School of Molecular and Life Sciences

Life-history characteristics and mortality estimates of two groupers, *Epinephelus areolatus* and *E. bilobatus*, off the north-western coast of Australia

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

The large majority of samples used in this study were collected via commercial fishing operations (i.e., 87%), or supplemented from various research surveys (i.e., 13%) directed by the Western Australian Fisheries and Marine Research Laboratories, Department of Primary Industries and Regional Development, Government of Western Australia (DPIRD). All samples were processed by DPIRD staff. In Western Australia, the Animal Welfare Act 2002 does not require DPIRD to obtain a permit to use animals for scientific purposes unless the species are outside the provisions of the Fish Resources Management Act 1994 and Fish Resources Management Regulations 1995. All sampling was undertaken in strict adherence to DPIRD Policy for the handling, use and care of marine fauna for research purposes.

Signature:

Date: 29.11.2023

Abstract

The yellowspotted rockcod, *Epinephelus areolatus*, and the frostback rockcod, *Epinephelus bilobatus*, are both relatively small epinephelids (<50 cm total length) inhabiting coastal waters in depths of up to 200 m, and 100 m, respectively. *Epinephelus areolatus* has an extremely widespread Indo-Pacific distribution, whereas *E. bilobatus* has a restricted distribution in the eastern Indian Ocean. Both species are harvested as part of the multispecies demersal commercial fisheries (i.e., the Northern Demersal Scalefish Managed Fishery, and the Pilbara Demersal Scalefish Fisheries), which operate along the broad continental shelf of north-western Australia. The estimated total annual landings derived from catch proportions are substantially higher for *E. areolatus* (113 t in 2019) than for *E. bilobatus* (5.3 t in 2021) across all years from these managed fisheries. This study is the first to investigate the age, growth, reproductive biology, and mortality of these two species, which has improved our understanding of their inherent vulnerability to overfishing in north-western Australia. The life-history characteristics and mortality estimates determined from this study are presented as separate chapters for *E. areolatus* (Chapter 2), and *E. bilobatus* (Chapter 3).

Samples of *E. areolatus* were collected between 2012 and 2018, and samples of *E. bilobatus* were collected between 2012 and 2022, from commercial catches and research surveys along the north-western Australia coast (~2000 km), comprising the Kimberley, Pilbara, and Gascoyne regions. Detailed histological examination of the gonads (*E. areolatus*, n = 1889; *E. bilobatus*, n = 1460), which covered a wide length and age range determined that both species were monandric protogynous hermaphrodites. A notable difference detected between the gonad histology was that *E. areolatus* had a much higher prevalence of non-functional spermatogenic crypts dispersed within the ovaries of mature functioning females than *E. bilobatus*. In the Pilbara region, the length and age at which 50% of the females attained sexual maturity was similar (< 10% difference) between species, i.e., 266 mm L_T and 2.7 years for *E. areolatus*, and 278 mm L_T and 2.5 years for *E. bilobatus*. Based on ovarian developmental stages from histological examination, and trends exhibited by the gonadosomatic indices of females, the spawning period for both species was protracted over 10-12 months of the year, with biannual peaks occurring around March and September. Although the gonadosomatic indices were lower for female *E. bilobatus* across the year, the length of each spawning peak was comparable between species, with the March peak occurring over 3–4 months, and the September peak limited to one month. The estimated lengths and ages at which 50% of female *E. areolatus* change sex to male in the Kimberley and Pilbara regions was 364 and 349 mm L_T, and 7.9 and 7.3 years, respectively. Similarly, 50% of female *E. bilobatus* changed sex to male at 336 and 342 mm L_T, and 6.2 and 6.5 years in the Kimberley and Pilbara regions, respectively.

The trends exhibited by the mean monthly marginal increments confirmed the annual formation of one opaque and one translucent zone growth zone in the sagittal otoliths of both species, with the opaque zone deposited during the cooler months and becoming delineated in September and October for both species. Thus, counting the growth zones from the sectioned otoliths of individuals of each species, in conjunction with a relative birth date, the time of year the growth zones are delineated, and a date of capture determined that both *E. areolatus* and *E. bilobatus* had a moderate longevity of approximately 20 years. Variations in growth were observed between each region in north-western Australia, which was not consistent between species. The growth of *E. areolatus* was not correlated with latitude. Instead, a parabolic relationship was evident, where the smallest mean length-at-age and fastest growth rates (k) occurred in the mid latitudes of the Pilbara. In comparison, *E. bilobatus* had no significant difference in growth rates (k) between the two warmer northern regions, but in the cooler and most southern region of its distribution this species attained a significantly larger mean length-at-age. Three values of natural mortality (M) were used to assess the level of fishing mortality (F) in the Kimberley and Pilbara commercial trap fisheries. For both *E. areolatus* and *E. bilobatus* all point estimates of fishing mortality (F) were less than M indicating at the time of sampling that the harvest of these species was sustainable. The life-history characteristics determined from this study infer a relatively high population productivity for these two groupers, and the estimates of fishing mortality suggest these species, that comprise a large proportion of the epinephelid catch from the multi-species tropical fisheries in north-western Australia, are currently being harvested sustainably under current management arrangements.

Acknowledgement of Country

We acknowledge that Curtin University works across hundreds of traditional lands and custodial groups in Australia, and with First Nations people around the globe. We wish to pay our deepest respects to their ancestors and members of their communities, past, present, and to their emerging leaders. Our passion and commitment to work with all Australians and peoples from across the world, including our First Nations peoples are at the core of the work we do, reflective of our institutions' values and commitment to our role as leaders in the Reconciliation space in Australia.

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Chapter 1: General Introduction

1.1 Groupers (Epinephelidae)

Groupers are a group of marine fish inhabiting tropical and subtropical reefs around the world (Craig et al., 2011). As adults they can attain maximum sizes from < 30 cm to > 2.5 m in length (Harmelin-Vivien and Harmelin, 2022). All groupers go through a bipartite life cycle, progressing from a pelagic larvae phase into a mostly sedentary benthic juvenile and adult phase (Félix-Hackradt, 2022). Being carnivorous, groupers feed on other fish, cephalopods and benthic invertebrates (Randall, 1987; Harmelin-Vivien and Harmelin, 2022) and have the capacity to shape reef assemblages through their typical top-down control of prey abundance and influence on abundances of lower trophic levels (Sadovy de Mitcheson and Liu, 2022).

Traditionally, groupers have been treated as the subfamily Epinephelinae within the family Serranidae (Craig et al., 2011). Genetic studies have suggested that the family Serranidae is polyphyletic, i.e., derived from more than one ancestral group (Smith and Craig, 2007; Craig et al., 2011). A molecular study by Smith and Craig (2007) has led to the subfamily Epinephelinae now being recognised as the monophyletic family Epinephelidae. The family consists of 16 genera and 163 species, with the genus *Epinephelu*s being the most speciose comprising 86 species (Craig et al., 2011).

Groupers are a highly valued and ecologically significant group, facing a range of threats worldwide. Intrinsic life-history traits, such as late maturation, extended longevity, formation of spawning aggregations (Sadovy de Mitcheson et al., 2013), slow growth rates (Morris et al., 2000), and exhibiting a variety of sexual strategies can make some grouper species more vulnerable to overfishing. Habitat destruction, fragmentation, and degradation, caused through anthropogenic influences such as, but not limited to, pollution, anchorages, coastal development, and aquaculture threaten the coastal and estuarine environments groupers commonly use as nursery grounds (Félix-Hackradt, 2022). Global warming is affecting coral reefs, with bleaching events becoming more frequent, further fragmenting healthy reef environments (Félix-Hackradt, 2022). Increasing ocean temperatures are also affecting larval durations and dispersal potential, as well as altering ocean circulation and stratification, potentially changing the recruitment patterns and gene flows of a species (Félix-Hackradt, 2022).

1.2 Reproductive biology of *Epinephelus* species

Groupers exhibit a range of sexual patterns, which can vary within genera. Gonochorism, hermaphroditism, and bi-directional sexual modes have all been confirmed within the genus *Epinephelus* (Sadovy de Mitcheson and Liu, 2008). Gonochoristic species remain and reproduce as either females or males throughout their life history (Sadovy de Mitcheson and Liu, 2008). *Epinephelus striatus* is the only known gonochoristic species from this genera, however this species may have a diandric development, but the overfishing of larger males may have removed any secondarily derived males (Sadovy and Colin, 1995; Sadovy de Mitcheson and Liu, 2008). Functional hermaphrodites have a proportion of individuals which will function as a female and a male over the course of their life (Sadovy de Mitcheson and Liu, 2008). Simultaneous hermaphroditic individuals reproduce as both sexes at the same time, or within a very short time frame, whereas sequential hermaphrodites change from one sex to the other (Sadovy de Mitcheson and Liu, 2008). Sequential hermaphrodites can be protandrous where they change from a functional male to a functional female, or protogynous where they change from a functional female to a functional male (Sadovy de Mitcheson and Liu, 2008). There are no reported cases of simultaneous hermaphrodites or protandry in the genus *Epinephelus* (Sadovy de Mitcheson and Liu, 2008).

Protogynous hermaphrodites have two developmental pathways: (i) monandric where all males are derived exclusively from functional females, i.e., all males are secondary; and (ii) diandric where there are two types of males, males that develop directly from the juvenile phase and males that develop from functional females (Sadovy and Shapiro, 1987; Sadovy de Mitcheson and Liu, 2008). Protogynous hermaphroditism with a monandric development is the predominant sexual pattern in the *Epinephelus* genera with confirmed cases in *E. marginatus* (Marino et al., 2001; Fennessy, 2006), *E. rivulatus* (Mackie, 2006), and *E. fuscoguttatus* (Pears et al., 2006). However, diandric development has also been confirmed within the genus, e.g., *E. andersoni* (Fennessy and Sadovy, 2002), and *E. coioides* (Grandcourt et al., 2009; Liu and Sadovy de Mitcheson, 2011). Bi-directional sex change is a form of sequential hermaphroditism where a species changes sex in both directions (Sadovy de Mitcheson and Liu, 2008). In the genus *Epinephelus*, this has only been recorded in *E. akaara* under captive rearing experiments, otherwise this species displays protogyny (Okumura, 2001).

Determining the sexual pattern in groupers is difficult and requires particular attention to histology throughout all life stages (Chan and Sadovy, 2002; Sadovy de Mitcheson and Liu, 2008). Conclusive evidence for protogynous hermaphroditism requires the detection of gonads in transition, i.e., containing degenerating vitellogenic ovarian tissue and proliferating testicular tissue (Sadovy de Mitcheson and Liu, 2008). Additional histological evidence is the presence of an ovarian lumen in the testes, atretic oocytes within testes, and peripheral sperm sinuses in the gonad wall (Sadovy and Shapiro, 1987). Bimodal length/age frequency distributions, displaying larger/older males and female biased sex ratios in the population structure, further support the conclusion of protogynous hermaphroditism. However, this is not a diagnostic criteria alone as it can be caused by other factors, e.g., growth rates, mortality rates, and spatial segregation (Sadovy and Shapiro, 1987).

1.3 Age-based life-history characteristics

Age data is a fundamental biological variable, it can be used to calculate longevity, growth, mortality, and productivity (Campana, 2001). Demographic parameters derived from age-based studies are capable of contributing to population dynamic models that estimate the impacts of fishing and evaluate the status of a fisheries resource. Age estimates are obtained by counting growth zones that are formed on the hard parts of a fish (e.g., scales, otoliths, vertebrae, fin rays), with the underlying premise that the periodic growth increments will be exhibited on a daily or annual basis within these structures (Manooch, 1987; Campana, 2001). Sagittal otoliths are most commonly used because they continue to accrete calcareous material throughout the fishes life irrespective of a marked reduction in somatic growth following maturation (Choat and Robertson, 2002). In relatively long-lived teleosts, it is frequently necessary to section the otolith transversely in order to distinguish the growth increments. Transverse sections of sagittal otoliths have been used to determine that Epinephelidae species can be relatively long lived (e.g., 72 years for the deep-water eightbar grouper *Hyporthodus octofasciatus*, DiBattista et al., 2018).

Fundamental to estimating the fishes age, is validating that the growth increments (annuli) are formed annually (Campana, 2001). Several methods exist to validate the annual deposition of growth zones in otoliths, with marginal increment analysis (MIA) the most common (Campana, 2001). The marginal increment is the distance from the most recently delineated opaque zone to the outer edge of the otolith, expressed as a proportion of the distance between the outer margins of the two most recently delineated opaque zones. Plotting the mean monthly marginal increment values will typically display a sinusoidal cycle during the year, indicating that the growth increment is deposited annually (Campana, 2001). To date, all age-based demographic studies of epinephelids, that have validated the growth increments in otoliths, have determined they are formed annually. MIA has demonstrated that the growth increments are formed annually in various species from the genus *Epinephelus* e.g*., E. marginatus* (Reñones et al., 2007), *E. niveatus* (Costa et al., 2012), *E. morio* (Renán et al., 2022), among others.

1.4 Demersal scalefish resources in tropical north-western Australia

In tropical north-western Australia the retained demersal scalefish resource consists of more than 60 teleost species, but the catches are dominated by the highly valuable families such as snappers (Lutjanidae), emperors (Lethrinidae) and cods (Epinephelidae) (DPIRD, 2023). The harvest of the demersal scalefish resource in this region is shared between the commercial, recreational, charter and indigenous sectors. Parts of the north coast region are extremely remote, hence recreational fishing effort is predominantly concentrated around populated areas and access points (e.g., boat ramps), and is mostly line-based from private boats or charter vessels (Wakefield et al., 2023). The reported total catch of demersal scalefish in the north coast region of Western Australia is notably lower from the recreational sector (41 - 63 t in 2020/21, see Ryan et al., 2022) than from the commercial sector during the same period (4258 t, see Wakefield et al., 2023).

The commercial multispecies demersal scalefish fisheries that operate in north-western Australia are: (i) the Northern Demersal Scalefish Managed Fishery (NDSF) in the Kimberley region, which is essentially a trap based fishery; and (ii) the Pilbara Demersal Scalefish Fisheries (PDSF) in the Pilbara region, permitting fish trawl, fish trap, and line gear types (Wakefield et al., 2023). The Pilbara fish trawl fishery operates between 50 and 100 m depth, the Pilbara trap fishery operates between 30 and 200m, the Pilbara line fishery extends into waters deeper than 200 m to the Exclusive Economic Zone (EEZ, 200 nautical mile limit), and Zone B of the Kimberley NDSF extends to ~200 m depth (Figure 1.1). Each fishery operates within designated boundaries under specific management arrangements, with input controls in the form of gear restrictions and effort allocations (Wakefield et al., 2023). In WA, fisheries resources are monitored and assessed by identifying specific 'indicator' species from the suite of retained species derived from a matrix of risk-based attributes (i.e., inherent vulnerability, risk to sustainability, and management importance see Newman et al., 2018a). These indicator species are monitored regularly, and their status is assumed to represent the status of the suite of species and therefore the resource (Newman et al., 2018a). The indicator species in the NDSF are goldband snapper *Pristipomoides multidens* and red emperor *Lutjanus sebae,* whereas in the PDSF, the indicator species are red emperor, rankin cod *Epinephelus rankini*, and bluespotted emperor *Lethrinus punctualtus* (Wakefield et al., 2023).

During 2021, seven vessels operated within the NDSF, landing 1,544 t of demersal teleosts and in the PDSF, there were two fulltime vessels operating in the trap fishery, two fulltime vessels operating in the trawl fishery and seven vessels (operating for 5 months each) operating in the line fishery, landing 662 t, 1928 t and 124 t of demersal teleosts respectively for each fishery (Wakefield et al., 2023). Within these multispecies fisheries, the cods and groupers (Epinephelidae other than *E. rankini*) comprise at least 10 species, and catches are typically reported together as a 'mixed cod' category in the compulsory catch and effort logbook returns provided by commercial fishers. Consequently, catches at a species level are derived from catch proportions recorded during fishery-independent surveys (Department of Primary Industries and Regional Development, WA, unpublished data). The stock status of the demersal scalefish resource in the northern bioregion, which, prior to this study, has been inferred from the stock status of the indicator species from the regions, which includes one species of the Epinephelidae in the Pilbara, was at an acceptable level during the 2021 fishing season in both the NDSF and PDSF (Wakefield et al., 2023). Past annual assessments of the NDSF resource have considered the stock level and fishing effort to be acceptable (see annual

Status reports of the fisheries and aquatic resources of Western Australia ranging between 2007 to 2021). In the PDSF, declining indicator species catch rates in addition with declining total catch rates between 2003 and 2008, in conjunction with red emperor (an indicator species of the region) exceeding reference levels for estimates of fishing mortality in 2007, triggered a voluntary reduction in trawl fishing effort in 2008, which was later legislated in 2009 (Newman et al., 2012b). Thus, since 2011/12 the stock level and fishing effort in the PDSF resource have been considered to be acceptable (see annual Status reports of the fisheries and aquatic resources of Western Australia ranging between 2007 to 2021).

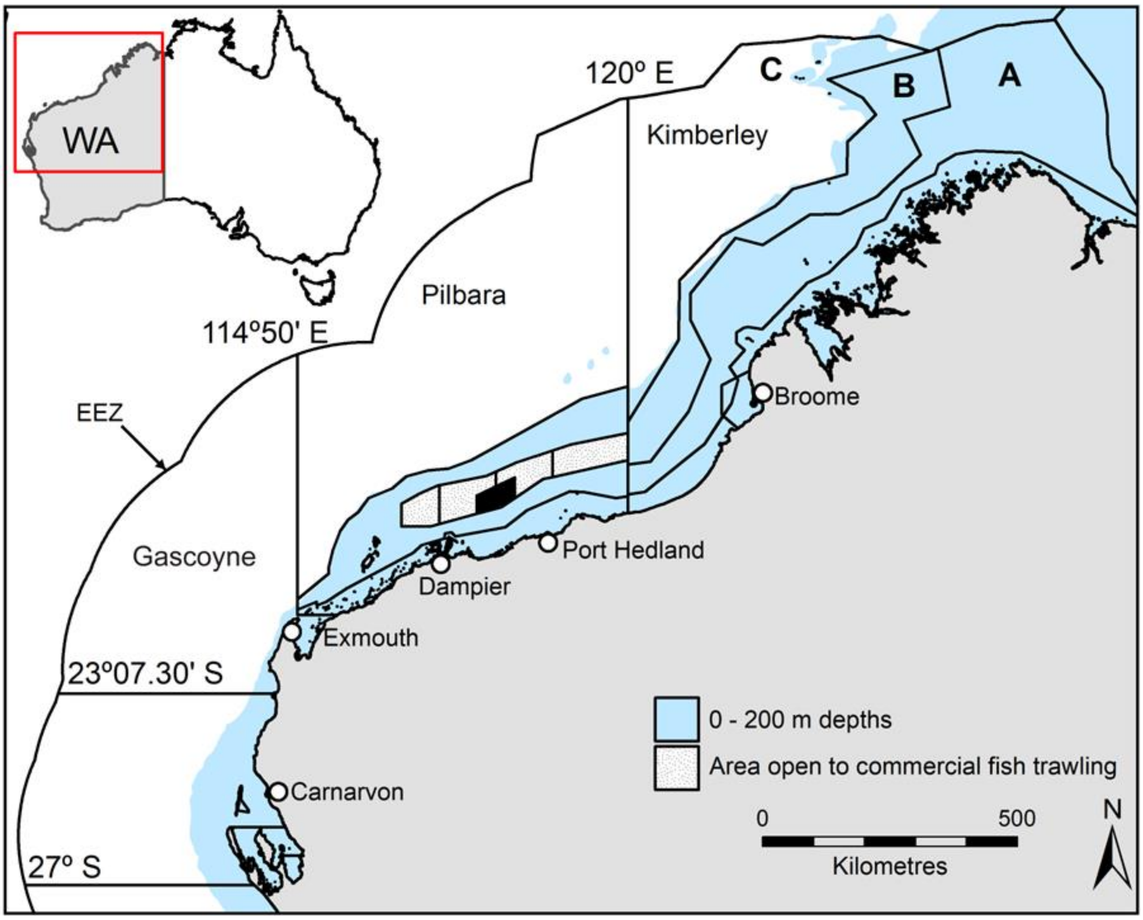

Figure 1.1 Management boundaries for the Northern Demersal Scalefish Fishery (NDSF) that operates in the Kimberley region, and the Pilbara Demersal Scalefish Fisheries (PDSF) in the Pilbara region on the north-west coast of Western Australia (WA, inset). In the Kimberley, zones A, B and C of the NDSF permit commercial trap fishing. In the Pilbara, areas open to commercial fish trawling (grey shade) are nested within the wider commercial trap fishery area (blue and grey shades), and areas closed to commercial fishing (black shade).

1.5 Aims and objectives

This is the first study investigating the age, growth and reproductive life-history of *Epinephelus areolatus* and *E. bilobatus*. For each species the specific objectives were to: (i) test the hypothesis that *E. areolatus* and *E. bilobatus* are protogynous hermaphrodites, and if that is proven correct determine whether each exhibits monandric or diandric development; (ii) estimate the length and age of sexual maturity, and sex change if applicable; (iii) determine the time of year and duration of the spawning period; (iv) validate that the growth zones in the sagittal otoliths are formed annually, and determine the time within the year that the growth zones become delineated using marginal increment analysis; (v) compare the von Bertalanffy growth curve parameters between the Kimberley, Pilbara, and Gascoyne bioregions in north-western Australia; and (vi) assess the level of fishing pressure within the Kimberley and Pilbara commercial trap fisheries by comparing estimates of fishing mortality (*F*) relative to those for natural mortality (*M*). Population productivity can be inferred, when the life-history traits determined for each species are considered holistically (i.e., a collection of favourable traits would deem a species productive). Knowledge of the life-history characteristics, inferred population productivity, and mortality estimates determined for these two species, which comprise a large proportion of the epinephelid catch from the multi-species tropical fisheries in north-western Australia, will facilitate an appraisal of existing management arrangements and aid managers when developing future management strategies for the sustainable harvest of *E. areolatus* and *E. bilobatus.*

1.6 Thesis structure

This thesis is organised into four chapters. Chapter 1 is a general introduction, and Chapter 4 is a general discussion bringing the study together. Chapters 2 and 3 are each data chapters focused on an individual species, investigating their age, growth and reproductive characteristics from which to infer their inherent vulnerability, and to determine estimates of fishing mortality relative to those of natural mortality from representative age compositions to provide an improved understanding of the sustainability of harvest levels under current management arrangements. A conceptual flow diagram (Figure 1.2) outlines the background and rationale, specific aims, and structure of this thesis. Both data chapters have been published as stand-alone papers in peer reviewed journals, subsequently there is some repetition of text and duplication of figures throughout the thesis.

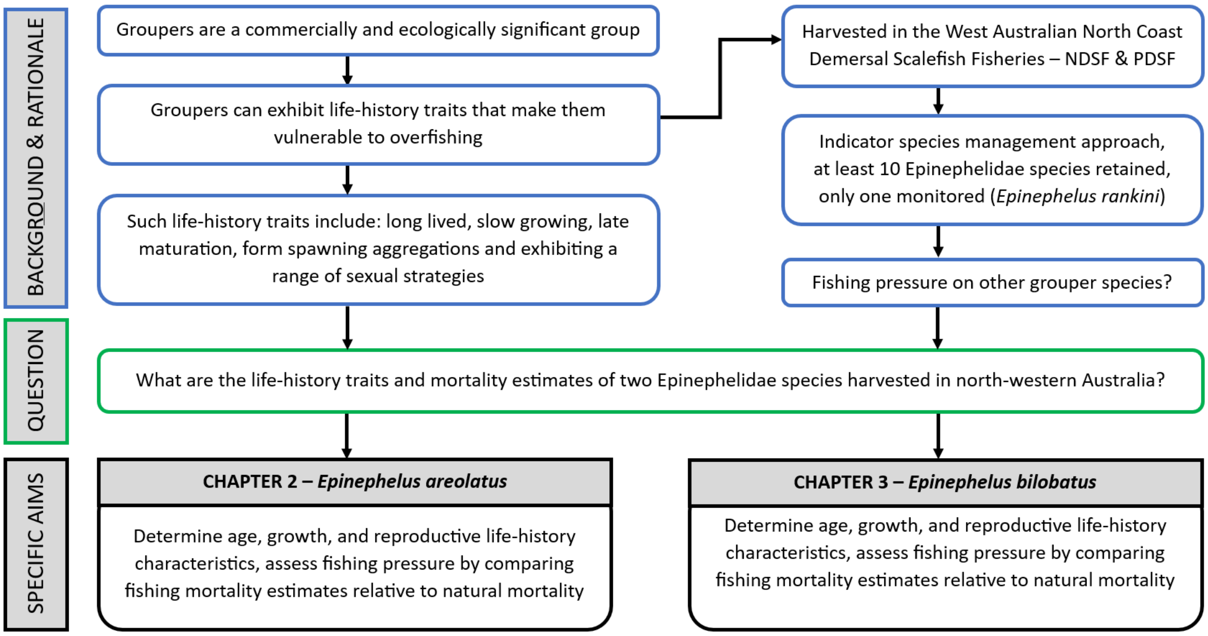

Figure 1.2 Conceptual flow diagram outlining the background and rationale, specific aims, and structure of thesis.

Chapter 2: Age, growth and reproductive life-history characteristics infer a high population productivity for the sustainably fished protogynous hermaphroditic yellowspotted rockcod (*Epinephelus areolatus*) in north-western Australia

2.1 Abstract

The yellowspotted rockcod, *Epinephelus areolatus*, is a small-sized grouper that is widely distributed throughout the Indo-Pacific, where it forms a valuable component of the harvest derived from multi-species fisheries along continental shelf margins. Samples of *Epinephelus areolatus* were collected from 2012 to 2018 from commercial catches and research surveys in the Kimberley, Pilbara and Gascoyne regions of north-western Australia to improve our understanding of the life history, inherent vulnerability and stock status of this species. Histological analysis of gonads (n = 1,889) determined that *E. areolatus* was a monandric protogynous hermaphrodite. Non-functional spermatogenic crypts were dispersed within the ovaries of 23% of mature functioning females, however these crypts were not observed during the immature female phase. The length and age at which 50% of females matured was 266 mm total length (L_T) and 2.7 years. The spawning period was protracted over 10-12 months of the year with biannual peaks at the start of spring and autumn (i.e., September and March) when the photoperiod was at its mid-range (i.e., 12.1 hours). Estimates of the lengths and ages at which 50% of *E. areolatus* change sex from female to male were very similar (i.e., < 5% difference) between the Kimberley and Pilbara regions, i.e., L_{50}^{sc} of 364 and 349 mm L_T and A_{50}^{sc} of 7.9 and 7.3 years, respectively. A maximum age of 19 years was observed in all three regions, but there was significant regional variation in growth. These variations in growth were not correlated with latitude, instead a parabolic relationship was evident, where the smallest mean length-at-age and fastest growth rates (k) occurred in the mid-latitudes of the Pilbara region. In the Kimberley and Pilbara regions, individuals were not fully selected by commercial fish traps until 5-6 years of age, hence, several years after reaching maturity. It is considered that these life-history characteristics infer a high population productivity, which underpins the sustainable harvest of this species, despite comprising the largest catches of all epinephelids in the multispecies tropical fisheries across north-western Australia.

