	(24,602)						
	n=3	n=1	n=1				
	Overall mean batch fecundity						
	87,244	157, 702	22,928				
Means	(49,898)	(56,377)	(24,715)				
	n = 26	n = 10	n = 8				
Mean fish size (cm FL)	38.3	38.9	38.9				
Mean fish weight (g)	Mean fish weight (g) 1053.2		1234.4				
Fish size range (cm) 25.5 - 51.0		30.0 - 46.5	34.0 - 49.0				

### **4.4 Discussion**

This study has revealed that although female *P. leopardus* are reproductively active over a four month period on the northern GBR, the time schedule of egg production in *P. leopardus* is tightly linked to lunar phase and time of day, and spawning is concentrated in two months. The majority of spawnings occurred in September – October during the new moon and first quarter moon phases. Sadovy et al (1994b) found a similar discrepancy between the spawning pattern of *Epinephelus guttatus* determined from gonadal assessment on broad and fine time scales. Data pooled across months and years revealed mature ripe ovaries for three months of the year. However, samples collected 1-4 times weekly during the spawning season revealed spawning activity was restricted to only a two week period each year (Sadovy et al 1994b).

The patterns in egg production only partly concurred with visual observations of aggregative spawning. Spawning aggregations were closely tied to the new moon but not the first quarter lunar phase. Aggregations occurred for three months, September – November, but generally the largest aggregations were in September and October (Chapter Two). The high percentage of females with hydrated oocytes and post-ovulatory follicles during the first quarter when spawning aggregations had dispersed, indicates that the coral trout were also spawning outside aggregations. Spawning frequency calculations also provide evidence that coral trout did not confine their spawning to aggregations. The calculation of spawning frequency based on the presence of post-ovulatory follicles, gives 30 spawns per year, whereas the maximum number of spawns possible in aggregations is 15 (Chapter Two).

A synthesis of the results from Chapters Two, Three and the present chapter suggests that coral trout maximised their spawning during the new moon phase during which time some individuals used aggregations to spawn. Females also continued to spawn into the first quarter phase, and during this period were not spawning in aggregations. There is some suggestion that it is the larger females that continue to spawn in the first quarter phase. Differences in the sizes of females spawning at different times, with larger females spawning over a longer season have been demonstrated in the northern anchovy (Parrish et al 1986).

Spawning frequency estimates suggest that females spawned every other evening during the new moon lunar phase, thus it might be expected that they move to the aggregation sites on alternative days. The tagging study showed females were present at the primary aggregation site at Elford Reef on alternative evenings, though they were also seen on single evenings and consecutive evenings (Chapter Three). The latter supports the simultaneous occurrence of young and old POFs in a few females.

The results suggest *P. leopardus* employ a combination of strategies to maximise their reproductive success. If aggregations are the most desirable means of spawning why do they not continue for longer? A precise cue to ensure individuals gather together (Robertson 1991) is provided by the new moon. The fact that the aggregations did not continue suggests other factors are also important. Possibly preferable currents and tidal flow for egg dispersal (Shapiro et al 1988, Johannes 1978) only occur during the new moon phase. Alternatively, females may be divided in the strategy that they adopt – some use primary sites, while others do not, and the analysis of the histological readings has pooled these two groups together. However, the lack of any spatial patterns in spawning activity does not support this interpretation.

If females are restricting their spawning activity during full moon/third quarter periods as the results suggest, they appear to do so by retaining oocytes in the earlier stages of vitellogenesis (yolk globule and yolk vesicle). The results show that females do not revert to a pre-vitellogenic state within the spawning season because in September-October 66-100% of mature females were in a vitellogenic state.

Within the lunar cycle of spawning activity, the final stage of oocyte maturation, hydration, exhibited a tightly defined diurnal pattern, resulting in spawning at sunset. This accords exactly with observations of spawning rushes, which were only seen during a ~30 min period spanning sunset (Chapter Two). The staging or ageing of post-ovulatory follicles proved extremely effective in revealing both the actual time of spawning and the estimation of spawning frequency. Their short life (~ 24 hrs) is well recognised as an accurate way of back-calculating spawning time (Davis and West 1993, Hunter and Macewicz 1985). In contrast, little is known about the length of time it takes for oocytes to hydrate or how long hydrated oocytes can be retained within the

ovaries. This study has shown that hydration takes 4-9 hrs to complete in *P*. *leopardus*, and that hydrated oocytes are not retained, but are either released or reabsorbed. Thus, hydrated oocytes are a good indication of imminent spawning, and therefore time schedules of hydrated oocytes provide an accurate means of determining spawning time in this species. In contrast, the presence of POFs gave a specific spawning cycle in *Lutjanus vittus* which was not detected when spawning was measured by the presence of hydrated oocytes (Davis and West 1993).

Notably, the dusk timing of spawning predominated during both new moon and 1<sup>st</sup> quarter lunar phases, i.e. when coral trout were spawning both in and out of aggregations. This suggests that dusk spawning is advantageous whatever the spawning mechanism. Dusk spawning is common in many pelagic spawners, especially the Serranidae (Sadovy 1996). Reduced risk of predation from diurnal planktivores has been proposed to explain dusk spawning (Chapter Two, Johannes 1978).

### **Fecundity**

This is one of the few studies to provide detailed time schedules of egg production and estimates of spawning frequency and annual fecundity in a tropical serranid. The temporal tracking of post-ovulatory follicles showed clearly that reproductive output in P. leopardus was not constant throughout the spawning season. Spawning frequency was greatest during the new moon and 1<sup>st</sup> quarter lunar phases, and in two months out of the four month season. The results provide evidence of the flexible spawning mechanisms employed by coral trout, and suggest that coral trout do not restrict their egg output to spawning in aggregations. Since spawning frequency and fecundity have rarely been measured in tropical serranids, it is difficult to assess the significance of the values obtained in the present study. On average females spawned 30 times a year, which is probably relatively high, particularly for a species that employs aggregative spawning. The latter tends to result in single or few spawning episodes where vast numbers of eggs are released (eg. Epinephelus striatus, Carter et al 1994). It should be noted that 30 spawns per year is an average estimate for the population, and does not provide information on whether all females spawn throughout the season, or indeed every year, as known in other marine species (Sadovy 1996). High spawning frequencies have been recorded in other tropical species. For example, the snapper

*Lutjanus vittus* was estimated to spawn 22 times a month, with an annual frequency of between 90 and 150 spawns per female (Davis and West 1993). *P. leopardus* was estimated to release an average of only ~ 23,000 eggs in one evening, which concurs with the 22,000-55,000 eggs recorded by Rimmer et al (1994). Relative fecundity (total fecundity/female weight) provides a standardised value for comparisons between species (Sadovy 1996). For *P. leopardus* relative fecundity was estimated at 2,485 eggs per gram body weight (mean female weight = 1,053g, mean length = 383 mm FL) in 1993, which is considerably greater than the highest relative fecundity (~1450 eggs) compiled for eight serranids by Sadovy (1996, Fig. 2.6). Further, the higher fecundity estimated in 1994 would yield a relative fecundity of 5,276 eggs per gram body weight.

Although females used spawning aggregation sites repeatedly (Chapter Three), 30 spawnings per season would not be possible if restricted to aggregations. Aggregations formed on average for 15 days per season (3 x 5 day new moon periods, Chapter Two), and females spawned on average every other night during the peak period. Therefore the average maximum number of spawnings per female at primary aggregation sites would be around eight. A maximum of eight resighting of one female at the primary aggregation site at Elford Reef over the season (Chapter Three) concurs with this calculation. *P. leopardus* appears to adopt other spawning mechanisms to generate the egg output measured in this study, by using spawning outside aggregations and by continuing to spawn into the  $1^{st}$  quarter lunar phase.

Thus, *P. leopardus* released its relatively small batches of eggs on average 30 times during the season, giving an overall estimated annual fecundity of between 2.6 and 4.7 million eggs. The range represents differences in laboratory techniques and years (1993 versus 1994). Considering the indirect approach in measuring fecundity, the several assumptions, and the large number of variables that affect egg output, these estimates can only be considered as approximate. For example, measurements of batch fecundity assume all hydrated oocytes will be spawned. However, since I found no evidence of residual hydrated oocytes (Farley and Davis 1998) or attric hydrated oocytes (Appendix I) this assumption is reasonable for *P. leopardus*. Fish size or weight are typically cited as major determinants of fecundity (Roff 1992, Plan Development Team 1990, Bagenal 1966). However, a poor relationship between these variables and batch fecundity was obtained for coral trout, suggesting other factors are influencing egg

output. As an example, for fish of size class 36-40 cm FL, batch fecundity estimates ranged from 38,000 to 189,870 eggs. Possibly the complex sexual ontogeny, social and/or nutritional factors influence the fecundity of individuals.

#### Fisheries management implications

The average size at first maturity (21 - 25 cm FL) was considerably smaller than expected from previous studies (Adams 1996: 28 cm FL; Ferreira 1995: 32 - 36 cm FL). As a result, this parameter was not well estimated in the present study because insufficient females of <25 cm FL were collected. The reason for this difference is not clear. Both Ferreira (1995) and Adams (1996) collected samples both north (Lizard Island region) and south (Townsville region) of the present study (Cairns region) and found no regional differences, therefore it seems unlikely that a population between their two study areas would differ substantially. A decrease in minimum size at first maturity as a response to high fishing pressure in Serranidae has been proposed by previous workers (Sadovy 1996, Koenig et al 1996, Huntsman and Schaaf 1994, Bannerot et al 1987). Fishing pressure has increased by around 30% on the Great Barrier Reef since 1995 (QFMA 1999). However, sampling for the present study took place in 1992 - 1993, Ferreira sampled in 1990 - 1992, and Adams (1996) sampled in 1995, therefore a recent increase in fishing pressure is not a plausible explanation. Similarly, the possibility that Cairns is a more heavily fished region than the others is not supported by commercial fishing log book data, which indicates fishing effort is greatest in the Townsville region (Mapstone et al 1996), and around five orders of magnitude greater than Cairns (Ayling et al in press). Locational differences in reproductive parameters that are unrelated to fishing have been demonstrated in other coral reef species (eg. Acanthurus nigrofuscus, Hart and Russ 1996). The ratio of  $L_{min}/L_{max}$  for females is often predictable in the Serranidae (Sadovy 1996). In the present study Lmin was 23.0 cm FL, and Lmax was 63.0 cm FL for females, giving a ratio of 37%, which lies at the bottom end of the range (33%-74%) reported by Sadovy (1996) for 27 species of serranid. This suggests that the coral trout of the present study mature relatively early compared with other serranids. The coral trout fishery on the Great Barrier Reef has been managed using a minimum size restriction since at least 1984

(pers. obs., Hancock 1992). Minimum legal sizes in fisheries management are usually equivalent to average size of first maturity ( $L_{50}$ , 50% of females are mature), on the basis that this allows females at least one spawning season (King 1995, Hill 1992). The coral trout fishery's minimum legal size is currently set at 38 cm TL (QFMA 1999), which is equivalent to around 34cm FL (pers. obs.), which would allow females 1-2 spawning seasons before they enter the fishery.

