University of Newcastle

The Reproductive Biology and Spawning Behaviour of *Chromis hypsilepis* (Pisces: Pomacentridae)

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BSc

Submitted in fulfilment of the requirements for the degree of Masters of Philosophy at The University of Newcastle, Australia.

March 2010

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Dedication

I dedicate my thesis to my Great Uncle Tom, may he rest in peace. He was a wonderful family man, and the reason why I had the ability to study and get my degree wherever and however I wished. Thank you, and let your soul be forever blessed for the things you did for the family and the world.

RIP Thomas Thewes

Acknowledgements

I owe my success to having listened respectfully to the very best advice, and then going away and doing the exact opposite. ~ GK Chesterton

I would like to thank my parents first. They financially supported my hopes to move to the other side of the world, and study. To my Mother who flew over with me on the first of the many long flights, helped me find a decent place to stay, and also stayed up late to talk to me online during those times when we were apart. She has also supported me through the many ups and downs that a thesis has, while always staying positive. I thank my family for all their moral support.

To my advisers, for all their help from the proposal to the final editing, all three were extremely helpful. To Bill Gladstone, for all our ups and downs, I thank you. Between the equipment failures, data collections, collection assistance, and MANY edits, you were a huge help, throughout the entire research project, and every step of the way. To Jason Morton, for the major assistance with the initial dissections and understandings of the research, to the statistical analysis, your assistance is one that could not have been done without. I thank you, and all your assistance from the start to the absolute finish of this research. And last but certainly not least, Doug Ferrell, I thank you. Between the many hours down in Cronulla, your assistance with time management and otolith sections were amazing. The words of encouragement to the checking of my ageing estimates, you went above and beyond anything I could have expected. My advisers were a critical part in the entire process, and for that, I will be forever grateful.

To my dive partners; the many that I have had over the past 2 years, a VERY large thank you. Especially to Steve and Shanti, for spearing most, if not all of my fish (except the 5 I ended up spearing, and I believe only by accident). I will never forgive you for making me watch the bate bag and fighting off eels and sharks in the process...

But for those who went diving with me, and for me, I thank you. This research could not have been completed without you. To all those who helped me in any way, it was much appreciated!

To the many at Cronulla Research Centre: Thank you. To the people and facilities, for all the materials, helpful guidance, and good times, thank you for assisting with the project; your experience and expertise were wonderful to learn from.

To the major financial contribution to my Great Uncle Tom, and the Legion Foundation, for their wonderful scholarship that supported my studies throughout everything; including that which got me to where I am today, I thank you the most.

To Terrigal Crown Plaza for their kind funding of the underwater cameras, I thank you.

To those who were the sanity through instant messages, emails, private messages, and the outings that de-stressed me, I thank you. To facebook and those friends on it... I thank you for all my time wasted, while I tried to regain some of my sanity.

I thank my wonderful partner, throughout our friendship and every stage of our relationship, I thank you. You were the support that I have never asked for but always received and wanted. For all the diving you did for me, with me, and risking your life, in some situations, in the process. I am eternally grateful for all you have done. LYFAD Dazz.

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Abstract

Fishes of the family Pomacentridae utilize a great range of reproductive strategies, although most research has focussed on tropical species. *Chromis hypsilepis* is a schooling planktivore that occurs on temperate rocky reefs from northern New South Wales to northern Tasmania. During the summer breeding season, large numbers of *C. hypsilepis* migrate to spawning aggregation sites. This spawning strategy is unusual in the family Pomacentridae and demersal spawning is very uncommon among fishes that form spawning aggregations. This study was undertaken to fill the gap in understanding of the reproductive biology of *C. hypsilepis* and to provide detailed descriptions of the reproductive behaviour as part of improving the understanding of this unusual reproductive strategy. Age, growth, sexual maturity and reproductive behaviour of *C. hypsilepis* were described from a population at Terrigal, New South Wales. The length and age at sexual maturity were also compared to a population at Jervis Bay, 200 km south of Terrigal. *C. hypsilepis* is gonochoristic and occurred in a 1:1 sex ratio. The use of bands in the sagittal otoliths of *C. hypsilepis* as a measure of age was validated with marginal increment analysis. A maximum age of 22.5 years was recorded. Males and females, respectively, attained sexual maturity at 75.2 and 89.2 mm SL at Terrigal, corresponding to ages of 1.9 and 2.0 years. Von Bertalanffy growth models showed that males and females attained 50% of their asymptotic length within 1-2 years. There was minimal variation between the Terrigal and Jervis Bay populations. Spawning behaviour was described using mounted underwater video cameras, recording up to 11 hours per day. Spawning was acyclic. Spawning occurred throughout the day, building up during the morning hours to a peak at midday and dramatically dropping in frequency throughout the rest of daylight hours. The results of this study are a necessary component of understanding the unique reproductive strategy of *C. hypsilepis*.

Abbreviations

T_{50}	Age at which 50% of population become sexually mature
cm	Centimetre
DPI	Department of Primary Industries
g	Grams
>	Greater than
≥	Greater than or equal to
GSI	Gonadosomatic Index
L_{50}	Length at which 50% of population become sexually mature
<	Less than
≤	Less than or equal to
MIA	Marginal Increment Analysis
Max	Maximum
µm	Micrometer
mm	Millimetre
min	Minimum
n	Sample size
sec	Seconds (time)
SE	Standard error
SL	Standard length
%	Percentage
W_{50}	Weight at which 50% of population become sexually mature

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Chapter 1.Introduction

1.1 Research Context

Chromis hypsilepis (family Pomacentridae) is a schooling planktivorous fish that occurs in temperate waters from northern New South Wales (NSW) to northern Tasmania and occasionally New Zealand (Francis, 1996; McGrouther, 2006). Recent research has revealed that *C. hypsilepis* has a reproductive strategy that involves the migration of mature males and females to a limited number of spawning aggregation sites, demersal spawning on a semi-lunar cycle, and paternal care of the developing embryos (Gladstone, 2007b). The potential benefits provided by the spawning aggregation site include reduced predation on spawning adults, developing embryos, and newly hatched larvae (Gladstone, 2007a). Many species of fish migrate and aggregate to spawn at a restricted number of locations during their spawning season (Claydon, 2004). However, this strategy is unusual within the family Pomacentridae. A more complete appreciation of the uniqueness of the reproductive strategy of *C. hypsilepis* is not possible because of gaps in understanding about its reproductive biology and behaviour. This is the situation for most species of pomacentrids in south-east Australia (Tzioumis and Kingsford, 1995).

The aim of this project was to describe the reproduction of *C. hypsilepis.* The research addressed the reproductive biology (gender system, length/weight at sexual maturity, longevity, geographic variations) and spawning behaviour (diel variation in spawning frequency, participation in spawning). Monthly collections of gonads and otoliths were done at Terrigal, NSW, Australia (33° 26’ 52.84” S, 151° 26’ 40.06” E) over sixteen months. Age was determined from otolith growth rings and annual periodicity of growth ring formation was validated by marginal increment analysis. Monthly collections of gonad samples, coupled with histological examination, were

used to verify that the species was gonochoristic. A smaller collection of fish from a more southerly location, Jervis Bay, NSW, Australia (35° 01' 44.74"S, 150° 42' 41.48" E), provided a geographic comparison of the age, length, and weight at sexual maturity. A combination of underwater observations and long-term remote video recordings were used to provide a detailed description of spawning behaviour, including diel variation in spawning frequency and the participation of individuals in spawning.

The remainder of this chapter provides a review of the literature on the reproductive biology of pomacentrid fishes and the available information on *C. hypsilepis*. Chapter two provides the results of research into the annual reproductive cycle of *C. hypsilepis* and sexual maturity. This chapter also includes the analysis of geographic variation of *C. hypsilepis*, between Jervis Bay, NSW and Terrigal, NSW. Chapter three addresses the spawning behaviour of *C. hypsilepis*, including descriptions of spawning, estimates of participation rates in spawning and time allocated to spawning, and diel and seasonal variations in spawning rates. Chapter four concludes and discusses the research and possibilities for applications and future research.

1.2 Pomacentridae

The family Pomacentridae (damselfishes) contains 348 species, within 28 genera (Allen *et al*., 2002), in four subfamilies: Amphiprioninae (anemone fish, 2 genera), Chrominae (damselfish, 5 genera), Lepidozyginae (with 1 monospecific genus, *Lepidozygus tapeinosoma*), and Pomacentrinae (damsels, 20 genera) (Jang-Liawa *et al*., 2002; Allen *et al*., 2006). *Chromis hypsilepis,* the subject of this study, is from the subfamily Chrominae, which consists of four genera: *Chromis* (75 species), *Dascyllus* (9 species), *Azurina* (2 species), and *Acanthochromis* (1 species) (Nelson, 1994). Australia has 141 species of pomacentrids (Allen *et al*., 2006), which accounts for 42%

of the world's pomacentrid species. The Indo-Pacific region contains up to 83% of pomacentrid species (Allen, 1996).

Pomacentrids are one of the most numerous groups of fishes on coral reefs in terms of numbers of individuals and numbers of species (Allen, 1975). Pomacentrids are usually small, with coral reef species commonly less than 100 mm SL, and temperate reef species growing to over 250 mm SL (Jang-Liawa *et al*., 2002). The Eastern Pacific species, *Chromis punctipinnis*, grows to 330 mm SL (Allen, 1991). While the majority of pomacentrid species are found on tropical reefs (Allen, 1991), many species are found in temperate, brackish, and freshwater environments (Allen, 1989). Three species, including one from Australia, are mainly estuarine fish, which occasionally will enter fresh water (Allen *et al*., 2002). The freshwater demoiselle, *Neopomacentrus taeniurus*, is an Australian species that occurs in coastal embayments in brackish waters, mangrove estuaries and in the lowermost reaches of freshwater streams (Allen *et al*., 2002). Damselfish are usually associated with coral or rocky reefs and therefore usually found near shore and in shallow water (mostly less than 25 m deep) (Jang-Liawa *et al*., 2002). The extremes of this include *Chromis struhsakeri,* which have a depth range between 99 and 183 m, while *C. viridis* has a range between only 1 and 12 m (Allen, 1991). While there is no evidence of a strictly pelagic pomacentrid species, most species have a pelagic larval phase (Kavanagh, 2000).

The diversity of damselfishes drops off rapidly with increasing latitude (Kingsford, 1999). Forty-six species have been recorded from NSW waters (Kuiter, 2000). Many tropical damselfish are transported southward on the East Australia Current as larvae but do not survive the harsh temperate winters (Figueira *et al*., 2009). Species like *Stegastes gascoynei, Stegastes fasciolatus,* and *Pomacentrus milleri* are just a few of the tropical species which have been seen in the temperate waters of NSW. *Parma* species are known to be the most abundant, as well as the largest, damselfish found in

the waters of NSW. In southern Queensland and NSW, the most abundant species is *Parma polylepis*. In northern-central NSW, *Parma unifasciatus and P. microlepis* are two of the most abundant species (Kingsford, 1999). *P. microlepis* is the most common on reefs in the central to southern NSW. *P. microlepis* is a permanently territorial species, with both genders holding their own territories on the reef; females leave their territories during the spawning season to enter into the males' territories for spawning. These territories are clearly defined and many species, including other *P. microlepis,* will be fought off if entering into the area. The spawning season is October through January, which is similar to that of the *C. hypsilepis.*

Feeding habits represented within the family Pomacentridae include planktivory, omnivory, and herbivory. *Stegastes nigricans* is an omnivorous species, consuming mainly algae over various size ranges, however small *S. nigricans* will consume invertebrates. *Cheiloprion labiatus* is the only known consumer of coral (Allen, 1991). Species in the subfamily Chrominae have a protrusible upper jaw, which is a typical feature of diurnal planktivores that permits the mouth to project to engulf prey without swimming forward (Sales, 1999). Allen (1991) reported that all species of *Chromis* are planktivores. Many species of *Chromis,* in large aggregations (Claydon, 2004), feed on zooplankton above reefs (Dinwiddie *et al*., 2006). One example, *Chromis chromis,* acts as an intermediary, transferring energy from the pelagic to reef environments through their consumption of zooplankton in open water and their excretion of energy-rich faeces at night onto the reef (Pinnegar and Polunin, 2006).

1.3 Pomacentrid Reproductive Biology

1.3.1 Spawning Seasons

The duration and timing of the spawning seasons varies among species of pomacentrids. Many species have one spawning season while others have two over

the year consisting of a primary and a secondary, or smaller, season. For example, *Eupomacentrus fuscus* spawns in three smaller seasons, in January, June, and September (Munro *et al*., 1973). *Chromis dispilus*, in New Zealand, spawns from early December to the beginning of April (Kingsford, 1985). *C. hypsilepis* in NSW spawns from early October through to the middle of February (Gladstone, 2007b). Distinct spawning seasons are present in many tropical species. *Abundefduf saxatilis* has a spawning season from April to November (Munro *et al*., 1973). The spawning season for *Acanthochromis polyacanthus* is from October to January (Thresher, 1985). None of the 25 species of pomacentrids reviewed by Gladstone and Westoby (1988) exhibited continuous year-round spawning. More recent work revealed that *Dascyllus trimaculatus* however, spawns year-round, with an increased frequency between June and August (Schmitt and Holbrook, 1999).

1.3.2 Spawning Cycles

The spawning cycle of a species is defined as the spawning frequency throughout the spawning season. The spawning cycle of pomacentrid fishes within the genus *Chromis* can be lunar (occur once around the new, full, or quarter moons), semi-lunar (occurring on both the new and full moons), periodic (occurring at regular intervals unrelated to the lunar cycle), or acyclic (no relationship with the lunar cycle or no clear pattern). Species with lunar spawning cycles include *Chromis cyanea* (Cole, 2008) and *C. mulitineata* at Punta de San Blas (Robertson *et al*., 1990). Species with semi-lunar spawning cycles include: *C. hypsilepis* (Gladstone, 2007a), *C. notata* in Koinoura (northern Kyushu) (Nakazono *et al*., 1979) and *C. viridis* (Cole, 2008).

Periodic spawning unrelated to the lunar cycle has been reported in *Chromis multilineata* in South Bimini (spawning 8 days after full moon) (Myrberg *et al*., 1967) and *C. dispilus* (spawning every 8-10 days) (Tzioumis and Kingsford, 1995). Acyclic

spawning, where there is no periodicity in the spawning activity (Tyler and Stanton, 1995) has been reported for *C. notata* at Mukaishima Island, Japan (Ochi, 1986).

Timing of spawning within the spawning season has also been shown to be related to fish size. In *Dascyllus ablisella* large females spawned earlier than smaller females (Asoh, 2003). This has positive effects on the reproductive success of the smaller individuals. Many smaller fish in a species will lay their eggs in nests which have eggs already in them. Larger clutch sizes of individual nest-guarding males are associated with more parental care and less filial cannibalism, consumption of one's offspring (Asoh, 2003). With the shorter development time, the eggs from the larger fish will have had time to start developing before the smaller fish has laid eggs in the male's nest, thus increasing the reproductive success of the species.

1.3.3 Spawning Strategies

Demersal eggs are produced by all pomacentrid species, which are guarded and tended until hatching (Allen *et al*., 2006). Most pomacentrid species demonstrate a two phase life span with a pelagic larval stage, and a benthic juvenile-adult phase. This stage allows for the dispersal of the larvae over large distances (Bernardi *et al*., 2003). The only species of pomacentrid without this dispersive larval stage is *Acanthochromis polyacanthus* (Doherty *et al*., 1995; Planes and Doherty, 1997).

Some spawning strategies reflect the non-spawning behaviours of species (Allen *et al*., 2006). Permanently territorial species, such as *Stegastes nigricans,* use their territories for feeding, shelter and spawning (Karino and Nakazono, 1993). Species of tropical *Chromis* (Cole, 2008) and *Dascyllus* are intermediate, using their territories for shelter and spawning, while also dependent on the water column for foraging.

At least twenty-one families of tropical reef fish (including pomacentrids) form spawning aggregations in a limited number of areas within their range (Russell, 2001). These courtship and spawning areas are known as traditional spawning sites, or fish

spawning aggregation sites, FSAS. A typical spawning location will have several key characteristics, including strong currents that ensure the dispersal of eggs and larvae away from the spawning site to ensure increased probability of larval dispersal (Russell, 2001). Courtship, spawning and nesting occur on rocky and sandy bottoms within the spawning sites (Hutchinson, 2006). Courtship behaviours can occur within or above the spawning territories. *Chromis hypsilepis* is non-territorial normally, establishing a territory only for the few months of the spawning season. *C. hypsilepis* spawns in aggregations after migration to the FSAS and only in a small section of the available reef (Gladstone, 2007b).

A behaviour, that some temporarily territorial species use, is to "cluster" (Allen and Gomon, 2008) in specific regions, which occurs regularly. This clustering is advantageous to both females and males. When the males group together at central territories, the clump becomes more visible to females, while reducing the predation risk to them as well. This grouping tends to be a hotspot for females to come to, which allows less attractive males to crowd around the more attractive males. This gives the less attractive males a chance to increase their spawning success while reducing conflicts among territorial males (Godin, 1997).

1.3.4 Gender Systems

Pomacentrid species can be hermaphroditic or gonochoristic. Individuals of hermaphroditic species can exist as functional males and females simultaneously throughout all or most of their lives (called simultaneous hermaphroditism). This gender system has not been reported for pomacentrids. In sequential hermaphroditism some species exist as females when small and change sex to become male at a larger size (called protogynous hermaphroditism) or exist as males when small and change sex to become female when large (called protandrous hermaphroditism). Gonochoristic species retain separate functional sexes throughout their life. The Amphiprioninae

subfamily is known to have the largest number of hermaphroditic species within the family Pomacentridae. Amphiprioninae exhibit protandrous hermaphroditism where, in group situations, the dominant male will change sex to become a dominant female when the female is removed or killed (Forsgren *et al*., 2002). Protandry occurs when male-male competition is less important in sexual selection. The change of sex is theorized to occur when the benefits from having a larger body size (and thus the higher fecundity associated with it), outweigh the benefits of being a male (Forsgren *et al*., 2002). Protogynous hermaphroditism within the family Pomacentridae is known only from the genus *Dascyllus* (Asoh, 2005). The majority of fish are gonochoristic (Yaron and Sivan, 2006) and within the family Pomacentridae, gonochorism has been verified in *Parma microlepis* (Tzioumis and Kingsford, 1999) and species of *Dascyllus* (Asoh, 2005)

1.3.5 Mating systems

The mating systems of pomacentrids include lek-like spawning aggregations, long-term monogamy, polygyny and polyandry (Nakazono *et al*., 1979). Spawning aggregations with solitary or communal spawning can occur with lek-like or promiscuous spawning strategies. Species of *Chromis, Neopomacentrus, and Abundefduf* exhibit lek-like spawning aggregations (Helfman *et al*., 2009). Lek-like spawning aggregations occur when males aggregate in specific areas for the purpose of courting females and spawning with them, while the females come to the area just for spawning (Godin, 1997).

Promiscuous mating is based on females and males mating with every opportunity and can be the cause of little to no sexual dimorphism (Godin, 1997). Males court any female that passes their territory. Promiscuous mating has been reported in *Pomacentrus nagasakiensis* (Moyer, 1975) and species in the genus *Eupomacentrus* (Godin, 1997). Allen (2006) observed that promiscuity has so far only

been observed in the pomacentrid fishes. Spawning by species which utilize spawning aggregation sites displays promiscuity-like characteristics. Males and females tend to be promiscuous in these situations; however, female promiscuity is not widely studied (Godin, 1997).

Polygamous mating systems occur in two main forms. When a female has several male partners, it is called polyandry. Within the family Pomacentridae this is only known to occur in the anemone fish subfamily Amphiprioninae. Females defend territories around the host anemones, a form of resource-defence polyandry (Godin, 1997). The dominant fish is a female, with many males around the anemone, where they lay and raise young inside the area protected by the anemone. One dominant female presides over many subordinate males, within one or two sea anemones (Godin, 1997).

Polygynous mating systems in the family Pomacentridae are exhibited by species in the genus *Dascyllus* including: *D. aruanus, D. marginatus, D. reticulates, D. albisella*, and *D. melanurus* (Fishelson, 1998).

Monogamous mating systems develop when the female territories are so large that the dominant male cannot suppress every other male within the territory. Monogamy is based on female-male pair bonds that are formed in two ways: continuous throughout the lives of the two individuals, or temporary for one act of spawning or the whole of the spawning season. Barlow (1984) defines monogamy in teleosts fishes as either repetitive spawning between two individuals, or mutually caring for young. Long-term monogamy is a rarity in the family Pomacentridae and has been reported for *Acanthochromis polyacanthus* (Kavanagh, 2000), and three species of anemone fishes: *Amphiprion bicinctus*, *A. frenatus* and *Premnas biaculeatus* (Fishelson, 1998).

1.3.6 Parental Investment

Parental investment theory provides an evolutionary explanation for the investment of time and energy by parents in their offspring, which potentially reduces their own survival and future reproductive success (Godin, 1997). For most pomacentrids, after successful courtship, the female will deposit eggs in nests males have created in their territories. The eggs, which have a tuft of adhesive filaments, will attach to the substratum (Hutchinson, 2006). After spawning, the female typically leaves the male's territory to either spawn with another male, or to obtain more energy (via feeding) for continued reproductive purposes (Tzioumis and Kingsford, 1995). Males are the primary care givers, defending the area from predators until the eggs hatch (Tzioumis and Kingsford, 1999; Asoh, 2003; Hutchinson, 2006). The costs to males from their egg care include reductions in their feeding rate of 24% and 85% (Robertson *et al*., 1990; Gladstone, 2007b).

Unique to the pomacentrids (and amongst reef-associated fishes), *Acanthochromis polyacanthus* has a long period of parental investment. Both parents care for around 100 fertilized eggs and the hatched larvae and juveniles until they grow to 28 to 40 mm in length, usually requiring several months (Barlow, 1981). Monogamous pairs, like those in the genus *Amphiprion*, care for their eggs, until hatching, when the larvae become dispersed in the plankton (Kavanagh, 2000). *Stegastes nigricans* exhibits minimal parental care. The fertilized eggs are scattered around the algae covered nests and the fanning action that many species use to clean the nest is minimal. The scattered pattern of eggs, which are attached to the filamentous algae, allows circulating water to supply oxygen (Karino and Nakazono, 1993).

1.4 *Chromis hypsilepis*

1.4.1 General Knowledge

Chromis hypsilepis (Figure 1.2) are found from northern NSW to northern Tasmania, Lord Howe Island and Norfolk Islands, and northern New Zealand, reaching their greatest densities on the rocky reefs of NSW (Kuiter, 2000; Gladstone, 2007b; Allen and Gomon, 2008). *C. hypsilepis* has a maximum length of 160 mm SL and is found within shallow depths to more than 40 m (Kuiter, 2000; Allen and Gomon, 2008). The species normally occurs in very large groups on reefs with outgoing currents along reef ridges (Kuiter, 2000; Allen and Gomon, 2008).

C. hypsilepis is known to have some commercial importance (New South Wales, 2007) (Figure 1.1). In the 2005-06 financial year more than 16,000 fish (representing 1,684 kg) were caught between Jervis Bay and Wollongong as by-catch. Accidental catch of this species has been sold in the Sydney fish markets (recorded from the 2001/2002 financial years onwards). *C. hypsilepis* were caught as by-catch from purse seines and hauling nets for garfish or general purpose fishing. In Wollongong alone, in the 2006-07 financial year, 1,137 kg were obtained. Future studies need to identify if the species is a reliable species for supplementary fish resources. The large abundance of *C. hypsilepis*, and its relatively young age at sexual maturity, suggests it could be used as a replacement for over-fished resources .

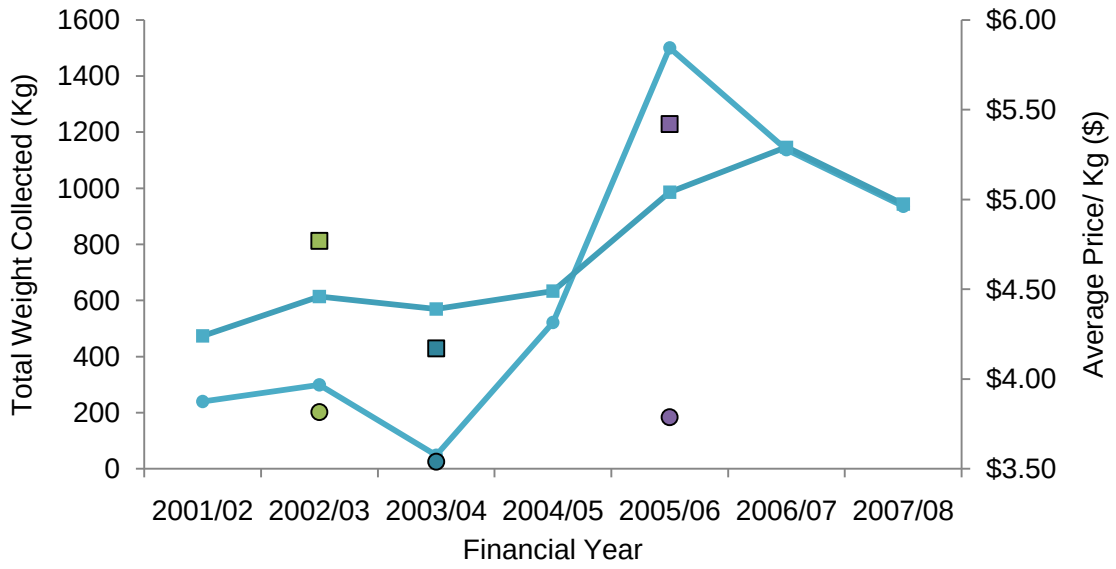


Figure 1.1 Catch records for *C. hypsilepis* by method. Graph shows total weight collected (Kg) ● and the average price per Kg ■ at 3 locations, with catch method indicated: Wollongong (◠ by purse seine; ▫ by hauling nets), Jervis Bay ▫ (by purse seine) , and Hawkesbury River ▫ (by hauling nets).

Large boulders cover more than 50% of the spawning aggregation site, which is similar to non-spawning sites (Gladstone, 2007a). The spawning aggregation site is unique in having more overhangs and crevices where *C. hypsilepis* spawn and stronger currents for a more rapid dispersal of newly hatched larvae to open water (Gladstone, 2007a).

Previous observations and studies of *C. hypsilepis* have touched on general observations of spawning (Tzioumis and Kingsford, 1995), spawning season and cycles, diel variation in spawning frequency (Gladstone, 2007b) and the unique characteristics of the spawning aggregation site (Gladstone, 2007a). *C. hypsilepis* prefer spawning aggregation sites with large over-hanging boulders and avoid exposed flat rocks when spawning (Gladstone, 2007a). *C. hypsilepis* were described as aggregation spawners in which spawning occurred on a semi-lunar cycle between early October and mid February (Gladstone, 2007b). Gladstone (2007a; b) also reported no

significant diel variation in spawning frequency, a significant reduction in feeding rate of brooding males, and egg hatching 4.5 days after spawning at 3-7 hours after sunset. The two studies (Gladstone, 2007a; b), were both completed over one spawning season in one location, Terrigal, NSW, Australia. The spawning observations were completed on multiple dives per day during one spawning cycle, and the finding of no significant diel variation in spawning frequency was based on observations at discrete time intervals (0600, 0830, 1130, and 1500 h). Spawning by *C. hypsilepis* has been reported only from one other study (Tzioumis and Kingsford, 1995), where it was described as synchronous with no predictable frequency. This finding differs from the semi-lunar spawning cycle reported by Gladstone (2007b).