Keywords: Epinephelidae, extended spawning period, maturity, otolith sections, reproduction, sex change

2.2 Introduction

Species belonging to the Epinephelidae represent a large portion of the catches in tropical and subtropical multi-species fisheries (Sadovy de Mitcheson et al., 2013). Due to resource limitations, the majority of multi-species fisheries are typically managed using single-species assessments of only a few indicator species within the targeted suite (Newman et al., 2018a). Hence, the life-history characteristics and status of many species within this targeted suite are often unknown. Life-history traits including maximum sizes, lengths and ages at maturity, growth rates, longevity, and reproductive strategies determine the inherent vulnerability of a species to exploitation (Coleman et al., 2000) and are critical inputs to population modelling. It is widely acknowledged that many larger grouper species possess life-history traits which make them highly susceptible to over exploitation. For example, the relatively long lifespan, slow growth rate, late maturity, protogynous sex change and aggregating spawning behaviour infers *Epinephelus fuscoguttatus* are highly susceptible to overfishing (Pears et al., 2006). Nonetheless, the sustainable management of tropical fisheries can be achieved under good governance regimes (e.g., limited entry, catch and effort controls, vessel monitoring systems, size limits, spatial closures) irrespective of the productivity of a single species within a multi-species complex. In Western Australia (WA), this is achieved using an indicator species approach (Newman et al., 2018a). This allows some species to be underexploited, as management is focused on the less productive more vulnerable species. Under this regime, species that are highly productive relative to the indicator species could sustain long periods of high catches (harvest).

The Epinephelidae consist of 16 genera and 163 species, with the *Epinephelus* genus being the most speciose, accounting for about half of these species (86 known species; Craig et al., 2011). Distributed worldwide, though primarily occurring in the tropics and sub-tropics, they are an economically valuable family supporting a number of large fisheries. Protogynous hermaphroditism is considered their most prevalent mode of reproduction (Sadovy de Mitcheson and Liu, 2008), consequently fisheries which are size selective can potentially reduce the reproductive success of a protogynous hermaphrodite species by removing larger individuals, thus altering the sex ratio toward less males (Vincent and Sadovy, 1998; Alonzo and Mangel, 2004). Owing to the species diversity within the Epinephelidae, it is expected that there would also be a diverse range of life-history traits and thus vulnerabilities to exploitation. Although the plasticity of life-history characteristics is apparent at the family level, variations in reproductive parameters and localised complexities also occur at the species level (Choat, 2012). Thus, at a species level, it is important to understand the age, growth and the reproductive aspects of their life-history strategies at a regional context, to be able to evaluate vulnerability and better inform assessments of stock status.

The yellowspotted rockcod or areolate grouper, *Epinephelus areolatus* (Forsskål, 1775), is a small epinephelid (< 50 cm total length) that has a widespread distribution throughout the Indo-Pacific region, ranging from South Africa northwards to the Red Sea and Persian Gulf, and extending east to Japan and then southwards to Fiji and Tonga, encompassing the Arafura Sea and northern Australia (Craig et al., 2011). However, it is absent from Micronesia, Polynesia and the islands of the western Indian Ocean (Craig et al., 2011). In WA, *E. areolatus* is found at least as far south as Carnarvon on the upper west coast (27°S), northwards to the border with the Northern Territory (129°E, Figure 2.1). This species is ubiquitous throughout its distribution occupying a range of habitats including rocky outcrops, low-relief limestone reefs, coral rubble, soft coral, seagrass beds and soft sediment habitats in depths of up to 200 m across the continental shelf (Craig et al., 2011).

The coast of north-western Australia, where *E. areolatus* is harvested, has a long history of fishing. Foreign trawl fishing fleets, particularly from Taiwan, heavily exploited the Pilbara region up until the establishment of management areas in 1987 (Wakefield et al., 2017c). At present, there are two commercial demersal scalefish fisheries operating along the north-western coast of WA. The Northern Demersal Scalefish Managed Fishery (NDSF) in the Kimberley region permitting trap and line fishing and the Pilbara Demersal Scalefish Fisheries (PDSF) in the Pilbara region permitting fish trawl, trap and line fishing (Newman et al., 2018b). Within these tropical multispecies demersal fisheries, the reported catches of small cods and groupers (Epinephelidae) are typically combined, comprising at least ten species. However, fishery-independent surveys demonstrated that *E. areolatus* represents about 73% of the small grouper catch in the Kimberley, and 93% in the Pilbara trap fisheries (proportions by numbers of fish, Department of Primary Industries and Regional Development WA, unpublished data). Based on proportions by weight, derived from length-weight relationships, the estimated annual catch of *E. areolatus* in 2019 was ~64 t and ~49 t, in the Kimberley and Pilbara fisheries, respectively.

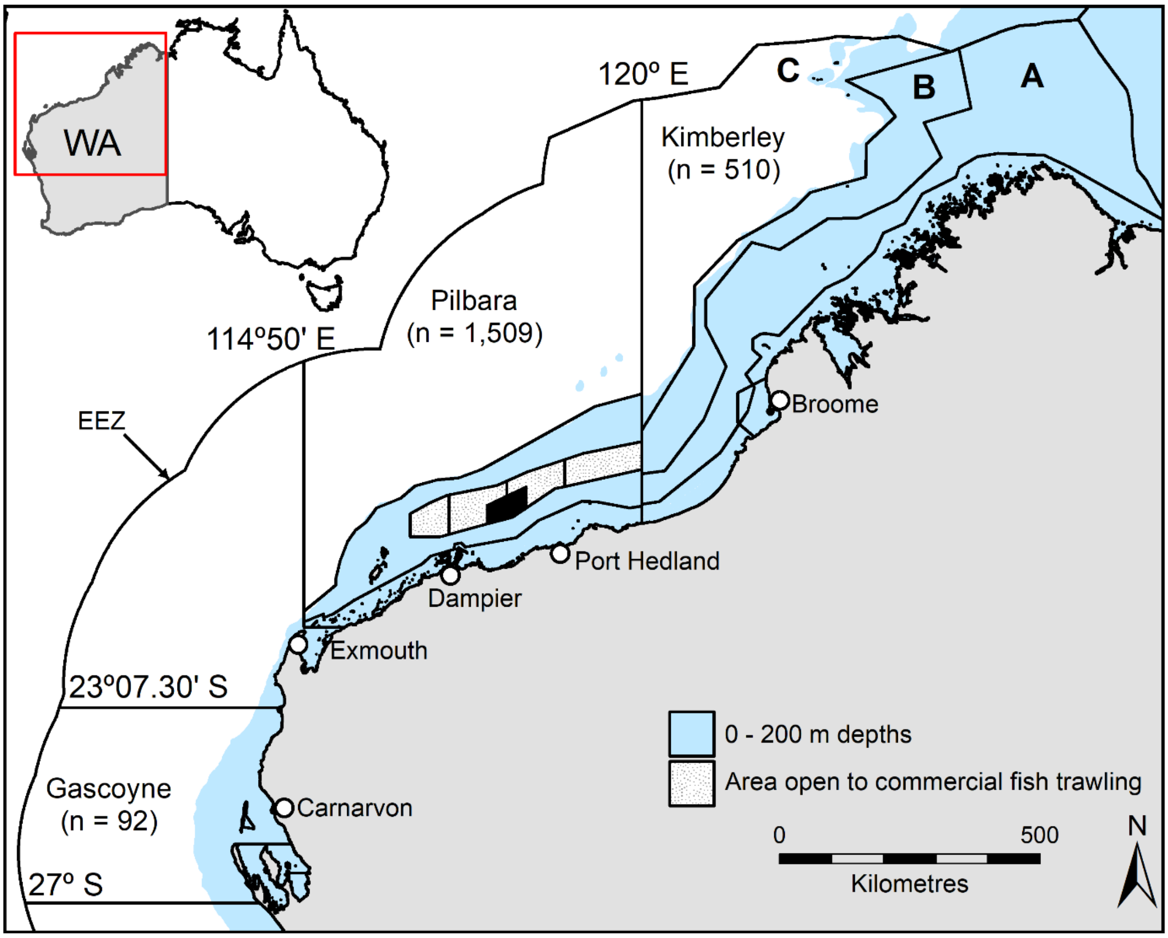

Figure 2.1 Management boundaries for the demersal scalefish fisheries on the northwest coast of Western Australia (WA, inset), sample sizes (*n*) of *Epinephelus areolatus* are shown for each region. In the Kimberley, zones A, B and C of the Northern Demersal Scalefish Managed Fishery (NDSF) permit commercial trap fishing. In the Pilbara, areas open to commercial fish trawling (grey shade) are nested within the commercial trap fishery boundary (blue and grey shade), and areas closed to commercial fishing (black shade). In the Gascoyne, areas open to commercial line fishing extend to the exclusive economic zone (EEZ, 200 nm from the coastline). The typical depth range for this species is 2 to 200 m (blue shade).

Despite a reasonable level of exploitation for this species in north-western Australia, information on their life history is scant. As part of an investigation into the longevity of multiple fish species in New Caledonia, a maximum age of 15 years for *E. areolatus*, was derived using transverse sections of otoliths (Loubens, 1980b); compared to 25 years in northern Oman (Nair and To, 2018). Scale reading has been used to estimate ages in an assessment of inshore commercial species in the Red Sea (Mahmoud et al., 2009); and Darar (1994) used length frequency analysis to derive estimates of von Bertalanffy growth parameters. Aspects of reproduction such as spawning period, maturity, fecundity and sex ratio have been reported in studies by Mahmoud (2009), Ahmad et al. (2011), Abdul Kadir et al. (2016) and Osman et al. (2018) in the Red Sea, Arabian Gulf and Malaysia. Differences in age and reproductive parameters between these studies may be attributable to regional variations, but could also be attributable to the methods and techniques employed to formulate results. For example, the calculation of the gonadosomatic index is likely to be considerably different between studies if immature individuals are not removed. Further, for a species that may display hermaphroditism, as is typical of epinephelids, gonadal histology over a wide range of length and age classes needs to be examined to confirm sexual ontogeny (Sadovy de Mitcheson and Liu, 2008). This would also improve the biological parameter estimates required for assessment purposes.

This is the first investigation of *E. areolatus* in Australia and the first to encompass all aspects of their life history in a single study. The specific objectives were to, (1) confirm that *E. areolatus* is a protogynous hermaphrodite, and to determine whether it is monandric or diandric; (2) determine the seasonality of the annual spawning period; (3) estimate the length and age at sexual maturity and sex change; (4) determine the extent to which the length and age compositions and growth vary between regions in north-western Australia; and (5) compare estimates of fishing mortality between the two managed trap fisheries operating in the Kimberley and Pilbara regions of north-western Australia. The life-history characteristics and mortality estimates determined from this study will contribute toward an improved understanding of the inherent vulnerability and sustainable exploitation of *E. areolatus*.

2.3 Material and methods

2.3.1 Sampling and somatic measurements

Samples of *E. areolatus* were collected from commercial catches and during research surveys in the Kimberley, Pilbara and Gascoyne regions of north-western Australia between 2012 and 2018 (Figure 2.1). Specimens were sampled from commercial catches taken by the NDSF (Kimberley trap; *n* = 70), the Pilbara Trap Fishery (*n* = 839), and the Gascoyne Line Fishery (*n* = 92); and from a fishery independent monitoring survey aboard a commercial trap fishing vessel in zone B of the NDSF during 2012 (*n* = 439). Additional specimens were collected from the Pilbara Fish Trawl Fishery (*n* = 549), and from various research surveys conducted in WA (n = 122) to improve sample sizes of smaller and larger individuals for age, growth and reproductive parameter estimates. Regular monthly samples were only taken from the Pilbara region to determine reproductive parameters. For each specimen, the total length (L_T) was measured to the nearest 1 mm, both sagittal otoliths were removed, cleaned and stored dry, and when possible, the whole wet weight (W_W) was measured to the nearest 0.1 g. An allometric length (L_T) versus weight (W_W) relationship for females and males combined was derived by fitting the power function, $W_W = a\ L_T\ b$, to the log transformed data using least squares regression. Log-transformation was determined to be appropriate based on examination of the residuals, parameters were back-transformed with correction for bias to the above form (Quinn and Deriso, 1999). In Western Australia, the Animal Welfare Act 2002 does not require the Department of Primary Industries and Regional Development (DPIRD) to obtain a permit to use animals for scientific purposes unless the species are outside the provisions of the Fish Resources Management Act 1994 and Fish Resources Management Regulations 1995. Nonetheless, all sampling was undertaken in strict adherence to the DPIRD Policy for the handling, use and care of marine fauna for research purposes.

2.3.2 Gonadal development and spawning period

The gonads of each fish were removed and weighed (W_G) to the nearest 0.01 g and a portion of the largest lobe was preserved in pH neutral 10% buffered formalin for histological examination (n = 1,889 analysed). Medial transverse sections of the preserved gonads were embedded in paraffin wax, sectioned at 5 µm, mounted on slides and stained in haematoxylin and eosin. Gonad histology was used to assign sex and reproductive development stage of all individuals, because determining a sexual pattern macroscopically in fish from species that exhibit hermaphroditism is problematic (Sadovy and Shapiro, 1987; Sadovy de Mitcheson and Liu, 2008).

The histological criteria used to determine gonad development stages were modified from classification schemes used for *Epinephelus rivulatus* and *Cephalopholis boenak* (Mackie, 2000; Chan and Sadovy, 2002; Table 2.1, Figure 2.2). The developmental stage of each gonad was classified according to the most advanced gametes present and their relative prevalence within the gonad (Chan and Sadovy, 2002; Erisman et al., 2010). The diagnosis of sexual pattern followed the criteria outlined for functional hermaphrodites by Sadovy and Shapiro

(1987) and Sadovy de Mitcheson and Liu (2008). The presence of spermatogenic crypts in a proportion of the female ovaries made determining the sexual function and developmental stage more challenging. The difficulty was distinguishing the gonads of functional females with crypts of spermatogenic tissue from gonads undergoing sexual transition (Chan and Sadovy, 2002; Liu and Sadovy, 2004). If a specimen contained a greater proportion of female tissue than male tissue and could not be classified as either transitional or bisexual, it was considered to be a functioning female with crypts of spermatogenic tissue. A proportion of males contained previtellogenic oocytes, however they could easily be distinguished from females by the dominance of spermatogenic crypts or disrupted tissue with empty or collapsed crypts.

Gonadosomatic indices (GSIs) were calculated for females from the Pilbara region with a L_T equal to or greater than the length at which 50% of females were mature ($L_T \geq L_{50}^{\mathrm{mat}}$) using the equation:

$$\mathrm{GSI} = 100\ (W_G\,/\,W_W).$$

Data for the GSIs and gonad developmental stages of females were pooled by month over the sampling period. The timing and duration of the spawning period was estimated based on annual trends in the mean monthly GSIs of females and the monthly prevalence (%) of ovaries that were either mature developed or in spawning condition (i.e., stages IV and V). The mean monthly sea surface temperatures (SST) were calculated over a 15-year period between 1999 and 2013 for the offshore waters of the Pilbara region of north-western Australia (~19.5°S, 118.5°E) and were obtained from the Reynolds SST database (Reynolds et al., 2007). The mean monthly daylight hours were calculated for Port Hedland (~20.3°S, 118.6°E, during 2013), located centrally in the Pilbara region, and were obtained from Timebie (www.timebie.com).

2.3.3 Estimates of sexual maturity and sex change

Estimates of the lengths and ages at which female *E. areolatus* from the Pilbara region attained sexual maturity were determined using data from the peak spawning months, i.e., January, February, March and September, based on mean monthly GSIs > 1.25 and > 60% of females containing mature or spawning ovaries (i.e., stages IV and V). The lengths at which

50% of females reached sexual maturity (L_{50}^{mat}) were estimated using a logistic regression to determine the relationship with length of the probability that a female during the spawning period possessed developing, developed, spawning or spent gonads (i.e., stages III - VI). It was therefore assumed that during the peak spawning periods, females with gonads of these stages would have had the potential to spawn, were spawning or had recently spawned, and that females with ovaries at stages I and II would have remained immature. The maturity ogive used was a re-parameterised form of the logistic equation (e.g., Punt and Kennedy, 1997; Hesp et al., 2004b; Wakefield et al., 2007):

$$\psi_L = \left\{1 + \exp\left[-\log_e(19)\frac{(L - L_{50}^{\mathrm{mat}})}{(L_{95}^{\mathrm{mat}} - L_{50}^{\mathrm{mat}})}\right]\right\}^{-1},$$

where ψ_L is the proportion of mature *E. areolatus* at a particular total length L_T, and L_{50}^{mat} and L_{95}^{mat} are the estimated lengths by which 50 and 95% of *E. areolatus* have attained sexual maturity, respectively. The L_{50}^{mat} and L_{95}^{mat} for the females and their 95% confidence limits (CLs), were determined by bootstrapping, where 2,000 sets of estimates of the logistic equation parameters were obtained from the analysis of data produced by random resampling, with replacement. The point estimates and 95% CLs of the proportions of mature fish in each length class were calculated as the median, 2.5 and 97.5 percentiles, respectively, of the 2,000 bootstrap estimates. The same approach was employed to determine the relationship between the proportion of mature females with respect to age, a, i.e.:

$$\psi_a = \left\{1 + \exp\left[-\log_e(19)\frac{(a - A_{50}^{\mathrm{mat}})}{(A_{95}^{\mathrm{mat}} - A_{50}^{\mathrm{mat}})}\right]\right\}^{-1},$$

where A_{50}^{mat} and A_{95}^{mat} are the ages by which 50 and 95% of individuals were mature.

Estimates of the lengths and ages at which *E. areolatus* changed sex from females to males were calculated separately for both the Kimberley and Pilbara regions. Data were included from all months sampled for all individuals where gonad histology was available to identify sex and gonad stages. The small number of individuals that were categorised as transitional were treated as males as sex change to a male was imminent and bisexuals were removed from this analysis as their functional direction was uncertain. Estimation of length and age at sex change used the same logistic equation and bootstrap analysis as that for estimating maturity (see above), but the length- and age-based parameters (L_{50}^{mat} and L_{95}^{mat}; A_{50}^{mat} and A_{95}^{mat}) were substituted for the length and age at which 50 and 95% of individuals change sex (i.e., L_{50}^{sc} and L_{95}^{sc}; A_{50}^{sc} and A_{95}^{sc}).

Table 2.1 Histological criteria used to determine the gonad development stages of *Epinephelus areolatus* (modified from Mackie (2000) and Chan and Sadovy (2002)).

Maturity stage	Microscopic description
I – Immature female	Gonads small with a thin outer wall. Lamellae organised and contain only primary growth stage oocytes (chromatin nucleolus and perinucleolus). No evidence of prior spawning (muscle bundles, vascular tissue, blood vessels and yellow brown bodies, Figure 2.2a).
II – Mature resting female	Primary growth stage oocytes not always well packed, often appearing disorganised within the lamellae with a thick ovarian wall. There may be evidence of prior spawning (muscle bundles, vascular tissue, blood vessels and yellow brown bodies). Spermatogenic crypts in the spermatocyte or spermatid stage may be present in low to moderate numbers. On occasion it can be difficult to differentiate immature ovaries from mature resting ovaries.
III – Mature developing female	Primary growth stage oocytes and cortical alveolar oocytes present in the lamellae. The cortical alveolar oocytes frequently have variable amounts of degeneration. Spermatogenic crypts in the spermatocyte or spermatid stage may be present in low to moderate numbers.
IV – Mature developed female	Lamellae dominated by yolk globule stage oocytes, but not always tightly packed. Primary growth stage oocytes and cortical alveolar oocytes present in low numbers. Some vitellogenic oocytes (<50%) undergoing atresia. Crypts of sperm tissue may be present in low to moderate numbers in the spermatocyte or spermatid stage.
V – Mature spawning female	Oocytes in all developmental stages present but migratory nucleus or hydrated stage oocytes dominate. A coalescence of the lipid droplets in the yolk globule and migratory nucleus oocytes was common. Post-ovulatory follicles often present. Vitellogenic oocytes (<50%) undergoing atresia were also common. Spermatogenic crypts in the spermatocyte or spermatid stage may be present in low to moderate numbers (Figure 2.2b and c).

VI – Mature spent female	Ovary disorganised containing pre-vitellogenic oocytes and degenerating cortical alveolar and yolk globule stage oocytes. Greater than 50% of yolk globule stage oocytes undergoing atresia (otherwise the ovary is classified as a mature developed female, stage IV). Crypts of sperm tissue may be present in low to moderate numbers in the spermatocyte and spermatid stages.
Inactive male	Gonads dominated by crypts of spermatogonia and spermatocytes, with small numbers of spermatids. Sperm sinuses not always visible. Previtellogenic oocytes common (chromatin nucleolus and perinucleolus stages).
Mature developing male	Lobules contain crypts with similar proportions of spermatogonia and spermatocytes together with spermatids and spermatozoa. Previtellogenic oocytes (chromatin nucleolus and perinucleolus) occasionally present.
Mature active male	Gonads dominated by spermatids and spermatozoa with lesser proportions of spermatogonia and spermatocytes. Ruptured crypts with mature sperm present in both lobule sinuses and large peripheral sinuses. Previtellogenic oocytes are rare (Figure 2.2d).
Mature spent male	Disrupted tissue with empty or collapsed spaces. Few spermatogenic crypts of any development stage. Previtellogenic oocytes rare.
B – Bisexual	Lamellae filled with similar proportions of immature ovarian and testicular tissue, i.e., previtellogenic oocytes often up to the cortical alveolar development stage (regularly degenerating), and crypts of spermatogenic tissue up to the spermatid development stage. Morphological evidence suggesting prior spawning as a female (muscle bundles, vascular tissue, blood vessels and yellow brown bodies) frequently apparent. Developing peripheral sperm sinus rare (Figure 2.2e).
T – Transitional	Degenerating vitellogenic oocyte stages and the presence of many spermatocyte and spermatid stage crypts. Previtellogenic oocytes present. Developing peripheral sperm sinus rare (Figure 2.2f).

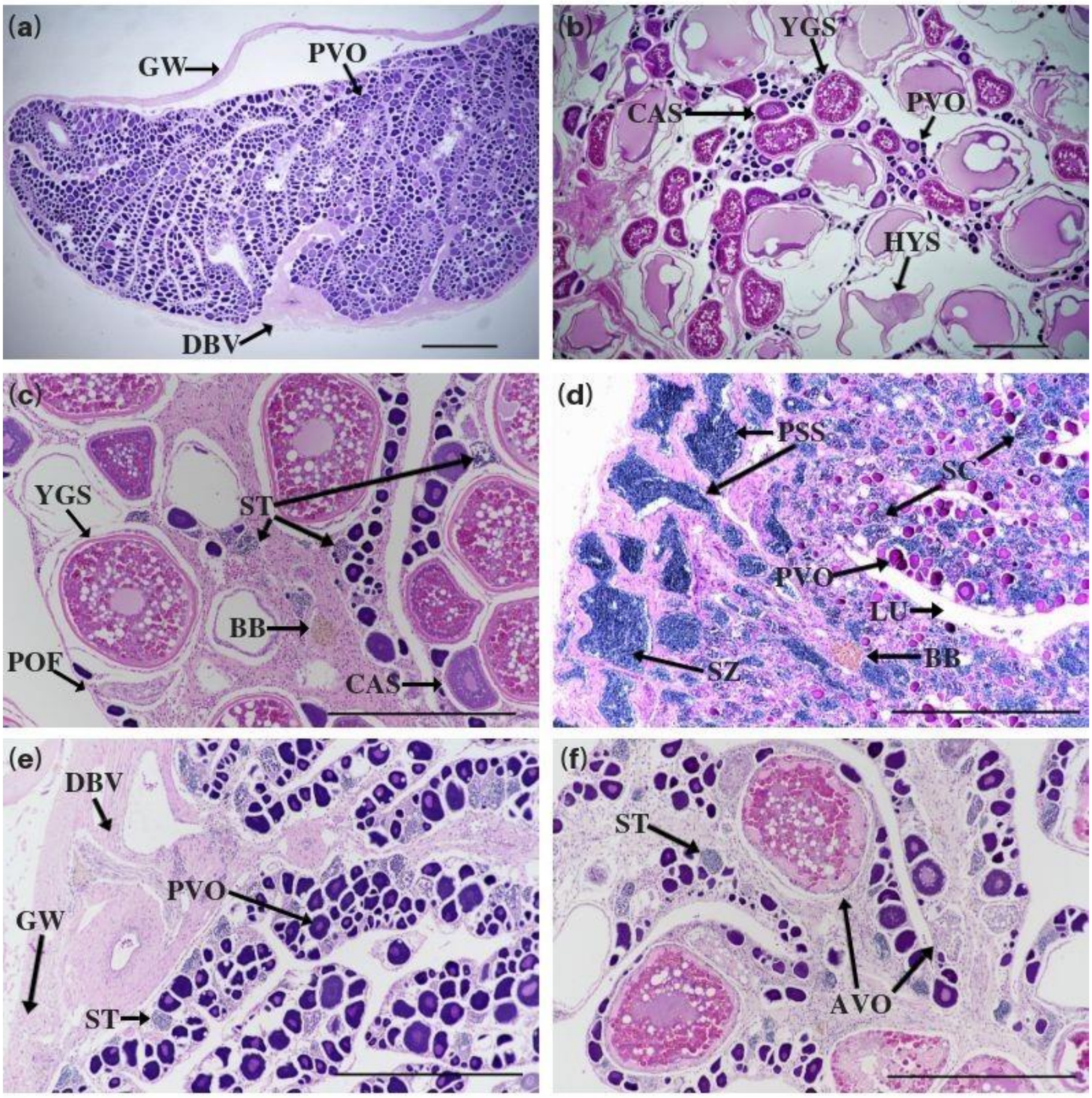

Figure 2.2 Histological features of gonad developmental stages of *Epinephelus areolatus*: (a) immature female; (b) mature spawning female; (c) mature functioning female with spermatogenic crypts; (d) mature active male; (e) bisexual; (f) transitional (AVO = atretic vitellogenic oocytes, BB = brown body, CAS = cortical alveoli stage oocytes, DBV = dorsal blood vessel, GW = gonad wall, HYS = hydrated stage oocytes, LU = lumen, POF = post-ovulatory follicles, PSS = peripheral sperm sinus, PVO = pre-vitellogenic oocytes, SC = spermatocytes, ST = spermatids, SZ = spermatozoa, YGS = yolk globule stage oocytes (scale bar: 500µm)).

2.3.4 Age and growth

Interpretations of annual growth zones in thin transverse sections of the sagittal otoliths of each *E. areolatus* were used to determine its age. Either the left or right otolith from each fish was embedded in epoxy resin and sectioned transversely through the primordium in an orientation perpendicular to the sulcus acusticus (see Wakefield et al., 2017a). Thin otolith sections (i.e., 180 – 250 µm) were cut for each otolith using a low-speed saw (Buehler Isomet) with a diamond tipped blade. Otolith sections for all *E. areolatus* < 100 mm L_T ($n = 24$) were further wet polished by hand using initially 30 µm and finally 5µm diamond impregnated paper to achieve an otolith section thickness of ~150 µm to improve the clarity for daily growth increment counts. Note for young-of-the-year individuals where the first annulus had not yet formed, daily ring counts could be determined, thus facilitating backdating to the spawning period and providing essential data to improve growth parameter estimates. All sections were rinsed in an acidic solution (i.e., 2% HCl) for ~20 seconds (see Gauldie et al., 1990), then again in water, and mounted on a glass slide with a cover slip using casting resin (see also Newman et al., 2015).