Unlike average size at first maturity, sex change is rarely considered in fisheries management regulations. Clearly, a minimum size restriction for a protogynous hermaphrodite, as currently in place for coral trout, can result in the selective removal of males which could result in stock sustainability problems caused by sperm limitation (Koenig et al 1996, Shapiro 1987). However, since the average size at sex change is 46 – 50 cm FL, and market forces in the commercial coral trout fishery are favouring small fish, males are not being highly targeted, as assessed by this study's coral trout population from Cairns. To thoroughly evaluate this issue, numbers and sizes of coral trout taken in the commercial fishery are required, but unfortunately commercial log books only records weights.

The female:male sex ratio may be predicted to increase in response to fishing pressure due to the removal of males, based on two assumptions: (a) that the species is a monandric protogynous hermaphrodite, that is one male type occurs, that derived from females (Ferreira 1995); (b) sex change occurs over a broad age/size range (Ferreira and Russ 1995) and is therefore probably controlled socially (Sadovy 1996, Shapiro et al 1993b), i.e compensatory mechanisms will operate in P. leopardus in response to fishing (Vincent and Sadovy 1998). Sex ratios for various populations of P. leopardus on the Great Barrier Reef are given in Table 4.4. There is no indication that the female:male ratio has increased over time, which might be expected with the increase in fishing pressure on the Great Barrier Reef (QFMA 1999). Sex ratios varied considerably. Unexpectedly, male biased ratios have been reported from three of four reefs in the southern Swains region (Table 4.4, Adams 1996). Some spawning aggregations of the coral trout *Plectropomus areolatus* have been found to be heavily biased towards males in Palau. This has been attributed to selective fishing of females which are more accessible in shallow waters (Johannes et al 1994). However, aggregation sex ratios need to be interpreted carefully because they can differ markedly

from non-aggregation sex ratios (Sadovy 1996). Sex ratio may be affected by a number of mechanisms or direct impacts, and, as such, the combination of effects should be considered. For example, earlier sex change will effectively increase female mortality thereby reducing the F:M ratio, but this may be offset by the selective removal of males (or vice versa).

**Table 4.4** Sex ratios for *P. leopardus* on the Great Barrier Reef. a = calculated as functional or operational sex ratios, i.e. mature males and females, no immature females or transitionals.

Location	Fished/Unfished	Sex ratio	n	Study, date of sampling
		(F:M)		(published date)
Wistari Reef –	Unfished	2.9 <sup>a</sup>	72	Goeden 1971-72 (1978)
Capricorns				
Townsville	Fished	1.99 <sup>a</sup>	202	Ferreira 1990-2 (1995)
Lizard	Fished	1.79 <sup>a</sup>	95	Ferreira 1990-2 (1995)
Lizard	Unfished	0.9 -1.4 <sup>a</sup>	80	Adams 1995 - 97 (1996 +
	Fished	1.0 - 1.4 <sup>a</sup>		unpubl.)
Swains	Unfished	0.7 - 0.2 <sup>a</sup>	80	Adams 1995 - 97 (1996 +
	Fished	1.6 - 0.3 <sup>a</sup>	ļ	unpubl.)
Townsville	Unfished	1.8 - 0.9 <sup>a</sup>	80 -	Adams 1995 - 97 (1996 +
	Fished	4.0 - 1.4 <sup>a</sup>	24	unpubl.)
Cairns	Fished	1.83 <sup>b</sup>	688	Samoilys 1992-3 (this study)

b = includes all males and females including immatures, but not transitionals.

Although, *P. leopardus* would be defined as a teleost with a relatively large and wide body size, and therefore be likely to produce large numbers of eggs in one spawning (*sensu* Choat and Bellwood 1991), its batch fecundity and spawning frequency indicate it conforms more closely to those teleosts that spawn frequently and produce small numbers of eggs (Sadovy 1996, Robertson 1991). *P. leopardus* appears to attain high annual fecundity by adopting various spawning mechanisms, which result in high spawning frequency per year, even though spawning is tightly coupled to seasonal, lunar and diel cycles. The high annual fecundity in *P. leopardus* may explain its relatively high natural abundance on the Great Barrier Reef compared with its conspecifics both in Australia and in the Pacific Islands (Johannes et al 1999, Samoilys et al 1995, Ayling and Ayling 1986).

#### Chapter Five: GENERAL DISCUSSION

## 5.1 Major findings

The spawning behaviour of *Plectropomus leopardus* provides a striking contrast to many other serranid fishes. This project found that *P. leopardus* formed well defined, and spatially and temporally predictable spawning aggregations. However, the use of such aggregations appeared to be fundamentally different from that of other epinepheline serranids, which appear to confine all spawning output to aggregations (reviewed by Domeier and Colin 1997). Consequently this has been the expected mode of reproduction in epinepheline serranids (Shapiro 1987). In contrast, *P. leopardus* exhibited flexibility and complexity in their spawning behaviour and employed other spawning mechanisms besides aggregative spawning.

Coral trout formed major (termed primary) spawning aggregations, at the same sites each year, and aggregations occurred during the new moon phase for three consecutive months each spawning season (Chapter Two). This pattern has been confirmed for a further five to six years at the same reefs in a separate study (Samoilys et al in prep.). Zeller (1998) also found that *P. leopardus* moved to spawning aggregation sites during the new moon phase. The present study thus confirmed the lunar aggregative spawning of *P. leopardus* on the Great Barrier Reef (GBR) first described by Samoilys and Squire (1994). These results are not surprising because many Serranidae aggregate at fixed sites to spawn, and this is often correlated with lunar phase (Domeier and Colin 1997, Sadovy 1996, Shapiro et al 1993b, Colin et al 1987, Shapiro 1987). However, the number of coral trout participating in aggregations was relatively low (a maximum of 128 fish in one aggregation). In addition, the species pair-spawned within a narrow time frame, ~ 30 min at dusk, with only a few spawning rushes observed per evening (Chapter Two). Further, the number of eggs released per spawning was low (~23,000 eggs, Chapter Four).

The movements of known individuals in relation to the primary spawning aggregation site at Elford Reef and the frequency with which they revisited the aggregation site (Chapter Three) provided evidence that aggregative spawning is not the dominant mode of spawning in *P. leopardus*. It was estimated that the aggregation site at Elford Reef was the only aggregation site within a  $6.5 \text{ km}^2$  area, and that it drew coral trout from an area of only  $1.5 \text{ km}^2$ , representing a population of ~ 3000 fish. However, less than 20 % of these fish used the site to spawn, which accounted for the low numbers of fish seen in aggregations. This contrasts with other serranids where numbers in spawning aggregations are in the hundreds (Domeier and Colin 1997). Further, evidence for spawning outside the primary aggregation sites was seen in the occurrence of spawning behaviour (courstship and male colours) at the secondary sites (Chapter Two).

Patterns in egg production provided further evidence that spawning mechanisms other than aggregative spawning existed. Egg production cycles only partly concurred with visual observations of aggregative spawning, and revealed that a considerable amount of the reproductive effort occurred outside spawning aggregations during the 1<sup>st</sup> quarter lunar phase (Chapter Four). Thus, coral trout spawned primarily during the new moon and 1<sup>st</sup> quarter lunar phases but used different mating strategies in these two lunar phases. I suggest that the combination of spawning mechanisms compensates for the relatively low batch fecundity in coral trout (~90,000 eggs). Indeed, spawning frequency was found to be high (Chapter Four). Low batch fecundity is presumably a function of being a protogynous hermphrodite and therefore females are relatively small, and the number of eggs released by females in a single spawning was low. This finding contrasts with the proposal that relatively large, wide-bodied fishes such as the Serranidae are able to produce large numbers of eggs in a single batch, and therefore spawn less frequently (Choat and Bellwood 1991), compared with the small, narrowbodied fishes such as the acanthurids, which compensate for low batch fecundity by having extended spawning seasons and rapid egg production (Sadovy 1996, Robertson 1991). The high spawning frequency in *P. leopardus* resulted in annual fecundities of 2.6 - 4.7 million eggs, which are at least twice as high as the relative fecundities of other serranids (Sadovy 1996).

### 5.2 The significance of a flexible mating system in P. leopardus

Aggregative spawning behaviour is typical of species with pelagic larvae that migrate to spawn (Sadovy 1996), such as many of the Serranidae (Domeier and Colin 1997). There is a growing body of literature that suggests the timing and location of these aggregations is a mechanism for pulling together adults that are normally widely dispersed (Robertson 1991, Robertson et al 1990, Colin et al 1987), rather than for any adaptive significance in terms of egg survival (Shapiro et al 1988, Johannes 1978). However, these theories need not be mutually exclusive (Samoilys and Squire 1994). In addition, aggregations may function as mechanisms for mate selection and provide cues for sex change through social interaction (Shapiro et al 1993b). Social control of sex change has been demonstrated in the basslet Anthias squamipinnis (Shapiro 1981), and the wrasse Labroides dimidiatus (Robertson 1972). However, these species live in tightly constrained haremic social units. Except when spawning, P. leopardus is usually widely dispersed, not territorial, and non-aggregative in its behaviour except occasionally when forming feeding parties (Samoilys 1997a, Samoilys 1987), a similar social structure to the Caribbean serranids Mycteroperca spp. (Coleman et al 1996, Carter et al 1994, Gilmore and Jones 1992), and the red hind Epinephelus guttatus (Sadovy et al 1994b). Therefore, spawning aggregations certainly serve to draw adults together, and this may facilitate mate selection and sex change (Chapter Two). However, if this is the case, P. leopardus cannot be dependent on spawning aggregations for these processes because only a relatively small proportion of the population used aggregations (Chapter Three).

Eggs released at primary aggregation sites may well experience lower mortality due to the physical characteristics of the site favouring offshore dispersal (Gilmore and Jones 1992, Shapiro et al 1993a, Colin and Clavijo 1988, Johannes 1988, Johannes 1978). However, such enhanced levels of egg survival would provide a relatively small contribution to overall egg survival in *P. leopardus* since few fish used the aggregation sites and coral trout spawned almost as frequently during the first quarter moon phase (Chapter Four). Despite the fact that aggregative spawning is common in a number of reef fish families not just the Serranidae (Domeier and Colin 1997, Colin and Clavijo 1988), the results of the present study question the often cited advantages of aggregative spawning. However, it is possible that eggs produced by coral trout that use

aggregations may contribute disproportionately more to future stocks. If this is the case, the question that then arises, is why does this mode of reproduction not spread through the population? Presumably additional factors are determining which spawning mechanism individual coral trout adopt.