Figure 1.2 *Chromis hypsilepis* at Terrigal, NSW

The aims of this study are to investigate the reproductive biology of *C. hypsilepis* and to determine the maturity parameters and the behaviours associated with their spawning habits. The maturity parameters will also include an examination of possible geographic variations. Diel variation in spawning frequency and spawning periodicities were observed using non-invasive video observations, while recording up to 11 hours of data within the spawning season, considerably extending the extent of observations over those previously reported (Gladstone, 2007b).

Chapter 2.Reproductive Biology

2.1 Introduction

2.1.1 Aims and Overview

The aims of this study are to determine aspects of the reproductive biology of *Chromis hypsilepis*. The age, length and weight at maturity were determined by examining fish collected over a period of 16 month study at Terrigal, NSW. Gonads and otoliths were collected and examined for maturity stages and age determination. The age at maturity of the population at Terrigal was determined through studying yearly rings on the hard surface of otoliths. The age was compared to the size of the specimen and the maturity stage of the gonad. Specimens were categorized by gender, standard length (mm), total length (mm), and weight. A geographic variation study was done in February 2008, comparing the age, length, and weight at maturity of *C. hypsilepis* at Terrigal to a different population in Jervis Bay, southern New South Wales.

2.1.2 Introduction

There are many techniques to determine the age of fish. These are radiocarbon uptake, RNA:DNA ratio, growth in a controlled environment, mark-recapture analysis, length-frequency distributions, and back-calculation from rings on hard structures such as bones, otoliths and scales (Moyle and Joseph J Cech, 2004). Growth rings are found in the otoliths and are observed, most commonly, through sectioning (Hernaman *et al*., 2000). The use of otoliths to age fish is one of the most commonly used analyses, with its modest sampling requirements and low costs (Campana, 2001).

Studies of the growth, age, and longevity of temperate species of the family Pomacentridae are rare. One species that has been studied is a local temperate

damselfish, *Parma microlepis*. *P. microlepis* deposit growth increments on an annual basis, before their spawning season, June to September (Tzioumis and Kingsford, 1999). The longevity and growth rate are large, compared to the few other damselfish studied. For example, growth rate of *Dascyllus albisella* (k= 0.23) was much less than *P. microlepis* (k= 0.41). *Pomacentrus moluccensi,* had a growth rate of k= 0.45, which is also similar to other tropical species of pomacentrids (Tzioumis and Kingsford, 1999).

The anemonefish, *Amphiprion percula*, was estimated to have a life expectancy of up to 30.8 years of which 14 years was spent as a female (Buston and Garcia, 2007). Other anemone fishes have been recorded with longevities of 18 years (*A. frenatus* and *A. perideraion*) and 12 years (*A. clarkii*) (Buston and Garcia, 2007). The maximum longevity of *Parma microlepis* was estimated to be 37+ years (Tzioumis and Kingsford, 1999). The tropical species *Pomacentrus wardii* and *P. moluccensis* have maximum ages of 10 and 17 years respectively (Tzioumis and Kingsford, 1999). Two temperate species, *Parma victoriae* and *Stegastes altus* have longevities of 15 years (Buston and Garcia, 2007). *S. fuscus* reached a maximum age of 15 years (Schwamborn and Ferreira, 2002), however, they also showed large variability in growth rate between individuals. *Chromis chromis* is a small reef fish with a maximum age of 9 years, which was attributed to the high mortality rate due to predation (Dulčić and Kraljević, 1995).

Few studies have reported geographic variation in longevity within the family Pomacentridae. Meekan *et al*. (2001) examined many small damselfish species in the Galapagos Archipelago and Panama. In the Galapagos Archipelago, this study found the maximum ages for *Stegastes acapulcoensis and S. arcifrons* to be >20 years and for *S. leucorus beebei* to be >15 years. In Panama, *S. acapulcoensis* reached a maximum age of 10+ years. *S. flavilatus* from Panama were relatively short lived,

reaching a maximum age of 4 years, while in Baja, individuals from Baja reached ages up to 10 years (Meekan *et al*., 2001).

There are different gender systems in pomacentrids. Most species of pomacentrids are gonochoristic (Tzioumis and Kingsford, 1999). Some pomacentrids change sex (hermaphrodism). Simultaneous hermaphroditic species are rare, and do not occur within the family Pomacentridae. However, some pomacentrids are sequentially hermaphroditic. Anemonefishes are protandrous, starting out as male and changing into a female at later stages. Protogynous species of pomacentrids, like *Dascyllus aruanus*, change from first being female to later being male in their lifetime (Helfman *et al*., 2009).

Many types of mating systems are represented in the family Pomacentridae including monogamy, promiscuity, and polygamy. With some species, like most *Chromis* species, spawning aggregations are used as a spawning strategy, increasing the likelihood of promiscuity. The small site-attached pomacentrid, *Pomacentrus nagasakiensis*, is a solitary species that mates in a promiscuous way. Most species in the Amphiprioninae subfamily exhibit polyandrous mating systems with harems (Helfman *et al*., 2009). Only one species is known to be monogamous in the pomacentrid family; *Acanthochromis polyacanthus*, which is a mouth brooder, which has one mate for the breeding season.

There are few examples of polymorphism in pomacentrids as their mating systems do not encourage sexual dimorphism or dichromatism (Feeley *et al*., 2009). *Parma microlepis* is permanently polychromatic, changing body colour between juveniles and mature males and females (Tzioumis and Kingsford, 1999). Some species are seasonally dimorphic, varying slightly during their spawning seasons (Helfman *et al*., 2009). Most species of pomacentrids are monomorphic, showing no distinguishable differences on the external forms of the males or females.

2.1.3 Overview and Chapter Objectives

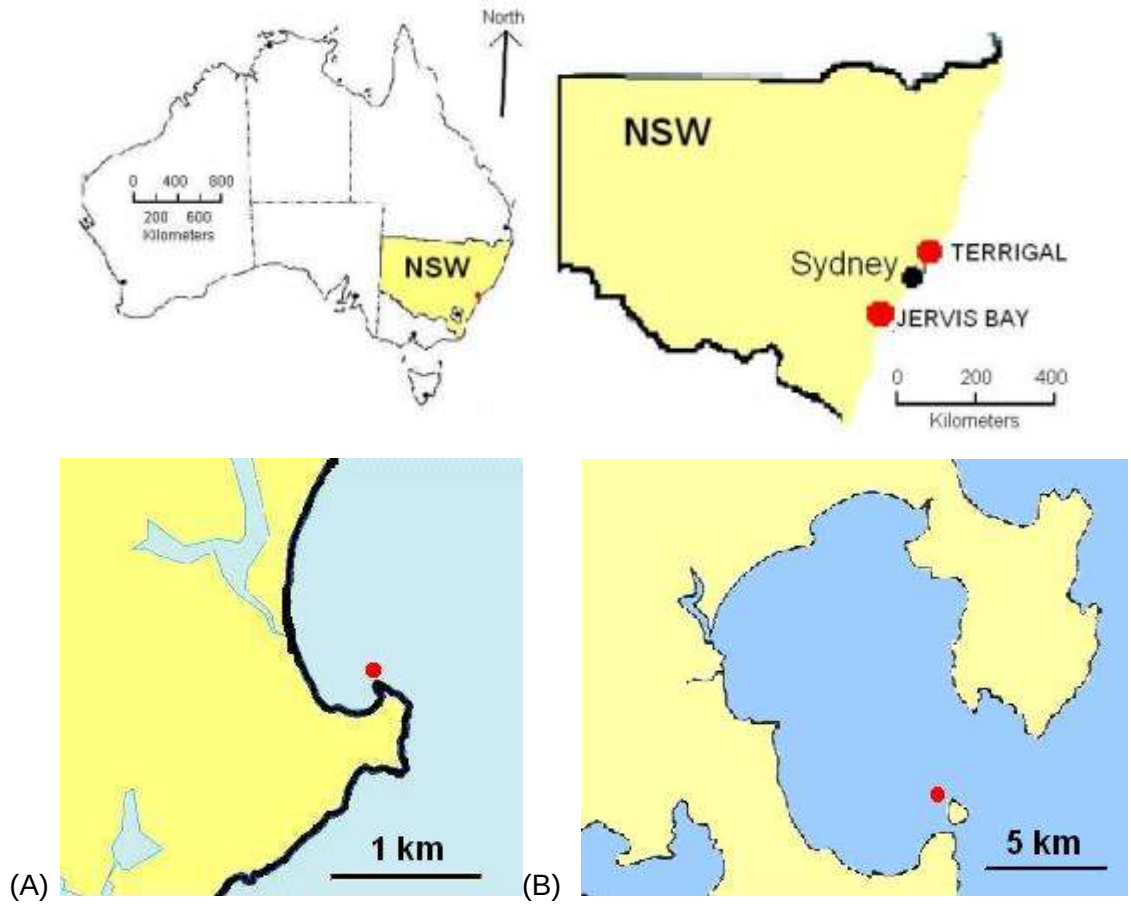
This chapter investigates the reproduction, growth and maturity of *C. hypsilepis* by describing the relationships between age and length, age and weight, age and GSI, and the relationship between size and age at maturity. The technique used for determination of age is counting otolith increments, and validating the yearly formations of the annuli using marginal increment analysis to determine longevity and growth rate.

2.2 Methods and Materials

2.2.1 Study Regions

The majority of the research occurred at Terrigal, New South Wales, Australia (33° 27' 00" S, 151°26' 00" E) (Figure 2.1). The site, known as One Spot City, has four types of habitats, which are described by Gladstone (2007b). This site starts with a shallow fringe reef dominated by algae at 3-5 m, followed by urchin-grazed barren boulders with abundant sea urchins (*Centrostephanus rodgersii*) at 5-18 m. Between 18 and 22 m, the reef is covered by encrusting species. Kelp (*Ecklonia radiata*) beds occur intermittently between depths of 10-15 m, between the various habitats.

A second study site was used at Bowen Island, Jervis Bay, NSW (35° 01' 44.74"S, 150° 42' 41.48" E) (Figure 2.1) in the Jervis Bay Marine Park. The site is a rocky reef habitat with a substratum of urchin grazed boulders at 5-12 m depth.



(A) (B)

Figure 2.1 Locations of study sites (A) Terrigal (B) Jervis Bay. Study sites are indicated by red dots and yellow shaded areas depict land.

2.2.2 Field Sampling

Twenty-five to 30 fish were collected monthly from April 2007 to August 2008 at Terrigal. Specimens were collected on SCUBA with hand spears with approval from the University of Newcastle's Animal Care and Ethics Committee (ACEC: 1054 0708) and the New South Wales Department of Primary Industries (DPI Permit Number: P07/0035). Collected fish varied in standard length (SL) from 82 mm to 165 mm (n= 478). A larger sample of fish was collected, in February 2008, when *C. hypsilepis* are known to spawn (Gladstone, 2007a) to determine the age at sexual maturity at Terrigal and Jervis Bay. Fifty-five fish (63-144 mm SL) were collected at Terrigal and 66 fish (68-157 mm SL) were collected at Jervis Bay.

2.2.3 Gonad Collection and Storage

Gonads were dissected from the fish collected during the monthly sampling and a macroscopic colour and stage analysis done. The gonads were described as a particular shade of one of four colours from a colour chart created for the study (Figure 2.2). This chart was created using a base of red, orange, yellow, and amber. The gender was then determined for each sample through macroscopic observations using the descriptions in Haddy and Pankhurst (1998) (Table 2.1). Adding to the descriptions, the stage 2 males were classified mature and recessed gonads, due to no stage devoted to recessed mature gonad states in males, as there were with the female stages. The gonads were preserved in a 10% formalin solution, and then transferred into 70% ethanol, weighed to the nearest 0.0001 g and stored.

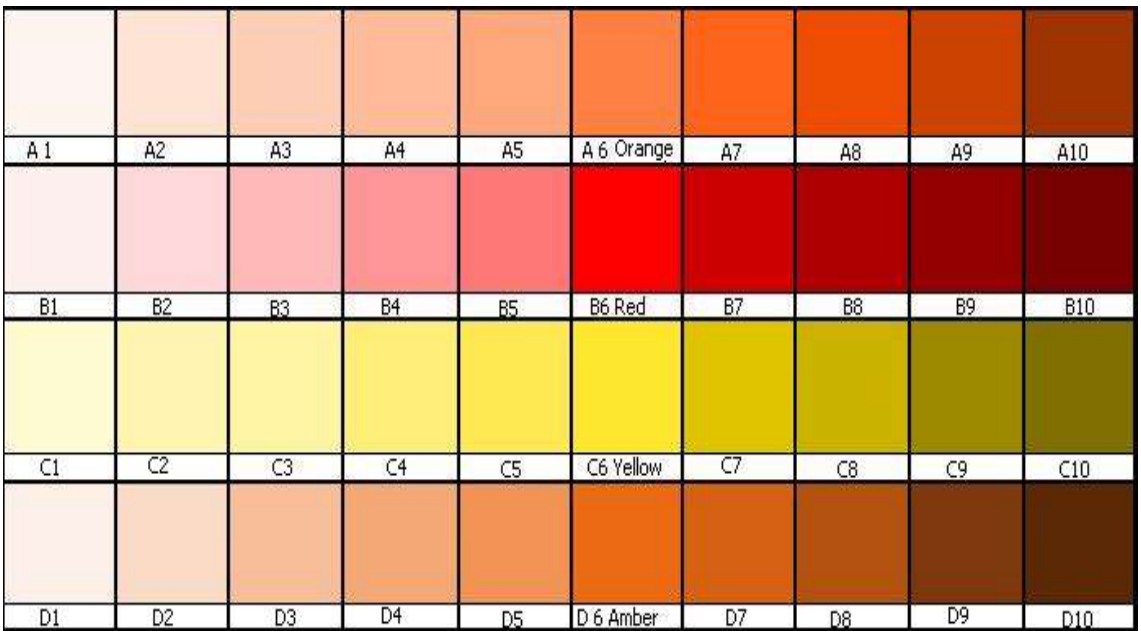


Figure 2.2. Colour swatch used to classify colour of macroscopic samples of the gonads of *Chromis hypsilepis*.

Table 2.1 Gonad stage chart from Haddy and Pankhurst (1998) used to describe the gonad stages of *Chromis hypsilepis* (please see text for slight modifications).

Stage	Classification	*Macroscopic appearances*	*Histological characteristics*
	Females		
1	Immature	*Ovary small clear threads*	*Previtellogenic oocytes*
2	Regressed	*Ovary small clear and orange*	*Cortical alveoli-stage oocytes appear*
3	Vitellogenic	*Ovary orange with opaque oocytes visible through epithelium*	*Oocytes in exogenous vitellogenesis*
4	Hydrated	*Ovary orange with hydrated oocytes visible through epithelium*	*Final oocytes maturation and hydration*
5	Ovulated	*Eggs in the oviduct can be extruded with gentle pressure*	*Hydrated oocytes in the oviduct, post-ovulatory follicles present*
6	Spent	*Ovary flaccid and bloody*	*Atretic vitellogenic oocytes but predominantly previtellogenic oocytes present*
	Males		
1	Immature	*Testis white threads*	*Spermatogonia and a few previtellogenic oocytes^*
2	Spermatogenic	*Testis firm and ivory white*	*Secondary spermocytes, spermatozoa*
3	Partially Spermiated	*Testis firm and ivory white with viscous milt in sperm duct*	*Spermatozoa predominant*
4	Fully Spermiated	*Testis firm and ivory white with free-flowing milt in sperm duct*	*Spermatozoa predominant*
5	Spent	*Testis grey to bloody and flaccid*	*Residual spermatozoa, reduced spermatocytes and increased connective tissue*

^ Oocytes in dorsal section of gonad in all male stages.

2.2.4 Gonad Processing

Gonads from fishes collected during February 2008 at Terrigal and Jervis Bay were sent to the Australia National University for embedding, sectioning, (5 µm), and staining (haemotoxylin and eosin). Sectioned gonads were observed, under a compound microscope, to determine their state at maturity (at x10 and x40 magnification) (Figure 2.3) and the development stages of individual gametes (at x100 magnification). The examination of the histological sections was done to confirm the macroscopic staging of gonads and sexing of individual fish, and to assess the gender system of *C. hypsilepis*.

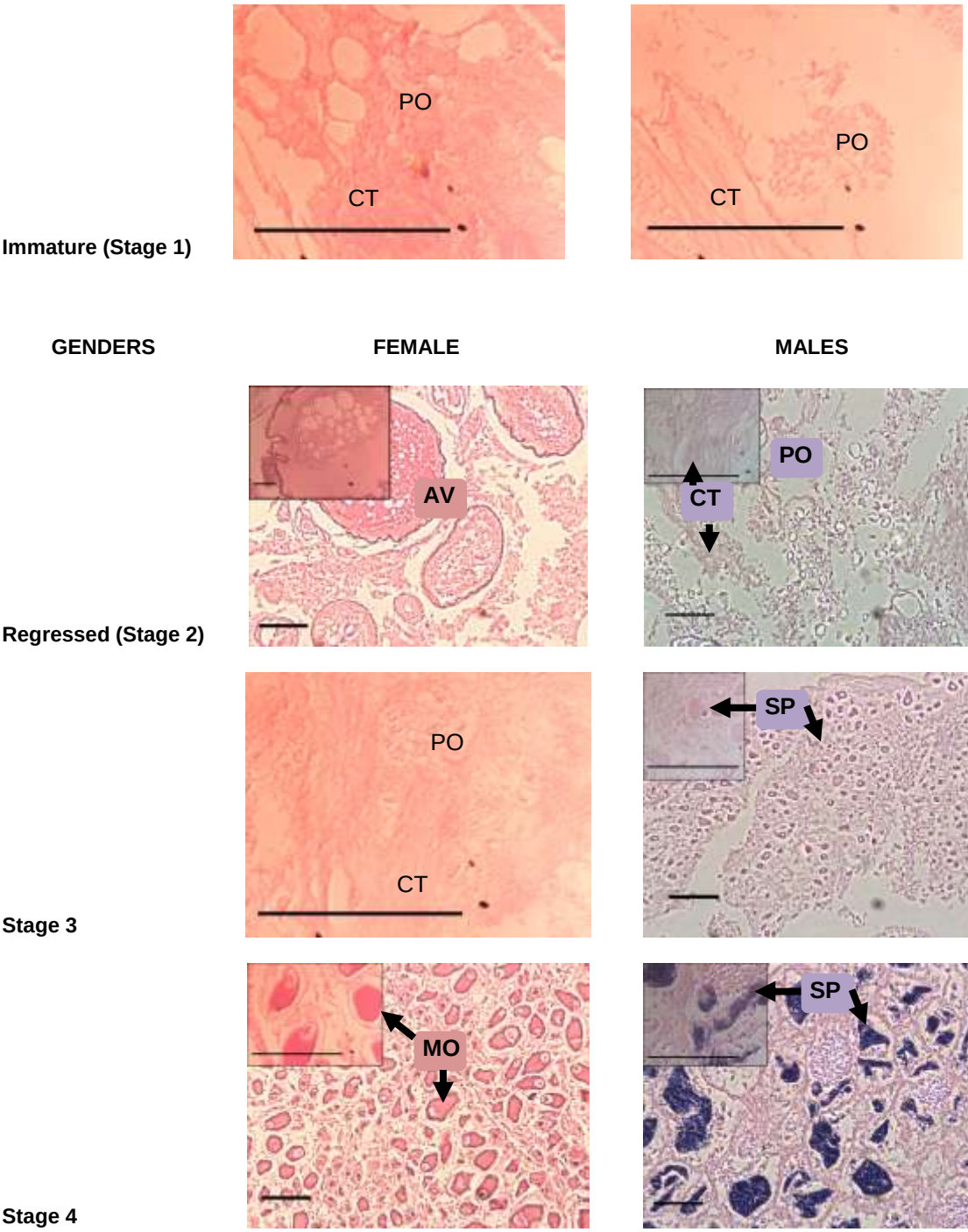
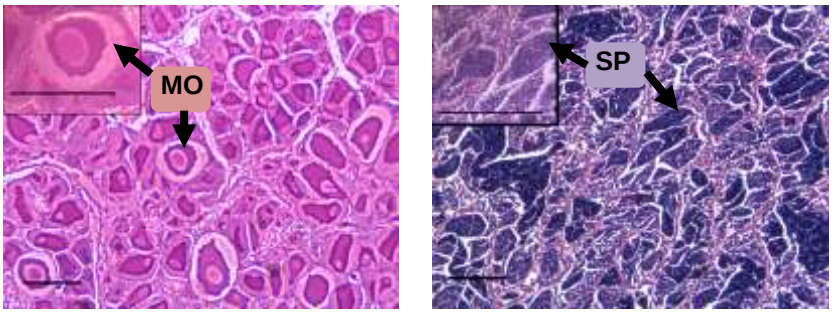


Figure 2.3 Gonad stages with scale bars = 1 µm. All images taken at 10x magnification with 40x image in corner unless otherwise noted. Stages correlate with corrected chart (Haddy and Pankhurst, 1998) data. Immature stage taken at 40x magnification. Connective tissue (CT); Previtellogenic oocytes (PO); Cortical alveoli- stage oocytes(CA); Mature oocytes (MO); Spermatozoa (SP); Atretic vitellogenic (AV).

Stage 5



Spent (Stage 6)

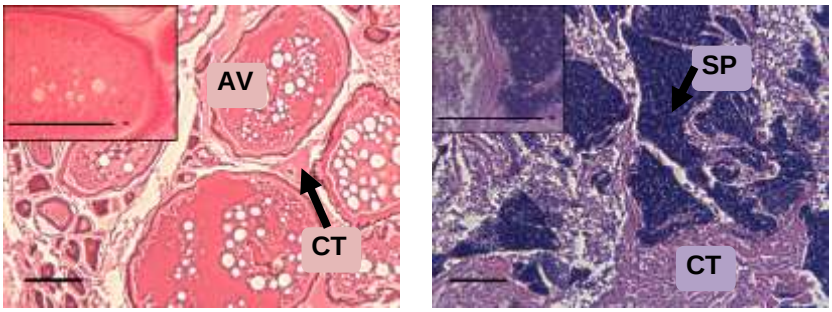


Figure 2.3 (continued) Gonad stages with scale bars = 1 µm. All images taken at 10x magnification with 40x image in corner unless otherwise noted. Stages correlate with corrected chart (Haddy and Pankhurst, 1998) data. Immature stage taken at 40x magnification. Connective tissue (CT); Previtellogenic oocytes (PO); Cortical alveoli- stage oocytes(CA); Mature oocytes (MO); Spermatozoa (SP); Atretic vitellogenic (AV).

Samples were described macroscopically, according to the NSW DPI standard chart (Haddy and Pankhurst, 1998). This chart was compared to the colour chart. The Haddy and Pankhurst (1998) chart had a description of colour features combined with each stage. Additional samples were obtained in Jervis Bay, in February 2008. Gonads were removed, weighed (to the nearest 0.001g) and a gonadosomatic index (GSI) was calculated, if gonads were of a measurable weight (Equation 2:1).

$$GSI\ (\%) = 100 \times [W_g / (W_f - W_g)]$$

W_g = wet weight of gonad

W_f = wet weight of fish

$W_f - W_g$ = somatic weight

Equation 2:1 Gonadosomatic Index

2.2.5 Otolith Collection and Storage

The sagittal otoliths were removed, washed and dried. The otolith pairs were examined for integrity and perfect shape (no cracks, breaks, or missing pieces) and one was obtained for sectioning. The selected otolith was weighed to the nearest 0.00001g. Otoliths were stored in labelled paper envelopes prior to age determination.

2.2.6 Processing of Otoliths

Otoliths were processed at the NSW Department of Primary Industries, Cronulla Fisheries Research Centre. The otoliths were first viewed by the naked eye, to determine if the otolith was complete and not fractured. Using a backlight, the focus of the otolith was marked with a dot using a fine tip pen. A block of Polyplex Clearcast Resin was created (Figure 2.4), and two rows of five otoliths were placed on each block, for a total of 10 otoliths per block. The dots ensured that sectioning occurred around the focus of each otolith. The otoliths were covered in resin to create a solid block. This block was then placed in an oven at 35°C for 12 hours, until the resin had hardened.

Figure 2.4 Otoliths in resin before sectioning

After the resin cooled, the blocks were set on a Gemmasta GS6 Trim saw, with a diamond blade, and cut in 700 µm sections. At least one cut was made before the dot, one surrounding the dot, and one after the dot. Otolith sections were cleaned with water and absolute ethanol, dried at room temperature and then set onto slides with resin. The slides were then sanded to ensure the best readability, using a wet/ dry polishing sander, to about 500 µm thickness. Cover slips were then added to the slides with resin and then dried.

2.2.7 Determination and Validation of Growth Rings

Using an Olympus compound microscope, with reflected light, otolith rings were distinguished for each sample, using the best otolith section from the three to five that were prepared. Digital images were taken using a Q-Imaging 5 megapixel microscope-mounted camera and then the opaque rings were counted. The outer edge and the core of the otoliths were marked to ensure readability and consistency of measurements for future studies. Images and data were stored in the DPI SQL server database written by William Robinson and James Craig. Marginal increment analysis (MIA) was done on all samples to determine the frequency of banding and the age at which the first band was deposited. MIA was done by measuring the distance between the last two visible opaque rings and then to the outer edge, through a DPI computer analysis in the database. The MIA is expressed as a percentage of the last completed ring (Figure 2.5).

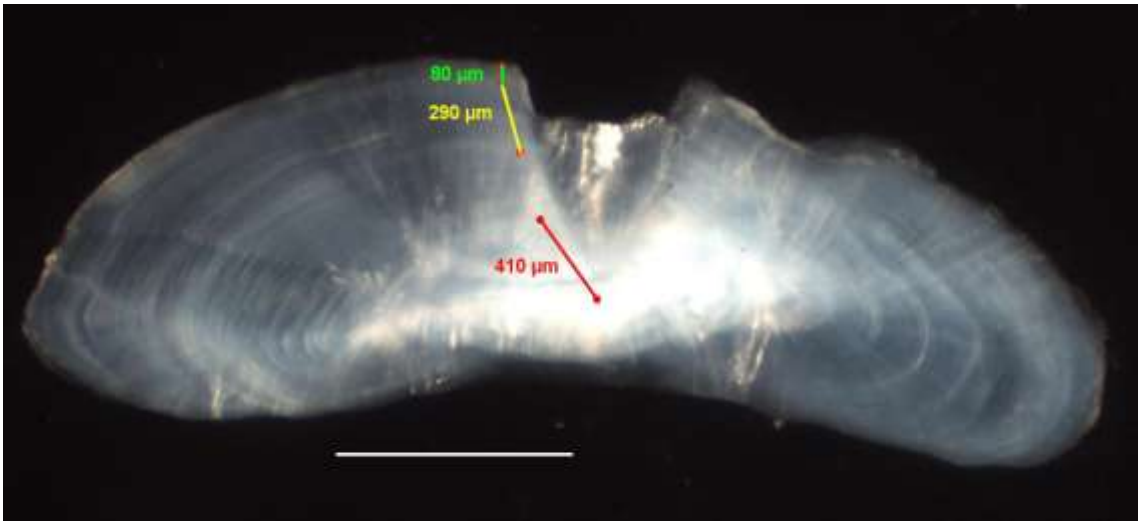


Figure 2.5 Measurements on otolith for MIA data. Red dots indicate the points of interest: core, 3 age rings, and outer edge. White bar signifies 1 mm.