Otolith sections were examined without knowledge of fish length or sex using a dissecting microscope under reflected light at 20-40x magnification. Ages were estimated by counts of the delineated opaque zones, and the outer margin of each otolith section was recorded as either being translucent or opaque. Two readers provided opaque zone counts for a subset of otoliths ($n = 50$) to ensure interpretation of growth zones were consistent, and that the level of ageing precision between readers was acceptable based on an Index of Average Percent Error (IAPE) < 5.5% (Beamish and Fournier, 1981). All otolith sections were then examined by both readers and when an opaque zone count differed an agreement was either made or the otolith was removed from further analysis ($n = 1,980$ aged).

Marginal increment analysis (MIA) was used to determine the timing of opaque zone formation and to validate that a single opaque zone is deposited annually in the otoliths of *E. areolatus*. The marginal increment of each otolith (i.e., the distance from the proximal edge of the outermost opaque zone to the periphery of the otolith) was measured on images of sectioned otoliths perpendicular to the opaque zones and adjacent to the ventral side of the sulcus acusticus using computer imaging software (Leica V4.13). This distance was expressed as a proportion of the distance between the proximal edges of the two most recently delineated opaque zones. Due to resource limitations and an extensive amount of literature that has demonstrated that growth zones in the otoliths of teleosts in this region are formed annually (e.g. Newman et al., 2012a; Ong et al., 2015; Ong et al., 2016; Ong et al., 2017; Ong et al., 2018; Wakefield et al., 2020a; Wakefield et al., 2020b; Taylor et al., 2021), annual trends in marginal increments were only determined for the modal age classes from the age compositions, i.e., the 5 and 6 year old cohorts.

The age for each *E. areolatus* was estimated using the birth date, the count of completed opaque zones and the time of year that the opaque zone is delineated. The birth date was considered to be 1 September, a period just before the onset of peak spawning, which was defined by trends in the annual GSI and microscopically staged gonads. This time of year, also coincided with the completion of opaque zone deposition in the otoliths (i.e., during the months (Jun-Sept) when water temperature was coolest).

The growth of *E. areolatus* from the Kimberley, Pilbara and Gascoyne regions were determined using the von Bertalanffy growth equation fitted to the length-at-age data. Considering, 1) the mean length of males was not positively correlated with age, and 2) the distributions of their lengths-at-age were comparable with those of females across the same age range; it is highly likely the growth of males is a continuation from that of the females. Thus, a von Bertalanffy growth curve was fitted to the lengths-at-age of all *E. areolatus* combined within each region. This approach is consistent with other studies that determined the growth of protogynous hermaphroditic teleosts (e.g. Lek et al., 2012; Wakefield et al., 2013; Wakefield et al., 2015). The form of the von Bertalanffy equation was $L_t = L_\infty(1-\exp^{-k(t-t_0)})$, where L_T is the predicted total length (mm) of fish at age t (years), L_∞ is the asymptotic total length (mm) at an infinite age, k is the growth coefficient, a constant that determines the rate at which L_T approaches L_∞ (year^{-1}), and t_0 is the hypothetical age (years) at which fish would have zero length. Growth curves were fitted using least sum of squares, and estimates of L_∞, k and t_0 were determined by bootstrapping, where 1,000 sets of parameters were obtained from the analysis of data produced by random resampling with replacement. The von Bertalanffy growth parameters and lower and upper 95% CLs were calculated as the median, 2.5 and 97.5 percentiles, respectively, of the 1000 bootstrap estimates. Considering the sample sizes for *E. areolatus* were relatively low from the Kimberley and Gascoyne regions, particularly for younger and smaller fish, a two parameter von Bertalanffy growth curve was used where the value of t_0 was fixed to that derived from the Pilbara region where an adequate number of juveniles were sampled (i.e., $t_0 = -0.35$). Likelihood ratio tests were used to determine whether the von Bertalanffy growth curves differed between regions (Cerrato, 1990).

2.3.5 Mortality and age-based selectivity

The instantaneous rate of total mortality (Z) for *E. areolatus* was estimated by fitting catch curves to the age composition data collected in 2012 from each of the two trap fisheries of north-western Australia, i.e., in the Kimberley and Pilbara regions. Although catch curve methods are based on the assumption of the population being in equilibrium, with levels of recruitment and mortality of fully-selected fish in the sample remaining constant over time, they are commonly applied to assess the exploitation level of species for which available data do not suggest these assumptions have been violated (Smith et al., 2012). For *E. areolatus*, the age composition data provided no clear evidence of inter-annual recruitment variability, or a change in mortality among the cohorts represented by each sample (see results). The fitted catch curve model was fundamentally the same as that described by Thorson and Prager (2011) and previously applied to an assessment of the giant ruby snapper (*Etelis boweni*) in north-western Australia (Wakefield et al., 2020b). It assumed a constant natural mortality (M) and allowed for the estimation of fishing mortality (F, where $Z = F + M$), and logistic selectivity at age, which simulation testing has shown to provide more accurate F estimates than catch curve models that assume a knife-edge selectivity (Thorson and Prager, 2011). The selectivity at age (S_a) was described as

$$S_a = \left\{1 + \exp\left[-\log_e(19)\frac{(a - A_{50}^{\text{sel}})}{(A_{95}^{\text{sel}} - A_{50}^{\text{sel}})}\right]\right\}^{-1},$$

where A_{50}^{sel} and A_{95}^{sel} denote the ages by which 50% and 95% of individuals are selected by the fishery, respectively.

Recognising the uncertainty around estimates of M generated by commonly-used empirical methods for fish (Then et al., 2015; Liu et al., 2020), three alternative values of this parameter were considered for *E. areolatus* in this study in order to evaluate the likely sensitivity of this parameter.. These were calculated from the maximum observed age (A) for this species and based on the theoretical assumption that some proportion of the population (x) will survive to this age, i.e.

$$M = -\log_e(x)/A.$$

Values for x of 0.001, 0.01 and 0.015 were applied to provide the three estimates of M, where those based on x values of 0.001 and 0.015 broadly correspond to M estimates derived from the empirical equations for fish described by Hoenig (1983) and Then et al. (2015), respectively, as

$$M = 4.899\, A^{-0.916} \text{ and}$$

$$M = \exp(1.46 - (1.01 \log_e(A))).$$

To account for uncertainty around each parameter estimated by the catch curve analysis (F, A_{50}^{sel} and A_{95}^{sel}), the model was fitted to each of 1000 sets of age composition data, randomly resampled with replacement from the samples collected in each fishery, to derive the median value and the lower and upper 95% CLs of these bootstrapped estimates.

2.4 Results

2.4.1 Gonadal characteristics and length and age compositions

Histological sections from a total of 1,889 *E. areolatus* were examined, with 1,304 females, 532 males, 24 bisexuals, 10 transitionals and 19 unknown specimens identified. All gonads contained a membrane lined central lumen and numerous lamellae. In ovaries, the oocytes were produced within the lamellae. In testes, sperm was never observed in the lumen, instead, large crypts of spermatids and/or spermatozoa were often visible in the peripheral sperm sinuses around the outer gonad wall (Figure 2.2d). Yellow brown bodies were found in mixed proportions through the gonads of mature females, males, bisexuals and transitional fish. A greater percentage of males contained yellow-brown bodies (98%) in their testes than females did in their ovaries (51%).

The smallest fish aged for which gonadal histology was also examined (117 mm L_T and 1.1 years), was an immature female (i.e., stage I). Immature females (n = 217) did not contain spermatogenic crypts, however, the ovaries of 248 (23%) mature functioning females (stages II - VI) did contain such crypts. Crypts were most often few in number and it was common for them to be aggregated around the dorsal blood vessel. Spermatogenic crypts in ovaries were observed in fish > 2 years old and 228 mm L_T in all mature female developmental stages and all months of the year. The testes of 61 (11%) males, ranging from 262 mm to 434 mm

L_T, and in ages from 4 to 14 years contained previtellogenic oocytes, in the chromatin nucleolus and perinucleolus stages.

The length and age frequency distributions of *E. areolatus* differed markedly between regions (Figure 2.3). The Kimberley sample was collected entirely from the commercial trap fishery (NDSF), which accounts for the paucity of fish < 240 mm L_T. During this study, smaller fish were only obtained from research surveys and catches taken by the commercial fish trawl fishery that operates in the Pilbara region. These samples of smaller individuals are also reflected in the higher numbers of one and two-year-old fish in the Pilbara region. A length and age frequency composition was not performed for the Gascoyne region due to the small sample sizes, and because many of these fish were not sexed histologically.

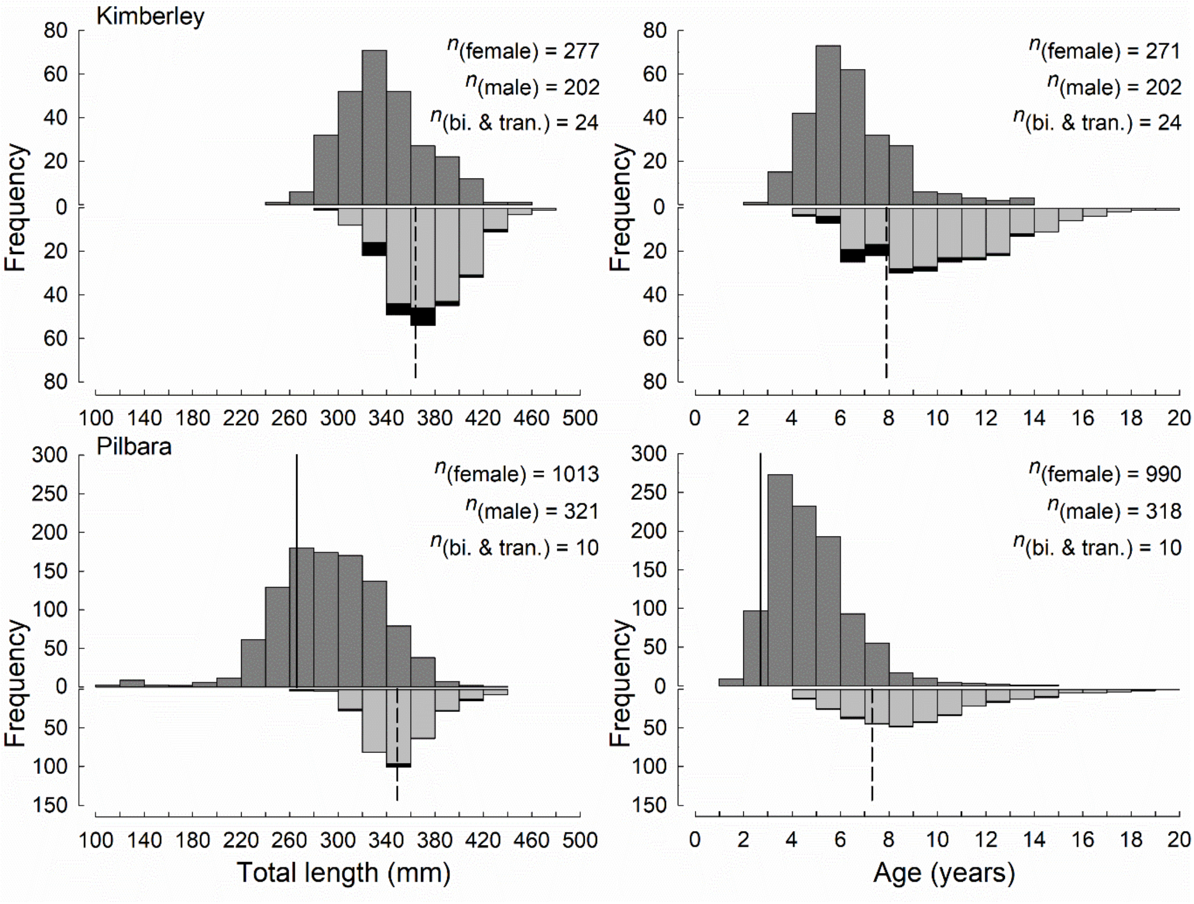

Figure 2.3 Length and age frequency compositions of female (dark grey bars), male (grey bars) and bisexual combined with transitional (black bars) *Epinephelus areolatus* collected in the Kimberley and Pilbara regions of Western Australia. The dashed lines represent estimated lengths and ages at which 50% of individuals changed sex (calculated separately for each region), and the solid lines represent the estimated lengths and ages at 50% maturity for females (from the Pilbara region only).

The length and age frequencies demonstrated that males were typically larger and older than females (Figure 2.3), and as such females were more abundant in the size intervals ≤ 360 mm L_T and ≤ 340 mm L_T in the Kimberley and Pilbara, respectively, and at age classes ≤ 8 years old. The maximum lengths recorded were males of 475 mm and 434 mm L_T in the Kimberley and Pilbara regions, respectively. The maximum ages recorded for both regions were 19 years, which were also males. Although the largest fish were predominantly males, some females attained large lengths, i.e., 441 mm and 430 mm L_T in the Kimberley and Pilbara regions, respectively. In the Kimberley, the smallest male was 304 mm L_T, whereas in the Pilbara, there were 4 males below 300 mm L_T, with the smallest being 262 mm L_T. In contrast, 546 females were recorded below 300 mm L_T in the Pilbara. The youngest males sampled were 4 years of age, occurring in both the Kimberley (n = 3) and the Pilbara (n = 12) regions.

Transitional fish (n = 10) were sampled from the Kimberley (n = 5) and the Pilbara (n = 5) regions, ranging in size from 314 to 405 mm L_T and from 5.1 to 12.7 years of age. Transitional fish (although uncommon) were present in seven months of the year i.e., March (n = 2), April (n = 1), June (n = 1), September (n = 1), October (n = 1), November (n = 3) and December (n = 1). Bisexual fish (n = 24) were more frequently encountered than transitional fish and were more prevalent in the Kimberley (n = 19) than the Pilbara (n = 5). Bisexual fish ranged in length from 294 to 429 mm L_T and 4.1 to 14.8 years of age, thus exhibiting analogous length and age ranges to those of transitional stage fish. Pooling of the transitional and bisexual fish revealed comparable length and age classes as to that of males (Figure 2.3). Bisexuals were most common in October (n = 16), but were also sampled in February (n = 3), April (n = 1), June (n = 1), and November (n = 3).

2.4.2 Annual spawning period and relation to day length and water temperature

Ovaries of *E. areolatus* at either the mature developed or spawning stages (IV and V) were observed in every calendar month. The percentage of stages IV and V ovaries increased from a low of 6% in July to 64% in September, then declined to ~20% in November and December. The highest proportions of mature and spawning individuals (i.e., > 79%) were recorded in the ensuing three months (i.e., January to March), before declining precipitously into the winter months (Figure 2.4). The trend in mean monthly GSIs paralleled those for the ovarian developmental stages, with two conspicuous peaks, i.e., in September (1.7) and March (2.4), and minima in June-July and November-December (< 0.6, Figure 2.4). Histological examination determined that multiple developmental stages of oocyte were present in mature ovaries throughout the spawning period, which is indicative of an indeterminate multiple batch spawner. Male GSIs were not calculated as parasite infestations of the gonads was often profuse (94% infestation in males compared with 38% in females) making male gonad weights inaccurate.

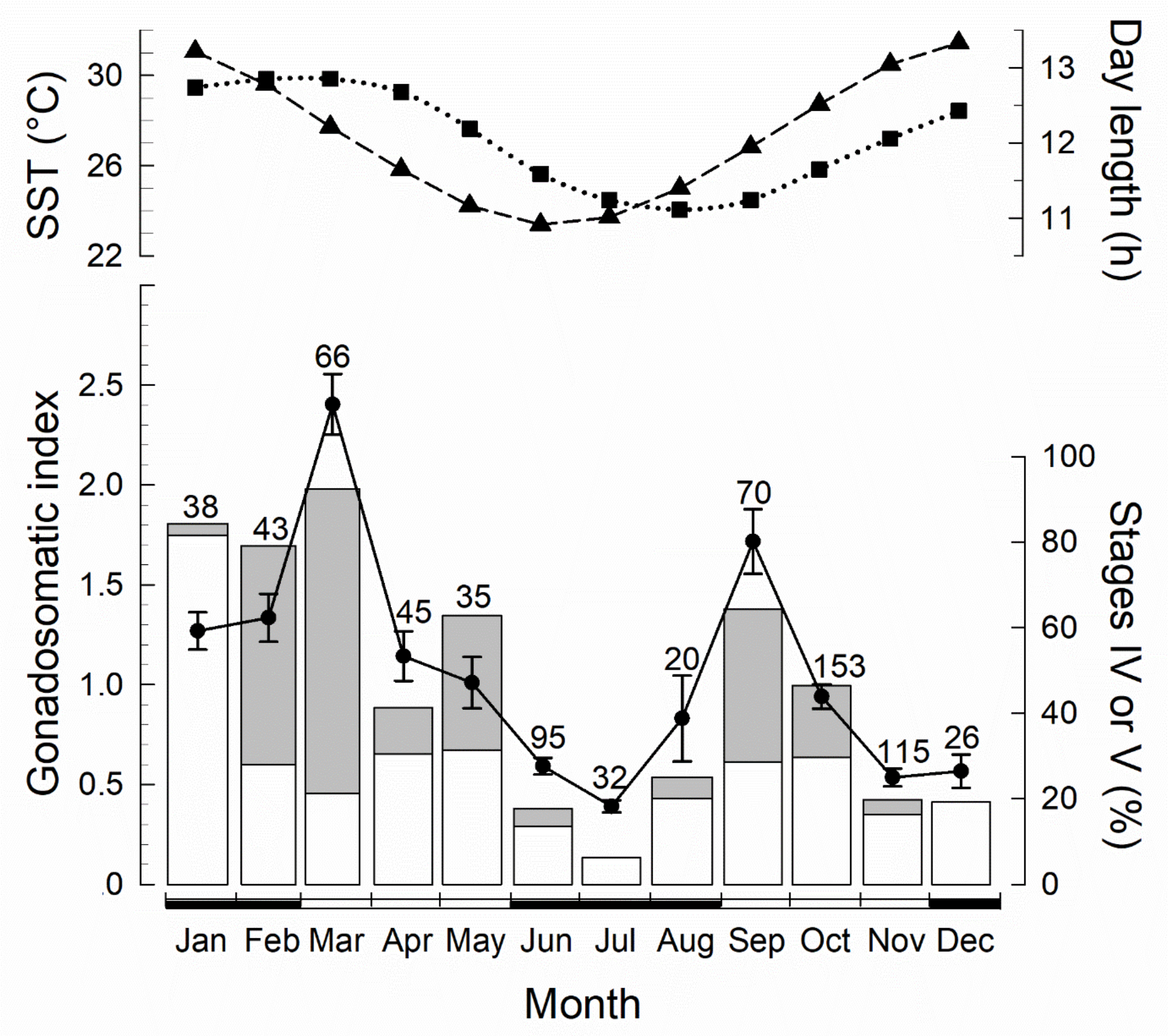

Figure 2.4 Mean monthly gonadsomatic indices (solid line with ± 1 SE) and monthly prevalence (%) of mature developed (stage IV, white bars) and spawning ovaries (stage V, grey bars) of female *Epinephelus areolatus* from the Pilbara region. The data have been pooled for corresponding months (sample sizes shown) and are limited to fish ≥ the length at 50% maturity (266 mm L_T). On the *x*-axis, black rectangles represent summer (Dec-Feb) and winter (Jun-Aug), and white rectangles represent autumn (Mar-May) and spring (Sep-Nov). Mean monthly sea surface temperatures (■) and mean monthly daylight hours (▲) off the north-western coast of Western Australia are shown above the histogram.

The biannual peaks in spawning in March and September corresponded with the two periods when the photoperiod is at its mid-range of ~12.1 hours, despite annual trends in photoperiod decreasing in length in March and increasing in length in September (Figure 2.4). Further, the months of least spawning activity (i.e., June-July and Nov-Dec) corresponded with the lowest and highest day lengths (*c.* 11 and *c.* 13 hours). In contrast, the biannual peaks in spawning corresponded with opposing phases of the annual water temperature cycle, i.e., close to the annual minima in September (~24°C) and maxima in March (~30°C, Figure 2.4).

2.4.3 Lengths and ages at maturity and sex change

During peak spawning months in the Pilbara region, histological analysis of gonads was performed on 261 females (i.e., 57 immature and 204 mature), and of these, ages were determined for 257 (i.e., 57 immature and 200 mature). The length range of mature females (possessing ovaries at stages III - VI) was 237 to 415 mm L_T, and the age range was 2 to 14 years. The estimated length and age at which 50% of female *E. areolatus* attained sexual maturity (i.e., L_{50}^{mat} and A_{50}^{mat}) in the Pilbara region was 266 mm L_T and 2.7 years (Table 2.2, Figure 2.5).

Table 2.2 Total lengths (mm, L_T) and ages (years) at which 50% and 95% (L_{50}^{mat}, L_{95}^{mat}, A_{50}^{mat} and A_{95}^{mat}, respectively) of female *Epinephelus areolatus* reach sexual maturity in the Pilbara region, and change sex in the Pilbara and Kimberley regions of north-western Australia (with 95% lower and upper confidence limits).

	L_{50}	L_{95}	L_{min}	n	A_{50}	A_{95}	A_{min}	n
Pilbara								
Maturity								
Estimate	266	320	237	261	2.7	5.0	2.1	257
(low, upp)	(257, 274)	(306, 336)			(2.4, 3.0)	(4.3, 5.9)		
Sex Change								
Estimate	349	410	262	1339	7.3	10.7	4.0	1313
(low, upp)	(346, 354)	(401, 421)			(7.1, 7.6)	(10.1, 11.4)		
Kimberley								
Sex Change								
Estimate	364	448	304	484	7.9	12.2	4.1	478
(low, upp)	(358, 371)	(430, 470)			(7.5, 8.3)	(11.2, 13.3)		

Note: L_{min}, the minimum total length of mature female fish in maturity estimates and non-female fish in sex change estimates; A_{min}, the minimum age of mature female fish in maturity estimates and non-female fish in sex change estimates; *n*, sample size.

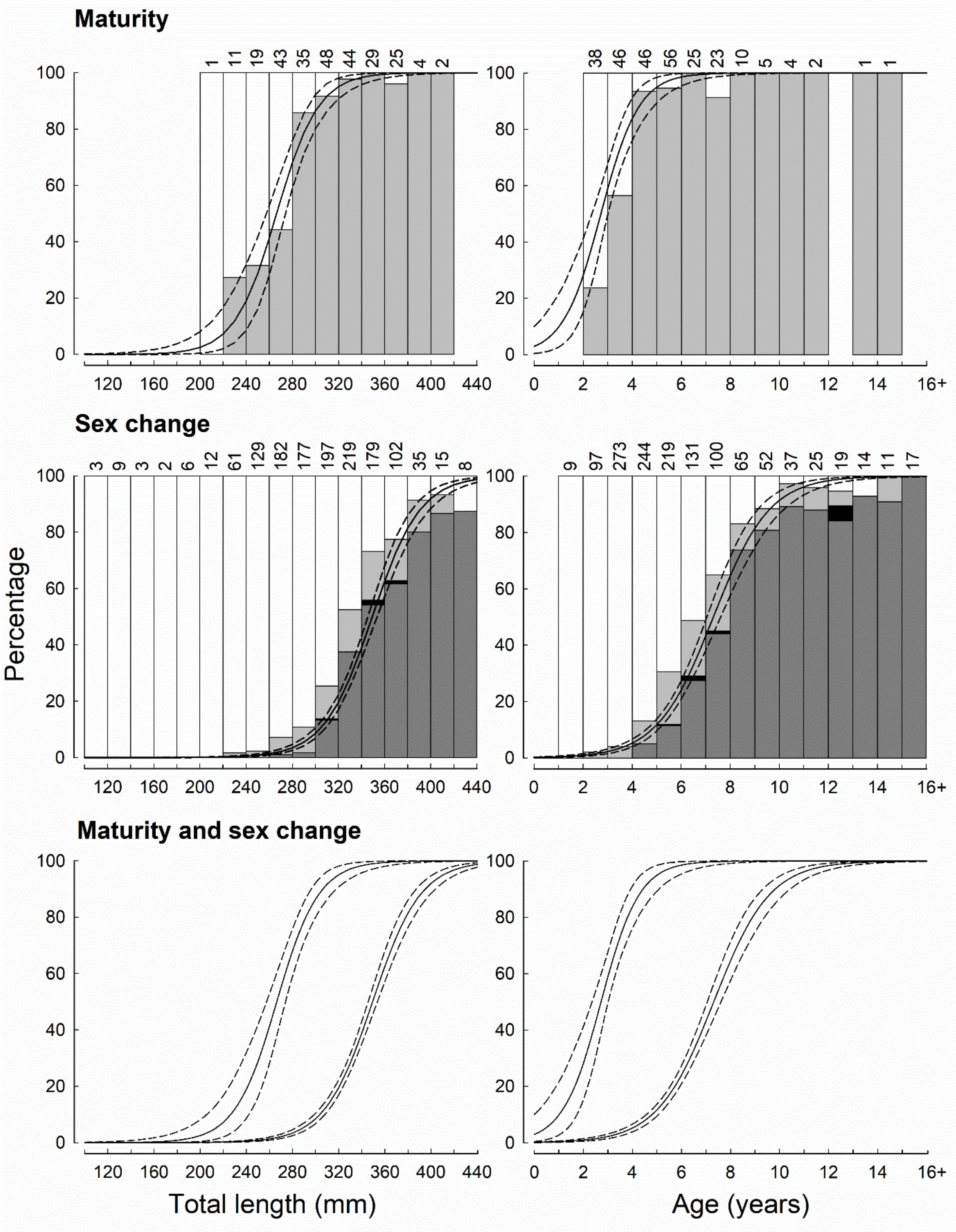

Figure 2.5 Percentage of *Epinephelus areolatus* (sample sizes shown above each class) from the Pilbara region of Western Australia that were immature or mature (top row) and female or male (middle row) in each length (left column) or age class (right column), fitted with logistic regressions (solid lines) and their 95% confidence limits (dashed lines). Maturity - immature females (stages I and II, white bars) and mature females (stages III - VI, grey bars); sex change - females (white bars), females with spermatogenic crypts (light grey bars), transitionals (black bars) and males (dark grey bars); maturity and sex change schedules compared for length and age (bottom row).

The prevalence of males increased with length and age in the Pilbara region (Figure 2.5). Males accounted for the majority of individuals among the largest length classes (i.e., 87% > 400 mm), and comprised by far the majority for individuals in each age class for over half of its lifespan (i.e., > 80% from 10 to 20 years of age, Figure 2.5). The prevalence of males in each length and age class was similar in the Kimberley region. Thus, the estimated lengths and ages at which 50% of *E. areolatus* change from female to male differed by < 5%, i.e., L_{50}^{sc} of 364 and 349 mm L_T and A_{50}^{sc} of 7.9 and 7.3 years, respectively (Table 2.2, Figures 2.5 and 2.6).