The finding that less than 20% of the population of *P. leopardus* around the primary aggregation site at Elford Reef were using that site to spawn, and that the site drew fish only a relatively short distance (~1 km, Chapter Three), is surprising. Other serranids migrate considerable distances to their spawning aggregation sites. For example, 110 km to 240 km is reported for *Epinephelus striatus* (Carter et al 1994, Colin 1992); and five to 18 km for Epinephelus guttatus (Sadovy et al 1994a, Bernett-Herkes 1975), and *P. leopardus* is certainly capable of moving several kilometres (Samoilys 1997a, Zeller 1997). Although much of the work on aggregative spawning in epinepheline serranids suggests that spawning is restricted to this particular mechanism (Domeier and Colin 1997), few studies (but see Sadovy et al 1994b, Shapiro et al 1993b) have examined egg maturity schedules to confirm that fish were not spawning at other times. In P. leopardus, certain individuals that did use the aggregation site, both male and female, did so frequently, both within the new moon period, and on subsequent new moons through the season. The reason that only certain individuals adopt this mode of spawning is not clear. Possibly the social structure of aggregations, and their spatial confines restrict the number of fish that can participate. With the males, there was a strong indication that only large dominant males (up to six) could establish territories at the site, which they defended throughout the season when the aggregations formed. Male territoriality at aggregation sites has been reported in a number of serranids: the Caribbean Mycteroperca phenax (Gilmore and Jones 1992), M. tigris (Sadovy et al 1994a) and M. venenosa (Colin 1992), and the Indo-Pacific Plectropomus areolatus (Johannes et al 1999). Presumably access to females is maximised in such aggregations, though only to those males that can establish territories. The reason that large numbers of females do not use the aggregation site may be linked to a number of factors such as: the energetic costs and/or predation risks involved in moving from their home sites (Samoilys 1997a, Zeller 1997) to the spawning site; high competition for mates, and possible lower fertilization rates associated with high mating-rate males (Warner et al 1995).

Dusk spawning was clearly demonstrated, both by the observations of spawning rushes at spawning aggregations (Chapter Two), and from the time schedules of hydrated oocytes and post-ovulatory follicles (Chapter Four). Dusk spawning is common in many pelagic species especially the Serranidae (Sadovy 1996, Colin 1992). It seems unlikely that changing light levels might provide cues for the final stages of egg maturation (Hoffman and Grau 1989) since hydration started in the early morning (0700 – 0900) and took 4 - 9 hours to complete (Chapter Four). The time course of hydration is therefore likely to be physiologically constrained. The suggestion that male courtship in aggregations in the afternoon and evening is an important trigger for hydration (Chapter Two) is not supported by the early morning initiation of hydration. The dusk timing of spawning occurred during both new moon (aggregative spawning) and 1<sup>st</sup> quarter (nonaggregative spawning) lunar phases. This suggests that dusk spawning is advantageous whatever the spawning mechanism. Reduced risk of predation from diurnal eggpredators has been proposed to explain dusk spawning (Johannes 1978), but there is little empirical evidence available to support this (Shapiro et al 1988). However, since dusk spawning occurred when coral trout were not aggregating to spawn during the 1<sup>st</sup> quarter moon phase, the results supports the risk of predation hypothesis. Dusk spawning did not fully avoid predation: planktivorous fusiliers (*Caesio* spp.) were observed feeding on eggs in spawning aggregations on up to 27% of the rushes (Chapter Two). Possibly the risk is greater during the day.

Strong ebbing tidal currents have been considered important for carrying eggs away from the reef to avoid predation (Johannes 1978). Although spawning aggregations of coral trout occurred during the relatively stronger spring tides (new moon), the majority of aggregations monitored in the present study occurred on flooding tides and when currents at the primary sites were rarely strong. The equally high proportion of spawnings during the 1<sup>st</sup> quarter phase further suggest that tidal currents are not an important factor in determining the timing of spawning in *P. leopardus*.

There has been much discussion on the significance of protogynous hermaphroditism and its implications to fisheries management. The suggested mechanism is a flexible social organisation which enables protogynous hermphrodites to maintain high spawning success, for example by maturing or changing sex earlier (Bannerot et al 1987). Empirical data from porgy in South Africa (Garratt 1986) and gag from the Gulf

of Mexico (Koenig et al 1996) support the predictions of Bannerot and co-workers. However, despite the occurrence of compensatory mechanisms (eg. earlier sex change) in these species, the mechanisms did not operate effectively when fishing mortality was high. Using population simulation modelling based on the biology of the gag (Mycteroperca microlepis), Koenig et al (1996) demonstrated that the resilience breaks down at high fishing mortalities because sperm eventually becomes limited. Thus, populations appear to behave like non-compensatory protogynous hermaphrodites (Vincent and Sadovy 1998). In contrast, Huntsman and Schaaf (1994) demonstrated, again using simulation modelling, that the reproductive capacity of groupers is dramatically reduced by fishing, whether they be gonochores or protogynous hermaphrodites. Various compensatory mechanisms, such as earlier sex change and earlier female maturation, only improved their resilience to fishing at high fishing mortalities, compared with gonochoristic populations. Huntsman and Schaaf (1994), whose simulation model was based on the biology of the graysby (Epinephelus *cruentatus*), maintain that the relative improvement in reproductive capacity at high fishing levels is inconsequential because most of the original reproductive capacity will have been lost by then. The contrast between the two simulation modelling studies is striking; possibly it reflects the difference in biology between Epinephelus cruentatus and Mycteroperca microlepis.

Flexibility in sex change and sexual maturity are suggested by the biology of *P*. *leopardus*. Socially induced sex change was proposed for *P. leopardus* whereby aggregations provide the cues (Samoilys and Squire 1994, Shapiro et al 1993b), however, as stated above, cues from within aggregations would only be accessed by less than 20% of the population, for around 60% of the time that they are reproductively active. The broad range in size at sex change in coral trout (Chapter Four, Ferreira 1995) supports the social induction model (Shapiro 1987). In addition, the average size at first maturity was lower in the population studied here (Chapter Four) compared with other studies (Adams 1996, Ferreira 1995). These changes in female maturity and sex change may be compensatory, in response to fishing. If so, the mechanisms are functioning within widely dispersed social units (Samoilys 1987), i.e. those coral trout (the majority) that employ non- aggregative spawning.

Although coral trout employ aggregative spawning, using fixed sites and times, less than 20% of the population appear to use this strategy, and the estimate could be closer to 14% (Chapter Three). Other less significant spawning sites were found during the new moon but they were less predictable in occurrence and numbers of fish were considerably lower. In addition, coral trout also spawned outside the aggregative period at other locations, possibly back in their home ranges, presumably in pairs or small groups. Further evidence of their variable spawning strategies was seen in the presence of bisexual individuals (fish with both hydrated oocytes and dorsal sperm sinuses filled with spermatozoa, Appendix I), though they were rare.

The present study indicates that *P. leopardus* adopts more than one mating strategy, unlike other serranids studied to date, though it is seen in other families such as the scarids (Robertson and Warner 1978) and labrids (Warner and Robertson 1978). Different matings ("non-random mating", Partridge and Halliday 1984) are common in a number of animals, and are likely to have different fitness consequences. It would be interesting to examine whether the differences in spawning strategies between females is reflected in their fecundities. If, for example, egg output was maximised during aggregations (females were observed spawning up to five times in one evening at aggregations, Chapter Two), then the relatively few females that use aggregations may contribute disproportionately more to the egg output of the population.

By adopting various spawning mechanisms coral trout increased their spawning frequency which resulted in high annual fecundities. A high spawning frequency and range of mating mechanisms would maximise egg output in the variable environment characteristic of coral reefs. I suggest that the flexible spawning strategies seen in *P. leopardus* greatly increases their ability to adapt to environmental change. This should improve their capacity to respond to exploitation.

#### 5.3 Fisheries management implications

Minimum legal size restrictions are only effective if post-release survival is high (King 1995). Despite the use of this management tool for the Great Barrier Reef coral trout fishery for at least 15 years, and tagging studies on *P. leopardus* (Davies 1996, Bienssen 1989), post-release survival has not been quantified, but has been assumed based on anecdotal information. The high rate of tag resightings (64%) in the tagging experiment and the continuous resightings of tagged individuals over a three month period (Chapter Three), similar to those in another tagging study on *P. leopardus* (Samoilys 1997a), provides some evidence that post-release survival rates in coral trout are high.

The size at first maturity measured in the present study (Chapter Four), 21 - 25 cm FL, indicates that the present minimum legal size of 38 cm TL provides ample opportunity for females to spawn once before entering the fishery. It is also the smallest size at first maturity recorded to date for *P. leopardus* on the GBR. If this is a compensatory response to fishing pressure (Vincent and Sadovy 1998), the result suggests recreational fishing effort must be high since commercial fishing effort is not greater in the Cairns region compared with other regions of the GBR (Ayling et al in press, Mapstone et al 1996). However, natural variation in reproductive parameters in response to locality cannot be excluded (eg. Hart and Russ 1996). Further, sex ratios were not strongly female biased, nor was there evidence of a significant reduction in average size at sex change, both indicators of compensatory mechanisms in response to high fishing pressure.

Spawning aggregations are frequently targeted by fishers (Beets and Friedlander 1999, Sadovy et al 1994a), and elsewhere this has led to the disappearance of spawning aggregations and the subsequent collapse of fisheries of groupers (Johannes et al 1999, Sadovy 1994a,b, Bohnsack 1989). In response, some countries have introduced spawning site closures, such as for the Nassau grouper in Bermuda, and for coral trout in Palau (Johannes et al 1999). Having reviewed the GBR line fishery management plan (QFMA 1996) the Queensland Fisheries Management Authority have recently published a Draft Management Plan (QFMA 1999) which includes the introduction of seasonal spawning closures, based on some of the findings in the present study (Turnbull and Samoilys 1997). Spawning season closures have frequently been used to

protect reproductively active commercially important fishes, such as spanish mackerel and barramundi on the east coast of Australia (Kailola et al 1993). Two nine day closures which coincide with the new moon period are proposed for coral trout: September - October in the northern GBR and October - November in the southern GBR (QFMA 1999). The effectiveness of such closures will be dependent on a number of factors. Firstly, that fishing effort does increase during the spawning season, and that fishers are targeting spawning aggregations. Commercial log book data show that there is an increase in effort and catch of coral trout during spring (Turnbull and Samoilys 1997, Mapstone et al 1996). Davies (1996) in a tagging study on P. leopardus also states that catch and CPUE were greater during the spawning season. This increase is likely to be linked to favourable weather conditions at that time (pers. obs.). Evidence for the targeting of spawning aggregations on the GBR is largely anecdotal. There is no evidence of lunar periodicity in catch, effort or CPUE in the commercial log book data (Turnbull and Samoilys 1997). However, to interrogate the commercial log book data for lunar patterns assumes that fishers record their catches accurately on a daily basis. Since the data remains un-validated this assumption is equivocal. Further, there is now evidence of the disappearance of one spawning aggregation on the GBR. The aggregation at the Elford Reef primary site was not present during the October new moon period from 1996 - 1998, and diary records from a charter boat operator suggest the disappearance was caused by targeted fishing by local commercial operators (Samoilys et al in prep.). Thus, although the evidence for targeted fishing of spawning aggregations is weak, the assumption that it may be occurring is precautionary from a fisheries management perspective.