2.2.8 Statistical Analyses

The hypothesis that males and females occur in a 1:1 ratio in the Terrigal and Jervis Bay collections was tested by a G-test (Sokal and Rohlf, 1995). The entire sample of fish collected at Terrigal between April 2007 and August 2008 was used whereas the February 2008 sample was used for the Jervis Bay analysis. Immature samples were included in the sampling for both males and females in both locations, as the immature samples could not be determined to be gender specific for statistical analysis. In addition, histological sections of gonads were examined for any evidence of sex reversal. Least squares linear regressions (of raw and ln-transformed data) were used to test the hypothesis that there were significant relationships between length and weight for each sex at Terrigal and Jervis Bay (Equation 2:2). Analysis of covariance was used to test the hypothesis that the slopes of the regression lines did not differ between males and females at each location and between locations for each sex. Length-frequency and weight-frequency distributions of males and females at Terrigal and Jervis Bay were compared by Kolmogorov-Smirnov tests, to evaluate the hypothesis that there was no sexual dimorphism in *C. hypsilepis*.

$$\ln(W) = b * \ln(L) + a$$

W= Weight

L= Length

ln= Natural Log

a= y intercept

b= x intercept

Equation 2:2 Linear Regression

Age (in years) was determined using ring counts and MIA (see Section 2.2.7). The age was related to the size (standard length mm) using the von Bertalanffy growth model, creating a growth model for each sex at each location (Terrigal, Jervis Bay). The age, in years, was also compared to the weight (g), using the same von Bertalanffy growth model comparison. Standard length (mm) was used in comparison with the gonad development classifications (Haddy and Pankhurst, 1998).

A von Bertalanffy growth curve equation was fitted to the length at age, and the weight at age data using the SOLVER add-in option within Microsoft Excel 2007. The von Bertalanffy growth equation is:

$$L_t = L_\infty [1-e^{-k(t-to)}]$$

L_t= length (mm SL) at age t (years)

L_∞= asymptotic length (mm SL) predicted by the equation

k= growth coefficient (year $^{-1}$)

t_0= hypothetical fish age (years) at 0 mm SL

Equation 2:3 von Bertalanffy Growth Curve Equation

Maturity ogives were determined by logistic regression with maturity ogives and 95% confidence intervals estimated by the SOLVER analysis on Microsoft Excel 2007 (Walker, 2005). Age was set in whole and partial years, determined by using estimated MIA data. Constants derived from the fitted logistic equation were used to estimate the length at which 50% of individuals become mature (L_{50}). The same analysis was also undertaken using age and weight data to determine the age and weight at which 50% of individuals were sexually mature. For comparison, calculations were also done to estimate the length, age, and weight at which 25% and 75% of fish were sexually mature.

L_{50} was determined by fitting logistic curves to the probability that fish of a specific length possessed mature gonads (based on the macroscopic and histological samples). The form of the equation was:

$$P_{50m} = \frac{\exp(a+b*L)}{1+\exp(a+b*L)}$$

P_{50m}= Proportion of mature fish at length L (mm)

L= Length

a & b= Constants

Equation 2:4 Logistic Curve

2.3 Results

2.3.1 Sex Ratio and GSI

A total of 520 fish were collected: 466 from Terrigal and 64 from Jervis Bay. Eighteen fish were identified as juvenile: 11 from Terrigal and 7 from Jervis Bay. Juvenile fish were identified as small fish with gonads not macroscopically mature or sex specific. At Terrigal, the relative proportions of males and females differed from month to month (Figure 2.6). The proportion of males increased during the spawning season. Overall at Terrigal 237 males and 218 females were collected (G= 0.79, p= 0.37). At Jervis Bay, 37 males and only 10 females were collected (G= 16.5, p <0.001).

The gonadosomatic index (GSI) was collected for the sampled months. June and November were excluded due to gonads being damaged during collection. An increase in GSI was seen during the spawning season (Figure 2.7). In December, the GSI grew to its highest peak of 10.4% and 3.9% in females and males, respectively.

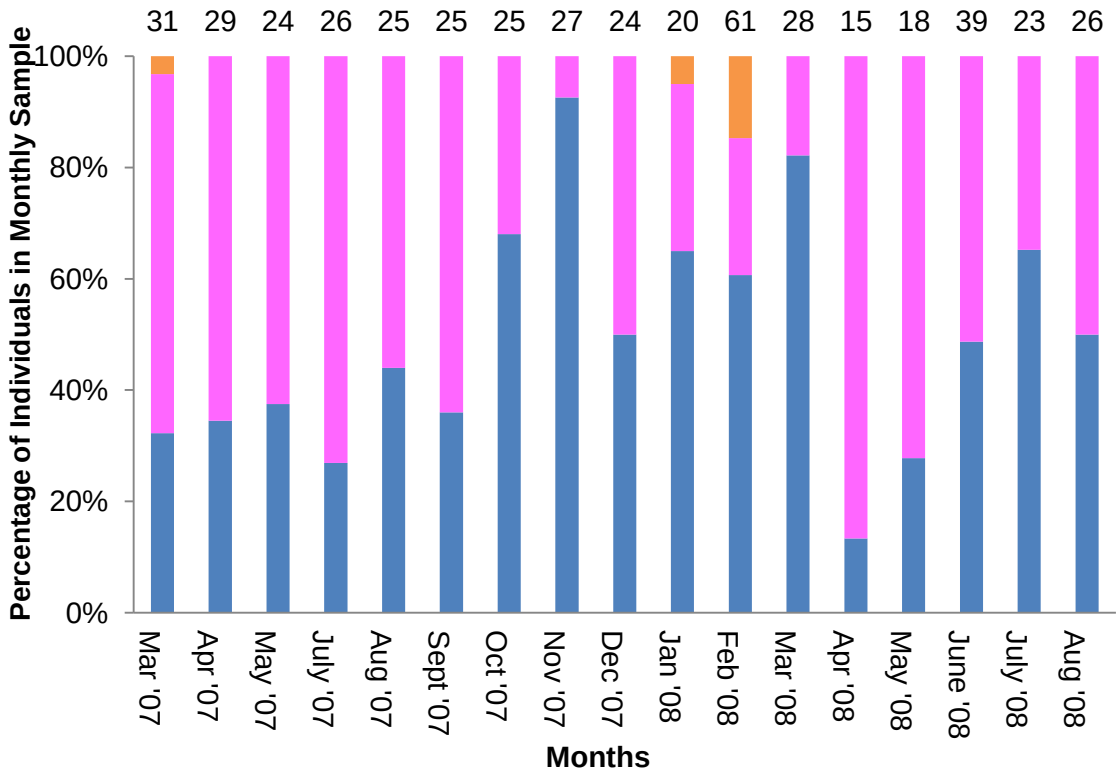


Figure 2.6 Relative proportions of males ■, females ■, and juveniles ■ in the sample of *Chromis hypsilepis* collected monthly at Terrigal between March 2007 and August 2008. Sample sizes for each month are shown. June 2007 sample was not obtained due to hazardous weather conditions.

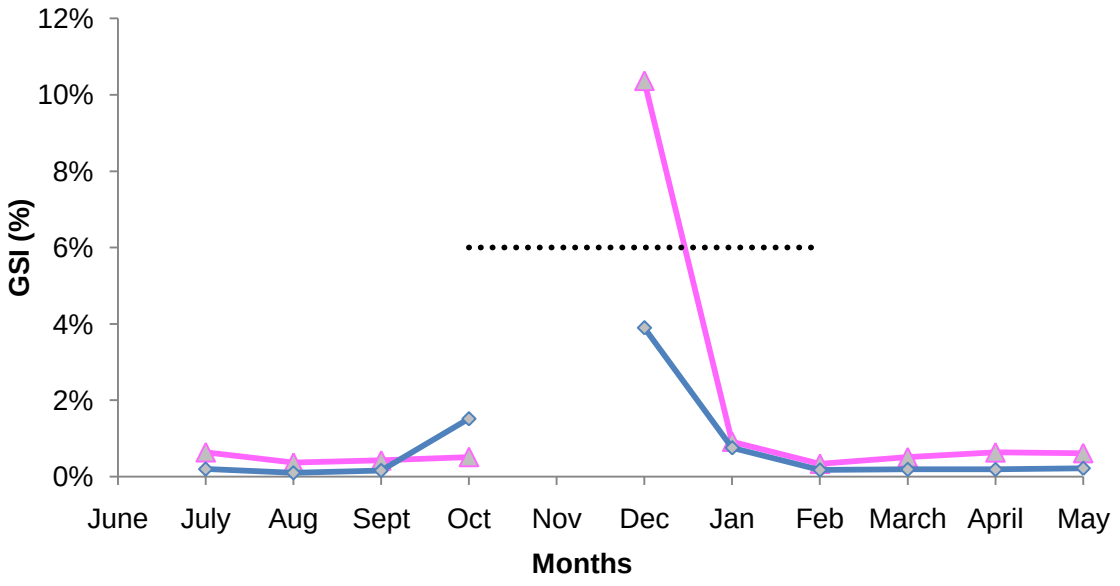


Figure 2.7 GSI data for Terrigal males ◆ and females ▲. June and November samples could not be calculated. Dotted line signifies the spawning season.

2.3.2 Length and Weight

Females at Terrigal were 82-164 mm SL, while males were 86-165 mm SL. The length-frequency distributions of males and females at Terrigal were significantly different (Kolmogorov-Smirnov D=0.17, p=0.003) (Figure 2.8A). This difference appears to reflect a larger number of males of 135-150 mm SL rather than the absence of any length class in either sex (Figure 2.8A). Juveniles at Terrigal were 63-102 mm SL. Jervis Bay males were 70-150 mm SL, while females were 72-148 mm SL. The length-frequency distributions of males and females at Jervis Bay were not significantly different (D=0.34, p=0.26) (Figure 2.8B). Juvenile fish at Jervis Bay were 68-83 mm.

The weight of male *C. hypsilepis* at Terrigal varied from 34.86 g to 179.01 g, compared to females at Terrigal that ranged from 30.7 to 174.78 g. There was no significant difference in the weight-frequency distributions of males and females at Terrigal (D=0.11, p=0.18) or Jervis Bay (D=0.34, p=0.26) (Figure 2.9). Jervis Bay females had a smaller weight range, weighing between 20.30 and 138.40 g, while the weights of males were varied between 16.20 and 135.20 g. The weight of juveniles from Terrigal varied between 13.30 and 67.00 g, compared to that of Jervis Bay juveniles that ranged from 17.00 to 29.90 g.

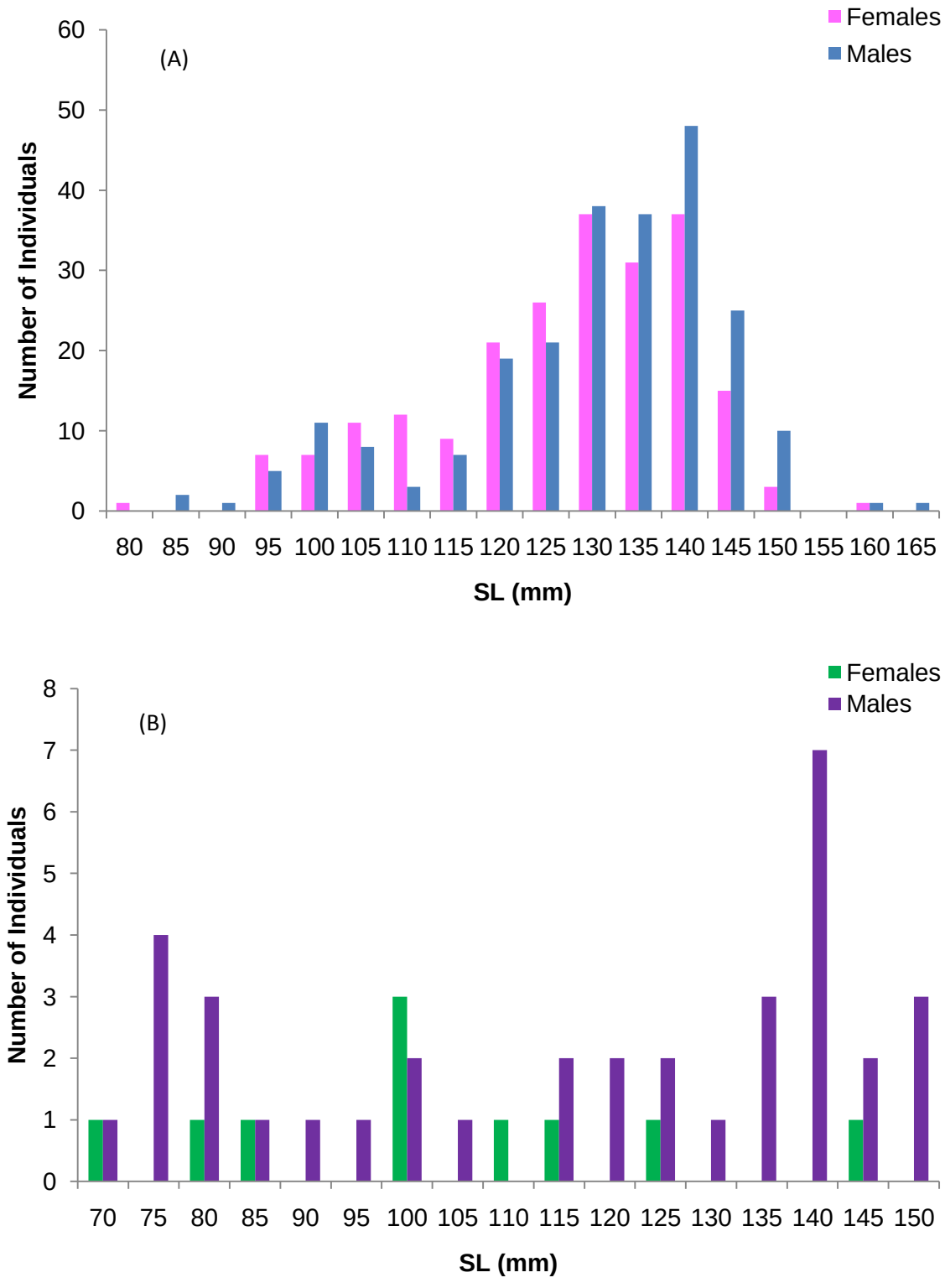
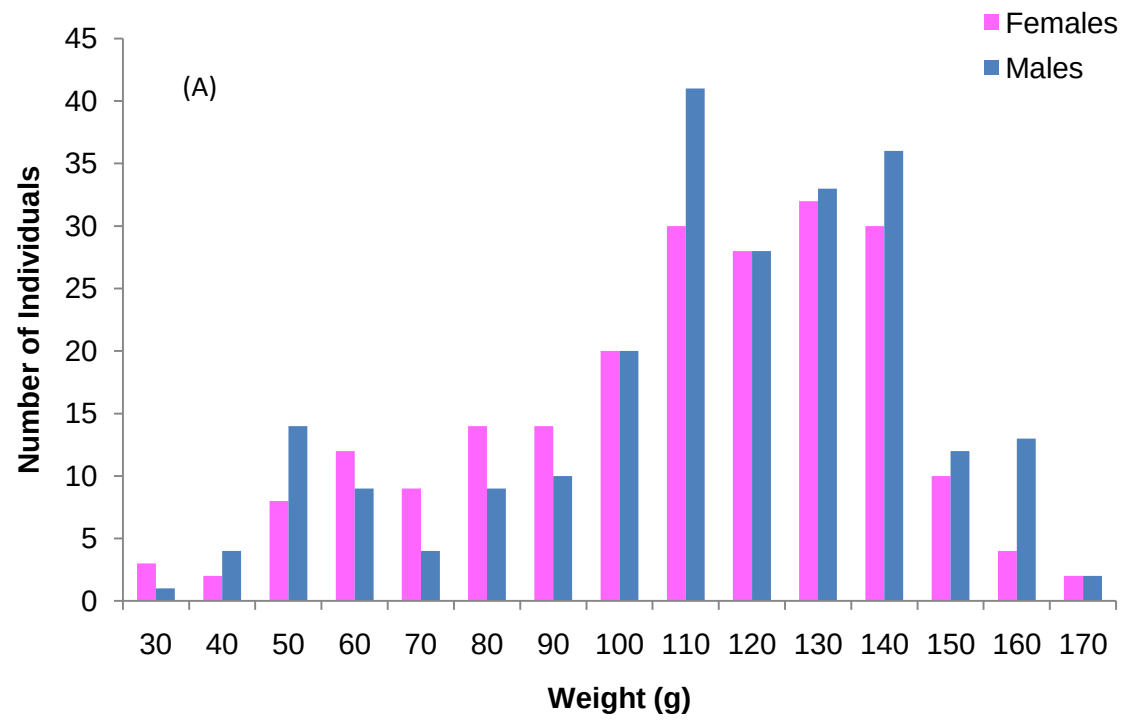


Figure 2.8 Length-frequency distributions of male and female *Chromis hypsilepis* at (A) Terrigal and (B) Jervis Bay.



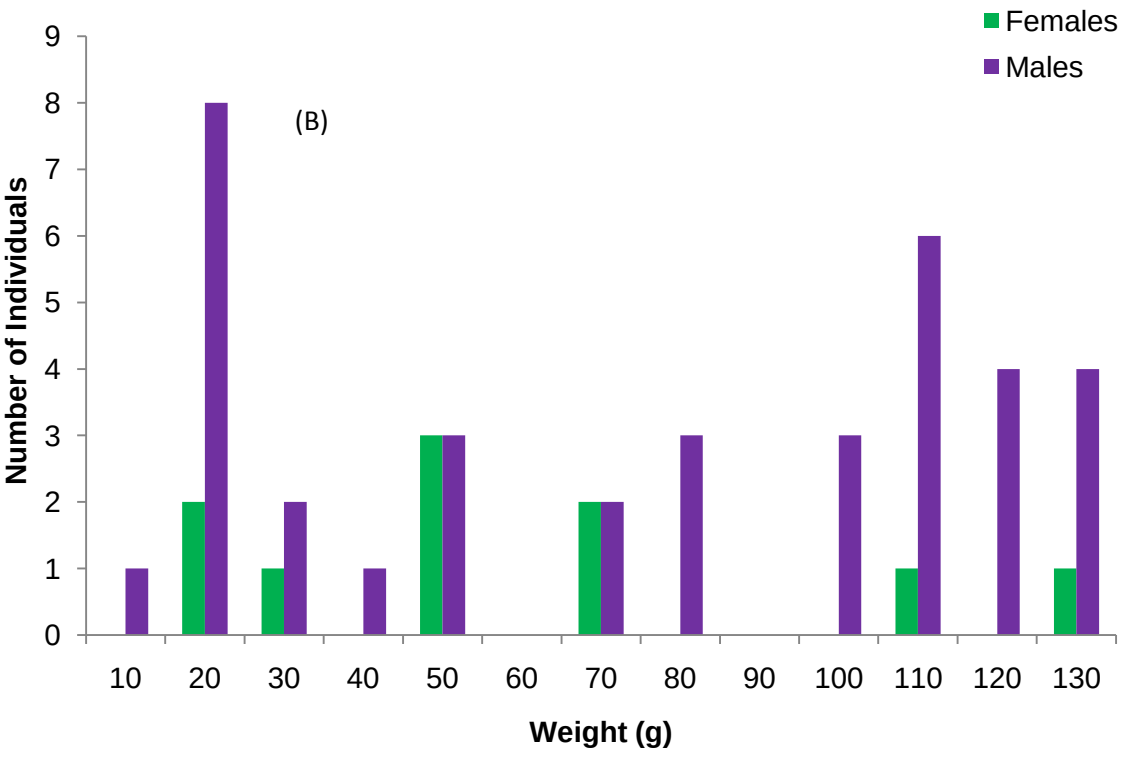


Figure 2.9 Weight-frequency distributions of male and female *Chromis hypsilepis* at (A) Terrigal and (B) Jervis Bay.

Length and weight of *C. hypsilepis* were significantly related in both sexes at both locations (Table 2.2). Natural log transformation of data slightly increased the R^2 values, compared to that of the raw data.

Transformed length-weight relationships for Terrigal males were described by the equation ln weight= 2.56(ln length) - 7.76, while the raw data relationship was described as weight= 1.98(length) - 143.2 (Figure 2.10). Terrigal females were described with raw data by weight= 2.06(length) - 151.1, transformed data was described as ln weight= 2.63(ln length) - 8.09 (Figure 2.11). Jervis Bay females were described by the raw data length-weight regression equation weight= 1.61(length) - 106.6 and the transformed data equation ln weight= 2.81(ln length) - 9.04 (Figure 2.12). The equation for males from Jervis Bay based on raw data is: weight= 1.521(length) - 97.32, while transformed data was described as ln weight= 2.67(ln length) - 8.42 (Figure 2.13) (Table 2.2).

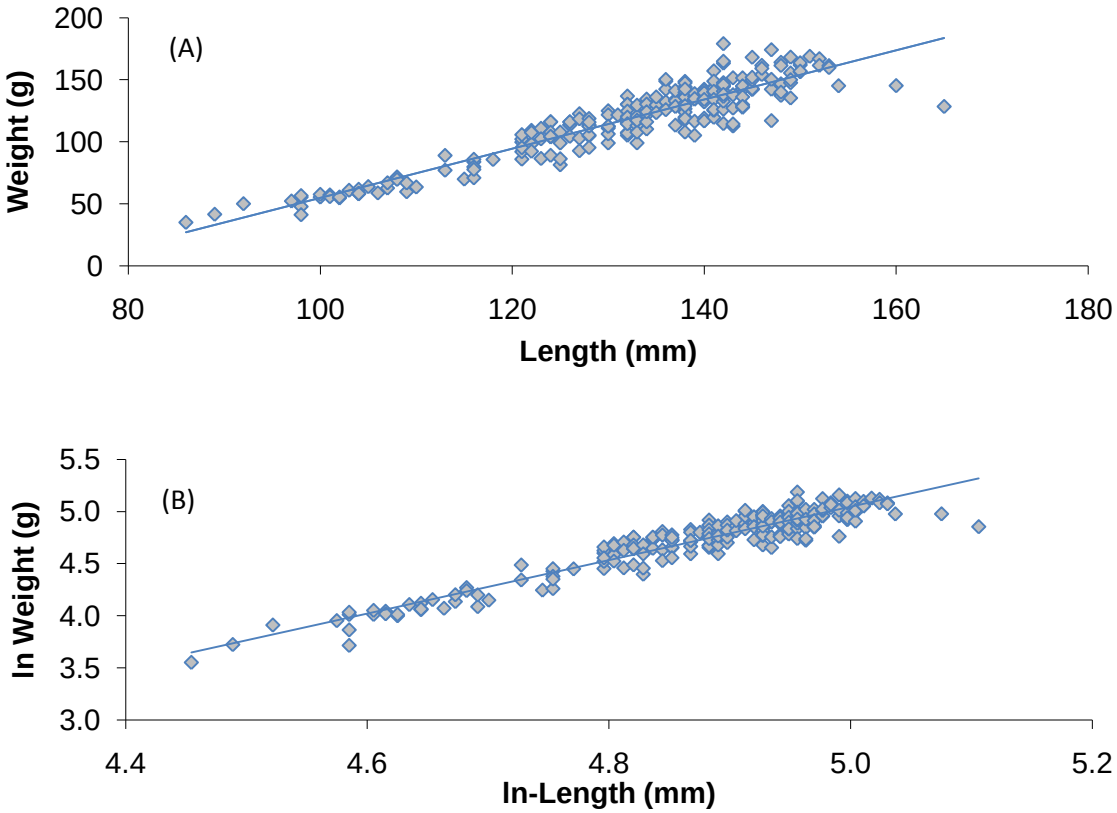


Figure 2.10 Length-weight regressions for Terrigal males based on raw (A) and ln-transformed (B) data

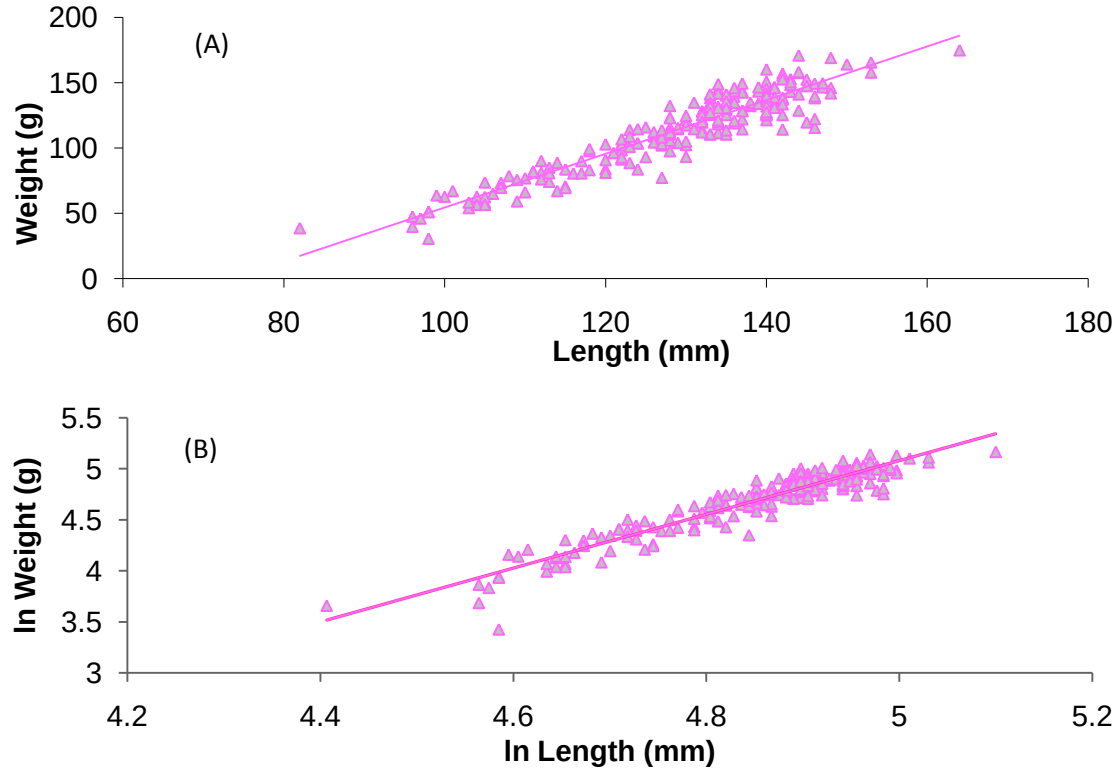


Figure 2.11 Length-weight regressions for Terrigal females based on raw (A) and ln-transformed (B) data