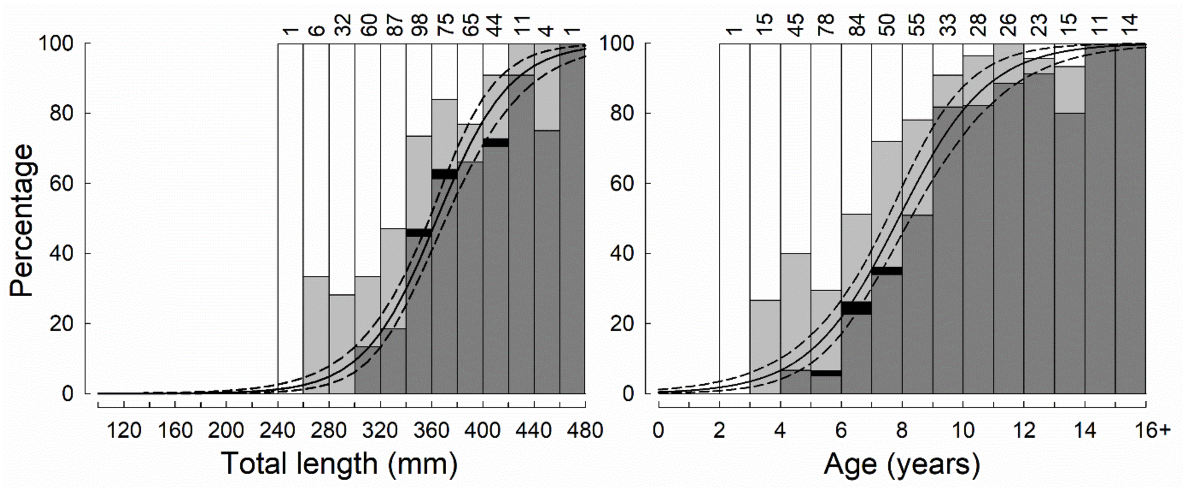

Figure 2.6 Percentage of *Epinephelus areolatus* (sample sizes noted above each class) from the Kimberley region of Western Australia that were female or male in each length or age class. Fitted with logistic regressions (solid lines) and their 95% confidence limits (dashed lines). Female (white bars), female with spermatogenic crypts (light grey bars), transitional (black bars) and male (dark grey bars).

2.4.4 Ageing precision and validation

The precision of opaque zone counts from transverse sections of otoliths of *E. areolatus* was within acceptable levels with an IAPE of 4.58% between two readers (n = 50, age range 2 to 18 years). Opaque zone counts between readers concurred for 46% of otoliths, differed by one for 40% of otoliths and differed by two for 14% of otoliths across all age classes. No opaque zone counts differed by more than two between readers.

The annual trends exhibited in the mean monthly marginal increments of *E. areolatus* otoliths with 5 and 6 opaque zones were highest during the austral winter, which coincided with otoliths possessing either a wide translucent or opaque outer margin (Figure 2.7). Mean monthly marginal increments declined precipitously during the austral spring, which coincided with the formation of a thin translucent zone on the outer margin of the otoliths. The mean monthly marginal increments then increased during the ensuing austral summer and autumn months, which coincided with all otoliths possessing a translucent outer margin (Figure 2.7). This trend demonstrates that a single opaque zone is deposited during the cooler winter months and a single translucent zone is deposited during the other months, and thus counting the number of opaque zones in sectioned otoliths provides an accurate method for ageing *E. areolatus*.

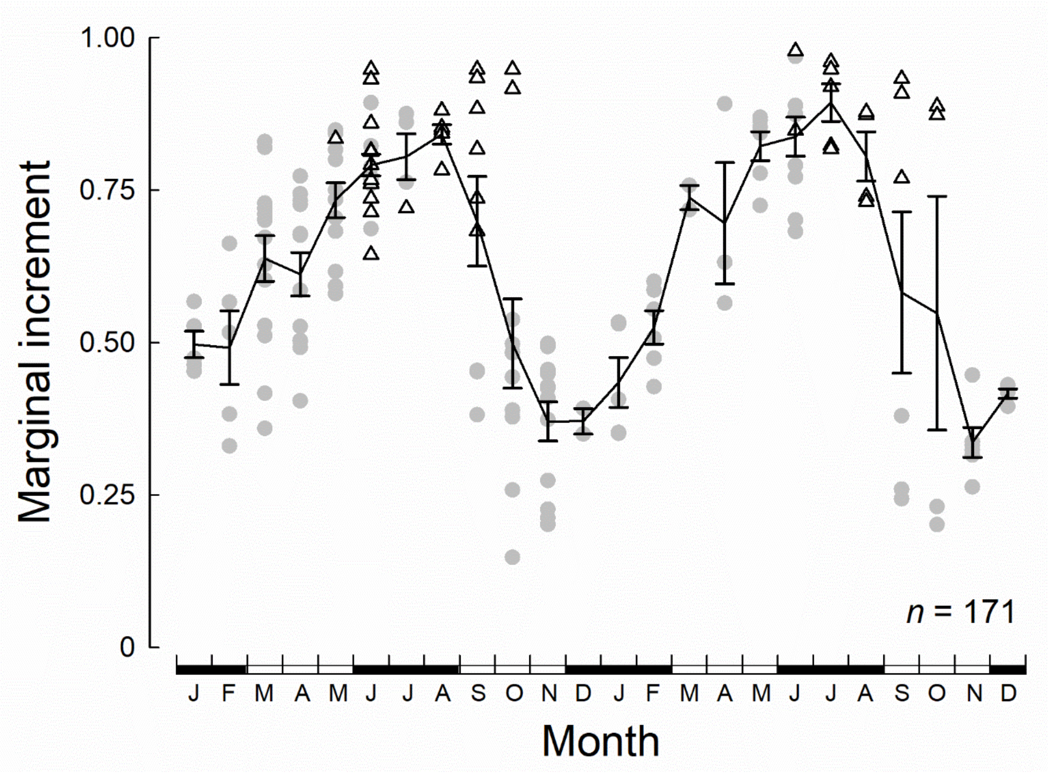

Figure 2.7 Individual (● translucent outer margins; △ opaque outer margins) and mean monthly (± 1 SE, lines) marginal increment values derived from sectioned otoliths with five and six delineated opaque zones displayed as consecutive calendar years for *Epinephelus areolatus* sampled from north-western Australia from April 2012 to May 2017. On the *x*-axis, black rectangles represent summer (Dec-Feb) and winter (Jun-Aug), and white rectangles represent autumn (Mar-May) and spring (Sep-Nov).

Daily growth increments were counted for 18 young-of-the-year *E. areolatus* that ranged in length from 34 to 76 mm L_T, that were caught on a single research survey during October and November in 2017 (*n* = 18). None of these fish contained an opaque zone and post settlement age estimates ranged from 15 days (34 mm L_T) to 87 days (72 mm L_T). The first annual opaque zone was found to be deposited in the otoliths of *E. areolatus* when fish were approximately 120 mm L_T.

2.4.5 Growth

The von Bertalanffy growth curve provided a good fit to the length-at-age data for females, males and unsexed individuals (i.e., gonadal histology was not possible) combined for the Pilbara region, with the small 0+ aged juveniles contributing toward a very reasonable estimate of t_0 of -0.35 (Table 2.3, Figure 2.8). Considering the absence of 0+ aged juveniles in the Kimberley and Gascoyne regions, the estimate of t_0 from the Pilbara was used as a constant when fitting the growth curves to the length-at-age data for the other two regions, resulting in a two parameter von Bertalanffy growth curve. The growth curves differed significantly among regions with differences attributable to both L_∞ and k ($p < 0.05$, Table 2.3). These variations in growth were not correlated with latitude, with *E. areolatus* attaining the largest length-at-age in the more southern latitude in the Gascoyne region, and the smallest length-at-age in the mid latitudes in the Pilbara region (Figure 2.8). For example, at a maximum age of 19 years the differences in estimated lengths among regions were 7.5% between the Pilbara and Kimberley regions (i.e., 358 vs 387 mm L_T, respectively), and 24.7% between the Kimberley and Gascoyne regions (i.e., 387 vs 454 mm L_T, respectively).

Table 2.3 von Bertalanffy growth parameters (estimates and associated 95% lower and upper confidence limits) for curves fitted to the total lengths (L_T) at age of *Epinephelus areolatus* from the Kimberley, Pilbara and Gascoyne regions of north-western Australia.

	L_∞ (mm)	k (yr^{-1})	t_0 (yr)	A_{max} (yr)	L_{max} (mm)	n
Kimberley						
Estimate	388	0.35	-0.35†	19.1	475	503
(low, upp)	(383, 393)	(0.33, 0.37)				
Pilbara						
Estimate	359	0.39	-0.35	19.8	434	1396
(low, upp)	(355, 363)	(0.37, 0.41)	(-0.43, -0.28)			
Gascoyne						
Estimate	455	0.30	-0.35†	19.0	504	78
(low, upp)	(440, 475)	(0.26, 0.34)				

Note: L_∞, hypothetical asymptotic length at an infinite age; k, growth coefficient; t_0, hypothetical age at zero length; A_{max}, maximum age; L_{max}, maximum length; n, sample size.
† t_0 is fixed in the Kimberley and Gascoyne region.

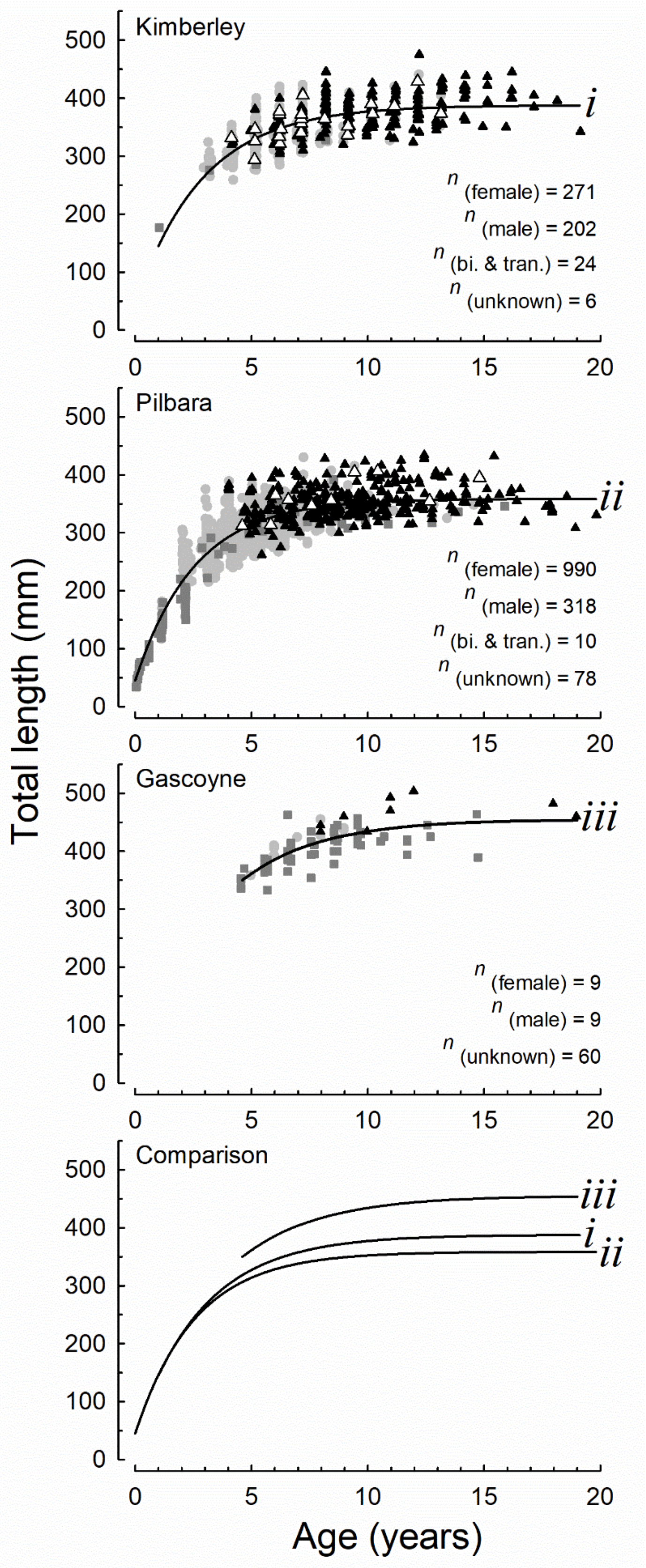

Figure 2.8 von Bertalanffy growth curves fitted to the lengths-at-age for female (●), male (▲), bisexual combined with transitional (△) and unknown sexes (■) of *Epinephelus areolatus* caught in the Kimberley, Pilbara and Gascoyne regions of Western Australia (sample sizes are shown, *n*).

2.4.6 Mortality and age-based selectivity

Representative age composition data collected for *E. areolatus* in 2012 from the trap fisheries in the Kimberley (*n* = 481) and Pilbara (*n* = 512) showed a similar age range (2-19 and 2-18 years, respectively), however, the sample from the Kimberley region comprised a higher relative proportion of older fish (>8 years, Figure 2.9). Catch curve estimates of selectivity showed that individuals become vulnerable to the trap fishing gear at a slightly older age in the Kimberley than in the Pilbara (i.e., ~6 and 5 years of age in the Kimberley and Pilbara respectively, Table 2.4, Figure 2.9). The median estimated age at which 50% of fish are selected by the trap fishery in the Kimberley was 4.5 years (95% CLs = 4.2-5.0 years), compared to 3.7 years (95% CLs = 3.5-3.9 years) in the Pilbara.

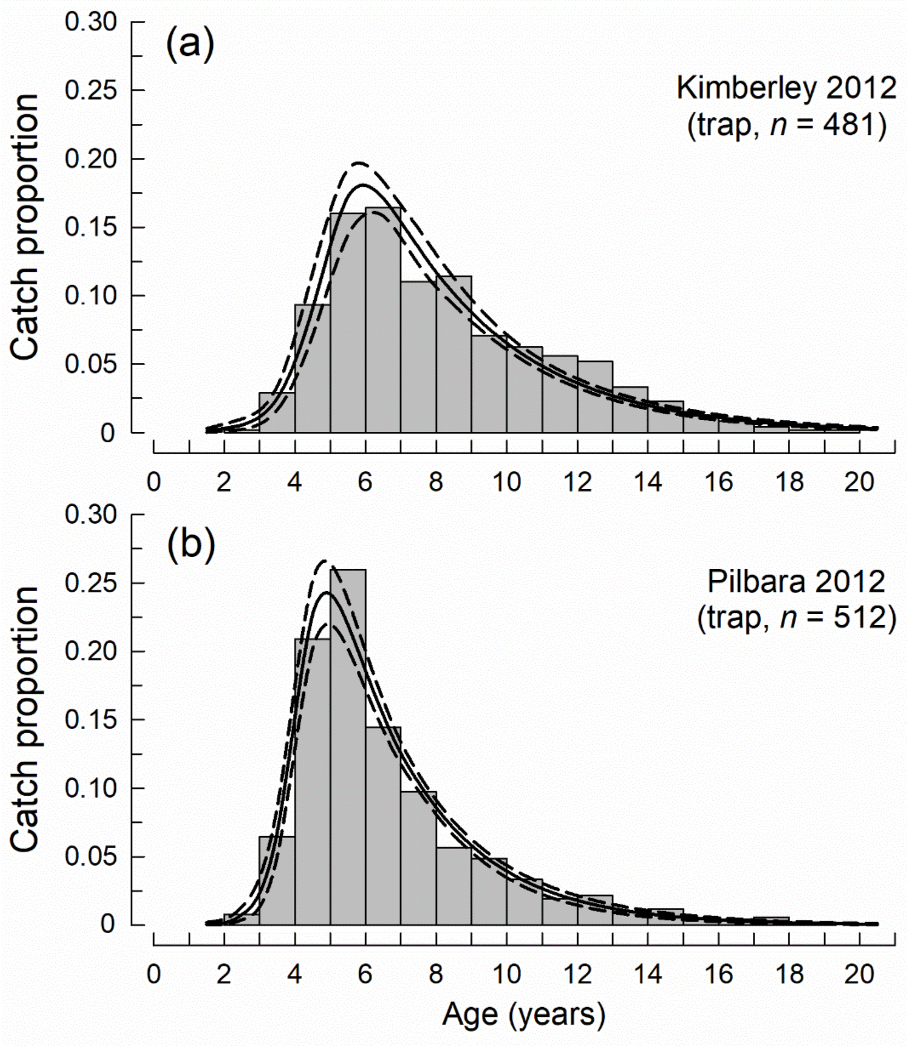

Figure 2.9 Catch curves assuming logistic selectivity (±95% CLs) fitted to the age compositions for *Epinephelus areolatus* collected by commercial trap fishing from: (a) the Kimberley; and (b) the Pilbara regions in north-western Australia during 2012 (sample sizes are shown, *n*).

Table 2.4 Estimates of fishing mortality (F) from catch curve analysis of fully selected fish and age-based selectivity parameters (A_{50}^{sel} and A_{95}^{sel}) with associated lower and upper 95% confidence limits for *Epinephelus areolatus* sampled from the Kimberley and Pilbara regions in north-western Australia during 2012.

	M=0.35	M=0.23	M=0.21			
	F (year^{-1})	**F (year^{-1})**	**F (year^{-1})**	**A_{50}^{sel} (years)**	**A_{95}^{sel} (years)**	**n**
Kimberley						
Estimate	*	0.07	0.09	4.5	6.2	481
(low, upp)	* *	(0.04, 0.10)	(0.06, 0.12)	(4.2, 5.0)	(5.5, 7.1)	
Pilbara						
Estimate	0.05	0.16	0.18	3.7	4.9	512
(low, upp)	(0.01, 0.10)	(0.12, 0.21)	(0.14, 0.23)	(3.5, 3.9)	(4.5,5.3)	

Note: F estimates were calculated based on three alternative values of natural mortality (M) (*$F < 0$. n = sample size).

The median estimate of Z for *E. areolatus* was significantly greater in the Pilbara (0.39 year^{-1}) than in the Kimberley (0.30 year^{-1}), with no overlap in the 95% CLs associated with the estimates from the two regions. Based on the assumptions that 0.1, 1.0 and 1.5% of fish survive to the maximum observed age of 20 years, estimates of M derived for this species in north-western Australia were 0.35, 0.23 and 0.21 year^{-1}, respectively. Although the uncertainty around each of these estimates of M was not evaluated, the highest value was not considered plausible for this species in the Kimberley as it exceeded the estimated value of Z to yield negative estimates of F (Table 2.4). Based on a moderate value for M of 0.23 year^{-1}, calculated assuming that 1% of fish survive to the maximum age, median estimates of F were 0.07 year^{-1} for the Kimberley and 0.16 year^{-1} for the Pilbara, with the upper 95% CLs both below M (Table 2.4). For the more conservative scenario based on a value for M of 0.21 year^{-1}, the upper 95% CLs associated with the Pilbara F estimate only slightly overlapped M, indicating a relatively low probability of overfishing occurring at the time of sampling.

2.5 Discussion

This is the first study to examine the reproduction, age, growth and mortality of the commercially important yellowspotted rockcod, *E. areolatus*, in Australia, which has sustained the largest catches of all epinephelids for at least the last two decades in the multispecies tropical fisheries across north-western Australia. This study also contributes towards the paucity of information on the life-history characteristics of this species throughout its distribution across the Indo-Pacific region, despite being heavily exploited in many regions (Craig et al., 2011). Small groupers such as *E. areolatus* constitute important fisheries resources throughout their distribution, particularly for small-scale fisheries throughout the tropical and subtropical Indo-Pacific region where they provide economic and/or cultural value (e.g. Newman et al., 2016). Currently, *E. areolatus* is harvested from continental shelf waters along the northwest coast of Australia, mainly in depths to 200 m by State (fish trawl, trap and line) managed multi-species commercial fisheries (Newman et al., 2018b). Catches from these demersal State managed fisheries are very biodiverse with the epinephelid component comprising at least ten species (Newman et al., 2001; Newman et al., 2018b). Commercial catches of *E. areolatus* across the NDSF (trap) and PDSF (fish trawl, trap and line fisheries combined) have remained relatively stable, for the past 10 years (2010–19), ranging from 42–66 t in the NDSF with a mean annual catch of 53 t, and from 20–49 t in the PDSF with a mean annual catch of 31 t (Department of Primary Industries and Regional Development WA, unpublished data). Overall, catches of *E. areolatus* have varied between 66 and 113 t per year across the State-managed fisheries since 2010, with an average annual total catch of 84 t.

Multiple lines of evidence including a comprehensive histological analysis of a wide length- and age-range of individuals determined that *E. areolatus* was a monandric protogynous hermaphrodite. The detection of ten individuals in a sexual transition phase, displaying degenerating vitellogenic stage oocytes and a proliferation of testicular tissue provided strong evidence that this species undertakes female to male sex change (Sadovy and Shapiro, 1987). The presence of a membrane–lined ovarian lumen and ovarian lamellar in the testes was also indicative that males have been derived from females. Hydrated oocytes were observed in the lumen of mature spawning females, yet this feature was not used for the transport of sperm in males. Rather sperm was transported via peripheral sperm sinuses which run longitudinally through the gonad wall and are developed by splitting the muscle layers of the ovarian capsule (Sadovy and Shapiro, 1987). The occurrence of remnant previtellogenic oocytes, in the chromatin nucleolus and perinucleolus stages, in a large proportion of the testes is also likely to indicate a protogynous sex change. However, this can be caused by other factors such as environmental conditions or an early bisexual phase (Sadovy and Shapiro, 1987). Yellow brown bodies were often apparent in mature females and males and it is likely that a proportion of these were atretic vitellogenic oocytes, demonstrating in the case of male fish a previous function as a viable female. Although justifiable, yellow brown bodies may also be the result of other processes such as parasitic encystation and sperm degeneration (Sadovy and Shapiro, 1987).

Bisexual gonads had a lamellae filled with similar proportions of immature ovarian and testicular tissue, with no certainty as to the direction of functionality. Transitional gonads by definition need to have degenerating vitellogenic stage oocytes and a proliferation of spermatogenic tissue, indicating previous maturity as a female and a direction of transition (Sadovy and Shapiro, 1987; Sadovy de Mitcheson and Liu, 2008). It is suspected that for *E. areolatus*, the bisexual phase may also be a sex changing phase occurring when the fish is not in spawning condition, hence there is no degeneration of vitellogenic stage oocytes needed to confirm a transition in function for hermaphroditism. The length and age range of bisexual fish was similar to that of transitional fish, with all bisexual and transitional fish having lengths and ages above the L_{50}^{mat} and A_{50}^{mat} for female maturity. The gonad morphology in some bisexual individuals suggested prior spawning as a female, with muscle bundles, vascular tissue, blood vessels and yellow brown bodies often evident. The perspective of bisexuals being fish undergoing sex change was also considered in a study of the protogynous hermaphrodite *Cephalopholis panamensis*, but noted that bisexuals cannot be used explicitly to determine sexual pattern (Erisman et al., 2010).

The bimodal length and age frequency distributions demonstrated that males represented the majority of larger and older individuals in the population structure, which is typical of monandric protogynous hermaphroditism. The youngest males observed were in their fourth year of life (n = 15), and thus older than the A_{50}^{mat} for females. (i.e., 2.7 years). In addition, extensive histological examination of individuals less than the age of the youngest male (n = 395 females) and length of the smallest male (n = 233 females), provides further compelling circumstantial evidence that *E. areolatus* begins life as a female.

The presence of females with non-functional spermatogenic crypts dispersed within the ovaries in low to moderate numbers was common and made the determination of functionality more difficult. This anomaly has also been observed among species of *Cephalopholis* (Siau, 1994; Chan and Sadovy, 2002; Erisman et al., 2010) and in *Epinephelus rivulatus* (Mackie, 2000). These fish were not considered to be in a bisexual or transitional phase because of the low proportion of spermatogenic tissue, and their presence in females of different maturity stages, even occurring in spawning females. It is plausible that some of these fish, although determined to be functional females, may have actually been in an early transitional phase.

The ovaries of 23% of the mature females (i.e., stages II – VI) contained crypts of spermatogenic tissue. Comparative studies of related species have reported higher rates, with almost 50% of mature females in *C. boenak* (Chan and Sadovy, 2002) and 46% of mature females in *E. rivulatus* (Mackie, 2000). As was the case with *C. boenak* (Chan and Sadovy, 2002), spermatogenic crypts were found in *E. areolatus* over a wide length and age range and in all months of the year. The significance of spermatogenic crypts in the gonads of female *C. boenak* was unclear (Chan and Sadovy, 2002), however, Mackie (2000) considered their presence in *E. rivulatus* to be an indication that the female was ready to change sex if the correct cues were provided. In the present study, it is considered their occurrence may be an adaptation to necessitate a rapid sexual transition to a male.

The trends exhibited by histologically staged gonads and GSIs of females demonstrated that female *E. areolatus* were recorded in spawning condition throughout the year, but were less prevalent in the winter months of June and July. Thus, the spawning period of *E. areolatus* in the Pilbara region was protracted from at least August to May with bimodal peaks in September and March. Asynchronous oocyte development was also evident inferring multiple batch spawning over a protracted spawning period. An extended spawning period of 10 months is consistent with the general pattern that smaller groupers tend to spawn over protracted periods (Sadovy, 1996). Year-round recruitment has been reported in some species of coral reef fish at lower latitudes in Papua New Guinea, however rates of recruitment were not constant across all months (Srinivasan and Jones, 2006). The essentially year-round spawning recorded for *E. areolatus* in north-western Australia suggests protracted annual recruitment and infers a relatively high level of population productivity.

The biannual peaks in spawning period coincided with both the mid-range annual cycles in photoperiod, in contrast to occurring at the opposing annual maxima and minima water temperatures. The protracted biannual spawning period recorded for *E. areolatus* in this study varies from those determined for this species elsewhere. Previous studies have only recorded a single annual peak in spawning with a duration of five to seven months in Malaysia, the Arabian Gulf, Red Sea and New Caledonia (Loubens, 1980a; Shapiro, 1987b; Mahmoud, 2009; Ahmad et al., 2011; Abdul Kadir et al., 2016). Although the spawning period of this species in other locations appeared restricted, the timing of the single annual peak did coincide with either the March (i.e., Malaysia, Abdul Kadir et al., 2016) or September (i.e., Arabian Gulf, Ahmad et al., 2011) peaks recorded in north-western Australia. It has been suggested by Choat (2012), that plasticity in the annual spawning periodicity of a tropical species is a reflection of regional evolutionary history associated with their local geography. For example, restrictions in annual spawning period are typically expressed in tropical species inhabiting locations associated with markedly smaller areas of preferred habitat, such as insular shelves, where spawning is limited to periods that facilitate egg and larval retention to maximise localised recruitment. In comparison, species inhabiting locations with much greater areas of preferred habitat for recruitment, such as wide continental shelf locations, typically exhibit a protracted spawning period to maximise broad egg and larval dispersal.

The estimated length and age at which 50% of female *E. areolatus* from the Pilbara region of WA attained maturity were 266 mm L_T and 2.7 years. Reproductive studies on this species in other locations have reported a range of estimates of maturity. In New Caledonia, Loubens (1980a) reported sexual maturation in females at 190 mm SL and an age of 2+; Abdul Kadir et al. (2016) reported an L_{50}^{mat} for females of 326 mm and 357 mm L_T within two regions of Malaysia; and in the Red Sea, Mahmoud (2009) reported female maturity at 235 mm L_T after the second year of life in Egypt, and Osman et al. (2018) reported female maturity at 255mm L_T and 2.23 years from the Gulf of Suez. These divergences could be the result of regional variance; however, they may also be the result of inconsistent methodologies when identifying and determining female stages, and/or in association with using different procedures for the calculation of female maturity schedules.