In summary, the proposed new moon spawning closures will protect *P. leopardus* when aggregating to spawn, and will therefore prevent targeted fishing of such aggregations. Further, although aggregative spawning makes up only a small portion of their reproductive output, 60% of spawnings occurred during the new moon, and 63% of spawnings occurred during September – October in the northern GBR. Therefore the proposed new moon closures will provide considerable protection to coral trout when spawning, which will enhance reproductive output (Beets and Friedlander 1999).

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# APPENDIX I

# Defining the reproductive biology of a large serranid *Plectropomus leopardus*

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# **1.0 Introduction**

There are three main objectives to this report. First, to provide a brief description of the ontogenetic and sexual development of gonadal tissue in the common coral trout, Plectropomus leopardus, as an introduction to the reproductive biology of a tropical protogynous hermaphrodite (section 2). Second, to provide details of the laboratory methods that we used to measure the reproductive stages and batch fecundity of P. leopardus (section 3). These were histology of gonad sections using haematoxylin and eosin stains, and counts of whole oocyctes, both standard procedures in reproductive studies. Third, to define the reproductive developmental stages in females and males, illustrated with colour plates, using detailed criteria to establish a standard for staging P. leopardus (section 4). We also discuss problems that we encountered in staging the gonads of P. leopardus, in section 5, to highlight some typical errors in histological interpretations, and provide some guidance in minimising these errors. Finally, in section 6, we provide brief concluding remarks. Much of this work is not new. We have relied heavily on previous reproductive work on P. leopardus by Ferreira (1995) and Adams (1996). Our aim is to synthesise information available from a variety of sources and provide a focussed laboratory guide for assessing the reproductive biology of a large exploited serranid on the Great Barrier Reef.

We have structured the report to reflect the objectives described above. We have also included a glossary to assist with terminology. In section 2 we have referred to a number of developmental stages with which the reader may not be familiar. These terms are fully described in section 4 where we define the developmental stages in detail.

# 2.0 Gonad development in Plectropomus leopardus

# 2.1 Maturity

*P. leopardus* is a protogynous hermaphrodite (Goeden 1978, Ferreira 1995), therefore a complete ontogenetic series in sexual development from very young immature fish to older sexually mature individuals, is only found in females. Males do not pass through an immature phase because they develop from mature females. Coral trout are considered to be monandric

(Ferreira 1993, 1995), that is males arise from one pathway: through sex change in females (Reinboth 1967). Such males are termed secondary males.

Females can be classified as either immature (juveniles) or mature depending on the ontogenetic stage of development. Minimum size or age at first reproduction may be used to define the stage at which females reach ontogenetic maturity. This has been estimated at 32-36cm FL and 2-3 years for *P. leopardus* by Ferreira (1995), and at 28cm FL and 1-2 years by Adams (1996). Mature females may have either inactive (resting) or mature (ripe) ovaries depending on the seasonal schedule of reproduction. Developmentally, this can be clearly defined by the presence of vitellogenic oocytes.

Individuals that are changing sex are termed transitionals and can be identified by a number of developmental characteristics co-occurring in gonads comprised of ovarian tissue. Fish which have recently changed from female to male will have partially developed testes, though they are nevertheless functional males. These are classified as incomplete males based on the proportion of testicular tissue in the gonad. Males in which the testes are fully developed are classified as complete (Adams 1996). This classification is somewhat arbitrary, but the developmental pathways for testicular tissue (spermatogenesis and spermiogenesis) are poorly correlated with seasonal schedules. Therefore, unlike females, stages of gametogenesis in males are difficult to use for defining maturity.

Pre-maturational sex-change, where males develop from immature females, may also occur in *P. leopardus*. Ferreira (1993) reports one male developing directly from an immature female for the con-specific *Plectropomus maculatus* based on the observation that "no signs of previous spawning were present and the macroscopic appearance of the gonad was that of an immature ovary". Ferreira (1995) also reported pre-maturational sex change in *P. leopardus*, though numbers of individuals were not provided. Adams (1996) reports for *P. leopardus* "one male that changed sex prior to maturing as a female". Neither authors provide details of the criteria used to determine that the female phase was juvenile. Pre-maturational sex change has been reported in other protogynous hermaphrodites (Sadovy and Shapiro 1987) such as the emperor *Lethrinus nebulosus* (Ebisawa 1990), the gobiid *Coryphopterus personatus* (Cole 1983), and the scarid *Calotomus spinidens* (Robertson et al 1982).

The sexual maturity or developmental stage of a gonad is defined by the stages of oocytes or spermatocytes present. However, this is somewhat complicated by the fact that *P. leopardus* are multiple spawners (Goeden 1978, Ferreira 1995). This means that oocytes and spermatocytes develop asynchronously (Ebisawa 1990, West 1990, Nagahama 1983, Wallace and Selman 1981). Thus the gonads of sexually mature females contain oocytes in various stages of development, from pre-vitellogenic to vitellogenic oocytes (Ferreira 1995). Similarly, males may contain sperm cells at various stages of development, from spermatocytes to spermatozoa, though spermatozoa are present for large portions of the year.

#### 2.2 Fecundity

Fecundity, or egg production, is usually expressed as annual fecundity: the number of eggs produced per year, by an individual, size or age class, population or stock. Counts of whole hydrated oocytes are used to measure fecundity. Annual fecundity is termed determinate in those species that produce all their eggs at one time such as haddock (*Melanogrammus aegelfinus*, Hislop et al 1978), whiting (*Merlangius merlangus*, Hislop 1975) and other species (Yamamoto 1956). In these species counts of hydrated oocytes at the time of spawning can provide a direct estimate of annual fecundity. In contrast, indeterminate fecundity is seen in fishes that continuously mature new batches of eggs throughout a typically protracted spawning season (Hunter et al 1985). This is recognised by the presence of oocytes in all stages of development in ripe females, as seen in *P. leopardus* (Goeden 1978, Ferreira 1995). Annual fecundity for species that spawn several batches within a season is less directly measured as a function of batch fecundity and the number of spawnings per year, or spawning frequency (Hunter and Macewicz 1985). Batch fecundity refers to the number of eggs produced in a single spawning batch (Hunter et al 1985).

Hydration represents the final vitellogenic stage prior to spawning. Hydrated oocytes are first seen within the ovarian lamellae. At ovulation they are released into the lumen. This should be considered when processing samples for oocyte counts. If ovulation has occurred oocytes may be lost from the lumen during tissue preparation (West 1990). It is therefore more accurate to measure batch fecundity of ovaries which are hydrated but have not yet ovulated. The latter is detected by the presence of free oocytes in suspension in preserved samples. With fresh samples (see below), this distinction is difficult and therefore batch fecundity measures will be based on hydrated oocytes that may be either within the lamellae or the lumen. A third

measures will be based on hydrated oocytes that may be either within the lamellae or the lumen. A third distinction is seen in samples obtained by "stripping" hydrated females. This technique, which involves applying firm hand pressure to the ventral surface of the fish (Rimmer et al 1994), is used to express eggs that have been released into the lumen (Figure 1). Counts from these samples will only give numbers of ovulated oocytes.



Figure 1. Expressing ("stripping") eggs from a ripe female Plectropomus leopardus.

# 3.0 Laboratory methods

# 3.1 Histology

Gonads were collected from Scott and Elford reefs on the northern Great Barrier Reef in 1992 and 1993 (Samoilys in prep.), and were preserved at sea in FAACC (Winsor 1984, 1991, 1994, see Appendix 8.2) within two hours of capture. For best results, samples should be transferred to 70% alcohol after one week of fixation. Here, histological processing of samples was conducted in the laboratory 6 - 18 months after capture without transferring samples to 70% alcohol. Although this is not recommended (Winsor pers. comm., Reilly pers. comm.) the appearance of the stained sections was clear, but there was a deterioration in the basophilic components of the tissue in some sections. This was due to the acid present in the FAACC. If samples are to be retained for long periods without transferring to alcohol, 10% phosphate buffered formalin is recommended rather than FAACC.

# 3.1.1 Preparation of tissue blocks

All preserved gonads were weighed to 1.0g. Those used for whole oocyte counts (see below) were weighed to the nearest 0.1g.

Tissue sections were taken from the right lobe (looking down on the dorsal surface of the fish). If the right lobe was damaged (ie. spear damage during sampling, poor dissection), the left lobe was sectioned and the discrepancy noted for those samples. Histological assessment of both lobes of *P. leopardus* found no differences between the two (Ferreira 1995), therefore this study standardised to the right lobe. Three sections, 2-4 mm in thickness, were made: Proximal (P), Medial (M), and Distal (D). Proximal sections were cut as close to the junction between the two lobes as possible. Medial sections were taken from the middle portion of the lobe where the lobe showed a uniform thickness (Figure 2). Distal sections were cut approximately 2 - 4 mm from the end of the gonad.



Figure 2. Cutting the medial transverse section from a *Plectropomus leopardus* gonad.

were placed in the same cassette. Large P, M and D sections that would not fit into the cassettes were separated either individually or as suitably sized pairs of tissue ie.[MD] sections together. Each cassette was marked with an 2B pencil ie. 523P, 546MD etc. The tissue cassette was then placed in 70% alcohol for a minimum of two hours.

Hydrated (see section 4) female gonads, including those that were stripped for oocyte counts (see below) were processed using agar moulds to hold the tissue together. The agar solution contained 3% Agar and 10% Formalin. The formalin preserves the agar (L.Winsor pers. comm.). Two – four millimetre sections (P,M,D) were cut for each gonad, as described above. Each section was then placed in melted agar in a metal mould. Having waited approximately 5 min for the agar to set, the agar block was removed from the metal mould and placed in a plastic casette, labelled, and processed normally (see below).