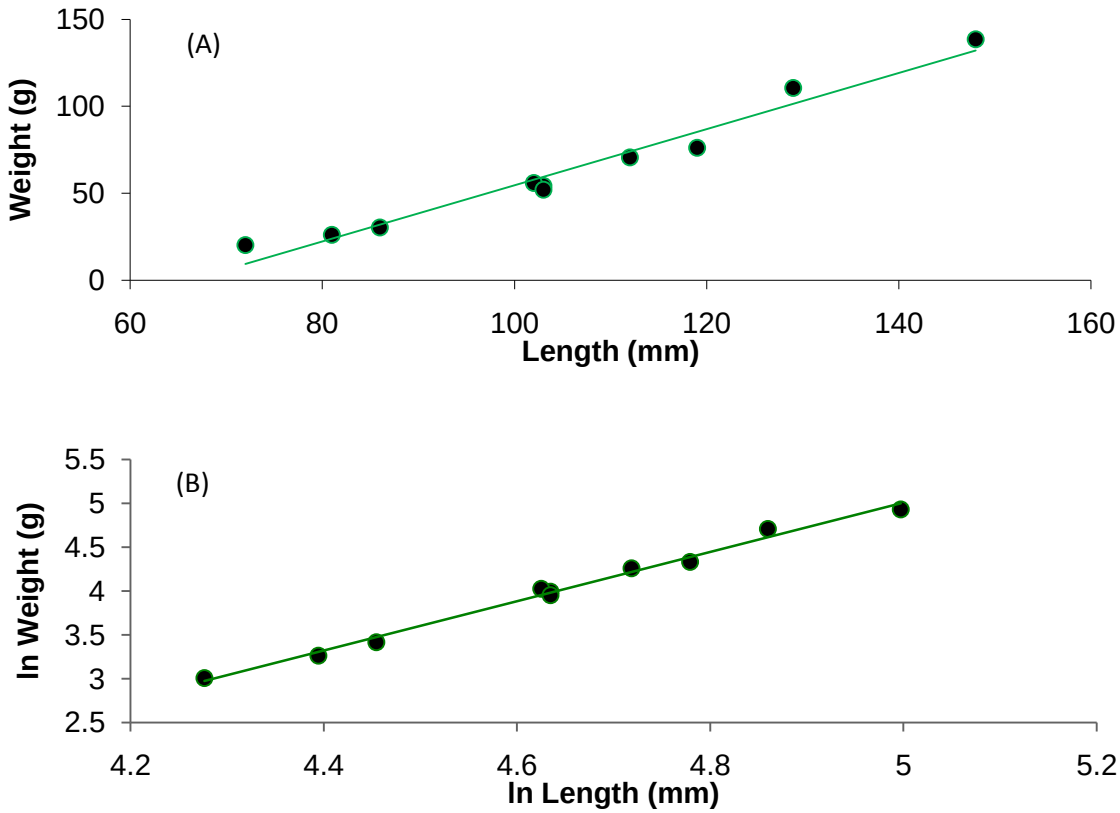


Figure 2.12 Length-weight regressions for Jervis Bay females based on raw (A) and ln-transformed (B) data

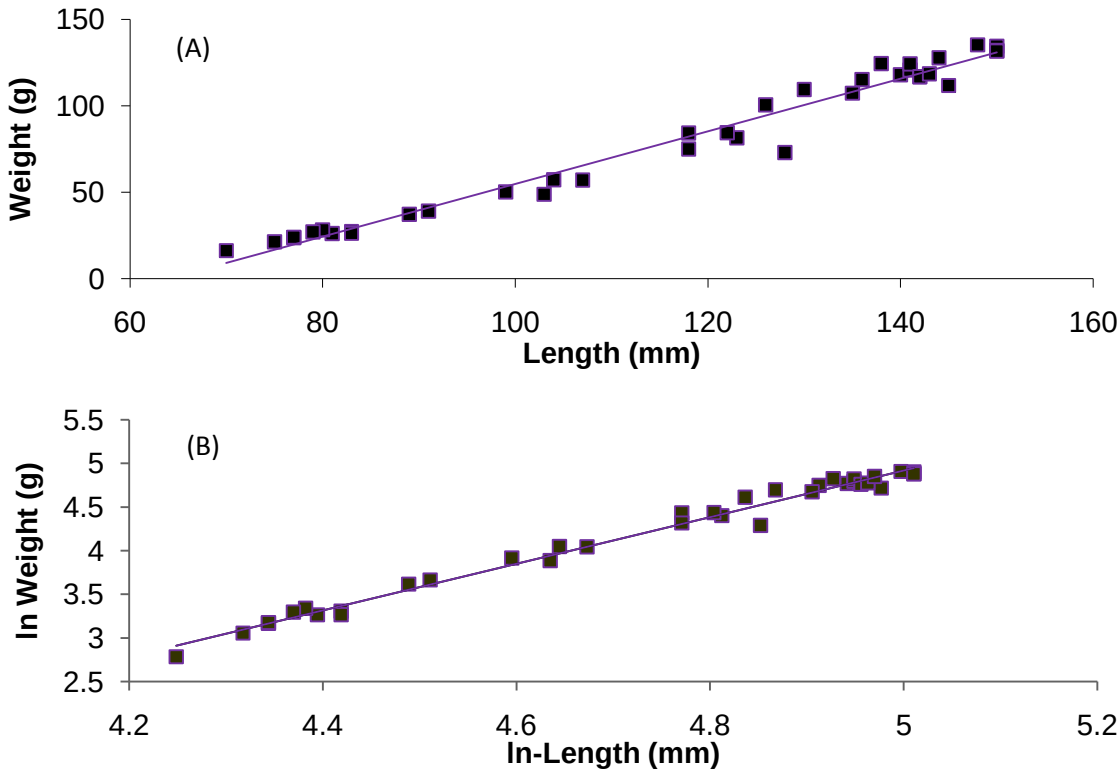


Figure 2.13 Length-weight regressions for Jervis Bay males based on raw (A) and ln-transformed (B) data

Table 2.2 Parameters for least squares linear regressions of the relationship between length (mm SL) and weight (g) for *Chromis hypsilepis* for raw and ln-transformed data (95% confidence intervals shown in parentheses). Parameters (a, b) are for the fitted equation Weight = b (Length) + a.

Raw data	n	a	b	*F*=	R^2
Terrigal Males	237	-143.23 (156.84,-129.61)	1.98 (1.88 - 2.08)	1449.37 (p< 0.001)	0.861
Terrigal Females	218	-151.15 (-164.38, -137.91)	2.06 (1.95,2.16)	1575.27 (p< 0.001)	0.879
Jervis Bay Males	37	-97.33 (-107.67, -86.99)	1.52 (1.43, 1.61)	1274.11 (p< 0.001)	0.973
Jervis Bay Females	10	-106.63 (-133.18, -80.08)	1.61 (1.37, 1.86)	227.50 (p< 0.001)	0.966
Transformed data	n	a	b	*F*=	R^2
Terrigal Males	237	-7.76 (-8.29, -7.24)	2.56 (2.45,2.67)	2189.38 (p< 0.001)	0.903
Terrigal Females	218	-8.09 (-8.68, -7.49)	2.63 (2.56, 2.78)	1791.53 (p< 0.001)	0.892
Jervis Bay Males	37	-8.42 (-8.93, -7.92)	2.67 (2.56, 2.78)	2569.71 (p< 0.001)	0.987
Jervis Bay Females	10	-9.04 (-10.05, -8.03)	2.81 (2.59, 3.03)	886.70 (p< 0.001)	0.991

The length-weight regressions of males and females for the raw data at Terrigal (t=1.03, p=0.31) and Jervis Bay (t=0.79, p=0.43) were not significantly different. The length-weight regressions of males and females for the ln-transformed data at Terrigal (t=0.87, p=0.39) and Jervis Bay (t=1.30, p=0.20) were not significantly different. The length-weight regressions based on raw data for males at Terrigal and Jervis Bay (t=6.83, p<0.001) and females at Terrigal and Jervis Bay (t=3.73, p<0.001) were significantly different. The length-weight regressions based on ln-transformed data for males at Terrigal and Jervis Bay (t=1.41, p=0.16) and females at Terrigal and Jervis Bay (t=1.55, p=0.12) were not significantly different.

2.3.3 Otolith Description

The sagittal otolith of *C. hypsilepis* (Figure 2.14) has an 'irregular marginal sculpturing-sectional shape' (Smale *et al*., 1995), with small serrated points on the

ventral side. The lateral side is convex, while the medial section, the section facing the middle of the fish, is a concave shape due to the sulcus groove in the middle of the surface. The sulcus opens to the ostial margin, showing no clear distinction between the two sections within the otolith's sulcus. In cross section the ventral rim has a well developed ridge-like edge, while the dorsal side is poorly developed. The rostrum of the otolith is only slightly protruding, with minimal presence of an antirostrum.

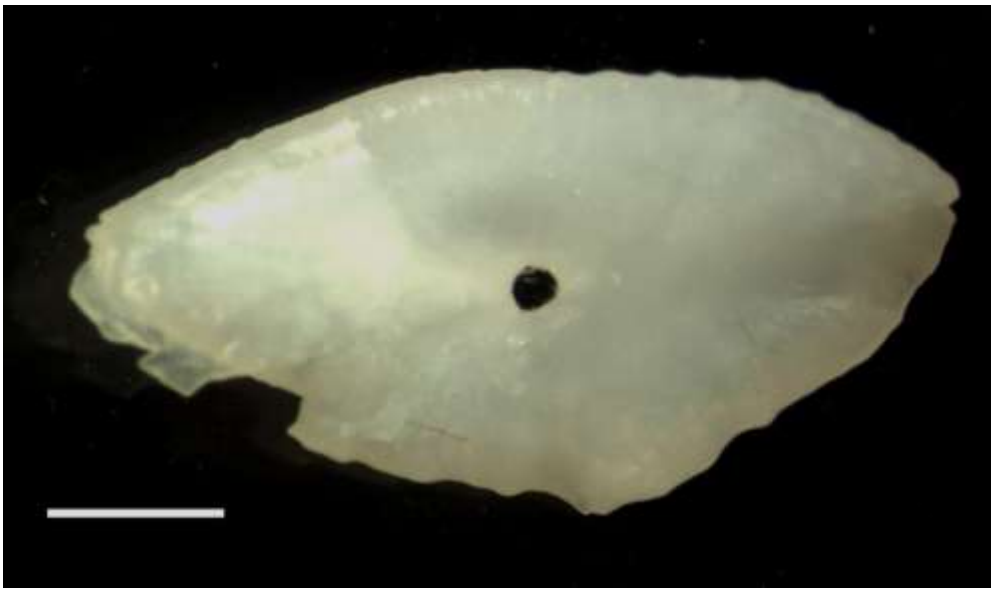


Figure 2.14 Otolith with scale (1 mm line). (8.2x magnification)

2.3.4 Ageing

The otoliths of *Chromis hypsilepis* possessed distinct alternating translucent and opaque bands (Figure 2.15). The mean monthly marginal increments were greatest in November-December (88.4%), decreased rapidly in January-February (52.2%) and increased slowly from March onwards (average 68%) (Figure 2.16A). A less pronounced trend was also apparent in fish tentatively aged 4-8 years (Figure 2.16B). The single decline and subsequent rise in the mean marginal increment (for fish of all ages and also for the subset of fishes aged 4-8 years) demonstrate that a single opaque band is formed annually in *C. hypsilepis* and that the number of opaque zones can be used to determine the age (in years) of individual fish.

For most mature specimens, excluding Jervis Bay females, ages ranged between 1.5 and 22.5 years (Figure 2.17). Males from Jervis Bay and Terrigal reached 22.2 and 21.5 years respectively, while females from Terrigal reached 22.5 years. Due to the limited numbers of Jervis Bay females, the age range was between 2.2 and 16.2 years. Juveniles ranged from 1.2 to 6.1 years at Terrigal and 2.2 to 5.2 years in Jervis Bay.

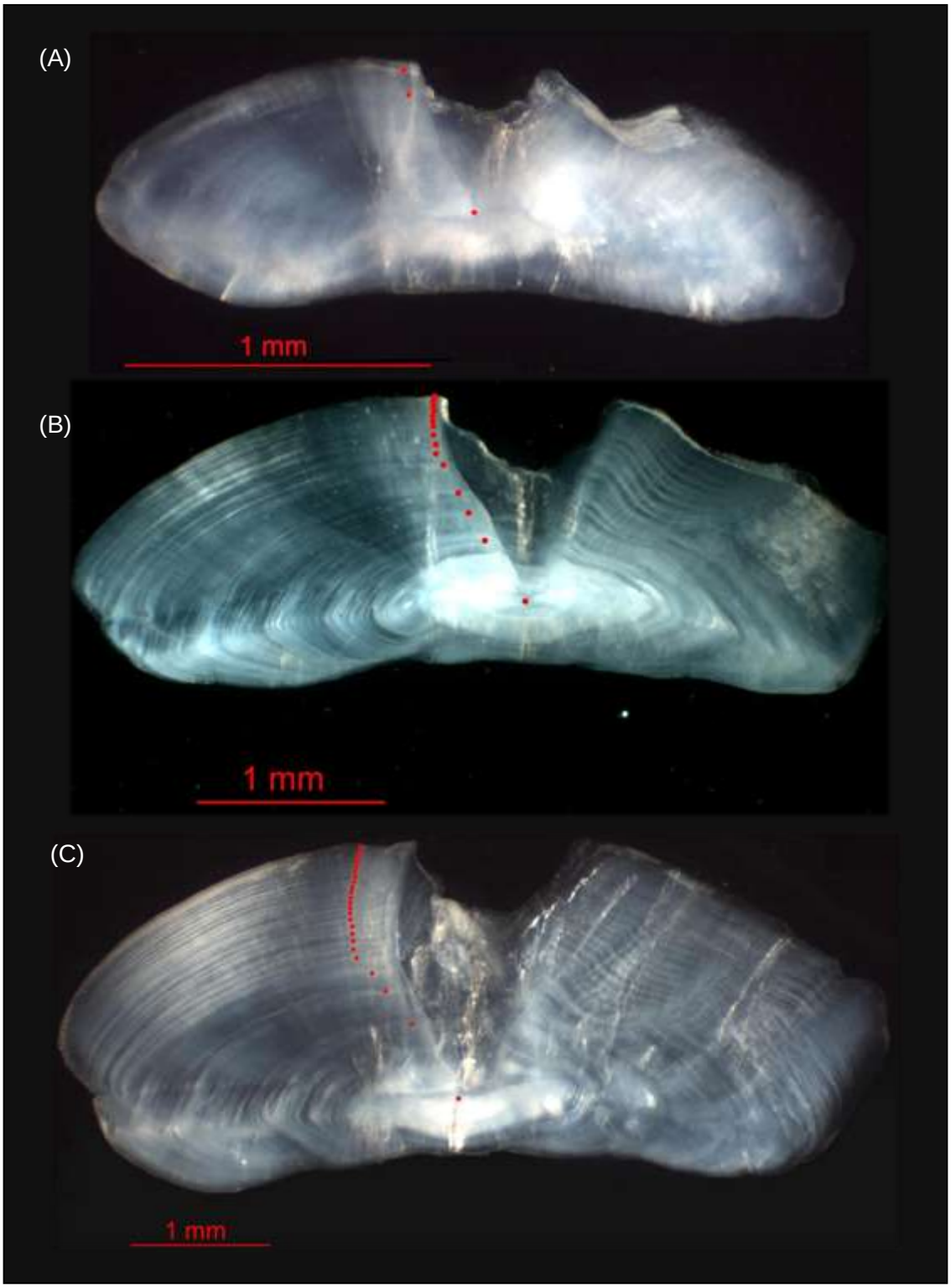


Figure 2.15 Sectioned otolith of a Terrigal (A) 1.5 year old female (4X magnification), (B) 9.2 year old male (2X magnification) and a (C) 22.5 year old female (2X magnification), all with markings. Scale is 1 mm.

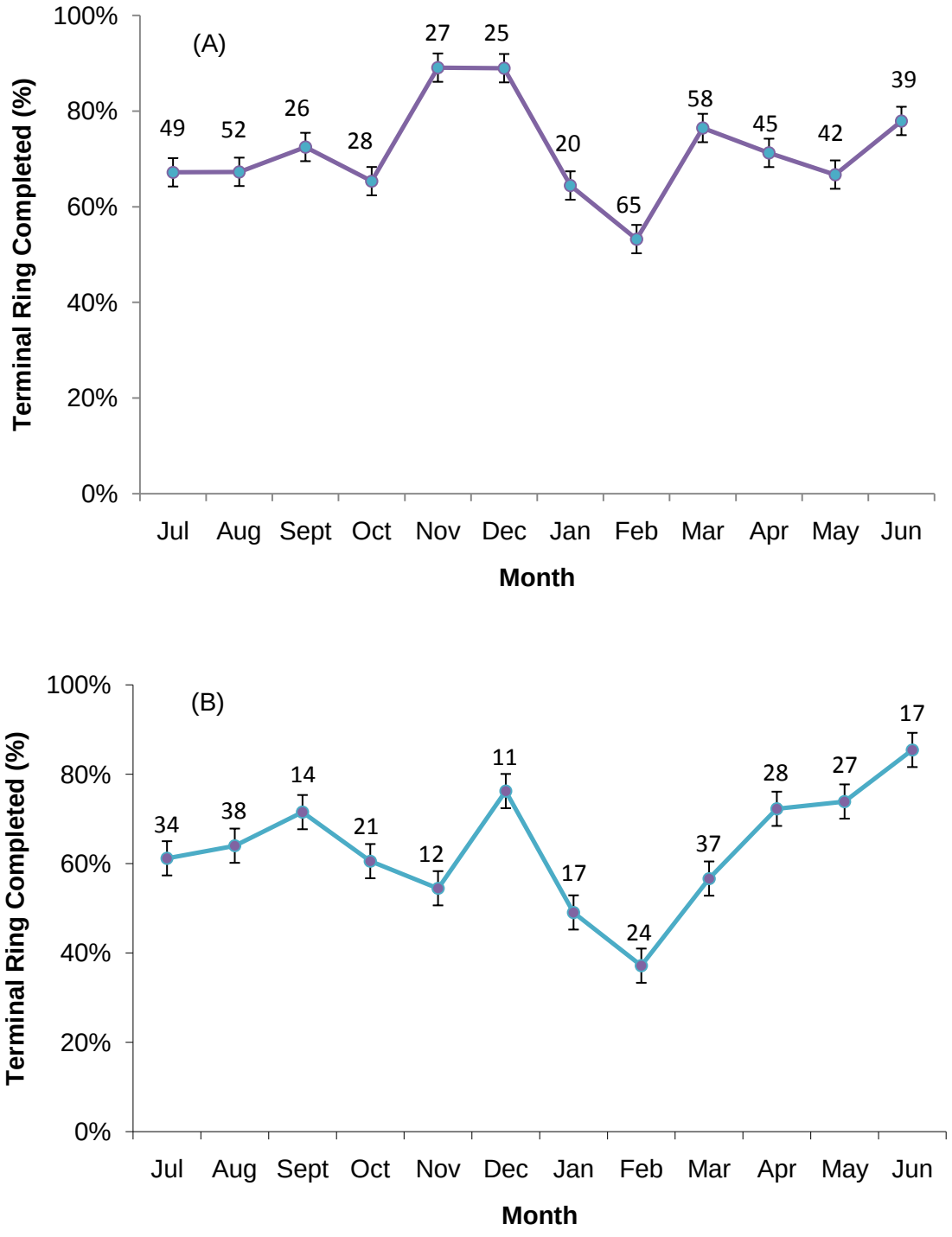


Figure 2.16 Mean monthly marginal increments (± standard error) on sectioned sagittal otoliths of *Chromis hypsilepis* for (A) all samples combined and (B) samples tentatively aged 4-8 years. Sample sizes for each month are shown above the mean value.

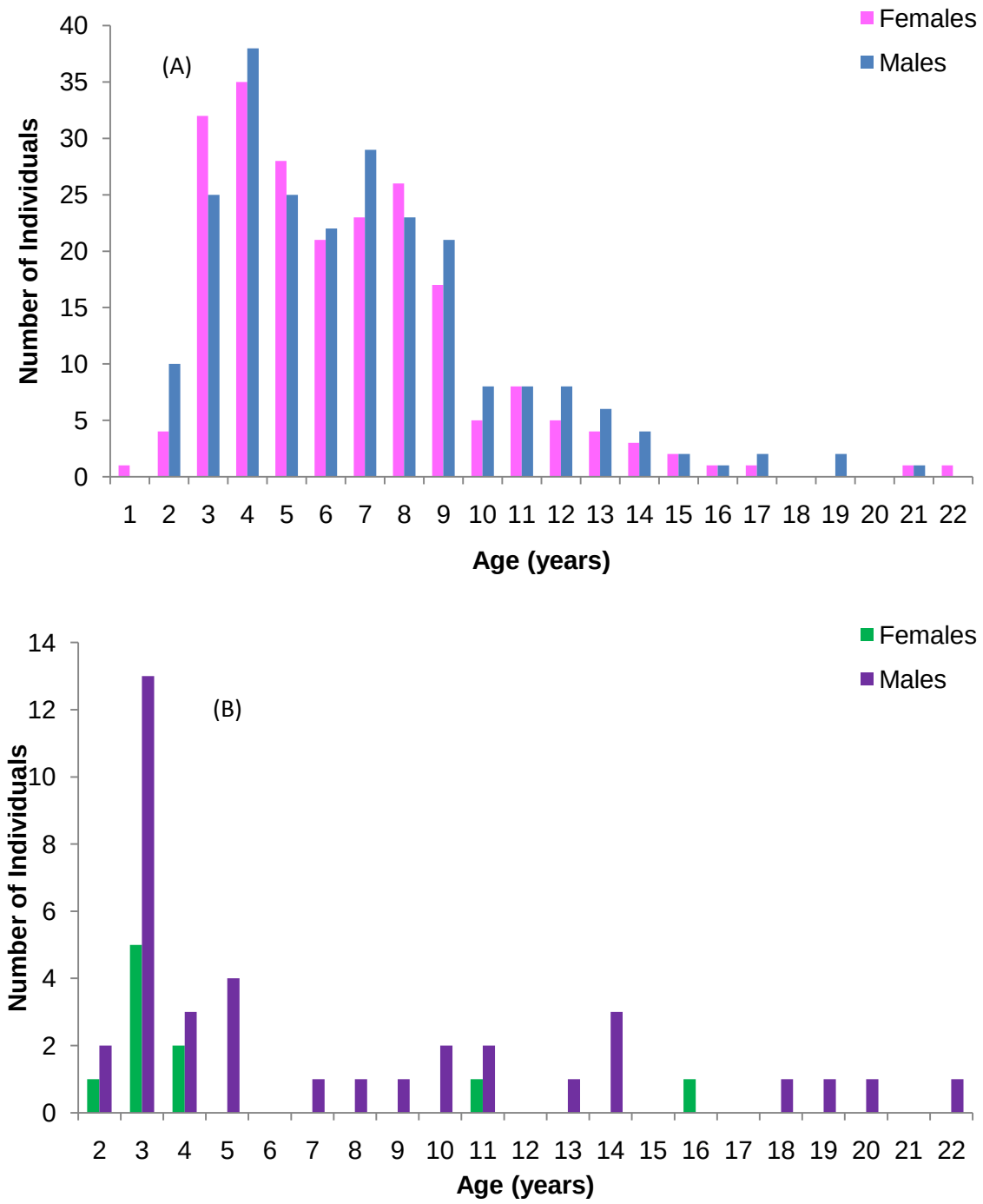


Figure 2.17 Age-frequency distributions of male and female *Chromis hypsilepis* at (A) Terrigal and (B) Jervis Bay.

2.3.5 von Bertalanffy Growth Curves

von Bertalanffy growth curves provided good fits to the length-at-age data for males and females at Terrigal (Figure 2.18). The coefficients of determination (R^2 values) for both sexes were satisfactory and similar (Table 2.3). The von Bertalanffy growth equations indicate that at Terrigal *C. hypsilepis* of ages 5, 10, 15 and 20 years attain lengths of 125.8, 142.5, 145.8, and 146.5 mm SL for males and 122.4, 140.2, 145.3, and 146.8 mm SL for females respectively. The von Bertalanffy growth curves provided less satisfactory fits to the length-at-age data for males and females at Jervis Bay (Figure 2.19) (Table 2.3) and for the weight-at-age data for males and females at Terrigal (Figure 2.22) (Table 2.4). The von Bertalanffy growth curves provided good fits for the weight-at-age data for males and females at Jervis Bay (Figure 2.23; Table 2.4). The equations indicate that at Jervis Bay *C. hypsilepis* of ages 5, 10, 15 and 20 years attain weights of 72.1, 112.3, 127.1, and 132.5 g for males and 54.3, 95.5, 129.3, and 156.9 g for females respectively.

The asymptotic lengths (mm) varied from 146.7-150.1 mm for all specimens, except Jervis Bay females, which were estimated to be 178.3 mm (Table 2.3). In the von Bertalanffy growth curves for the relationship between weight and age (Figure 2.21), Jervis Bay females were exceptionally large with an asymptotic weight of 281.6 g, while for other specimens it was estimated to be 158 g for Terrigal males and females and 135.7 g for males in Jervis Bay (Table 2.4).

The von Bertalanffy growth equations indicate that male *C. hypsilepis* of ages 5, 10, 15 and 20 years attain lengths of 125.8, 142.5, 145.8, and 146.5 mm SL at Terrigal and 111.6, 137.3, 145.8, and 148.7 mm SL at Jervis Bay respectively (Figure 2.20). Males at Terrigal reached half their asymptotic length within the first year, while Jervis Bay males grew slower, reaching half their asymptotic length by the second year. The asymptotic lengths for males were very similar between the two populations, 146.7 mm

at Terrigal and 150.1 mm at Jervis Bay. Female *C. hypsilepis* of ages 5, 10, 15 and 20 years attain lengths of 122.4, 140.2, 145.3, and 146.8 mm SL at Terrigal and 101.7, 126.9, 143.9, and 155.2 mm SL at Jervis Bay, respectively (Figure 2.21). The asymptotic lengths of females were 147.4 mm SL at Terrigal and 178.3 mm SL at Jervis Bay. Jervis Bay females reached half of their asymptotic length after the third year, while Terrigal females reached half of their asymptotic length within the first year.

The von Bertalanffy growth equations indicate that female *C. hypsilepis* of ages 5, 10, 15 and 20 years attain weights of 101.5, 139.0, 151.5, and 155.6 g at Terrigal and 54.3, 95.5, 129.3, 156.9 g at Jervis Bay respectively (Figure 2.24). At the maximum sampled ages (16.2 years), Jervis Bay females would reach 147.0 mm SL and 136.4 g, compared to Terrigal females which reached 22.5 years, 147.1 mm SL and 156.5 g. At the maximum sampled age of 21.3 years, Terrigal males reached 146.6 mm SL and 157.6 g, while Jervis Bay males reached 22.2 years 149.2 mm SL and 133.7 g.

Male *C. hypsilepis* of ages 5, 10, 15 and 20 years attain weights of 109.5, 146.6, 155.3, and 157.4 mm SL at Terrigal and 72.1, 112.3, 127.1, and 132.5 g at Jervis Bay, respectively, indicated by the von Bertalanffy growth equations (Figure 2.25). Half of the asymptotic weight was reached by males by 3.5 years at Terrigal and 4.5 years at Jervis Bay. Females reached half their asymptotic weight by 3 years at Terrigal and 17 years at Jervis Bay.