The lengths and ages at which 50% of *E. areolatus* are estimated to change sex from female to male were 364 mm L_T and 7.9 years in the Kimberley and 349 mm L_T and 7.3 years in the Pilbara. Other studies of this species have not predicted values of sex change for any direct valid comparison, however, based on the prediction that sex change occurs at ~80% of maximum length (Allsop and West, 2004; Wakefield et al., 2015), *E. areolatus* would be expected to change sex at 380 mm L_T in the Kimberley and 347 mm L_T in the Pilbara. Hence, this empirical life-history invariant relationship is supported by this study. All the lines of evidence described in this study demonstrate that individual *E. areolatus* first mature as a female, and subsequently later in life change sex to a male, confirming a monandric protogynous sexual pattern.

The detection of transitional individuals was rare, with only 10 individuals (0.54%) identified. The low proportion of transitional individuals sampled is consistent with other studies of epinephelids, which have typically observed < 3% (Liu and Sadovy, 2004). If the process of sex change is rapid, as suggested by Mackie (2000), the observation of fish in a transitional phase would thus be less frequent. It is largely unknown when and what triggers individuals to change sex. During post-spawning periods steroid levels are low and this may be a prerequisite to initiate sex change (Frisch, 2004). In this study, transitional fish were encountered across seven months of the year, which is likely to be the result of the prolonged spawning period. Pooling transitional and bisexual individuals together resulted in a 91% occurrence outside of the two peak spawning months, and a 68% occurrence collectively in October and November, however these two months also had the highest sampling rates. It is feasible that subsiding steroid levels after spawning activity maybe a precondition required for sex change. Environmental and/or social cues are also expected to influence the timing of sex change (Sadovy and Shapiro, 1987; Fennessy, 2006). The presence of large females indicates that not all females are automatically changing sex when males are removed as the size advantage hypothesis suggests. A potential explanation is that a reproductive advantage exists for females to defer changing sex if the larger female is more fertile than the smaller female (Yamaguchi et al., 2013), this has also been termed size-fecundity skew (Muñoz and Warner, 2003; Muñoz and Warner, 2004). Although, sequential hermaphroditism as a reproductive strategy is expressed in many marine fishes, the specific mechanisms for sex change in *E. areolatus* remain unclear.

MIA confirmed that one opaque and one translucent growth zone is formed annually in the otoliths of *E. areolatus*. Assigning a birth date for *E. areolatus* was problematic because it spawns for most of the year. Nonetheless, since a spawning peak occurred in September, it was assumed that this period likely resulted in a higher proportion of recruits and thus represent a majority of individuals in the population. Age and growth characteristics demonstrated *E. areolatus* has a moderate life span of up to 20 years and reached, on average, 77% of the asymptotic length after the first four years of life. Most growth consequently occurs at early ages and diminishes as the asymptotic length is reached, signifying that length cannot be used as an accurate indicator of age.

The Kimberley, Pilbara and Gascoyne regions of north-western Australia represent a continuous coastline spanning a wide latitudinal range (15°S to 24°S), which provides a particularly good model for exploring the ways in which the growth characteristics of a tropical species vary throughout its distribution and in relation to water temperature. In cooler latitudes the metabolic theory of ecology (MTE) predicts a species will typically attain, on average, larger body sizes, live longer and grow slower early in life than in warmer latitudes, exhibiting a counter gradient relationship between growth and water temperature (Kozłowski et al., 2004; Wakefield et al., 2017b). However, the growth of *E. areolatus* did not conform with the trends in growth characteristics predicted by the MTE, instead this species exhibited a parabolic relationship where the smallest mean length-at-age and fastest growth rates (k) occurred in the mid-latitudes of the Pilbara region. Departures from growth patterns consistent with MTE have been recorded along the western coast of Australia, including the converse relationship between growth and latitude (Cossington et al., 2010), or a parabolic relationship with reduced growth performance toward the extents of a species latitudinal distribution (i.e., negative edge of range effects, Wakefield et al., 2017b). However, this study represents the first example of a teleost species exhibiting increased growth performance toward the latitudinal extents of its distribution along the extensive coastline of Western Australia (i.e., positive edge of range effects).

The relative exploitation levels of fish populations can be determined by comparing values of fishing mortality (F) to standard reference points based on ratios of M. Wise et al. (2007) suggested F_{target} (i.e., equal to two thirds of M) as a level where F would be optimal and $F_{\text{threshhold}}$ (i.e., equal to M) as a level for additional management action. Applying these performance measures to the current study, the estimated value of F in 2012 for *E. areolatus* from the Kimberley region was below F_{target} based on all three alternative values of M, indicating harvest rates are at an acceptable level of fishing mortality in this region. In the Pilbara region, the estimates of F were between the F_{target} and $F_{\text{threshhold}}$ levels for the two lower values of M (i.e., worse case scenarios), indicating that harvest rates are also currently at an acceptable level in this region. The above evidence indicates that the current level of fishing mortality is unlikely to cause overfishing on the spawning biomass of *E. areolatus* in Western Australia, and thus cause these stocks to experience recruitment impairment.

The differences between the age compositions sampled between the Kimberley and Pilbara trap fisheries can be attributed to differences in total mortality rates. These differences may relate to the area fished by the PDSF being smaller and having a longer history of commercial fishing, than the NDSF. In addition, the PDSF resource is shared with a demersal fish trawl fishery, which has different gear selectivity and removes a larger proportion of smaller and younger fish. However, considering the age at which 50% of *E. areolatus* were retained in the NDSF and PDSF (i.e., 4.5 and 3.7 years, respectively) is much greater than their estimated age at maturity (i.e., A_{50}^{mat} of 2.7 years), individuals have the capacity to spawn and reproduce for at least a year on average before being selected by the trap fishing gear. This buffer between age-at-maturity and selectivity of the fishing gear likely provides a significant contribution toward the sustainable exploitation of this species in north-western Australia. Hence, the trap design and mesh size (50 mm square weld mesh) within these multi-species trap fisheries (see Newman et al., 2011) appear to be set at an appropriate level for the continued sustainable harvest of *E. areolatus*, by facilitating the escapement of smaller individuals.

2.5.1 Conclusions and management implications

This study has provided an improved understanding of the life-history parameters of *E. areolatus* in north-western Australia and will serve as a baseline enabling more informed assessments of inherent vulnerability and stock status into the future. As a result of the high population productivity of *E. areolatus* evidenced through year-round multiple batch spawning (maximising egg and larval dispersal), spawning across and along a wide continental shelf, early maturation, and attaining maturity before full selection into the trap fisheries, this species has sustained high levels of catches over an extended period, supporting the largest catch of any epinephelid in the multispecies tropical fisheries of north-western Australia. Moreover, it is important to note that the population productivity of *E. areolatus* is high (as a consequence of the population characteristics described above), in comparison to the indicator species used for management such as the red emperor, *Lutjanus sebae* (Newman et al., 2001; Newman and Dunk, 2002; Newman et al., 2018a). In addition, the estimates of *F* for *E. areolatus* in the Kimberley and the Pilbara regions indicated that the current levels of fishing are sustainable, however, periodic monitoring would ensure an acceptable stock status is maintained. The sustainable harvest of *E. areolatus* in north-western Australia is due to a combination of their resilient life-history traits and an effective multi-species management approach, making them one of the few reported epinephelids to have sustained long periods of exploitation.

Chapter 3: Life-history characteristics and mortality of the protogynous hermaphroditic frostback rockcod (*Epinephelus bilobatus*) from the eastern Indian Ocean

3.1 Abstract

The frostback rockcod, *Epinephelus bilobatus*, is a small epinephelid (< 50 cm total length) with a limited distribution in the tropical eastern Indian Ocean (i.e., north-western Australia and parts of Indonesia). Although its fishery harvest in Western Australia is low (< 6 t year^{-1}), its life history is not well known (i.e., reproductive biology, growth, and natural mortality) preventing assessment of its inherent vulnerability to overfishing. Samples of *E. bilobatus* were collected from ~2000 km of the north-western Australian coast, comprising the Kimberley, Pilbara, and Gascoyne regions, between 2012 and 2022 to determine its life-history characteristics. Detailed histological analysis of gonads (n = 1460) identified that *E. bilobatus* is a monandric protogynous hermaphrodite. The length and age at which females matured was 278 mm L_T and 2.5 years in the Pilbara region, and the lengths and ages at which they changed to males was 336 and 342 mm L_T and 6.2 and 6.5 years in the Kimberley and Pilbara, respectively (i.e., less than 5% variation between regions). Based on histology, female *E. bilobatus* were in spawning condition in all months. However, the mean monthly gonadosomatic indices and proportions of developed/spawning females were highest (i.e., > 0.95 and 40%, respectively) for 5 months of the year (i.e., January to April and September). Marginal increment analysis confirmed the annual formation of one opaque and one translucent zone in the sagittal otoliths of this species. *Epinephelus bilobatus* had a moderate longevity (~20 years) and attained a larger mean length-at-age in the cooler southern Gascoyne region compared to the more northern Kimberley and Pilbara regions. Three values of natural mortality (M) were used to assess the sensitivity of fishing mortality (F) in the Kimberley and Pilbara commercial trap fisheries, with all point estimates of $F < M$, indicating that at the time of sampling, the harvest of this species over a long period (> 20 years) was sustainable for these contemporary fisheries.

Keywords: age, Epinephelidae, growth, maturity, reproduction, sex change, spawning season

3.2 Introduction

The Epinephelidae (groupers) are distributed worldwide, though they primarily occur in the tropics and sub-tropics, and importantly are among the most highly valued reef fish species globally. Consisting of 16 genera and 163 species, groupers are biologically diverse exhibiting a range of life-history strategies (Craig et al., 2011). Many groupers are susceptible to fishing pressure as a consequence of particular life-history traits, including extended longevity, late maturation and the formation of spawning aggregations (Sadovy de Mitcheson et al., 2013). Nonetheless, these traits need to be considered at a species level, as it has been observed that smaller groupers can be highly productive, less selected by fishing gears, and consequently exhibit a lower vulnerability to overexploitation (Boddington et al., 2021). Protogynous hermaphroditism is the prevalent reproductive mode within the Epinephelidae, though this assumption needs to be verified as there is evidence of a range of sexual patterns present even within genera (Sadovy de Mitcheson and Liu, 2008). Sex changing species present a challenge for sustainable management, as size-selective fisheries can potentially reduce their reproductive success by targeting and removing larger individuals and thus altering the population sex ratio (Vincent and Sadovy, 1998; Alonzo and Mangel, 2004). Not only is plasticity present at the family level, but localised complexities can result in intraspecific variation further generating a variety of reproductive strategies at the species level (Choat, 2012). Thus, reliable species-specific data encompassing the age, growth, and reproductive biology, and an understanding of how these characteristics vary at a regional and latitudinal scale, is essential for stock assessment parameter inputs. Hence, improving risk-based management advice and for the formulation of robust management practices.

The frostback rockcod, *Epinephelus bilobatus* (Randall and Allen, 1987), is a small epinephelid (< 50 cm total length) inhabiting rocky benthos and coral reefs to depths of at least 100 m (Craig et al., 2011; pers. obs). It is a tropical eastern Indian Ocean species, with a limited distribution on the north-west coast of Australia from the Houtman Abrolhos Islands, Western Australia (WA), to the Cobourg Peninsula, Northern Territory, and extending north from Australia into the West Papua province, Indonesia (Bray, 2018). Very little is known about *E. bilobatus* and it is uncertain whether it is common within its range or if its distribution extends further (Craig et al., 2011). *Epinephelus bilobatus* is one of nine species of ‘reticulated groupers’ that have a rounded caudal fin and a network of close set dark brown spots separated by pale interspaces (other reticulated groupers include: *E. faveatus, E. hexagonatus, E. macrospilos, E. maculatus, E. melanostigma, E. merra, E. quoyanus and E. spilotoceps*; Heemstra and Randall, 1993; Craig et al., 2011). These species are all similar in appearance and have historically been confused and misidentified in the literature and in museum collections (Heemstra and Randall, 1993; Craig et al., 2011).

North-western Australia has a long history of fishing activity and up until the establishment of management areas in 1987, foreign fishing fleets heavily exploited this region (Wakefield et al., 2017c). At present, primarily two commercial multispecies fisheries harvest *E. bilobatus* in north-western Australia: (i) the Northern Demersal Scalefish Managed Fishery (NDSF) in the Kimberley region, permitting fish trap and line gear types; and (ii) the Pilbara Demersal Scalefish Fisheries (PDSF) in the Pilbara region, permitting fish trawl, fish trap, and line gear types (Newman et al., 2021a). Within these multispecies fisheries, the cods and groupers (Epinephelidae other than *E. rankini*) comprise at least 10 species, and catches are typically reported together, as such, catches at a species level are derived from catch proportions recorded during fishery-independent surveys (Department of Primary Industries and Regional Development, WA (DPIRD), unpublished data). Based on these fishery independent data, *E. bilobatus* is estimated to comprise 4.9% by numbers and 2.7% by weight (derived from length-weight relationships) of this mixed grouper catch in the NDSF, and 3.0% by numbers and 2.2% by weight in the PDSF. Hence, the estimated annual landing of *E. bilobatus* in the NDSF and PDSF in 2021 was ~3.8 t and ~1.5 t, respectively. Reported boat-based recreational catches of *E. bilobatus* are low in the Kimberley, Pilbara and Gascoyne regions (e.g., < 100 fish retained in 2020/21; Ryan et al., 2022). Small-scale commercial and subsistence fisheries harvest *E. bilobatus* at unknown levels in Indonesia (Russell, 2018). However, fishing pressure is not considered to be a major threat to *E. bilobatus*, as reported by the IUCN red list assessment, and thus it has a listing of 'Least Concern' (Russell, 2018).

This is the first study to investigate the age, growth, reproductive biology and mortality of *E. bilobatus*. The specific aims of this study were to: (1) determine the reproductive mode of *E. bilobatus*; (2) if confirmed to be hermaphroditic, to estimate the length and age at sexual maturity and sex change; (3) determine the annual spawning period; (4) validate the annual deposition of growth zones in the sagittal otoliths using marginal increment analysis; (5) compare the length and age compositions and growth parameters between regions in north-western Australia; and (6) compare fishing mortality estimates relative to natural mortality

(M) to assess levels of fishing pressure in the Kimberley and Pilbara regions of north-western Australia. Knowledge of these life-history characteristics and mortality estimates will contribute toward understanding the inherent vulnerability of *E. bilobatus* and better inform managers regarding strategies for their sustainable harvest.

3.3 Methods

3.3.1 Sampling and somatic measurements

A total of 1,521 samples of *E. bilobatus* were collected from the Kimberley, Pilbara, and Gascoyne regions of north-western Australia (Figure 3.1), between 2012 and 2022. Specimens were sampled from the commercial fish trap catches taken by the NDSF (n = 362, predominantly zone B, see Figure 3.1), the PDSF (n = 574) and from fishery independent monitoring surveys aboard commercial trap fishing vessels in zone B of the NDSF during 2012 (n = 12). A further 193 samples were collected from research surveys that employed the same fish traps as those used in the commercial fisheries (Kimberley n = 27, Pilbara n = 119, Gascoyne n = 47). To improve the sample sizes of smaller and larger individuals for age, growth, and reproductive parameter estimates, additional specimens were collected from catches taken by the Pilbara Fish Trawl Fishery (n = 224), research survey trawls (Kimberley n = 26, Pilbara n = 12, Gascoyne n = 1), research opera trap surveys (Pilbara n = 6), line fishing (Kimberley n = 4, Pilbara n = 25, Gascoyne n = 11), and from spear fishing (Pilbara n = 70, Gascoyne n = 1). Regular monthly samples were only taken from the Pilbara region to determine the annual spawning period. For each specimen, the total length (L_T) was measured to the nearest 1 mm and when possible, the whole fish weight (W_W) was recorded to the nearest 0.1 g. The sagittal otoliths were extracted, cleaned, and stored dry for further preparation to determine ages. To estimate the weights of all individuals and account for any specimens that were unable to be sampled whole, an allometric relationship between length (L_T) and weight (W_W) for sexes combined, $W_W = a\ L_T\ b$, was derived from log transformed data using least squares regression (Quinn and Deriso, 1999). Log-transformation was applied based on examination of the residuals, and parameters were back-transformed with correction for bias to the above form (Quinn and Deriso, 1999). In WA, the Animal Welfare Act 2002 does not require DPIRD to obtain a permit to use animals for scientific purposes unless the species are outside the provisions of the Fish Resources Management Act 1994 and Fish Resources Management Regulations 1995. Nonetheless, all sampling was undertaken in strict adherence to DPIRD Policy for the handling, use and care of marine fauna for research purposes.

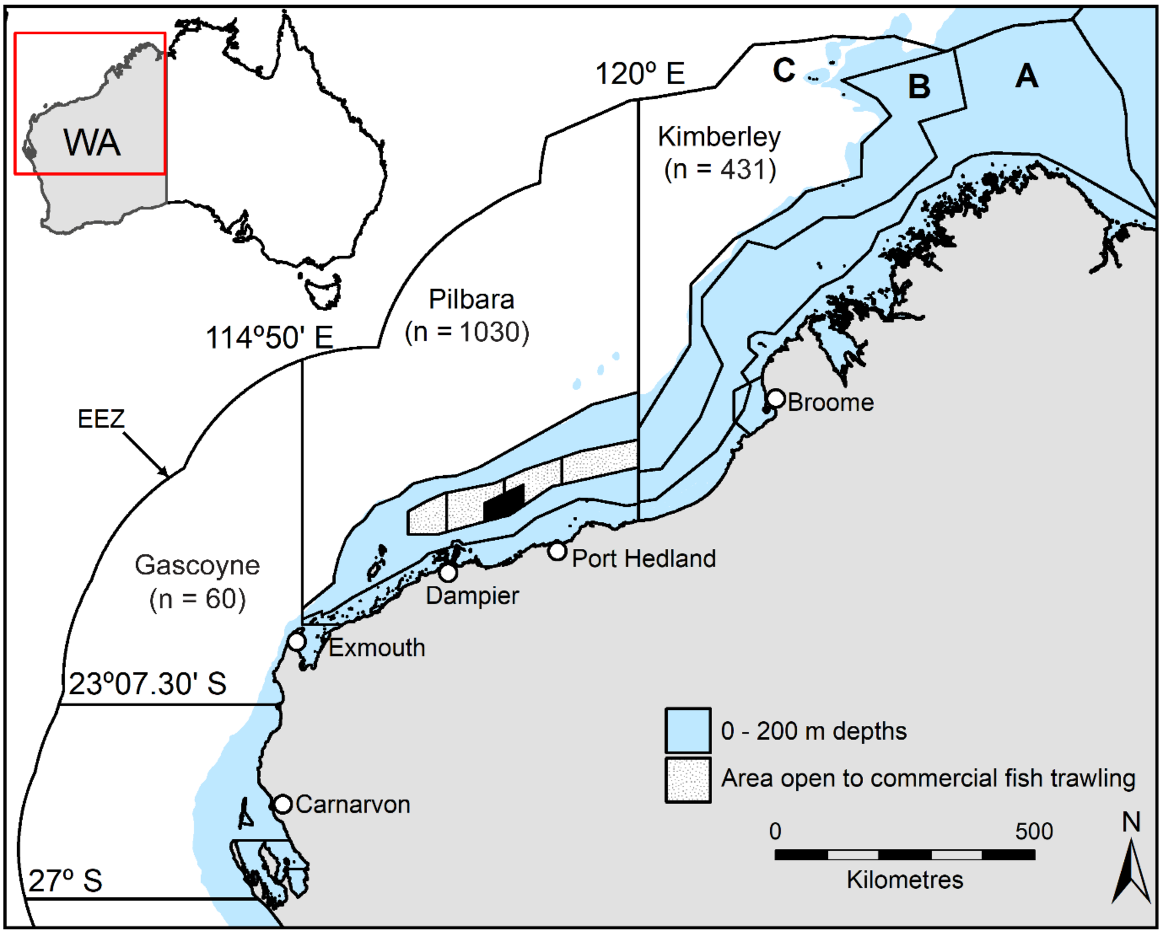

Figure 3.1 Sample sizes (*n*) of *Epinephelus bilobatus* collected from each region of north-western Australia, and the management boundaries for the Western Australian (WA, inset) demersal scalefish fisheries in this area. In the Kimberley, zones A, B and C of the NDSF permit commercial trap fishing. In the Pilbara, areas open to commercial fish trawling (grey shade) are nested within the wider commercial trap fishery area.

3.3.2 Reproductive biology

Macroscopic staging of gonadal development in hermaphroditic species is unreliable, thus sex and reproductive stage of specimens were determined only for those for which histological sections could be obtained (*n* = 1460; Sadovy and Shapiro, 1987; Sadovy de Mitcheson and Liu, 2008). The gonads of each of those specimens were removed and weighed to the nearest 0.01 g (W_G) and a portion of the largest lobe was preserved in 10% formalin. Medial transverse sections of these preserved gonads were embedded in paraffin wax, sectioned at 5 µm, mounted on glass slides and stained using haematoxylin and eosin, and examined microscopically using transmitted light. The sex and reproductive development stage were classified using a modified version of the histological criteria outlined by Boddington et al. (2021) for *E. areolatus* (Table 3.1, Figure 3.2), with the primary difference between these reproductive schemes being the rare occurrence of spermatogenic crypts present within functional ovaries. The presence and relative abundance of the most advanced gametes within the gonad was used to classify the development stage of each gonad (Chan and Sadovy, 2002; Erisman et al., 2010), and the diagnosis of sexual pattern followed the criteria outlined for functional hermaphrodites by Sadovy and Shapiro (1987) and Sadovy de Mitcheson and Liu (2008).

Gonadosomatic indices (GSIs) were calculated for mature female fish (i.e., $L_T \geq L_{50}^{\text{mat}}$) from the Pilbara region using the equation: GSI = 100 (W_G / W_W). Specimens from different years were pooled by month and the spawning period was estimated from the annual trends in the mean monthly GSIs, and the monthly prevalence of females containing ovaries that were either developed or spawning (i.e., stages IV or V). The mean monthly sea surface temperatures (SST), and mean monthly daylight hours reported in Boddington et al. (2021) for the northwest coast of WA were adapted and applied for the relevant time series in this study.

The estimated length and age at sexual maturity for female *E. bilobatus* were only able to be estimated from the Pilbara region, and were derived from samples collected during peak spawning months (i.e., January to April, and September), when monthly GSIs were highest (> 0.95), and the percentage of females containing ovaries that were either developed or spawning (i.e., stages IV or V) were greatest (> 40%). The length and age based maturity schedules were determined using the same analyses as outlined in Boddington et al. (2021). Where the lengths at which 50% of females reached sexual maturity (L_{50}^{mat}) were estimated using a logistic regression to determine the relationship with length of the probability that a female during the peak spawning period possessed developing, developed, spawning or spent gonads (i.e., stages III - VI). It was therefore assumed that during these peak spawning periods, females with gonads of these stages would have had the potential to spawn, were spawning or had recently spawned, and that females with immature or resting ovaries (i.e., stages I and II) would have remained immature. The maturity ogive used was a re-parameterised form of the logistic equation (e.g., Punt and Kennedy, 1997; Hesp et al., 2004b; Wakefield et al., 2007):

$$\psi_L = \left\{1 + \exp\left[-\log_e(19)\frac{(L - L_{50}^{\mathrm{mat}})}{(L_{95}^{\mathrm{mat}} - L_{50}^{\mathrm{mat}})}\right]\right\}^{-1},$$

where ψ_L is the proportion of mature *E. bilobatus* at a particular total length L_T, and L_{50}^{mat} and L_{95}^{mat} are the estimated lengths by which 50 and 95% of *E. bilobatus* have attained sexual maturity, respectively. The L_{50}^{mat} and L_{95}^{mat} for the females and their 95% confidence limits (CLs), were determined by bootstrapping, where 2000 sets of estimates of the logistic equation parameters were obtained from the analysis of data produced by random resampling, with replacement. The point estimates and 95% CLs of the proportions of mature fish in each length class were calculated as the median, 2.5 and 97.5 percentiles, respectively, of the 2000 bootstrap estimates. The same approach was employed to determine the relationship between the proportion of mature females with respect to age, a, i.e.:

$$\psi_a = \left\{1 + \exp\left[-\log_e(19)\frac{(a - A_{50}^{\mathrm{mat}})}{(A_{95}^{\mathrm{mat}} - A_{50}^{\mathrm{mat}})}\right]\right\}^{-1},$$

where A_{50}^{mat} and A_{95}^{mat} are the ages by which 50 and 95% of individuals were mature. Estimates of the lengths and ages at which *E. bilobatus* changed sex from female to male were calculated for the Kimberley and Pilbara regions separately. Data from all months were included where gonad histology was available to determine sex and developmental stage. Transitional fish (changing from functional female to functional male) were pooled with males, as sex change was considered imminent, and bisexual fish (gonads comprise immature ovarian and testicular tissue) were removed from this analysis as their functional direction was uncertain. Estimation of the length and age at sex change used the same logistic equation and bootstrap analysis as that for estimating maturity (see above), but the length- and age-based parameters (L_{50}^{mat} and L_{95}^{mat}; A_{50}^{mat} and A_{95}^{mat}) were substituted for the length and age at which 50 and 95% of individuals changed sex (i.e., L_{50}^{sc} and L_{95}^{sc}; A_{50}^{sc} and A_{95}^{sc}).

Table 3.1 Histological criteria used to determine the gonad development stages of *Epinephelus bilobatus* [modified from Boddington et al. (2021)].

Maturity stage	Microscopic description
I – Immature female	Gonad is small with a thin outer wall. Lamellae is organised and consists only of chromatin nucleolus and perinucleolus stage oocytes. No evidence of prior spawning (i.e., muscle bundles, vascular tissue, blood vessels and yellow-brown bodies not present, Figure 3.2a).
II – Mature resting female	Chromatin nucleolus and perinucleolus stage oocytes present, not always well packed, often appearing disorganised within the lamellae with a thick ovarian wall. Evidence of prior spawning (i.e., muscle bundles, vascular tissue, blood vessels and yellow-brown bodies present). Spermatogenic crypts in low numbers up to spermatid stage seldom present.
III – Developing female	Chromatin nucleolus, perinucleolus, and cortical alveolar stage oocytes present in the lamellae. Cortical alveolar oocytes frequently have degeneration. Spermatogenic crypts in low numbers up to spermatid stage seldom present.
IV – Developed female	Lamellae dominated by yolk globule stage oocytes, not always tightly organised. Chromatin nucleolus, perinucleolus, and cortical alveolar stage oocytes present in low numbers. Some vitellogenic stage oocytes (< 50%) undergoing atresia. Spermatogenic crypts in low numbers up to spermatid stage seldom present. (Figure 3.2b).
V – Spawning female	Oocytes in all developmental stages present but migratory nucleus or hydrated stage oocytes dominate. A coalescence of the lipid droplets in the yolk globule and migratory nucleus oocytes common. Post-ovulatory follicles often present. Vitellogenic oocytes (<50%) undergoing atresia common.
VI – Spent female	Ovary disorganised containing chromatin nucleolus, perinucleolus, cortical alveolar (not always present) and yolk globule stage oocytes. Greater than 50% of the yolk globule stage oocytes undergoing atresia. Spermatogenic crypts in low numbers up to spermatid stage seldom present.