Eggs that had been stripped from hydrated females (see below) were also processed for histological examination, to confirm that they were hydrated. Using a pipette, a sample of eggs was placed in the top of a syringe in a syringe unit (after Cook and Hotchkiss 1977, Figure 3). Excess formalin was removed with the pipette. Melted agar was then added and stirred gently to mix the eggs but keep them near the bottom of the syringe. Having waited approximately 5 mins for the agar to set the syringe was then ejected. The top half of the agar block was removed with a razor blade and discarded. The bottom 3-4mm of agar block was then placed in a plastic cassette with label and processed normally (see below).



Figure 3. Diagram of a syringe unit (after Cook and Hotchkiss 1977) used for processing ovulated eggs histologically.

# 3.1.2 Processing of tissue

Gonads were routinely processed to paraffin wax using an automatic tissue processor (Winsor 1994). This machine dehydrates the samples, passing them through a graded series of ethyl alcohol; three changes of absolute ethyl alcohol; two changes of xylene; and finally impregnates them with paraffin wax. The cassettes were then placed in Labec Vacuum Setup for 30 minutes at 50 kPa, to complete the impregnation of the tissues with paraffin wax and remove any remaining air bubbles trapped within the tissue. The tissue samples were then embedded in paraffin wax using metal moulds, paying particular attention to the orientation of the individual samples (Figure 4). Tissue samples were then sectioned using a rotary microtome (American Optical (AO)). Sections were cut at  $5\mu$ m (after Ferreira, 1993, 1995).



Figure 4. Embedding tissue sections in wax blocks at an embedding centre.

A ribbon of sections was cut from each block, and floated out on a waterbath set at  $40 \degree C$  and containing gelatine to aid adhesion of the section to the glass microslide. Two to three sections were then recovered onto microslides. Temperatures >40 ° C were found to be too hot for the very large ripe females. All microslides were cleaned in alcohol prior to mounting of the sections. The orientation of the proximal, medial and distal sections on the microslide (Figure 5) reflected the arrangement of tissue samples set during embedding (see above).



Figure 5. Example of position of different tissue sections on a glass microslide, to facilitate recognition.

Slides were placed in a jar containing approximately 5 mls of formalin and the jar placed in an oven  $(60^{\circ} C)$  for 30 minutes. The heat causes formalin to form a vapour which aids adhesion of tissue sections to the microslides. Microslides were then removed from the formalin vapour jar, placed in a slide rack, and returned to the oven  $(60^{\circ} C)$  to dry for a minimum of 1hr 30min (usually overnight) prior to staining.

Sections were routinely stained using Mayer's Haematoxylin and Young's Eosin-Erythrosin stains (Winsor 1991, 1994) until the desired cytological elements were clear in the tissue section. Haemalum is basophilic and stains nuclei blue. Eosin is acidophilic and stains cytoplasm pink-red. Staining involved a routine process where tissue sections are dewaxed and taken to water, stained, then dehydrated and cleared. Histoclear, which contains the transition solvent limonene (Winsor 1994), was used as the clearing agent. Stained slides were mounted with a coverglass using DePex mounting medium.

#### 3.1.3 Reading histological sections - assigning stages

Slides were read in random order to avoid possible biases in interpretation associated with prior knowledge of collection time and fish size. Gonads were assigned to a developmental stage based on the most mature cell(s) present, regardless of how many there were (West 1990, Sadovy et al 1994). It is unusual for the most mature cells to be rare. For example, in the present study, in defining running ripe or hydrated females, 7 females out of a total of 117 running ripe females were found with only 1-2 hydrated oocytes.

The proximal, medial and distal sections were examined from 742 gonads. The sex structure of the population is shown in Table 1. Bisexuals were individuals which possessed both female and male cells capable of spawning at the same time (see section 4.2). Differences in development stage along the length of the gonad were not found by Adams (1996, n=30) who also examined longitudinal and saggital sections, and apparently were not examined by Ferreira (1993, 1995). To conform with Ferreira's (1993, 1995) and Adams (1996)'s work on coral trout reproduction, the medial section was taken as the standard, and different stages or cells in the proximal or distal sections were noted separately.

**Table 1.** Sex structure of population determined by reading all three sections (D,M,P) or by reading the medial section alone (M).

Sex	D, M, P sections	M section only
Female	440	445
Transitional	66	61
Male	243	243
Bisexual	3	3

Readings of proximal, medial and distal sections differed for 129 fish (17%, Table 2). These differences were analysed in terms of the error associated with reading the medial section only. The most accurate reading is that which detects the most advanced stage and any other cells used for defining reproductive status (e.g. post-ovulatory follicles).

Of the 129 fish that showed differential development along the length of the gonad, ten differed with respect to sex (1.3 % of the population) and these were all transitionals. Of the ten individuals, five would have been incorrectly sexed (classified as females) if only the medial section was read (Table 2). Thus, in terms of sexing a population, error rates are low (1.3%), but in terms of detecting transitionals within a population, the error associated with using medial sections only is relatively high (8.2%).

**Table 2.** Errors associated with reading medial sections alone when sexing and staging coral trout gonads. Number refer to the number of fish from a total sample of 742 individuals. Percentage error relates to the overall uncertainty or potential for error based on the number of gonads with a non-uniform development. \* = based on using all three sections.

Category	Total number in sample*	Number with non- uniform development		Number incorrectly staged if medial section read	
		Number	%	Number	%
All	742	129	17.4	N/A	N/A
Sex – all	742	10	1.3	5	0.7
Sex - Transitionals	66	10	16.3	5	8.2
Stage – Females	440	7	1.6	3	0.7
Stage – Males	243	0	0	0	0
Most mature cells – Female	440	14	3.2	6	1.4
Most mature cells – Transitionals	66	1	1.5	1	1.5
Most mature cells – Male	243	0	0	0	0
POFs	117	17	14.5	3	2.6

With respect to staging (e.g. Resting versus Ripe in females, see section 4.1 below), only females showed differences between medial and other sections (no stages were defined for transitionals, see section 4.2 below). Stages differed between sections for seven females (Table 2). Differences were seen between Running ripe and Ripe stages (n=5), for example hydrated oocytes not present in all sections, and between Resting and Ripe (n=2), for example yolk globule stage oocytes appearing in distal or proximal sections with only pre-vitellogenic cells in the medial section. Of these, three individuals (0.7% of all females) were less developed in the medial section compared with the proximal and/or distal sections, and would therefore have been incorrectly staged.

Within stages, the most mature cells were distributed unevenly in 14 females (excluding the 10 female/transitionals described above) and one transitional. Thirteen females differed within the

ripe stage (e.g. yolk globule vs migratory nucleolus stages) and one differed within the resting stage. In each case reading the medial section only would have classified 6 of these females incorrectly, i.e. the most mature cell would not have been detected (Table 2).

Other differences were minor and related to the non-uniform presence of cells such as atretic oocytes, post-ovulatory follicles, brown bodies and parasites. For example, of the 117 females with post-ovulatory follicles, only 3 would have been incorrectly classified as not having recently spawned if only the medial section was read (Table 2).

Despite these differences between sections, there was no consistent pattern in sexual development along the length of the gonad. For example, there was no gradation in development from proximal to medial to distal. On the basis of the low error rates between section readings it was concluded that taking the medial section as the standard reading was acceptable. Nevertheless, additional sections are recommended as a back- up because coral trout gonads are frequently infected with parasites which can engulf large sections of the gonad.

# 3.2 Whole oocyte counts for batch fecundity estimation

Counts of whole hydrated oocytes for measures of batch fecundity were done in volumetric or gravimetric sub-samples (Hunter et al 1985). Both techniques are described below. The gravimetric method was used on a subsample of hydrated ovaries collected and preserved in 1993 for maturity staging. The volumetric method was used for fresh ovaries (collected in 1994) and for samples of eggs that had been expressed (stripped) from freshly caught hydrated females in 1993. Fresh ovaries were collected and processed in 1994 because it was difficult to process the preserved samples from 1993 without damaging the oocytes. This problem was identified by Lowerre-Barbieri and Barbieri (1993), who developed a new technique for separating eggs prior to preservation. The method requires fresh ovaries, and therefore we obtained a small sample to trial this technique in 1994.

#### 3.2.1 Gravimetric method

Twenty-six hydrated ovaries (preserved in FAACC) that had been collected during the new moon period in the afternoon or evening of the 1993 spawning season were selected for oocyte counts. Total gonad weight (to 0.1 g) was obtained prior to any sectioning (see above). From each gonad three transverse tissue blocks approximately 2mm in width were cut as subsamples. One block was taken from each of the proximal, medial and distal sections of the left lobe. Preferably, batch fecundity should be measured in both lobes to test for differences between lobes, but was not done here because the right lobe had already been dissected for histology (see above). Therefore, the estimates are relative estimates, standardised to the left lobe, and should be interpreted as such. Tissue samples were large enough (approximately 10mg) to contain 100 - 200 oocytes (Hunter et al 1985). Each section was weighed to 0.1g. The tissue sample was placed on a slide and covered with 33% glycerol for  $\sim 15$  min. Oocytes were then teased apart and pipetted onto a raceway for counting under a dissecting microscope at 10-15X. Only the hydrated oocytes were counted. Hydrated oocytes are large, white and contain an oil droplet, characterisitics seen in the hydrated oocytes obtained from the stripped females (see below). Oocyte counts per gram were extrapolated to the whole gonad weight, then averaged from the three blocks (sub-samples), to estimate the total number of hydrated oocytes per gonad.

#### 3.2.2 Volumetric Method

The two samples that were processed volumetrically were prepared as follows:

## Fresh ovaries

Ten fresh gonads were collected in 1994 and each whole ovary weight was measured to 0.1g. Gonads were then cut into right and left lobes and each lobe weighed to 0.1g. After separation of the oocytes from the ovary membrane (see below) the empty gonad sac was also weighed to 0.1 g. Any oddities in the tissue such as parasitism were noted.

Oocytes were separated from the left lobe while being held over a collection sieve. The sieve was made from a piece of nylon plankton net (250  $\mu$ m mesh) attached securely to the end of a

15 cm diameter PVC pipe, with a depth of 15 cm (Figure 6, after Lowerre-Barbieri and Barbieri, 1993). The width and depth of the pipe allowed manipulation of the gonad without spillage.



Figure 6. Diagram of the mesh sieve used to separate hydrated oocytes in fresh gonads.