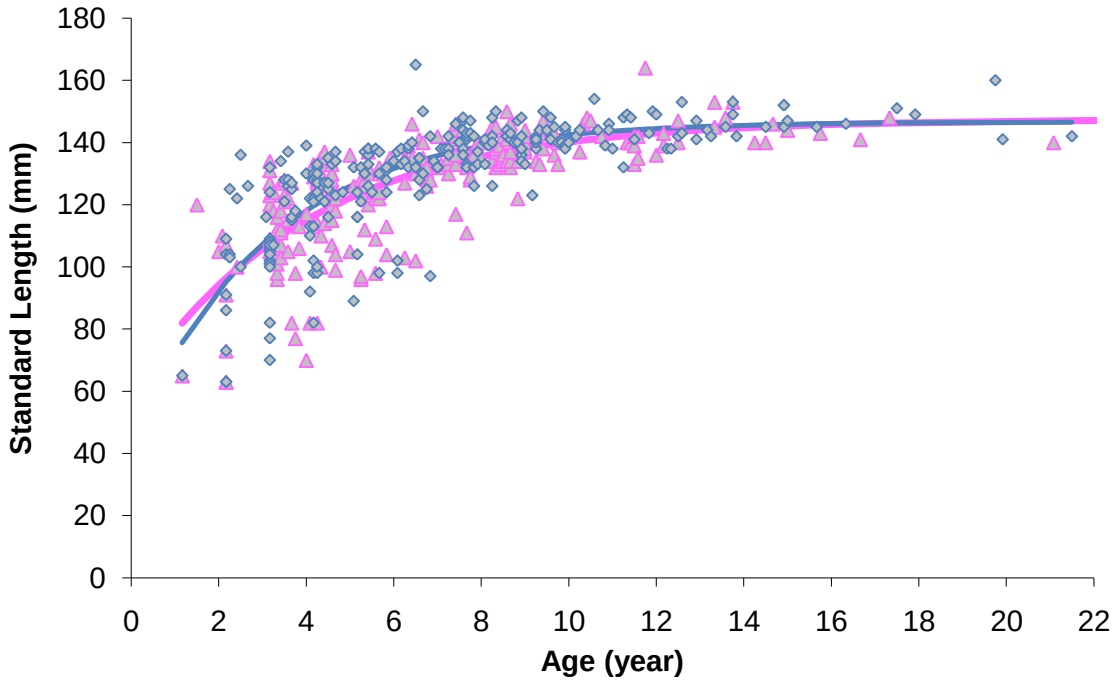


Figure 2.18 von Bertalanffy growth curve, SL (mm) vs. Age (years); Terrigal males ◇ vs. females △

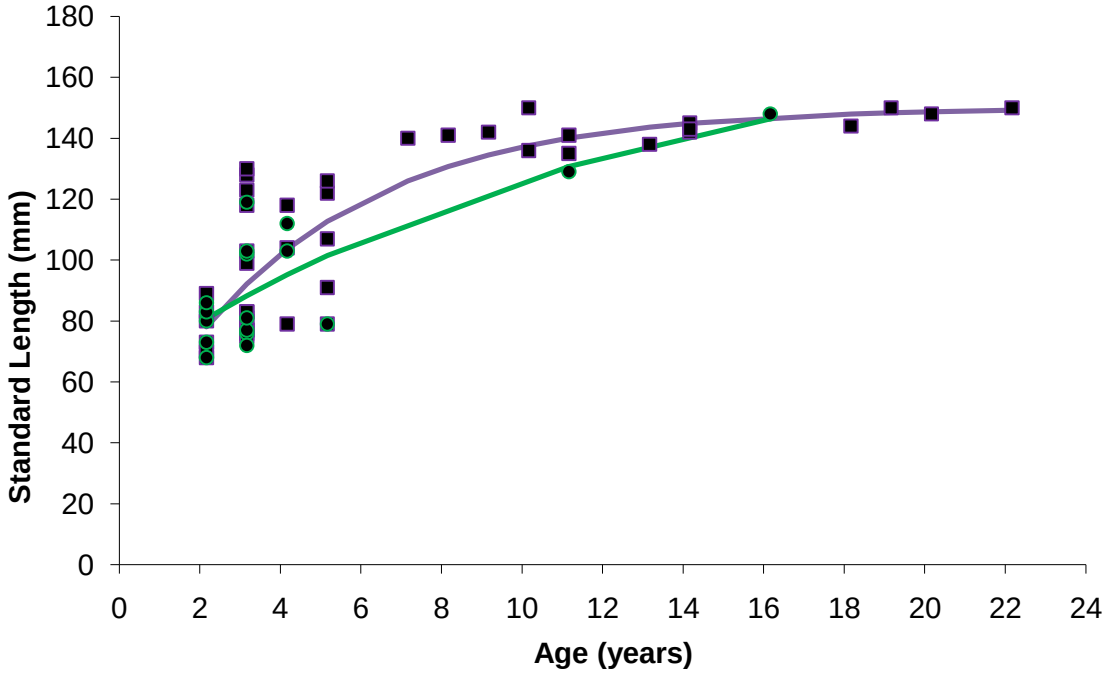


Figure 2.19 von Bertalanffy growth curve, SL (mm) vs. Age (years); Jervis Bay males ■ vs. females ●

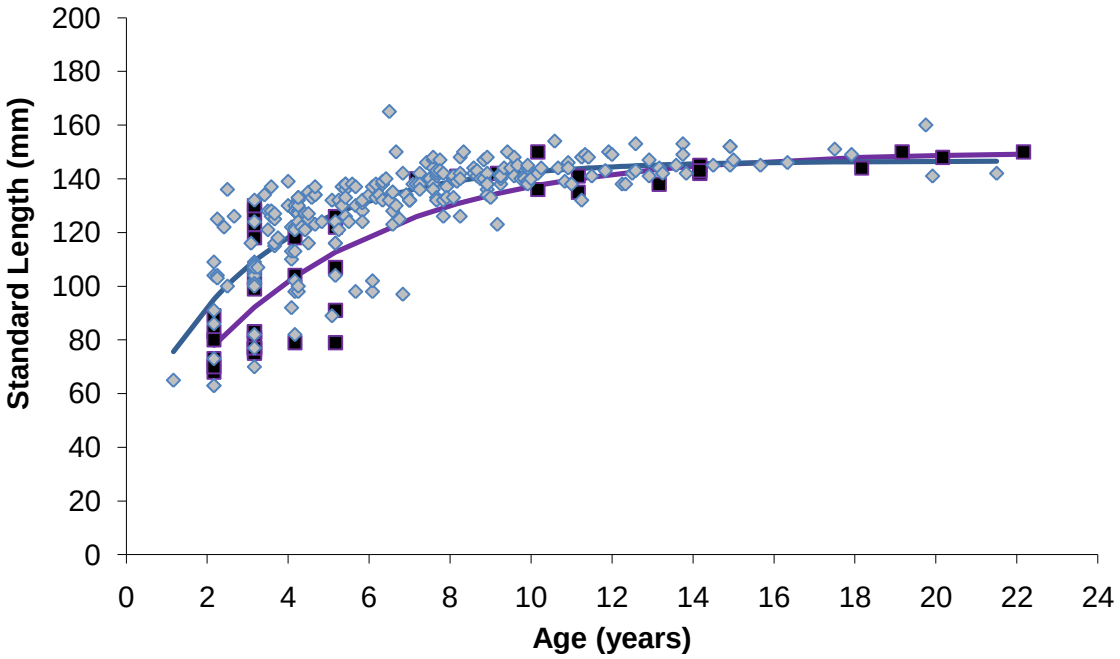


Figure 2.20 von Bertalanffy growth curve, SL (mm) vs. Age (years); Terrigal males ◇ vs. Jervis Bay males ■

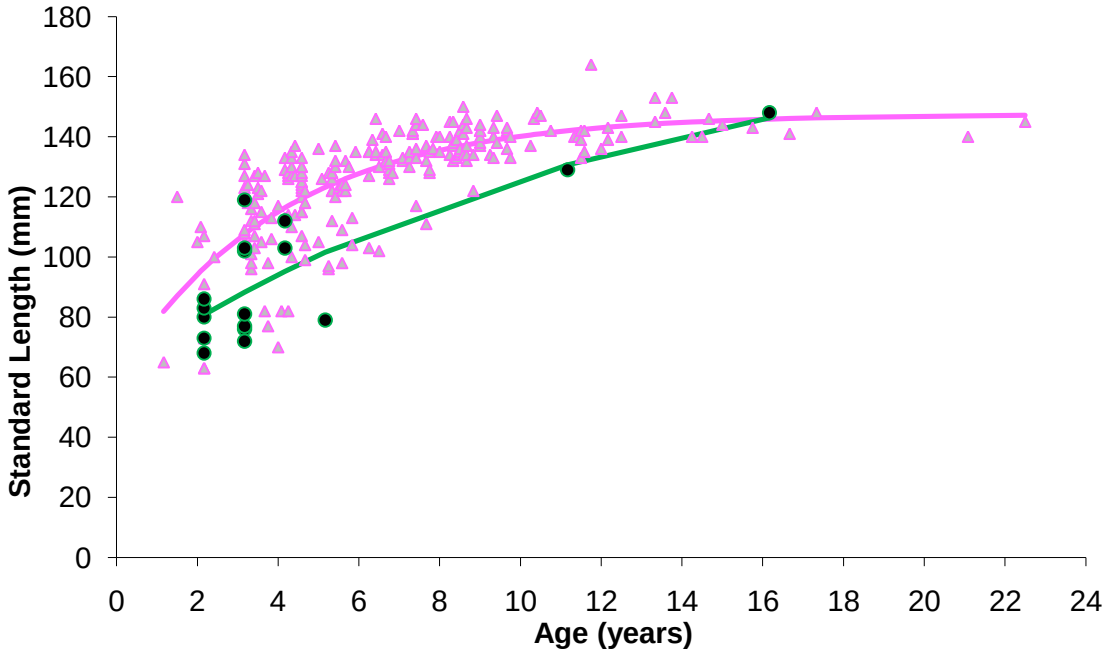


Figure 2.21 von Bertalanffy growth curve, SL (mm) vs. Age (years); Terrigal females △ vs. Jervis Bay females ●

Table 2.3 von Bertalanffy growth parameters for the length (SL mm) at age (years)

	L_∞	k	t_0	R^2	n
Terrigal Males	146.7	0.32	-1.09	0.61	247
Terrigal Females	147.4	0.25	-2.09	0.57	228
Jervis Bay Males	150.1	0.22	-1.19	0.16	44
Jervis Bay Females	178.3	0.08	-5.56	0.42	17

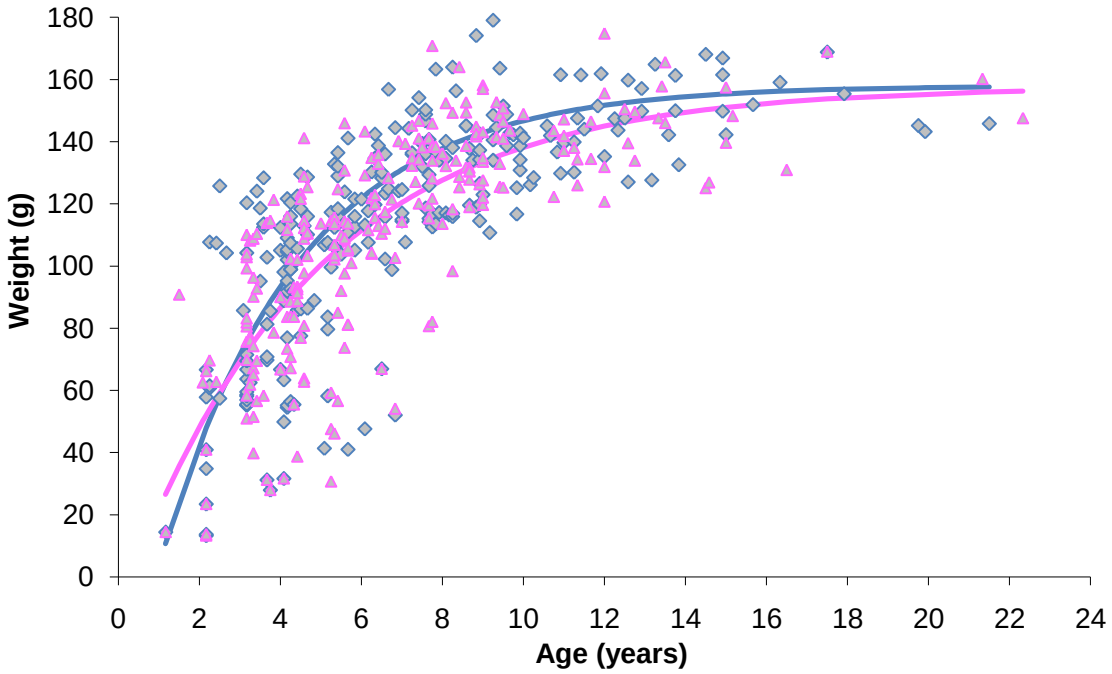


Figure 2.22 von Bertalanffy growth curve, Weight (g) vs. Age (years); Terrigal males ◇ vs. females △

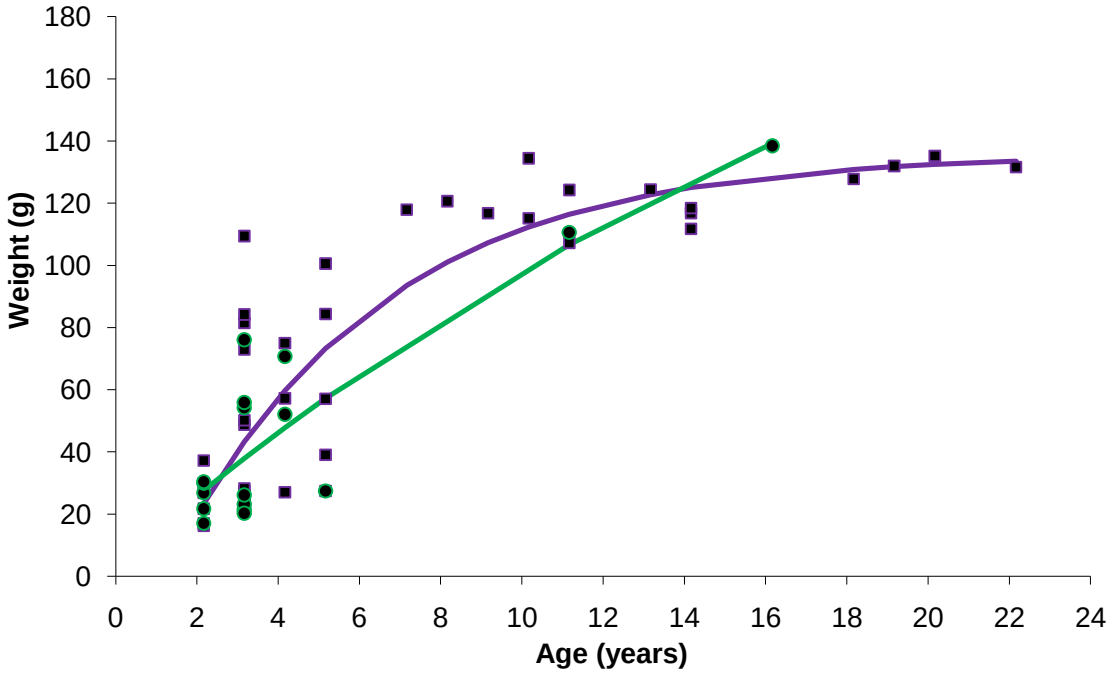


Figure 2.23 von Bertalanffy growth curve, Weight (g) vs. Age (years); Jervis Bay males ■ vs. females ●

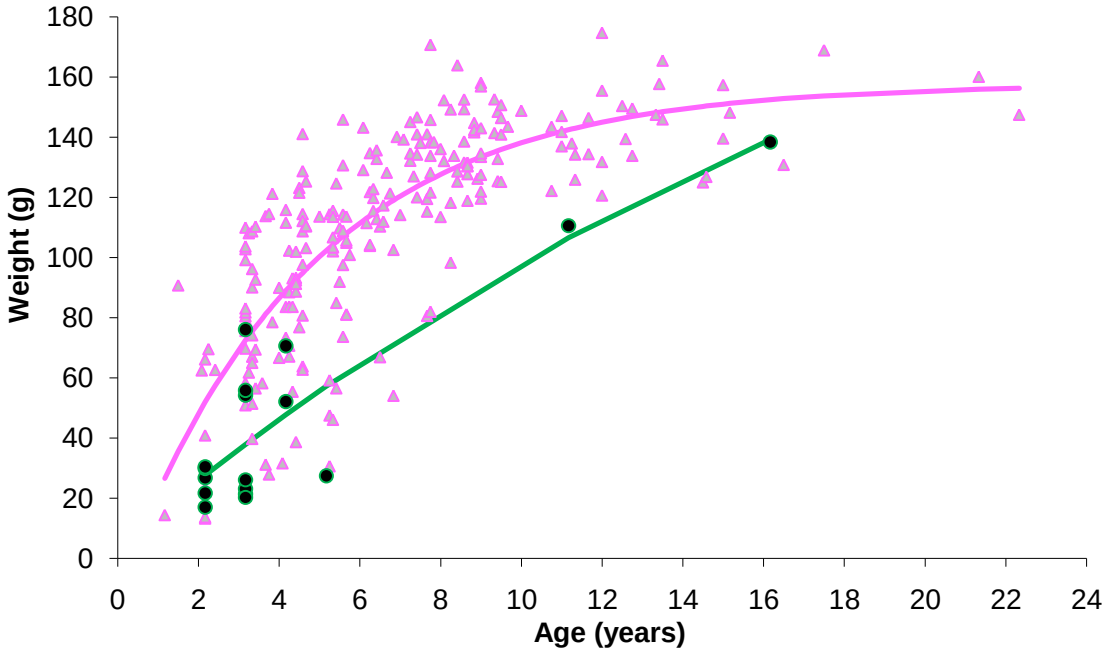


Figure 2.24 von Bertalanffy growth curve, Weight (g) vs. Age (years); Terrigal females ▲ vs. Jervis Bay females ●

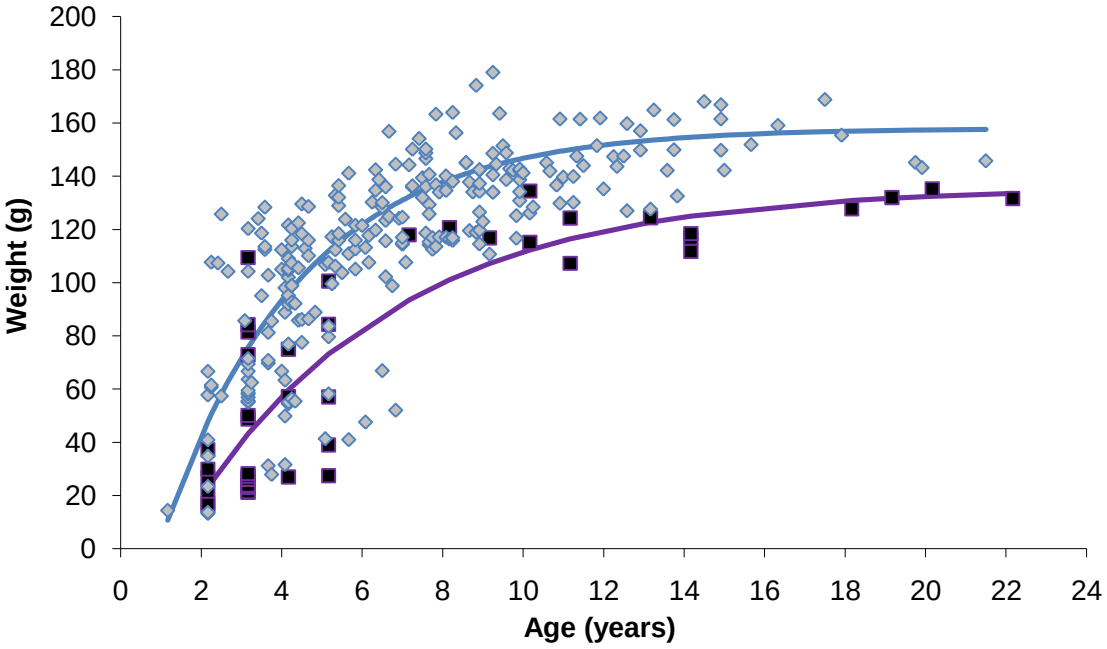


Figure 2.25 von Bertalanffy growth curve, Weight (g) vs. Age (years); Terrigal males ◆ vs. Jervis Bay males ■

Table 2.4 von Bertalanffy growth data, Mass vs. Age

	W_∞	k	t_0	R^2	n
Terrigal Males	158.0	0.29	0.93	0.45	247
Terrigal Females	157.7	0.22	0.31	0.30	228
Jervis Bay Males	135.7	0.20	1.21	0.68	44
Jervis Bay Females	281.6	0.04	-0.36	0.69	17

2.3.6 Maturity Parameters

The state of sexual maturity was determined through macroscopic examination of the gonad. This macroscopic examination included colour, size and other features. Colour was found to be unreliable macroscopic indicator of sexual maturity, due to limited variation in colour occurring among the different gonad maturity stages (Figure 2.26) and variation in the colour of gonads within a stage (Table 2.1).

Macroscopic examination found that mature gonads were present in individuals throughout the year (Figure 2.27). Males showing stage 2 (recessed) gonads were found most frequently, with stage 2 gonads being present between January and October. Stage 4 gonads were found in males between November and March. Stage 5 gonads were sound in males between December and February. Males with stage 3 gonads were apparent at the beginning of the spawning season, November, and were present until April (Figure 2.27A). Females showed a similar pattern, showing stages 2 and 3 between May and October (the non-spawning season). Stage 4 ovaries were only found in November and all ovaries examined in December, were stage 5. Stage 6 ovaries were present between January and March (Figure 2.27B).

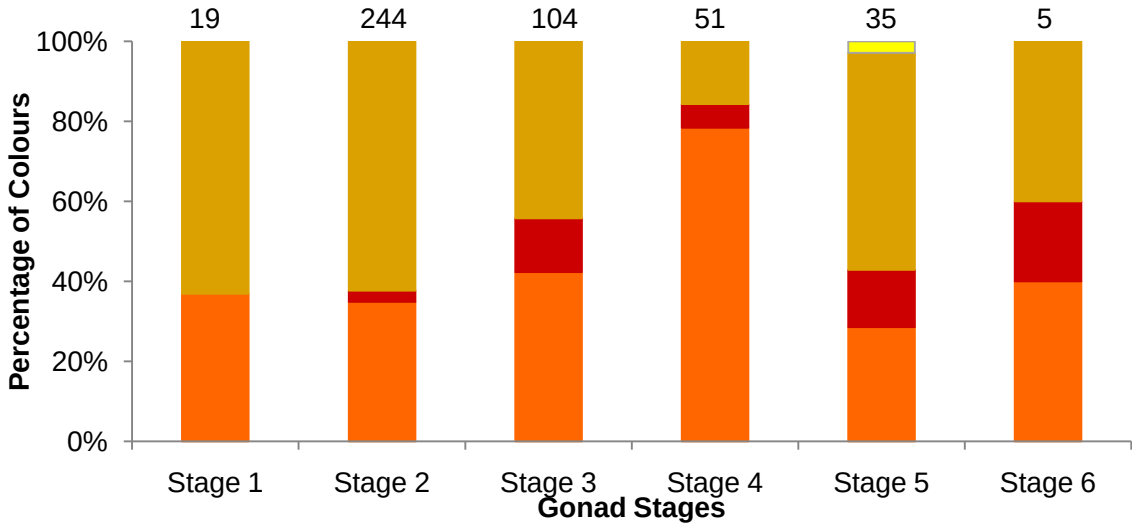


Figure 2.26 Colour divisions within the described stages for both males and females; colours divided: Orange, Red, Amber, and Yellow; Number of individuals examined for each stage is labelled above.

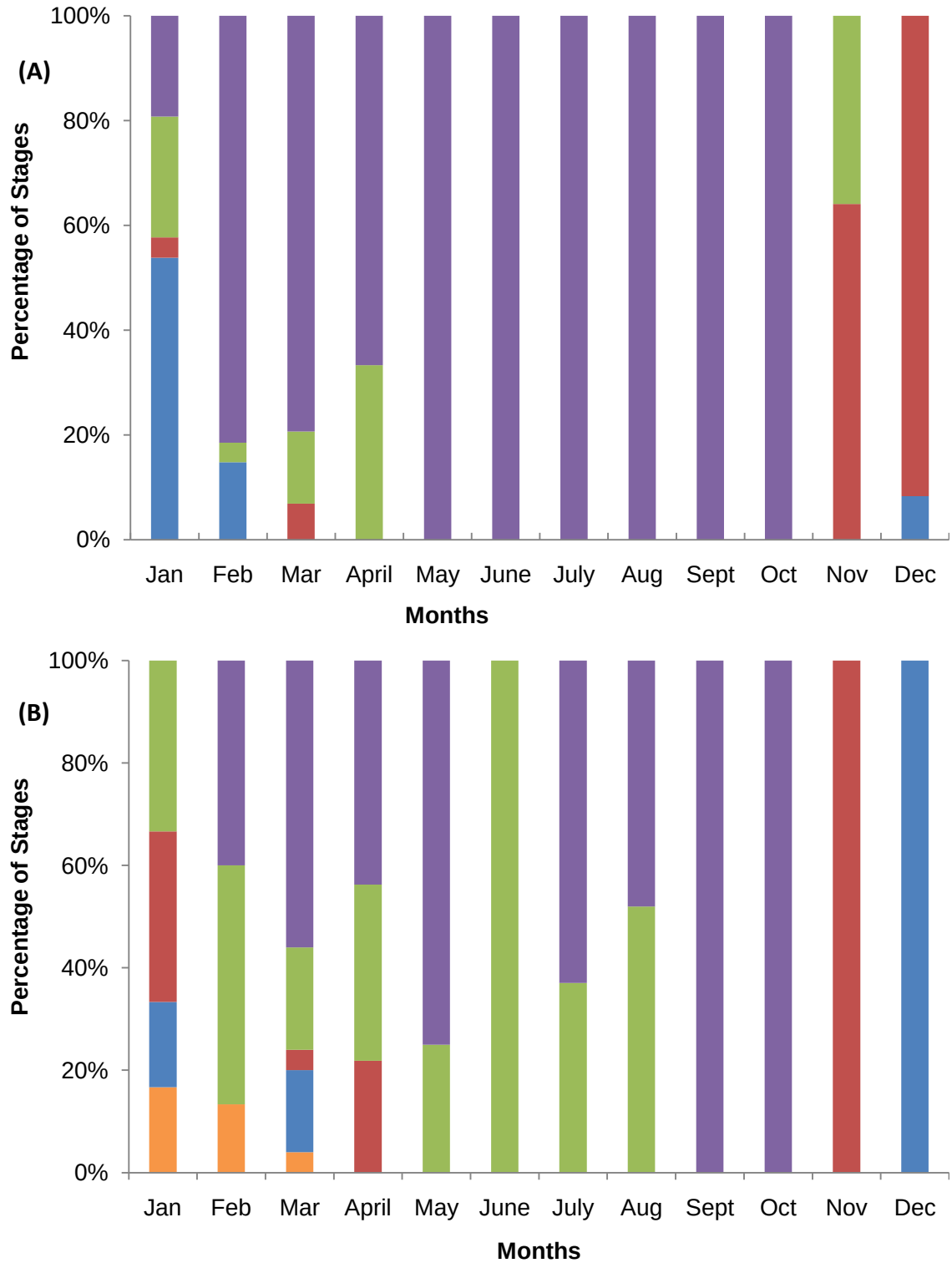


Figure 2.27 (A) Terrigal males and (B) Terrigal females gonad stages per month; Gonad stages: Second, Third, Fourth, Fifth, and Sixth

Length maturity ogives showed that both Terrigal and Jervis Bay males both had an L_{50} of 75.2 mm SL (Figure 2.28), while females from Jervis Bay had an L_{50} of 83.0 mm SL (Figure 2.29; Table 2.5). Terrigal females had an even larger L_{50}, at 89.2 mm SL (Figure 2.29). Juveniles from Terrigal were <102 mm SL, while Jervis Bay juveniles were <83 mm SL. Males and females at Terrigal had a W_{50} of 36.8 g and 40.2 g, respectively, while Jervis Bay males and females had a W_{50} of 21.0 g and 31.3 g, respectively (Figure 2.30; Figure 2.31; Table 2.6). Juveniles weighed <65.8 g at Terrigal and <29.9 g at Jervis Bay. Fifty per cent of fish at Terrigal attained sexual maturity at 1.9 years for males and 2.0 years for females. Fifty percent of fish at Jervis Bay attained sexual maturity at 2.4 years for males (Figure 2.32) and 2.8 years for females (Figure 2.33; Table 2.7). Terrigal juveniles were <4.3 years, except for one at 6.1 years, while Jervis Bay juveniles were <3.2 years, with the exception of one at 5.2 years.