Inactive male	Gonads dominated by crypts of spermatogonia and spermatocytes, with small numbers of spermatids. Sperm sinuses not always visible. Previtellogenic oocytes common in the chromatin nucleolus and perinucleolus stages. Atretic vitellogenic oocytes are rare and do not co-occur with previtellogenic oocytes.
Developing male	Lobules contain similar proportions of crypts with spermatogonia and spermatocytes, in addition to some with spermatids and spermatozoa. Previtellogenic oocytes common in the chromatin nucleolus and perinucleolus stages. Atretic vitellogenic oocytes are rare and do not co-occur with previtellogenic oocytes.
Active male	Gonads dominated by spermatids and spermatozoa with lesser proportions of spermatogonia and spermatocytes. Enlarged and ruptured crypts with mature sperm present in both lobule sinuses and large peripheral sinuses. Previtellogenic oocytes common in the chromatin nucleolus and perinucleolus stages. Atretic vitellogenic oocytes are rare and do not co-occur with previtellogenic oocytes. (Figure 3.2c).
Spent male	Disorganised tissue with empty or collapsed spaces. Few spermatogenic crypts of any development stage. Previtellogenic oocytes common in the chromatin nucleolus and perinucleolus stages. Atretic vitellogenic oocytes are rare and do not co-occur with previtellogenic oocytes.
B – Bisexual	Lamellae filled with similar proportions of immature ovarian and testicular tissue, i.e., previtellogenic oocytes in the chromatin nucleolus and perinucleolus stages and on occasion the cortical alveolar stage, in conjunction with crypts of spermatogenic tissue up to the spermatid development stage. Circumstantial evidence of prior spawning as a female present (i.e., muscle bundles, vascular tissue, blood vessels and yellow-brown bodies). Peripheral sperm sinus often present and developed. (Figure 3.2d).
T – Transitional	Degenerating vitellogenic stage oocytes and the presence of many crypts with spermatocyte and spermatid stages. Previtellogenic oocytes present in the chromatin nucleolus and perinucleolus stages and occasionally the cortical alveolar stage. Peripheral sperm sinus generally absent, but occasionally developing. (Figure 3.2e).

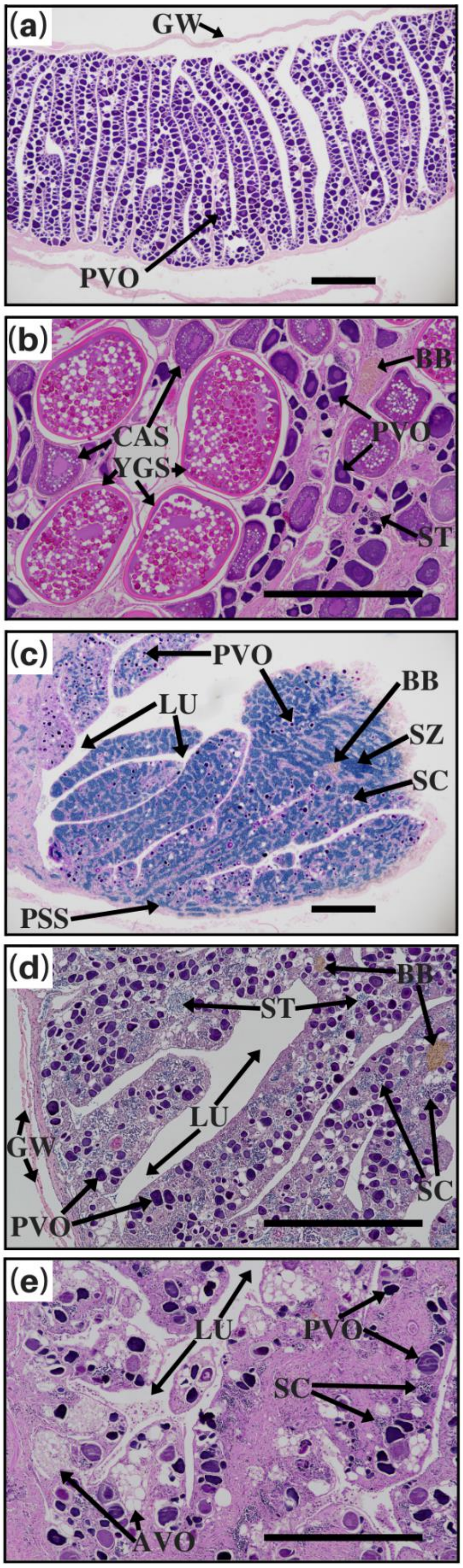

Figure 3.2 Histological features of gonad developmental stages of *Epinephelus bilobatus*: (a) immature female; (b) developed female with spermatogenic crypts; (c) active male; (d) bisexual; (e) transitional (AVO = atretic vitellogenic oocytes, BB = yellow-brown body, CAS = cortical alveoli stage oocytes, GW = gonad wall, LU = lumen, PSS = peripheral sperm sinus, PVO = pre-vitellogenic oocytes, SC = spermatocytes, ST = spermatids, SZ = spermatozoa, YGS = yolk globule stage oocytes (scale bar: 500µm).

3.3.3 Age and growth

Interpretations of alternating opaque and translucent growth zones in thin transverse sections of the sagittal otoliths of each *E. bilobatus* were used to estimate ages. One sagittal otolith from each specimen was embedded in epoxy resin and sectioned transversely through the primordium perpendicular to the sulcus acusticus (see Wakefield et al., 2017a). Two thin sections were cut (~230 µm) from each otolith using a low-speed Isomet saw (Buehler Ltd) with a diamond tipped blade. To improve the clarity of daily increments in very small individuals (i.e., < 100 mm L_T, $n = 8$), the transverse otolith sections were wet polished by hand using 30 µm then 5 µm diamond impregnated paper to achieve an otolith section thickness of ~150 µm. All sections were then rinsed in 2% HCl for ~20 seconds (see Gauldie et al., 1990), then rinsed in water, allowed to dry, and mounted on a glass slide with a cover slip using casting resin.

Thin otolith sections of each *E. bilobatus* were examined under reflected light at 20-40x magnification without any knowledge of their length or sex. The age of each fish was determined by counting delineated opaque zones, and the growth zone on the outer margin of each section was classified as either translucent or opaque. Ageing precision was determined by comparing opaque zones counts for a subset of otoliths ($n = 71$) from two experienced readers (CW & SN). The level of ageing precision was deemed acceptable based on an Index of Average Percent Error (IAPE) < 5.5% (Beamish and Fournier, 1981). All otolith sections were then examined by a primary reader (CW, $n = 1461$ aged). Sixty specimens were not aged because their otoliths were damaged or their otolith sections were deemed unreadable, and six of the aged fish had no length, but had gonads so were used in the reproductive analysis.

Marginal increment analysis (MIA) was used to validate that a single opaque and translucent growth zone was deposited annually in the otoliths of *E. bilobatus*. Considering numerous age-based studies on teleosts that inhabit the same waters as those sampled for *E. bilobatus*, have validated that growth zones in sectioned otoliths are formed annually (Stephenson and Hall, 2003; Wakefield et al., 2020a; Boddington et al., 2021), MIA was only examined for age cohorts around the mode in the overall age composition (i.e., those with 3 or 4 delineated opaque zones). MIA was expressed as the proportion of the distance between the outer edge of the most recently delineated opaque zone to the periphery of the otolith, to the distance between the outer edges of the two most recently delineated opaque zones. Marginal increments were measured along an axis from the primordium to the crista inferior adjacent to the sulcus acusticus. The measurements were made on images of the sectioned otoliths taken using a video camera (Nikon DS-Fi3) employing imaging software (Leica Application Suite V4.13). Annual trends in mean monthly marginal increments for *E. bilobatus* with 3 or 4 delineated opaque zones were used to determine that a single opaque and translucent growth zone was deposited in the otoliths annually.

The decimal age of each *E. bilobatus* was determined using the count of delineated opaque zones derived from sectioned otoliths, the time of year when opaque zones are typically deposited, a relative birth date (i.e., 1 September) and their date of capture. Daily increment counts, prior to the formation of the first annulus, improved estimates of ages for very small fish, which better informed early growth and subsequent growth parameters (i.e., t_0).

The von Bertalanffy growth equation was fitted to the length-at-age data to determine the growth of *E. bilobatus* from the Kimberley, Pilbara, and Gascoyne regions, separately. Considering the mean lengths of males were not positively correlated with age and the distribution of male lengths within each age class were comparable to those of females ages across the same range, it is highly likely that the growth of males is a continuation from that of females. For this reason, a single von Bertalanffy growth equation was fitted to the lengths at age of all individuals combined within each region, consistent with other growth studies of protogynous hermaphroditic teleosts (Lek et al., 2012; Wakefield et al., 2013; Boddington et al., 2021). The form of the von Bertalanffy equation was $L_t = L_\infty(1 - \exp^{-k(t-t_0)})$, where L_t is the predicted total length (mm) of fish at age t (years), L_∞ is the asymptotic total length (mm) at an infinite age, k is the growth coefficient, a constant that determines the rate at which L_t approaches L_∞ (year^{-1}), and t_0 is the hypothetical age (years) at which fish would have zero length. Growth curves were fitted using the least sum of squares, and estimates of L_∞, k and t_0 were determined by bootstrapping, where 1000 sets of parameters were obtained from the analysis of data produced by random resampling, with replacement. The von Bertalanffy growth parameters and lower and upper 95% CLs were calculated as the median, 2.5 and 97.5 percentiles, respectively, of the 1000 bootstrap estimates. Due to poor representation of relatively smaller and younger individuals from the Kimberley and Gascoyne regions, and a relatively low overall sample size from the Gascoyne, a two-parameter von Bertalanffy growth equation was fitted in these regions with t_0 fixed to that derived from the Pilbara region (i.e., $t_0 = -0.2$). The resulting growth curves for each region were compared using a likelihood ratio test (Cerrato, 1990).

3.3.4 Age-based selectivity and mortality

The instantaneous rate of total mortality (Z) for *E. bilobatus* was estimated using a modified catch curve analysis with age-based logistic selectivity for representative age composition data collected from the commercial trap fisheries in the Pilbara in 2012 ($n = 337$) and the NDSF in 2014 ($n = 338$). This analysis assumed that each population was in a state of equilibrium, i.e., recruitment and total mortality were constant across years. The numbers of *E. bilobatus* in each cohort in the age compositions suggested there was very little inter-annual recruitment variability or change in total mortality among years. The fitted catch curves used are similar to those described by (Thorson and Prager, 2011), and have been applied in previous assessments in north-western Australia to *Etelis boweni* (Wakefield et al., 2020b) and *Epinephelus areolatus* (Boddington et al., 2021). It assumed a constant natural mortality (M) and allowed for the estimation of fishing mortality (F, where $Z = F + M$), and logistic selectivity at age, which simulation testing has shown to provide more accurate F estimates than catch curve models that assume a knife-edge selectivity (Thorson and Prager, 2011). The selectivity at age (S_a) was calculated as:

$$S_a = \left\{1 + \exp\left[-\log_e(19)\frac{(a - A_{50}^{\mathrm{sel}})}{(A_{95}^{\mathrm{sel}} - A_{50}^{\mathrm{sel}})}\right]\right\}^{-1},$$

where A_{50}^{sel} and A_{95}^{sel} are the ages at which 50% and 95% of individuals were selected by the fishery, respectively.

The instantaneous rate of natural mortality (M) was assumed constant and calculated using the maximum observed age (A_{max}, years) of *E. bilobatus* in north-western Australia using the equation:

$$M = -\log_{\mathrm{e}}(x)/A_{\mathrm{max}},$$

where x is an assumed theoretical proportion of individuals that will survive to the maximum observed age. Recognising the importance of the parameter M and the difficulty of reliably estimating its value, three alternative values of this parameter were considered. Values for x

of 0.001, 0.01 and 0.015 were applied to provide three estimates of M (i.e., representing 0.1%, 1.0% and 1.5% survival to the maximum age), with values of 0.001 and 0.15 broadly corresponding to M estimates derived from the empirical equations for fish described by Hoenig (1983) and Then et al. (2015), respectively, as

$$M = 4.899\,A^{-0.916} \text{ and}$$

$$M = \exp(1.46 - (1.01\log_e(A))).$$

To account for uncertainty around each parameter estimated by the catch curve analysis (F, A_{50}^{sel} and A_{95}^{sel}), the model was fitted to each of 500 sets of age composition data, randomly resampled with replacement from the samples collected in each fishery, to derive the median value and the lower and upper 95% CLs of these bootstrapped estimates.

The F values for *E. bilobatus* were compared to target ($F = 0.67M$), threshold ($F = M$) and limit ($F = 1.5M$) established biological reference points (Wise et al., 2007; Williams et al., 2015). These data were then evaluated in relation to the likelihood (probability) of potential future levels of depletion (based on AS 4360 / ISO 31000 standards, see Fairclough et al., 2021), which consider a level of F between target and threshold infers a medium or acceptable level of risk; a level of F between threshold and limit infers a high or undesirable level of risk, while a level of F that exceeds the limit reference value infers a severe or unacceptable level of risk.

3.4 Results

3.4.1 Histological characteristics of gonads

A total of 1521 *E. bilobatus* were sampled, of which gonad histology was examined for 1460 individuals. Based on histology, 970 individuals were female, 431 were male, 26 were bisexual, 5 were transitional, and 28 were unclassified, with all gonads containing a membrane lined central lumen and numerous lamellae. Different stages of oocytes and sperm cells were located within the lamellae of ovaries and testes, respectively (Figure 3.2). Crypts of spermatids and spermatozoa ruptured and joined, and sperm were transported via intralobule sinuses to sperm sinuses in the outer gonad wall. Macrophage aggregates (yellow-brown bodies) were present in all sexes with varying prevalence (i.e., females 55%, males 99%, bisexuals 100%, transitionals 100%).

The majority of functioning females (i.e., 98% of stages II-VI ovaries sampled) only contained ovarian tissue, with the gonads of the remaining 2% (n =18) also possessing spermatogenic crypts in low numbers, up to the spermatid stage. Crypts were never observed in immature (stage I) or spawning females (stage V). The smallest and youngest female observed with spermatogenic crypts possessed spent ovaries (stage VI) and was 281 mm L_T and 3.5 years old. Males with primary growth stage oocytes in the chromatin nucleolus and perinucleolus stages were common (n = 103, 24% of all males) and ranged in length from 282 to 410 mm L_T, and in age from 2.8 to 13.4 years. Functioning males with atretic vitellogenic stage oocytes in the testes were rare (n = 9), and when present such oocytes were in very low numbers (typically only one or two) and never observed concurrently with primary growth stage oocytes. The males that had atretic vitellogenic oocytes occurred in all developmental categories and ranged in length from 307 to 393 mm L_T, and from 5.5. to 14.9 years of age.

The presence of parasite infestations and granulomas within the gonads of both females (49%) and males (87%) made interpreting some gonad histology difficult. The challenge was distinguishing and identifying if certain granulomas were enclosing the fishes own cells (i.e., atretic oocytes) or if it was protecting itself from the damage caused by past or present parasite activity. It is assumed the majority of the granulomas were a result of parasitic activity rather than atretic oocytes, because parasite infestation was prevalent during gonad dissection. Nonetheless, for specimens where the cause of the granulomas could not be determined with certainty, the functioning sex was still apparent, and they were thus included in the analyses.

3.4.2 Length-weight relationship, and length and age compositions

A broad length (i.e., 27 to 424 mm L_T) and weight (i.e., 0.2 to 1057.2 g W_W) range of *E. bilobatus* were sampled across all regions, and the resulting combined length-weight relationship for all sexes was $W_W = 0.00001379\ L_T^{\ 2.987}$ ($r^2 = 0.97$, n = 1352).

The length frequency compositions of *E. bilobatus* were very similar between the Kimberley and Pilbara regions (Figure 3.3), with unimodal distributions for both females and males. The mode in length frequencies for females was 280-320 mm L_T for both the Kimberley and Pilbara regions, with the modes for males also very similar between these two regions (i.e., 300-340 vs 320-360 mm L_T, respectively, Figure 3.3). The age frequency distributions for females of this species in the Kimberley and Pilbara regions both exhibited a single mode at 3 years, whereas the ages of males had a relatively broader unimodal distribution (Figure 3.3). Length and age frequency compositions were not examined for the Gascoyne region due to low sample sizes.

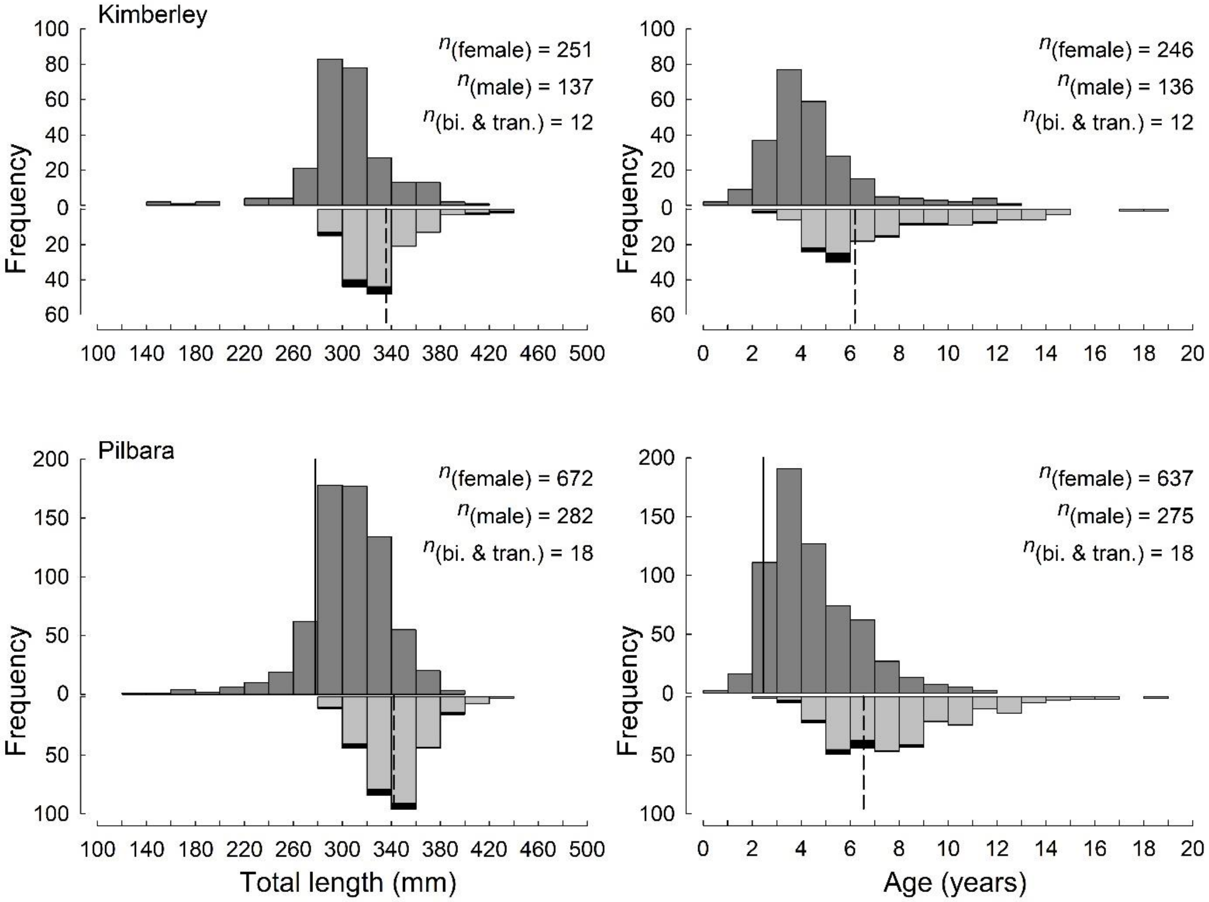

Figure 3.3 Length and age frequency compositions of female (dark grey bars), male (light grey bars) and bisexual combined with transitional (black bars) *Epinephelus bilobatus* collected in the Kimberley (above) and Pilbara (below) regions of Western Australia. Solid lines represent estimated lengths and ages at 50% female maturity, dashed lines represent estimated lengths and ages at 50% sex change.

Despite overlap in the length and age frequency distributions between sexes, females were more abundant in the smaller and younger classes, and conversely males were more abundant in the larger and older classes (Figure 3.3). The smallest and youngest fish from each region were females of 146 mm and 133 mm L_T and 0.94 and 0.97 years from the Kimberley and Pilbara, respectively. The smallest males sampled were 282 mm L_T (6.6 years of age) from the Kimberley and 289 mm L_T (6.4 years of age) from the Pilbara, and the youngest were 2.9 years (311 mm L_T) and 2.8 years (331 mm L_T) from these regions, respectively. In comparison, there were 210 females sampled that were smaller than the smallest male (n = 40 Kimberley, n = 170 Pilbara), and 120 females sampled that were younger than the youngest male (n = 29 Kimberley, n = 91 Pilbara). The largest and oldest fish collected from each region were males, measuring 435 mm (14.2 years) and 424 mm L_T (10.2 years), and 18.1 years (372 mm L_T) and 18.2 years (372 mm L_T) from the Kimberley and Pilbara regions respectively.

Transitional fish were uncommon (n = 4 Kimberley, and n = 1 Pilbara), and their lengths and ages ranged from 303 to 430 mm L_T and from 5.4 to 11.2 years. Each transitional fish was encountered during a different month of the year (i.e., February, March, June, August, and November). Bisexual fish were also relatively uncommon (n = 8 Kimberley, n = 17 Pilbara) and their lengths and ages were analogous to those of transitional fish, ranging from 291 to 406 mm L_T and from 2.9 years to 9.4 years. Bisexual fish were sampled in most months of the year.

3.4.3 Maturity and sex change

The estimated length and age at which 50% of female *E. bilobatus* reached sexual maturity in the Pilbara region (i.e., L_{50}^{mat} and A_{50}^{mat}) was 278 mm L_T and 2.5 years (Table 3.2; Figure 3.4). The lengths (n = 61) and ages (n = 57) of immature females (i.e., stages I and II) in this region ranged from 148 to 352 mm L_T and from 1.0 to 9.4 years. Whereas, the lengths (n = 144) and ages (n = 136) of mature females (i.e., stages III, IV, V and VI) ranged from 262 to 373 mm L_T and from 2.4 to 11.4 years.

The prevalence of males increased with length and age in both the Kimberley and Pilbara regions (Figures 3.4 and 3.5). Males accounted for ≥ 50% of all individuals from the

Kimberley region in all length classes greater than 320 mm L_T and age classes older than 5 years. In the Pilbara region, males reached 100% prevalence in all length classes above 400 mm L_T (n = 7) and age classes above 12 years (n = 27). The estimated lengths and ages at which 50% of females transitioned to males differed by less than 5% between regions, i.e., L_{50}^{sc} = 336 vs 342 mm L_T and A_{50}^{sc} = 6.2 vs 6.5 years in the Kimberley and Pilbara regions, respectively (Table 3.2; Figures 3.4 and 3.5).

Table 3.2 Total lengths (mm, L_T) and ages (years) at which 50% and 95% of female *Epinephelus bilobatus* reach sexual maturity in the Pilbara region, and change sex in the Pilbara and Kimberley regions of north-western Australia (with 95% lower and upper confidence limits).

	L_{50}	L_{95}	L_{min}	n	A_{50}	A_{95}	A_{min}	n
Pilbara								
Maturity								
Estimate	278	316	148	205	2.5	5.3	1.0	193
(low, upp)	(272, 283)	(303, 333)			(1.9, 2.8)	(4.3, 7.2)		
Sex Change								
Estimate	342	400	133	955	6.5	10.5	1.0	913
(low, upp)	(339, 347)	(390, 412)			(6.3, 6.9)	(9.7, 11.3)		
Kimberley								
Sex Change								
Estimate	336	431	146	392	6.2	11.8	0.9	386
(low, upp)	(327, 347)	(400, 474)			(5.6, 6.9)	(9.9, 14.0)		

Note: L_{min}, the minimum total length; A_{min}, the minimum age; n, sample size.

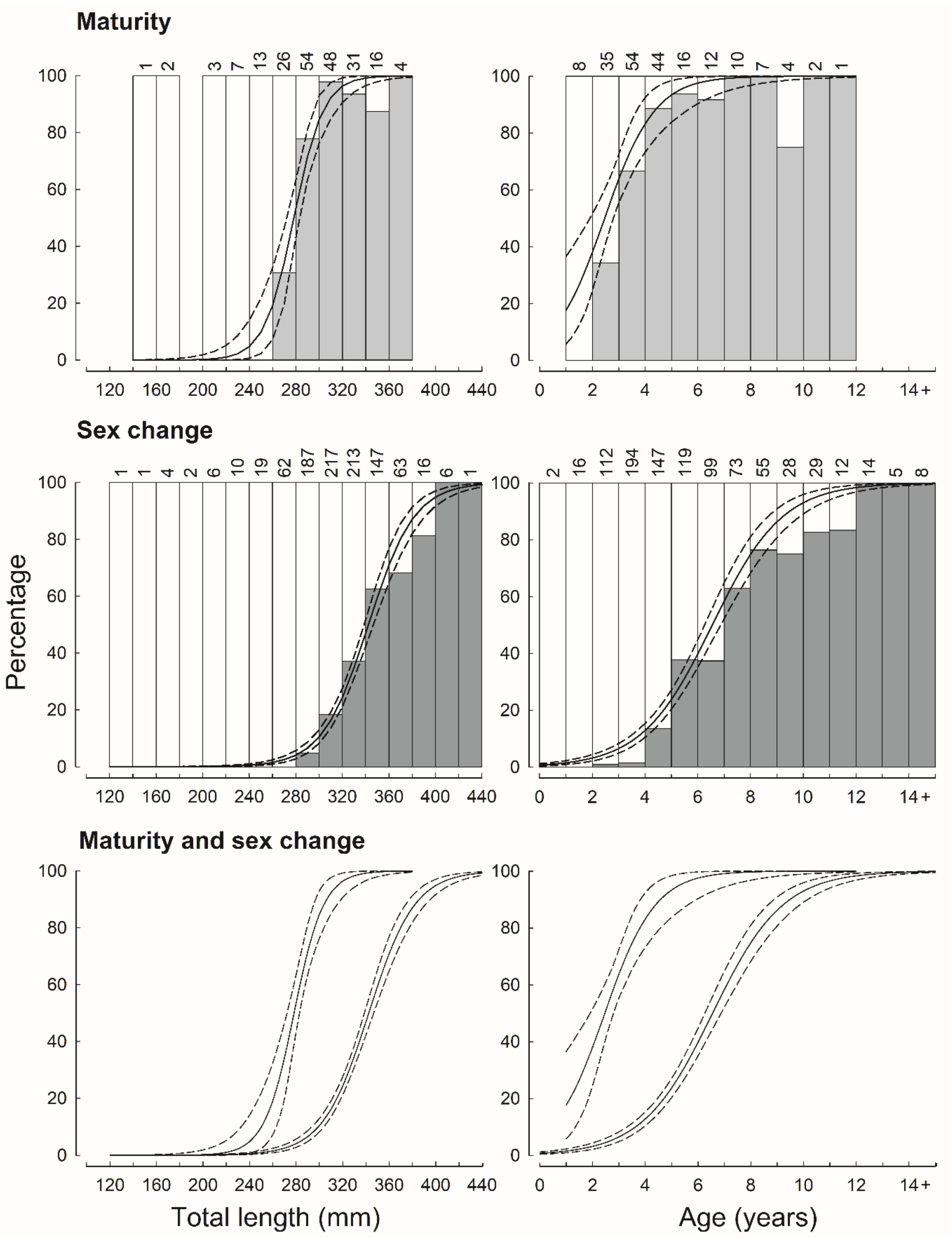

Figure 3.4 Length (left) and age (right) percentage frequencies of *Epinephelus bilobatus* with immature (stages I and II, white bars) or mature ovaries (stages III – VI, light grey bars, above); and females (white bars) or transitionals/males (dark grey bars, middle) from the Pilbara region, fitted with logistic curves (solid lines) and 95% confidence limits (dashed lines). Sample sizes shown above each class. Maturity and sex change ogives compared for length and age (below).