Separating oocytes from the ovarian tissue first involved cutting a longitudinal slit with a scalpel down the length of the lobe. Holding the opened lobe over the 250  $\mu$ m mesh sieve, the oocytes were then dislodged by directing a strong, fine jet of water at the ovarian tissue. A length of 1 cm diameter soft plastic tubing attached to a standard laboratory faucet provided the jet of water. Typically, each lobe took between 10 and 15 minutes to be cleared of oocytes. Parasitised samples took a little longer to clear as connective tissue around the parasites tended to hold the oocytes together.

Once all the oocytes were removed from the ovary, they were then washed into a funnel using the 250  $\mu$ m mesh plankton net as a filter. The oocytes were then transferred to containers where they were preserved in 2% neutrally-buffered formalin.

#### Stripped eggs

Eight females were stripped in 1993 to express ovulated hydrated oocytes (Figure 1). The eggs were immediately placed in 10% formalin which turns them white and hard (more so than FAACC). The gonad was also fixed and preserved in 10% formalin for histological processing (see above). Traditionally Gilson's fluid has been used as a preservative for egg counts because it hardens the oocytes and chemically separates them from the ovarian stromal tissue (Hunter et al 1985, Lowerre-Barbieri and Barbieri 1993). However it has substantial problems of oocyte shrinkage and destruction of hydrated oocytes, and it is also extremely toxic (Hunter et al 1985, Lowerre-Barbieri and Barbieri 1993). The destruction of hydrated oocytes makes it particularly inappropriate for fecundity estimation.

The freshly separated oocytes preserved in 2% buffered formalin (1994 samples) and the oocytes from the stripped females in 10% buffered formalin (1993 samples) were counted using the volumetric method described by Hunter et al (1985). The total volume of eggs was first measured to 1.0 ml in a graduated cylinder. Buffered formalin was added to round up the volume to a convenient number. To subsample, the oocytes were gently stirred to suspend them as evenly as possible in the formalin. 1.0 ml of the suspended oocytes was then extracted quickly with a Gilson pipette and placed in a glass raceway. Speed was important to obtain an evenly distributed subsample and to prevent blocking of the pipette. The total number of oocytes in the raceway was counted with a dissecting microscope at 10-15X. The oocytes were then replaced in the jar and the process repeated to obtain  $\geq$ 3 replicate subsample counts from each ovary. As in the gravimetric method, oocyte counts per 1.0 ml were extrapolated to the whole volume, then averaged across sub-samples to estimate the total number of hydrated oocytes per gonad.

Note that the present study did not compare batch fecundities between right and left lobes and therefore, extrapolating egg counts from the left lobe to the total gonad weight or volume may be innacurate. However, a paired t-test comparison between left and right lobe weights collected in 1994 revealed no significant differences between the lobes (t = -1.181, df=9, p=0.268).

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# 4.0 Developmental stages of maturity defined

The maturity stages defined as a standard for histological interpretation of *P. leopardus* gonads are listed and described in the following tables. A description of female and male gametogenesis also follows. The definitions have been developed from a sample of 752 gonads collected from Scott and Elford Reefs in 1992-1993, with considerable reference to previous work on *P. leopardus* by Ferreira (1994,1995) and Adams (1996), and to other relevant texts (Smith 1965, Yamamoto et al 1965, Yamamoto 1969, Nagahama 1983, Sadovy and Shapiro 1987, Ebisawa 1990, Sadovy and Colin 1995, Burton et al 1997). Assessment was made from the medial section of the gonad which gave 445 females (of which 35 were immature), 243 males, and 61 transitionals (see section 3.1.3, Table 1). The number of individuals of each maturity stage in the present study are also shown in the tables of definitions.

Since coral trout are multiple spawners the gonads contain oocytes and sperm cells at various stages of maturity. For females the stage of maturity is quite clearly defined by the most mature oocytes present (see section 3.0). Other criteria typical of each stage which assist in defining female maturity stage are also listed. For the transitionals and males, other criteria, such as features indicative of previous spawning as a female, and the development of the dorsal sperm sinus, are used to define sexual maturity, together with sperm cell stage. The diagnosis of male maturity schedules, however, is problematic because spermatozoa are present in male testes for long periods of the year (Samoilys in prep.).

# 4.1 Females

Female maturity stage can be clearly defined by whether the oocytes are vitellogenic or previtellogenic (Table 3, section 4.1.2). Further definitions relate to hydration, the final stage of maturity prior to ovulation, and atresia, the process of degeneration (section 4.1.2). Final oocyte maturation and ovulation are not necessarily associated (Nagahama 1983). However, in *P. leopardus* it was extremely rare to find hydrated oocytes that had become atretic (residual). Most atretic oocytes were from the preceding yolk globule stage suggesting that once hydration occurs ovulation will follow. Note that Ferreira (1993, 1995) did not differentiate ripe and running ripe into separate stages as defined here (Table 3). For *P. maculatus* Ferreira (1993), defined the ripe stage with either yolk vesicle, yolk globule or hydrated oocytes present. Ferreira (1995) varied this slightly for *P. leopardus* with yolk vesicle and early (primary) yolk globule oocytes defined as ripening and late (tertiary) yolk globule and hydrated oocytes defined as ripe.

The criteria for distinguishing between immature females and mature resting females are not infallible. This problem is discussed in section 5.

**Table 3.** Female gonadal development stages for *P. leopardus*, abbreviations refer to Plates. Defining characteristics are shown in bold italics. All oocyte cells that may occur are listed in developmental order (early stage first). Other criteria are useful aids in assigning gonads to a developmental stage. Numbers in parentheses refer to total number of individuals in sample.

Developmental Stage	Plate	Oocyte Stages	Other Criteria
Immature, IM (35)	1	<ul> <li>Pre-vitellogenic oocytes:</li> <li>oogonia, oo</li> <li>chromatin nucleolus, cns</li> <li>early perinucleolus, eps</li> <li>late perinucleolus, lps</li> </ul>	no sign of prior spawning: • no brown bodies • thin gonad wall • compact, lamellae well packed
Resting, RE (134)	2	<b>Pre-vitellogenic oocytes</b> (as above)	brown bodies (bb) common (102 fish) but not always present thick gonad wall lamellae not compact, often vaculated
Ripe, RI (127)	3	<ul> <li>Vitellogenic oocytes:</li> <li>yolk vesicle, yv</li> <li>early and late yolk globule, yg</li> <li>migratory nucleus stage, mns</li> </ul>	may have atretic oocytes (ao), post-ovulatory follicles (pof) or brown bodies from previous spawning
Running ripe, RR (117)	4	Hydrated oocytes, hy	post-ovulatory follicles and atretic oocytes may be present
Spent, SP (32)	5	Atretic vitellogenic oocytes, ao Pre-vitellogenic oocytes, eps, lps	lamellae disrupted and disorganised vascularised, vaculated, though not in early Spent stage brown-bodies generally present



Plate 1.Immature female (see Table 3). cns = chromatin nucleolus stage oocyte, eps= early perinucleolus stage oocyte, lps = late perinucleolus stage oocyte, gw = gonad wall.



Plate 2.Resting mature female (see Table 3). bb = brown bodies. Other abbreviationsas in Plate 1.



**Plate 3.** Vitellogenic or ripe female (see Table 3). yv = yolk vesicle stage oocyte, yg = yolk globule stage oocyte, mns = migratory nucleus stage oocyte.



**Plate 4.** Hydrated or running ripe female (see Table 3). hy = hydrated oocyte.



Plate 5.Spent female (see Table 3). ao = atretic oocytes of stages 1 (young) and 2(medium), vac = vacuoles, bb = brown bodies. Pre-vitellogenic stages as in Plate 1.



**Plate 6.** Very early post-ovulatory follicle (pof1) and late post-ovulatory follicle (pof4) in a running ripe female (see Table 4). eps = early perinucleolus stage oocyte, yg = yolk globule stage oocyte, n = nuclei of granulosa cells.

## 4.1.1 Post - ovulatory follicles

When hydrated oocytes are released (ovulated) into the lumen they leave a ruptured follicle termed the post-ovulatory follicle (POF). These follicles have a brief life; they generally last around 24hr in the tropics because their rate of absorption is determined by water temperature (West 1990). In *P. leopardus* POFs were found to last around 24hr (Samoilys in prep.). The stages of disintegration of POFs can be distinguished, in other words POFs can be aged (Hunter and Macewicz 1985) to provide a more precise indication of time of spawning and to calculate spawning frequency. Based on the descriptions of *Engraulis mordax* (northern anchovy) POFs by Hunter and Macewicz (1985), four stages were recognised for *P. leopardus* (Table 4). Note that POFs breakdown completely after the Late stage and cannot be distinguished. The position of the nuclei are illustrated in Figure 7.



Figure 7. Position of nuclei in post-ovulatory follicles (see Table 4).

# Table 4. Post - ovulatory follicle stages

	PLATE	DESCRIPTION				
STAGE		Folds	Nuclei	Other		
Very early, 1	6	many and very convoluted	apical, clearly visible	2 follicle membranes: thecal layer + granulosa layer		
Early, 2	7	many and convoluted	apical or central, clear	granulosa cells clearly defined		
Mid, 3	8	fewer and not distinct	basal or pycnotic, but some may still be central or apical	granulosa cells less defined		
Late, 4	6	none - compact	pycnotic or not visible	messy looking and contracted		

# 4.1.2 Description of female gametogenesis

(abbreviations relate to Tables and Plates)

# Oogenesis (pre-vitellogenic stage):-

# Oogonia (oo):-

Small round cells, in nests, with separated chromosomes in nucleus (Stage 1, Ferreira 1994, Adams 1996).

# Chromatin nucleolus stage (cns):-

Chromosomes no longer well separated, and form a network in nucleus (Stage 2 Ferreira 1994, Stage 1 Adams 1996).

# Early perinucleolus stage (eps):-

Perinucleolus stains very darkly (Stage 3 Ferreira 1994, Stage 2 Adams 1996).



Plate 7.Early post-ovulatory follicle (pof2) in a ripe female (see Table 4).Abbreviations as in Plate 6. Note nuclei are apical and central.



Plate 8.Mid post-ovulatory follicle (pof3) in a ripe female (see Table 4).Abbreviations as in Plate 6.

## Late perinucleolus stage:-

Perinucleolus stains more faintly (Stage 4 Ferreira 1994, Stage 2 Adams 1996).

# Vitellogenesis (vitellogenic stage = "ripening", or 2° growth phase):-

# Early yolk vesicle stage (yv):-

Yolk vesicles appear in the cytoplasm, dark lipid globules appear, granulosa cells' nuclei become differentiated.

#### Late yolk vesicle stage (yv):-

Lipid globules are more abundant and stain darkly; difficult to distinguish from early yolk vesicle stage, and were combined here (both vesicle stages: Stage 5 Ferreira 1994, Stage 3 Adams 1996).