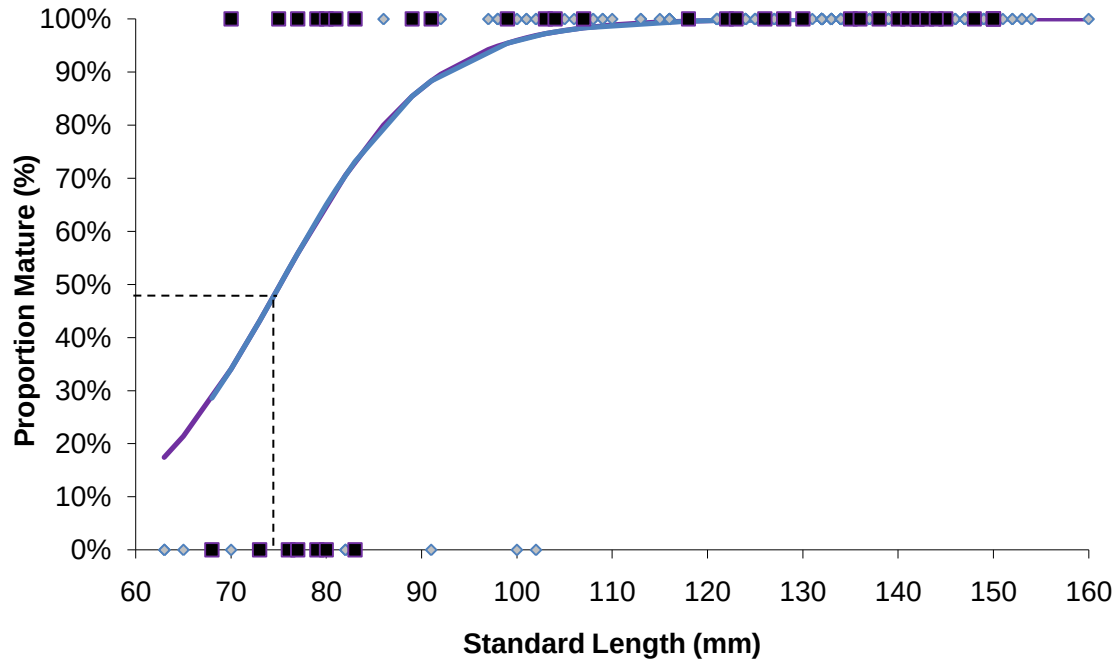


Figure 2.28 SL maturity ogives, Terrigal males ◇ vs. Jervis Bay males ■

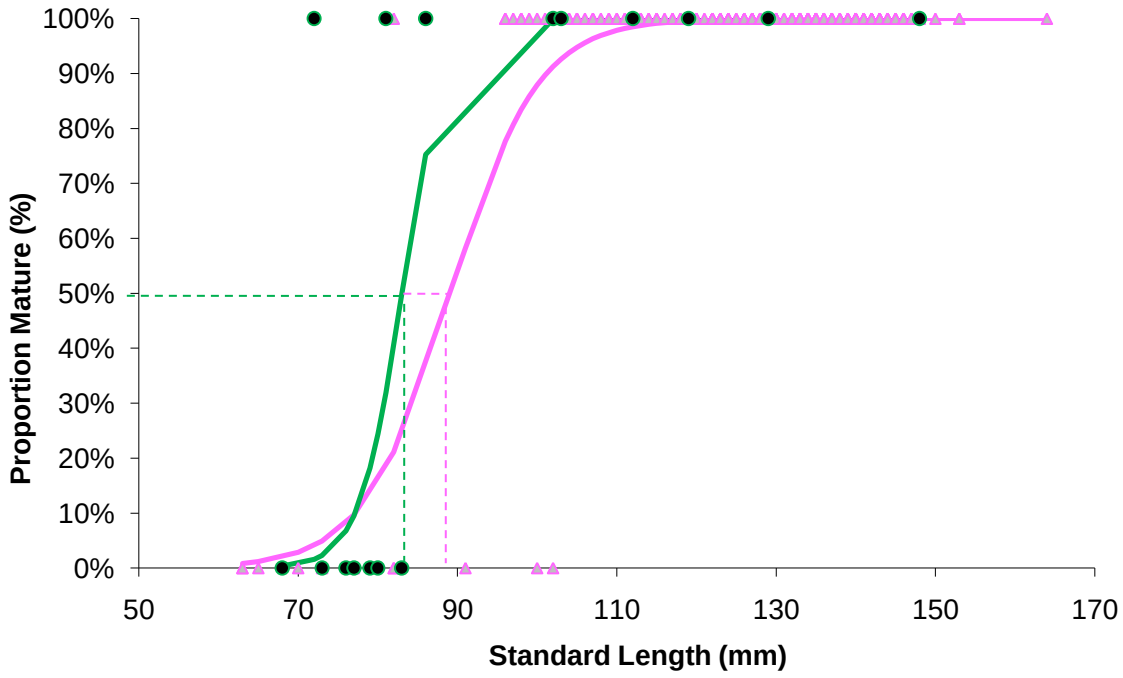


Figure 2.29 SL maturity ogives, Terrigal females △ vs. Jervis Bay females ●

Table 2.5 Standard length ogive data

	Parameters		Length Estimates (mm)		
	a	b	L_{25}	L_{50}	L_{75}
Terrigal Males	-9.61	0.13	66.6	75.2	83.8
Terrigal Females	-16.36	0.18	83.2	89.2	95.2
Jervis Bay Males	-9.61	0.13	66.6	75.2	83.8
Jervis Bay Females	-31.02	0.37	80.1	83.0	86.0

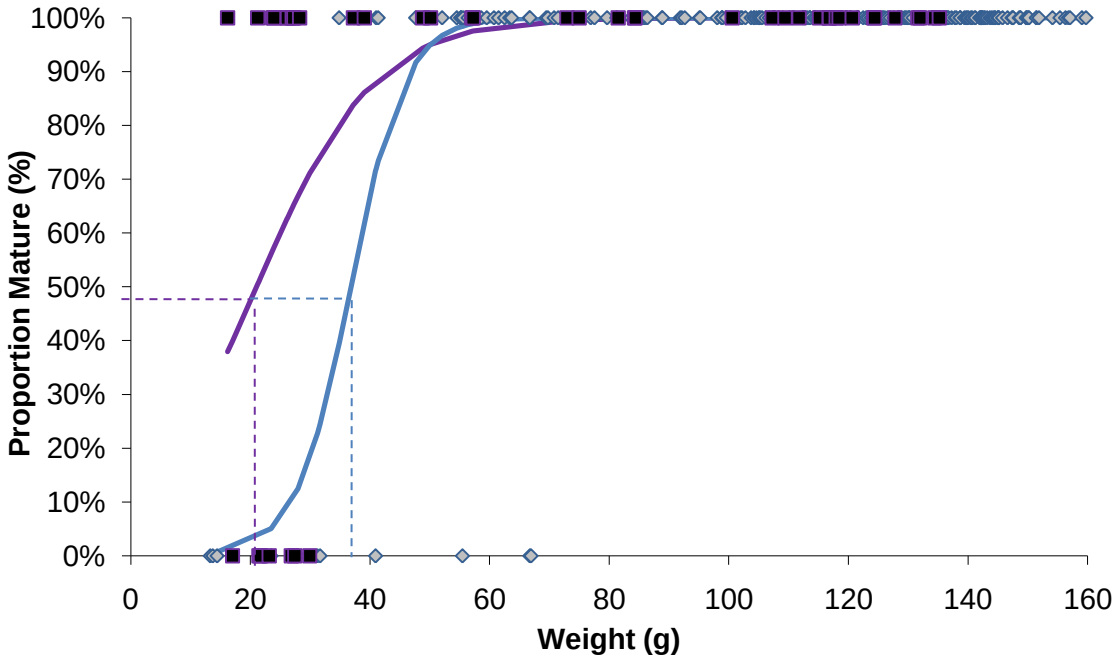


Figure 2.30 Weight maturity ogive, Terrigal males ◇ vs. Jervis Bay males ■

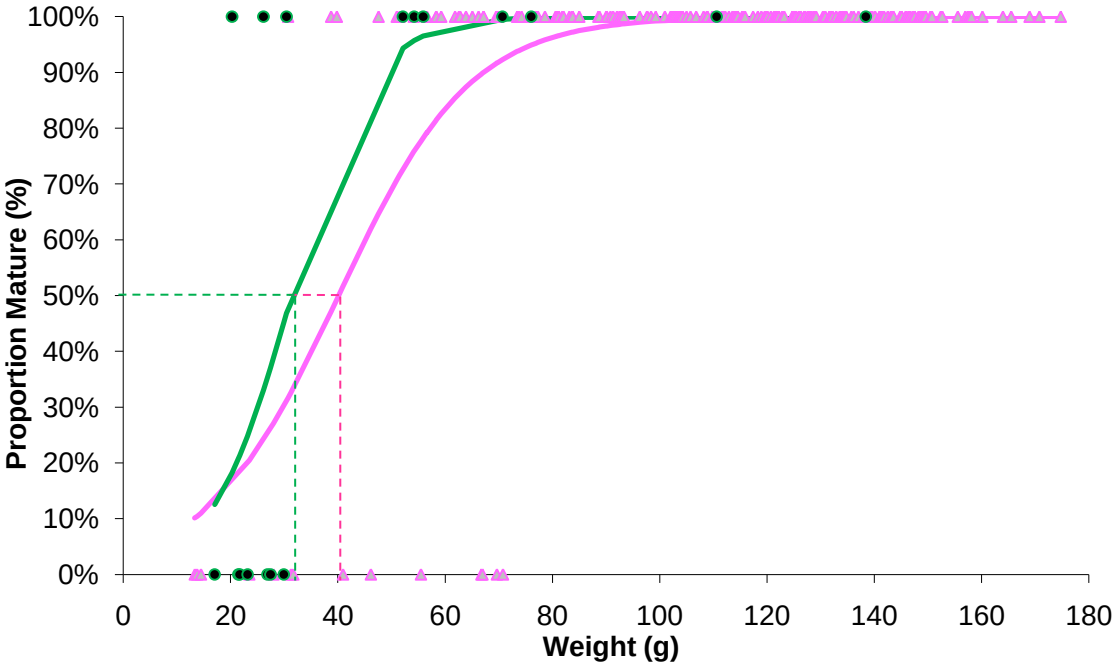


Figure 2.31 Weight maturity ogive, Terrigal females △ vs. Jervis Bay females ●

Table 2.6 Weight maturity ogive data

	Parameters		Weight Estimates (g)		
	a	b	W_{25}	W_{50}	W_{75}
Terrigal Males	-8.12	0.22	31.8	36.8	41.8
Terrigal Females	-3.27	0.08	26.7	40.2	35.6
Jervis Bay Males	-2.14	0.10	10.2	21.0	31.9
Jervis Bay Females	-4.25	0.14	23.2	31.3	39.5

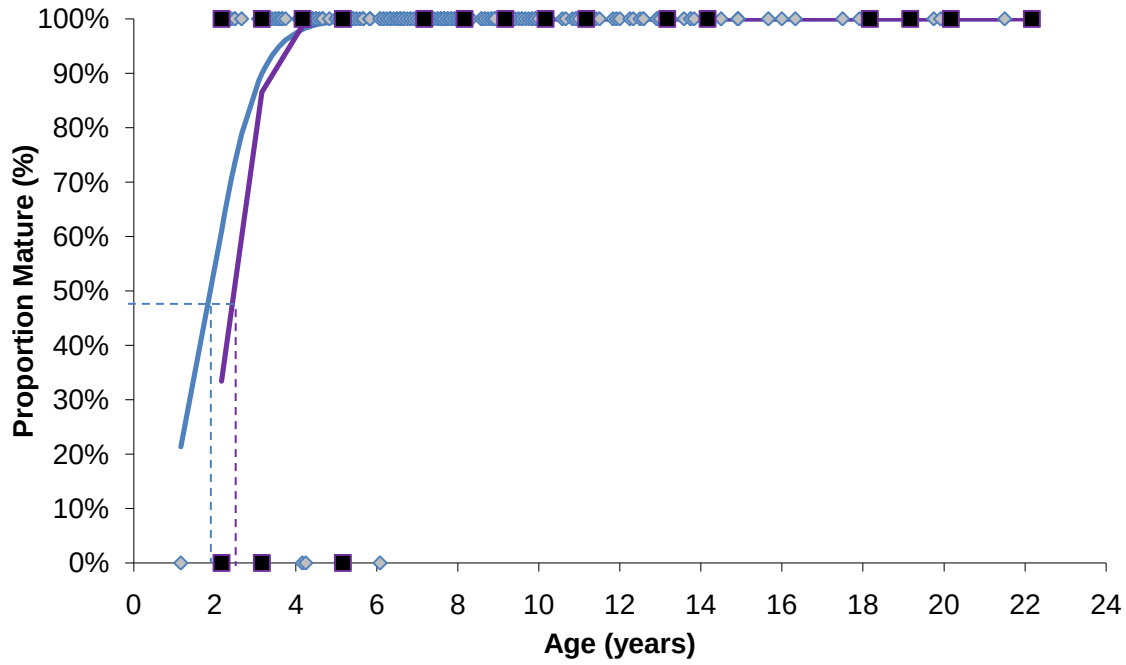


Figure 2.32 Age maturity ogive, Terrigal males ◇ vs. Jervis Bay males ■

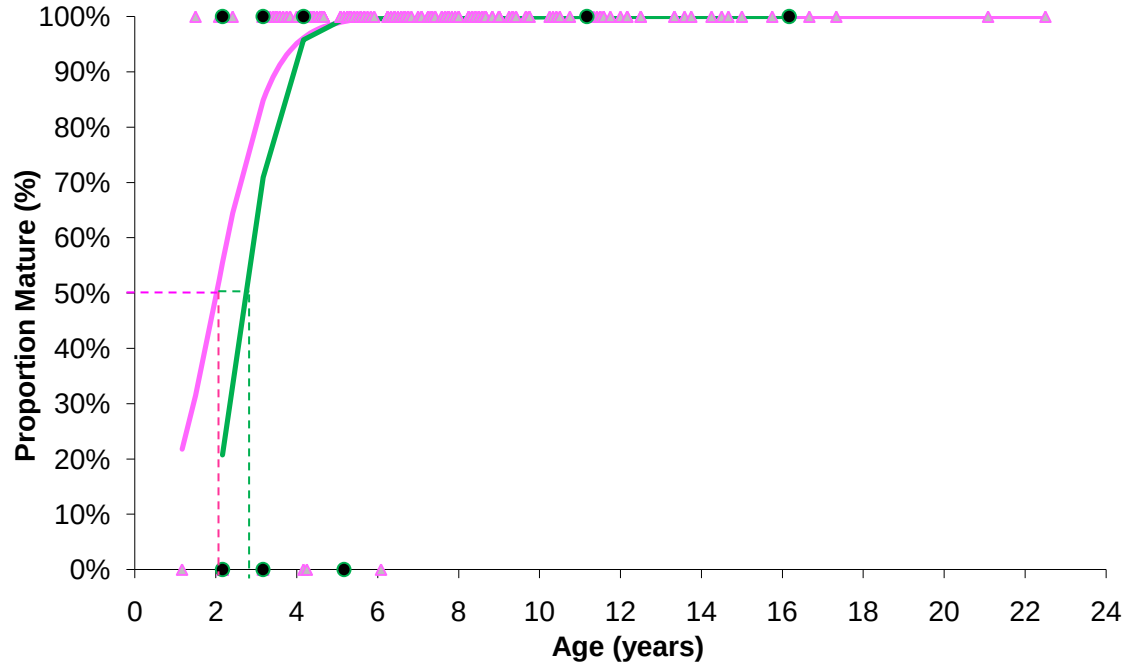


Figure 2.33 Age maturity ogive, Terrigal females △ vs. Jervis Bay females ●

Table 2.7 Age maturity ogive data

	Parameters		Age Estimates (years)		
	a	b	T_{25}	T_{50}	T_{75}
Terrigal Males	-3.35	1.75	1.3	1.9	2.5
Terrigal Females	-3.03	1.50	1.3	2.0	2.8
Jervis Bay Males	-6.21	2.55	2.0	2.4	2.9
Jervis Bay Females	-6.18	2.23	2.3	2.8	3.3

2.4 Discussion

2.4.1 Gender System

This study showed that *Chromis hypsilepis* is gonochoristic. The sex ratio was 1:1 for the specimens collected at Terrigal. The sex ration of the population in Jervis Bay differed however, catching the fish in the spawning season can account for this difference. The fish caught in the spawning season were primarily male. This most likely occurred because at the time of sampling fish were defending territories and therefore easier to catch. During the spawning season, females are either feeding in the water column or freely swimming around the large aggregation site. The length and weight frequency distributions showed little differences between the sexes, and none consistent with the occurrence of sex change. The histology and macroscopic examination of gonadial tissue showed a clear difference between the two genders. *Parma microlepis* is also a gonochoristic species with a 1:1 ratio of sampled populations, shown by the histology (Tzioumis and Kingsford, 1999). Pomacentrids are mostly gonochoristic (Tzioumis and Kingsford, 1999), however there are a few cases, in one subfamily, *Amphiprion*, there are hermaphroditic species (Helfman *et al*., 2009).

Chromis hypsilepis was not identified as sexually dimorphic or dichromatic, however personal observations suggest the occurrence of three temporary colours. *C. hypsilepis* has been identified in a silver, almost white tone, a dark, almost black tone, and a blue-green colour. There is a difference in colour of *Parma microlepis*, with the males being a dark brown-black colour, while females are between light brown to yellow (Tzioumis and Kingsford, 1999). *Hypsypops rubicunda,* a temperate water pomacentrid, is polychromatic, with three different colour phases in juveniles and mature males and females (Clark, 1970).

2.4.2 Sexual Maturity

Juvenile specimens could not be identified as male or female, by macroscopic observations. Length and weight were more reliable indicators of sexual maturity than age. Samples of the same young age could be classified as either juvenile or mature. The youngest male specimen, in Terrigal, was 2.2 years old, 34.9 g and 86 mm SL; this sample is a year older than the youngest juvenile identified, but is 23 mm longer and 21.6 g heavier. The youngest Terrigal female was 1.5 years old and was 17.4 g heavier and 19 mm longer. The small collection of young fish was due to the method of collection being concentrated on the medium to large sample sizes.

2.4.3 Growth

Many pomacentrids share similar growth rates to *C. hypsilepis*. *Dascyllus albisella* has a growth rate that is similar (k= 0.23) (Tzioumis and Kingsford, 1999) to that of the female *C. hypsilepis* at Terrigal. *C. hypsilepis* males at Terrigal grew at a slightly faster rate than the females at both locations, similar to the growth of male (k= 0.25) and female (k= 0.23) *C. chromis* (Dulĉić and Kraljević, 1995). The growth rate range for *Pomacentrus mollucensis* is between 0.45 and 0.6 (Fowler, 1990), which is higher than that of *C. hypsilepis,* and similar to *P. wardi* (k= 0.51) (Fowler and Doherty, 1992). Jervis Bay females have a very low growth rate (k=0.08), which is lower than the lowest recorded growth rate of *Stegastes fuscus* (k= 0.19) (Schwamborn and Ferreira, 2002). The relatively slow growth pattern for *S. fuscus* was unique to the size of the fish, which makes the slower growth of the female *C. hypsilepis* at Jervis Bay even more unique. The small sample size of females collected at Jervis Bay suggests that these conclusions regarding comparative growth rates should be treated with caution until further studies are done. Buesa (1987) found that species <50 cm have a faster growth rate than those >50 cm; this is not the case with *C. hypsilepis* females from Jervis Bay or *S. fuscus* (Schwamborn and Ferreira, 2002).

A large proportion of the growth of *C. hypsilepis* occurs in the first two years of their lives. Half of their asymptotic length is reached within the first year at Terrigal by males and females, while Jervis Bay males and females reached half of their asymptotic length in their second and third years respectively. By comparison, *Parma microlepis* reached half of its asymptotic length in the first two years (Tzioumis and Kingsford, 1999). *C. chromis* is described as a slow growing species, growing to 63% of the maximum size in 8 years and showing geographic variation depending on ecological conditions, e.g. water temperature (Dulčić and Kraljević, 1995). The growth rate of fish is highly dependent on environmental factors water temperature, levels of dissolved oxygen and ammonia, salinity, and photoperiod (Moyle and Joseph J Cech, 2004). The colder climates will bring slower growth patterns, and thus will vary the growth-maturity curve. This is apparent in a comparison of the growth coefficients of the Jervis Bay and Terrigal populations. *Parma microlepis* has been studied in various places around central NSW, with a slightly higher growth rate (k= 0.45) (Tzioumis and Kingsford, 1999) to that of *P. microlepis* obtained in Sydney, NSW (k=0.41) (Tzioumis and Kingsford, 1999).

The significance of early rapid growth has been suggested to be a life history strategy for early maturation and long life span. This is a response to unpredictable recruitment success (Leaman and Beamish, 1984; Longhurst, 2006). Although there are no data on the variation in recruitment of *C. hypsilepis*, it is possible that this species has evolved this life history strategy of investing in reproduction rather than growth as a means of ensuring sufficient offspring are produced over a long life to ensure the reproductive success of individuals. Buesa (1987) investigated 37 tropical species, with the findings that the smaller the maximum length, the faster the growth rate. This is apparent in *Chromis hypsilepis* (present study), *C. chromis* (Dulčić and Kraljević, 1995), *Parma microlepis* and *P. wardi* (Tzioumis and Kingsford, 1999)

2.4.4 Age

Sections of sagittal otoliths with counts of opaque bands were found to be a reliable technique for estimating the age of *C. hypsilepis*. The marginal increment analysis (MIA) revealed that bands were completed in December, and a new band started in January. The average marginal increment in February 2008 was similar between Jervis Bay and Terrigal samples, suggesting that there is no geographic variation in band formation. Bands are completed in the summer season which is also in the middle of the breeding season. Some pomacentrids are known to have new band formations within the summer season (Fowler and Doherty, 1992). *Stegastes fuscus, Pomacentrus moluccensis* and *P. wardi* deposit new bands during the dry season, when the water temperature is higher (Schwamborn and Ferreira, 2002). However, those like *Parma microlepis*, form new bands before their spawning cycle, occurring in the winter months (Tzioumis and Kingsford, 1999)

2.4.5 Geographic Variation

Weights varied between the two populations, with individuals reaching a maximum of 179.01 g and 138.40 g at Terrigal and Jervis Bay, respectively. Length data had a similar pattern to that of the weight. Fish at Terrigal had a maximum length of 165 mm SL for a 9.5 year old male; while fish at Jervis Bay had a maximum of 150 mm SL from three males aged 10.2, 19.2 and 22.2. Jervis Bay females exhibit the higher T_{50}, at 2.8 years. The length of the Jervis Bay population was affected in the growth coefficient (k), the lower k value created a curve that was set slightly apart from the Terrigal population which shows a longer growth rate to reach the asymptotic length (L_{∞}). The Terrigal population also had a growth coefficient which was greater than Jervis Bay's population, for weight as well. Jervis Bay females (n= 17) showed an

exceedingly large asymptotic length and weight, not consistent with the other populations. This is most likely due to the low numbers of the samples obtained.

While the two populations are only 200 km apart, they experience different water temperatures and water conditions. Jervis Bay has a colder water period, below 18° C, starting in June and ending in November, while Terrigal has a shorter period of colder water, between July and October (Powter, 2006). Weight gained by Terrigal populations seemed to be higher, suggesting that food sources are more available for Terrigal than for the population in Jervis Bay.

2.4.6 Conclusion

This study has described some aspects of the reproductive biology of *Chromis hypsilepis*. As hypothesized, *C. hypsilepis* was found to be gonochoristic with the two sexes occurring in a 1:1 ration and with minimal variation in their physical appearance. Males and females attained maximum ages of 21.3 and 22.5 years respectively. Growth bands in the sagittal otoliths were found to be a reliable indicator of age, with this finding validated by marginal increment analysis. The existence of seasonal spawning was confirmed by GSI and histology examination of gonads. There was minimal variation in growth parameters between the study population at Terrigal and a population at Jervis Bay.

Chapter 3.Reproductive Behaviour

3.1 Introduction

3.1.1 Overview and Aims

Chromis hypsilepis is a demersal spawner that undertakes all reproduction in large spawning aggregations. It has an austral spring-summer breeding season (October-February), a semi-lunar spawning cycle and has no significant diurnal variation in spawning frequency (Gladstone, 2007a; b). This information is based on limited observations mainly over a single spawning season (2004-05) (Gladstone, 2007a; b) and observations of spawning frequency at discrete time intervals during the day. The aims of this study were to determine the spawning behaviours of *C. hypsilepis* and expand the observations of Gladstone (2007a; b). The data for this study were gathered largely by a novel method of continuous underwater video recording (covering up to 11 hours per day) of fish at the spawning aggregation site, without human disturbance. The major findings of this study were that, contrary to previous findings, spawning did not follow any lunar pattern and spawning varied substantially throughout the day with an increased frequency observed in the middle of the day. Spawning males, who represented only 6% of the population, spent most of their time spawning (56%), while the majority (around 90%) of the population was swimming around the aggregation.

3.1.2 Introduction

Spawning aggregations are critically important phenomena in the population replenishment of many species of fish (Gladstone, 2007a; b). Many studies have described the behaviour of fishes at spawning aggregation sites and their spawning behaviours (Claydon, 2004). The majority of these studies have focussed on pelagic

spawners from the tropics (Munro *et al*., 1973; Johannes, 1978; Foster, 1989; Tyler and Stanton, 1995; Domeier and Colin, 1997; Asoh, 2003), with few examples of aggregations of demersal spawners (Swerdloff, 1970; Russell, 2001; Cole, 2008), and even fewer examples of demersal spawners from temperate regions (Tzioumis and Kingsford, 1995; Gladstone, 2007b). Our understanding of the behaviour of fish in spawning aggregations is therefore very limited, and this limits our understanding of the factors underlying the evolution of this reproductive strategy.

Fishes aggregating for spawning may incur several energetic costs arising from: migration to and from the spawning site, reduced feeding while migrating, territorial defence, courtship, spawning, and parental care. Species that spawn at aggregation sites reach them after migrating from hundreds of metres (e.g. pomacentrids) to hundreds of kilometres (Claydon, 2004). The feeding rates of males engaged in egg care are reduced by 24 to 85% (Robertson *et al*., 1990; Gladstone, 2007b). The need for spawners to recover from the costs of spawning has been hypothesized as an explanation for the existence of cycles of spawning activity (Robertson *et al*., 1990; Gladstone, 2007b). However, these costs are poorly understood for most species with spawning cycles. Further understanding of the potential total costs of reproduction in fishes that aggregate for spawning will provide a better test of the cost of reproduction hypothesis as an explanation for spawning cyclicity.

Little information is known on the spawning behaviour of *C. hypsilepis*. They have been studied to describe the preference for nesting habitat of males, which was determined to be the undersides of over-hanging boulders, while they avoided exposed flat rock surfaces for nesting (Gladstone, 2007a). The spawning aggregation selected a habitat which was dominated by hanging boulders for this reason (Gladstone, 2007a). There was no preference for spawning sin crevices, ledges immediately below an overhang, or vertical walls of boulders that were close to vertical boulder walls (Gladstone, 2007a). Their spawning acts occur on the semi-lunar cycle, similar to

Chromis viridis (Cole, 2008). *C. hypsilepis* showed no signs of spawning frequency change over the day (Gladstone, 2007a).