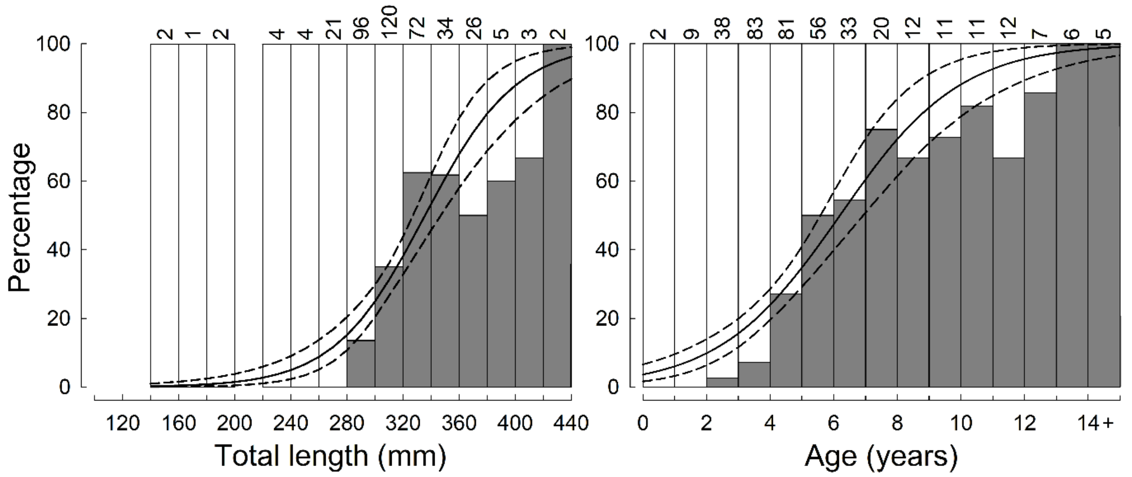

Figure 3.5 Length (left) and age (right) percentage frequencies of females (white bars) or transitional/male (dark grey bars) of *Epinephelus bilobatus* from the Kimberley region, fitted with logistic curves (solid lines) and 95% confidence limits (dashed lines). Sample sizes shown above each class.

3.4.4 Spawning period and its relation to day length and sea surface temperature

Female *E. bilobatus* greater than the L_{50}^{mat} that possessed either developed (stage IV) or spawning (stage V) ovaries were recorded in all months. However, the prevalence of females with stage IV or V ovaries was greatest (> 40% of fish sampled) for five months of the year, during the austral mid-summer to mid-autumn (i.e., January to April) and at the start of spring (i.e., September). In comparison, the prevalence of females with stage IV or V ovaries were lowest in mid-winter (i.e., 8% in July) and late spring (i.e., 1% in November, Figure 3.6). These months with a high prevalence of developed and spawning females corresponded with the highest mean monthly GSIs (> 0.95, Figure 3.6). Histological examination of mature ovaries determined all stages and size distributions of oocytes were present throughout the spawning period, which indicated that *E. bilobatus* is an indeterminate multiple batch spawner.

The biannual peaks in mean monthly GSIs and highest monthly prevalence of developed and spawning ovaries corresponded with the two annual periods when day length were at a mid-range of ~12 h. However, the longer four-month peak in spawning from January to April coincided with the period when day lengths were declining from an annual maxima, compared to the September spawning peak which coincided with an increasing trend in daylength (Figure 3.6). Conversely, the months of least spawning activity coincided with a photoperiod that was either at, or close to, an annual minima (i.e., ~11 h in June and July) or maxima (i.e., 13 h in November). The biannual peaks in reproductive activity also corresponded with opposing periods in annual water temperatures, i.e., close to the annual minima in September (~24 °C) and maxima in February and March (~30 °C, Figure 3.6).

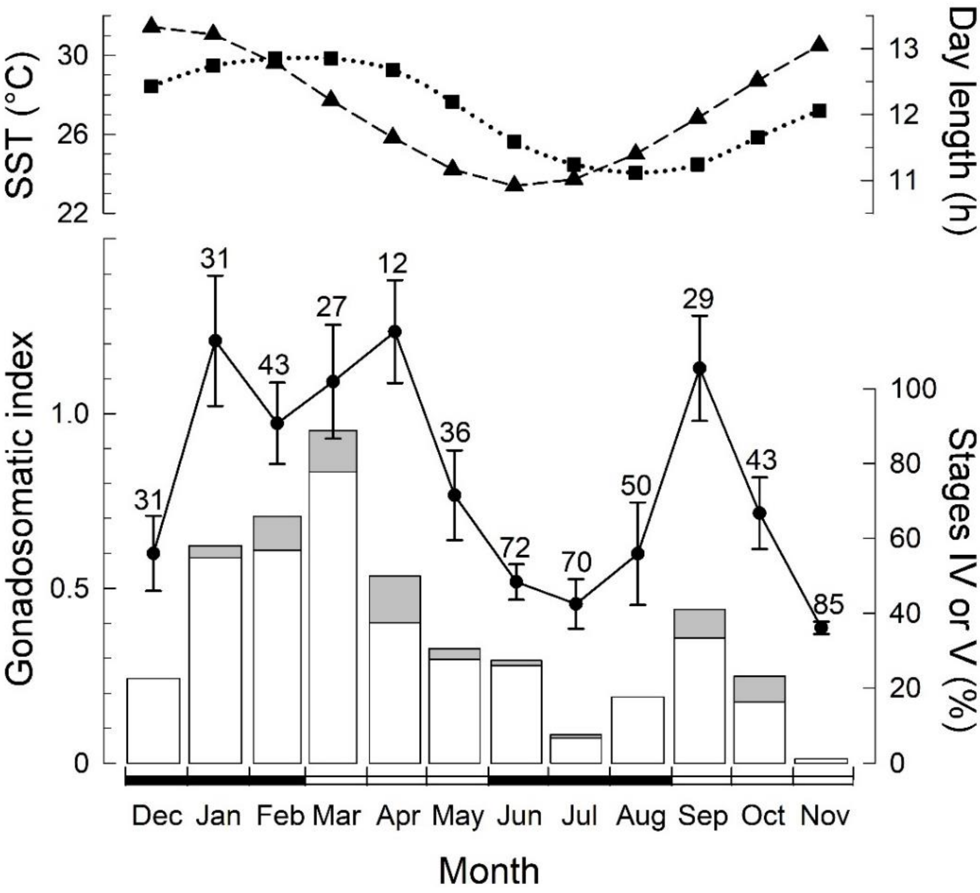

Figure 3.6 Mean monthly sea surface temperatures (■) and mean monthly daylight hours (▲) off the north-western coast of Western Australia (top) and mean monthly gonadsomatic indices (solid line with ± 1 SE) and monthly prevalence (%) of developed (stage IV, white bars) and spawning ovaries (stage V, grey bars) of female *Epinephelus bilobatus* from the Pilbara region (bottom). The data have been pooled for corresponding months (sample sizes shown) and are limited to fish ≥ the length at 50% maturity (i.e., 278 mm L_T). On the *x*-axis, black rectangles represent summer (Dec-Feb) and winter (Jun-Aug), and white rectangles represent autumn (Mar-May) and spring (Sep-Nov).

3.4.5 Aging precision and validation

Opaque zone counts from transverse sections of otoliths were examined independently by two readers, and the precision between counts was deemed acceptable based on an IAPE of 3.5% (i.e., < 5.5%, n = 71, range 2-14 years). Opaque zone counts between readers were the same for 66.2% of otoliths, differed by one for 29.6% of otoliths, and only one opaque zone count differed by more than two.

Mean monthly marginal increments derived from sectioned otoliths with three or four delineated opaque zones displayed an annual cyclic trend (Figure 3.7). Marginal increment values were highest when opaque zones were forming on the outer margin of the otolith during the austral winter (i.e., May to September). A precipitous decline in mean marginal increment values occurred in October during the austral spring when the newly formed opaque zone became delineated, and a thin translucent margin formed on the periphery of the otoliths. Over the ensuing seven months from October to April, the translucent outer margin became wider prior to the formation of another opaque zone (Figure 3.7). This trend demonstrates that one opaque followed by one translucent zone is deposited annually in the sagittal otoliths of *E. bilobatus* and thus counting the number of opaque zones in sectioned otoliths is an accurate method for determining age.

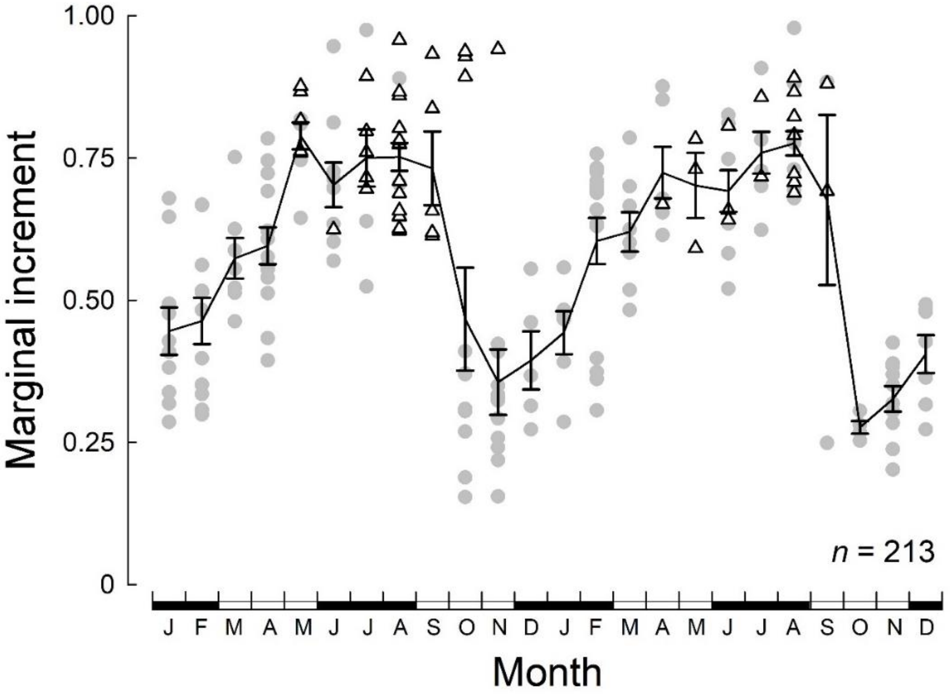

Figure 3.7 Individual (● translucent outer margins; △ opaque outer margins) and mean monthly (± 1 SE, lines) marginal increment values derived from sectioned otoliths with three and four delineated opaque zones displayed as consecutive calendar years for *Epinephelus bilobatus* sampled from north-western Australia. On the *x*-axis, black rectangles represent summer (Dec-Feb) and winter (Jun-Aug), and white rectangles represent autumn (Mar-May) and spring (Sep-Nov).

3.4.6 Age and growth

The von Bertalanffy growth curve provided a good fit to the length-at-age data for all fish from the Pilbara region, where a larger number of smaller and younger fish were sampled.

These younger juveniles included fish with ages determined from daily growth increments and ranged in age from 22 to 110 days (27 to 82 mm L_T, $n = 7$), which resulted in a very reasonable estimate of t_0 of -0.2 (Table 3.3; Figure 3.8). The likelihood ratio test demonstrated that the two parameter von Bertalanffy growth curve for the Kimberley region was not significantly different to the three parameter von Bertalanffy growth curve for the Pilbara region ($P = 0.089$, Figure 3.8), and thus a growth curve that combined length-at-age data for both regions was appropriate (Table 3.3). This combined growth curve for the two northern regions (i.e., Kimberley and Pilbara) differed significantly to the two-parameter growth curve for the more southern Gascoyne region ($P < 0.05$), where *E. bilobatus* had a greater estimated length at ages after the fish reached 3 years of age (Figure 3.8). For example, the estimated lengths at 5, 10 and 20 years were 326, 345 and 347 mm L_T in the two northern regions combined, compared to 350, 383 and 387 mm L_T in the southern Gascoyne region (i.e., ~10% larger at the maximum age). In the Gascoyne region, the asymptotic length (L_∞) was greater and the growth coefficient (k) was lower compared to the warmer northern regions (Table 3.3), suggesting the growth of this species was relatively slower initially (i.e., < 3 years of age) but attained a larger size in the cooler higher latitudes toward the southern extent of its range.

Table 3.3 von Bertalanffy growth parameters (estimates and 95% lower and upper confidence limits) for curves fitted to the total lengths (L_T) at age of *Epinephelus bilobatus* from the Kimberley, Pilbara, and Gascoyne regions of north-western Australia.

	L_∞ (mm)	k (yr^{-1})	t_0 (yr)	A_{max} (yr)	L_{max} (mm)	n
Kimberley						
Estimate	346	0.53	-0.20†	20.2	435	418
(low, upp)	(340, 352)	(0.49, 0.57)				
Pilbara						
Estimate	347	0.54	-0.20	18.2	424	979
(low, upp)	(344, 351)	(0.50, 0.58)	(-0.37, -0.11)			
Gascoyne						
Estimate	387	0.45	-0.20†	18.0	461	58
(low, upp)	(373, 402)	(0.39, 0.53)				
Kimberley and Pilbara combined						
Estimate	348	0.52	-0.29	20.2	435	1397
(low, upp)	(345, 352)	(0.47, 0.55)	(-0.49, -0.18)			

L_∞, hypothetical asymptotic length at an infinite age; k, growth coefficient; t_0, hypothetical age at zero length; A_{max}, maximum age; L_{max}, maximum length; n, sample size.
† t_0 is fixed in the Kimberley and Gascoyne region.

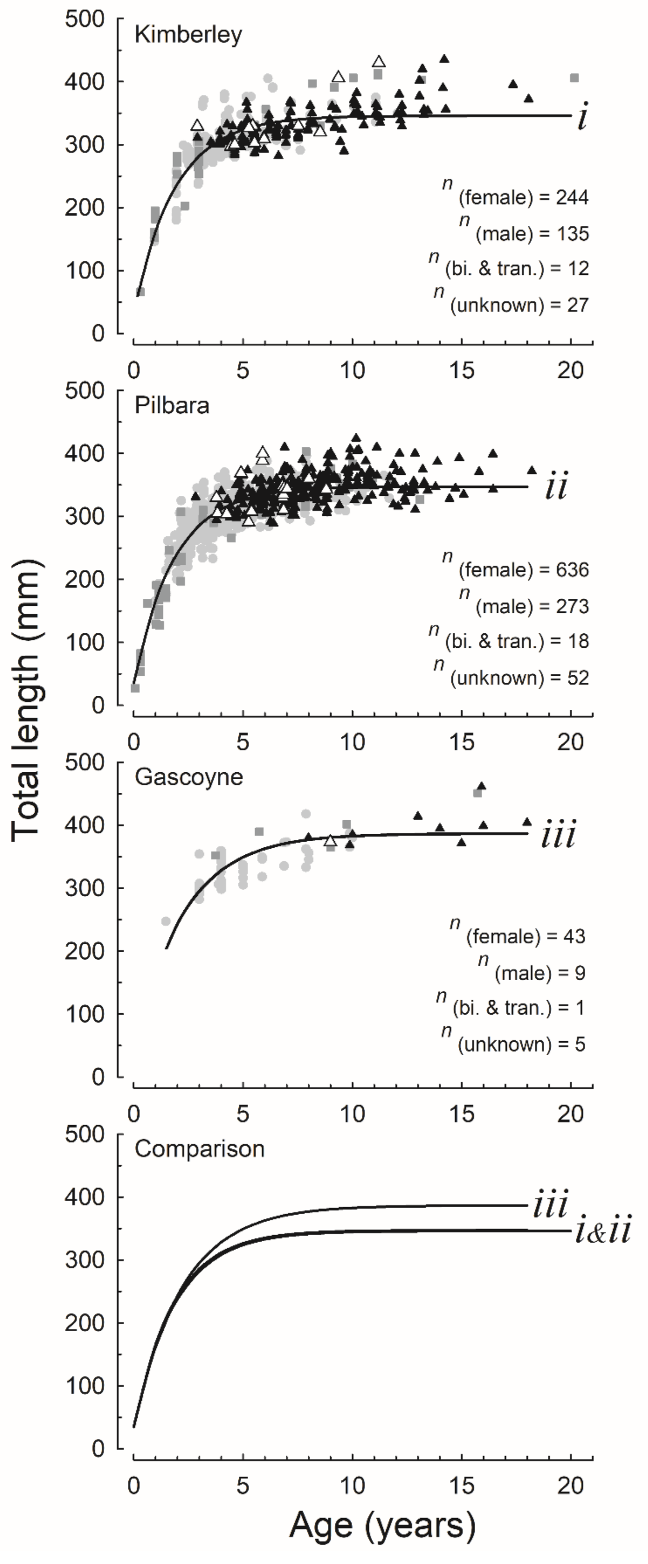

Figure 3.8 von Bertalanffy (vB) growth curves fitted to the lengths-at-age for female (●), male (▲), bisexual/transitional (△), and unknown sexes (■) of *Epinephelus bilobatus* caught in the Kimberley, Pilbara and Gascoyne regions of Western Australia (sample sizes are shown, *n*). vB growth curves compared (below)

3.4.7 Age-based selectivity and mortality

Representative age composition data for *E. bilobatus* collected from the commercial trap fishery in the Kimberley in 2014, and Pilbara in 2012, had the same age range (i.e., 2 to 18 years) and similar sample sizes (Figure 3.9). However, estimates of selectivity at age differed between the two regions with the age at which 50% of fish were selected by the commercial fish traps being 2.5 years vs 3.8 years in the Kimberley and Pilbara, respectively (Table 3.4). Estimates of the instantaneous rate of total mortality were slightly higher in the Pilbara (Z = 0.40) than in the Kimberley (Z = 0.36), however the 95% CLs for these estimates overlapped between regions.

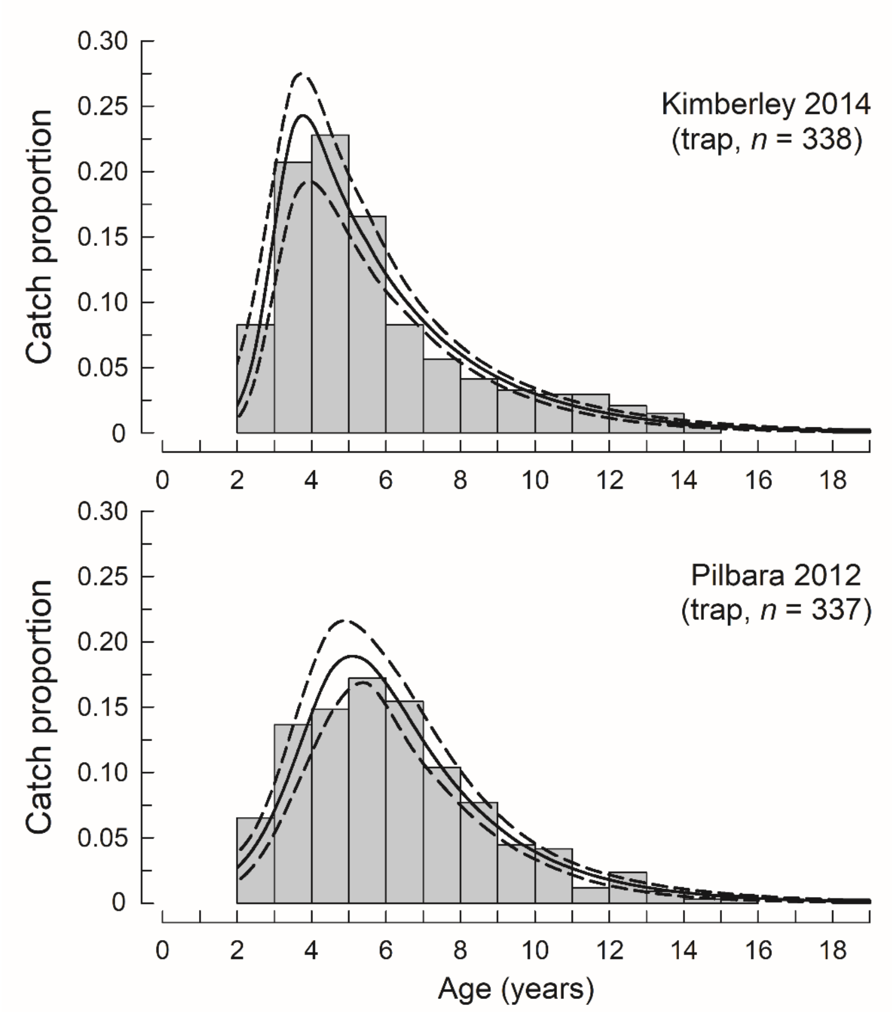

Figure 3.9 Catch curves assuming logistic selectivity (±95% CLs) fitted to the age compositions for *Epinephelus bilobatus* sampled from the commercial trap fisheries in the Kimberley (in 2014, above) and Pilbara (in 2012, below) regions of Western Australia.

Table 3.4 Estimates of fishing mortality (F) from catch curve analysis of fully selected fish and age-based selectivity parameters (A_{50}^{sel} and A_{95}^{sel}) with lower and upper 95% confidence limits for *Epinephelus bilobatus* sampled from the Kimberley region in 2014 and the Pilbara region in 2012.

	$\boldsymbol{M}$**=0.34**	$\boldsymbol{M}$**=0.23**	$\boldsymbol{M}$**=0.21**			
	$\boldsymbol{F}$ **(year^{-1})**	$\boldsymbol{F}$ **(year^{-1})**	$\boldsymbol{F}$ **(year^{-1})**	$\boldsymbol{A_{50}^{\mathrm{sel}}}$ **(years)**	$\boldsymbol{A_{95}^{\mathrm{sel}}}$ **(years)**	$\boldsymbol{n}$
Kimberley						
Estimate	*	0.13	0.15	2.5	3.5	338
(low, upp)	* *	(0.08, 0.19)	(0.10, 0.21)	(2.1, 3.0)	(2.7, 4.5)	
Pilbara						
Estimate	0.05	0.17	0.19	3.8	6.0	337
(low, upp)	(0.01, 0.21)	(0.11, 0.26)	(0.13, 0.28)	(3.2, 4.6)	(4.8, 7.6)	

Note: F estimates were calculated based on three alternative values of natural mortality (M).
* Values not considered biologically plausible; *n*: sample size.

Based on the assumption that 0.1%, 1.0% and 1.5% of *E. bilobatus* survive to a maximum age of 20 years, estimates of natural mortality (*M*) were 0.34, 0.23 and 0.21 year $^{-1}$, respectively. The highest rate of *M* was not considered biologically plausible for the Kimberley region as it exceeded the estimated value of *Z* to yield negative estimates of *F*. Applying a moderate level of natural mortality ($M = 0.23$ year $^{-1}$), based on 1.0% survival to the maximum age, resulted in an estimated *F* of 0.13 and 0.17 year $^{-1}$ in the Kimberley and Pilbara regions, respectively (i.e., $F \approx F_{\mathrm{target}}$ for both regions). In comparison, a more conservative value of natural mortality ($M = 0.21$ year $^{-1}$), based on 1.5% survival to the maximum age, resulted in a higher estimate of *F* in both regions, i.e., 0.15 and 0.19 year $^{-1}$ in the Kimberley and Pilbara, respectively (i.e., $F_{\mathrm{target}} < F < F_{\mathrm{threshold}}$ for both regions). The upper 95% CLs for *F* from both estimates were below or equal to *M* in the Kimberley region and above *M* in the Pilbara region (Table 3.4).

3.5 Discussion

This is the first study of the reproduction, age, growth, and mortality of *E. bilobatus*, across most of its range along the continental shelf of north-western Australia. This species is harvested in these waters by state managed multispecies commercial and recreational fisheries (i.e., fish trap, trawl, and line) in low quantities compared to other demersal teleosts (Newman et al., 2021b). Due to resource limitations and the large number of commercially and recreationally important species along the WA coastline, studies of the biology and stock status of populations of many individual species, such as *E. bilobatus*, are rarely possible. Instead, indicator species have been selected based on a matrix of risk, (i.e., inherent vulnerability, current risk to wild stock, and management importance, see Newman et al., 2018a), and stocks of these indicator species are monitored and assessed regularly. The status of these indicator species is assumed to represent that of the suite of exploited species within that fisheries resource, including *E. bilobatus* (Department of Fisheries, 2011; Newman et al., 2018a). The life-history information and mortality estimates from this study, improve our understanding of the population dynamics of *E. bilobatus,* and suggested overfishing was not occurring at the time of sampling ($F \leq {\sim}M$), and also provides evidence that the indicator species monitoring approach implemented in WA has been effective and paradigmatic for the management of second tier non-indicator species.

3.5.1 Evidence for protogynous hermaphroditism in *Epinephelus bilobatus*

Multiple lines of evidence based on detailed histological analysis of a wide length and age range of juveniles and adults determined that *E. bilobatus* was a monandric protogynous hermaphrodite (i.e., all males are derived from adult females). Strong evidence confirming this reproductive mode was the presence of fish with transitional gonads ($n = 5$; 0.35% of classified gonads) that simultaneously possessed degenerating vitellogenic stage oocytes and proliferating crypts of spermatocytes and spermatids (Sadovy and Shapiro, 1987). The rare occurrence of transitional fish is consistent with other studies of epinephelids, where transitional fish typically represent < 3% of the overall samples (Liu and Sadovy, 2004). Gonad morphology demonstrated that males were secondarily derived as the testes contained an ovarian-like lumen and lamellar organisation, remnant from the previous female phase. Further, peripheral sperm sinuses developed within the gonad wall for the transport of sperm. Yellow-brown bodies can be caused by a range of factors including atresia of oocytes (Agius and Roberts, 2003), it is therefore plausible that some of the yellow-brown bodies present within the male gonads (99% prevalence) further denote secondary male development.

Marked variations in the length and age distributions between females and males should not be used primarily to diagnose sexual pattern (Sadovy and Shapiro, 1987). However, when considered with other lines of evidence, such as histological characteristics of gonads, the length and age frequency compositions were consistent with *E. bilobatus* exhibiting monandric protogynous hermaphroditism. The absence of any males below the length and age of 50% female maturity (i.e., 278 mm L_T and 2.5 years) provides further circumstantial evidence that *E. bilobatus* begins life as a female.

The presence of oocytes in the testes of functioning male *E. bilobatus* were identified in two forms, firstly, the common occurrence of previtellogenic oocytes in the chromatin nucleolus and perinucleolus stages; and secondly, the rare occurrence of a small number of atretic vitellogenic stage oocytes. Primary growth stage oocytes can sometimes remain in the testes of epinephelids after sex change (Sadovy de Mitcheson and Liu, 2008). However, this can also be the result of other factors, including detrimental environmental conditions or an earlier bisexual phase (Sadovy and Shapiro, 1987). Functioning males containing a small number of atretic vitellogenic oocytes within the testes has been observed in *Cephalopholis boenak* (Chan and Sadovy, 2002), and Nolan et al. (2017) classified this characteristic as intersex in *Epinephelus adscensionis*.