## Yolk globule stages (yg):-

There are primary, secondary and tertiary yolk globule stages and it is difficult to tell these apart. The stage depends on the position of the nucleus. In these stages the yolk vesicles are coalescing. The nucleus is not always seen in section, it depends on the position of the section (Stages 6 & 7 Ferreira 1994, Stage 4 Adams 1996).

Note: yolk globule stage oocytes will not necessarily proceed to full maturation (hydration) – see atretic stage below.

# Migratory nucleus stage (mns):-

Yolk globules coalesce to form large empty spaces, typically 1-2, and the nucleus completes its migration to the cell membrane. This stage represents the start of hydration (Nagahama 1983, West 1990).

#### Hydrated oocyte stage (hy):-

The yolk globules have all coalesced and separated from the cell membrane. The centre of the cell appears all the same colour (pale pink) and the radiata is translucent and thinner (Stage 8 Ferreira 1994, Stage 5 Adams 1996).

#### **Degenerating stage:-**

There is a variety of degenerating cells present in female coral trout which aid in staging the gonads. These are described below. The Spent stage (Table 3) is defined by the presence of atretic oocytes but only once the gonad has reverted to a pre-vitellogenic state. Atretic oocytes are often seen in ripe females, and therefore cannot be used alone to define the Spent stage.

# <u> Atresia :-</u>

Atretic mature oocytes are collapsing un-ovulated oocytes. If the oocyte does not ovulate, the granulosa cells become hypertrophic and then phagocytic. They appear messy and the cell membrane is thin, disintegrating and the nucleus irregular. Atresia is not always a post-spawning phenomenon (Ebisawa 1990); it also occurs during sex change: mature eggs are phagocytosed first (Ferreira 1993, 1995). Similarly, mature oocytes may be unused for other reasons, these will also degenerate into atretic oocytes. Atretic oocytes are common in *Plectropomus leopardus*, suggesting that re-absorption of ripe oocytes is energetically preferable to ovulation if certain spawning conditions are not met.

Three stages, or ages, of atretic oocytes were recognised in *P. leopardus*, following the definitions of Hunter and Macewicz (1985). Two stages are illustrated in Plate 5.

## Post-ovulatory follicle (pof) stage:-

When the hydrated oocyte is released the follicle remains, and looks collapsed. It consists of the granulosa layer of the oocyte cell membrane which can be clearly distinguished for a short time. The follicle then degenerates over approximately 24 hrs (Samoilys in prep.) during which the granulosa layer becomes less and less distinct.

#### <u>Brown-bodies:-</u>

Atretic oocytes give rise to brown bodies which appear as yellow/brown "blobs" with an oily appearance. Brown bodies can also be seen macroscopically on whole gonads (including preserved ones) as brown dots along their length. Brown bodies are present in spawning and post-spawning females and in both resting and post-spawning males. In the latter they may be hormonal structures that originated from atretic oocytes.

## Fragmented (reabsorbed) previtellogenic oocytes:-

These are degenerating early oocytes. Ferreira (1993) reports they are commonly seen in male gonads, but they are difficult to distinguish from laboratory processing artifacts (Adams pers. comm.).

## 4.2 Transitionals

The key feature which identifies transitionals is the presence of spermatic tissue in a gonad consisting largely of ovarian tissue (Table 5). Sadovy and Shapiro (1987) describe the sperm tissue as proliferating and the ovarian tissue as degenerating. However, in early transitional *P. leopardus* the sperm crypts are small and few, and the ovarian tissue is not yet degenerating. In fact, male cells may occur only as spermatogonia which are difficult to detect. Thus, transitionals were defined here as gonads in which the male cells had at least completed spermatogenesis (see below), ie. spermatocytes had formed. The absence of spermatozoa in the dorsal sperm sinus (Hastings 1981) was used to distinguish transitionals from incomplete (early) males. Fragmenting and re-absorbing pre-vitellogenic oocytes are features listed by Ferreira (1993, 1995) and Adams (1996) for transitionals, but they are difficult to distinguish from artifacts caused by laboratory preparation (Adams pers. comm.). Other features listed in Table 5 draw on Ferreira (1993, 1994, 1995) and Adams (1996) and the present study.

Three bisexual fish were also identified in the present study. They were rare (0.4 % of the population) and have not been documented for *P. leopardus* before. These individuals were distinguished by the possession of both female and male cells capable of spawning at the same time. They contained hydrated oocytes and the dorsal sperm sinus was filled with spermatozoa (Table 5).

Table 5. Transitional and bisexual gonadal development stages. Criteria are separated into those that define the stage (Defining criteria) and into others (Other features) that are useful aids in assigning gonads to a developmental stage. Strongly defining characteristics are shown in bold italics. Numbers in parentheses refer to total number of individuals in sample. Abbreviations refer to Plates.

Developmental stage	Defining criteria	Other features
Transitional, T (61) Plate 9	dorsal sperm sinus (dss) not fully formed; precursory dss may be present but contains no sperm cells gonad consists largely of ovarian tissue but sperm crypts are present	fragmentation and reabsorption of pre- vitellogenic oocytes may occur brown bodies common (40 fish), but not always present yolk vesicle or yolk globule oocyte stages rare* (4 fish) atretic oocytes seen rarely (3 fish) sperm crypts typically at periphery of gonad wall sperm cells usually present: spermatogonia sg, spermatocytes sc, spermatids st spermatozoa sz, are rare and then only in crypts
Bisexual, B (3) Plate 10	dorsal sperm sinus filled with spermatozoa hydrated oocytes	gonad resembles a running ripe female with vitellogenic oocytes including hydrated oocytes (Table 3), except it contains a fully developed dorsal sperm sinus filled with spermatozoa

\* In this context Ferreira (1993, 1995) refers to transitionals and females interchangeably.

# 4.3 Males

Both ontogenetic and sexual development must be considered when staging male coral trout gonads. Two ontogenetic stages were defined: incomplete (recently changed sex) and complete (Table 6), which give an approximation of how recently the male changed sex. Sexual stages (Table 7) are seen in both incomplete and complete males. Again, criteria listed in Tables 6 and 7 draw on Ferreira (1993, 1994, 1995) and Adams (1996), the present study and some standard texts (Wallace and Selman 1981, de Vlaming 1983, Nagahama 1983).



**Plate 9.** Transitional gonad (see Table 5). st = spermatids in sperm crypts, pdss = position where dorsal sperm sinus will form, eps = early perinucleolus stage oocyte, lps = late perinucleolus stage oocyte.



**Plate 10.** Bisexual gonad showing both hydrated oocytes (hy) and the dorsal sperm sinus (dss) filled with spermatozoa (sz). Other abbreviations as in Plate 3.

Table 6. Male ontogenetic stages of development. Criteria are separated into those that define the stage (Defining criteria) and into others (Other features) that are useful aids in assigning gonads to a developmental stage. Strongly defining characteristics are shown in bold italics. Numbers in parentheses refer to total number of individuals in sample. Abbreviations refer to Plates.

Developmental stage	Plate	Defining criteria	Other features
Incomplete ripe male, I (43)	11	dorsal sperm sinus, dss, formed and filled with spermatozoa sz gonad dominated (>50%) by pre-vitellogenic ovarian tissue spermatozoa	lamellae not yet lobular- typical of complete male atretic oocytes may still be present spermatogonia sg, spermatocytes sc, spermatids st, may be present
Complete ripe male, C (187)	12	gonad dominated (>50%) by spermatogenic tissue dorsal (dss) and central (css) sperm sinuses filled with sperm cells spermatozoa	sperm crypts join to form large intra-lobular sinuses, css pre-vitellogenic oocytes may still be present spermatogonia, spermatocytes, spermatids may be present


**Plate 11.** Incomplete male (see Table 6). Gonad dominated (>50%) by pre-vitellogenic ovarian tissue. dss = dorsal sperm sinus, sz = spermatozoa. la = lamellae still female in shape. Other abbreviations as in Plate 1.



**Plate 12.** Complete male (see Table 6). Gonad dominated (>50%) by spermatogenic tissue. css = central sperm sinus, st = spermatids, sc = spermatocytes, bb = brown bodies. Other abbreviations as in Plate 11.

Table 7. Male sexual stages of gonadal development. Criteria are separated into those that define the stage (Defining criteria) and into others (Other features) that are useful aids in assigning gonads to a developmental stage. Strongly defining characteristics are shown in bold italics. Numbers in parentheses refer to total number of individuals in sample. Abbreviations refer to Plates. Note Spent and resting males may be incomplete or complete.

Developmental stage	Plate	Defining criteria	Other features
Ripe , R	11, 12	dorsal sperm sinus, dss, formed and filled with	atretic and pre-vitellogenic oocytes may still
(230)		spermatozoa, sz	be present
		central (css) sperm sinuses filled with spermatozoa	spermatogonia sg, spermatocytes sc,
			spermatids st, may be present
Spent, S	13	spermatozoa rare and only in crypts	other (early stage) sperm cells uncommon
(4)		vascularised, vs, and well developed stromal	dorsal sperm sinus, dss, empty or collapsed
		<i>tissue,</i> sr	with some spermatozoa
		disorganised	brown bodies bb, common/abundant
Resting male, R1	14	spermatozoa not present or rare	dorsal sperm sinus empty or collapsed with
(post-spawn)		vascularised and well developed stromal tissue	some spermatozoa
(7)		compact	brown bodies common/abundant
			other (early stage) sperm cells common
Resting male, R2	15	spermatozoa rare and only in crypts	other (early) sperm cells dominate
(pre-spawn)		little or no vascularisation	dorsal sperm sinus empty
(2)		little or no stromal tissue	brown bodies common/abundant



**Plate 13.** Spent male showing typical disorganised appearance of gonadal tissue, extensive stromal tissue (sr) and vascularisation (vs) (see Table 7). bb = brown bodies, sz = one crypt with spermatozoa, st = spermatids.



**Plate 14.** Post-spawning resting male (R1), showing typical compact appearance of gonad lobules (lo) (see Table 7). sg = spermatogonia, p = parasites. Abbreviations as in Plate 12.



**Plate 15.** Pre-spawning resting male (R2) showing little vascularisation and little stromal tissue (see Table 7). Abbreviations as in Plates 12 and 14.

#### 4.3.1 Description of male gametogenesis

#### *Spermatogenesis*:- (spermatogonia → spermatocytes)

#### Spermatogonia:-

These are the largest male cells and they occur in a crypt. They resemble oogonia, but they do not develop a chromatin nucleus. The lack of clear, distinctive morphological features between oogonia and spermatogonia is well known (Reinboth 1980).

#### 1° spermatocyte stage:-

Large nucleus, small amount of cytoplasm.

2° spermatocyte stage:-Nuclei well separated and stain darkly.