Kingsford (1985) studied the spawning period of *Chromis dispilus* which extends from early December to the beginning of April. *C. hypsilepis* spawns between October through the middle of February (Gladstone, 2007a). *C. viridis* spawned between November and mid to late January (Cole, 2008). Spawning cycles were observed during the spawning season. *C. hypsilepis* and *C. viridis* were both found to have a semi-lunar spawning cycle (Gladstone, 2007a; Cole, 2008). Cole (2008) also reviewed the spawning cycles of other Pomacentridae, focusing on the *Chomis* genus. *C. notata, C. multilineata, C. dispilus, C. cyeanea, and C. hypsilepis* were all noted to exhibit spawning synchrony, which varied between moderate and high.

Diurnal variation in spawning frequency among species of Pomacentridae within the spawning day has been minimally studied. Cole (2008) recorded the observations of the spawning synchrony during the morning hours of the spawning season and could not comment on variations in frequency throughout the day. Gladstone (2007b) observed the spawning of *C. hypsilepis* at 0600, 0830, 1130 and 1500 hours, and found no significant variation throughout the day.

The aims of this study are to expand and verify Gladstone's (2007b) observations on the semi-lunar cyclicity and lack of diurnal variation in spawning frequency of *Chromis hypsilepis* by using more intensive observations. The study describes and quantifies reproductive and other behaviours of *C. hypsilepis* at the spawning aggregation site and the proportion of fish engaged in reproductive behaviour. The results provide a more comprehensive assessment of the cost of reproduction in *Chromis hypsilepis*.

3.2 Methods and Materials

3.2.1 Study Area

This study was undertaken at Terrigal, NSW Australia between October 2008 and February 2009. The study area is described in detail in Section 2.2.3.

3.2.2 Video Recording

Two types of underwater video cameras were developed to provide continuous recording over at least a 12 hr period. In the first system a “Securview” lipstick camera was connected to a “SanDisk V-Mate” video memory card recorder (within an underwater housing) and the system was powered by 12 and 6 volt batteries. The system was developed and provided by Dr M Lowry (NSW Department of Primary Industries, Port Stephens). Difficulties with this system (incomplete recordings, power failures, short circuits) led to a second system being developed. This consisted of a “Traveler HD” 5 mega pixel camcorder powered by a 12-volt battery. A transformer was used to reduce the battery power from 12 to 5 volts to run the camera. Video recording was done on 16GB SDHC memory cards. The underwater housings were mounted in a frame (Figure 3.1) that was secured (via chains) to the substratum. The cameras were positioned, by divers, within the spawning aggregation site and pointed towards boulders where fish were expected to spawn. Two trials of different positions were required in order to provide a field of view that included a sufficiently large number of spawning fishes. The camera was able to view an area 2 metres high by 3 metres wide at a distance of 2 metres from the camera.

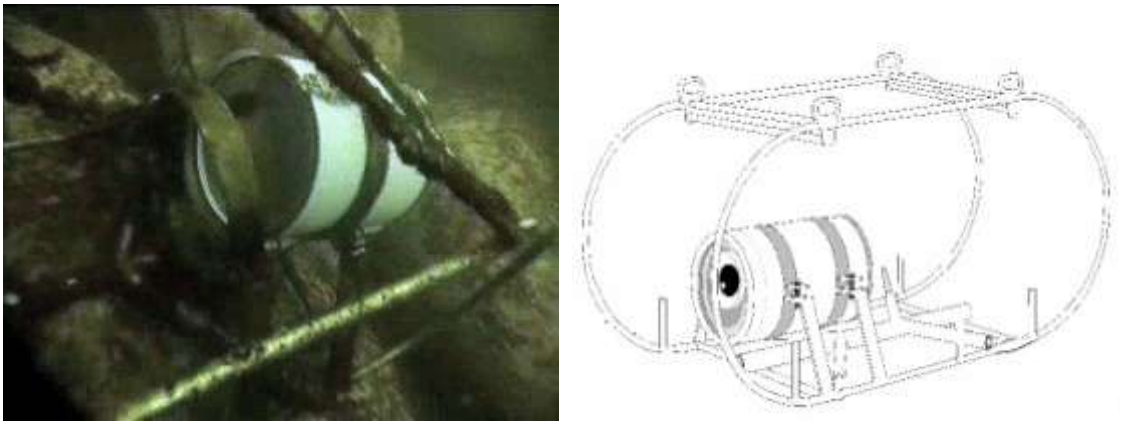


Figure 3.1 Underwater video camera mounted, via chains, in a frame at 8 metres depth. Video camera set up inside the blue housing. With schematic of the frame and housing set up.

Fish were videotaped for up to 12 hours and over 4 days around the semi lunar cycle. Video recording occurred the day before the full/new moon, the day of the full/new moon, and two days following the full/new moon. Due to dangerous water conditions and technical difficulties, various dates were not sampled, or replaced with other sampling. On two days, the 6th and 7th of November 2008, spawning observations were made using SCUBA.

3.2.3 Video Analysis

Between October 2008 and February 2009, 106 hours of underwater videos were recorded and analysed. Video recordings were analysed by playing back through a laptop computer using VLC Media Player software. The relationship between spawning and the lunar cycle was determined by noting the date on which spawning occurred and the lunar phase. The following observations/ behaviours were noted for each video recording (i) *C. hypsilepis* absent; (ii) "hanging", which included swimming, feeding, and sometimes guarding; (iii) "territorial behaviour", and (iv) "spawning". Territorial behaviours included chasing other *C. hypsilepis* through or out of the area of spawning, "visiting" other territories, and guarding the eggs from predators. The frequency of these behaviours was also compared between the beginning, middle and

end of the spawning season. Other general observations that were made included the identity of other fish species that used the area of the spawning aggregation and the responses of *C. hypsilepis* (avoidance, predation attempts on *C. hypsilepis*, or no response to the other species).

3.2.4 Diurnal Variation in Spawning

Spawning occurred all day on 22nd December 2008. Diurnal variation in spawning frequency was quantified by noting the frequency of spawning per 5 minute interval between 0730 h and 1755 h. The variation in spawning frequency was also correlated with the times of sunrise, the sun at the highest and sunset to investigate if the sun positioning was a determining factor in the frequency of spawning.

3.2.5 Spawning Behaviour

Video recorded on 22nd December 2008 was observed to describe the spawning of *C. hypsilepis.* The activities were divided into four behaviours: (1) guarding and nesting included males caring for the nests or chasing fish away from the nest, (2) swimming and floating included *C. hypsilepis* swimming or floating around the aggregation without other behaviours associated with it, (3) chasing was defined as a male *C. hypsilepis* chasing conspecifics or heterospecifics unrelated to egg care, and (4) spawning was determined to be the act which is described in section 3.5.3. Two sampling methods were used to describe these behaviours. Focal-animal sampling (Lehner, 1996) was used to quantify the proportion of time spawners engaged in behaviours (1)-(4) throughout the day of spawning. Four sections of video tape (each of 15 minutes duration) were randomly selected from the day-long recording. The four sections of video tapes started at: 0820 (morning), 1205 (early afternoon), 1525 (late afternoon), and 1740 (evening). The focal animal was a male that occupied a territory, primarily within the video. In addition, instantaneous sampling of behaviours (Lehner,

1996) was done to determine the frequency of described behaviours among all fishes present in the field of view. Instantaneous sampling was completed by randomly capturing 20 screen shots from the day-long video and counting the number of individuals engaged in the behaviours described above. Snapshots were chosen and divided evenly throughout the day, into morning (0730-0959) early afternoon (1000-1229), late afternoon (1230-1459), and evening (1500-1755).

3.3 Results

3.3.1 Spawning Cycle

Out of 20 days of observations, spawning occurred on 7 days (Figure 3.2). Spawning did not appear to be correlated with the lunar cycle. Spawning occurred more frequently in the beginning of the spawning season, in October 2008, rather than the middle or end of the spawning season. January was not sampled. Spawning was not observed in February.

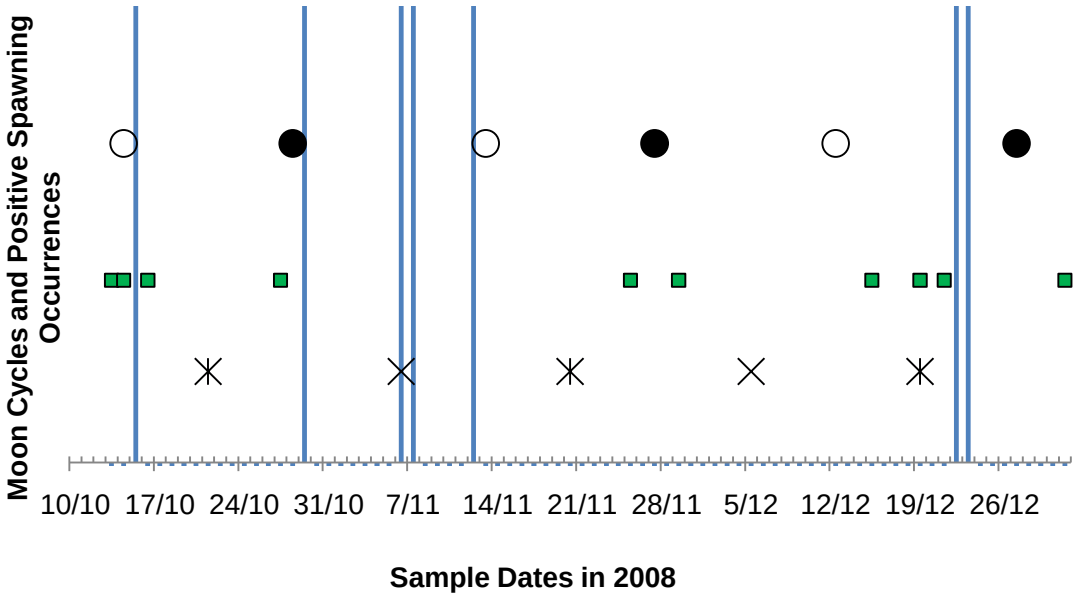


Figure 3.2 Spawning occurrences compared to the lunar cycle. Spawning observed ▮, Full Moon ○, New Moon ●, First Quarter ×, and Third Quarter ✳. Days which had videos, but no spawning was observed, are identified ■.

3.3.2 Diurnal Variation in Spawning Frequency

A total of 3,298 spawning occurrences were observed throughout the day-long recording. These represented individual spawning passes over the substratum by males and bouts of egg deposition by females. Spawning frequency increased throughout the morning, reaching a peak (n= 493 per hour) at 1200, which represented 15% of the daily total. After 1200, spawning decreased dramatically, with the exception of a second peak between 1500 and 1530. Spawning frequency was lowest at 1400, 1600 and 1700 hours, with frequencies as low as 3 spawning acts within 5 minutes, compared to the highest of 55 spawning acts over a period of 1205 to 1210 (Figure 3.3). The spawning peak during the 1200 hour coincided with the time when the sun was highest.

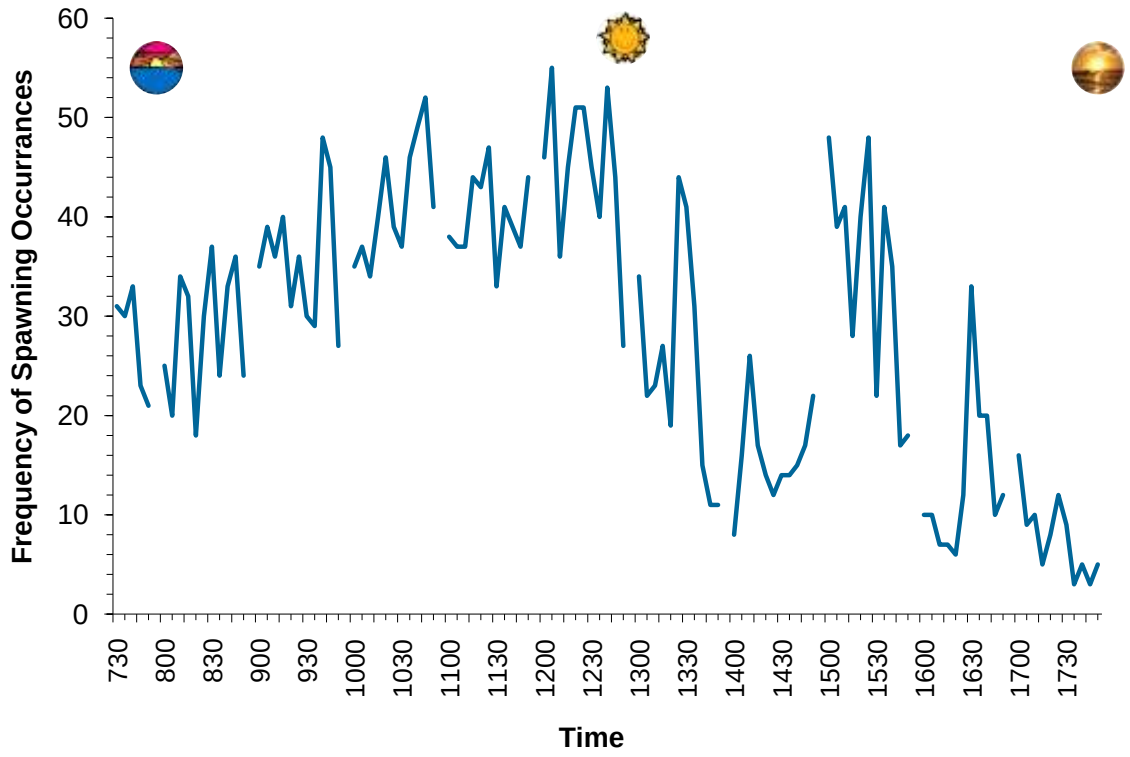


Figure 3.3 Frequency of Spawning during the hours of 7:30 to 17:55 in 5 minute intervals and sunrise , sun at the highest point in the sky , and sunset is also marked on the graph .

3.3.3 Reproductive and Other Behaviours

The individuals that were swimming included juveniles, females waiting to spawn, and other non-spawning individuals. The males formed groups in small areas surrounded by sections where boulders peak. The groups were not characterized by obvious feature, such as size or colour phase. However, when an "outsider", (either another *C. hypsilepis* or another species) entered into the group one of the males guarding the territory would chase it away. Most male *C. hypsilepis* stayed close to the substratum, while juveniles or females were swimming around the population. In one instance recorded, the male that was being investigated went to "visit" another's territory for over 5 minutes before returning. As this species has no identifiable markings, it is not certain that the same fish came back into the territory.

There were a few species which interrupted the spawning or pre-spawning behaviours of *C. hypsilepis*. Although these species did not attack *C. hypsilepis* they consumed the eggs or investigated the clutch of eggs. *Cheilodactylus fuscus* (Appendix 5) interrupted the pre-spawning behaviours of male *C. hypsilepis* by entering into the territory and causing the *C. hypsilepis* to retreat away into protective coverings. The *Ch. fuscus* did not attack the *C. hypsilepis* however, they consumed the eggs within the nests.

Focal-animal sampling showed that individuals spent 55.7% of their time spawning and 40.5% guarding eggs (Figure 3.4A). The remaining 3.8% was devoted to chasing conspecifics away from their territories. No obvious feeding was observed by males guarding the nests. The time spent on these activities varied throughout the day (Figure 3.4B). Spawning was highest in the middle of the day, while guarding was greatest in the morning and the evening. Chasing also increased in the evening, but was practically non-existent in the morning. Most of the chasing occurred in the evening, after most of the spawning had slowed.

Individual spawning occurrences lasted for varying amounts of time during the different times of the day. Spawning events lasted anywhere between 2 sec and 86 sec (mean ± SE= 15 ± 3 sec) in the morning, to 32 ± 4 sec in the early afternoon (range= 5-73 sec). The late afternoon and evening varied tremendously, between an average of 31 ± 13 sec and 11 ± 4 sec seconds respectively. The longest spawning event was in the late afternoon, 220 sec. Durations of spawning in the evening varied between 2 and 75 sec.

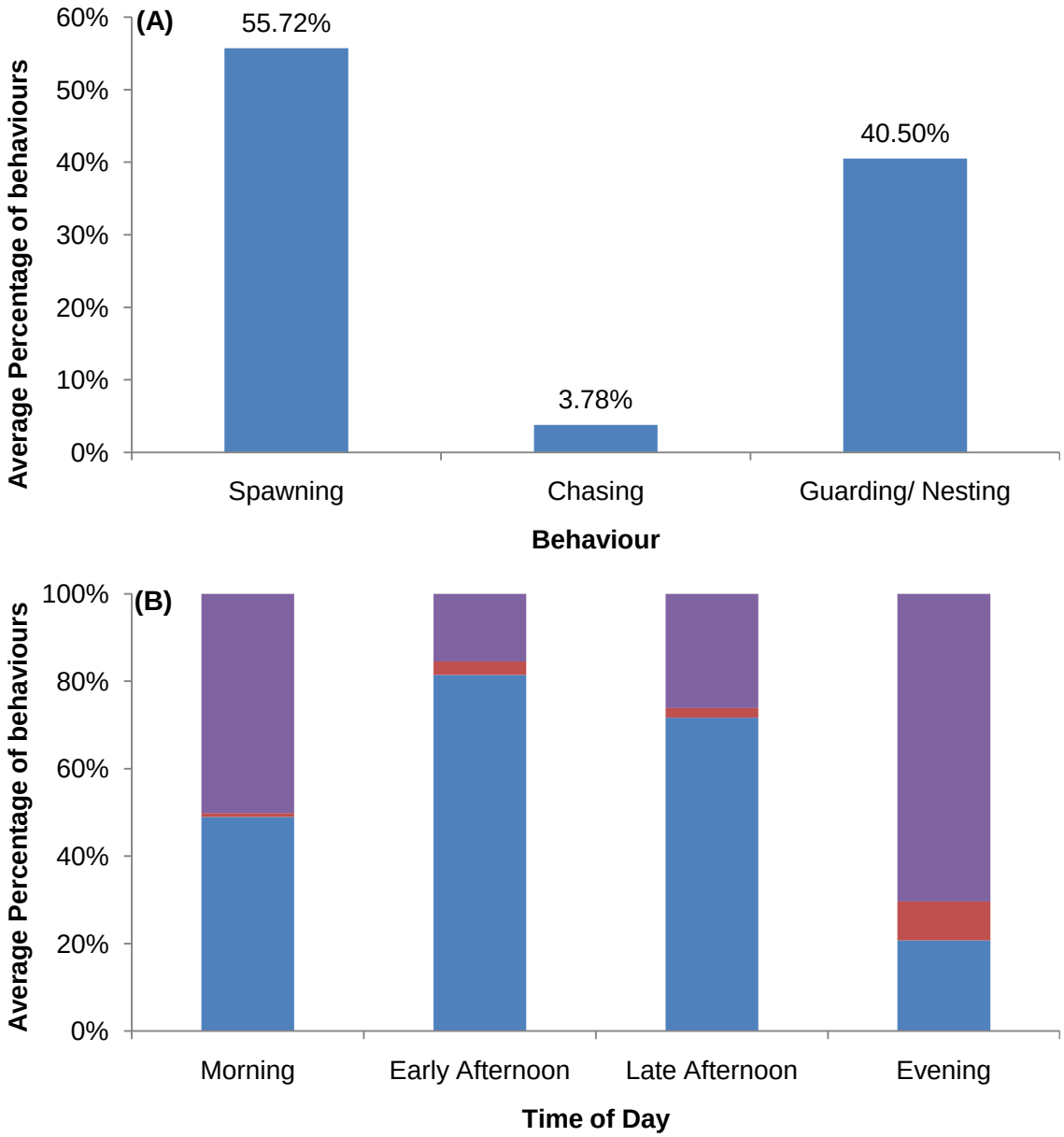


Figure 3.4 Activity during the day, with Focal Animal Sampling; dividing the day (A) averaged and (B) divided throughout the day, with Spawning, Chasing and Guarding/ Nesting

Instantaneous sampling was completed on 20 snapshots from random sections of the day (Figure 3.6). The behaviours of a total of 420 individuals were categorized across the 20 snapshots. Chasing behaviour (i.e. chasing or being chased) represented only 0.5% of all behaviours recorded. Guarding and nesting was the next lowest, representing 1.7% of recorded behaviours. Spawning represented 6.4% of the observed behaviours. The population was observed to be swimming or floating for the remainder of the 91.4% of behaviours recorded (Figure 3.5A).

The day was also divided into the morning, early and late afternoon, and evening, to show the different activities throughout the day, and the changes that occurred throughout the day (Figure 3.5B). In the evening, spawning and swimming/ floating were the only activities observed. The occurrences of spawning varied from 9.5% in the morning, to 3.6% in the afternoon, and up to 6.6% in the evening. The majority of the day, varying from 90 to 96%, was spent swimming or floating through the aggregation. Guarding and nesting only occurred during the morning, while chasing only occurred during the afternoon.

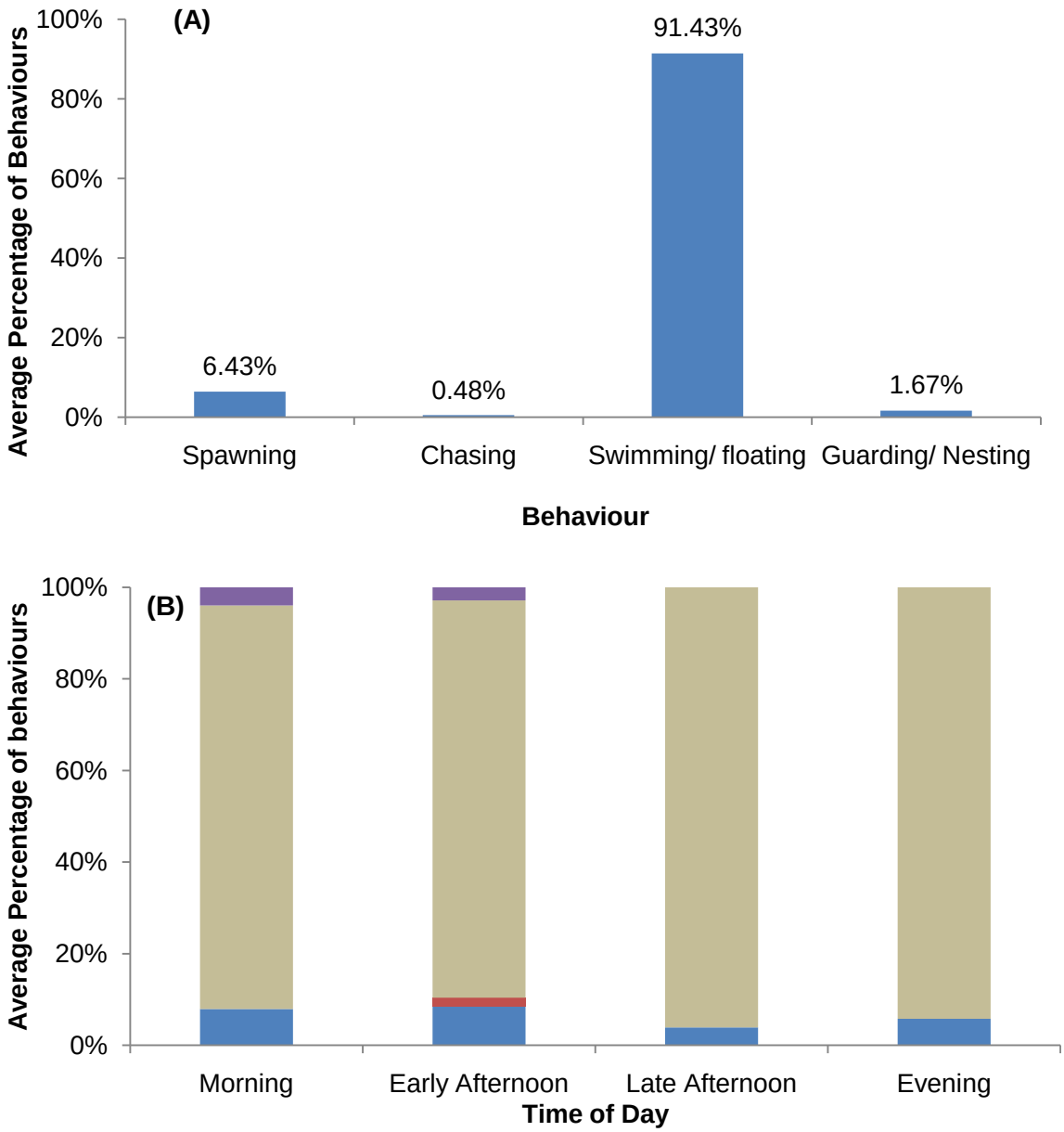


Figure 3.5 Instantaneous sampling of spawning aggregation (n= 420) throughout the day (A) averaged; (B) dividing the day, with Spawning , Chasing , Swimming/ Floating , and Guarding/ Nesting

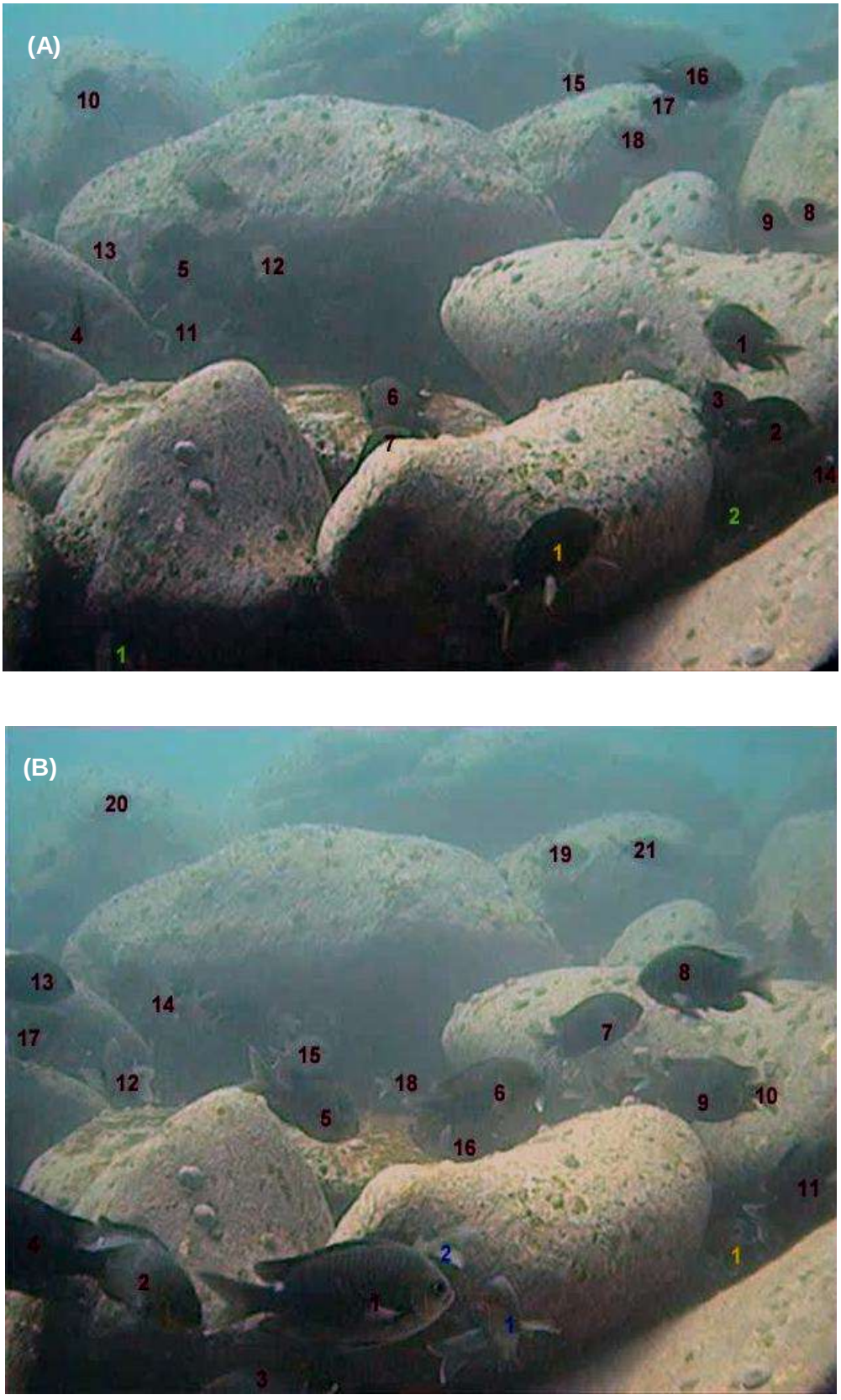
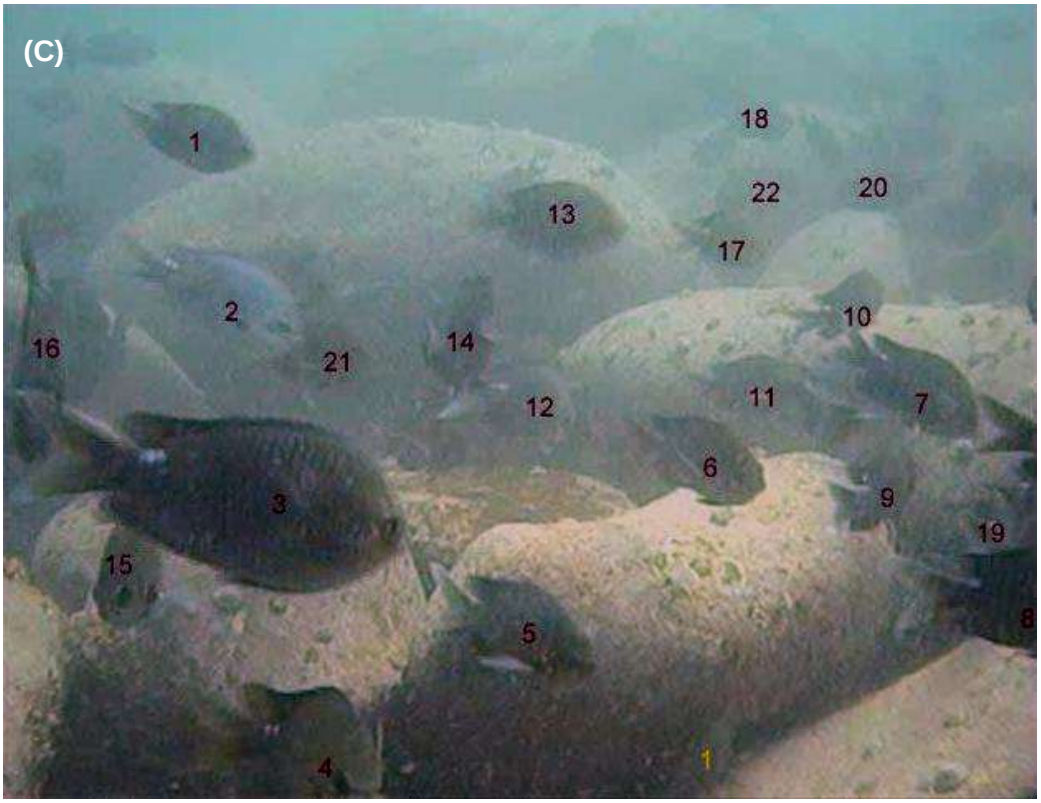


Figure 3.6 Behavioural observations of *Chromis hypsilepis* in the (A) morning, (B) early afternoon, (C) late afternoon, and (D) evening. Numbers coloured to indicate behaviour: swimming (1), spawning (1), chasing (1), and guarding/ nesting (1).