The presence of non-functional spermatogenic crypts in low numbers (up to the spermatid stage), dispersed within the ovaries of mature females was rare (2%), in comparison to other studies, e.g. ~50% in *Cephalopholis boenak* (Chan and Sadovy, 2002), 46% in *E. rivulatus* (Mackie, 2000) and 23% in *E. areolatus* (Boddington et al., 2021). All ovaries containing crypts of non-functional spermatogenic tissue in *E. bilobatus* were above the estimated length and age at which 50% of females reached sexual maturity (i.e., L_{50}^{mat} and A_{50}^{mat}), and these individuals were not classified as bisexual or transitional due to the low quantity of spematogenic tissue and the dominance of oocytes at various maturity stages. Lowerre-Barbieri et al. (2011) propose that long before transitioning, sequential hermaphrodites will typically display small amounts of non-functional terminal sex gametes. The present study considers the small number of functioning females with crypts of non-functional spermatogenic tissue as either the early stage of the transition process, and/or individuals being ready for a rapid transition.

An unclear definition of the transitional phase can lead to a misdiagnosis of sexual pattern (Sadovy de Mitcheson and Liu, 2008). Transitional fish were defined as individuals with gonads exhibiting the breakdown of vitellogenic stage oocytes and the proliferation of spermatogenic tissue, indicating previous function as a mature female and the direction of sex change (Sadovy and Shapiro, 1987; Sadovy de Mitcheson and Liu, 2008). The gonads of both bisexual and transitional individuals contained previtellogenic oocytes, which were typically in the chromatin nucleolus and perinucleolus stages but could include cortical alveolar oocytes. The inclusion of cortical alveolar oocytes within the primary growth stage follows the affirmation by Grier et al. (2009) that this step in oocyte development occurs just prior to the commencement of secondary growth (i.e., vitellogenesis). Sadovy de Mitcheson and Liu (2008) further assert that the presence of cortical alveolar oocytes cannot be used as an indication of past or future female maturity. Bisexual fish did not have secondary growth oocytes present, instead the lamellae were filled with similar proportions of immature ovarian and testicular tissue and the functional direction was uncertain. It is suspected that bisexual individuals are transitioning during a period when the fish are not in spawning condition and, therefore, cannot meet the definition of transition required to confirm sexual pattern. This is supported by all bisexuals being above the L_{50}^{mat} and A_{50}^{mat} for female maturity along with morphological evidence suggesting prior spawning as a female, such as muscle bundles, vascular tissue, blood vessels and the presence of yellow-brown bodies. The viewpoint that bisexual individuals are a transitional phase that occurs out of spawning condition was also considered for *E. areolatus* (Boddington et al., 2021) and *Cephalopholis panamensis* (Erisman et al., 2010). However, as noted in these studies, bisexuals could not be used to diagnose sexual pattern. Bisexual and transitional fish were encountered across 10 months of the year and bisexual gonads were most frequent (52%) during the short period of least spawning activity during June and July. The time of sex change may be initiated post spawning when steroid levels are low (Frisch, 2004), and/or at a time when environmental and/or social cues are provided (Sadovy and Shapiro, 1987; Fennessy, 2006). However, the influence of these intrinsic and extrinsic factors on the timing of sex change is difficult to correlate for species that exhibit protracted year-round spawning (e.g., *E. areolatus*, Boddington et al., 2021).

3.5.2 Spawning period

The year-round presence of developed and/or spawning ovaries, asynchronous oocyte development, and the annual trends in mean monthly GSIs demonstrated that *E. bilobatus* has a protracted biannual spawning period with a short peak in early spring (i.e., September) and longer peak in mid-summer to mid-autumn (i.e., January to April). These biannual peaks in spawning both coincided with the same daylength (i.e., ~12 h) within the annual cycle in photoperiod, but at opposing periods of high and low water temperatures. The protracted spawning period is consistent with the general pattern that smaller grouper species tend to spawn over prolonged periods (Sadovy, 1996). A year-round spawning period with biannual peaks in reproduction corresponding with mid-cycle daylengths was also observed for *E. areolatus* from the same geographical region (Boddington et al., 2021). Srinivasan and Jones (2006) observed year-round recruitment at varying rates in some species of coral reef fish at lower latitudes in Papua New Guinea.

3.5.3 Lengths and ages at maturity and sex change

The estimated lengths and ages at which 50% of female *E. bilobatus* reached sexual maturity in the Pilbara region was 278 mm L_T and 2.5 years. In general, groupers mature at 40-60% of their maximum length (Shapiro, 1987a; Wakefield et al., 2015). Based on the life-history invariant relationship between maturity and growth (i.e., $L_{50}^{\text{mat}}/L_{\text{max}}$), this species matured at 65.6% of its maximum recorded length in this region. This relationship was consistent with that recorded for *E. areolatus* from the same location (61.3%; Boddington et al., 2021), but slightly higher than that recorded for other smaller groupers, i.e., 50.9% for *E. rivulatus* (Mackie, 2000) and 56.7% for *C. boenak* (Chan and Sadovy, 2002).

There was very little regional difference in the lengths and ages at which 50% of *E. bilobatus* were estimated to change sex (i.e., < 2%, L_{50}^{sc} = 336 and 342 mm L_T and A_{50}^{sc} = 6.2 and 6.5 years for the Kimberley and Pilbara regions, respectively). These similarities in the lengths and ages at sex change were consistent with growth trajectories between these two regions, such symmetries can be explained by the life-history invariant relationships that predict growth and reproductive schedules (Charnov, 1993). As such, L_{50}^{sc} occurred at 77.2% and 80.1% of the maximum recorded lengths in the Kimberley and Pilbara regions, respectively. This life-history invariant relationship was consistent with another small grouper from north-western Australia (i.e., *E. areolatus*), where $L_{50}^{\text{sc}}/L_{\text{max}}$ = 76.6% and 80.4% in the Kimberley and Pilbara regions, respectively (Boddington et al., 2021). The prediction that on average sex change will occur at 80% of the maximum length (Allsop and West, 2003; Wakefield et al., 2015) maybe useful to approximate estimates of the length of sex change in analogous grouper species for which life-history information is limited.

3.5.4 Validation of ageing methods and growth

MIA confirmed the annual formation of one opaque and one translucent growth zone in the sagittal otoliths of *E. bilobatus*. This species has a moderate longevity of ~20 years and is relatively fast growing reaching, on average, 82% of the asymptotic length at 3 years of age. The metabolic theory of ecology (MTE) predicts that in cooler latitudes, a teleost species will typically attain a larger body size, live longer, and grow slower early in life (i.e., the rate at which the asymptotic length is approached, *k*) than in warmer latitudes (Kozłowski et al., 2004; Wakefield et al., 2017b). Despite *E. bilobatus* having a wide latitudinal range in WA (i.e., ~14 - 27°S), this study found no significant differences in the growth and longevity of this species across most of its geographic range in the two warmer northern regions (i.e., 14.0 - 21.5°S in the Kimberley and Pilbara regions). However, in the cooler and most southern region of its distribution, at the edge of its geographic range (i.e., > 21.5°S in the Gascoyne region), this species reached a significantly larger length-at-age. Thus, it is difficult to disentangle whether the trends in age and growth exhibited in this study are consistent with an edge-of-range effect, and/or correspond with those predicted by the MTE (e.g., Pörtner et al., 2001; Osovitz and Hofmann, 2007; Wakefield et al., 2007). It is acknowledged that a relatively smaller sample size was collected from the Gascoyne region, which may have influenced the growth parameter estimates, thus additional samples may need to be collected for future studies. Many shallow water teleosts in WA with distributions that span significant latitudinal ranges and temperature gradients have shown trends in age and growth traits that are consistent with the temperature-size rule predicted by the MTE (Wakefield et al., 2020a). Departures from such trends predicted by the MTE have also been exhibited along this coastline, including no significant differences (e.g., Hesp et al., 2004a), a converse relationship (e.g., Cossington et al., 2010), or a parabolic relationship with increased or decreased growth performance toward the extents of a species latitudinal distribution (i.e., edge of range effects, Wakefield et al., 2017b; Boddington et al., 2021).

3.5.5 Selectivity and fishing mortality

The estimated age at which 50% of *E bilobatus* were retained in the Kimberley and Pilbara trap fisheries was 2.5 and 3.8 years, respectively. In comparison, the estimated age at 50% maturity in the Pilbara was 2.5 years, indicating that, in the Pilbara trap fishery, a large proportion of individuals have likely spawned prior to capture, which would contribute toward their sustainable harvest. The absence of fish below 2 years of age being captured by these multispecies trap fisheries (see Newman et al., 2011), indicates the mesh size (50 mm square weld mesh) facilitates escapement for these younger and smaller individuals.

The exploitation levels of a fish stock can be assessed by comparing estimates of fishing mortality (F) to biological reference points based on ratios of natural mortality (M). For a demersal species with a longevity greater than 10 years, Wise et al. (2007) established reference points for fishing mortality rates as F_{target} (i.e., $F = 0.67M$) as an optimum exploitation level and $F_{\text{threshold}}$ (i.e., $F = M$) as a level for additional management action. Due to the direct effect of M on the calculation of reference points and its large influence on other mortality parameters (Maunder et al., 2023), three values of M were considered. Firstly, applying the highest value for M (most optimistic scenario) in the Pilbara trap fishery, F was below F_{target}. Secondly, applying a moderate value for M, point estimates for F were below F_{target} in the Kimberley trap fishery and slightly higher than F_{target} in the Pilbara trap fishery. Finally, applying the lowest value for M (i.e., least optimistic scenario), F estimates in both trap fisheries were between F_{target} and $F_{\text{threshold}}$. Considering all point estimates of F were below the three estimated values of M (i.e., $F_{\text{threshold}}$) for both regions, there was a relatively low probability of overfishing occurring at the time of sampling. This indicates that at the time of sampling the level of F in relation to international recognised biological reference values (Wise et al., 2007; Williams et al., 2015) denoted a medium or acceptable level of risk to stock sustainability under current management arrangements, and therefore no additional management action would have been required.

3.5.6 Conclusions and management implications

This study has improved our understanding of the life-history characteristics of *E bilobatus* from north-western Australia and will provide a baseline for future assessments of stock status. *Epinephelus bilobatus* is a relatively small (< 50 cm) monandric protogynous hermaphrodite, with life-history traits suggesting relatively high population productivity, including relatively fast growth, moderate longevity (~20 years), early maturation, and year-round spawning. They have maintained a long history (> 20 years) of low catches as byproduct mixed with other small grouper species, in the multi-species demersal scalefish fisheries in the NDSF and PDSF. Fishing mortality estimates concluded that *E. bilobatus* is currently being fished sustainably in these multispecies trap fisheries, implying that the indicator species management approach has been effective and paradigmatic for this species. Although periodic monitoring would be beneficial to directly confirm ongoing sustainability of *E. bilobatus*, the current indicator species approach will likely indirectly manage to maintain a sustainable population.

Chapter 4: General Discussion

4.1 Summary of key findings

This thesis described the life-history characteristics and determined mortality estimates of two groupers, *Epinephelus areolatus* and *E. bilobatus* off the north-western coast of Australia. Life-history studies of this magnitude, across multiple fisheries covering a large latitudinal range, are rarely possible. This general discussion identifies the key findings established for each species in chapters 2 and 3, considers the management of hermaphroditic species, and reviews parts of the study which could use further investigations and possible future directions (outlined in a conceptual flow diagram, Figure 4.1).

Epinephelus areolatus and *E. bilobatus* are both relatively small groupers attaining similar adult sizes (< 50 cm total length) and ages (~20 years). *Epinephelus areolatus* has a very broad Indo-Pacific distribution, whereas *E. bilobatus* has a narrow distribution in the eastern Indian Ocean (i.e., north-western Australia and parts of Indonesia). Both species comprise a valuable component in the commercial multispecies demersal fisheries in north-western Australia. Based on proportions by weight from the mixed cods landed in the NDSF and PDSF combined, the estimated 2021 annual catch of each species was ~ 99 t and 5 t for *E. areolatus* and *E. bilobatus*, respectively (derived from fishery-independent surveys and length-weight relationships, DPIRD, unpublished data).

Histological analysis of the gonads of *E. areolatus* and *E. bilobatus* determined both species exhibited monandric protogynous hermaphroditism (i.e., all males are secondarily derived from adult females). The presence of non-functional spermatogenic crypts dispersed within the ovaries of mature functioning females was a primary difference observed between the reproductive strategies of *E. areolatus* (23% presence) and *E. bilobatus* (2% presence). The length and age at which 50% of females matured in the Pilbara was similar for both species, i.e., 266 mm L_T and 2.7 years for *E. areolatus* and 278 mm L_T and 2.5 years for *E. bilobatus*. A protracted annual spawning period extending over 10-12 months, containing biannual peaks separated by a period of markedly lower spawning activity during winter (June and July) was recorded for both species. The lengths (L_{50}^{sc}) and ages (A_{50}^{sc}) at which 50% of females changed sex to male was similar between regions (i.e., < 5% variation between the Kimberley and

Pilbara) for both species. In the Kimberley, L_{50}^{sc} was 364 and 336 mm L_T and A_{50}^{sc} was 7.9 and 6.2 years for *E. areolatus* and *E. bilobatus* respectively, and in the Pilbara, L_{50}^{sc} was 349 and 342 mm L_T and A_{50}^{sc} was 7.3 and 6.5 years for *E. areolatus* and *E. bilobatus*, respectively.

Marginal increment analysis determined that one opaque and one translucent zone is deposited annually in the sagittal otoliths of *E. areolatus* and *E. bilobatus*. A parabolic relationship was observed for the growth characteristics exhibited by *E. areolatus* in relation to latitude, and both *E. areolatus* and *E. bilobatus* exhibited faster growth rates and attained a larger mean length-at-age in the cooler more southern Gascoyne region. Comparing fishing mortality estimates to biological reference points based on ratios of natural mortality ascertained that there was a relatively low probability that either *E. areolatus* or *E. bilobatus* were being overfished in the Kimberley or Pilbara trap fisheries at the time of sampling.

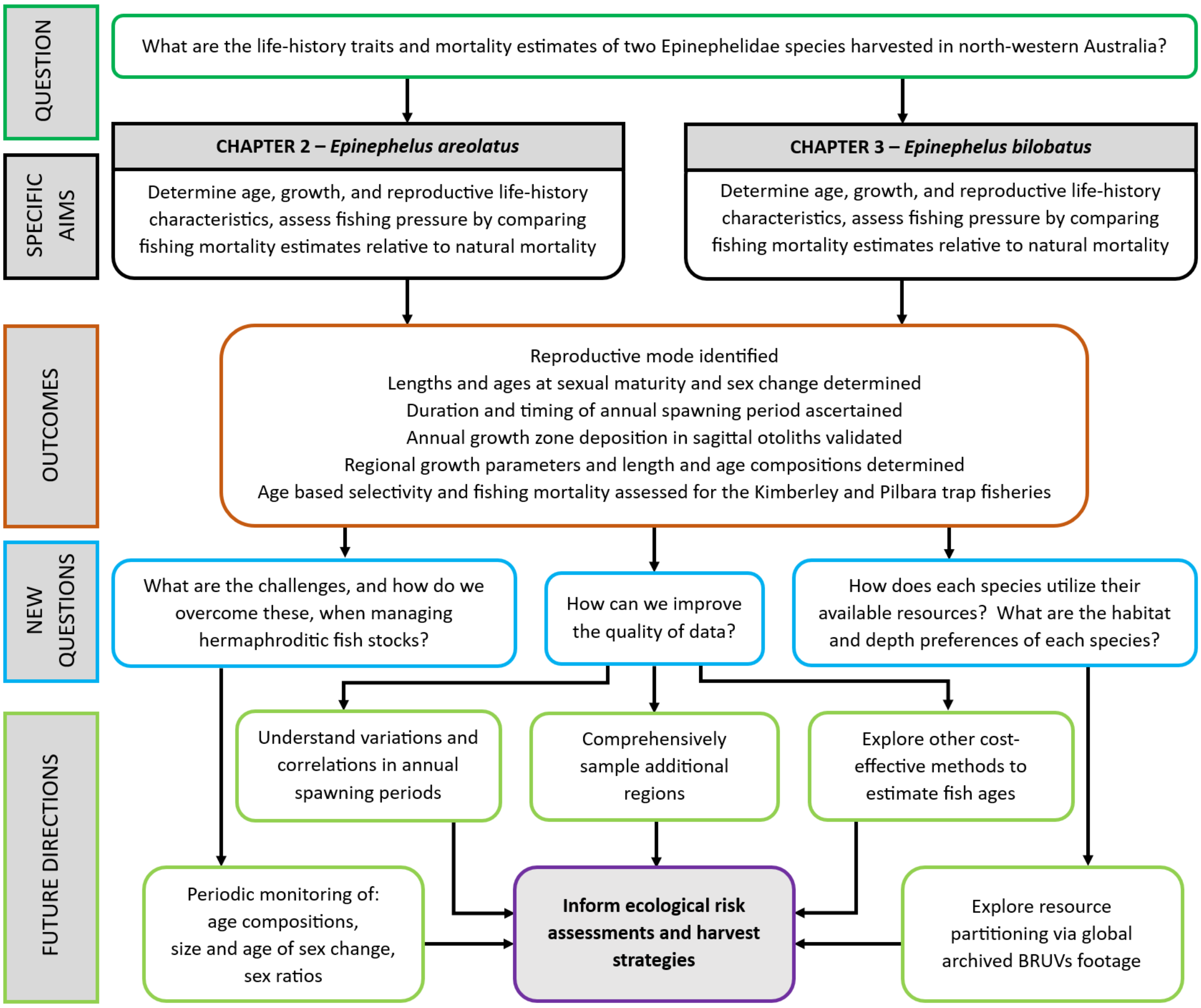

Figure 4.1 Conceptual flow diagram outlining thesis outcomes and future directions.

4.2 Management of protogynous hermaphrodites

Reliable data on the reproductive biology of a species is essential for sound management practices. Protogynous species such as *E. areolatus* and *E. bilobatus* present a challenge for management because size selective fisheries can potentially reduce their reproductive success via targeting and removing the larger older individuals (Vincent and Sadovy, 1998; Alonzo and Mangel, 2004). The removal of males can cause the sex ratio to become increasingly female biased creating the potential for sperm limitation (Vincent and Sadovy, 1998; Sadovy de Mitcheson and Liu, 2022), which can reduce fertilisation rates and eventually decrease population growth (Alonzo and Mangel, 2004; Provost and Jensen, 2015; Robinson et al., 2017). The significance of altered sex ratios and its potential impact on fertilisation rates is one of the uncertainties encountered in stock assessment models of protogynous fish (Brooks et al., 2008; Provost and Jensen, 2015).

A recent review by Provost and Jensen (2015) established that the effects of fishing on hermaphroditic species cannot be generalised. Instead, the impacts of fishing are likely dependent on species-specific life-history characteristics. For instance, there is known variability among species exhibiting sex specific behaviours (e.g., some species display aggressive male behaviour when guarding females or defending territories) and certain species undertake sexually dimorphic growth (e.g., to compete with existing males some hermaphrodites have a burst of growth after sex change), which can contribute to catchability and make one sex more vulnerable to fishing pressure (Provost and Jensen, 2015). Thus, effective stock assessment models tailored for protogynous species need to consider species specific life-history traits, changes in the sex ratio over time, changes in the size and age of sex change overtime and account for any sex selectivity by the fishing gear (Provost and Jensen, 2015).

Increased fishing pressure on a protogynous hermaphrodite can induce changes to life-history traits, such as decreasing the size and age of maturity and sexual transition (Hamilton et al., 2007; Robinson et al., 2017). Plasticity in the timing of sex change arises because females change sex earlier in life, when they are younger and smaller, to compensate for the selective removal of males (Robinson et al., 2017). This can conceivably lead to a reduction in female fecundity, due to the reduced overall body sizes of the enduring mature female population

(Robinson et al., 2017). The present study determined the length and age parameters for maturity and sex change for both *E. areolatus* and *E. bilobatus*, these life-history parameters can be used as reference points for future studies, to determine changes (if any) in these parameters relative to fishing exploitation.

In the future, if adverse changes were detected for either the maturity and/or sex change life-history parameters for these species, a management approach for protogynous fish, that aims to protect the larger reproductive individuals is to set minimum and maximum size limits (Hamilton et al., 2007). Minimum size limits aim to prevent recruitment overfishing, whereas maximum size limits aim to not only protect the large males and hopefully prevent sperm limitation, but also protect the large females with higher fecundity (Hamilton et al., 2007). Such size limit restrictions are in place for the protogynous grouper *E. fuscoguttatus* in Queensland, Australia (Pears, 2012).

4.3 Limitations of research and future directions

This study has improved our understanding of the life-history characteristics of *E. areolatus* and *E. bilobatus* from north-western Australia. The large sample sizes across a very wide length and age range, combined with gonad histology for each individual, permitted quality estimates of age, growth, reproduction, and mortality parameters for each species. The life-history data provided here will assist when considering and developing management plans for these regions and fisheries into the future. While this study was well-executed meeting all objectives, parts of the study which could have been improved upon, and possible future research directions are described below.

Sample sizes in the Gascoyne region

Sample sizes must be considered when developing studies on life history. Samples of both species were collected across their large geographical range on the WA coastline, though, due to resource limitations, a greater emphasis was placed on the sample collection from the Kimberley and Pilbara regions. This was attributable to the large commercial demersal fisheries operating within these regions which facilitated a large part of the sample collection, as well as satisfying the objective to collect representative age compositions from each of the commercial trap fisheries in north-western Australia. In the Gascoyne region, sample sizes are relatively lower than those in the other regions and consequently only the von Bertalanffy growth parameters were determined for each species from this region. Thus, additional sampling in this region of both *E. areolatus* and *E. bilobatus*, across a wide length and age range could improve upon the growth parameter estimates, as well as provide reproductive parameters and age compositions from a relatively lower exploited region, enabling a comparison to the other regions (i.e., the Kimberley and Pilbara).

Determining spawning period

Trends in the mean monthly GSIs combined with the monthly prevalence of mature developed (stage IV) and spawning ovaries (stage V) were used to determine the spawning periods of *E. areolatus* and *E. bilobatus* in the Pilbara region. The GSI data presented for these species, in chapters 2 and 3, were combined for each month across all years. This method is acceptable and was a necessity because the resolution in the data to plot GSIs separately across years was not available. To better understand variations in the annual spawning period and correlations with temperature and photoperiod, a time series of GSIs across multiple years would be beneficial. For sex changing species, such as *E. areolatus* and *E. bilobatus*, gonad histology is essential to determine sex, as macro-staging of gonads is unreliable and would certainly affect the calculation of GSIs. Consequently, the extra expense of processing and analysing additional gonad histology has limited the regions in which GSIs could be determined (i.e., GSIs were not determined for the Kimberley or Gascoyne regions). Hence, variations in the timing of gonadal recrudescence and the duration of the spawning period among regions requires further investigation for these two epinephelids.

Non-lethal epigenetic ageing for sampling fisheries closed areas

In the Pilbara region, the PDSF permits fish trawl, fish trap and line gear types. Each fishery operates within designated boundaries under specific management arrangements. A Targeted Fishery Closure (TFC) area (~2,450 km^2, see Figures 1.1, 2.1, and 3.1) was established in 1998, which is closed to all commercial fishing activities (due to its remoteness > 100km offshore effectively limits access for recreational fishers), and is located in the middle of the PDSF to maximise any potential for dispersal of eggs and larvae (Langlois et al., 2021). After only 12 years of protection, the relatively large TFC area has been shown to contribute a significantly greater biomass and abundance of inherently less productive (e.g., slow growing, longer lived) fishery targeted species (Langlois et al., 2021). The age composition data provided in this study, was derived from counting the annual growth increments in sectioned otoliths of commercially caught *E. areolatus* and *E. bilobatus*, which is a long-established fish ageing method. Unfortunately, this method is expensive, labour intensive, subject to reader experience bias, and necessitates the fish to be euthanised (Weber et al., 2022). Removing the otoliths from fish which have been commercially harvested is not problematic (Mayne et al., 2023), however in other contexts such as for aging threatened species or obtaining ages of fish from protected areas, otolith extraction could be considered undesirable (Mayne et al., 2021). An emerging alternative aging method is a molecular-based process by means of epigenetic clocks, which could be adopted to collect samples from the Pilbara TFC area. For each specimen collected, genetic material is extracted, and an epigenetic clock is developed from DNA methylation at cytosine-phosphate-guanosine (CpG) sites (Weber et al., 2022; Mayne et al., 2023). Age is subsequently predicted by calibrating the epigenetic clocks with known ages (Mayne et al., 2023), which for *E. areolatus* and *E. bilobatus* would be from age estimates derived from the otolith collections outside the TFC area, such as those from this study. Inclusion of ages for *E. areolatus* and *E. bilobatus* from the TFC area would be more representative of the region and thus improve upon the estimates of mortality at the stock level. Further, once an epigenetic clock is established, this technique could offer a non-lethal, high throughput and cost-effective age predictor (Mayne et al., 2023).

Resource partitioning between study species

This study established that *E. areolatus* and *E. bilobatus* share many similar life-history traits. At the time of sampling, both species were being fished sustainably (i.e., all point estimates of $F < M$) in the north-western Australia managed demersal fisheries, nonetheless annual catches of *E. areolatus* are considerably higher than *E. bilobatus* (DPIRD, unpublished data). Based on these higher catches and the extended natural distribution of *E. areolatus*, it is assumed that *E. areolatus* is considerably more productive than *E. bilobatus*. This study did not investigate the ecology of these two epinephelids, although subtle differences were observed in habitat preferences (e.g., *E. areolatus* was not encountered in depths < 20 m whereas *E. bilobatus* was frequently sampled in this depth). A consideration for future research would be to investigate the ecology of these two, and potential other epinephleids, using data from the large stereo-BRUVs (baited remote underwater stereo-video systems) data bank collection available via the Global Archive. The information obtained from this would not only allow an assessment of how each species utilises the available resources but would also allow the identification of any habitat and/or depth preferences of importance for each life stage. This information would be important for informing ecological risk assessments.

4.4 Conclusions

Epinephelus areolatus and *E. bilobatus* are equivalent sized groupers, attaining comparable maximum ages, that share many similar life-history traits. The life-history strategies determined in this study for each species (Chapters 2 and 3) infer relatively high population productivity and are not characteristic of those which can make a species vulnerable to overfishing (i.e., these species were not determined to be slow growing, late maturing, or exceptionally long lived). At the time of sampling, fishing mortality estimates concluded that *E. areolatus* and *E. bilobatus* were being fished sustainably in the NDSF and PDSF multispecies fisheries, implying that the indicator species management approach has been effective for the sustainable management of these species. Whilst periodic monitoring would be informative to directly confirm ongoing sustainability, it seems the indicator species management approach is indirectly maintaining sustainable populations of the second-tier non-indicator species. A study of the life history and mortality of these second-tier species, like the current study, is unlikely to be conducted periodically. Nevertheless, the comprehensive results from this study will be able to be used in future models to inform managers regarding the status of *E. areolatus* and *E. bilobatus*, as well as other second-tier species that exhibit similar life history traits.

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