#### *Spermiogenesis:-* (spermatocytes -> spermatozoa)

#### Spermatid stage:-

Spermatids have small nuclei, are close together, occur in crypts, and they stain dark red (with haematoxylin/eosin).

#### <u>Spermatozoa:-</u>

Spermatozoa are spermatids with tails which stain blue (haematoxylin); the tails stain pink (eosin) though these can be difficult to see. Spermatozoa in sexually mature males occur in crypts which have joined to form sinuses.

# 5.0 Problems in defining stages

## 5.1 Distinguishing between immature females and mature resting females

One of the most critical problems in staging *Plectropomus leopardus* gonads was the difficulty in distinguishing between immature females and mature resting females. Clearly, this is critical for establishing minimum size at first maturity, a vital population characteristic in exploited species used to determine minimum legal size in fisheries management (Hill 1992). There is a minimum legal size for the *P. leopardus* fisheries on the Great Barrier Reef, which has been in place at least since 1984 (Samoilys pers. obs.) though no records exist as to when it was introduced (Hancock 1992).

Gonad wall thickness (Sadovy and Colin 1995, Burton et al 1997) is a useful guide (Table 3) but it is not completely reliable because wall thickness can vary in different sized females, through poorly treated samples, or through differences in sectioning. Similarly the presence of brown bodies is indicative of a resting mature female, however not all resting mature females will have brown bodies, although the majority do (Table 3). Ferreira (1994) proposed that the relative proportion of early and late pre-vitellogenic stages may help in separating resting from immature females, but it seems unlikely that such differences could be distinguished from ontogenetic changes in oocyte development. Sadovy and Colin (1995) used the presence of prominent intralamellar muscle bundles in *Epinephelus striatus* as evidence of prior spawning. Unfortunately, such bundles were not apparent in *P. leopardus*. Preliminary work on histological stains for detecting lipofuscin which is present in mature females suggests such techniques may provide a more conclusive tool for distinguishing between mature resting females and immature females (Adams 1996).

In the present study the difficulty in detecting immature females with certainty is illustrated as follows. Of the 35 Immature females identified in a total sample of 169 pre-vitellogenic females, 14 were difficult to define with complete certainty, using the characteristics of gonad wall thickness and the presence/absence of brown bodies. Four of these fish were taken during September – October and therefore if they were mature females they were unlikely (66-100%, Samoilys in prep.) to have been in a pre-vitellogenic state. A further four were taken in November when the likelihood of being vitellogenic drops to 58-61% (Samoilys in prep.). Based on these likelihoods these 8 females were retained as Immatures. Note, than an

additional individual of 48.5 cm FL originally defined as immature was unlikely to be so at that size, and was therefore re-classified as Resting. It was also vaculated which is characteristic of resting females. In summary, the remaining six (17%) of the 35 (or 3% of the pre-vitellogenic females) were classified as Immature, but with no great certainty.

# 5.2 Defining male stages

In contrast to females, development stages in male coral trout gonads were much harder to define and were not closely linked to the spawning season. Ripe males were found throughout the year, including March and July which are outside the spawning season (Samoilys 1997), though sampling was incomplete (Samoilys in prep.). Resting or spent males were uncommon (n = 13 out of 243). The definitions proposed here for spent and resting (both pre and post-spawning) male coral trout differ from those of Ferreira (1993, 1995) and Adams (1996) and are an attempt to provide unambiguous definitions. The definitions described here are put forward as a proposal and require further testing. Discussions with Samantha Adams greatly facilitated the proposed definitions. The primary criteria we have used are the presence of spermatozoa, the presence of stromal tissue or vascularisation, and the appearance of the gonad.

If spermatozoa are present the male is considered to be capable of spawning and is therefore ripe unless the spermatozoa only occur in crypts and then rarely (1-2 crypts). In this case the male is considered to be either resting or spent (Transitionals also fall into this category). To distinguish between resting and spent males we have used the presence of stromal and vascular tissue and the appearance of the gonad. Spent and post-spawning resting males are the most difficult to distinguish because they both have well developed stromal and vascular tissue. However, spent gonads are disorganised, whereas resting gonads are compact (post-spawning) or organised (pre-spawning). Two types of resting males were apparent: pre-spawning resting males were characterised by gonads which were not vascularised and did not contain stromal tissue and were not compact. They only differed from ripe gonads by their lack of spermatozoa.

The phenomenon of pre-maturational sex change, or fish that have become male prior to maturing as a female, has received little attention in this report (see section 2), because criteria were not established to define these individuals. This requires further research.

# **6.0 Conclusions**

Assigning discrete stages to a continuous variable is always problematic. However, the ability to classify gonads into maturity stages provides a basis for quantitative evaluation of reproductive state. This is particularly useful for comparing the reproductive biology of populations through, for example, time and space, or in relation to impacts such as fishing. It is perhaps less useful for quantitative comparisons between species where differences in functional morphology of gonads are probably more interesting and revealing.

The usefulness of clearly defined developmental stages for quantifying the precise timing of spawning and spawning frequency is obvious, and has been demonstrated for female *P. leopardus* (Samoilys in prep.). What remains is to assess the usefulness of the male stages for examining spawning patterns in male *P. leopardus* in greater detail. Further, the application of the gonadal developmental stages proposed here to other Serranidae would also be an interesting avenue of study.

This report has highlighted some of the difficulties in assigning developmental stages to fish gonads. One of the most pressing needs is the development of reliable staining techniques for distinguishing between immature and mature resting females. This is vital if we are to accurately determine minimum size at first maturity in these important commercial species. In addition, the phenomenon of pre-maturational sex change in *P. leopardus* requires further investigation. There has been no research done on the juvenile developmental stages of coral trout gonads. This could provide interesting insights into the developmental pathways in coral trout reproduction, which would elucidate the proposal that diandric male *P. leopardus* may exist in the southern Great Barrier Reef (Adams et al in review).

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# 8.0 Appendix

# 8.1 Glossary of terms and features for describing Plectropomus leopardus gonads

## 8.1.1 Female structural terms

*Lumen* = cavity which collects ovulated eggs ready for emission

Lamellae = term used to describe the structure of ovarian tissue (see lobules below)

*Nests* = small round space containing oogonia

## 8.1.2 Male structural terms

Crypts = small, round space containing spermatogonia (early stages)

Lobules = term used to describe the structure of testicular tissue; lobules are small lobes, similar in form to the ovarian lamellae from which they have developed - this is a feature of monandric protogynous hermaphrodites (Sadovy & Shapiro 1987)

Sinus = long, elongate space containing spermatozoa (late stage). One main dorsal sinus runs along the length of the gonad. There are central sinuses also. Sinus walls stain dark

Vestigial lumen = remains of ovarian lumen, into which lobules project

#### 8.1.3 General terms

*Basophilic* = "base loving" - stains acidic substances; the blue stain of Haematoxylin is an example - it stains spermatids, spermatozoa and nuclei

Cytoplasmic inclusions = organelles in the cytoplasm e.g. yolk vesicles etc.

*Diandric* = where there are two types of males in hermaphrodites: primary males, which develop directly from juveniles, and secondary males which develop from females (see monandric)

Eggs = oocytes that are at least ovulated (West 1990) - use for spawned gametes, otherwise the term oocyte is used

*Functionality* = as male or female

Gametogenesis = gonadal development

*Gonad wall* = wall of gonad; dorsal wall is usually thicker because it is attached to lateral body wall or mesenteries on dorsal side

Granulosa = outermost membrane of oocyte, see radiata

*Hypertrophic* = become bigger (a pathological term), e.g. granulosa cells of oocyte follicle

*Monandric* = one type of male in protogynous hermaphrodites, which develop from the female (see diandric)

*Multiple stages of oocytes/sperm cells* = Presence of multiple stages in the one gonad is logically an indication of multiple spawning

Ovulation = release of hydrated oocyte from its follicle

*Perinucleolus* = membrane round egg nucleus; becomes less distinct with maturity

*Pycnotic* = broken down

*Radiata* = membrane of oocyte next to granulosa layer

Stromal tissue = supportive tissue in gonads e.g. between crypts - common in spent males

*Vaculated* = several empty spaces

Vascularised = new capillaries, veins and blood vessels developed in tissue

# 8.2 Chemicals and materials used

# FORMALDEHYDE-ACETIC ACID-CALCIUM CHLORIDE FIXATIVE (FAACC) (extract from Winsor 1991, 1994)

formaldehyde (40%)100	ml
acetic acid (glacial)50	ml
calcium chloride (anhydrous)10	g
OR calcium chloride (dihydrate)13	
tap water	ml
рН	1.9-

# 2.0

Specimens are fixed for a minimum of 24 hours at ambient temperature, but preferably a week (formaldehyde takes about a week to fix tissue components). No deleterious effects have been observed in tissues stored for about 2 months in the fixative.

FAACC fixative was designed by L. Windsor at JCU in 1981 specifically as a substitute for BOUIN'S PICRO-FORMAL-ACETIC ACID FIXATIVE, widely used as a fixative for fish gonads.

Bouin's (1897) PFAA fixative has several disadvantages which include cost, need to wash fixed specimens to remove picric acid prior to storage or processing (adding to cost), poor long term storage characteristics (loss of nuclear staining in stored tissues), hazards of picric acid (explosive when dry; causes dermatitis), poor field fixative - containers invariably leak, invariably resulting in yellow-stained field notes and personal gear! Tissues fixed in Bouin's

exhibit acidophilia (very eosinophilic in an H&E stain), though this can be overcome by reducing the formaldehyde to 4%. The cost of Bouin's fixative and subsequent ethanol washing is 5.6 times that of FAACC.

FAACC fixative has none of the disadvantages of Bouin's PFAA fixative. It is simple, relatively inexpensive, stable and non-staining. Tissues fixed in FAACC do not require washing in ethanol, and have good balanced staining characteristics. The appearance of stained FAACC fixed tissues closely approximates that of tissues fixed in Bouin's, and also that of gonads fixed in DAVIDSON'S FIXATIVE (used by some fish laboratories).

FAACC has been used for gonad fixation of a variety of fish species which include Coral trout, Red-throat emperor, Whiting, Anchovy, Spanish Mackerel, Black Marlin, Sail fish, Grunter, Threadfin and Parrot fish. It is also used as a gonad fixative for Giant clams, oysters, squid and cuttlefish.

In FAACC the formaldehyde is a non-coagulant cross-linking protein fixative. The acetic acid precipitates DNA and is included to off-set hardening due to formaldehyde, providing a reasonable texture for microtomy. Calcium (as calcium chloride) fixes certain lipids (eg. lecithin) and probably behaves as an additive fixative for some proteins (the role of calcium in fixatives is presently poorly understood).