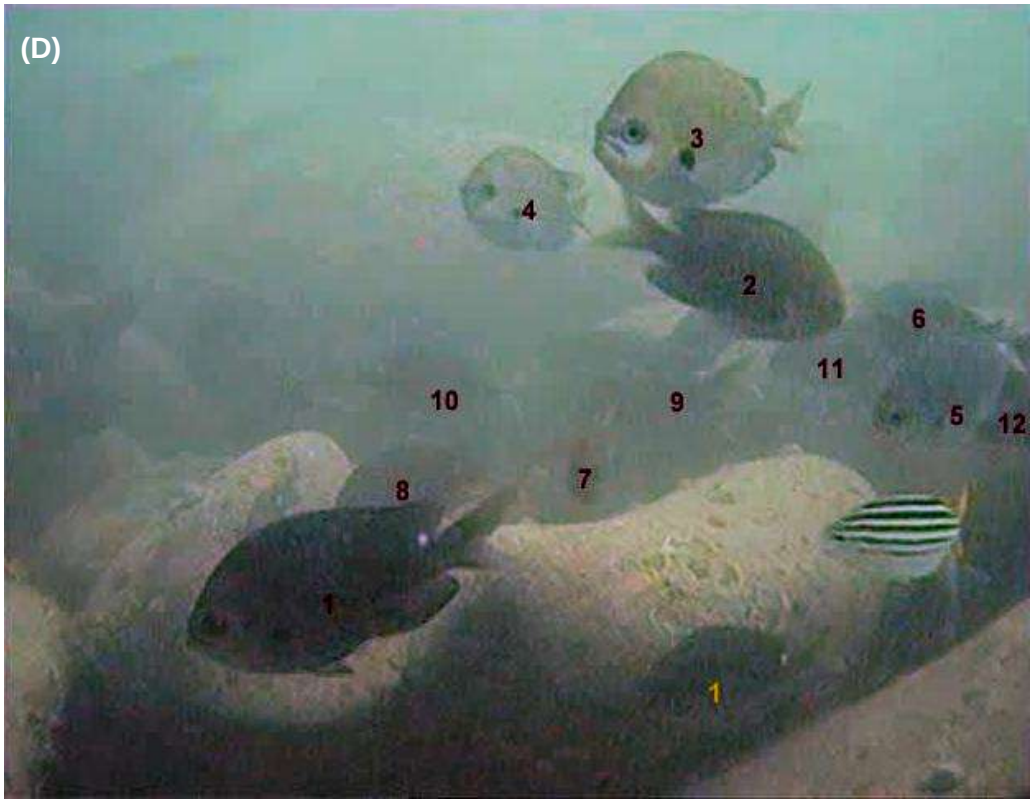


Figure 3.6 continued Behavioural observations of *Chromis hypsilepis* in the (A) morning, (B) early afternoon, (C) late afternoon, and (D) evening. Numbers coloured to indicate behaviour: swimming (1), spawning (1), chasing (1), and guarding/ nesting (1).

Many species occurred within the spawning aggregation site and their presence did not disturb the behaviour of *C. hypsilepis*. *Girella tricuspidata* is known to be a schooling fish, which comes into the spawning ground to graze in large schools which vary in sizes (Appendix 5). Species that were regularly observed included *Atypichthys strigatus* (Appendix 7), *Trachinops caudimaculatus (*Appendix 8*), Schuettea scalaripinnis* (Appendix 9), and *Pempheris* spp. (Appendix 10). Transients within the aggregation site included *Enoplosus armatus* (Appendix 11), common squid (*Sepioteuthis australis*) (Appendix 12) and crested Port Jackson sharks, *Heterodontus galeatus,* (Appendix 13), which are also common in the surrounding area. The most common of the transient species was the eastern blue groper, *Achoerodus virdis* (Appendix 14). *C. hypsilepis,* were not alarmed or aggravated by these species and continued without interrupting normal spawning behaviours.

3.4 Discussion

3.4.1 Spawning Behaviours

Spawning was defined as clearly distinct acts of spawning, described best by Russell (1971). Spawning was defined as “both fishes were close alongside and orientated head to tail, or, sometimes facing in the same direction. Both fishes then moved with a rapid quivering motion alongside each other, skimming the substrate and making 1 to 4 passes over the nest” (Russell, 1971). Spawning of two *C. hypsilepis* included this act within the male’s territory and squirming was seen, while fish moved along the rock substratum. The number of times *C. hypsilepis* passed over the nest was more than the 1 to 4 times that were recorded for *C. dispilus* (Russell, 1971). Each spawning pass made by *C. dispilus* lasted for 2 to 3 seconds during the spawning events observed by Russell (1971). In contrast, spawning by *C. hypsilepis* varied between 2- 220 seconds.

C. hypsilepis also formed male groups amongst the boulder substratum. This is for protection of spawning territories and nests. There is also a chance that this could lead to genetic flow, due to the "visits" that were seen in the male territorial areas. Although it is not conclusive in the video analysis, this could be one way for the large numbers of males, in the territorial groupings, to increase the genetic flow. The hypothesis that younger males or males without territories will sometimes come into the territories of those with nests, to covertly fertilize the eggs, could be used here in another way. In the videos no other males were seen attempting to enter the territory, despite the presence of large numbers of males around the territory.

Many of reproductive behaviours reported for other *Chromis* species were also observed for *C. hypsilepis*. *C. hypsilepis* was identified as a territorial species by Gladstone (2007b), and this was verified in this study. Males were observed guarding the nests for about 40% of their time, according to the focal-animal sampling. Most *Chromis* species exhibit territorial behaviours (Swerdloff, 1970). *C. viridis* was described to have a "aggregation-formation phase" (Cole, 2008), which is the first phase that occurs in *C. hypsilepis* when they start the spawning process. Aggregations are formed for *C. hypsilepis* a month or more before the spawning acts first occur (Gladstone, 2007b). The behaviour of *C. caeruleus* was recorded to have similar behaviours to that of other *Chromis* species, which included a "signal jump" and "skimming" (Swerdloff, 1970). The "signal jump" also appears in *C. viridis* (Cole, 2008), but was missing from the spawning behaviours of *C. hypsilepis.* *C. hypsilepis* does display the "skimming" behaviour described by Swerdloff (1970). Skimming is also described in Russell's (1971) observations of spawning *C. dispilus.*

3.4.2 Spawning Frequency and Lunar Cycle

This study found no relationship between spawning frequency and the lunar cycle. The seven days on which spawning occurred did not correlate with the semi-

lunar cycle, or any other type of lunar cycle. According to Gladstone (2007b), *C. hypsilepis* spawned on the semi-lunar cycle, however, the intervals between spawning events varied throughout the spawning season. Other damselfish, including *Parma microlepis* and *Chromis dispilus* (Tzioumis and Kingsford, 1995), exhibited synchronized spawning that did not appear to correlate with a lunar cycle. Results reported in this study for *C. hypsilepis* are similar to those other species. Variation between the present and previous studies (Gladstone, 2007b) could reflect variation between the years, due to spawning occurring at different times throughout the spawning season. Most pomacentrids have spawning cycles which do not correlate with the lunar cycle, making the possibility of this pattern plausible for *C. hypsilepis*. This can also be accounted for the study being concentrated on the information gained in observing one spawning season.

3.4.3 Diel Spawning Frequency

The spawning frequency changed throughout the day. Gladstone (2007b) concluded that there was little change in frequency throughout the day. Gladstone (2007b) recorded spawning frequency four times per day over three days, on SCUBA, and found no significant diurnal variation. In this study however, underwater observations showed that spawning frequency increased gradually throughout the morning, but dropped off dramatically after 1230. After midday, the frequency of spawning events observed over 5 minute intervals was highly variable- ranging from as high as 48 to as low as three. Spawning frequency decreased after midday and into the evening. Although large spikes occurred at 1500 h, this was uncommon in the overall observations of the aggregation. This study has a technological advantage over previous studies with the underwater video recordings. The recordings were constant observations with minimal breaks between each hour. The previous studies have observed spawning frequencies on three days, sampling four times for 30 minute

observation periods. These observations were broken up with 2 to 3 hours break in between observations.

3.4.4 Cost of Reproduction

Spawning, guarding and nesting behaviours were undertaken by only a small proportion of fish at the aggregation site. For spawners, spawning was the primary activity, contributing to 56% of their observed behaviour. As males spent a large amount of their time (40%) guarding their nests, the amount of time allocated for feeding was minimal (4%). *C. hypsilepis* is noted for the large amount of parental care performed by the male. No noticeable feeding took place by the spawning fish. Feeding was not observed in the instantaneous spawning sampling. Therefore, a great amount of time is devoted to reproduction by nesting males. Nesting males also incur a cost of reproduction, from guarding the nests. As nest guarding was a large amount of their time, 40%, the amount of time allocated for feeding was minimal. Gladstone (2007b) quantified the cost for guarding males using feeding rate. The feeding rates for guarding males were reduced by 85% compared to those individuals without nests. The large number of spawning events throughout the day, the continual spawning through the day, the time devoted to reproduction, and Gladstone's (2007b) results on reduced feeding rate show the large cost of reproducing, for the spawning males. The cost is not great for the entire population due to the limited amount of fish spawning. Assuming the population is between 3,500 and 33,000 individuals (Gladstone, 2007b), the number of spawning individuals would be between 210 and 1,980.

3.4.5 Conclusion

The spawning behaviours of *Chromis hypsilepis* were both similar to, and different from, the spawning of other *Chromis* species. Using advanced equipment and data collecting methods, spawning occurrences were observed in more detail and were

more precisely defined. Spawning did not correlate to the lunar or semi lunar cycle. The diel spawning frequency changed throughout the day, while slowly increasing till midday, then dropping off dramatically in the afternoon and evening. Spawning represented a major investment for spawners, in terms of the amount of time devoted to spawning and egg defence.

Chapter 4. Conclusion

4.1 Overall Findings

While *Chromis hypsilepis* has some traits of its reproduction characteristic of the family Pomacentridae (e.g. gonochorism, demersal spawning, parental care of fertilized eggs), there are many unique features of its reproductive biology and spawning behaviour. Gladstone's (2007b) study described the strategy of spawning aggregations used by *C. hypsilepis*. Although all pomacentrid species are demersal spawners, few of them aggregate to spawn and most aggregating species are pelagic spawners (Claydon, 2004). *Parma microlepis* is a temperate species and a demersal spawner (Tzioumis and Kingsford, 1999), which occurs with *C. hypsilepis* on similar rocky reefs around NSW. The other unique feature (for aggregating species) displayed by *C. hypsilepis* is its continuous spawning throughout the day.

The population of *Chromis hypsilepis* at the aggregation site, according to Gladstone (2007a), is estimated to be in the tens of thousands. However, it was unclear from Gladstone's (2007b) observations whether male *C. hypsilepis* spawned in their territories with multiple females. The present study found, using novel underwater video recordings, that male *C. hypsilepi*s spawned with multiple females and that this species therefore has a polygamous mating system. This study expanded earlier calculations about the potential costs to males from reproduction (Gladstone 2007a) by finding that males do not feed while spawning, engage in a large number of spawning acts during the day, spawn continuously throughout the day, and spend more than 50% of their time in spawning-related activities. The proportion of spawning individuals at the spawning aggregation site was found to be very low, representing only 6.4% of individuals sampled. The majority of the individuals in the aggregation, not coming down to spawn, mainly swam through the spawning aggregation site. The swimming

group did not seem to be actively or aggressively trying to spawn. Many individuals, which were of a suitable size to spawn (see chapter 2), were seen swimming in the water column above the spawning males' territories. With these estimations, less than 2,000 individuals will be spawning during the spawning season, while over 27,000 individuals are gathered at the aggregation site, assuming a population of about 30,000 *C. hypsilepis* (Gladstone, 2007a).

Sexual maturity was attained at approximately the same age for males at both locations, at 2 years. The females differed in their age of sexual maturity, with Terrigal females reaching maturity around 2 years of age, while the Jervis Bay females reached maturity at around 3 years of age. The weight at which individuals attained sexual maturity (W_{50}) varied between the populations, with *C. hypsilepis* at Jervis Bay reaching W_{50} at a lower weight than that of the Terrigal population. While the average weights of males and females at Jervis Bay were not significantly different, the females and males reached maturity (W_{50}) at 21 g and 31 g respectively. *C. hypsilepis* at Terrigal reached their W_{50} at 36.8 g for males and 40.2 g for females. The length-frequency distributions of males and females were similar at Jervis Bay but differed at Terrigal. The differences between males and females in their L_{50} were 14 mm at Terrigal and 8 mm at Jervis Bay.

This study found that the sagittal otoliths of *Chromis hypsilepis* could be used for ageing and validated their use through marginal increment analysis. *C. hypsilepis* is a long-lived species. The maximum longevity of the species (22.5 years) is less than *Parma microlepis* (37 years), which is also a temperate damselfish species (Tzioumis and Kingsford, 1999). Temperate species are generally known to reach older ages than the tropical species of the same family (Tzioumis and Kingsford, 1999). The tropical species *Chromis chromis* (Dulčić and Kraljević, 1995) and *Stegastes rectifraenum* (Meekan *et al*., 2001) reached ages of half the age of the temperate species, only reaching 9 and 10 years, respectively. *S. flavilatus* is a relatively short-

lived species with longevity varying between Baja (19 years) and Panamá (4 years) (Meekan *et al*., 2001).

4.2 Future Studies

The results reported in this study on the spawning cycle of *Chromis hypsilepis* differed to previous published observations (Tzioumis and Kingsford, 1995; Gladstone, 2007b). Differences between the current and previous studies could relate to differences in sampling frequency, methods of observation, or reflect local and inter-annual variation in spawning cyclicity. Further research needs to be completed over many spawning seasons to determine the existence, or otherwise, of lunar spawning cycles. The study needs to be completed over the entire spawning season, and spawning observations need to be completed throughout the middle of the day, when spawning is at the peak frequency.

This study covered only two areas on the coast of NSW, which are relatively close together (200 km) and minimal geographic variation was observed. *C. hypsilepis* occur throughout temperate NSW. Future observations need to be made at more widely separated locations, such as northern NSW, New Zealand, and southern NSW, to confirm the minimal geographic variation in reproductive biology reported here.

This study also raised several questions around further understanding of the reproductive strategy of *C. hypsilepis*. An important question for future research is where *C. hypsilepis* come from for spawning, and where they feed at times outside of the spawning season. Understanding the area from which fish migrate is critical for developing an understanding of the geographical significance of the spawning aggregation site. This would require individuals to be tagged at the spawning aggregation site and for areas to be surveyed for tagged individuals at increasing distances from the aggregation site.

The finding that only 6.4% of individuals at the aggregation site engaged in spawning, and that most of the non-spawners were sexually mature, suggests the need for further research to understand the factors that determine which individuals engage in spawning and the frequency of spawning by individuals throughout the spawning season. This could also be done by a tagging study that followed known individuals throughout the spawning season to determine their frequency of spawning.

A common feature of polygamous mating systems, of the type described here for *C. hypsilepis*, is great variation in individual reproductive success. Future research could look at means of quantifying reproductive success, its variation among males at the aggregation site, and the factors responsible for the observed variation. The present study serves as a basis for this by its findings for males on the relationships between length and age, and weight and age.

The day-long video recordings suggested that males might aggregate in loose groups. Further research, on the groups established within the aggregation, is needed and whether these groups affect the spawning frequencies or probabilities of spawning or genetic variation.

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Appendixes

Spawning Pictures

Appendix 1 Two fish together- spawning 22 December 2008

Appendix 2 Spawning against rock

Aggregation Photographs

Appendix 3 Aggregation of *Chromis hypsilepis*

Appendix 4 continued Aggregation of *Chromis hypsilepis*

Predatorial Species Photos

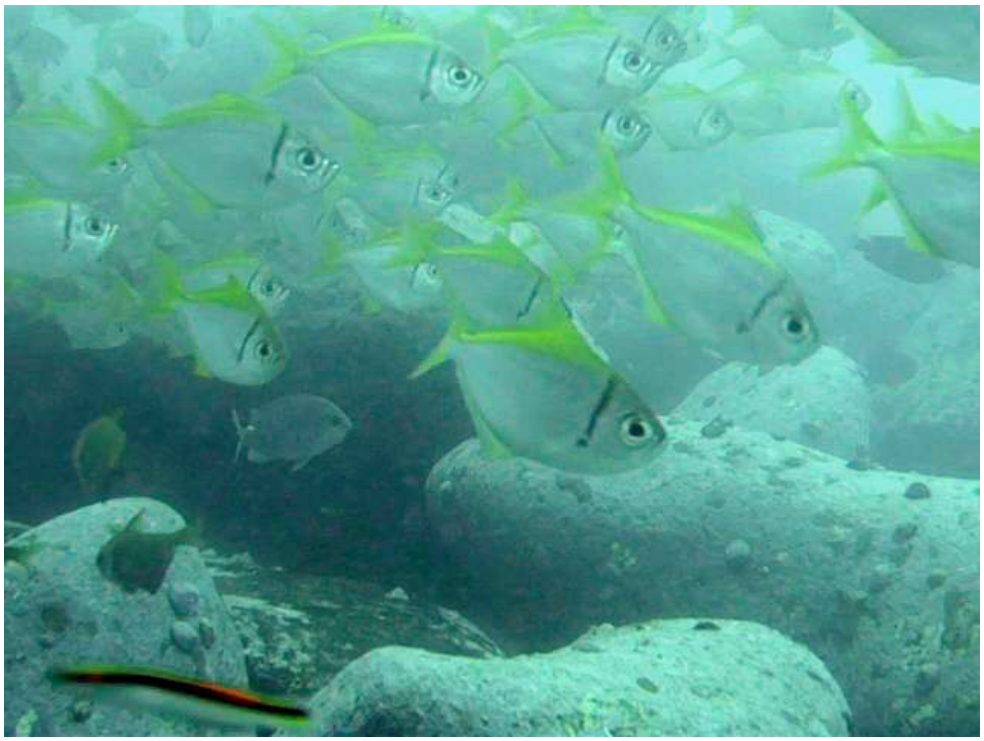
Appendix 5 Predatory on spawning ground; Red Morwong *Cheilodactylus fuscus*, and *C. hypsilepis*

Appendix 6 Blackfish, *Girella tricuspidata*, scraping the rocks for algae and other particles, including *C. hypsilepis* eggs, for consumption.

Cohabitating Species Photos

Appendix 7 Australian Mado, *Atypichthys strigatus*, around spawning sites

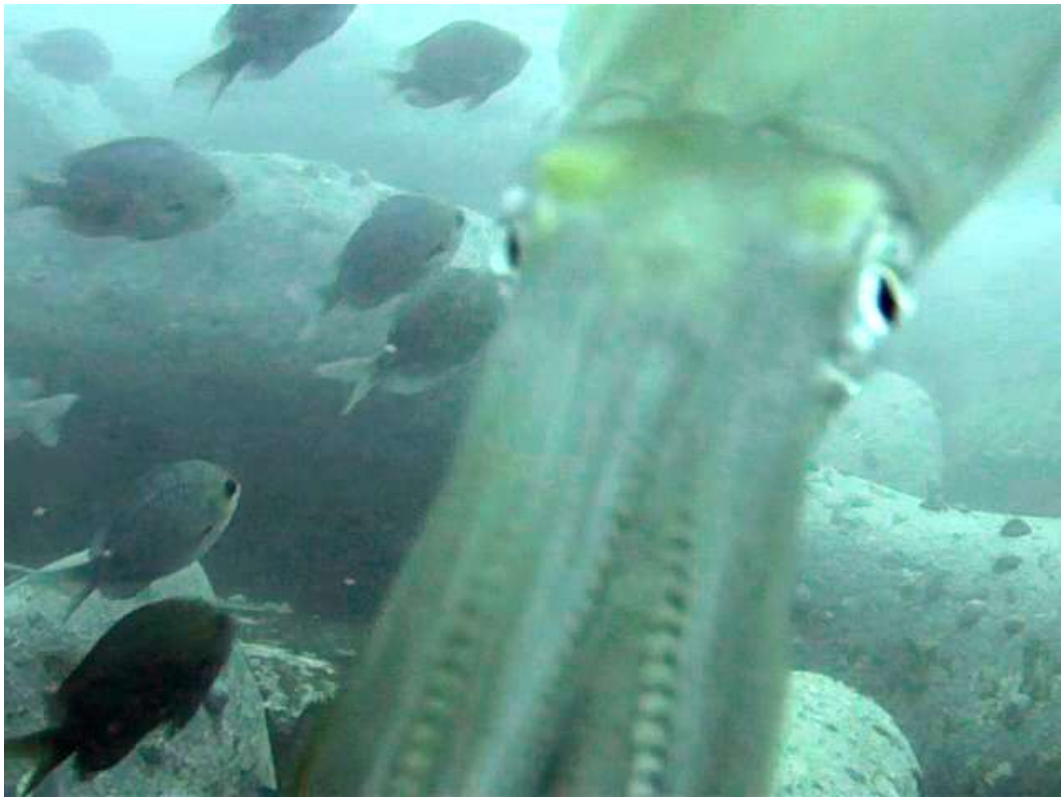
Appendix 8 Southern Hulas, *Trachinops caudimaculatus*, swimming around the aggregation site.



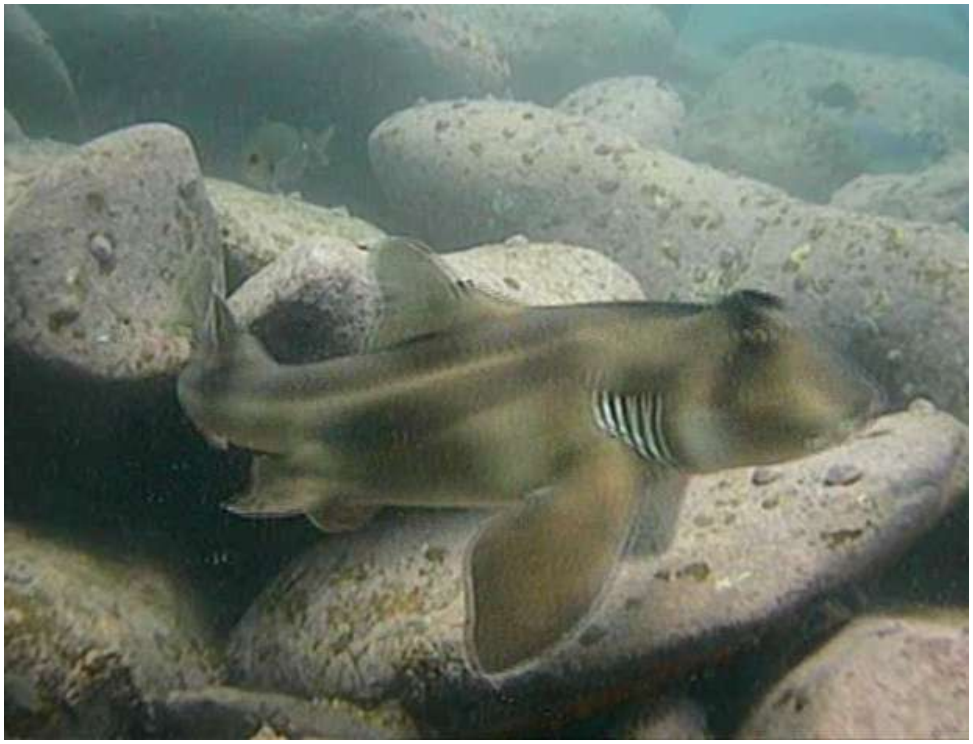
Appendix 9 Eastern Pomfred, *Schuettea scalaripinnis*

Appendix 10 Small- scale Bullseyes, *Pempheris compressa* around spawning grounds is one type of *Phempheris* species which cohabitates with *C. hypsilepis.*

Appendix 11 Old Wives, *Enoplosus armatus,* around the spawning grounds



Appendix 12 Squid examining the camera



Appendix 13 Crested Port Jackson shark, *Heterodontus galeatus swimming through aggregation*

Appendix 14 Eastern Blue Groper, *Achoerodus viridis*, in brown female stage.